II International Symposium on the Pomegranate

Edited by:
P. Melgarejo, D. Valero

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These Proceedings include 63 scientific papers presented at the II International Symposium on the Pomegranate, held in Madrid (Spain) from 19 to 21 October 2011.

Production and commerce of pomegranate and its derived products have experienced significant growth everywhere in the world. Progress has been made in cultivation techniques and some traditional problems have been solved, although new problems such as *Xanthomonas axonopodis* attacks constitute a present threat. New public and private institutions in different countries are working to improve the production and quality of the pomegranate, and new varieties are being commercialized. The cultivar is a determining factor in issues related to nutritional composition and bioactive compounds with antioxidant activity.

At the same time, significant advances in postharvest technologies have been made in the past years, specially focused on the use of technologies with natural compounds of great efficiency in maintaining the quality and safety of pomegranate fruit during prolonged storage periods.

Special importance is attached to the advance in the industrialization processes through the technologies for processing ready-to-eat arils. In the past years, new protocols for quality evaluation using sensory analysis have been developed. With respect to the relationship between pomegranate and health, recent clinical studies report that pomegranate juice or extract can reduce the progression of prostate cancer, although more studies are needed to confirm these experimental results.

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Preface

The current Proceedings include the scientific papers presented at the II International Symposium on the Pomegranate. The Symposium was organized by the Department of Plant Production and Microbiology and the Department of Food Technology of the University Miguel Hernández (Spain), and by the Mediterranean Agronomic Institute of Zaragoza, of the Centre International Centre for Advanced Mediterranean Agronomic Studies (IAMZ-CIHEAM). The Symposium was held in Madrid from 19 to 21 October 2011 within the framework of Fruit Attraction, IFEMA.

The researchers and technicians involved in pomegranate are aware of the importance of this fruit, its genetic resources as well as its health implications. The total number of papers in this volume is 63, distributed into 6 subtopics. According to the scientific papers, production and commerce, not only of the fresh product but also of pomegranate-derived products, have experience significant growth everywhere in the world. Progress has been made in cultivation techniques and some traditional problems have been solved, although new problems such as *Xanthomonas axonopodis* attacks (bacterial-type disease) constitute a present threat in Indian orchards and also in the rest of the world. On the other hand, new public and private institutions in different countries are working to improve the production and quality of the pomegranate, and as a result new varieties are being commercialized.

In addition, the cultivar is a determining factor in issues related to nutritional composition and bioactive compounds with antioxidant activity. At the same time, significant advances in the postharvest technologies have been made in the past years, specially focused on the use of technologies with natural compounds of great efficiency in maintaining the quality and safety of pomegranate fruit during prolonged storage periods. These technologies have shown their effectiveness in reducing the chilling injury symptoms when the fruit is stored under refrigeration conditions.

Special importance is attached to the advance in the industrialization processes through the technologies for processing ready-to-eat arils, which have been developed at industrial scale to assure the overall quality and safety of the product. In the past years, new protocols for quality evaluation using sensory analysis have been developed. With respect to the relationship between pomegranate and health, recent clinical studies report that pomegranate juice or extract can reduce the progression of prostate cancer, although more studies are needed to confirm these experimental results.

Dr. Pablo Melgarejo and Dr. Daniel Valero

Inaugural conference

The pomegranate tree in the world: Its problems and uses

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Abstract. The pomegranate tree has been known since ancient times and has traditionally been cultivated in the Near East, later spreading to the rest of Asia and the Mediterranean. It is now cultivated in all five continents: firstly, because its cultivation offers exceptional prospects, it is profitable, it can be grown in arid regions, it has less hydric requirements than other crop types and it can grow and produce in conditions which would not be possible for other more important fruit trees to do profitably; secondly, because, in the last ten years, it has been found to have properties which can prevent and possibly cure some illnesses. Knowing and obtaining new varieties which can provide crops over a longer period of time and therefore meet market demands better, with quality fruits for different uses, as well as the discovery of its numerous nutritious, pharmacological, functional and cosmetic properties has led to this fruit tree becoming more and more in demand by farmers and its fruits and derived products becoming more used by consumers. Undoubtedly, of all the factors mentioned above which have aroused such a great interest in this crop throughout the world, it is the potential of its fruits to prevent/cure illnesses that has most influenced its cultivation. This factor has led to a great interest for the industrialization of its fruit (juices, tubs of grains, dehydrated seeds, rind, etc.), which has contributed remarkably to the increase of crop areas and a rise in farmers' income. Other products being commercialized are extracts for manufacturing capsules, obtaining oils or for manufacturing cosmetic products. All these new products are becoming widely accepted among consumers and industrialists who see this species as a new market opportunity. The world surface area dedicated to the cultivation of pomegranate trees is more than 300,000 ha and world production is probably higher than 3,000,000 t; the main production countries being India, Iran, China, Turkey and the USA.

Keywords. Pomegranate tree – *Punica granatum* – Production – Industrial processing – Marketing.

I – Origin, botany and biodiversity

1. Origin

The pomegranate tree has been cultivated since ancient times and is one of the biblical fruit trees, such as the vine, the olive tree or the palm tree (also cited in the Koran). According to the Russian scientist Vavilov its origin centre is Centre IV, the Near East, which includes the interior of Asia Minor, Transcauscasus, Iran and the highlands of Turkmenistan, and is the centre for other fruit trees such as, fig, apple, palm, quince, cherry, almond, hazelnut, chestnut, etc., as well as other plant species (Sánchez-Monge, 1974). Its cultivation spread from this region to the rest of the Mediterranean countries, India and China. The Spanish took it to America, where, especially in the last 15 years, it has become of great importance and the cultivated surface is continually expanding.

2. Botany

The pomegranate tree (*Punica granatum* L.) belongs to the Myrtales order and to the Punicaceae family, which comprises only the genus *Punica* L. (Font Quer, 1959). The two best known species of this genus are:

Punica granatum L.: the pomegrante tree, grown for its fruits.

Punica nana L.: the dwarf pomegranate tree, for ornamental use whose fruits are not edible. Sometimes this species is considered as a variety of *P. granatum*, called *P. granatum* var. nana L.

Other authors (Zukvskij, 1950; Moriguchi *et al.*, 1987; Guarino *et al.*, 1990) have pointed out that the *Punica* genus includes two species: *P. protopunica* Balf. [endemic to the island of Socotra (Yemen)] and *P. granatum* (Mars, 1998), presuming that the former is involved in the origins of the cultivated pomegranate tree (*P. granatum* L.).

Punica granatum L. is a diploid species whose somatic number is 2n = 16, and haploid chromosomes = 8 (Westwood, 1982) or 2n = 16 or 18 (Mars, 1998).

3. Biodiversity

Its centre of origin is the Near East (Vavilov's Centre IV) (Fig. 1), spreading to different regions where it is cultivated and where it has a wide genetic diversity as a consequence of the propagation from its seeds, which are scattered by man, by birds or other animals, and germinate easily.
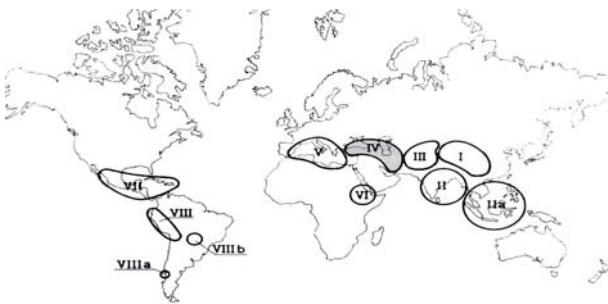
Fig. 1. Centres of origin and diversity of cultivated plants, according to Vavilov. *Source: Sánchez-Monge (1974).*

The wide diversity that the species presents is apparent from the number of varieties described in different countries of the East, the Mediterranean area and the West. In fact, Spain has the main germplasm bank in Europe with more than 104 accessions, although there are also large collections in other countries such as, India, Turkmenistan, Iran, etc.. For example, in India there is one with more than 760 accessions or in Turkmenistan, whose Experimental Station for Plant Genetic Resources, created in 1934, has 1,117 accessions (Mars, 1988).

II – Economic importance

1. Importance of its cultivation in Spain

Spain is the main producer and exporter of pomegranates in Europe. Spanish yield, 22,311 t (MMARM, 2010), is fundamentally based in the province of Alicante (91.8%) in three municipalities, Elche, Albatera and Crevillente in order of importance, which reflects its socio-economic importance for these areas. However, these official figures do not coincide with those declared by producers and exporters; the most important association of pomegranate producers and traders declared they had yielded and traded 45,000 t in 2010, which greatly differs from official figures.

The pomegranate tree is able to tolerate soils and saline water, soils with poor drainage and also shows an extraordinary resistance to drought. It has the minimal requirements which, in certain periods, are necessary to obtain quality fruits and abundant crops.

2. Evolution of its cultivation in Spain

Compared to other fruit trees, the pomegranate tree has not shown a remarkable increase in the last few decades, during which a decrease in the surface dedicated to their cultivation has been registered for certain periods. Figure 2 shows both the evolution for the total regular cultivates area and the total producing area. Since 1970, the curves for the planted surface and the production surface have separated, with figures of around 100 ha in 1975. Between 1970 and 1980 the cultivated area decreased from 2,000 to 1,500 ha., but since 1980 there has been a high increase, reaching a historical peak of 3,300 ha in the year 2000 and in 2009 the cultivated area reached 2,285 ha (MAPA (1999); MMARM (2010).
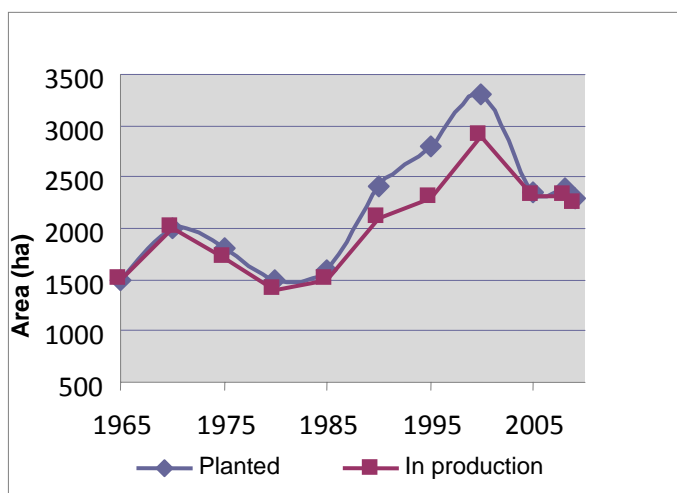
Fig. 2. Evolution of national cultivated and producing area (1965-2009).
Sources: MAPA (1999); MMARM (2010).

This increase is mainly due to the progressive evolution of the price obtained by farmers; illustrated in Fig. 3.

Farmers who belong to trading cooperatives or Agricultural Transformation Companies usually receive around 0,06-0,10 € more per kilogram, regardless of the variety cultivated.

With respect to prices received by farmers (Fig. 3), without taking into account the price of the different varieties, it should be noted that the price indicated in the graph does not correspond to the weighted average value.

The average national yield: 10,005 kg/ha (MMARM, 2010), is very low, although there are plantations whose production potential reaches 30,000 t/ha.

The main varieties grown in Spain are 'Mollar' and 'Valenciana' and more recently the variety 'Wonderful' has been introduced, which is one of the most cultivated and well-known variety in the world, with the exception of the numerous varieties cultivated in Iran, India or China.

In 2009, the average price received by Spanish farmers for their pomegranates was 0,60 €/kg (MMARM, 2010). The values of Spanish production are shown in Fig. 4.
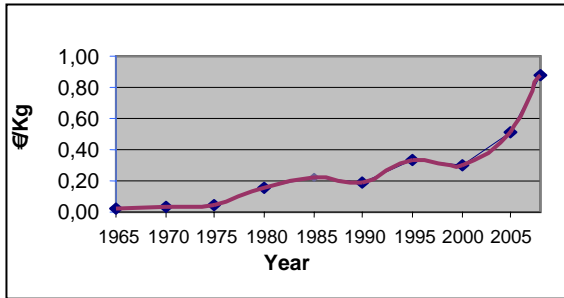
Fig. 3. Average price received by farmers (€/Kg) for the period 1965-2009.
Sources: MAPA (1999); MMARM (2010).
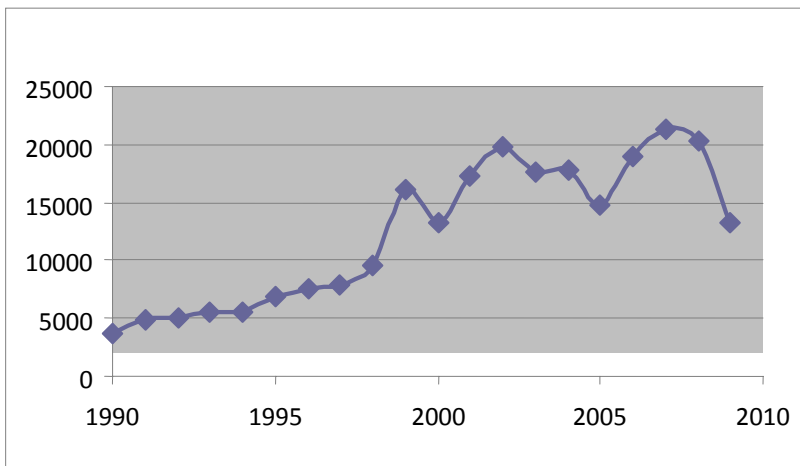
Fig. 4. Field price (thousands of €) of Spanish pomegranate production (1990-2009).
Source: MMARM, 2010.

3. Evolution of Spanish production and trade

In the context of the evolution of national pomegranate markets, the two that most stand out are 'Mercamadrid' in Madrid and 'Mercabarna' in Barcelona. The quantity sold on the national market is less than 45% of the total amount traded, and a greater percentage is exported (Melgarejo and Salazar, 2003).

The export figures corresponding to 2009, presented by ICEX (2010) on their website, do not appear to coincide with the real exports. For this reason, a survey was carried out in 2010 for some of the pomegranate export companies, and 13 of them alone were found to have exported 16,916 t in 2009. Therefore, considering that other export companies in the provinces of Alicante and Murcia were not consulted, it was estimated that exports in 2009 must have been

higher than 18,000 t. Figure 5 illustrates the evolution of Spanish pomegranate exports based on the data available up to 1998, and the estimations for 2009.

4. Pomegranate exports and imports

As seen in Fig. 5, pomegranate exports have experienced a huge increase from 1965 to 2009 (2010 survey), going from 4,500 to 18,000 t.

Monthly pomegranate imports for the period 2002-2009 (ICEX, 2010) have increased notably, reflecting the market growth for this fruit, which is becoming more and more appreciated by consumers. The monthly analysis of imports during 2009 (ICEX, 2010) indicates the countries we import this fruit from and the amount (Figs 6 and 7).
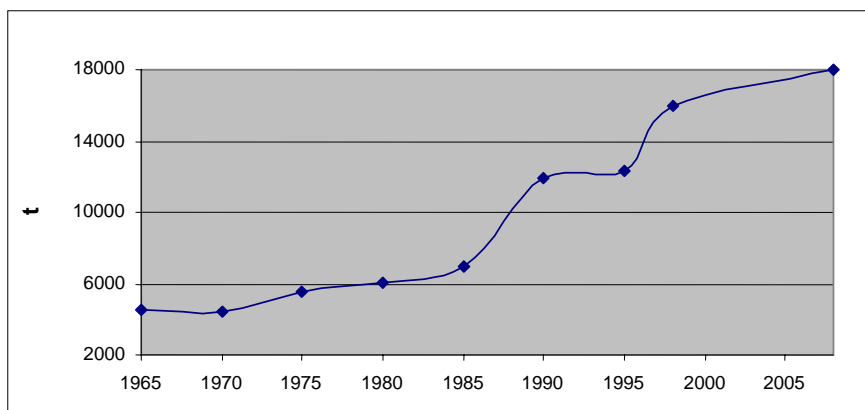
Fig. 5. Pomegranate exports for the period 1965-2009 (t). *Source: Melgarejo and Salazar (2003); 2009: Own estimation.*

Fig. 6. Pomegranate imports for the period 2002-2009 (t).

	Marru	Perú	Turqu	Chile	Israel	India	Otros
☐ Serie1	99	236	38	90	356	57	1
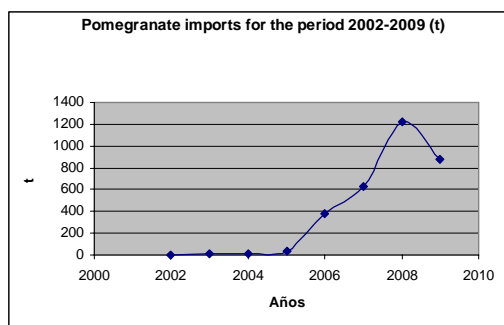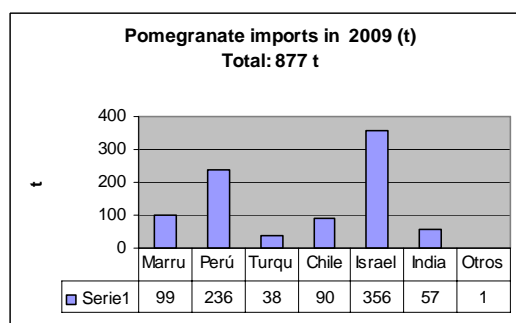
Fig. 7. Pomegranate imports in 2009 (t). Total: 877 t.

5. World pomegranate production

In spite of the fact that in general this sector in Spain has experienced a good period, if steps are not taken to promote the consumption of this product, the increase in production could

negatively affect the price received by farmers. In 2009, Spain exported more than 18,000 t of pomegranates (estimation from our survey), which would mean an export percentage of around 70% of the production. On the other hand, Spain now has many serious competitors in the European and world market such as, Iran, India or Turkey (leading producers) or other countries that have recently joined this sector (Chile, Peru and Argentina) who export to the USA and Europe. Some countries on the American continent are growing considerably, especially the USA, which exports to different countries. Other countries like South Africa and Australia also appear on the list of pomegranate producers. In California pomegranates are harvested from August to mid-November, as in Spain. The USA exports (17,000 t) to Japan and also to Canada, Mexico and England, and 80% of its production is for processing juice, which reached 0,35 $/kg in 2006, (Simonian, 2007) – a very interesting price for its industrial processing. Another area of reference in South America is Chile, which exports to the USA and Europe; its exports are increasing rapidly – harvesting here from March to April.

There are no reliable data about world cultivated area and pomegranate production; probably because it is considered a minor fruit tree. From the data provided by different researchers and associations, an estimation was made about world pomegranate production (Fig. 8). The data provided does not, therefore, correspond to an exact year and constitutes an estimation based on different sources (Elfalleh, 2011; Chambers IV, 2011; Droguoudi, 2011; Syouf, 2011; Gozlekçi, 2011; Mirdheghan, 2011; Haidioui, 2011; Frank, 2011; MacLean, 2011). The estimated productions of some countries are grouped in 'Others'.
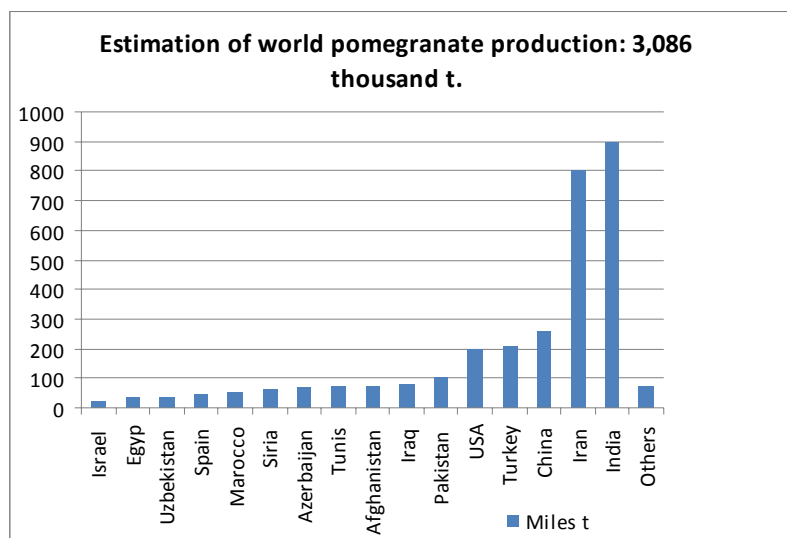
Fig. 8. Estimation of world pomegranate production: 3,086 thousand tonnes.

The total area dedicated to pomegranate cultivation in the world is well above 300,000 ha, of which more than 76% is found in five countries (India, Iran, China, Turkey and the USA) (Fig. 8). However, the countries that fall behind (Spain, Egypt and Israel), with a surface between16,000 and 2,400 ha, are the ones that have developed much more in exports, research, market development and new varieties (Quiroz, 2009).

We should give a special mention to India where bacterial blight, a disease caused by *Xanthomonas axonopodis* pv. Punicaceae*,* is causing serious damage to crops. According to different sources and the National Research Centre on Pomegranate, this bacteria, considered

to be of little importance a few years ago, is capable of causing yield losses 30% to 90%. This pathogen is currently affecting exports from India notably.

4. Crop perspectives

Due to the high increase that this crop has experienced in the last few years, it has become apparent that steps need to be taken towards reducing the risk associated with a faster growth in production than in consumption, which can endanger the economy of agricultural exploitations and companies that trade in this sector. However, the revolution in research studies and in the development of new products has given a boost to this millenary crop, providing incentives for obtaining new varieties and their technological development. This, in turn, enables this crop to be maintained profitably; either through new marketing methods for the fresh product or through derived products, which have aroused a lot of interest among scientists and consumers.

In some areas of Spain, where pomegranates are not traditionally grown, such as in the region of Extremadura and some Andalusian provinces, trials are being carried out on large pomegranate plantations. This is done either as an alternative to other fruit trees or to widen the production calendar and the marketing campaign. Therefore, making a better use of industrial facilities and opening up new markets. Given the profitability of this crop, a similar situation is occurring in other parts of the world, such as in the state of Florida (USA), where they have been establishing commercial plantations since 2009 following the example of California (Castillo, 2011). They are even considering substituting citric groves for pomegranate orchards.

III – Plant material and cultivation techniques

1. Plant material

The genetic diversity of the species is wide, but studies on plant material selection and characterization are very recent. Throughout history man has always tried to select the best individuals; the quality of plant material is a current problem which was referred to by Columela (1 AD.) in his V book "The pomegranate and its remedies", where he highlighted the problems of split damage, acidity and seed hardness. Alonso de Herrera (1513) in his article "General Agriculture" outlined the same problems described by Columela, but highlighted its easy adaptation to all types of soils and climates, indicating the advantages and unsuitability of each one of them and classifying pomegranate into sweet, sour and soursweet. He also described some crop techniques, the medicinal properties of the flowers, which doctors have named then *Balaustías* and are known in pharmacy as *flores granati*, as well as the juice, the rind and fruits. However, scientific studies about this species were really scarce until 30 years ago, although more recently there has been an extraordinary scientific interest in its properties for treating many problems which affect human health.

Its resistance to salinity makes it especially suitable for cultivation in arid and semi-arid regions. This resistance to salinity (Sánchez-Capuchino, 1986) is only equalled, by the fig tree (*Ficus carica* L.), prickly pear *(Opuntia ficus-indica* L.) and the jujube (Z*iyzphfus vulgaris* L.), species cultivated in Levant and the Southeastern Spain, and only surpassed by the date palm (*Phoenix dactylifera* L.) which has maximum resistance. It is equally resistant to iron chlorosis, which is only equalled by the fig tree and in some cases by the olive tree (*Olea europea* L.).

In Spain, two varietal groups of commercial interest stand out – the "Valencianas" and "Mollares", although different studies have highlighted that outside these groups there is an interest in an abundance of Spanish varieties. The "Mollar" group is the most important, comprising many individuals characterized for their excellent quality, good productivity and harvesting period between 25 September and 15 November. The "Valencianas" group, also

comprising a great number of varieties, is characterized for its early harvesting period, between 5 August and 20 September, and its significantly smaller trees. There are also other less known varietal groups, which are grown locally and whose cultivation is very old, with many different denominations such as, Piñonencas, Piñón Tierno, Casta del Reino, Albar, San Felipe, etc. As with the Mollar and Valenciana groups, within these generic denominations there are diverse individuals whose characteristics sometimes have little in common.

Traditional problems of seed hardness, acidity or split damage have been overcome with the use of clones and adequate cultivation techniques. However, the fundamental problems today are: the use of varieties which are large and have a red interior and exterior; and in particular how to increase the annual average yield, whose do not surpass 11 t/ha. In this context, it is apparent that through current knowledge of cultivation techniques and new plant material selection, both the quality of fruit and yields can be increased notably.

On the other hand, besides native plant material cultivated in Spain in the last few years, new varieties of different origins have been introduced, such as 'Wonderful' (several clones) and others. More than 500 varieties of pomegranate in the world have been cited, which clearly illustrates its genetic and therefore, the possibilities for obtaining new individuals which would meet the demands from the different sectors which use this fruit.

Photograph 1. Clon C 26.

Photograph 2. Valenciana fruit.

The 'Wonderful' variety, one of the most widely grown in the world (USA, Israel, Greece, Chile, etc.), has sour to soursweet seeds with a woody kernel, depending on the harvesting period. It is an attractive red and is suitable for industrial use but not for fresh consumption; its yield is usually medium to low, and is not generally higher than 18,000 kg/ha.

Currently, not only can varieties be selected for cultivation because of their organoleptic qualities and their unpleasant taste for fresh consumption, but they should also be selected for their aptitudes for industrial processing such as, for nutritional use or for the prevention or cure of illnesses - as used to be in cultures such as the Hindu.

The program for selecting and obtaining new varieties in Spain began during the eighties of the last century by the Department of Plant Science at the Polytechnic School of Orihuela (University Miguel Hernández of Elche), which has now been extended to other state and private centres. This means that soon new varieties will be marketed to meet consumer, industrial and productive demands. Some of these new varieties are estimated to have a potential yield of more than 40,000 kg/ha.

2. Cultivation techniques

The main problems that affect this species generally arise from the lack of selection from the existing plant material, and the lack of research teams which dedicate time to their study. In the last few years, however, as a result of the revaluation of this fruit, there has been an increase in the number of research teams which study plant improvement, cultivation techniques and the properties of the fruits for dietary and medicinal uses. Consequently, traditional cultivation techniques have been improved and many advances have been made; for example, in propagation and cultivation, fertigation, snake control or the use of plastic mulching for the soil.

Photograh 3. Rouge fruits (Marrakech).

Photograph 4. PTO7 fruits.

It is usual for many technicians and farmers to consider the pomegranate tree as a marginal species and easy to manipulate. However, reality is quite different and, if they have a good knowledge of the plant material, its destination, cultivation techniques, conservation and manipulation techniques and industrial processing techniques, crops can be obtained within a wider harvesting period than traditionally done with better quality fruits, crops that are as important as those from other fruit trees, fruits which are suitable for each type of use or destination, and finally as high a profitability as for the more valued fruit trees. Furthermore, this fruit tree should be considered as having exceptional characteristics for preventing and/or curing illnesses, a fact which should be diffused and which would undoubtedly place it in a very different position to the one it is in now.

Photograph 5. Clon C11.

Photograph 6. Clon C26.

The problems and actions for increasing the profitability of pomegranate cultivation and how to exploit it are the focus of new studies about genetic improvement, improving cultivation techniques extending the production and marketing period, increasing the size of trading companies, enabling them to keep up to date technologically and to have greater control over supply, incentives for research on obtaining new industrial products (of nutritional, pharmacological and cosmetic interest), and to diffuse the benefits that the different parts of the pomegranate provide for humans. All of this would not only permit the exploitation of the better quality fruits, but also of the fruits that have an undesirable size calibre defect or are not in good condition.

IV – Industrial processing and obtaining food products

Until 15 years ago, the only industry in Spain for processing pomegranates was for producing a fresh product – not even any natural pomegranate juice could be found in the supermarkets. Some years earlier there had been a small production of pomegranate juice, but the only product that was generally sold with the name of pomegranate was just an artificial drink obtained from essences, which did not come from this fruit,. However, there are many traditional recipes for its exploitation; it has been used in the pharmaceutical and chemical industries, and liqueurs from this fruit have been commercialised.

Currently, the main use of the pomegranate is for its fresh consumption, although in recent years there has been a continual increase in the industrial production of juices and extracts from its different parts. Its ornamental use is also becoming more and more important, using both its fresh and dry fruits as a plant. As an ornamental plant, in many cases the adult trees *Punica granatum* L. from old plantations are usually used, and in other cases, the dwarf trees (*Punica granatum* var. Nana) are used, as well as the so called 'double flower' pomegranate trees.

The tree has also been used since ancient time, for its wood , flowers and root bark, which is one of the most used parts, and which traditionally is known to contain 5 alkaloids (pelletierine, pseudopelletierine, isopelletierine, pseudopelletierine and methyl pelletierine) in quantities that vary between 0.5 and 0.9%, which are also found in a lesser quantity in the trunk and branch wood bark. Tanaka *et al.* (1985) later discovered a new alkaloid called Punicafolin. The use of the bark as a vermifuge has been extensive, since it also contains tannins mixed with the alkaloids, and therefore, it reduces intestinal absorption. This remedy was already known in Egypt more than 2,000 years ago. There are many other remedies described from different parts of the pomegranate.

Different products of nutritional, pharmaceutical or cosmetic interest can be obtained from the fruit; they are: whole seeds for fresh consumption or prepared in different ways, juices, syrups, alcoholic drinks, jams, jellies, dehydrated seeds, nutritional fibre, dry rind for making infusions (as an astringent or as a vermifuge) and pomegranate oil. Both the seeds and the rind contain different products which are useful for the prevention of illnesses, and have interesting properties for diet: fatty acids, anthocyanins, tannins, punicalagins, ellagic acid, vitamins, minerals, crude fibre, sugars, organic acids, etc.

1. The pomegranate as a functional food

A simple definition of functional foods is generally described as follows: "Foods which are consumed as part of a normal diet and contain biologically active components which benefit health and reduce the risk of illnesses". Among these foods, we can highlight those that contain certain minerals, vitamins, fatty acids or dietary fibre, foods which have had biologically active substances added to them such as, phytochemicals or other antioxidants and probiotics which have live cultures of beneficial microorganisms.

The pomegranate can be described as a fleshy berry fruit which generally contains 8 carpels

where the seeds are found (edible portion), which represent between 58 and 75% of the fruit, depending on the varieties. The carpel membranes and the rind make up about 25-42%. The woody portion of the seeds varies between 5 and 15%. The whole fruit is made up of approximately 80% of water.

The seeds have a woody consistency, a fleshy or pulpy testa with a prismatic shape, no albumin, straight embryo, and cotyledons wrapped around one another. They are pink, deep red or white and are very juicy. The fact that we usually eat the seeds whole (with the woody part) is an important differential aspect in comparison to other fruits, which we do not eat the seed of. The seeds are rich in water, sugars, crude fibre, polyunsaturated fatty acids (two essential), vitamin C and potassium, and are low in sodium and calories. It is the ellagitannins and the anthocyans which give it its antioxidant properties.

In a recent study about quality parameters, sensorial analysis and the volatile composition of 9 cultivars of Spanish pomegranates, 21 volatile compounds were found in fresh pomegranate juice, including, aldehydes, monoterpenes and alcohols. The most abundant compounds were hexanal, limonene, trans-2-hexanal and cis-3-hexanol (Melgarejo *et al.* 2011). Some of the most significant compounds to be found in the pomegranate are described below.

A. Anthocyans

Anthocyans are considered as responsible for the red colour of the pomegranate and its seed, which is an important quality attribute. Red depends on the concentration of the anthocyans and on the type of anthocyan. 6 anthocyans have been identified in the pomegranate as being responsible for the colour of the pomegranate juice: delphinidin 3-glucoside and 3.5-diglucóside; cyanidin 3-glucoside and 3,5-diglucoside y pelargonidin 3-glucoside and 3.5-diglucoside (Du *et al.*,1975). The presence of these phenolic compounds lies in their antioxidant action (they protect them against free radicals, delaying the aging process of the cells). For the last few years, this aspect has been an important area of study in a great number of fruits, including the pomegranate.

In a study of 6 cultivars from the 'Mollar' group, it was observed that the cultivar induces differences in the chemical composition of the juice. Glucose and fructose were the dominant sugars. The predominant organic acid was citric acid and the main anthocyan was cyanidin 3-glucoside, followed by diglucoside, cyanidine3, 5-diglucoside (Legua *et al.*, 2011).

Photograph 7. Juice samples for analysis.

The free radical capturing activity of these flavonoids was demonstrated by Espín et al., 2000. This means that 10% of the antioxidant capacity of pomegranate juice is due to the presence of these polyphenols (Gil et al., 2000). The antioxidant capacity of pomegranate juice is three times higher than that of red wine and green tea (Gil et al., 2000).These compounds can be also used as natural colouring by adding them to other foods.

B. Fatty Acids (FA)

We are more than aware of the importance of unsaturated fatty acids in diets. The pomegranate is a fruit rich in seeds that contain a woody portion of between 5% and 15% which is rich in fibre and fat. With respect to FA, one of the differences between the pomegranate and other fruits lies in the fact that we eat the seeds. The woody part contains fat which vary between 37 and 143 g/kg of the fruit (Melgarejo *et al.* 1995). This fatty content and its composition in fatty acids is a quality parameter for the consumer, especially the ratio *saturated fatty acids/unsaturated fatty acids*. The composition of essential fatty acids (linoleic, linolenic and araquidonic) are of great importance especially for their content in polyunsaturated fatty acids, which, as they greatly reduce the levels of HDL-cholesterol, plays an important role in the prevention of cardiovascular diseases and other heart problems (Grande 1988; De Hoya and Mata 1989). The composition of fat and fatty acids of the seeds not only helps us to establish the chemotaxonomic relations between the varieties studied (Sunder Rao *and* Sino, 1992 and Onyencho Hettiarachchy, 1993), but they are also of great interest for diet and the prevention of some illnesses. The study about the fat and fatty acid composition of 5 Spanish varieties of pomegranate (Melgarejo *et al.*, 2001; Hernández *et al.*, 2011), grown under homogenous conditions (2 sweet from mid-season – ME16 and MA2, one sweet early – VA1, one sour sweet - PTO8, and one sour, BA1), gave the following results (Table 1).

Table 1. Total fat content (g/kg dry matter) and fatty acid composition of 5 Spanish varieties of pomegranate

	ME16	MA2	VA1	PTO8	BA1
Lipids[1] (g/kg)	80.92±18.2	79.97±22.66	68.97±11.49	130.95±89.3	104.9±7.31
(%) Palmitic ($C_{16:0}$)	3.83±0.96	4.08±1.25	3.63±0.33	4.30±1.66	2.99±0.14
(%) Stearic ($C_{18:0}$)	2.38±0.89	1.93±0.28	1.6±0	2.62±1.05	1.64±0.16
(%) Oleic ($C_{18:1}$) (9)	4.82±1.97	5.83±3.56	4.39±1.13	5.70±2.65	4.09±0.10
(%) Oleic ($C_{18:1}$)(10)	1.09±0.53	1.02±0.43	0.84±0.21	0.79±0.19	0.61±0.049
(%) Linoleic ($C_{18:2}$)	7.74±3.63	8.54±5.6	7.3±2.07	7.48±3.46	4.98±0.13
(%) Punic[2] ($C_{18:3}$)	66.76±15.4	75.28±11.06	78.5±1.96	75.39±11.39	79.29±0.077
Saturates	6.21	6.02	5.23	6.92	4.63
Monounsaturates	5.91	6.85	5.23	6.49	4.7
Diunsaturates	7.74	8.54	7.3	7.48	4.98
Triunsaturates	66.76	75.28	78.5	75.39	79.29
Unsaturates	80.41	90.67	91.03	89.36	88.97
Saturates/unsaturates	0.077	0.066	0.057	0.077	0.052

[1]Values are averages from three determinations during two consecutive campaigns (1995 and 1996). The number in brackets which follows the monounsaturated fatty acid indicates the double bond position.
[2]Punic acid $C_{18:3}$ (linolenic) with double bonds in position 9, 11 and 13, without determining the geometrical configuration.

Punic acid was shown to have anti-atherogenic effects after a 4 week application in patients who received 400 mg twice a day, in comparison with those who received a placebo with a statistically significant HDL_C reduction, at 95% confidence. Seric cholesterol, LDL cholesterol,

glucose concentration and the variables of corporal composition did not experience any changes (Mirmiran *et al.*, 2010). The iodine index is around 164.

Oil extraction from seeds can be achieved through several procedures. Currently, the cold pressed procedure in a screw press does not exceed 50-60% of the total content, while extraction through supercritical CO_2 can reach 98% seed oil, and this is therefore the method most used (Sánchez, 2009). At present, several brands of pomegranate oil are marketed. It has a f 266.7 cps viscosity (soya has 42.4 cps) (Sánchez, 2009), and therefore, as a thick oil, it is adequate for cosmetics. Its properties for topical and internal uses are as indicated in Table 2.

Table 2. Uses and properties of pomegranate oil

Topical use	Internal use
Regeneration of epidermis	Chemoprotective effect
Anti-inflammatory effect	Reduction of lipose tissues in the body
Anti-carcinogenic properties	Normalizer of lipid metabolism and cardiovascular protection

Source: Sánchez, 2009.

C. Crude fibre (CF)

The CF content in Spanish pomegranates varies between 5 and 22%, and those whose crude fibre content is lower than 9% have a soft kernel. The edible portion of this fruit can be considered as a source of natural fibre.

Fibre is found in the woody portion of the seeds. Drinks or extracts that do not contain this part will not have this fraction of fibre.

D. Tannins

Tannins are substances found in plants and have a polyphenol structure, an astringent flavour and are soluble in water, alcohol and acetone. They are used to tan skins because of their capacity to precipitate proteins. The pharmacological actions they present come from their capacity to form complexes and to precipitate metals, alkaloids and proteins (Arango, 2010):

(i) They are astringent and anti-diarrhoeal, and include and precipitate proteins present in secretions.

(ii) Antimicrobial and anti-fungus.

(iii) Antidotes for poisoning from alkaloids and heavy metals. Toxicity is low and arises from the possible gastric intolerance and constipation that they can cause.

Pomegranate rind contains hydrolyzable tannins called ellagitannins. As they are hydrolyzed in the body produce ellagic acid. It is a substance which promotes apoptosis (natural cell death) of cancerous cells without harming normal cells. It is a potent antioxidant and anti-carcinogen, which protects cells against the harm caused by free radicals and inhibits DNA mutations. Other functions are (Sánchez, 2009): improved capillary activity and strengthening of membranes, softens skin and improves elasticity, reduces retinopathy and improves vision, reduces varicose veins, helps improve cerebral functions and combats inflammation in arthritis.

Ellagitannins can be transformed into what could be the most active anti-inflammatory compound in healthy subjects who consume pomegranates. While for inflammation of the colon, the effects could be due to the non-metabolized fraction of related ellagitannins (Larrosa *et al.*, 2010).

Tannins are found in the pericarp of the fruit and also in pomegranate rind (Cortex Fructus Granati), and are used against diarrhea.

Currently, different products obtained from pomegranate rind are being marketed. They are extracts with functional properties which possess punicalagins and ellagic acid. Some of these extracts have concentrations from 40 to 90% of ellagic acid.

Punicalagin is the polyphenol with greatest molecular weight, which is hydrolyzed to ellagic acid and is metabolized in the intestinal tract giving urolithins. The punicalagins are compounds which have the most antioxidant capacity, or free radical captors, and are responsible for approximately 50% of this activity in pomegranate juice, followed by other hydrolyzable tannins (33% of the total activity), and to a lesser extent ellagic acid (3%) (Gil et al., 2000; García-Viguera et al., 2004)

Main functional properties of punicalagins are (Sánchez, 2009): (i) powerful antioxidant effect; (ii) anti-carcinogen; and (iii) protector of cardiovascular system.

Recent studies about the organic acid composition in pomegranate juice show citric and malic presence (Melgarejo et al., 2000), and the presence of quinic acid has also been described as the second in importance (Poyrazoglu *et al.*, 2002; Hernández *et al.* 2011).

The dried rind has been also used for making extracts, and is used fresh as a food for livestock as it has been shown that weight gain and conversion rate improve from the functional effect of its active components (Sánchez, 2009).

2. Products derived from pomegranates

Some of the products marketed are the following:

(i) Juices, widely marketed in different countries.

(ii) Liqueurs, "wines" and vinagers.

(iii) Grains in tubs (minimally processed). Commercial production is incipient but with a great future.

(iv) Dehydrated seeds. They are marketed in Spain and in other countries and constitute an interesting option because of their cooking possibilities and easy conservation.

(v) Jams, preserves, jellies, liquors and other drinks; they have been traditionally homemade and have a great future in the markets.

(vi) Pomegranate extracts. This section could include a wide range of extracts, used as cosmetics, food supplements, dietetic supplements and nutraceuticals

(vii) Others.

Recent scientific and medical research indicate that pomegranate juice has anti-bacterial, anti-virus, anti-carcinogen and anti-inflammatory substances, as well as substances which control cholesterol and prevent cardiovascular problems.

However, it is still too early to accept some assertions about the properties of pomegranate based compounds, since some studies have been carried out *in vitro* and not on humans. Some studies on humans have already achieved positive results. On the website: http://www.healthdiaries.com/eatthis/11-health-benefits-of-pomegranate-juice.html, eleven health benefits from pomegranate juice are given (fight against breast cancer, prevention of lung cancer, slowing down of prostate cancer, maintenance of stable levels of PSA in men, protection from neonatal brain, prevention of osteoarthritis, prevention of Alzheimer disease, reduction in cholesterol, decrease in blood/artery pressure, prevention of dental plaque and reduction in cardiovascular risk).

Photograph 8. Minimally processed.

Photograph 9. Jam and liqueur.

Photograph 10. Cosmetics.

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Session 1
Economics and marketing

Economic prospects of pomegranate growing in the Spanish Mediterranean region

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Abstract. The objective of this paper is to analyze origin price trends, as well as to determine the profitability of Spanish pomegranate farms through the evaluation of investments. This analysis is performed in parallel with mandarin, since citrus is one of the main tree crops with which it competes and shares territory. Although farmers in the Spanish Mediterranean have preferred to choose citrus in recent decades, its drop in value is encouraging the choice of alternative tree crops, and there will be areas, like the one studied here, where the interest in species with more favorable prospects, such as the pomegranate, will increase. This is possible if demand grows, supported by the notion that it is a healthy or functional food.

I – Introduction

Pomegranate growing is chiefly centered in the southeastern Spanish Mediterranean region. Both surface area and production boomed nationally in the early eighties, leveling off in subsequent years (MARM, 2011). Alicante is the province with the strongest tradition in pomegranate cultivation and this is where most Spanish pomegranates have been grown for many years (CAPA, 2011). This tree crop is well established in the main pomegranate growing regions due to the fact it is well adapted to the agro-ecological conditions. The pomegranate is resistant to the hot dry climate and poor soils, tolerating calcareous soils with a degree of salinity (Melgarejo and Martínez-Valero, 1992). It is clearly an agronomic survivor in these areas, and has always coexisted with other intensively grown tree crops, such as citrus. Currently, international demand and a good market position in Spain (Mira, 2011) provide highly favorable expectations for this tree crop; however, this is dependent on its viability and profitability in the field.

Within this context, the objective of this paper is to analyze price trends, as well as to determine the profitability of pomegranate farms through the evaluation of investments. This analysis is performed in parallel with mandarin, since citrus is one of the main tree crops with which it competes and shares territory. Taken together this indicates the economic prospects one may expect of Spanish pomegranate growing.

II – Economic analysis of Spanish pomegranate growing

The analysis of pomegranate cultivation, and its comparison with mandarin, will be addressed taking into account the technical and economic aspects summarized in Table 1. On the one hand, an analysis has been made of the variation in prices received by farmers, and secondly, the profitability derived from the business after an investment project, such as tree crop planting and subsequent husbandry.

Table 1. Technical and economic aspects included in the viability analysis

	Pomegranate (Caliber C14)		Mandarin
Farm size (ha)	5		5
Planting distance (metros)	4x3.5		5.5x3.5
No. trees/ha	620		520
Study area	Southern Alicante		Northern Murcia
Data sources			
Varieties analyzed	Pomegranate "Mollar de Elche"	Pomegranate "Valenciana"	Manderin "Clemennules"
Data source	Statistics (CAPA, 2011) and questionnaire answered by technicians and producers		
Prices obtained. Updated June 2011			
Prices	Post-harvest	Post-harvest	Pre-harvest
Study period	1986-2010 (24 yrs)	1980-2010 (21 yrs)	1989/90-2010/11 (21 harvests)
No. of observations	205	104	146
Investment data			
Investment (€/farm)	83,661		134,196
Production interval (kg/tree)	3.5 - 44 [†]		3 - 75.72

[†] Harvest "Mollar de Elche", the "Valenciana" variety is considered with half this average yield.

1. Price trends

The prices received by farmers, which represent the main component of agricultural income, decreased and likewise there was no improvement in the payment of quality selection, which may indicate a setback for Spanish Mediterranean fruit growing and citriculture (Caballero *et al.,* 2010).

Figure 1 shows the price trends of the two species analyzed, pomegranate and mandarin, including the trend curves, with significant quadratic fitting. The equations show that the price trend has been upward for pomegranates. The "Valenciana" variety has fetched an average price, over the series, of 0.824 €/kg with an increase of 6.5%. However, the situation of the "Mollar of Elche" variety has been much better, which although starting off from lower values, 0.52 €/kg, has risen by almost 49%, representing an annual increase of over 2%. The situation has been quite different for the "Clemennules" mandarin, which is the most representative variety of the Spanish citrus industry, with the whole series fetching an average price of around 0.37 €/kg, with a decrease of 19%, *i.e.,* a loss in value close to 1% per annum.

2. Profitability analysis for pomegranates and mandarins

The financial analysis involving the determination of income generated by the project activity minus the necessary out-payments, updated, determine the cash flows throughout the duration of the project (a total of 25 years for the two crops). The values introduced for both types of farms are listed below:

Investment: Involving the building of an irrigation reservoir, installing a drip irrigation distribution network in fields, machinery and tools, as well as all plantation costs.

Fig. 1 a) "Valenciana" pomegranate

Data, harvests from week 37 (1989) to week 40 (2010)

Fig. 1 b) "Mollar de Elche" pomegranate

Data, harversts from week 46 (1986) to week 50 (2010)

Fig. 1 c) "Clemennules" mandarin

Data in fortnight, harvests from 1989 to 2011

Price trend equations:
Fig. 1a) $y = 0.00003 X^2 - 0.0026X + 0.8377$
Fig. 1b) $y = 0.00002 X^2 - 0.0029X + 0.5042$
Fig. 1c) $y = -0.00002 X^2 + 0.0013 X + 0.4226$
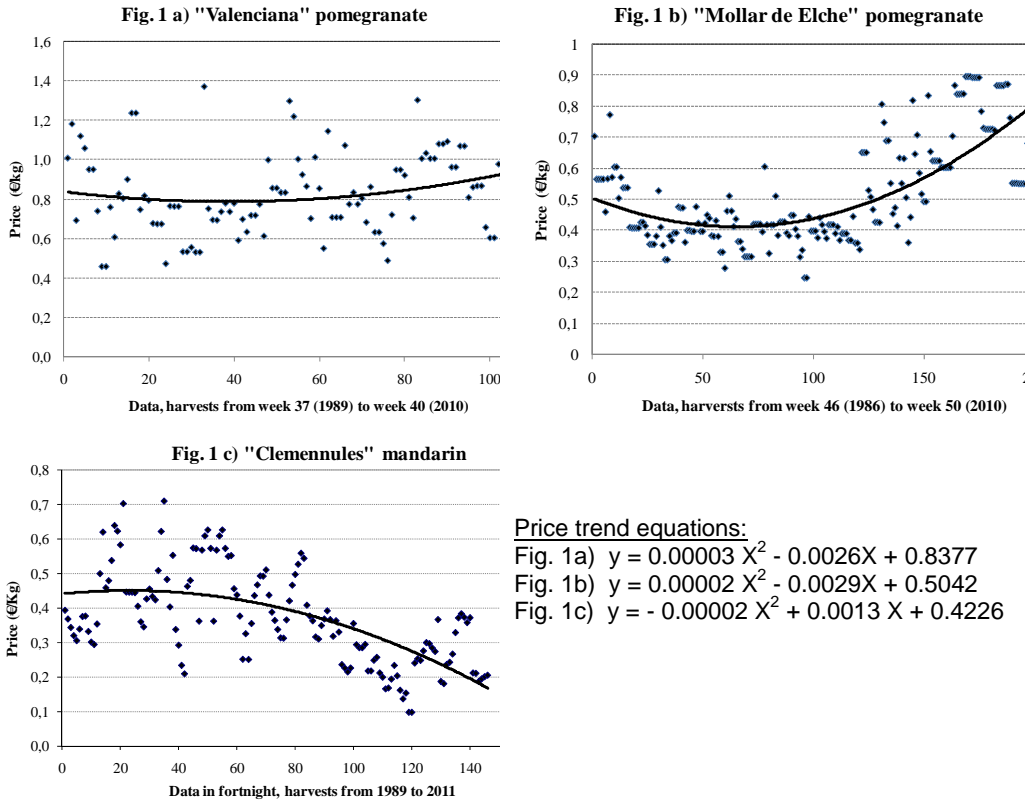
Fig. 1. Price trends for mandarin and pomegranate in the Spanish Mediterranean region.

Regular expenses: These are the operating and maintenance costs of the tree crop (water, fertilizers, plant protection products, etc.). These values increase with the age of the trees and are related to the onset of production.

Regular income: Proceeds from the sale of harvests at market prices.

Non-recurring/extraordinary expenses: These involve the renewal of fixed assets whose useful life requires their replacement before the investment project has terminated.

Extra income: obtained from the sale of modernized fixed assets.

The *Net Present Value* (NPV), which indicates the absolute profit that the project would give the farmer (Romero, 1992). The *Internal Rate of Return* (IRR) and *Benefit to Investment Ratio* (BIR) are also estimated, measuring the return provided by the activity itself and the relative gain provided by each euro spent, respectively. Once the cash flows have been estimated and updated with a discount rate of 4%, investment analysis results are shown in Table 2.

The pomegranate shows good returns for the two main varieties at current average prices, and although these would diminish were prices to go down they would continue to maintain a return similar to the mandarin, a tree crop that has always competed in terms of territory and resources.

Table 2. Reference prices and results of the investment analysis for pomegranate and mandarin in Spain

		Price (€/kg)	NPV (€)	IRR (%)	BIR
Pomegranate	Average Price "Valenciana"	0.824	91,407	8.29	1.09
	Average price "Mollar"	0.52	172,561	11.79	2.06
	Adverse price Mollar[†]	0.45	70,881	7.77	0.85
Mandarin	Average price "Clemennules"	0.37	250,852	13.42	1.87
	Normal price [††]	0.28	55,594	6.83	0.41

[†]Adverse price for pomegranates, estimated at a decline of 13%.
[††]Normal price for mandarin at present, according to the experts consulted.
Source: Own data.

III – Conclusion

If the price trends are maintained, the viability and continuity of the two species seem to differ greatly. Although farmers in the Spanish Mediterranean have preferred to choose citrus in recent decades, its drop in value is encouraging the choice of alternative tree crops, and there will be areas, like the one studied here, where the interest in species with more favorable prospects, such as the pomegranate, will increase. Without doubt, steady and guaranteed prices that are equal to or better than the current ones would seem to be decisive for pomegranate growing. This is possible if demand grows, supported by the notion that it is a healthy or functional food. In the production areas, an effort should be made to achieve a greater presence and availability of this fruit on the markets (home and abroad) something that is feasible, as a business structure has been established and is ready to extend the campaign, along with plant improvement and innovation programs, the use of cold storage and suitable means of transport.

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Analysis of the production structure and crop costs of pomegranates in Spain

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Abstract. The importance of the pomegranate is growing with increasing worldwide consumer demand, which is stimulated due to this fruits are considered a functional food, its technological improvements in processing and conservation and a wide range of new industrial uses of the fruit (packed arils, juices and extracts). The increased significance of this fruit in world trade is stimulating improvements in farming techniques and commercialization. This paper reviews the production structure of pomegranates in Spain, highlighting both the more favorable and limiting aspects. An analysis of the costs of pomegranate production at representative farms in Alicante province has been performed in order to identify more direct inputs into the economy of the farms which can contribute to their viability and continuity in the medium term. The first finding to be emphasized is that variable costs alone account for over 80% of total costs. Labor is the largest cost (over 32% of total costs), mainly due to the operations of pruning, thinning and harvesting. The irrigation water cost is over 17% due to the high average price of this natural resource in the area. The current average prices in the two main Spanish pomegranate varieties ('Mollar de Elche' and 'Valenciana') exceed the minimum threshold to offset the costs.

I – Introduction

Pomegranates (*Punica granatum* L.) are one of the traditional fruits cultivated in Spain, where Alicante province stands out over the rest with 84% of the surface and 90% of national production. Spain is the largest European Union exporter of pomegranates. The importance of the crop is growing with increasing worldwide consumer demand, which is stimulated by several factors: (i) pomegranates are considered a functional food; (ii) technological improvements in processing and conservation; and (iii) a wide range of new industrial uses of the fruit (packed arils, juices and extracts). The increased significance of this fruit in world trade is stimulating improvements in farming techniques and commercialization. This paper reviews the production structure of pomegranates in Spain, highlighting both the more favorable and limiting aspects. The paper concludes with an analysis of the costs of pomegranate production at representative farms in Alicante province, in order to identify more direct inputs into the economy of the farms which can contribute to their viability and continuity in the medium term.

II – Commercial production structure and status of the Spanish pomegranates

Spain cultivated 2,387 ha of pomegranate groves in 2008, of which 84.4% were concentrated in Alicante province, especially in the Bajo Segura and Bajo Vinalopó areas (MARM, 2011). Commercial pomegranate orchards in Alicante province increased in the first half of the 20th century. For example, only 1,207 ha (69.4% of national total) of pomegranate orchards were

cultivated in Alicante province in 1940. The 20,890 tons produced in Alicante in 2008 represented just over 90% of the total (Fig. 1). Spain is currently Europe's largest producer, highlighting its good export potential (more than 50% of its total production). Germany, England, Holland, France and Italy are the most significant destinations of Spanish pomegranate exports.

●●●●●● Prod. Alicante (tn)　━ ━ Prod.Spain (tn)　━━━ Surf. Alicante (ha)　━━━ Surf. Spain (ha)
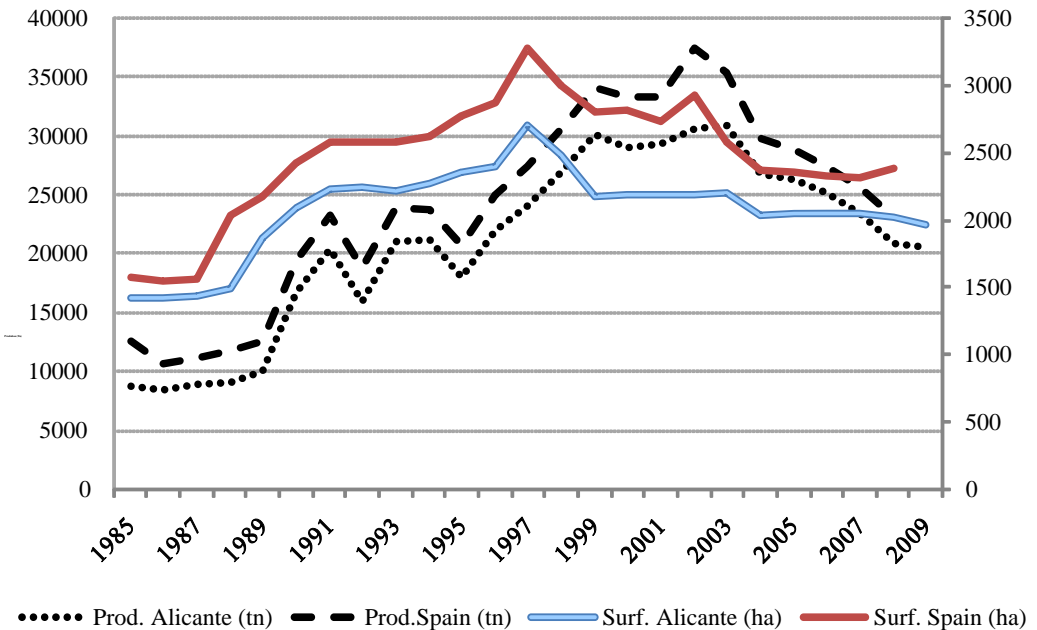
Fig. 1.　Pomegranate surface and production in Spain and in Alicante province. *Source: MARM, 2011.*

An important factor in renewing the Pomegranate production system is the implementation of efficient irrigation systems, since fresh water is a scarce natural resource. During the last decade, drip irrigation was implemented in many farms to replace flood irrigation, although in 2004, 83% of the pomegranate groves were still irrigated by flooding techniques.

Some unfavorable aspects of the production structure include the advanced age of farmers, the lack of generational change and the structure of small farms, with an average plot size of about 1.4 ha.

Strengths that characterize pomegranate cultivation in Spain include: (i) recent crop research and technological developments; (ii) drip irrigation expansion; (iii) provision of cold storage; (iv) post-harvest improvements to extend the traditional campaign; and (v) good network logistics and commercial-producing regions.

III – Analysis of production costs

Production cultural operating costs for pomegranate trees has been taken from data collected by a survey of farms in the main pomegranate producing municipalities in Alicante province

(Elche, Albatera and Crevillente). After reviewing the usual workplan of crop farms, each of the inputs used have been economically quantified. Calculation methodology has followed the pattern of budget enterprise (Caballero *et al.*, 2004). Table 1 specifies the cost of producing a hectare of pomegranates ('Mollar of Elche' cultivar) in mature orchards at a spacing of 5x4 m. This variety is harvested beginning in October. The farm of reference has a production area of five hectares with irrigation infrastructure and vehicles compatible with this size. The cultural practices represent products and materials considered typical of a well-managed orchard in the region. Drip irrigation is applied, with an average amount of 4,800 cubic meters of water. The growers distribute the fertilizer through the irrigation system. Although it is a family farm, crop management is professional; this is the reason why all labor has been computed at market price. The share of national insurance cost is included in Insurances (section 2.4).

Table 1. Spanish pomegranate production costs in 2011[†]

	Cost (€/ha)	% of total costs
1. Variable costs (VC)	**5875.46**	**80.9**
1.1. Irrigation water	1201.75	16.6
1.2. Fertilizers	1110.00	15.3
1.3. Insecticides, fungicides, herbicides and traps	544.90	7.5
1.4. Variable costs of owned machinery	602.61	8.3
1.5. Total manual labor cost	2288.20	31.5
1.6. Rental of machinery and labor to blend the trimings	128.00	1.8
2. Fixed costs (FC)	**1110.29**	**15.3**
2.1. Fixed costs of owned machinery	275.85	3.8
2.2. Amortization of the planning cost	144.00	2.0
2.3. Amortization of installations (irrigation systems and permanent installations)	216.00	3.0
2.4. Taxes and insurance	474.44	6.5
TOTAL COSTS WITHOUT OC (1+2)	**6985.75**	**96.2**
3. Opportunity costs (OC)	**275.51**	**3.8**
3.1. Capital interest of the planting cost	72.00	1.0
3.2. Capital interest of current assets	117.51	1.6
3.3. Capital interest on installations (irrigation systems and fixed installations)	86.00	1.2
TOTAL COSTS WITH OC (1+2+3)	**7261.26**	**100.0**
Cost per Kg without opportunity costs (Profitability threshold for net margin)	0.35	
Cost per kg with opportunity costs (profitability threshold for profit)	0.36	

[†]Farm of reference: 'Mollar de Elche' variety, 5 ha, drip irrigation. Average production of reference: 20,000 kg/ha.

The first finding to be emphasized is that variable costs alone account for over 80% of total costs. Labor is the largest cost (over 32% of total costs), mainly due to the operations of pruning, thinning and harvesting in particular, as well as work performed by the employer. The irrigation water cost is remarkable due to the high average price of this natural resource in the area. In addition, there are outstanding costs charged to the machinery itself and the burden of social security contributions on the labor required.

The final figures also include minimum thresholds of profitability, based on an average production of 20,000 kg/ha. The average price per kilogram must exceed 0.35 Euros in order to offset the variable and fixed costs (the threshold for profit), and a price of 0.36 Euros per kilo is required to compensate for all costs, including the opportunity costs.

IV – Conclusions

The current average prices in the two main Spanish pomegranate varieties ('Mollar de Elche' and 'Valenciana') exceed the minimum threshold to offset the costs, which allows an optimistic vision of the pomegranate future over the short term. The optimization of water, fertilizers, and the degree of increased mechanization of this crop will remain essential elements to keep costs at acceptable levels, especially if they have to compete with pomegranates from the southern Mediterranean countries which operate with lower costs. We will face new business challenges that Spanish pomegranate production must meet. Farmers must continue efforts to improve production, optimize all cultural practices and control all aspects which could increase production costs.

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The prices in Europe of pomegranates and arils

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Abstract. World pomegranates demand increased through the beginning of the 21st century, probably mainly due to the publication of research findings on the fruit's contribution to human health. A lack of dependable national statistics prevents us from estimating world trade. A paired time-series of price data by exporting and importing country in Europe is available, offering useful information to producers, traders and investors. The information allows an analysis of: (i) seasonality of fruit and aril prices by country of origin and European market; (ii) sensitivity of the trade to economic crises such as experienced in 2008; and (iii) estimation of supplier prices and comparison by hemispheres. The results are invaluable for producers and traders, as well as regional and national plannners.

Keywords. Pomegranate – Arils – Wholesale prices – Price distribution – Price differenciation – Europe – Export – Import.

I – Introduction

The recent global trend of increased demand for pomegranates, whether as fresh fruit or as derived products, is growing at an impressive pace. It is considered to be the outcome of the many favorable effects on human health these products were found to possess.

The global published information on the trade of pomegranates and their derivatives offers only a partial picture. This is due to the garden character of production: small scale production in the largest producing countries (India, China and Iran). The fact that less than 10 percent of total production is internationally traded exposes the industry to potentially serious fluctuations: it is easy to trigger significant change in quantities entering the market by diverting even a small portion of production from domestic consumption in the large production areas to the international marketplace.

This report is based on the analysis of data available from readily accessible sources, including weekly data of the International Trade Center (ITC) on wholesale prices of pomegranates in a large number of urban markets in Europe, and on information about packaged pomegranate arils sold in supermarkets of the United Kingdom. In processing the data we calculated average monthly prices collected from 1,587 observations between individual buyer and seller countries in Europe.

If we have any confessed economists among our readers, they probably want to be apprised of the following information:

(i) The study covers the period from the beginning of 2002 through July, 2011, with the exception of the data for August to December in 2003.

(ii) Unfortunately, we have no quantity data to accompany the price observations, either from countries of supply or from countries of demand.

(iii) Nor do we have information on prices relative to fruit size – a dimension that expresses consumer preferences in various countries or – at least – consumption patterns.

(iv) The prices used are nominal, i.e. in current Euros for each of the ten years of observations.

II – General time related pomegranate price trends

Figure 1 demonstrates the general price trend of pomegranates in Europe. Considering the missing observations in 2002 and 2011, which cause an upward bias in the average annual price, pomegranate prices seem to have maintained a very steady level over time, fluctuating between €2.50 and €3.50 per kilogram.
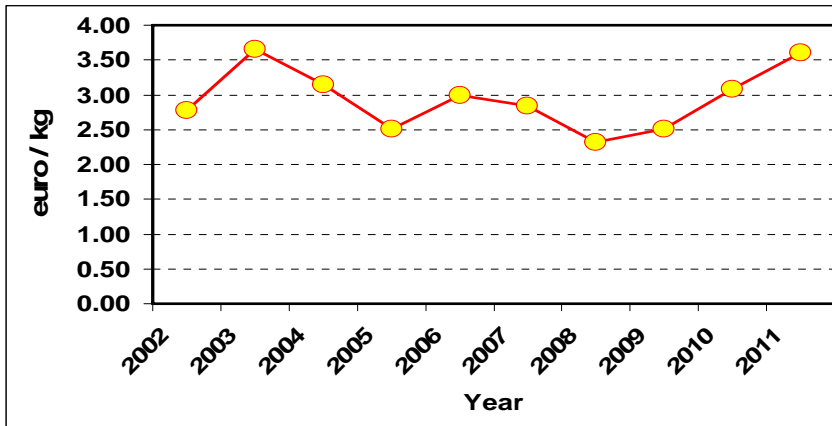
**Fig. 1.   Pomegranates price trend: average annual wholesale price, 2002 – 2011
(in 2003 and 2011, only January-July).**

1. Monthly price distribution

There are significant monthly differences in pomegranate prices during the main harvest season of the northern hemisphere, i.e. between September and the following January (including two or three months of supply from cold storage), and the main supply season of the southern hemisphere – from March through June.

Figure 2 accentuates the difference between the average price of €2.33/kg in the "regular" i.e. traditional season and €3.66/kg during the off-season. The difference of €1.43/kg is apparently due to the small quantities of pomegranates arriving in Europe from the southern hemisphere. This occurs in spite of the large variety of competing fruits available on the market.

Identification of supplies according to the ripening seasons in the two hemispheres allows the sellers to work out a strategy.   Thus, for example, arranging to begin supplying fruit in the northern hemisphere a month earlier – say in August (or perhaps even in July), by developing earlier ripening varieties, should reward the sellers with premium prices. Similarly, the suppliers of the southern hemisphere are also challenged to extend their marketing season into July and August, for example, by extending the cold storage period.

2. Regional price differentiation in Europe

The European demand for pomegranates is not homogeneous. This is clear from an analysis of prices paid for the fruit in the different countries. Europe was divided into sub-regions for the purpose of making a differential price analysis: Western Europe, including France, Belgium and the Netherlands, Central Europe, with Germany, Switzerland and Austria, the north – Denmark, Finland, Sweden, the South – Spain and Italy, and the UK, which was analyzed separately.
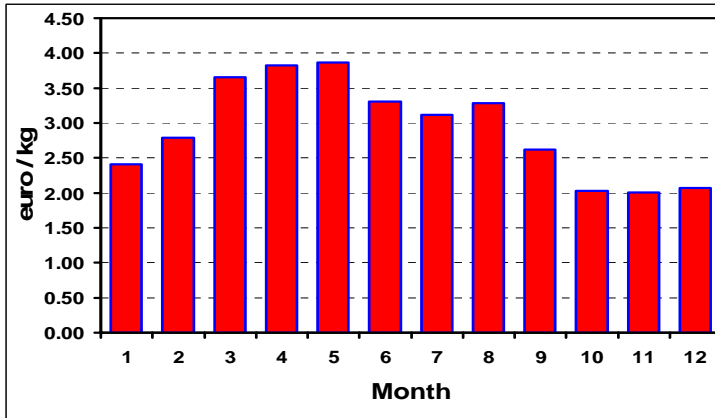
Fig. 2. Pomegranates monthly distribution of wholesale prices.

Although Europe of the third millennium is a continent without borders, different prices are set from country to country, and with respect to this, it is notable that as far as Spain is concerned, it is not at all clear whether what determines the imports might be the need for raw material for industrial processing. In this case, the quality of the imported fruit is not the highest.

An interesting picture develops from examination of Fig. 3 the price differences among the countries of the continent are not great but having said that two interesting points stand out:

(i) In the countries of the north – Scandinavia – the prices are significantly high and attract suppliers to ship large quantities in that direction;

(ii) In contrast, in Britain during most months of the year the prices set are considerably lower than those found in the European continent. This may be due to the British being traditional buyers of Indian pomegranates, where the fruit is characteristically not large and of Indian varieties.
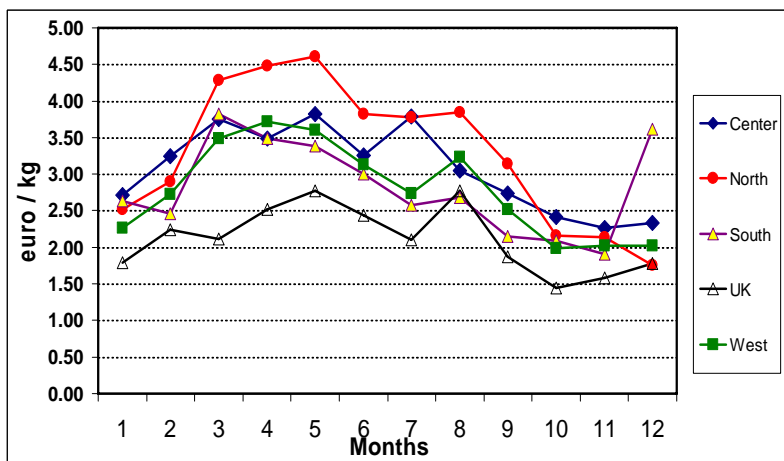
Fig. 3. Pomegranates regional monthly wholesale price distribution.

3. Prices to exporters

Figure 4 illustrates price behavior according to the country of origin. Southern hemisphere suppliers enjoy premiums over northern producers, with the exception of India which ships its unique evergreen varieties over 8 months of the year, thus benefiting from off-season price premiums.
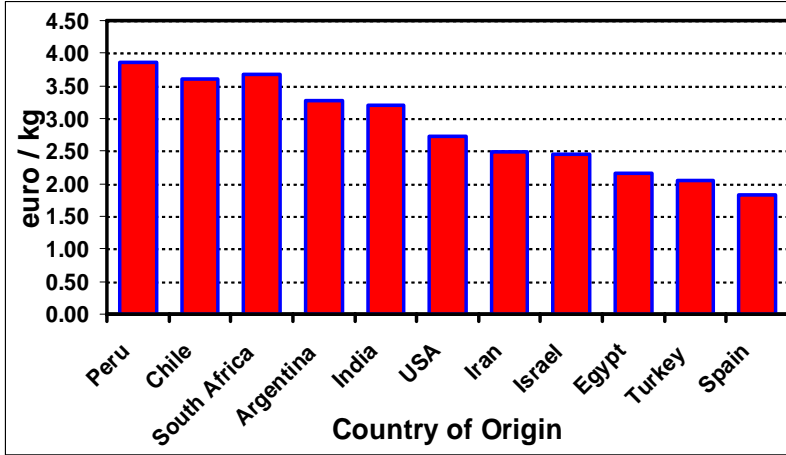
Fig. 4. Pomegranates: prices received by supplier countries.

4. The 2008 financial crisis and its effect on pomegranate prices

In the light of the financial crisis of 2008, a question may arise as to the sensitivity of pomegranate prices to difficult conditions in the economy. To obtain an insight into this issue, a comparison was made of prices before (i.e. August 2002 through July 2008) and after the crisis (Fig. 5).
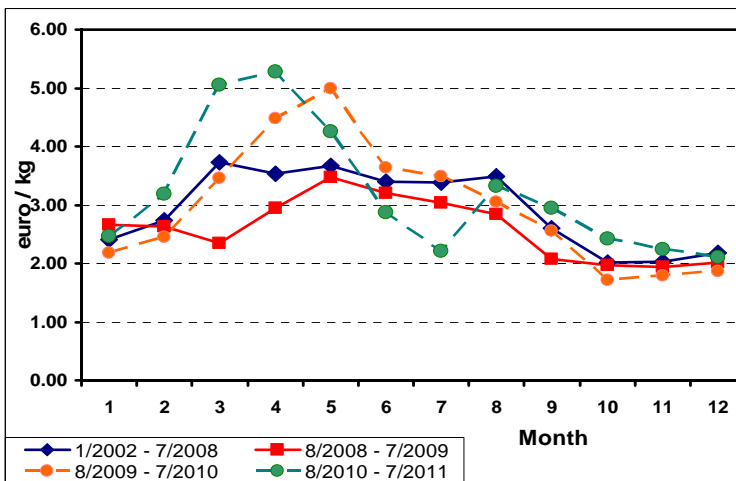
Fig. 5. Pomegranates: monthly distribution of prices before and after 2008.

A significant decline was noted, although "only" of 16 percent and then only for a single season. We say "only" because during the same period, the prices of other fruits dropped between 25 and 30 percent. In the following season (August 2009 – July 2010) prices recovered almost totally (-4.6%). In the year after, they again rose above the levels of the previous years by 9-10 percent.

5. Retail price levels for arils in British supermarkets

Pomegranate arils, chilled or frozen, are a relatively new product in the pomegranate industry. Packaged and ready-to-eat, it is a typical convenience product and its prices are accordingly high.

Figure 6 indicates that the retail prices of this relatively new product – calculated on the basis of Euros per net kilogram – run between €14 - 16 per kg. Considering that at least three kg of quality fruit are needed to produce one kg of arils, if the cost of the raw material does not exceed €1/kg, the picture obtained is optimistic. Feasibility studies in a number of countries indicate that it is clearly profitable to invest in the plant and equipment required for a fully operational production setup.
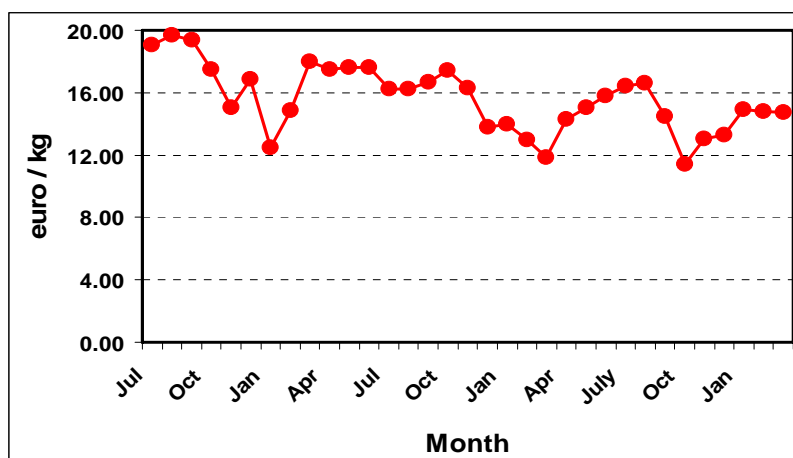
Fig. 6. United Kingdom: prices of pomegranate arils.

III – Conclusions

The foregoing comments sum up an almost complete decade of observing the pomegranate industry and the factors affecting it. We now have a detailed picture of price behavior in Europe over ten years. It is possible to conclude from the information gathered that the economic climate is very favorable to pomegranate producers and shippers. Furthermore, presently available information allows producers to plan marketing strategies and commercial initiatives of varying character. It also supplies those in charge of project management or purveyors of other support services, current information in real time, enabling them to advise and assist in the timely formulation of rational business decisions.

A comprehensive industrialization of the processing of the pomegranate fruit The key to its economic viability

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Abstract. The past 10 years have witnessed major changes in the pomegranate industry. Specifically, a worldwide significant increase in the pomegranate planted area, an increase in fruit production, an increase in export quantities and an increase in the stored and processed fruit. Two factors are primarily responsible for these changes: (i) an increasing demand for the pomegranate fruit driven by a substantial body of published results of research on the characteristics of the pomegranate fruit, suggesting that the fruit has both, antioxidant and anti-inflammatory properties, helping reduce the risk of prostate cancer and artery plaque. And (ii), the unique development of a machine for automatic extraction of the arils, thus, facilitating the use of ready–to-eat arils and/or development of various postharvest processing lines, such as juice and wine production and development of various pharmaceutical and nutraceutical and cosmetic products derived from the arils kernels. While the current increase in fruit production continues and more "players" are joining in, there is no guarantee that the economic viability of the pomegranate industry is ensured. In fact, past experience have shown that if increased production is not matched by a similar increase in demand, prices of the fruit are liable to come down; competition will increase; small growers will be forced out of business and we shall witness (as seen in the past) uprooting of existing orchards with declined economy. The pomegranate industry is still undergoing a process of evolution from specialty to commercial crop. Thus, to ensure its sustained economy, a system approach has to be adopted addressing all the factors associated with the industrialization of the processing. Unfortunately, many growers do not even make use of existing technologies and do not initiate R&D to develop new processing technologies addressing the unique characteristics of the pomegranate fruit. Thus, for example, the arils extracting machine is yet not fully commercialized. Likewise, the unique freezing technology is still not fully utilized and very little is done to develop commercial products from the various components of the fruit, such as: the arils, the rind, the arils kernels and the juice. The potential is high, but unless a system approach is adopted, the fruit will remain a minor crop globally.

Keywords. Pomegranate – Arils – Processing – Freezing – Juicing.

I – Introduction

The past 10 years have witnessed major changes in the pomegranate industry. Specifically, a worldwide significant increase in the pomegranate planted area, an increase in fruit production, an increase in export quantities and an increase in the stored and processed fruit.

Two factors are primarily responsible for these changes. First, an increasing demand for the pomegranate fruit driven by a substantial body of published results of research on the characteristics of the pomegranate fruit, suggesting that the fruit has both, antioxidant and anti-inflammatory properties, helping reduce the risk of prostate cancer and decreasing artery plaque. The second factor is the development of a unique machine for automatic extraction of the arils, thus, facilitating the use of ready-to-eat arils and/or development of various postharvest processing lines, utilizing the arils as raw material for the production of quality juice

and wine and development of various pharmaceutical, food additives and cosmetic products derived from the arils kernels and the fruit's peel.

While the current increase in pomegranate production continues and more "players" are joining in, there is no guarantee that the economic viability of the pomegranate industry is ensured. In fact, past experience have shown that if increased production is not matched by a similar increase in demand, prices of the fruit are liable to come down; competition will increase; small growers will be forced out of business and we shall witness (as seen in the past) an uprooting of existing orchards with declined economy.

The pomegranate industry is still undergoing a process of evolution from specialty to commercial crop. Many potential consumers have not yet been exposed to this unique fruit and its many advantages. Yet, fruit production is increasing steadily, new orchards are being planted and yield per area is increasing through the introduction of better orchard management systems and introduction of new varieties, which provide almost a year around supply. Thus, to ensure the pomegranate sustained economy, and matching the growing supply with corresponding demand, a system approach has to be adopted addressing all the factors associated with the industrialization of the processing of the fruit. Unfortunately, many growers do not even make use of the existing technologies and do not initiate R&D to develop new processing technologies addressing the unique characteristics of the pomegranate fruit. Thus, for example, the arils extracting machine is yet not fully commercialized. Likewise, the unique freezing technology is still not fully utilized and very little is done to develop commercial products from the various components of the fruit, such as: the arils, the rind, the arils kernels and the juice. The potential is high, but unless a system approach is adopted, the fruit will remain a minor crop globally.

II – Proposed integrated scheme

The potential for the utilization of the pomegranate fruit illustrate schematically the complete processing possibilities of the pomegranate fruit (Fig. 1).
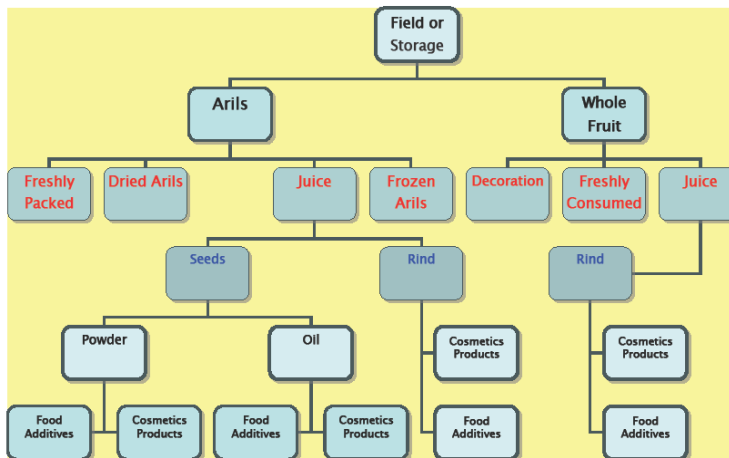
Fig. 1. Pomegranate fruit utilization scheme.

While the schematics portray a wide array of processing technologies, there are at present only three basic technologies which are partially used and are a far cry from a comprehensive processing scheme. These are the arils extraction technology, the frozen arils technology and

juice production. Thus, assuming that fruit production is going to increase in the foreseeable future, the only way to sustain the continued viability of the pomegranate industry is by increasing the demand for the fruit and its derivatives, the result of the various processing systems.

The current state of the art with the arils extraction technology is given in Fig. 2, that of the freezing technology in Fig. 3 and juice extraction in Fig. 4. These technologies are currently being used in 10 countries.

Unfortunately, all the other processing channels represent only a small fraction from the huge potential market in the food additives industry, medical products and the cosmetic industry. Thus, for sustained viability these additional processing channels need to be integrated into one cohesive processing scheme.

Fig. 2. Arils extraction technology: A patented principle of operation (i) Current Field Capacity: up to 56 fruits per minute; (ii) Operates in 10 countries with over 30 installations.

Fig. 3. Frozen aril technology: (i) Keep the nutrition as of fresh; (ii) Available all year round.

Fig. 4. Juicing technology. High-quality juice through extracted arils technology: (i) No bitter taste; (ii) Easy to consume; (iii) Can be frozen and de frozen without being concentrate; (iv) Available all year.

III – Conclusions

The pomegranate fruit has a tremendous potential, based on its unique characteristics. The awareness of its many advantages has led to a dramatic increase in its production worldwide – a trend which continues even at present. However, if this trend will not be matched up by a similar increase in the demand, the viability of this industry is likely to witness a major setback. Thus, only a concerted effort to integrate all factors associated with the processing of the fruit would ensure the viability of this industry. The technologies are already available and it is now a matter of implementing the various technologies.

The Argentinean experience in the cultivation of 1000 ha of pomegranates (5 provinces) Test of varieties and management of crop

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Abstract. In the year 2007 the commercial pomegranate plantation at scale started in Argentina destined for exportation. At present, the pomegranate project has 1000 hectares implanted in different latitudes, in provinces such as Salta, San Juan, Córdoba, Entre Ríos and Formosa, where the nursery is located. The Genetics is Israeli in its origin for the open varieties like Wonderful clones 100 and 101, Acco and, other varieties have Royalty such as Emek, Kamel, Shany Yonay and Shir; the first two varieties are the ones that are commercially planted in greater proportions. The crops are managed with densities of 416 plants per hectare, with drip irrigation and fertirrigation and plastic mulching over the line. The fertilization used is basically N, P and K. The plants are pruned and are shaped as an open vase multitrunk without trellising systems. The expected time for harvest is March April at its peak. This year 2011 the first commercial harvests have started.

Keywords. Argentina – Pomegranate – Varieties – Crop – Genetics – Israel.

I – Pomegranates in Argentina. current situation

There are pomegranate plants scattered across the country, implanted by European settlers. But it was at the end of the 90's that the first commercial plantations started.

At the beginning of 2007 Tikagroup is created in order to give advice to and support agricultural and agroindustrial investments, focusing especially on pomegranates. In order to do this, Tikagroup produces and multiplies different varieties of pomegranates in their nursery in Formosa, with vegetal material of Israeli origin. Pioneers in the production of pomegranates at great scale in Argentina, Tikagroup gives advice from the selection of the farm or field to the placement of the product in the market. In this way, Tikagroup has developed and promoted the production in order to export premium quality fruit to the Northern hemisphere in counter season. At present, the pomegranate project has 1000 hectares implanted in different latitudes, in provinces such as Salta, San Juan, Córdoba, Entre Ríos and Formosa, where the nursery is located.

II – Management of the crop

All the projects carry out studies of resources and a comprehensive planning prior to the setting up of the farms, including a survey of topography, soils, water supply, provision of energy, access roads and, subsequently, planning of the units of production in terms of drainage, roads, windbreakers, systems of irrigation and planting. There is drip irrigation with 1 lateral per line with non compensating emitters with rates of 2 lt/ hour of nominal output and an irrigation rate, which depending on the area, goes at the top between 7 and 9 mm/day, fertirrigation is used, water supply from underground wells with pressure and volume controllers, mulching on the lines and all the technology rendered appropriate and convenient for the best production according to cost/benefit and profitability. The planting is carried out both in spring as in autumn with 1-year-old-plants in pots of 6 lts and they are pruned as soon as they are implanted or

before that in the nursery. The formation of the tree chosen is multi trunk without trellising in an open vase system with 4-6 principal branches and at least two layers of production, keeping the plant for manual harvest. The spacing chosen is 6 x 4 and 6 x 3 that is equivalent to densities of 416 and 555 plants per hectare. The production of plants is done under strict health standards, quality and certifications. The commercial varieties at scale so far are Wonderful and Acco.

The crop is fertilized using liquid or solid fertilizers mainly to incorporate them through drip irrigation. The fertilization has to be based on the size of the tree, variety and objective (vegetal or fruit), applying the basic elements which are nitrogen (N), phosphorus (P) and potassium (K). In mature plantations (base): nitrogen (N): 180-200 un; phosphorus (P): 40-60 un; potassium (K): 360-400 um; ratio: N / K: 1 / 1.7-2.

Weed control: this operation is very important in most of the areas. It is based on the use of mulching on the line to keep it clean without using herbicides and, on the use of herbicides on the laterals of the mulching and mechanical control between lines.

Control of pests and diseases: in Argentina there is no incidence of important pests or diseases. Still in some areas there are aphids, whiteflies and scale insects from other fruit trees. There are few plants with *Phytophtora* after a year of planting affecting a small proportion. As prevention, metalaxil was applied in the irrigation system, and mainly humic and fulvic acids are supplied to improve the root system and the affected plants are eliminated and replaced. With regards to pests, only leaf-cutter ants are detected chlorpiriphos and fipronil used. Besides this, it is necessary to control the Mediterranean fruit fly as it is a host species and in Argentina there are only two areas declared free of it; and then, a comprehensive pest control will be carried out with monitoring and application of the required pesticides when reaching damage thresholds.

Pruning: it is of upmost importance for the forming of the tree to prune the trees as explained above in an open vase multi trunk system so the following stops are carried out: pruning for the formation of the trees during the first years, pruning for renovation, pruning to thin cracked fruits, green or summer pruning to allow the light to penetrate and removal of suckers at the base of the neck.

Harvest: the tree starts producing after the third vegetative cycle, i.e. fourth calendar year, and it enters the cycle of productive period at year 6. The expected estimated yields are: at the 4th year 15 tons; at the 5th 25 tons; at the 6th 35 tons. The harvest has been verified for March April, but it is expected to be from the beginning of March till the end of April depending on areas and varieties.

III – Projects, places, planted surfaces

Gross area 1100 hectares – Net area 950 hectares:

   (i) Salta – Dragones: 500 ha base 300 ha planted (250 ha planted in 2009; 50 ha planted in September 2011). Next 250 ha in following years.

   (ii) Córdoba – Cruz del Eje: 500 ha base with 300 ha planted **(**90 ha planted in 2009; 100 ha planted in 2010, and 80 ha in 2011). In this province there are two neighboring projects next to the main productive units of 25 ha and 5 ha, respectively.

   (iii) San Juan – Retamito – Campo Grande del Acequión: 375 net ha**.** In this place there are 4 projects of implanted pomegranates. 95 ha planted in 2008, 65 ha planted in 2009 and 175 hs planted in 2010.

   (iv) Entre Ríos – Concordia: 15 ha. In this area smaller pilot project was started comprising 15 ha; 5 ha planted in April 2010 and 10 ha in 2011.

In 2011 the first commercial harvests of pomegranates took place in Argentina. Several

analyses were performed on the pomegranate juice which showed very good qualities; average degrees brix (14 to 20 ºbrix) and acidity between 18-22; and soluble solids: 175. Anyway, these results are expected to improve as the fruit is still young.

Varieties. The production of *pomegranate* plants is carried out with material coming from Israel, identified and certified by ARO (Agricultural Research Organization), Institute Vulcani, under the inspection of SENASA:

   (i) Registered open varieties: Wonderful, Acco, Hercovitz (116).

   (ii) Registered varieties with Royalty: Emek, Kamel, Shany Yonay, Shir.

IV – Tests

1. Garden of introduction

In the different areas a series of tests were carried out with several varieties. The aim was to evaluate the performance of all the varieties mentioned above.

ACCO **–** Early red cultivar. Peel color: red to pink. Uniformly spread all over the peel and it appears very early in the fruit development. It gets its total color before the arils ripen and the fruit reaches its final size. Low sensitivity to sunstrokes. Aril color: dark red. Soft seeds, big arils, high juice content, suitable for the extraction of arils. Ripening date in Israel: second half of August. Thin peel. Taste: sweet, no sourness. Size of fruits: 350-450 grams. Yield: 30 ton/ha.

®EMEK **–** Early cultivar Registered variety (patent). Full red peel color. It gets full color before the arils reach the final size. Low sensitivity to sunstrokes. Aril color: red. Soft seeds, big arils, high juice content. Hardness of seed: medium. Ripening date in Israel: beginning of August (early – Israel). Peel thickness: medium. Size of fruits: 450-600 grams.

®KAMEL **–** Registered variety (patent). Late red cultivar very similar to "Wonderful". The color of the peal is dark full red, very strong and uniform that appears very early in fruit development. It is not sensitive to sunstroke. Size of arils: big. Aril color: dark red. Medium seed softness. Hardness of ripe seeds is medium. Ripening date in Israel: mid to end of September. Peel thickness: medium. Taste: sour-sweet. High content of juice. Size of fruits: 500-800 grams.

®SHANI YONAY **–** Registered variety (patent). More polygonal and flatter than the Akko variety. Size of fruits: 350-500 grams, it ripens during the second half of August. Peel color: full red, it gets full color before the arils ripe and the fruit reaches the final size. Low sensitivity to sunstroke. Soft seeds, big arils, high juice content.

116- (HERCOVITZ) **–** Mid season red cultivar. Aril color: red. Medium softness of seeds. Ripening date in Israel: mid September. Peel thickness: medium. Taste: sour-sweet. Size of fruits: 450-600 grams.

WONDERFUL **–** Late variety. It ripens in mid- October. Size of fruits: 500-800 grams. Peel color: dark red, yet not full. Sensitive to sunstroke. The arils are dark red. They are tangy; hardness of ripe seeds is medium. It is the most popular variety in Israel and in USA. It is the favorite and most well- known variety among consumers.

Several clones are introduced, but only a few of them have top quality.

2. Development of phenological stages in pomegranate varieties. Test taken as example: Cruz del Eje (Córdoba)

Apart from the commercial varieties on the farm, Wonderful and Acco, a garden of introduction

was set up where 6 additional varieties were implanted. The outstanding points from the phonological stages are taken into consideration:

Sprouting: after wintertime, in the middle of August, the temperature started to rise. From 8/19 to 8/28 sprouting occurred and the differentiation of leaves started, being Wonderful the first, and Shani (8/26) and Emek (8/28) the last ones. The period of sprouting includes 4 sub-periods: - coming out of the first leaves (D), division of leaves (D2), growing of leaves (D3) and enlargement of internodes (D4). According to the tests, this period lasts around 45 days in all varieties and results in the flowering (F), although the incidence of new sprouts is not stopped during the vegetative period.

Flowering (F): the coming out of buds is around the second half of September, from 9/16 for Wonderful; 9/21 for 116, Acco, Shany and, at the end of September for Kamel and Emek. The flowering encompasses several stages-types: coming out of buds (E), swollen calyx (E2), opening of calyx (E3) and open flower (F). Said state takes places between 10/05 (Wonderful) and 10/12 (Shany), and lasts from 7 to 20 days. Four flowerings could be clearly observed during the green period, the most important ones in terms of production took place between October and the middle of November.

Fruit set: From the second fortnight and up to the end of October, the fruit set (H) takes place, the varieties Wonderful and Shany are the earliest, then 116 and the last ones, Acco and Kamel. This stage takes between 20 and 30 days, leading to the development of the fruit(J).

Session 2
Plant material and breeding

Diversity of pomegranate (*Punica granatum* L.) germplasm in Spain

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Abstract. Twenty-nine pomegranate (*Punica granatum* L.) accessions were studied to determine the overall degree of polymorphism. Cluster analysis showed a considerable phenotypic and genetic diversity in the local pomegranate germplasm. The cluster analysis produced a dendrogram with four main clusters, showing accessions closely related and simultaneously very different from others. The geographic origin was a determinant criterion for cultivars clustering.

Keywords. Diversity – Pomegranate – *Punica* – Spain – Accession – Characterization.

I – Introduction

There is growing interest in pomegranate not only because it is pleasant to eat, but also because it is considered to be a functional product of great benefit in the human diet, as it contains several groups of substances that are useful in disease prevention (Melgarejo and Martínez, 1992; Melgarejo and Artés, 2000; Melgarejo and Salazar, 2002, Cam *et al.*, 2009). Prospections for local pomegranate germplasm were undertaken since 1992. Many local types were inventoried and described (Melgarejo, 1992). The collection original containing 59 accessions collected from different growing regions in the country were established. They represent about 16 local denominations (Melgarejo, 1992).

This paper describes the diversity observed in local germplasm when evaluated under uniform conditions. The main objectives of this work were to determine the overall degree of polymorphism of the characters used in morphometric studies.

II – Material and methods

1. Plant material

Areas prospected and germplasm collecting procedures adopted were reported in Melgarejo (1992). Twenty-nine accessions, representing 9 denominations, were included in the present study (Table 1). They are represented by adult trees maintained in the same collection at Alicante in Southeast of Spain (Melgarejo, 1993).

2. Characters studied

Studies were based on characteristics of the fruit and of the seeds, as well as of the leaves and flowers. Morphometric measurements and chemical analyses carried out on samples of 20 mature fruits, in 25 seeds, 50 leaves y 25 flowers of every variety. The study was realized for three consecutive years, measuring up the following variables.

Table 1. Names, abbreviations and origin of pomegranate accessions evaluated

Code	Accession	Location†	Code	Accession	Location†
AB1	Albar de Blanca 1	Blanca (Murcia)	ME17	Mollar de Elche 17	Elche (Alicante)
ADO2	Agridulce de Ojós 2	Ojos (Murcia)	ME18	Mollar de Elche 18	Elche (Alicante)
ADO3	Agridulce de Ojós 3	Ojos (Murcia)	ME19	Mollar de Elche 19	Elche (Alicante)
BA1	Borde de Albatera 1	Albatera (Alicante)	ME20	Mollar de Elche 20	Elche (Alicante)
BO1	Borde de Ojós 1	Ojos (Murcia)	ME21	Mollar de Elche 21	Elche (Alicante)
CRO1	Casta del Reino 1	Ojos (Murcia)	MO3	Mollar de Orihuela 3	Orihuela (Alicante)
CRO2	Casta del Reino 2	Ojos (Murcia)	MO4	Mollar de Orihuela 4	Orihuela (Alicante)
MA1	Mollar de Albatera 1	Albatera (Alicante)	MO5	Mollar de Orihuela 5	Orihuela (Alicante)
MA2	Mollar de Albatera 2	Albatera (Alicante)	PTO3	Piñón tierno de Ojós 3	Ojos (Murcia)
MA3	Mollar de Albatera 3	Albatera (Alicante)	PTO4	Piñón tierno de Ojós 4	Ojos (Murcia)
ME11	Mollar de Elche 11	Elche (Alicante)	PTO5	Piñón tierno de Ojós 5	Ojos (Murcia)
ME12	Mollar de Elche 12	Elche (Alicante)	PTO6	Piñón tierno de Ojós 6	Ojos (Murcia)
ME13	Mollar de Elche 13	Elche (Alicante)	PTO7	Piñón tierno de Ojós 7	Ojos (Murcia)
ME14	Mollar de Elche 14	Elche (Alicante)	PTO8	Piñón tierno de Ojós 8	Ojos (Murcia)
ME16	Mollar de Elche 16	Elche (Alicante)			

†Melgarejo (1992).

A. Fruit

Fruit weight (FW), expressed in g; Equatorial diameter (FD1), expressed in mm; Calyx diameter (FD2), expressed in mm; Fruit height without calyx (FL1), expressed in mm; Total fruit height (FL2), expressed in mm; Calyx height (FL3), expressed in mm; Number of carpels (Nc); Rind and carpels weight (PcMc), expressed in g; Skin thickness (Ec), expressed in mm, the measurements were performed on two opposite faces in the equatorial zone; Seeds yield (Rs) = [FW-(PcMc)/FW]x100 (%).

Diameters, fruit height and skin thickness. Fruit weights and Rind and carpels weight were taken (Sartorius Model BL-600) with an accuracy of 0.1 g.

B. Seed

Maximum width (Sw) and length (SL); Seed weight (SW), determined by a precision weighing device (Mettler AJ50) with an accuracy of 0.0001 g; Juice content (JV), using an electric extractor and a seed sample of 100 g; Total soluble solids (TSS) (ºBrix), determined by an Atago N-20 refractometer at 20ºC; Acidity expressed as citric acid (A), determined by acid–base potentiometer and pH; Maturity index (MI = TSS/A). Three repetitions per clone and year were carried out.

The parameters measured in the woody portion of the seeds were: Maximum width (w) and length (l); Weight of the woody portion (wpw) of each seed; Woody portion index (wpi), determined from the wpw/SW ratio 100 (%).

C. Leaf

The surface of the leaves was assessed by an image digital analyzer device (Digital Image Analysis System, Delta-T). The measured variables were: LW: width of the leaf (mm); Ll: length of the blade (mm); Lt: total length of the leaf (mm); Lp: length of the petiole (mm); LS: leaf surface (mm^2).

D. Flower

The measured variables were: FD: diameter of the flower (mm); FL: length of the flower (mm);

NP: number of petals; NS: number of sepals; LP: length of the petals (mm); AP: width of the petals (mm); LE: length of the style (mm); NE: number of stamens.

The lengths were measured by a digital caliper/caliper (Mitutoyo) with a 0.01 mm accuracy. The fruits weight, determined by a precision weighing device (Sartorius BL 600) with an accuracy of 0.01 g.

3. Statistical analysis

Mean values registered for each parameter were used to perform a clustering of cultivars into similarity groups using the Ward´s, Method Squared Euclidean. Data processing was performed using the PASW Statistics 18 (SPSS Inc., Chicago, USA).

III – Results and discussion

The cluster analysis produced a dendrogram with four main clusters (Fig. 1).
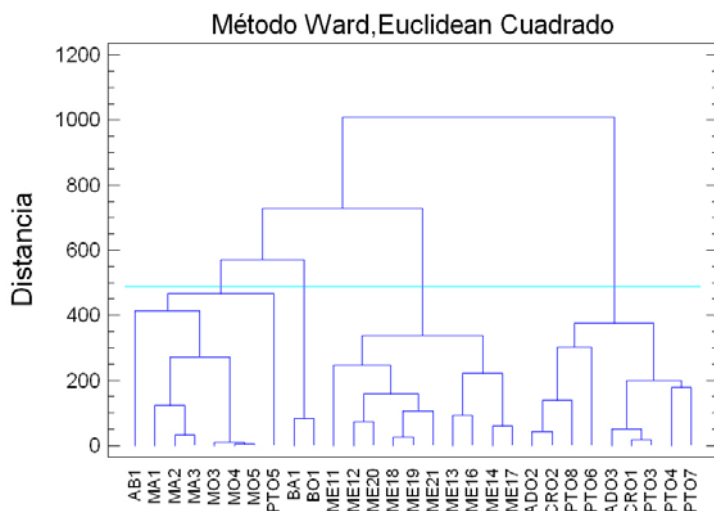
Fig. 1. Cluster analysis grouping of 29 Spanish pomegranate cultivars. See Table 1 for cultivars names abbreviations.

The first cluster (I) is the most heterogeneous group and is composed by 8 varieties proceeding from several localities by fruits of average-big size and sweet juice. The second cluster (II) grouped cultivars BA1 and BO1 characterized by to present fruits of average-big size, high index of woody portion and acid juice. The third cluster (III) includes the varieties of the group varietal ME (10 accessiones). All they have fruits of average size, with low acidity of the juice and in general high indexes of maturity (Fig. 1).

The last group (IV) is composed by 9 varieties, all of them proceeding from the same geographical zone. The varieties of the cluster IV are characterized for presenting fruits and seeds of big size. Also it is interesting to indicate that in the Spanish analyzed varieties, four groups were obtained from an analysis cluster (Fig. 1), remarkably coinciding all grouped varieties with their geographical origins but the PTO5 (Fig. 1). While these results agreed with those reported in for other fruit tree species (Barbagollo *et al.*, 1997), they clearly differed from the classification established by Mars and Marrakchi (1999).

IV – Conclusions

The study revealed considerable phenotypic (and presumably genetic) diversity among pomegranate accessions, showing more proximity between varieties of the same geographical area.

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The Pomegranate National Collection of Afghanistan

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Abstract. The Perennial Horticulture Development Project – Afghanistan is a project in support to the Ministry for Agriculture of Afghanistan. The main goal is the strengthening of the perennial horticulture through the development of a highly standardized nursery sector and the adoption of local genetic resources. Pomegranate has been surveyed in a wide area of the Afghan territory and collected during the first phase of the project (2006-2010). The *in situ* collection holds 60 different labeled and recorded varieties located in various provinces. These accessions have been propagated and planted in year 2009 in two different duplicated *ex situ* collections. Besides a first characterization of the *in situ* collected material reported in the PHDP Germplasm "*In-Situ*" Collection Database, all the clones are being fully characterized and evaluated following international descriptor lists and standards.

Keywords. *Punica granatum* – Germplasm – Repository.

I – Introduction

Afghanistan is an area of diversification for many fruit tree species, among which pomegranate. The areas of major diffusion of scattered wild populations and of productive orchards are the Provinces of Kandahar, Balkh, Farah, Kapisa, Samangan, Nagharhar and Heart (Samadi, 2008). The total production in 2007 was of about 500,000 t (Source: Ministry of Agriculture, Irrigation and Livestock) on a surface of about 42,000 hectares, contributing for about 2% of the total horticultural production of Afghanistan (Glozer and Fergusson, 2008). In the last few years the demand of pomegranate is increasing and new orchards have been established. About 50,000 t were exported as fresh fruits in 2009.

Samadi (2008) described 48 cultivars coming from various Provinces of Afghanistan, and differently characterized by colour, flavor and seed hardiness. Few cultivars are sweet and "seedless", while most of them are medium-sweet to sweet taste and bring hard seeds. "Kandahari" and "Bedana" (which means "seedless") are considered two excellent cultivars (Glozer and Fergusson, 2008). The propagation of pomegranate is traditionally carried on by cuttings collected from productive trees, nevertheless a formal and traced procedure is lacking. As a result, orchards are not homogeneous and different varieties are grown together in the same orchard. This aspect may be considered as positive in a subsistence fruitculture, since genetic and phenotypic variation ensures to obtain a certain amount of production notwithstanding possible adverse climatic events, and the disruption of diseases and pests. Nevertheless, in an advanced fruitculture heterogeneous orchards are an obstacle.

II – The Perennial Horticulture Development Project (PHDP)

In 2006 the European Commission-EuropeAid Programme funded the Perennial Horticulture

Development Project (PHDP) (www.afghanhorticulture.org) in support to the Ministry of Agriculture, Irrigation and Livestock of Afghanistan. A second phase was supported by EC for the period 2011-2015. As stated in the project web pages "*The specific objective of the project is to develop a demand oriented and export led perennial horticulture industry*". A major purpose of the project is to develop the nursery sector of this country in order to strengthen and to qualify fruit production. The main activities regard the establishment of a traced nursery system based on the propagation of true-to-type local varieties. The adopted steps can be summarized as follows: (i) individuation of superior trees in productive orchards; (ii) cataloguing and definition of the *in situ* National Collection; (iii) propagation from the *in situ* original mother plants; (iv) establishment of the *ex situ* National Collection; (v) characterisation and evaluation; and (vi) foundation of traced mother stock nurseries (MSN).

III – The Pomegranate National Collection of Afghanistan

During the years 2006-2008 different areas of the country were surveyed in order to individuate and collect superior accessions of fruit tree species, including pomegranate. Local experts, nurserymen and fruit growers participated actively to this search. The main criteria to select the tree samples to be introduced in the "*In situ*" National Collection were based on the principle that the high market value genotypes and also some outstanding genotypes should be collected. This activity resulted in the field registration and labelling of 956 *in situ* accessions of different species, 60 of which were pomegranates Passport data and when possible a first characterisation was carried on Fig. 1.

Fig. 1. **Passport data and pictures of "Kandahari May Khush" (Clone AFG6056) from the PHDP *In situ* Collection Database (see http://afghanistanhorticulture.org/Germplasm.aspx).**

The *ex situ* Pomegranate National Collections were established as duplicates in Nangarhar and Kandahar Provinces in 2009; they hold 59 Afghan varieties and 20 imported cultivars, as listed in Table 1. Each collection consists of 6 replicated trees of all clones. When available, phenological data, such as flowering and ripening time of fruits were collected in 2010 and 2011. A descriptor list was defined taking into account the Descriptor List of Pomegranate (EC GENRES29 Project, 1996) and other descriptors lists (Bellini *et al.*, 2007). The standardised characterisation and evaluation, which regarded all the organs of the tree, and especially fruits, started in year 2011.

The Pomegranate National Collection is considered the official repository of this species and it represents an authorised source of propagation material to constitute mother stock nurseries, from where high quality and traced cuttings will be used for the production of true-to-type saplings by the private nursery sector.

Table 1. List of the accessions of the Pomegranate National Collection (AFG = domestic clones; IMP = imported cultivars)

AshkShoter-AFG0872	Kandahari-AFG5019	Sherinak-AFG6058	Wonderful-IMP7163
BaluchKhani-AFG4082	Kandahari-AFG6057	ShinaDanadar-AFG6064	Mae-IMP7164
Bedana-AFG0345	Kandahari-AFG6066	SorDoublPosti-AFG0389	Eve-IMP7165
Bedana-AFG0383	KandahariMayKhush-AFG6056	SorKhog-AFG0382	PurpleHeart-IMP7166
Bedana-AFG0762	LalBaqliDana-AFG4067	SorZodRas-AFG0385	Sakerdze-IMP7167
Bedana-AFG5018	Mahali-AFG0884	Sorkhak-AFG0296	Al-sirin-nar-IMP7168
Bedana-AFG6053	ManayiSur-AFG0384	Sorkhak-AFG0859	ApseronskiKrasnyj-IMP7169
Bedana-AFG6060	MayKhosh-AFG0873	Sorkhak-AFG0878	NikitskiRanni-IMP7170
Bedana-AFG6061	MayKhosh-AFG6065	SpinKhog-AFG0386	Zubejda-IMP7171
Bocha-AFG6068	MayKhoshShinki-AFG5025	SpinKhog-AFG0563	Crab-IMP7172
Bum-AFG0387	MayKhoshSpin-AFG5022	SpinTrush-AFG0388	Cranberry-IMP7173
Danadar-AFG0761	NazekPost-AFG0561	Tashkurghani-AFG0860	Palermo-IMP7174
DanadarSor-AFG5020	PandPost-AFG0562	Tashkurghani-AFG6063	MedovyiVahsha-IMP7175
Fakhi-AFG4081	Pastaki-AFG6067	Tor-AFG0390	Ariana-IMP7176
Kabli-AFG5021	Qohi-AFG0295	Turosha-AFG6054	Girkanets-IMP7177
Kabuli-AFG6059	SabzakDaniSafid-AFG4079	Turosha-AFG6069	Sumbarskii-IMP7178
Kabutak-AFG0297	SabzakDaniSorkh-AFG4080	TuroshaShinki-AFG0763	Mayatadzhy-IMP7179
KagaShin-AFG5024	Sherinak-AFG0302	TuroshaTaki-AFG6062	Andalib-IMP7180
KagaSpin-AFG5023	Sherinak-AFG0861	ZerAnar-AFG0298	Sirenevyi-IMP7181
Kandahari-AFG0862	Sherinak-AFG6055		Kopetdag-IMP7182

During 2011-2013 it is planned to complete the characterisation and evaluation of the *ex situ* collected accessions. All the clones will be described and a list of recommended varieties of pomegranate for Afghanistan will be released. Data will be used to compare clones in order to identify them and to discard duplicates and fill possible gaps.

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Breeding Mexican pomegranates to improve productivity and quality and increase versatility of uses

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Abstract. Pomegranate is a marginal fruit crop in Mexico, practiced by small farmers and focused on the national market. The Mexican varieties bear during summer, they are probably segregants of Spanish and American pomegranates, obtained through sexual propagation and informal introduction, they are maintained in gardens and family orchards. Mexico also regularly imports pomegranates from USA under the frame of commercial treaties. INIFAP has been supporting a Genetic Resources and Breeding program since 2002 pursuing the following objectives: to obtain new pomegranate cultivars suitable for export – Wonderful type–, to extend the harvest season into fall and to enhance fruit quality, among others. We have characterized the national germplasm and obtained selections adequate to supplement the local market, others with ornamental value and some with outstanding functional properties, advances that will be discussed in this presentation.

Keywords. Commercial varieties – Segregants – Breeding programme – Gene reservoir – Germplasm bank – Local market – Export.

I – Introduction

The pomegranate probably arrived in Mexico during the XVI y XVII centuries, introduced from Spain by the catholic friars involved in evangelization of the new territories. It was one of the species regularly included in the backyard orchards of the monasteries and missions, they were planted to provided fresh fruit to their tenants (Mondragon and Juárez, 2009). The germplasm base grew with the contributions of migrants of arab origin and the continuous exchange with USA, the natural market for Mexican products and the sink of migrant labor.

The easiness of sexual propagation may explain the presence of numerous segregants of Spanish pomegranates. Along with ornamental appeal, high adaptability favored the spread of pomegranates in gardens and family orchards of central and north central Mexico. Today solitary trees and small family orchards can be found in semiarid, subtropical and even in humid tropical sites of 16 states, however only Oaxaca, Hidalgo and Guanajuato report commercial production. These states provide 62% of the fresh pomegranate consumed in México City, Guadalajara and Monterrey, the main urban centers of the country. Official statistics reported 689 ha and 6,910 ton of annual production in 2010 (SIAP, 2011). Mixed cultivation of pomegranate with cereals, forage and leguminous crops is the rule, the plant is also found in the boundaries of field plots, providing an additional income for small farmers. Therefore pomegranate can be considered a marginal fruit crop in Mexico when compared to other commercial species like guava (23,000 ha), peach (35,000) or cactus pear (53,000) (SIAP, 2011; Gallegos and Mondragon, 2011).

In contrast to the Middle East and the Mediterranean growing areas which produce pomegranate in fall, Mexican pomegranates have adapted to bear fruit in summer – late June to early September, maturing the fruit during the short dry spell or "canícula" inserted in the bimodal rainfall pattern of the highlands. Still, there are some genotypes bearing fruit in early

summer or fall, but the quality of the fruit is not satisfactory. The bulk of the Mexican pomegranate is consumed as fresh fruit, either whole or as loose arils, it is widely available though in small quantities: Large supermarkets offer the produce without discriminating origin or varieties. Non-significant volumes of acidic pomegranates are used as raw material to manufacture liquors at cottage level in the western states of Jalisco and Colima. Under the free trade agreement (NAFTA) with North America a number of food products based on or containing pomegranate juice or arils –refreshing beverages, juices, cereals, etc. are available in the Mexican market.

Surprisingly enough, the most important use for pomegranate arils during season in Mexico is as an ornament or garnish of the special dish "chiles en nogada" (stuffed ancho peppers covered with a creamy nutty dressing) offered in restaurants from July to September, to celebrate independence. The dressing is sprinkled with fresh parsley and pomegranate arils arranged to resemble the colors of the Mexican flag, green-white-red.

Imports of fresh pomegranate from USA during fall have increased, large fruits arranged in fancy packing ensure high prices ($4-8 US/kg) at high-end supermarkets. Lower quality fruit is also available in popular supermarkets ($3-4 US/kg), a large difference on prices when compared to the 0.50 to 0.8 US/kg offered for the national product in summer.

The Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP) included pomegranate in its research programs since 2002, to rescue a naturalized plant resource and to diversify the portfolio of options for growers in semiarid regions. The aim is to characterize and enhance the Mexican germplasm with new cultivars accepted by the international markets, and to extend the harvest season in order to reduce imports. We are also aware that the growers involved in pomegranate cultivation need crop technology suitable to their conditions. In this paper we describe the importance of pomegranate and the progress and prospects of genetic enhancement in Mexico.

II – Pomegranate growing areas

Table 1 presents a the description of outstanding sites for pomegranate production, they share some common features; they are scattered across Central Mexico, orchards are usually less than 0.5 ha, located in places receiving less than 700 mm of annual rainfall, insufficient for commercial production unless supplementary irrigation is provided. A common problem of these areas is the occurrence of significant volume of rainfall at the late stage of ripening, associated to fruit cracking. Mexico also has large tracts of land with Mediterranean climate suitable for pomegranate expansion in the north, and interest on the crop is rising in Sonora, Baja California and Chihuahua.

Table 1. Basic agroclimatic features of outstanding areas of pomegranate production in Mexico.

Location	Altitude (masl)	Climate	Soil type	Annual rainfall (mm)
Apaseo el Alto, Gto.	1850	Semiarid	Vertisol, Feozem	642
Tecozautla, Hgo.	1700	Subtropical	Feozem	517
Metztitlán, Hgo.	1320	Subtropical	Feozem	378
Tasquillo, Hgo.	1655	Subtropical	Feozem	344
Metzquititlán Hgo.	1380	Subtropical	Feozem	497
Tehuacán, Pue.	1676	Semiarid	Litosol	473
Venado, SLP	1790	Semiarid	Coluvial y aluvial	493
Tecomaxtlahuaca, Oax.	1680	Temperate	Cambisol cálcico	580
Cuatro Cienegas, Coah.	740	Semiarid Mediterranean	Xerosol, litosol, Yermosol, vertisol	246

III – Pomegranate genetic resources

1. Comercial varieties

The most common varieties available in the Mexican market are:

Apaseo (Fig. 1). Originary from Apaseo el Alto, Gto. Trees of bushy growth habit –numerous stems. Deciduous tree which looses foliage at the end of fall, flowering occurs in april, when the risk of late frost is over. Highly productive >200 fruits per tree. Average fruit size >300 g, that can reach 700 if properly thinned, they are ready for harvest from July to early septembre. Thick epidermis somewhat sensitive to cracking specially under heavy rains. The aril is bright red, with sweet juice, seeds of intermediate size and moderate hardness. The yield of clean arils is approximately 50% of the total fruit weight.

Tecozautla (Fig. 1). Originary from Tecozautla, Hgo. Caduceous trees of bushy growth habit. Tolerate well slightly alkaline and heavy soils. Productive, long lived reports of trees older than 40 years are available. Highly productive > 200 fruits/tree. Well adapted to Hidalgo, Queretaro and Guanajuato states. Flowers open at the end of March and April, ahead of the risk of late frosts, fruit ripening occurs from the end of June and early August. Intermediate to large fruits (>250 g), round, yellow-orange skin with a slight pink-reddish blush. Respond well to thinning. Up to 60% of aril yield. Deep-red arils, sweet juice with small and soft seeds. A choice cultivar to combine with peach production.

Fig.1. Apaseo (left) and Tecozautla (right) pomegramate varieties.

2. Segregants of Spanish type and other origins

This type of pomegranates are widely found in Central Mexico. Fruits ripen in summer presenting variations of green, yellow and orange skin with or without reddish blush. The fruits vary in size, they are presented to the consumer as a mix of varieties. Arils are predominantly red and sweet. Usually the fruits are locally marketed. This pool represents a gene reservoir for adaptation to specific environments, as well as quality traits prized by local and regional consumers, they were developed through informal selection and continuous cultivation during more than four centuries.

3. Germplasm bank

INIFAP maintains a field collection –unique in the country- containing 2218 plants of varied ages; 13.5% are adult productive plants, and the rest are juvenile plants obtained from hybridizations carried out during 2009 and 2010. The orchard is located at Celaya, Gto. (20° 35´

52´´ N; 100 ^{o} 49´ 28´´ W) at 1,767 m of altitude. The climate of the site is warm semiarid, receiving 600-700 mm of annual rainfall. Regarding the genetic origin the collection contains samples of commercial varieties, segregants of American, North African and Middle East origin, as well as segregants of Wonderful variants (Fig. 2). From this pool we selected a group of six candidates for registration, they include selections for fresh fruit consumption, ornamental use and dry flower production.

Fig. 2.   A sample of fruit variability of pomegranates of the Spanish type, and segregants obtained from foreign accessions, compared to the local Cv, Apaseo (right, upper right) all share the summer bearing habit.

IV – Breeding objectives

To extend the harvest season. The actual demand of pomegranate covers July to early September, a season expected to extend into fall driven by the continuous imports of American fruit. Therefore it is imperative to develop late bearing varieties, a short term goal is to extend it at least a month from September 15 to October 15, when the American pomegranates arrive.

To improve fruit quality. Mexican consumer prefers large pomegranates >250 g –similar trend of other fruits (Gallegos and Mondragon 2011), associated to premium prices. The arils can be red to dark red, but should be sweet with low acidity. Soft and small seeds are also a plus. According to the market trend, packed loose arils are becoming common in supermarkets, therefore it is necessary to develop genotypes with large "yield" of loose arils, Tolerance to fruit cracking will increase revenues to farmers and industrial processors and reduce health risks.

Generation of Wonderful-type pomegranates with the summer and early fall bearing habit. Mexican pomegranates are yellow-orange with a reddish blush when ripe, well accepted in the national market. However the export market is taken by the Wonderful variety. The program attempts to combine the early bearing habit of the Mexican and the attractiveness of the Cv. Wonderful, aiming at a specific window of opportunity in the American market.

V – Breeding strategy

Collection and characterization of the national germplasm. Focused on genotypes of evident agronomic value and suitable for fresh consumption as well as industrial processing.

Introduction of selected foreign germplasm. Introduction of selected germplasm bearing traits not available in the national pool, according to the objectives of the program.

Hybridization and selection. The best individuals are included in a dynamic hybridization program, Up to now we have the F1 and selfs on the field in the juvenile phase.

Agronomical, chemical and functional characterization. A complete description of plant productivity, phenology, chemical and functional properties is included in the process. In some specific cases in vivo essays will be performed.

Propagation and evaluation. Advanced selections are propagated and tested in traditional production areas. We started evaluation of our selections in new potential areas (Sonora, Aguascalientes, Chihuahua) in 2010.

Documentation and registration. Descriptors are complete for the traditional varieties, four selections for fruit production, one with ornamental value and two for dry flower production. They will be protected under the current Mexican legislation.

VI – Progress

Table 2 summarizes the basic features of the fruits of the first generation of improved pomegranates developed for the Bajío and similar regions, sweet and sub-acid (sa) with potential industrial value are included.

Table 2. **Fruit traits of pomegranate selections obtained by INIFAP**

Selection	Diam. (cm)	Calix length (cm)	Peel thickness (mm)	Fruit weight (g)	Aril weight (g)	Seed weigth (g)	Total sugar (ºBrix)	Seed hardness
37-12 sa	8.6	2.1	5.1	395.6	192.1	-	19.2	Intermediate
39-2 sa	9.3	2.5	5.6	501.6	218.6	10.9	16.6	Intermediate
34-20	8.4	2.8	6.35	315.8	125.1	7.5	17.7	Soft
Jerecuaro	8.7	2	2.7	343.5	203.8	-	17.2	Soft
33-17	8.6	1.6	2.3	326	207	7.5	17.5	Intermediate
34-15	8.6	2	2.6	288.7	261.3	8.8	14.5	Soft
Tecozautla	9.1	2.3	5	407	225.3	8.2	16	Soft
33-12	9.2	2.3	4	375	262	-	15.6	Intermediate
Apaseo	8.3	-	-	289.2	182	10.1	11.5	intermediate

VII – Versatility of uses

The program has developed selections specific for dry flower production, they flower profusely with orange, red and deep red colors, they have been characterized (Meillon 2010; Reynoso *et al.*, under review), a field trial is underway to optimize production. According to our preliminary data it is possible to produce at least 1 kg of dry flower per tree/year. High density planting, training and pruning could increase dry flower yield to highly profitable levels.

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Morphological and physiological characteristics in pomegranate cultivars with different yields

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Abstract. Variations in yield, percentage of hermaphrodite flowers, leaf mass to area ratio, leaf area, stomatal density, leaf gas-exchange and stem water potential (Ψ_{stem}) characteristics were studied in four pomegranate accessions. Yield was greatest in 11005 and Kallisti, compared with 11019 and 11021 accessions. Similarly, percentage of hermaphrodite flowers was greatest in 11005, followed by Kallisti which may be related with greater yields. In addition 11005 had highest photosynthetic rate and leaf area, but leaf mass:area was lowest. Intrinsic water use efficiency was highest, and stomatal conductance and transpiration were usually lowest in 11005 and Kallisti, a characteristic that may enable water conservation. Significant greater stomatal density was also found in Kallisti, which may suggest for a flexible stomata regulation to water deficit conditions. There were no significant differences among the studied genotypes in Ψ_{stem}.

Keywords. Hermaphrodite flowers – Leaf gas exchange – Leaf mass:area ratio – Stem water potential – Stomatal density.

I – Introduction

Understanding the relationship between yield and morphological and physiological characteristics is an important objective in crop breeding. Yield is the most important economic characteristic in pomegranate and is often recorded to vary greatly in different pomegranate genotypes. The abundance of hermaphrodite flowers would have a profound impact on yield since this will affect the number of fruit produced. Leaf gas exchange and leaf morphological characteristics have also been documented to affect yield in many species, however little is known for pomegranate. The present study aims to investigate various morphological and physiological parameters that may be related with increased yield in pomegranate.

II – Materials and methods

The experiment was contacted on the pomegranate accessions 11029 (named as Kallisti), 11005, 11019 and 11021, grown in a collection orchard at the Pomology Institute. The trees were 9 years old and planted in a 5 x 1.5 m distance in a randomised block design of six trees per genotype in two replicate blocks per tree. Mature fruits were harvested when most of their colour was red and total yield was measured. The numbers of hermaphrodite and male flowers were measured in 40 open flowers, three times during the flowering period, and the mean percentage of hermaphrodite flowers was calculated. Measurements of leaf area, leaf mass to area ratio (LMA), and chlorophyll content were made in leaves gathered from the middle part of young shoots in morning of August 19, 2010, from three trees of each accession. Leaf area was measured using a tracing technique in 30 leaves, and LMA was measured from the dry weight and leaf area of 10 leaves. Chlorophyll was measured in 9 leaves after extraction in 96% (v/v) ethanol for 48 h in the dark. Stomatal density was measured in artificial replicas of nail varnish

in the central region around the midrib of lower epidermis. For each leaf impression, five fields of view were selected for analysis.

Leaf gas exchange analysis was carried out with a LI-6400 portable gas exchange system (LI-COR Biosciences, Lincoln, USA). Measurements were carried out employing a PPFD of 1,700 µmol m^{-2} s^{-1}, c$_a$ of 350 µmol m^{-2} s^{-1} and leaf temperature of 28ºC. The parameters determined were CO_2 carbon assimilation (Pn) and stomatal conductance (g$_s$) following Von Cammerer and Farquhar (1981). The ratio of Pn/g$_s$ was used as an estimate of short-term (instantaneous) leaf water use efficiency (WUE). Measurements were taken on the 5th of July, and 5th of August, from 9:00 to 11:00 hours, on six replicate leaves.

Stem water potential (Ψ_{stem}) was measured with a pressure chamber (Plant Moisture System, Skye instruments Ltd, Powys, UK). Leaves were previously placed in a black polyethelene bag wrapped in aluminium foil for at least 90 min before measurements to allow leaf water potential to equilibrate with stem water potential. Leaves were placed in the chamber within a few seconds after excision. Measurements were made in the next day after the gas exchange measurements, from 8:30 to 10:00 hours, on six replicate leaves.

Statistical analyses were performed using SPSS (SPSS Inc., Chicago, USA). Data were subject to ANOVA or MANOVA, and then significant differences between individual means were determined using the Duncan's multiple range test at the 5% level.

III – Results and discussion

Yield was greatest in Kallisti and 11005, compared with 11019 and 11021 (mean values 19.2 *vs* 8.3 kg, respectively), and may have resulted from a greater percentage of hermaphrodite flowers (63.8% in 11005, 37.6% in Kallisti, compared with 15.1% in 11019 and 11021), which suggests for a greater yield potential (Table 1).

Table 1. Mean values (±SE) of total yield and various morphological, and physiological characteristics in four pomegranate genotypes

	Kallisti	11005	11019	11021
Total yield (kg)	20.0 ±1.5 a	18.3 ±3.7 a	7.9 ±1.2 b	8.6 ±1.1 b
% Hermaphrodite flowers	37.6 ±9.7 b	63.8 ±6.1 a	13.4 ±2.1 c	16.7 ±3.8 c
Leaf mass per unit area (LMA, g m^{-2})	92.8 ±4.0 a	79.9 ±4.2 b	84.4 ±3.9 ab	94.0 ±4.1 a
Leaf area (cm^2)	5.5 ±0.3 c c	7.8 ±0.3 a a	6.8 ±0.2 b b	6.8 ±0.2 b b
Photosynthetic rate (Pn, µmol m^{-2}s^{-1})	9.5 ±0.7 b	13.0 ±0.6 a	9.0 ±0.7 b	10.0 ±0.6 b
Stomatal conductance (g$_s$, mmol m^{-2} s^{-1})	138.2 ±8.1 b	138.9 ±8.5 b	207.1 ±10.4 a	179.7 ±8.3 a
Transpiration rate (E, mmol m^{-2} s^{-1})	4.6 ±0.1 c	4.7 ±0.2 bc	6.3 ±0.3 a	5.7 ±0.2 ab
Water use efficiency (Pn/g$_s$, µmol mol^{-1})	84.2 ±10.3 b	96.0 ±16.4 a	49.9 ±6.5 c	58.2 ±4.3 bc
Stomatal density (stomata mm^{-2})	149.9 ±4.1 a	101.0 ±7.1 c	122.6 ±2.0 b	68.8 ±3.1 d
Total chlorophyll $_{mass}$ (mg g^{-1} DM)	6.4 ±0.6	7.0 ±0.5	7.7 ±0.2	6.9 ±0.6
Total chlorophyll $_{area}$ (mg dm^{-2})	5.9 ±0.5	5.6 ±0.4	6.5 ±0.2	6.5 ±0.5
Stem water potential (Ψ_{stem}, MPa)	-1.15 ± 0.08	-1.24 ±0.07	-1.15 ±0.05	-1.08 ±0.04

Different letters denote significant differences at P ≤0.05.

Increased levels of Pn was also found in 11005, compared with the rest studied accessions and may be related with the greatest assimilate demand from fruit (Drogoudi and Ashmore, 2000) and/or better developed photosynthetic mechanism. Leaf area was also greatest in 11005,

which may have provided greater availability of photosynthetic assimilates to accommodate yield. Total chlorophyll content expressed per dry mass or leaf area did not differ among the studied genotypes.

Leaf mass per unit area is an indicator of leaf thickness and the degree of mesophyll development within a leaf blade and is often related with greater photosynthetic capacity (Le Roux *et al*., 2001). In the present study, although 11005 had highest Pn values and leaf area, LMA was lowest (Table 1). The present results coincide with the study of Mediavilla *et al.* (2001) where in intraspecific comparisons, found that Pn were higher in low LMA leaves, and may be due to a higher proportion of leaf nitrogen in the photosynthetic machinery. Reduced thickeness (in low LMA leaves) should also tend to decrease the path length from the stomata to cell wall surfaces reducing gaseous diffusion resistance (Mediavilla *et al*., 2001).

Intrigliolo *et al.* (2011) found that different irrigation treatments in pomegranate induced considerable differences in Pn and g_s, whereas there were no significant differences in Ψ_{stem} in spring and autumn, suggesting an near-isohydric behaviour of pomegranate trees. Similarly, in the present study there were no significant differences among the studied genotypes in Ψ_{stem} although they varied in Pn, g_s and WUE.

Intrinsic water use efficiency (Pn/g_s) was highest, and g_s and E were usually lowest in 11005 and Kallisti, a characteristic that may enable water conservation. Significant greater stomatal density was also found in Kallisti, which may suggest for a flexible stomata regulation to water deficit conditions.

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Pomegranate improvement through clonal selection and hybridization in Elche

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Abstract. Spain is one of the most important pomegranate producers and exporters in the Mediterranean Basin. In spite of various pomegranate cultivars grown, sweet and soft seeds 'Mollar de Elche' and 'Valenciana' are the main varieties-population. This paper reports the activities in progress on pomegranate germplasm and breeding in the last ten years at Valencian Agricultural Research Institute (IVIA) in Elche. A collection of 35 accessions collected from different parts of Alicante was established and characterised. The collection has been implemented with new accessions. Hybridization between 'Mollar de Elche' and other foreign varieties was done in order to obtain darker skin and early ripening. Crossbred progenies were selected and they are being examined in an experimental orchard.

Keywords. *Punica granatum* – Germplasm – Accessions – Fruit characteristics.

I – Introduction

Spain is one of the most important pomegranate producers and exporters in the Mediterranean Basin, where the species has a long history. Commercial pomegranate cultivation is concentrated in the South-East, mainly around the South of Alicante province. In this area, pomegranate became a crop in the 19th century. Later, the pomegranate cultivation decayed during the drought period happened at the beginning of 20th century and re-emerged about seventy years ago, when irrigation facilities and water supply were available.

Most of cultivars known today are selections from an unknown origin (Holland, 2009). 'Mollar de Elche' and 'Valenciana` are the most widely spread cultivars in the local area. 'Mollar de Elche' sweet and soft seeded, is the Spanish pomegranate most known in the world. There is variability within this variety-population (Martinez, 2006), therefore it is recommended to select the best clones in order to optimize the potential of the crop in new pomegranates orchards.

Very few varieties have been developed by systematic breeding programmes despite the need of meeting the demands of local and international consumer, processors, growers and exporters. Breeding new varieties are being achieved by conventional approaches through hybridization what generally involves selection of parents, hybridization and selection of superior genotypes. Development of varieties by hybridization and selection has been reported in Israel, India, China, Turkmenistan and Azerbaijan (Bar-Ya'akov, 2009; Jalikop, 2010). The hybrids exhibited distinct morphological features.

 Attractive colour is one of the most important sensory characteristics of fruits. For instance, dark colour is a desirable fruit character for fresh consumption and export purpose in Europe. Other desired traits are red peel and early ripening. 'Mollar de Elche' has pink peel and middle ripening time.

The objectives of this study are to find out local pomegranate genotypes, characterize them and to evaluate initial breeding results. In this paper we report the activities in progress carried out on pomegranate germplasm since 2001 at Valencia Agricultural Research Institute (IVIA) in Elche.

II – Materials and methods

Pomegranate accessions were selected from locally grown pomegranate trees found in commercial orchards in 2001. They were raised from cuttings and planted in an experimental orchard in Elche. New accessions have been collected up till now from Valencia, Murcia and Alicante. In addition to the local collection, 11 foreign introduced cultivars were planted. The climate in the South-East region of Spain is semi-arid, dry and hot in summer. Annual precipitation in Elche is less than 280 mm, mainly in October.

Pomological and morphological characteristics for these accessions were recorded. The variables measured were: thorniness, tree vigour, suckering tendency, leaf length, maximum width leaf, fruit weight (g), fruit equatorial diameter (mm), diameter of calyx (mm), total height of fruit without calyx (mm), skin colour, thickness of skin at equatorial area (mm), skin weight plus carpel endocarpic weight (g), seed length (mm), tegmen length (mm), seed width (mm), tegmen width (mm), seed weight (g), tegmen weight (g), seeds firmness (achenes), harvest maturity, eating quality and flavour were investigated. The pH, titrable acidity, and soluble solid content were measured. The pH measurements were performed using a pH-meter (GLP 21; Crison Ltd, Spain). Total titrable acidity (TA) was determined potentiometrically using 0.1 M NaOH to the end point of pH 8.1 and expressed as grams of citric acid per litre. The soluble solid content (SSC) was measured by refractive index as °Brix with an Atago N1 refractometer (Tokyo, Japan). The ratio, also known as the maturity index, was calculated as the relation between TSS and TA. Peel and juice colour measurements were determined using a Minolta CR-400 tristimulus colour spectrophotometer (Osaka, Japan) according to the CIELAB convention. Juice colour was performed in glass cells of 2 mm path length (CT-A22).

Fertilization is provided through the irrigation system. Trees are grown 4 m apart along the row and 4.5 m between the rows. Four clones per accession are planted.

1. Breeding plot

Following the characterization we selected cultivars for the establishment of a breeding project aimed to improve existing cultivars. Breeding began in 2002 with pollinations between parents with desirable phenotypes. Normal hermaphrodite type flower was effected for crossing purposes after emasculation of flowers shortly before anthesis. Inter-specific hybrids between 'Mollar' and 'Wonderful' were successfully developed. The seedlings are planted in a distance of 0.5 m from each other. The plot is irrigated from April to October. After examining the tree for at least two successive years, the non promising seedlings are discarded. Once a desirable plant is located it is propagated vegetatively by cuttings.

III – Results and discussion

1. Pomegranate accessions

A collection of 35 accessions collected from Valencia, Murcia and Alicante was established and characterized. These accessions differ from each other by a diverse range of features such as fruit size, peel colour, aril colour, growth habit and yield The characterization resulted in 19 accessions from 'Mollar de Elche' , and 6 accessions from ´Valenciana' . The main traits of the selected types are shown in Table 1. CM49 was the most productive, CM55 has the darker colour arils and CM63 produced the biggest fruit size. Among the Mollar types, the average fruit weight was 330 g, fruit width was 87.25, total soluble solids 16.5%, and acidity 0.21. 'Valenciana' clone CV111 has lower weight tegmen/aril ratio.

A molecular genetic identification base on microsatellites markers is being in progress in order to fingerprint all the accessions.

Table 1. Juice yield, total soluble solids (TSS), titrable acidity (TA), index maturity ratio (IM) and pH from selected pomegranate accessions

Accessions	Juice yield (g 100 g^{-1})	TSS (ºBrix, 20ºC)	TA (g citric acid 100 g^{-1})	IM (TSS/TA)	pH	Fruit weight (g)
CM-63	37.37	16.8	0.20	80.89	3.87	369.81
CM-55	37.23	17.53	0.19	89.22	4.22	367.23
CM-49	38.56	17.73	0.21	85.21	3.86	337.21
V-111	42.13	15.43	0.20	74.20	3.94	285.96
V-120	40.37	15.27	0.28	54.62	3.75	280.12
V-116	39.46	15.30	0.21	71.33	3.88	265.69
Wonderful	34.35	16.55	1.41	11.73	3.28	324.86

2. Pomegranate improvement

Breeding began in 2002 through crossing of selected genotypes. Hybridization between 'Mollar de Elche' selected genotypes and other foreign varieties was done in order to obtain sweet fruits, red arils but darker skin and to enlarge the ripening season. Although, sweet fruits with soft seeds, resistance to cracking and sunburn are also requested. Selection of appropriate parents from 'Mollar de Elche' accesions from our collection was done. Inter-specific hybrids between 'Mollar' and 'Wonderful' were successfully developed. The hybrids exhibited variability in colour, pH, maturity index and morphological features (Table 2) . Seedlings were classified as sweet, sweet-sour and sour, juicy and table fruit, soft and hard-seeded. A high degree of variation for fruit characters was observed. Into the progeny, 16% of fruits were sweet, 18% were soft tegmen, 76% were darker peel than Mollar, 78% were more red arils than Mollar and 6% had excellent eating quality. After data, from at least two successive years the non promising seedlings were discarded.

Table 2. Peel colour values (L*,a*,b*,Chroma, Hue angle), pH, total soluble solids (TSS), titrable acidity (TA) and ratio (TSS/TA) from selected pomegranate Mollar x Wonderful hybrids

Accessions	Peel colour					pH	TSS	TA	TSS/TA
	CIE*L**	CIE*a**	CIE*b**	*C**	*h**				
H7/26	40.28	32.41	10.86	34.18	18.53	2.94	14.57	0.39	37.04
NH5/6	48.06	50.19	29.67	58.30	30.59	3.68	14.13	0.40	35.36
NH6/9	58.70	44.27	25.99	51.34	30.42	3.35	14.97	0.53	28.25
H2/19	49.13	49.20	27.93	56.57	29.58	3.29	12.87	0.55	23.57
NH1/9	49.18	48.00	29.33	56.25	31.43	3.38	13.37	0.53	25.23
NH4/7	56.14	41.32	27.95	49.89	34.08	3.55	12.90	0.51	25.12
H10/29	39.77	43.85	20.55	48.43	25.11	2.96	13.07	0.39	33.50
NH1/5	46.54	52.24	27.46	59.02	27.73	3.18	14.90	0.44	34.21
H1/18	43.96	50.46	29.48	58.44	30.29	3.77	12.87	0.28	46.03
H10/18	49.28	52.14	26.02	58.27	26.52	2.91	13.80	0.42	33.17
H10/33	42.74	50.64	27.40	57.58	28.42	3.05	13.10	0.39	33.31
Mollar	64.97	23.96	31.71	39.74	52.93	3.87	16.66	0.21	80.11
Wonderful	54.11	36.23	29.76	46.89	39.40	3.28	16.55	1.41	11.73

The desirable plants are selected and propagated vegetatively by cuttings. The preselected genotypes are under evaluation in multi-location experimental orchards. The seedlings are planted in a distance of 2.5 m from each other. In order to reduce several undesirable traits, backcrosses were carried out. Currently 600 F2 cross pollinated pomegranate seedlings are under evaluation.

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A preliminary survey on pomegranate (*Punica granatum* L.) genotypes localized in Apulia region, Southeastern Italy

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Abstract. Little information is available about the pomegranate genotypes present in Italy and in particular in Apulia (Southeastern region of Italy). A two-year study (2008-2009) was carried out in order to evaluate morphological, organoleptic and chemical parameters of 8 pomegranate genotypes (4 sweet and 4 sour) localized in some small orchards. Significant differences were observed among the 8 genotypes for many of the parameters investigated.

Keywords. Sweet – Sour – Morphological measurements – Polyphenols – Antioxidant activity.

I – Introduction

Some studies have been published on the morphological and biochemical characteristics of pomegranate fruits in various Mediterranean countries (Barone *et al.*, 2001; Drogoudi *et al.*, 2005; Tzulker *et al.*, 2007; Martínez *et al.*, 2006) but no information is available about the genotypes present in Apulia region (Southeastern of Italy), where no hectares result officially under cultivation (ISTAT, 2010).

The objective of this research was to analyze morphological, organoleptic and chemical parameters of 8 genotypes of pomegranate localized in some orchards in order to better characterize genotypes that may be used for cultivation or for breeding programs in the next future and as a perspective of further development of pomegranate cultivation in the region.

II – Materials and methods

The collection of pomegranate fruits was conducted in the years 2008 and 2009 from adult trees located in private small orchards. For both years harvesting time ranged from mid-end September to mid October. Four genotypes were sour and four were considered sweet. The genotypes analyzed were: Common Triggiano (ComTri), Modugno Triggiano (ModTri), Common Molfetta (ComMol), A dente S. Giorgio (AdeSgi), Sour Molfetta (SouMol), Sour Ostuni (SouOst), Sour S.Giorgio (SouSgi), Sour Triggiano (SouTri).

Morphological measurements (Mars and Marrakchi, 1999; Martínez *et al.*, 2006) and organoleptic and chemical analyses were carried out on samples of 15 mature fruits per genotype and per year. Total phenolic contents (TPC) of the pomegranate juices were assayed according to Folin-Ciocalteu method. The antioxidant activity was measured with a spectrophotometric method by using DPPH (Brand-Williams *et al.*, 1995) and slightly modified. The antioxidant activity was expressed as TEAC and AEAC.

III – Results and discussion

The average weight of the fruit was 338.7 g and a significant difference was observed between the two years (317.7 g, in 2008 and 359.7 g, in 2009). The weight of the fruit ranged from a minimum of 168.9 g (SouMol) to a maximum of 574.9 g (SouOst), as shown in Table 1. Mean fruit weight was similar to that measured for some Spanish cultivars (Martínez *et al.*, 2006). The mean weight of the arils was lower than that obtained for new varieties in Spain, 505 mg (Martínez *et al.*, 2006) and for Iranian accessions, 444 mg (Sarkhosh *et al.*, 2009) but are close to the mean weight of Greek accessions (Drogoudi *et al.*, 2005).

SouOst presented the highest juice volume (72.2 cm^3) and SouTri the least (65.2 cm^3); the mean value was 67.8 cm^3, higher than the value reported in Spain, 59.13 cm^3 for new varieties (Martínez *et al.*, 2006). The highest °Brix (Table 1) value was surprisingly measured in a sour genotype, SouMol (18.0), and the lowest in a sweet genotype, ComTri (14.7). The °Brix mean value of the apulian genotypes was 16.0, very similar to what reported for Spanish varieties (Martínez *et al.*, 2006). Total acidity ranged from 5.4 (ComMol) up to 25.0 g/l (SouTri). The results for the morphological and organoleptic characteristics indicated a significant difference among the 8 examined genotypes. AdeSgi seemed the most interesting for the fresh market both for the size of fruit and arils.

Table 1. Mean values of the morphological and organoleptic characteristics of the fruits

Genotype	Fruit weight (g)	Aril weight (mg)	Seed weight (mg)	Juice volume (cm^3/100g)	°Brix	Total acidity (g/l citric acid)
SouTri	374.2	367.1	21.2	65.2	16.3	25.0
ComTri	267.2	435.1	21.9	70.8	14.7	4.3
ModTri	226.5	335.5	23.6	65.8	15.3	5.7
ComMol	173.5	426.9	21.7	69.5	16.4	5.4
SouMol	168.9	277.4	20.8	66.2	18.0	21.7
SouOst	574.9	403.9	23.5	72.2	14.8	19.6
SouSgi	543.4	323.4	23.1	65.5	16.8	23.6
AdeSgi	381.3	519.1	24.9	67.3	15.5	5.5

The content of total polyphenols (Table 2) determined by HPLC ranged from 6.4 (AdeSgi) to 97.1 (SouMol) mg/l. The content of total polyphenols determined by Folin–Ciocalteu method (Table 2) was higher than the values obtained by HPLC and varied from 303 (SouOst) to 1328 (SouSgi) mg of gallic acid equivalent/l of juice. Our values are similar to polyphenols values recently reported for Chilean genotypes (676-1280) (Sepúlveda *et al.*, 2010). Antioxidant activity of pomegranate juices varied from 8.0 (ComTri) to 17.7 (SouMol) and from 6.0 (ComTri) to 13.7 (SouMol), respectively for AEAC and TEAC (Table 2). Sour genotypes presented the highest antioxidant activity even higher than red wine and green tea (6-8 TEAC) and similar to values (12-14 TEAC) reported for juice of 'Wonderful' (Gil *et al.*, 2000). Vitamin C content (Table 2) ranged from 89 (SouOst) to 236 (SouMol) mg/l, values much higher than the values (13-52 mg/l) reported in the Greek accessions (Drogoudi *et al.*, 2005). In conclusion, significant differences have been observed among the pomegranate genotypes. Considering all the morphological, organoleptic and chemical parameters analyzed, some genotypes are worthy to be considered either for the fresh market or for the juice industry.

Table 2. Polyphenols, antioxidant activity and vitamin C content of the juice

Genotype	Polyphenols (HPLC-UV) (mg/l)	Total Polyphenols (mg/l GAL)	Antioxidant activity (mM AEAC)	Antioxidant activity (mM TEAC)	Vitamin C (mg/l)
SouTri	17.2	788.3	11.3	9.3	144.3
ComTri	12.8	1020.0	8.0	6.0	156.0
ModTri	11.0	1020.0	10.7	8.0	144.7
ComMol	13.2	630.7	10.9	8.3	156.7
SouMol	97.1	960.7	17.7	13.7	236.3
SouOst	6.6	303.0	10.5	8.0	89.0
SouSgi	57.3	1328.0	16.0	12.0	192.0
AdeSgi	6.4	436.3	10.3	7.7	105.0

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La culture du grenadier (*Punica granatum* L.) au Maroc

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Abstract. In Morocco, pomegranate (*Punica granatum* L.) has been considered for long time a secondary fruit tree. In the last years, its culture is extended over different regions in more than 4000 ha. Today, Morocco is one of the world's important pomegranate production countries. The total production is 58000 tons. It's well concentrated in the center and the north regions. The total of production is consumed in local markets and very little quantities (<0.5%) are exported. Fruits are consumed fresh or processed into juice. Local varieties are numerous and well adapted to agroecological conditions. Their denominations reflect mainly origin area, the shape or the fruit color. Interchange of plant material was very frequent between regions. These varieties are maintained in two germplasm collections in Beni Mellal and Meknès. The local germplasm is characterized by a large diversity. However, little is known about the genetic diversity organization in this crop.

Keywords. Pomegranate – Local varieties – Maroc.

I – Introduction

Le grenadier (*Punica granatum* L.) est l'une des espèces fruitières les plus anciennement cultivée au Maroc. Cependant, il a été considérée pendant longtemps comme une espèce secondaire. Cette espèce largement cultivée dans le pays semble être ubiquiste étant donné ses grandes potentialités adaptatives aux conditions environnementales (sols et climats). Durant ces dernières décennies, le grenadier prend de plus en plus de l'importance et sa culture est passée du caractère traditionnel pour se développer en vergers commerciaux.

Malgré ses caractéristiques nutritionnelles intéressantes, le grenadier reste sous-exploité au Maroc. Il est consommé principalement en fruit frais. Quant à la valorisation de la production par sa transformation, on note l'absence des unités industrielles qui peuvent améliorer la rentabilité de ce secteur. Les sous-produits (écorce, pulpe, etc.) sont sur tout utilisés en médecine traditionnelle pour soigner des brulures de l'estomac et en teinturerie.

En termes de recherche scientifique, cette espèce reste très peu étudiée au Maroc bien qu'elle a le potentiel de valoriser et de diversifier la production fruitière dans plusieurs régions du pays.

II – Situation de la culture du grenadier au Maroc

Au Maroc, la culture du grenadier a pris un grand développement ces dernières années. Actuellement, la superficie totale occupée est d'environ 4625 ha. La production de grenadier continue à augmenter considérablement, elle est estimée à 58000 tonnes atteignant un rendement moyen de 12 t/ha (MAPM, 2005). La majeure partie de cette production est destinée au marché local. Les quantités exportées restent faibles et ne dépassent pas 0,5% de la production totale.

Le grenadier se place parmi les espèces fruitières ayant une importance économique majeure, notamment dans les régions de Beni Mellal, Marrakech, Settat, Taounate, Nador, Meknès, Fès

et certaines oasis du sud (Walali *et al., 2003* ; Oukabli, 2004). La région de Beni Mellal au centre du pays est considérée comme la première zone productrice des grenades. Elle contribue avec plus de 45% de la production nationale (28800 t pour 1410 ha). Le rendement moyen obtenu dans cette région est de 20 t/ha contre une moyenne national de 12,5 tonnes à l'hectare (ORMVAT, 2002).

Au Maroc, la plupart des vergers du grenadier sont du type traditionnel et de petite taille. La culture est conduite en plantation régulière, seule ou associée à d'autres espèces comme l'olivier et la vigne. Il est adapté au climat sub-aride et continental et peut tolérer des températures de –12°C l'hivers et 42°C l'été. C'est une espèce qui s'accommode de sol très variés avec une préférence pour les terres d'alluvions profondes ou argilo-limoneuses à forte rétention en eau (Walali *et al.*, 2003).

III – Variétés de grenadier cultivées au Maroc

Il existe actuellement un grand nombre de variétés de grenadier au Maroc et probablement beaucoup de synonymie d'appellation. Ces variétés portent des dénominations différentes, attribuées selon la forme du fruit (Ounk Hmam), la couleur de l'épiderme (Sefri, Grenade rouge et Grenade jaune) ou la zone (Bzou). L'échange de matériel végétal entre différentes régions est très fréquent.

Deux groupes de variétés sont cultivées au Maroc :

(i) Les grenades à pépins doux consommées en frais : Le Sefri de Beni Mellal, les grenades rouges et jaunes de Marrakech, le Kharaji de Bzou, le Mesri de Meknès, Laroussi de Fès et Zhéri d'origine tunisienne.

(ii) Les grenades acides à pépins durs destinés généralement à la transformation : On distingue Wonderful, Negro, Monstruoso, et Dwarf semi evergreen.

Les variétés Sefri, Grenade rouge et Grenade jaunes sont les variétés les plus importantes qui sont cultivées dans le pays. Pour préserver le matériel génétique de cette espèce au Maroc, deux collections ont été établies : la collection d'Ahl Sousse à Beni Mellal et la collection de l'INRA à Meknès.

IV – Contraintes

La culture du grenadier au Maroc connaît actuellement quelques problèmes qui affectent la qualité des fruits et la rentabilité de la culture :

(i) La grande variabilité du matériel végétal rend le choix variétal assez problématique et pourrait engendrer une grande hétérogénéité au niveau de la production.

(ii) L'éclatement des fruits qui peut avoir plusieurs origines est un des principaux problèmes de la production de cette espèce.

(iii) Ravageurs et maladies : Parmi les principaux ravageurs et maladie du grenadier, il y a le puceron Aphis punicae qui colonise les jeunes pousses printanières et contribue à une forte altération qualitative et quantitative de la production (Fakhour et Sekkat, 2006). Une maladie fongique entraîne la pourriture du fruit dont les graines deviennent noires et impropres à la consommation. Les dégâts sont importants dans les zones fortement humides (Walali et al., 2003 ; Oukabli, 2004).

(iv) L'absence de station de conservation des fruits rend très difficile l'étalement de la période de commercialisation des grenades et occasionne des pertes énormes.

(v) L'exploitation des sous-produits du grenadier reste très limitée et à caractère traditionnel.

V – Conclusion

Le grenadier est une espèce commercialement très intéressante. Les fruits sont très recherchés localement et les possibilités d'exportation sont très importantes. L'avenir de la culture du grenadier au Maroc est lié à la solution des problèmes de sélection et d'amélioration variétale, à la maîtrise des techniques de conservation et à l'organisation professionnelle par la création d'association regroupant les producteurs.

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Characterization of six varieties of Moroccan pomegranate

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Abstract. This study evaluated the characteristics of six pomegranate varieties ("Sefri", "Ounk Hman", "Ruby", "Rouge Marrakech", "Bouaâdime" and "Jaune Marrakech"), being all indigenous to four provinces in Morocco where the species shows high variability. A morphological and organoleptic characterisation of the fruits and of the edible portion of the seeds were investigated. Some chemical characteristics of the juice, including total soluble solids, pH, acidity, crude fiber and maturity index, were assessed. The results of the study reveal that the Moroccan varieties present an average top weight to 430g/fruit and one big caliber. The assessment of pomegranate chemical compositions implies the great potential of Moroccan cultivars for both fresh market and fruit processing.

Keywords. Characterization – Fruit – Seeds.

I – Introduction

Pomegranate (*Punica granatum* L.) is an important tree of the tropical and subtropical regions of the word which is valued for its delicious edible fruit. It is cultivated in Iran, Afghanistan, India, Mediterranean countries, (Morocco, Spain, Turkey, Tunisia and Egypt) and Middles-East countries are the main regions of pomegranate cultivation and production (Jbir *et al.*, 2008; Melgarejo *et al.*, 2009). In Morocco the total production exceeded 58000 tn in 2005 and one surface of the 4625 ha (MAPM, 2005). Beni Mellal is the first region in Morocco in production and surface (1410 ha and 28800 tn) (ORMVAT, 2002). In recent years, there has been an explosion of interest in pomegranate fruit, because it is considered to be a functional product of great benefit in the human diet as it contains several groups of substances that are useful in disease risk reduction (Martínez *et al.*, 2006; Çam *et al.*, 2009a). In spite of various pomegranate cultivars grown in different regions of the Morocco, few published results on the properties on the cultivars in the literature are available, therefore, the aim of this work was to determine the physico-chemical characteristics, as well as the total soluble solids and titratable acidity, two parameters whose ratio defines the taste of pomegranate juice the six varieties grown in Morocco in order to gain more knowledge about the juicy potential of the fruits.

II – Material and methods

1. Plant material

Six varieties of pomegranate: "Sefri", "Ounk Hman", "Ruby", "Rouge Marrakech", "Bouaâdime" and "Jaune Marrakech", were collected from four provinces in Morocco. Fifteen kg of each variety were picked at commercial ripening. Once in the laboratory the pomegranate with defects (sunburns, cracks, cuts and bruises in peel) were discarded.

2. Characterization of the fruit and the seeds

From each cultivar and replication, 20 pomegranates were randomly picked every single year. The following fruit characteristics physical were studied: fruit weight, equatorial diameter, calyx diameter, fruit height without calyx, total fruit height, calyx height, number of carpels, skin thickness. Later the juice was extracted using an electric extractor and a seed sample of 100 g. Total soluble solids (TSS) was determined in triplicate from the juice obtained for each sub-sample with a digital refractometer Atago N1 (Atago Co. Ltd., Tokyo, Japan) at 20°C, and expressed as % (°Brix). Total acidity (TA) was also determined in triplicate in each sub-sample by automatic titration (877 Titrino plus, Metrohm) with 0.1 N NaOH up to pH 8.1, using 1 ml of diluted juice in 25 ml distilled H_2O, and results expressed as g citric acid per L^{-1}. Maturity index (TSS/TA). Up to date the following classification has been established for Spanish varieties (Melgarejo, 1993): Sweet varieties: MI=31-98; Sour-sweet varieties: MI=17-24 and Sour varieties: MI=5-7. Moisture percentage of pulp was determined by dried in a hot air oven at 50ºC until constant weight. Four repetitions per variety were carried out. Crude fiber (CF) contents were determined by a digestor Ankon220 fiber analizer model A220 made in USA, following the official methodology established by the Spanish Ministry of Agriculture, Fisheries and Food (MAPA, 1993).

III – Results and discussions

The average weight of the fruits of the Moroccan studied varieties changes inside a range understood between the 430.81 g "Rouge Marrakech" and the 535.06 g "Sefri" (Table 1), qualifying of big size, according to the criteria used for the Spanish varieties by Melgarejo (1993). The higher calibre of fruit was shown of the variety "Sefri", not presenting statistically significant differences with "Ounk Hman", "Ruby" and "Jaune Marrakech", whereas the lower calibre was shown by "Rouge Marrakech" (Table 1).

Table 1. Mean values of principal morphological parameters of the fruits

Cultivar	Pf (g)	D1 (mm)	D2 (mm)	L1 (mm)	L2 (mm)	L3 (mm)	Nc	Pc+Mc (g)	Ec (mm)	Rs (%)
'Sefri'	535.0b	100.44b	20.48a	87.80cd	99.65ab	11.85a	7.06c	207.76a	3.5a	60.98c
'Ounk Hman'	481.28ab	96.58ab	23.30b	84.14abc	97.92ab	13.78a	6.45b	205.85a	4.27ab	57.49bc
'Ruby'	504.36b	96.85ab	21.12ab	89.15d	103.65b	14. 51a	6.28ab	223.56a	4.25ab	55.96ab
'Rouge Marrakech'	430.81a	93.92a	22.02ab	82.10ab	94.33a	12.23a	5.9a	197.01a	4.93b	54.79ab
'Bouaâdime'	435.45a	92.85a	20.90ab	80.75a	95.18a	14.42a	6.45b	202.89a	3.66a	53.20a
'Jaune Marrakech'	484.15ab	96.48ab	21.67ab	85.07bc	97.13a	12.27a	6.60b	213. 84a	4.78b	55.74ab

The values followed by the same letter show no statistically significant differences (P < 0.05).
Pf: fruit weight; D1: equatorial diameter; D2: calyx diameter; L1: Fruit height without calyx; L2: Total fruit height;
L3: Calyx height; Nc: Number of carpels; Ec: Skin thickness; Rs: Seeds yield; Pc+Mc: Skin weight + membranes

Table 2 showed pomegranate qualitative traits. TSS contents significantly differed among the evaluated varieties, ranging from 15.2º Brix to 17.6º Brix for "Sefri" and "Bouaâdime", respectively. This TSS interval range agreed with those reported from other collections grown in different regions around the world (Chace *et al.*, 1981; Khodade *et al.*, 1990; Barone *et al.*, 1998, 2001; Fadavi *et al.*, 2005; Martínez *et al.*, 2006; Calín-Sánchez *et al.*, 2010; Dafny-Yalin *et al.*, 2010; Tehranifar *et al.*, 2010; Cristofori *et al.*, 2011). TSS assessment is not only important for juice quality evaluation, but for determining also the suitability of cultivars for pomegranate winemaking (Seser *et al.*, 2007). Regarding total acidity contents (TA), remarkable differences were found among Moroccan cultivars. "Bouaâdime" scored the highest content by far (4.7 g l^{-1} of citric acid), whereas all the

others just ranged from 2.1 to 3.3 g l^{-1}. Moroccan TA contents were similar to those showed by Spanish, Italian and Iranian cultivars (Martínez *et al.*, 2006; Tehranifar *et al.*, 2010; Cristofori *et al.*, 2011). The acidity content definitively plays an important role in the perception of fruit quality. Data from other collections around the world suggested that the TA content and pomegranate taste depend on climate and growing conditions (Dafny-Yalin *et al.*, 2010). The taste and flavor of pomegranates clearly rely on the maturity index (MI) (Martínez *et al.*, 2006; Çam *et al.*, 2009b). The MI significantly varied among Moroccan cultivars (Table 2). Whereas "Sefri" showed a MI of 73.25, "Bouaâdime" MI was 37.42. According to Martínez *et al.* (2006), Moroccan pomegranate varieties were grouped as sweet. Likewise, previous studies reported variable ranges of maturity indexes (Martínez *et al.*, 2006; Çam *et al.*, 2009b; Sarkhosh *et al.*, 2009). So it can be stated that pomegranate juice maturity index was basically conditioned by the cultivar factor. Since the evaluated Moroccan varieties showed high TSS contents, they are suitable for both fresh market and juice processing.

Table 2. Chemical properties of pomegranate juices from evaluated Moroccan cultivars

Cultivar	pH	TSS (ºBrix)	Acidity (g/l)	Moisture (%)	Maturity index	Crude fiber (%)
"Sefri"	5.15 a	15.28 a	2.14 a	81.45 c	73.25 cd	0.910 a
"Ounk Hman"	4.73 a	16.10 bc	2.21 a	80.90 c	77.64 d	0.999 ab
"Ruby"	5.46 a	16.55 c	3.01 bc	79.17 b	59.67 bc	1.786 c
"Rouge Marrakech"	4.98 a	15.95 b	2.53 ab	82.25 c	66.14 cd	0.964 ab
"Bouaâdime"	4.05 a	17.60 d	4,71 d	77.04 a	37.42 a	2.133 d
"Jaune Marrakech"	4.49 a	15.67 ab	3.33 c	80.82 bc	48.17 ab	1.051 b

The values followed by the same letter show no statistically significant differences (P < 0.05).

IV – Conclusions

Statistically significant differences were observed among the evaluated Moroccan pomegranates. The assessment of pomegranate chemical compositions implies the great potential of Moroccan cultivars for both fresh market and fruit processing.

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Chemical composition of pomegranate (*Punica granatum* L.) cultivars grown in Croatia

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Abstract. Pomegranate is among the oldest cultivated sub-tropical fruit species in the coastal part of Croatia, traditionally grown in central and southern Dalmatia. More recently, its fruits have gained interest for their nutritional values. The edible part of pomegranate fruit contains considerable amounts of vitamins, polysaccharides, polyphenols and minerals. One of the most important quality characteristics of pomegranate fruit are the arils and juice, which are primarily associated with anthocyanin pigments. Fruit samples of seven domestic pomegranate cultivars (Barski slatki, Ciparski rani, Crveni rani, Dividiš, Konjski zub, Sladun, and Šerbetaš) and wild pomegranate were collected from a productive pomegranate orchard in Metković (Croatia) (43°01'N 17°65 'E). The results on the main fruit quality traits (total soluble solids, total acidity, total sugar contents, total phenolic content, antioxidant activity and anthocyanins content) indicated significant statistical differences among cultivars.

Keywords. Pomegranate – Total soluble solids – Total acidity – Anthocyanins – Phenols.

I – Introduction

Pomegranate (*Punica granatum* L.) has been cultivated since ancient times through the Mediterranean region, Asia, Africa and parts of Europe (Morton, 1987). Pomegranate fruits are important for human health because of high antioxidant capacity and a high content of polyphenols and anthocyanins (Gil *et al.*, 2000).

In Croatia, pomegranate has been traditionally grown in Mediterranean part mainly in small orchards and gardens. The objective of this research was to analyse chemical properties of main pomegranate cultivars grown in the Mediterranean part of Croatia.

II – Material and methods

Fruit samples of seven pomegranate cultivars (Barski slatki, Ciparski rani, Crveni rani, Dividiš, Konjski zub, Sladun and Šerbetaš) and wild pomegranate were collected at the time of harvest in 2009 in productive pomegranate orchard in Metkovic (43°01'N longitude and 17°65 'E latitude).

The total soluble solids (TSS, °Brix) was determined using an optical refractometer, titratable acidity (TA, % of citric acid) was measured according to the AOAC method and total sugar content was determined by Luff-Schoorl method (AOAC, 1995). Maturity index (MI) was expressed as TSS/TA. Total phenolics were determined using Folin-Ciocalteu method (Singleton and Rossi, 1965). Antioxidant activities were determined with DPPH (Brand-Williams *et al.*, 1995) and TOSC (MacLean *et al.*, 2003) method. The analysis of anthocyanin content was carried out using Agilent 1200 HPLC system, using C18 column (150 x 3.5 mm; particle size 5 μm).

III – Results and discussion

Total soluble solids (TSS) of studied cultivars ranged from 12.5° to 15.0° Brix (Table 1). Cultivar Barski slatki had the highest TSS compared to other studied cultivars, except Šerbetaš and Wild. Turkish cultivars obtained TSS from 14.7%-17.9% (Ozgen *et al.*, 2008). Cultivars in this study belong to a group of sweet cultivars except cultivar Dividiš which belongs to a group of sweet-sour cultivars according to Onur and Kaska (1985). Titratable acidity of wild pomegranate (2.2%) was significantly higher than in other studied cultivars (Table 1). The MI depended on the cultivar, and in investigated cultivars varied from 11.5 in Dividiš to 33.9 in Konjski zub (Table 1). Higher TA resulted with the lowest MI (6.9). The highest TSC had cultivars Šerbetaš (13.7%) and Crveni rani (13.0%), where as the lowest one had cultivar Sladun (10.5%) (Table 1). In sweet Spanish cultivars TSC ranged from 11.96 g/100g to 15.89 g/100g (Melgarejo *et al.*, 2000).

Table 1. Total soluble solids (TSS), titratable acidity (TA), maturity index (MI), total sugar content (TSC), total phenolic content (TPC), DPPH, TOSC and total anthocyanin content (TAC) of seven Croatian pomegranate cultivars and wild pomegranate (means±SD)

Cultivar	TSS (°Brix)	TA (%)	Maturity Index (MI)	TSC (%)	TPC mg GAE/l	DPPH (%)	TOSC µmol Trolox eq/kg	TAC (Total anthocyanin content) mg/100g
Barski slatki	15.0±[*]0.2a[**]	0.592±0.03c	25.7±0.9b	12.1±0.2 b	2603.0±124.1abc	67.7±1.2ab	425.8±10.1	214.8±20.9a
Ciparski rani	14.1±0.2b	0.429±0.01d	33.3±1.1a	12.0±0.1 b	2902.0± 47.2a	73.6±1.8a	450.1± 4.1	228.0±10.0a
Crveni rani	14.1±0.1b	0.423±0.01d	33.5±1.0a	13.0±0.4 a	2948.7±161.1a	68.8±2.7ab	410.9±12.7	130.1±7.8b
Dividiš	13.1±0.5cd	1.141±0.04b	11.5±0.1c	11.3±0.3 b	1985.6±170.3d	56.3±5.0c	436.4±13.2	13.2±6.4d
Konjski zub	12.5±0.4d	0.369±0.00d	33.9±0.6a	11.9±0.4 b	2443.4± 85.6bc	63.1±2.2bc	404.9± 5.0	28.6±3.2d
Sladun	13.9±0.1bc	0.431±0.00d	32.3±0.3a	10.5±0.1 c	2327.9±182.2cd	63.8±6.4bc	434.8± 6.0	79.1±16.4c
Šerbetaš	14.4±0.2ab	0.464±0.01d	31.4±0.3a	13.7±0.5 a	2851.9± 99.2ab	67.1±1.7ab	420.0±16.6	203.1±15.0a
Wild	14.8±0.4ab	2.164±0.08a	6.9±0.3d	11.3±0.1 b	2261.1±202.7cd	63.5±1.8bc	427.5±17.5	42.1±3.1cd

[**] Different letters within column indicate significant differences by *LSD* test at *P*≤0.05.

In this study, TPC differed significantly between cultivars and varied from 1985.6 mg/l to 2948.7 mg/l (Table 1). Gil *et al.* (2000) found that TPC of the cultivar Wonderful was 2117 mg/l, TPC of pomegranates from Turkey was reported from 1245 to 3436 mg/l (Ozgen *et al.*, 2008). Differences between cultivars were found for DPPH but not for TOSC values (Table 1), presumably due to differences in reaction kinetics between these two methods and their interaction with radicals. In our study, DPPH averaged 65.5% and is higher compared to 36.9% that Tezcan *et al.* (2009) reported in their study of commercial juices, which have higher activity than experimental juices obtained by pressing the arils (Gil *et al.*, 2000).

The TAC of investigated cultivars varied significantly, the lowest values were recorded for Konjski zub and the highest TAC was found for Barski slatki, Ciparski rani, and Šerbetaš (Table 1).

IV – Conclusions

This study showed variation in some of the chemical and antioxidant characteristics of pomegranate cultivars grown in Croatia. It is important to further evaluate and conserve local genetic materials.

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The Pomegranate, *Punica granatum* L.: sustainability and improvement of biodiversity in Apulia, Italy

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Abstract. Nowadays the name and the image of pomegranate are widely used: it can be found as a name in several commercial activities such as banks, agritouristic farms, cultural and sporting associations; its presence in different commercial brands symbolizes "abundance". The plant is used for decorating the landscape and the aesthetics of ancient and modern villas, as well as in the dyeing industry and, since ancient times, has been celebrated by Orazio and Virgilio; this plant has been revalued from a pharmaceutical and nutritional point of view (in the Mediterranean diet). Thanks to its nutritional properties and high value of vitamins and antioxidants, it can be included among the nutraceutical products such as citrus fruit and actinidia (for the content in Vitamin C), almonds and other dried fruit (for the presence of Omega 3) and the olive oil (in the prevention of the cardio-vascular diseases).

Keywords. Food – Nutraceutical – History – Culture.

I – History and spread

Pomegranate is native to Persia and Afghanistan, spontaneously grows from South Caucasus to Punjab and is widely cultivated in Far East and in the Mediterranean countries. Rich in history and romance, the fruit has been always used in different ways; it has been described in the Egyptian mythology and art, praised in the Old Testament of the Bible and in the Babylonian Talmud and was brought by the caravans of the desert because it was refreshing and bracing. It spread in Central and Southern Asia, from India to Iran, in the 1st century a.C. and in 1416 has been reported as growing. It has been widely cultivated in all India, even in dry areas of South-eastern Asia, in Malaysia, in Eastern India and tropical Africa.

The most important growing regions are Egypt, China, Afghanistan, Pakistan, Bangladesh, Iran, Iraq, India, Burma and Saudi Arabia. There are some commercial orchards in Israel, in the coastal plain and the Jordan Valley. Currently, in many other Mediterranean countries, it represents a highly successful cultivation, particularly in Spain and Turkey. The latter hosted the first International Congress ISHS on Pomegranate in 2006.

The pomegranate tree gave the name to the city of Granada. It is a legendary tree with an ancient tradition since, for thousands of years, has been synonymous with fertility for all cultures that have been seduced by its fruits, rich in attractive red seeds, as an expression of life plenty. Not by chance, the fifteenth and sixteenth centuries painters often put a pomegranate in the hand of Child Jesus, referring to the new life brought by Christ. In Coptic art the pomegranate tree is present as symbol of resurrection; in ancient Greece, this plant was sacred to Juno (wife of Jupiter) and Venus (goddess of love); Roman brides used pomegranate branches to weave their hair; in Asian tradition, its broken fruit represents abundance and good luck. The considerable number of its seeds inspired many legends: in Vietnam the fruit breaks in two parts and brings hundred babies, the Turkish brides let it fall on the ground since it's known that they will have as many babies as the number of seeds which came out from the broken fruit. In

Dalmatia, according to the tradition, the groom has to transfer a pomegranate tree from the garden of his father-in-law to his own garden. According to an Indian belief, pomegranate juice counteracts infertility, while in the language of flowers it expresses ardent love. Very rich in vitamins, since thousands of years is a source of salvation for the people of arid areas of Asia and is considered ad the king of the fruits because of its particular crown shaped petiole. In ancient times, it was highly regarded for its therapeutic properties; 4000 years ago, Egyptians knew the vermicide properties of the root of pomegranate (Seeram *et al.*, 2006). In Europe, at the beginning of nineteenth century, the bark of the root was widely used to fight tapeworm; indeed, the modern analysis has confirmed the presence of highly effective alkaloids against tapeworms.

II – Description and use

Pomegranate (*Punica granatum* L.) belongs to the Punicaeae family, which includes only one gender and two species. Its stem can reach five meters height, is very branchy and twisted, with red-grey bark and thorny branches. The leaves are deciduous, oblong, mostly opposite, stiff and shiny. The scarlet flowers blossom at the ends of the branches, from May to July. The fruit is a large leathery berry, round and yellow-orange, divided in 7-15 internal cavities, in which the seeds are placed, enveloped in a sour or sweet pulp, juicy and transparent (the aril). As for many fruit species, pomegranate varieties differ in their taste, ranging from sweet to sour and this is related directly to the quality and quantity of the organic acids and sugars in the fruit. The fruit ripening occurs in the fall. Pomegranate is often grown as an ornamental species in gardens and terraces in warmer regions, its fruits and flowers are used to decorate tables and dishes. Nevertheless, this plant deserves greater attention: its fruits are rich in vitamin A and B, have antioxidant properties (Gil *et al.*, 2000) and contain anticarcinogenic, antimicrobial, antiviral and antiatherosclerotic. Recently the pomegranate juice has been taken into consideration for its cardio-vascular benefits. The fruit contains plenty of tannin, which has astrictive properties. Other than being effective against tapeworms, pomegranate is cooling, diuretic and tonic. The bark of the fruit, rich in tannin, is still used in North Africa and Orient for tanning leather. From dry peel a good colour can be obtained: a distinctive yellow-green which has been even found in some Egyptian tombs. If some iron is present, this colour turns into a suitable black paint to make ink, and the flowers, as well, can be used to prepare a red ink. The fruit, other than being an uncommon dessert, can be the main element for some gourmet jellies, refreshing drinks, sorbets and jams. Pomegranate juice can be used for the preparation of sweets and meat dishes. Lately, several research institutions in various Mediterranean countries characterized pomegranate germplasm collections both from a morphological and a biochemical point of view (Muleo *et al.*, 2008). The parameters considered for the varietal assessment are: productivity, ripening period, seed internal tegument consistency, fruit size, pulp and peel colour, juice acidity, total sugars (°Brix), resistance to biotic and abiotic stress and antioxidant capacity. Great importance is given to ornamental varieties. Indeed, flowering pomegranate varieties include many double flower varieties, suitable for growing in pots, with white, yellow, orange or dappled flowers.

III – Pomegranate in Apulia

Scarce information is available about the genotypes present in Apulia region (Italy). In particular, national data of 2009 indicate only 8 hectares cultivated to pomegranate and no hectares officially result under cultivation in the region (ISTAT, 2010), nevertheless, thanks to the pedo-climatic conditions, pomegranate could be an interesting and promising crop (Ferrara *et al.*, 2011). With appropriate economic supports which promote neglected species, as expected by the recent EU legislation, pomegranate in Apulia may occupy a wider area, restoring marginal lands (marshes and brackish) that are not occupied by more popular crops in the region. An advisable task could be the recovery of ecotypes, distributed throughout the

Apulia territory (according to a first phase of land exploration), which over the years have been naturally diversified (often deriving from seedlings) and whose spread in different areas was and still is made at household and company level, for several uses (food, ornamental in villas, gardens, botanical gardens and to decorate typical regional farms). In the past, pomegranate was cultivated in some wet areas of Apulia for commercial purposes, related both to food and industrial uses, in particular dyeing activity (Russo, 2009). One example is from Massafra, a village in the province of Taranto; here is a street named "via delle concerie" (literally "street of the tanneries") in memory of the dyeing activity for different types of tanning (textiles, leathers, etc.). Lately the interest for this species has increased, because of its various attitudes (fresh consumption, ornamental, agro-industrial, pharmaceutical, cosmetic and nutraceutical): a more effective application of the EU rules which provide for each Italian region a reference office for the protection of biodiversity across the territory, will ensure greater protection for this neglected species. The preservation of pomegranate biodiversity in Apulia has interesting cultivation perspectives, because of the rich genetic variability; the enhancement of biodiversity could determine an economic interest both for the product as fresh consumption, that Italy imports from Spain, and the use as an ornamental plant, since in the regional germplasm different ecotypes have been identified, some characterised by small fruit, others by very big fruit, as well as bigger flowers and different plant sizes ideal for ornamental use. Research funded by CNR is ongoing and soon there will be the phases of collection and storage extra situ of regional biodiversity, for its morph-qualitative characterization and to point out possible homonyms and synonyms.

IV – Future perspectives

In several countries the production has increased, as well as the spread, because of its positive aspects, but it needs to be supported by innovative bio-agricultural technologies. Genetic improvement, agronomic management and suitable post-harvest techniques play an important role, as well as the processes of extraction of the edible part; the application of appropriate packaging techniques is required in order to keep intact the quality of the product. Recently EU, in community projects and in national operational programs regarding food and health themes, stressed the importance on nutraceutical aspects of food products; pomegranate, thanks to both its nutritive and antioxidant value (because of the content in vitamine C), can be fully included among the nutraceutic fruits.

Acknowledgements

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Session 3
Cultivation techniques

The cultivation of Pomegramate cv. Wonderful in Chile

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Abastract. The pomegranate plantations in Chile have increased exponentially in the last decade taking fresh pomegranate exports from 200 t/year in 2007 to 2500 t/year in 2010. Practically 100% of the pomegranate production in Chile was based on the Wonderful variety which fulfils the requirements of intense red colour of the skin and the flesh inside demanded by the destination markets supplied by Chile: Europe and Russia. This flourishing industry in Chile has faced several challenges related to appropriate planting areas, growing and post-harvest techniques for fruit destined for export and industrialisation and to the large proportion of fruit unsuitable for export. The presentation will detail these challenges as well as the technical advances in cv. Wonderful growing that have been made thanks to the collaboration between producers, exporters and research centres in Chile.

Keywords. Chile – Growing – Wonderful.

I – Introduction

Chile started producing and exporting a limited amount of cv. Wonderful fresh pomegranates to the European market fifteen years ago. Ten years went by before the export, and companies involved in exporting pomegranates exponentially increased (Fig. 1), encouraged by the burst in the demand for red pomegranates by the European markets. Such increase in exports was sustained by a boom of pomegranate plantations in Chile, reaching a total of 724.6 ha in the country, according to the last data available (Table 1). This trend continues nowadays, especially with the recent opening of the United States market in 2010 (system approach), therefore the actual area planted with pomegranates in Chile is very likely well above this figure. Until now, virtually all of the plantations of pomegranates in Chile have continued to be of the cv. Wonderful which fits the market requirements, namely: deep red colour of skin and arils and large size. Although this late ripening cultivar reaches high yields in other countries, in Chile yields tend to be relatively low (15-25 t ha^{-1}). Such low yields are attributable to an inadequate initial knowledge about the edapho-climatic requirements of the crop and its technical management. Because of the high market prizes commanded by Chilean pomegranates until 2010, growing pomegranates under sub-optimal climatic conditions and applying inadequate orchard management still resulted in positive economic outcome. This scenario is slowly changing with the concurrence of Peruvian pomegranates and the exponential increment in the offer of Chilean fresh pomegranates (Fig. 1).

II – Growing Wonderful pomegranates in Chile: Achievements and challenges

In Chile the pomegranate industry has been developed both by the private sector and through research and development projects executed by the University of Chile (UCH) in collaboration with several growers and agro-industries and co-financed by governmental agencies such as INNOVA-Chile, FIA (Fundación para la Innovación Agraria) and FONDEF (Fondo de Fomento al Desarrollo Científico y Tecnológico).
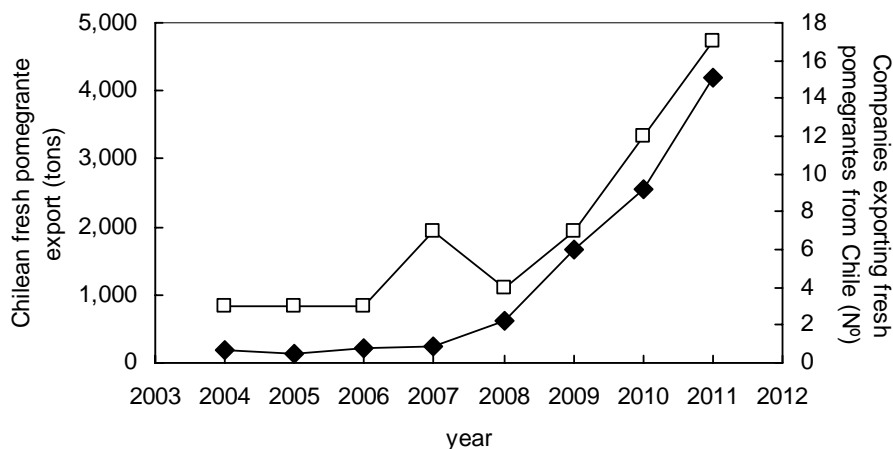
Fig. 1. Amount of fresh pomegranates exported from Chile between 2004 and 2011 and number of companies exporting them.

1. Optimal production zones

Many plantations of Wonderful pomegranate orchards in Chile have been placed in zones with sub-optimal climatic conditions, such as high autumn rain probability (Regions south of Valparaíso) which fall before the harvest of this late cultivar has been completed (or even initiated) resulting in a high incidence of fruit cracking. Also plantations near the coast, with milder summer and winter temperatures and frequent foggy days in spring, have shown to be inadequate for fresh pomegranate production. In this zones bloom, and consequently harvest, tend to spread in time, resulting in a significant proportion of fruit that does not fulfil its ripening process or develops very poor skin colour. Moreover, the incidence of fungal disease affecting the fruit, due to infections promoted by condensation during bloom, severely increases under costal climatic conditions of Chile. We now know that the best conditions for growing pomegranates in Chile are in the interior of the transversal valleys (Andes foothills) of the northern cultivation zones: especially the Atacama and Coquimbo Regions, which therefore concentrate 83% of the country's pomegranate production area (Table 1). These zones present long, dry and hot summers, high solar irradiance, slightly colder winters and nights than coastal regions; and dry spring and autumn, which all favour the production of high quality fresh pomegranates.

Table 1. Distribution of pomegranate plantations (ha) in different administrative Regions of Chile. Number between brackets indicates the year for which the data were obtained

Region (from north to south)						
Atacama (2011)	Coquimbo (2011)	Valparaíso (2008)	Metropolitana (2010)	O'Higgins (2009)	Maule (2007)	Country (Total)
215.5	387.7	22.8	72.7	10.5	15.4	724.6

2. Orchard management

Through a project co-financed by INNOVA-Chile several orchard management practices have been studied in commercial farms of growers associated to the project. The results have

allowed constructing the seasonal macro-nutrient extraction curves of new and producing pomegranate orchards under the local conditions. The irrigation requirements, along with periods in which controlled water deficit can be applied have also been quantified in this project. The adjustment of fruit load and its effect on fruit size, yield and the incidence of biennial bearing have also been established. Chemical thinning trials for reducing the presence of late fruits, which do not achieve adequate size and colour, have given interesting results indicating that ethephon sprays in late bloom efficiently thin out such fruits and may also contribute to a better red-colour development of the fruit skin. Trials for limiting sun scald damage to fruits have shown that the use of shading nets and paper bags most efficiently reduce this problem, as compared to different doses and frequencies of kaolin sprays.

3. Challenges

Planting frames and orchard design vary widely in Chile with frames ranging from low (4 x 6 m) to high (2 x 3.5 m) density plantations with trees formed to spindles, open vase and multi-trunk shapes. Trellising systems also vary from none to the use of sophisticated multi wire trellises. Although trials for assessing these different orchard designs have been initiated, they need to be evaluated in the long term. Control strategies for diseases, mainly fungi affecting the fruits in postharvest and quarantine plagues such as mealy bugs remains to be developed. The loss of exportable fruits to fruit cracking and sun scald as well as mechanical damages can account for more than 40% of the whole production in many orchards in Chile. Methods for controlling and preventing such damages are urgently needed and are currently being studied. A project involving the Chilean fruit growers association (Fedefruta), the agriculture and animal husbandry service (SAG) and UCH; and financed by INNOVA-Chile will attempt to tackle these challenges. The development of alternatives for adding value to pomegranates, mainly arils and juices, is also currently being developed by the industry and the UCH and should be able to absorb the fruit not achieving fresh fruit market requirements. The use of new varieties has also been undertaken and should enlarge the production area and harvest season in Chile.

III – Conclusions

The best areas and basic techniques for growing pomegranates in Chile have been developed in a collaborative effort of growers, the UCH and government funding agencies. Some aspects such as orchard design, pest, disease, sun scald and fruit cracking control remain to be developed. The technology transfer of the results generated in the abovementioned research and development projects is an important challenge for the future success of Chilean pomegranate industry.

Acknowledgments

Regulated deficit irrigation in pomegranate (*Punica granatum*) trees. Yield and its components

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Abstract. An experiment has been carried out during two seasons with the objective of identifying pomegranate yield responses to water restrictions applied during different phenological periods. In mature trees, five irrigation treatments where applied including trees watered at 100% and 50% of tree water needs (ETc) during the entire season, and other three regulated deficit irrigated (RDI) treatments with severe restrictions applied only during certain phenological periods. Results showed that all the deficit irrigated treatments allowed increasing water use efficiency (i.e. yield/water applied). An early season water stress, increased the number of fruit collected at harvest, with the drawback of reducing fruit weight if restrictions were prolonged during all the season. On the other hand, temporal water restrictions applied in the mid summer lead to a very small effect on final fruit weight. Results indicate the potential that RDI has in Pomegranate trees as a tool to cope with water scarcity in this crop.

Keywords. Fruit disorders – Stem water potential – Water stress.

I – Introduction

Pomegranate trees are considered as a culture crop tolerant to soil water deficit (Holland *et al.* 2009). Because of this, in Spain, its culture is concentrated in the south east, where fresh water available for agriculture is very scarce.

Regulated deficit irrigation (RDI) was developed in the 1980s as a strategy to save water and improve the productivity of fruit trees (Behboudian and Mills, 1997). RDI is based on reducing water applications only in those phenological periods when yield is less sensitive to soil water limitations. The effectiveness of RDI depends upon the identification of critical periods for tree performance. In this sense, to the best of our knowledge, there have not been field trials that have explored pomegranate tree responses to different irrigation regimes applied during the season. The objectives of the present research was then to study the effects of time of RDI imposition on tree performance.

II – Material and methods

1. Experimental orchards

The experiment was performed during the 2009 and 2010 seasons in a commercial mature pomegranate tree orchard (*Punica granatum*, L cv. 'Mollar de Elche') at Elche, Alicante, Spain, (38ºN, elevation 97 m). The soil was sandy-loam with an effective depth over 120 cm. Trees

were planted in 2000 at a spacing of 5 x 4 m and average tree shaded area was 48% of the soil allotted per tree. Average trunk diameter was 18.2 cm.

Trees received 100, 40 and 80 kg ha^{-1} year^{-1} of N, P_2O_5 and K_2O, respectively. Agricultural practices followed were those common for the area. Weather was recorded at an automated weather station near the orchard. Meteorological variables measured included solar radiation, air temperature, air humidity, wind speed and direction and rainfall. Precipitation and reference evapotranspiration (ETo) during the growing season (April to October) were 111 and 141, and 811 and 940 mm in 2009 and 2010, respectively

2. Irrigation treatments

Drip irrigation was applied with eight emitters per tree delivering 4.0 l h^{-1} each and were located in a single line parallel to the tree row. Irrigation treatments as depicted in Fig. 1 were:

(i) *Control*, where irrigation was scheduled in order to replace 100% of the estimated crop evapotranspiration (ETc). Crop evapotranspiration was estimated as product of reference evapotranspiration (ETo) and crop coefficient (Kc). ETo was calculated with hourly values by the Penman-Monteith formula as in Allen *et al.* (1998). The Kc values increased from an initial value of 0.32 used in March to a maximum value of 0.74 used in July, August and September according to previous recent findings obtained in the same plot (Intrigliolo *et al.* 2011).

(ii) *Sustained deficit irrigation (SDI)*, where water was constantly applied at 50% of control regime.

(iii) *Regulated deficit irrigation (RDI) RDI$_{spring}$*, where irrigation was applied at 25% of the control irrigation from the beginning of May 9 to end of June coincident with the flowering, fruit set and first exponential phase of fruit growth. During the rest of the season irrigation was applied at 100% ETc.

(iv) *RDI$_{summer}$* where irrigation was applied at 25% of the control irrigation from beginninf of July to end of August coincident with a linear fruit growth phase. During the rest of the season irrigation was applied at 100% ETc.

(v) *RDI$_{fall}$*, where irrigation was applied at 25% of the control irrigation by the end of August until leaf fall. Earlier in the season irrigation was applied at 100% ETc.

The reductions in the amount of water applied during the deficits were achieved by reducing irrigation duration, while frequency of irrigation was always the same for all treatments. Irrigation frequency changed over the season with all treatments irrigated once a week in early spring and autumn and five times a week during summer.

The experimental design was a randomized complete block, with four replicates per treatment. Each plot had three rows, with 8 trees per row. In each experimental unit, 1-2 central trees of the middle row were used for data collection.

3. Determinations

Actual irrigation volumes applied were determined using in-line water meters installed on each experimental plot.

Fruit from each tree were harvested in two-three commercial picks carried out by mid October, beginning of november. Yield and fruit number per tree were quantified and average fruit weight was determined by weighting and counting fruit from two harvest bins (approximately 20 kg per bin). Tree water use efficiency (WUE), was computed as (yield: irrigation + effective rainfall). The effects of the different irrigation treatments on two common fruit disorders for pomegranate

trees (fruit sun-burn and cracking) was evaluated by computing the percentage of fruit affected by these disorders over the total number of fruit collected per tree.

Stem water potential was measured in four trees per treatment with a pressure chamber, in two leaves per tree (total of 8 leaves per treatment). Mature leaves from the north face near the trunk, were enclosed in plastic bags covered with silver foil at least two hours prior to the measurements, which were carried out between 12:00 and 13:00 h solar time, approximately every week.
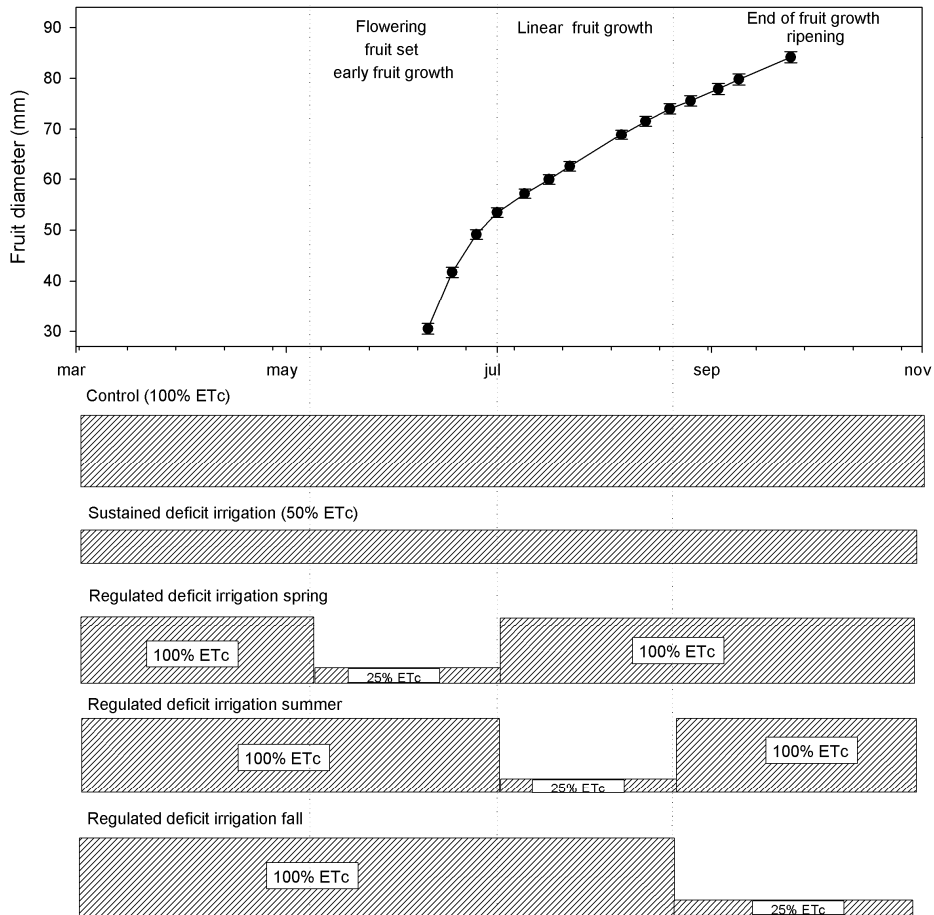
Fig. 1. Irrigation treatments applied during the 2009 and 2010 seasons. Water applications were based on percentage over the estimated crop evapotranspiration (ETc).

3. Data analysis

The effects of the irrigation regime on yield and its components was evaluated by analysis of variance using the general linear models "GLM" procedure of the SAS software (version 9.0; SAS Institute, Cary, NC). Since for most of the yield components, the treatment by year interaction was not statistically significant at $P < 0.05$, data presented are averages for both seasons.

III – Results and discussion

During the course of both season, there was a general decrease trend in Ψstem. Thus, even in the control trees, Ψstem decreased from values around –0.8 MPa registered in early spring, to values around –1.5 MPa obtained in the middle of the summer (Fig. 2). This was in part due to the higher evaporative demand as the season advanced. A similar behaviour has been reported in other fruit trees under drip irrigation such as a plum (Intrigliolo and Castel 2004) and it might be also due to a greater proportion of the roots in the dry part of the soil particularly towards the end of the season.

The SDI trees maintained during the course of the experiment the lowest Ψstem values, reaching a minimum values of –2.3 MPa by the end of the 2009 season (Fig. 2). In general, the RDI trees had lower plant water status than the Control during the period of water restrictions. However this effect was more noticeable in the RDI treatments with restrictions in the summer and fall (Fig. 2).
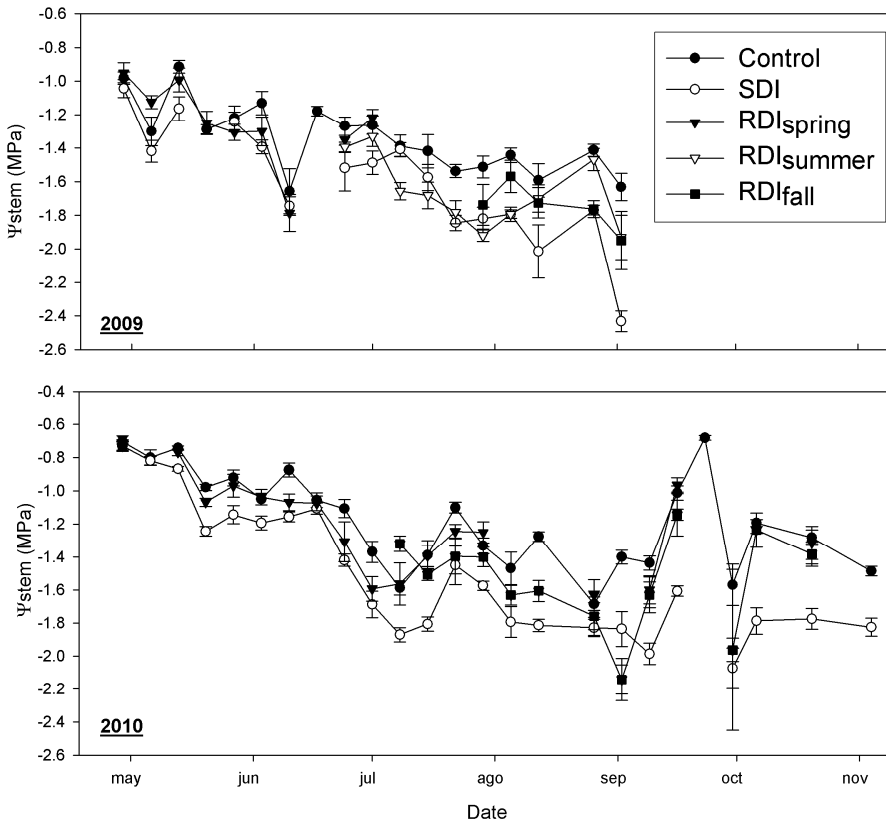
Fig. 2. Seasonal pattern in 2009 and 2010 of midday stem water potential (stem), of the different irrigation treatments. The error bars indicate the standard error.

Irrigation water volumed applied in the control treatment were, as an average for the two seasons, 427 mm (Table 1). On the other hand, in the SDI treatment irrigation applied was only 219 mm. Among all the RDI treatments, the one with the lower amount of water applied (- 23%

with respect to the control) was the RDI_{summer}, since this treatment had water restrictions during the time of the year with higher irrigation requirements due to the high evaporative demand.

Despite 46% less irrigation was applied in the SDI treatment than in the control, similar yield values were registered in both treatments (Table 1). As a consequence, the SDI treatment lead to an increase of water use efficiency (WUE) of 41%. The drawback was the reduction observed in average fruit weight that was of 20%. However, it should be noted that this decrease in fruit fresh weight at harvest was most likely due to not only the water stress exprienced by the SDI treatment but also due to the fact that the SDI regime lead to an increse in the number of fruit per tree collected (Table 1). In the SDI trees, there was then a heavier tree crop level and hence an higher fruit sink demand. The SDI strategy can be reccommended in those cases where prices for irrigation (water+energy) are high, or also for those productions focused to delivery pomegranate fruit to the industry.

Table 1. Yield and its components in the different irrigation treatments. Data presented are averages of two seasons (2009 and 2010)

	Irrigation (mm)	Yield (t ha^{-1})	WUE (kg m^{-3})	No. of fruit tree^{-1}	Fruit weight (g)	Fruit sun-burn (%)	Fruit cracking (%)
Control	427	22.5a	3.9a	136a	339a	2.4	13.0
SDI	229	22.1ab	5.5c	166b	274c	4.6	16.9
RDI$_{spring}$	362	24.0a	4.6b	153ab	320b	3.2	9.5
RDI$_{summer}$	327	21.6ab	4.4ab	137a	321b	4.7	13.0
RDI$_{fall}$	341	19.4b	3.8a	116c	345a	4.5	14.0

WUE, water use efficiency.
Within each column, different letters indicate statistically significant differences at P<0.05.

The fact that in the RDI_{spring}, that had water restrictions early in the season during flowering and fruit set, also had higher crop level than the rest of treatments (Table 1), indicates that a certain degree of water stress suffered by trees during spring might increase the tree bearing capacity. This behaviour observed in pomegranate is very different than what for instance observed in Citrus trees, where water stress during flowering and fruit set decreases tree crop level (González-Altozano and Castel 1999).

In both the RDI_{spring} and RDI_{summer} there was only a small reduction in fruit weight (–6%) with respect to the control trees. This reduction was lower than the amount of water saved in these treatments with respect to the control ones, thus both RDI regimes allowed increasing tree WUE. Particularly, the RDI_{spring} where water stress experienced was milder than in the RDI_{summer} (Fig. 1), seem to be a very convenient strategy for the application of deficit irrigation.

Te RDI_{fall} regime did not affect fruit weight, but it lead to a lower tree crop level. Further investigations are in due course to elucidate the possible reasons for this reduction in tree crop level caused by water restrictions applied late in the growing season.

It is very important to highlight that the different irrigation regimes did not affect the appereance of fruit disorders, since there were not statistically significant differences among treatments for both variables, fruit sun-burn and cracking (Table 1).

IV – Conclusions

Deficit irrigation is a feasible strategy to apply in pomegranate trees in case of water scarcity or high water prices. Contrarringly to other fruit tree crops, an early season water stress, increased

the number of fruit collected at harvest, with the drawaback of reducing fruit weight if restrictions were prolonged during all the season. On the other hand, temporal water restrictions applied in the mid summer, during the linear phase of fruit growth, lead to a very small effect on final fruit weight, allowing for 23% water savings.

Acknowledgements

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Efficacy and residues of selected insecticides for control of cotton aphid (*Aphis gossypii)* and mealybug (*Planococcus citri*) in pomegranates

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Abstract. Mealybug *(Planococcus citri* Risso) and aphids (*Aphis gossypii* Glover and *Aphis punicae* Passerini) are the most important pests of pomegranate production in Alicante (Spain). Due to the minor crop status of pomegranates, registered pest control chemicals for this crop are scarce. Experimental field efficacy of new conventional insecticides and alternative sprays against these sucking pests was assessed. Experiments to deal with these pests were carried out in randomized block design with three replications during 2009-2010, in two experimental pomegranate orchards in the Southeastern region of Spain. Other management practices and natural enemies of these pests were identified and quantified during the crop period. Results of the experiments suggest that the population of cotton aphid was resistant to Pirimicarb. New generation insecticides (Imidacloprid, Flonicamid, Acetamiprid) were more effective in controlling aphids. Moderately effective control can be achieved with soap, citrus oil and other tolerance exempt products, or some combination of these, but more than two applications may be necessary. Pesticide residue levels in ripening fruit after treatments were analysed.

Keywords. Pest management – Residue levels decline – Natural enemies.

I – Introduction

Pomegranate (*Punica granatum* L.) is one of the most important fruit crop in the south of Alicante (Southern Spain) where more than 90% of national production originates. Pomegranate pests and their management strategies differ greatly, depending on climate, countries and cultivars. Whereas pomegranate trees are attacked by more than 90 species of insects in India (Balikai *et al.*, 2011), in Spain most of these species have not been recorded. Literature on pomegranate pests in Spain is not extensive, but it is known that infestation by sucking pests like aphids and mealy bugs is more common in pomegranates in this Mediterranean area (Toledo *et al.*, 2000). Aphid infestation occurs after shooting, during flowering and fruiting stages of the crop, thereby reducing the vigour of the plant through the excretion of honeydew on the leaves and the development of sooty mould which covers the surface of leaves and fruits.

The available pesticides for use on pomegranates are expanding in other non UE countries. Because of the minor crop status of pomegranates, pesticides authorized and registered available for pest control on pomegranates in Spain are very scarce. Nowadays, Pirimicarb is the only conventional insecticide authorized for aphids control in Spain. Experimental trials with new active ingredients registered in other fruits can show their efficacy and perhaps their use can be extended to pomegranates. New products are being introduced into the market, many of which are safer to humans and the environment than older pesticides. The purpose is to ensure that producers of minor crops have an adequate range of pest control products (both traditional pesticides and biopesticides) in order to competitively produce safe and wholesome agricultural products. Natural enemies can contribute to the reduction of pest populations, but their use was not sufficient to clean up all present aphids and prevent fruit damage in experimental trials in Spain (Bartual *et al*, 2010). The level of pesticide residues in fresh fruit is considered an

important internal quality parameter. In the case of fruit destined for export, the maximum residue level (MRL) standards for the destination country should be met.

The aim of the present study was to evaluate natural enemies, and the efficacy and residue levels of new generation insecticides and registration exempt products for control of the most important sucking pests of pomegranates in Spain.

II – Material and methods

Studies were conducted during 2009-2010 on mature pomegranate groves located in Elche and Albatera (Alicante). Aphids and natural enemies were identified and quantified weekly during the crop period. In the experimental orchards, adult trees of 'Mollar de Elche', the most common cultivar in Spain, were sprayed with eleven insecticides in order to study their field efficacy. The pesticides selected for the current study include compounds from various pesticide classes which are authorized for citrus or other fruits in Spain. Aphid control treatments included five insecticides, Acetamiprid, Flonicamid, Imidacloprid, Pimetrozina and Pirimicarb (04/27/2010, Elche plot); of which only Pirimicarb has been approved for use on pomegranates in Spain until now, and three alternative products (04/29/2010, Albatera plot), neem extract, citrus extract and potassic soap; the last two products from MRL regulations. Mealybug control treatments (07/12/2010, Elche plot) included three insecticides (Chlorpyrifos, Chlorpyrifos-methyl and Fenoxicab). A randomised block design with 12 trees per treatment and three replicates was performed. Each experimental unit had three rows, with 4 trees per row, with at least one unsprayed bay between plots. A motorised mist was used to apply from 2 litres (for aphid control) to 3 litres (for mealybug control) of spray per tree (1000-1500 litres/ha) respectively, so that the spray on the fruit was at the point of runoff. A control with only water spray was maintained for comparison. At all application times fruit were dry at the start of spraying, there was no wind, and temperatures were between 18 and 23°C. The number of nymphs and adult aphids was counted from twenty terminals shoots, twenty flowers and twenty pieces of fruit in six trees per treatment. No rain fell for at least 2 days after application for all trials. An analysis of variance of angular transformed values was used to determine significance levels of mortality between treatments.

Eight fruit per plot were sampled for analysis of residue remaining on the fruit. For decline residue levels analysis of Chlorpyrifos, Clorpirifos-metyl and Fenoxicab, residue levels were calculated at 0, 7, 14, 21 and 28 days after spraying (09/04/2010). Evaluation of insecticide residue levels in ripening fruit was performed by Agri-Food Laboratory of 'Conselleria de Agricultura' (CAPA), using established and validated methods. Multi-residue analysis employed high performance liquid chromatography (HPLC/MS/MS) for Acetamiprid, Flonicamid, Fenoxicab, Imidacloprid, and Pimetrozina, and gas chromatography with mass spectrometric detection was used (GC/MS/MS) for Pirimicarb, Chlorpyrifos, and Chlorpyrifos-methyl. Instrumental limits of quantification (LOQ) for these active ingredients are 0.01.

III – Results and discussion

1. Aphids

Adults and nymphs of *Aphis gossypii* and *Aphis punicae*, were monitored during the assay, as well as a few individuals of *Aphis spiraecola*. The peak activity of pomegranate aphids was observed during the second fortnight of April. At first, *Aphis gossypii* appears in shoots and floral buds and causes different kinds of damage during April, whereas *Aphis punicae* appears in May and June and affects flowers and fruits. Aphidiids (Aphidiidae) parasitoids were recorded within the samples of *Aphis gossypii* and *Aphis punicae*. Other natural enemies of pests monitored were large lady beetles and *Scymnus* spp. (Coccinellidae), syrphid maggots (Syrphidae), green

lacewings (Chrysopidae) and *Aphidoletes aphidimyza* (Cecidomyiidae). Biocontrol contributed to reduce the pest populations but it was not sufficient to clean up all present aphids, and fruit damage was recorded in some untreated control areas. Ants aggravated this situation because they protected the aphids from their natural enemies.

At the Elche experimental plot, all insecticide treatments gave significantly (P<0.05) higher levels of protection as compared to untreated fruit (Table 1). Higher efficacy was shown by Imidacloprid, Acetamiprid and Flonicamid at 7, 14 and 21 days after spraying (04/27/2010).

Table 1. Efficacy of different treaments on pomegranate aphids, *Aphis punicae* Passerini and *Aphis gossypii* Glover in Elche plot. Application date 04/27/2010

Treatment	Active ingredient	Concentration	Dose per 100 litres	Per cent reduction in aphid population			% fruit damaged by stains or sooty mould
				DAT 7	DAT 14	DAT 21	
T1	Pirimicarb	50 %	60 ml	64.12b	26.25b	27.52b	70
T2	Imidacloprid	20 %	60 ml	97.32a	88.75a	57.54a	4
T3	Acetamiprid	20 %	30 g	95.41a	79.58a	67.11a	8
T4	Pimetrozina	50%	40 g	63.33b	24.59b	6.32bc	72
T5	Flonicamid	50 %	13 g	98.25a	82.53a	50.33ab	11
T6	Untreated control	---	---	3.11c	2.52c	5.22c	90

Evaluation dates: 4 May (7 DAT), 11 May (14 DAT), and 18 May (21 DAT). Angular transformed values were used to determine significance levels of mortality between treatments. Means followed by same letter within columns are not significantly different (95%, test LSD).

At the Albatera experimental plot (04/29/2010 with exempt products), moderately effective control was achieved with soap, citrus oil and neem, however a citrus extract and soap combination showed significantly different results, reaching more than 68% efficacy (Table 2). The relative importance of major variables such as sunlight, humidity, temperature or water pH range, may influence these results.

Table 2. Efficacy of different treatments on pomegranate aphids, *Aphis punicae* Passerini and *Aphis gossypii* Glover in Albatera plot. Application date 04/29/2010

Treatment	Product treatment	Conc.	Dose per 100 litres	Per cent reduction in aphid population			% fruit damaged by stains or sooty mould
				DAT 7	DAT 14	DAT 21	
T7	Potassic Soap	ND	200 ml	24.32 bc	41.25 b	9.52 cd	48
T8	Citrus extract	ND	300 ml	36.15 b	52.74 b	36.34 bc	58
T9	Neem oil (azadirachtin)	3.2 %	100 ml	27.85 bc	53.49 b	13.33 cd	86
T10	Citrus extract + Soap	ND	300+200 ml	68.20 a	71.21 a	74.14 a	17
T11	Untreated control	---	---	16.52 c	20.88 c	7.41 d	92

Evaluation dates: 6 May (7 DAT), 13 May (14 DAT), and 20 May (21 DAT). Angular transformed values were used to determine significance levels of mortality between treatments. Means followed by same letter within columns are not significantly different (95%, test LSD).

The current results suggest that the combination of citrus extract and soap works more effectively than the use of each of them separately against the aphids, but more than two

applications may be necessary. Pesticide residues were not detected (Table 3) in fruit after harvest (10/07/2010).

Table 3. Pesticide residue levels (mg/kg) in harvested pomegranates (10/07/2010)

Insecticides	Classification	Dosage (g ai ha^{-1})	MRLs in EU	Residue (mg.kg^{-1})
Neem extract	Azadirachtin	32	0.01[†]	<LOQ
Acetamiprid	Nicotinoids	60	0.01[†]	<LOQ
Flonicamid	Pyridine	65	0.05[†]	<LOQ
Imidacloprid	Nicotinoids	120	1	<LOQ
Pirimicarb	Carbamate	300	1	<LOQ
Pymetrozine	Not Established	200	0.02[†]	<LOQ

[†]Indicates lower limit of analytical determination. Pesticides Web Version - EU MRLs (08/19/2011) Instrumental limit of quantification (LOQ) is 0.01. Treatment 04/27/2010.

2. Mealybug

The number of mealybug infested fruitS following application of pesticides was not significantly different from the untreated fruit 7 days after application (data not shown) due to the low level of the pest population in some experimental units. This occurrence confirms the recommendation made in Integrated Pest Management, that treatments should be applied only on those areas of the orchard with incidence of mealybug if they are well defined.

Insecticide residue decay levels are presented for each trial in Table 4. For the three formulations sprayed for mealy bug control, the dissipation of methyl-chlorpyrifos in pomegranates was faster than Chlorpyrifos and Fenoxicarb. The residues were long lasting, with a slow degradation rate. On ripening day (10/07/2010), only Fenoxicarb residues were detected in the marketable fruit. No samples contained residues that exceeded the MRLs set by the European Union (Table 4).

Table 4. Pesticide residue levels (mg/kg) in pomegranates at 0, 7, 14, 21 and 28 days after spraying (09/04/2010)

Insecticides	Dosage (g ai ha^{-1})	MRLs in EU	Residue (mg.kg^{-1})				
			DAT 0	DAT 7	DAT 14	DAT 21	DAT 28
Chlorpyrifos	1440	0.05[†]	0.20	0.06	0.02	<LOQ	<LOQ
Chlorpyrifos-methyl	1344	0.05[†]	0.17	0.02	<LOQ	<LOQ	<LOQ
Fenoxycarb	150	0.05[†]	0.11	0.04	0.03	0.03	0.02

[†]Indicates lower limit of analytical determination. Pesticides Web Version - EU MRLs (08/19/2011). Instrumental limit of quantification (LOQ) is 0.01.

IV – Conclusions

The results obtained in this trial have given us information about the natural enemies of aphids found in the SE Spanish pomegranate orchards. However, the untreated trial areas showed an elevated percentage of fruit damage from aphids and mealybugs, which could necessitate the use of phytosanitary treatments, especially those which respect auxiliary fauna.

To increase the availability of pest control tools to pomegranate growers, it seems appropriate to authorize some of the newer generation products used in this trial (Imidacloprid, Flonicamid, Acetamiprid) which have proven more effective than Pirimicarb. Significantly effective results have been obtained with the combination of citrus extract and potassium soap. This application could be useful in organic orchards or to obtain lower fruit residues in the event that more than one pest control treatment is required. Among the traditional products used against mealybug, metal clorpirifos demonstrated a more rapid dissipation curve.

New pest control assays must be performed in pomegranate. Priority must be given to the use of biological control methods, alternative pesticides and newer active ingredients especially those which respect beneficiary insects.

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Threat of bacterial blight on pomegranate in India – Mitigation by an integrated approach

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Abstract. Pomegrante (*Punica granatum* L.), so called "fruit of paradise" is one of the major fruit crops of arid region. It is mainly grown in states of Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu. Bacterial blight of pomegranate, caused by *Xanthomonas axonopodis* pv. *Punicae*, considered to be a minor disease, was reported as bacterial leaf spot in the 1950s. At present this disease is observed on leaf, stem and on fruits, and has been responsible for the removing of many orchards in south India. Recently this disease was also observed in Rajasthan. A survey was conducted in North Karnataka during 2008-09 and 2009-10. Disease severity on the trees was 29.8 and 19.3% during 2008-09 and 2009-10 respectively. The disease was very severe in Mrig bahar (severity of 20.8%). Demonstrations were conducted for three years (2008-09 to 2010-11) in farmers fields over 6 locations involving various components like selection of hasta bahar treatment, proper training, sanitation, use of micro nutrients, use of organics and use of antibiotics along with copper compounds. The results indicated that before adopting the orchard integrated control management measures, the observed severity on the trees was up to 69%, and it was brought down to 3.0% in orchards where measures were adopted over three years. The average yield levels were 10 tons/hectare in demonstration plots. In orchard with nor integrated control, the disease severity on trees was up to 33.1% with average yield levels of 5.2 tons per hectare. Hence yield increased 4.8 tons, which worth Rs. 4.8 lakhs (1 lakh = 10^5), when compared to non-adopted orchards.

Keywords. Pomegranate – Bacterial blight – Integrated disease management – Causes of epidemics.

I – Introduction

Pomegranate (*Punica granatum* L.) is a good table fruit growing well in tropical and sub-tropical region of the world belongs to Punicaceae family.

During the last five to six years, farmers are facing a severe threat from bacterial blight disease. During recent years, the disease has reached its alarming stage bringing substantial damage to the crop and heavy losses to the farmers.

II – Early history and development of the disease

Hingorani and Mehta (1952) observed bacterial leaf spot disease in pomegrante for the first time in India. Ramesh Chand noticed this disease on leaves, nodes and fruits at IIHR Bangalore, during 1989. The bacterium was first noticed in some farms in Bellary district in the 1980; it started spreading rapidly in the early 2000s and took epidemic proportions in the last 4 to 5 years. It has caused severe damage and destroyed 90% of the cultivated area in the districts of Bagalkot, Belgaum, Bellary, Bijapur, Chitradurga, Gulburga, Koppal, Raichur, and Tumkur. As per Horticulture Department report, many farmers have resorted to uprooting of the trees across growing areas thereby causing a revenue loss of about Rs. 200 crore (at an average price of Rs 50,000 per tonne; 1 crore = 10^7) in Karnataka (Giridhar, 2008). This disease also had an outbreak in the year 2007 in pomegranate orchards of South Africa (Petersen *et al.,* 2010).

Table 1. Average severity of bacterial blight over the years in Northern Karnataka (India)

Name of Districts	2008-09					2009-10					2010-11				
	No. of fields	L[†]	F[†]	S[†]	SOT[†]	No. of fields	L	F	S	SOT	No. of fields	L	F	S	SOT
Bijapur	25	47.4	11.6	33.8	19.6	65	28.6	8.7	32.2	15.9	20	36.1	27.5	32.6	11.7
Bagalkot	12	38.1	70.9	69.0	66.7	51	41.5	18.4	38.0	23.5	21	42.2	23.8	39.7	23.7
Koppal	20	35.1	3.8	28.9	13.5	108	41.8	14.3	19.4	17.4	30	36.7	29.2	36.1	21.8
Bellary	26	43.8	13.5	35.5	19.2	35	51.6	12.7	38.4	20.4	30	51.0	28.0	40.4	19.4
Total	83	41.1	24.9	41.8	29.8	259	40.8	13.5	32.0	19.3	101	41.5	27.1	37.2	19.1

[†]L: Severity on leaves; F : Severity on fruits; S = Severity on stems; SOT: Severity on trees (10 L + 70 F + 20 S)

Table 2. Bahar wise disease severity in Karnataka during 2009-10

Name of Bahar	Period of survey	Total No. of fields	Severity on			Severity on Tree
			Leaf	Fruit	Stem	
Ambe	Jan-May	34	31.4	10.8	25.3	15.8
Mrig	June-Aug	132	40.9	15.7	30.2	20.8
Hasta	Sept-Dec	79	53.8	11.1	25.6	17.3

III – Present practices and its impact on the outbreak of bacterial blight of pomegranate

(i) Use of diseased seedling for planting.

(ii) The recommended spacing between the plant and rows is 4.5 x 4.5 meters is not followed.

(iii) Many farmers take crop soon after 1 year or 1 ½ year of planting.

(iv) Many farmers take up bahars (pruning) soon after the harvest of crop, without keeping the prescribed rest period of the tree.

(v) Wrong pruning methods and time of pruning.

(vi) Farmers are unaware of the role of sanitation in management of bacterial blight.

(vii) Many farmers are not supplying the required nutrients in a balanced manner at different growth stages of the crop.

(viii) Most of the farmers are using the pruning knife without any treatment.

(ix) Farmers are unaware that insect menaces increase the severity of disease.

(x) Frequent and unnecessary spraying.

(xi) Identification of bacterial blight of pomegranate.

(xii) After pruning, many farmers are not treating with bourdaeux paste.

(xiii) Movement of workers from diseased fields into healthy fields.

IV – Integrated management of bacterial blight of pomegranate

(i) Select disease-free planting material.

(ii) Growing of tall trees all along the orchard.

(iii) Use recommended dose of NPK along with FYM and bioagents.

(iv) Recommended plant spacing of 4.5 x 4.5 m or 4.0 x 4.5 m.

(v) Orchard sanitation:

- Application of microbial consortia at 25 g each PSB, Trichoderma, Pseudomonas and azospirrilium / plant along with farm yard manure.

- It is proved that insects like anar butterfly, ants, aphids, blister beetle, and larvae of fruit borer can disseminate bacteria; hence management of insects is a must.

- A minimum period of 4-6 months rest.

- Pruning should be taken during September-October months.

- Avoid unnecessary spraying of chemicals.

- Disinfection of pruning tools with a 2.5% sodium hypochlorite solution.

- Spray 1% Boudreaux Mixture before pruning and after pruning.

- Use of 100 g beaching powder at the base of the plant.

- Stem smear with streptocycline 0.5 g + Copper oxychloride 3 g + Red oxide 200 g/l of water.

- Use of a talc based formulation of Pseudomonas fluorescens at 10 g/l of water as a spray.

- Prophylactic spray of streptocycline at 0.05% + Copper oxychloride at 0.2%, followed by - Zinc sulphate 0.1% + Boron 0.1% + Magnesium sulphate 0.1% + Calcium sulphate 0.1%.

- Remove weeds in the orchard specially Tridex and Acharanthus spp.

- Mass eradication programme.

It has been clearly observed that by adopting integrated diseases management (IDM) practices the severity of the bacterial blight can be reduced to 5%. The yields are 9-12 tons /ha in orchards where IDM was practiced, but only 4-5 tons/ha were obtained in non-adopting orchards (Table 3).

Table 3. Disease severity and yield of pomegranate before and after adopting the IDM practices

Demo. sites	Demonstration plots							Farmer plots	
	Severity on tree (%)				Yield/ha (t)			Severity on tree (%)	Yield/ha (t)
	Before adopt.	After adopt.							
		08-09	09-10	10-11	08-09	09-10	10-11		
Hebbal[†]	69.1	3.4	2.5	2.4	12.00	12.00	7.0	33.1	4.0
H.B. Halli	18.3	1.4	1.5	1.4	10.50	12.00	14.0	21.4	6.5
Hanumasagar	26.2	--	--	5.2	--	--	9.0	28.1	5.0

[†]Severe attack by anthracnose.

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Status and management of anthracnose of pomegranate in Karnataka State of India

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Abstract. Pomegranate is extensively cultivated around the Mediterranean and other parts of world including India. It is regarded as the "Fruit of Paradise".The most popular varieties in India are Ganesh, Mridula, Arakta, Bhagwa (Kesar). Successful cultivation of pomegranate in recent years has met with different problems such as pests and diseases. Among the various fungal diseases, anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc., is one of the most serious disease of pomegranate, remaining latent in early stages of fruit development and reducing fruit quality to a greater extent. Propagules of pathogen cause lesions and decay of the fruit. The study was carried out in Karnataka State (India). The survey on the disease in the field showed the extent of anthracnose affecting the crop and quality of the fruits in different locations. The disease was widespread particularly in rainy season/high moisture conditions and incidence and severity disease were higher in Bagalkot district, followed by Koppal, Bijapur Gadag and Raichur districts. Studies on cultural, morphological, and physiological features of the pathogen showed maximum growth on Potato dextrose broth on the 12th day after incubation at 27±1ºC. *C. gloeosporioides* exhibited diversity with respect to cultural characters like type of the growth, mycelial colour, pigmentation and sporulation with maximum growth on Potato dextrose agar. The different days of incubation of culture filtrates of *C. gloeosporioides* differed in their action to inhibit the seed germination, root and shoot elongation of sorghum seeds and induction of phytotoxic symptoms on tomato seedlings. Among the tested fungicides, bioagents and botanicals, iprobenfos, propiconazole, carbendazim + mancozeb and *T. viride* were superior in inhibiting the mycelial growth of the fungus in vitro. carbendazim + mancozeb at 0.3 per cent and propiconazole at 0.1 per cent concentration were effective in reducing the percent disease index of anthracnose under field conditions.

Keywords. *Colletotrichum gloeosporioides* – Disease index – Antifungals.

I – Introduction

Pomegranate (*Punica granatum* L.) regarded as "Fruit of Paradise" is one of the most adaptable subtropical minor fruit crops. In India, it is regarded as a "vital cash crop", grown in an area of 150,000 ha with a production of 1,100,000 tons. Among the different states growing pomegranate, Maharashtra is the largest producer occupying 2/3rd of total area in the country followed by Karnataka, Andhra Pradesh, Gujarat and Rajasthan. Karnataka State has the distribution of cultivating pomegranate under tropical condition in an area of 12,042 ha with a production of 129,547 tons The crop is prone to many fungal diseases. Among various fungal diseases, anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is one of the most serious disease of pomegranate. Athracnose affects both quality and marketability of fruits. In the present investigation various aspects on anthracnose of pomegranate (*Punica granatum* L.) were undertaken during the period 2009 to 2010 with reference to survey and surveillance of disease, cultural, morphological, physiological aspects of pathogen, and management of disease by fungicides, bioagents, botanicals.

II – Materials and methods

Roving survey was conducted during 2009 and 2010 in all the cropping seasons viz., Mrigbahar, Hastbahar and Ambiabahar and observations were recorded on leaves and fruits by

following a 0 to 5 scale. The efficacy of six non systemic (one combi) and six systemic fungicides was tested against *C. gloeosporioides* under in vitro conditions at 0.1, 0.2 and 0.3 per cent concentration, whereas systemic fungicides were tried at 0.05, 0.1, 0.15 per cent concentrations. Antifungal effects of seven plant extracts were tried at 10, 20, and 30 per cent concentration by poisoned food technique. Four bioagents *Bacillus subtilis, Pseudomonas fluorescens, Trichoderma viride* and *T. harzianum* were evaluated for their efficacy through dual culture technique.

Evaluation of fungicide / bio agent experiment was conducted in an orchard at Bandi village, Taluk Yalburga, Koppal district, during 2010 Ambiabahar. The variety Kesar was used and sprayed with different fungicides, and bioagents. The experiment included nine treatments and one check with three replications. The percent disease index (PDI) and per cent disease reduction over control (PDC) was calculated and angular transformed data were analyzed statistically.

III – Results and discussion

1. Incidence and severity of the disease

Results of the survey revealed that fruits were more vulnerable to the attack by anthracnose than leaves as evidenced by more disease severity on fruits, irrespective of season, location and variety (Table 1). Among the different districts under survey, maximum and minimum severity of the disease on fruits was observed as in Bagalkot (28.76 PDI), Raichur (19.99 PDI) districts respectively. In general, the disease incidence and severity varied from season to season in different agro-climatic zones and varieties, which may be due to variation in pathogen, varieties and climatic conditions during mrigbahar (June-November).

Table 1. Severity of anthracnose of pomegranate measured as mean percent disease index (PDI) in major areas of northern Karnataka during 2009-10

Sl. No.	District	Mean PDI		Variety	Mean PDI	
		On leaf	On fruit		On leaf	On fruit
1.	Bagalkot	23.21	28.76	Araktha	26.39	33.61
2.	Bijapur	19.86	-	Ganesh	19.59	27.05
3.	Gadag	19.10	22.95	Kesar	20.97	25.90
4.	Koppal	21.84	23.50	Ruby red	20.33	26.33
5.	Raichur	14.36	19.99	Sindhoor	22.33	28.37

2. Disease management

A. In vitro evaluation of systemic-non systemic fungicides, botanicals and bioagents against C. gloeosporioides

The systemic, non systemic fungicides, botanicals and bioagents have been tested at different concentrations and effective concentrations are presented in Table 2. Among the systemic fungicides, iprobenfos showed 87.99% inhibition of mycelial growth of fungus and was followed by propiconazole (87.10%) at 0.15 per cent concentration while the lower per cent inhibition of mycelial growth was recorded by carbendazim (62.09). These results are in agreement with those of Prashanth (2007). However, in case of non-systemic fungicides at 0.3% concentration

carbendazim + mancozeb showed 75.10% inhibition of mycelial growth of fungus followed by captan with 60.77%, and the least inhibition was recorded in copper oxychloride.

Table 2. In vitro evaluation of systemic-non systemic fungicides, botanicals and bioagents against Colletotrichum gloeosporioides, measured as percent disease inhibition (PDI) of mycelial growth

Systemic fungicides (at 0.15% conc.)	PDI	Non-systemic fungicides (at 0.3% conc.)	PDI	Botanical extracts (at 30% conc.)	PDI	Bioagents	% inhibition[†]
Azoxystrobin	62.77 (52.43)[†]	Captan	73.88 (59.49)[†]	Datura leaf	61.70 (51.72)[†]	*B. subtilis*	53.88 (46.63)[†]
Carbendazim	62.09 (51.98)	Carbendazim + mancozeb	81.88 (64.79)	Eucalyptus leaf	5.27 (13.17)	*P. fluorescens*	67.0 (54.64)
Difenconazole	67.21 (55.05)	Copper Oxychloride	1.55 (7.26)	Garlic bulb	50.00 (44.98)	*T. harzianum*	72.47 (57.67)
Hexaconazole	64.55 (53.42)	Chlorothalonil	20.32 (26.33)	Ginger rhizome	33.33 (35.23)	*T. viride*	86.82 (67.85)
Iprobenfos	87.99 (69.67)	Mancozeb	22.99 (28.63)	Neem leaf	10.83 (19.19)		
Propiconazole	87.10 (69.47)	Propineb	29.10 (32.35)	Onion bulb	43.32 (41.14)		
				Tulasi leaf	0.70 (4.78)		
C.D (1%)	0.59		0.84		0.84		4.04

[†]Dual plate.
[††]Arcsin transformed values.

In tested bioagents, *T. viride* was found to be best in inhibiting mycelial growth of *C. gloeosporioides* (86.82%) followed by *T. harzianum* (72.47%) and *P. fluorescens* (67%), and the least per cent inhibition of mycelial growth was observed in *B. subtillis* (53.88%).

Testing of plant extracts showed fungistatic nature at higher concentration (30%). Two plant extracts viz. Datura leaf extract (61.7%), garlic extract (50%) showed ≥ 50% inhibition of mycelial growth, while almost no inhibition of mycelialll growth was noticed in tulasi leaf extract (0.70%).

B. Management of the disease in orchard

By utilizing the in vitro information a field experiment was planned and executed during ambiabahar 2010 (Jan - May). Eight different fungicides (five non-systemic, one combi product and two systemic) one bioagent (*Trichoderma viride*) and an untreated control were evaluated for their efficacy in disease control on pomegranate diseased leaves, flowers and fruits (Table 3). The results after seven sprays revealed that propiconazole at 0.1% concentration was significantly superior over other fungicides, where as iprobenfos (0.2%), carbendazim (0.2%) and difenconazole (0.1%) remained statistically on par with each other. Jamadar and Patil (2007) identified iprobenfos aganist anthracnose. Among non-systemic and combi fungicides, combi product like carbendazim + mancozeb at 0.3% concentration was significantly superior where as captan and mancozeb were less effective.

Table 3. Effect of chemicals and bioagents on severity of anthracnose of pomegranate and fruit yield

Treatments	Conc.	PDI[†] on fruits	PDC[†] fruits	Fruit yield (t/ha)
Carbendazim	0.2%	4.00 (11.53) [††]	82.30	5.89
Difenconazole	0.1%	4.00 (11.53)	82.30	5.85
Hexaconazole	0.1%	6.50 (14.70)	71.23	5.37
Iprobenfos	0.2%	2.41 (8.89)	89.23	5.97
Propiconzole	0.1%	1.20 (6.31)	94.58	6.28
Captan	0.3%	4.83 (12.69)	78.54	5.67
Mancozeb	0.3%	6.33 (14.56)	71.91	5.33
Carbendazim + mancozeb	0.3%	0.83 (5.18)	96.22	6.35
Trichoderma viride	10 g/l	17.00 (24.33)	24.97	4.63
Control	-	22.66 (28.41)		2.57
S. Em. ±		0.72		0.12
CD at 5%		1.53		0.27

[†]PDI: percent disease inhibition; PDC: per cent disease reduction over control.
[††]Arcsine transformed values.

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Usefulness of maximum diurnal trunk shrinkage as a continuous water stress indicators of pomegranate (*Punica granatum*) trees

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Abstract. The objective of this research was to asses the feasibility of using maximum diurnal trunk shrinkage (MDS) obtained by means of LVDT sensors, as a plant water stress indicators for pomegranate trees. The experiment was carried out with mature trees grown in the field under three irrigation regimes: (i) control (well watered trees); (ii) trees continuously deficit irrigated at 50% of the control regime (SDI); and (iii) trees that had a summer water stress cycle being irrigated at 25% of the control rates only in July and August (RDI). Control trees maintained lower MDS values than the SDI ones. In the RDI treatment, as water restrictions began, there was a slow increase in MDS, in correspondence with a decrease in stem water potential (Ψstem). When water was returned at full dosage, the RDI recovered to MDS and Ψstem values similar to the control. However, lower MDS for a given values were observed as the season advanced. The magnitude of differences between well watered and deficit irrigated trees was much larger in the case of MDS than for Ψstem. The tree-to-tree variability of the MDS readings was more than four times higher than for Ψstem.

Keywords. Deficit irrigation – LVDT – Stem water potential.

I – Introduction

Pomegranate trees are considered as a culture crop tolerant to soil water deficit (Holland *et al.* 2009). Because of this, in Spain, its culture is concentrated in the south east, where fresh water available for agriculture is very scarce. Nowadays, irrigation scheduling is often based on the FAO method where crop evapotranspiration (ETc) is estimated using the reference evapotranspiration (ETo) times the crop coefficient (Kc), according to the procedure suggested by Allen *et al.* (1998). However, Kc values for *Punica granatum* are not listed in FAO water use book by Allen *et al.* (1998). Hence, alternative methods need to be applied for an efficient irrigation scheduling of *Punica granatum*.

Plant water status information can be used to determine when to irrigate. Recently Intrigliolo *et al.* (2011) evaluated the usefulness of stem water potential (Ψstem) and leaf gas exchange as water stress indicators for pomegranate trees. However, because this measurements cannot be easily automated there is a need to look for other tools to continuously monitoring plant water status. In this sense trunk dendrometers have been widely assessed in fruit trees to monitor plant water status (Férnandez and Cuevas 2010). From trunk diameter variations (TDV) maximum diurnal trunk shrinkage (MDS) can be obtained and it has been shown that MDS has the potential to serve as plant water stress indicator (Férnandez and Cuevas 2010). This is because MDS is normally higher in plants with soil water deficit than in well irrigated trees.

However, before using MDS as a parameter to schedule irrigation, its usefulness as water stress indicator for pomegranate trees must be evaluated.

The objective of the experiment was to asses, for the first time for pomegranate trees, the usefulness of MDS as a water stress indicators. In trees under three irrigation regimes, the seasonal variations of MDS was compared with midday stem water potential measurements.

II – Material and methods

1. Experimental orchards

The experiment was performed during the 2010 season in a commercial mature pomegranate tree orchard (*Punica granatum*, L cv. 'Mollar de Elche') at Elche, Alicante, Spain, (38ºN, elevation 97 m). The soil was sandy-loam with an effective depth over 120 cm. Trees were planted in 2000 at a spacing of 5 x 4 m and average tree shaded area was 48% of the soil allotted per tree. Average trunk diameter was 18.2 cm.

Trees received 100, 40 and 80 kg ha^{-1} $year^{-1}$ of N, P_2O_5 and K_2O, respectively. Agricultural practices followed were those common for the area. Weather was recorded at an automated weather station near the orchard. Meteorological variables measured included solar radiation, air temperature, air humidity, wind speed and direction, air temperature and humidity and rainfall. Precipitation and reference evapotranspiration (ETo) during the growing season (April to October) were 111 and 811 mm, respectively.

2. Irrigation treatments

Drip irrigation was applied with eight emitters per tree delivering 4.0 l h^{-1} each and were located in a single line parallel to the tree row. Irrigation treatments were:

(i) Control, where irrigation was scheduled in order to replace 100% of the estimated crop evapotranspiration (ETc). Crop evapotranspiration was estimated as product of reference evapotranspiration (ETo) and crop coefficient (Kc). ETo was calculated with hourly values by the Penman-Monteith formula as in Allen *et al.* (1998). The Kc values increased from an initial value of 0.27 used in March to a maximum value of 0.77 used in July, August and September according to previous recent findings obtained in the same plot (Intrigliolo *et al.* 2011).

(ii) Sustained deficit irrigation (SDI), where water was constantly applied at 50% of control regime.

(iii) Regulated deficit irrigation (RDI) where irrigation was applied at 25% of the control irrigation from July 9 (day of the year DOY 190) to September 3 (DOY 246) coincident with a linear fruit growth phase. During the rest of the season irrigation was applied at 100% ETc.

The reductions in the amount of water applied during the deficits were achieved by reducing irrigation duration, while frequency of irrigation was always the same for all treatments. Irrigation frequency changed over the season with all treatments irrigated once a week in early spring and autumn and five times a week during summer.

The experimental design was a randomized complete block, with four replicates per treatment. Each plot had three rows, with 8 trees per row. In each experimental unit, a central trees of the middle row were used for data collection.

3. Determinations

Trunk diameter variations were measured with six linear variable differential transformers (LVDT, Schlumberger Mod. DF-2.5) per treatment. On each experimental tree a sensor was

fixed to the main trunk by a metal frame of Invar (a metal alloy with a minimal thermal expansion) located about 20 cm from the ground on the north side. Prior to installation the transformers were individually calibrated by means of a precision micrometer (Verdtech SA, Spain). The typical output coefficient was about 83 mV mm^{-1} V^{-1}. The resolution of trunk diameter measurements including all sources of variation (calibration, non-linearity, excitation and output voltage recording and thermal changes) was about 10 μm. From TDV the maximum daily shrinkage (MDS) was calculated as the difference between the maximum diameter reached early in the morning and the minimum reached normally during the afternoon. All sensor data were automatically recorded every 30 s using a data logger (model CR1000 connected to an AM16/32 multiplexer programmed to report mean values every 30 min). The system was powered by batteries. Data collection lasted from June 11 (DOY 162) till November 1 (DOY 305). However, during the experiment there were three periods (June 28 to July 1, August 24 to August 26 and September 21 to September 30) when data were not obtained due to battery power failures.

Stem water potential was measured in the same trees instrumented with LVDT sensors with a pressure chamber, following the procedures described by Turner (1981), in two leaves per tree (total of 8 leaves per treatment). Mature leaves from the north face near the trunk, were enclosed in plastic bags covered with silver foil at least two hours prior to the measurements, which were carried out between 12:00 and 13:00 h solar time, approximately every week. During the course of the entire experiment determinations were carried out in the Control and SDI treatments. On the other hand, in the RDI trees, Ψstem was only measured during the RDI cycle and until 45 days after the end of the water restriction period.

4. Data analysis

The effects of the irrigation regime on Ψstem and MDS was evaluated by analysis of variance using the general linear models "GLM" procedure of the SAS software (version 9.0; SAS Institute, Cary, NC).

III – Results and discussion

The experiment was carried under contrasting evaporative demand regimes. During the summer (till day of the year, DOY 250) daily ETo values were around 5 to 6 mm, except for three days, which values were around 7 mm. On the other hand, from DOY 250 onwards, ETo had a clear decreasing tendency (Fig. 1A). Over the course of the experiment, rainfall was scarce, and only more noticeable by the end of the experiment (Fig. 1A).

When TDV data collection started, the SDI treatment had already been irrigated at 50% of the Control regime. This explains why the SDI trees since the beginning of the experiment had statistically significant ($P<0.05$) lower Ψstem values than the control trees (Fig. 1B). On the other hand, initial differences in MDS, were not statistically significant ($P>0.05$, Fig. 1C). Differences in Ψstem and MDS between control trees and SDI ones statistically significant at $P<0.05$ by the middle of the summer (DOY 190 to 230). At the end of the experiment, there were still statistically significant differences in Ψstem between SDI and the control trees, but MDS of the SDI trees decreased as the evaporative demand became lower and differences with respect to the MDS value of the control trees were small and not statistically significant ($P>0.05$).
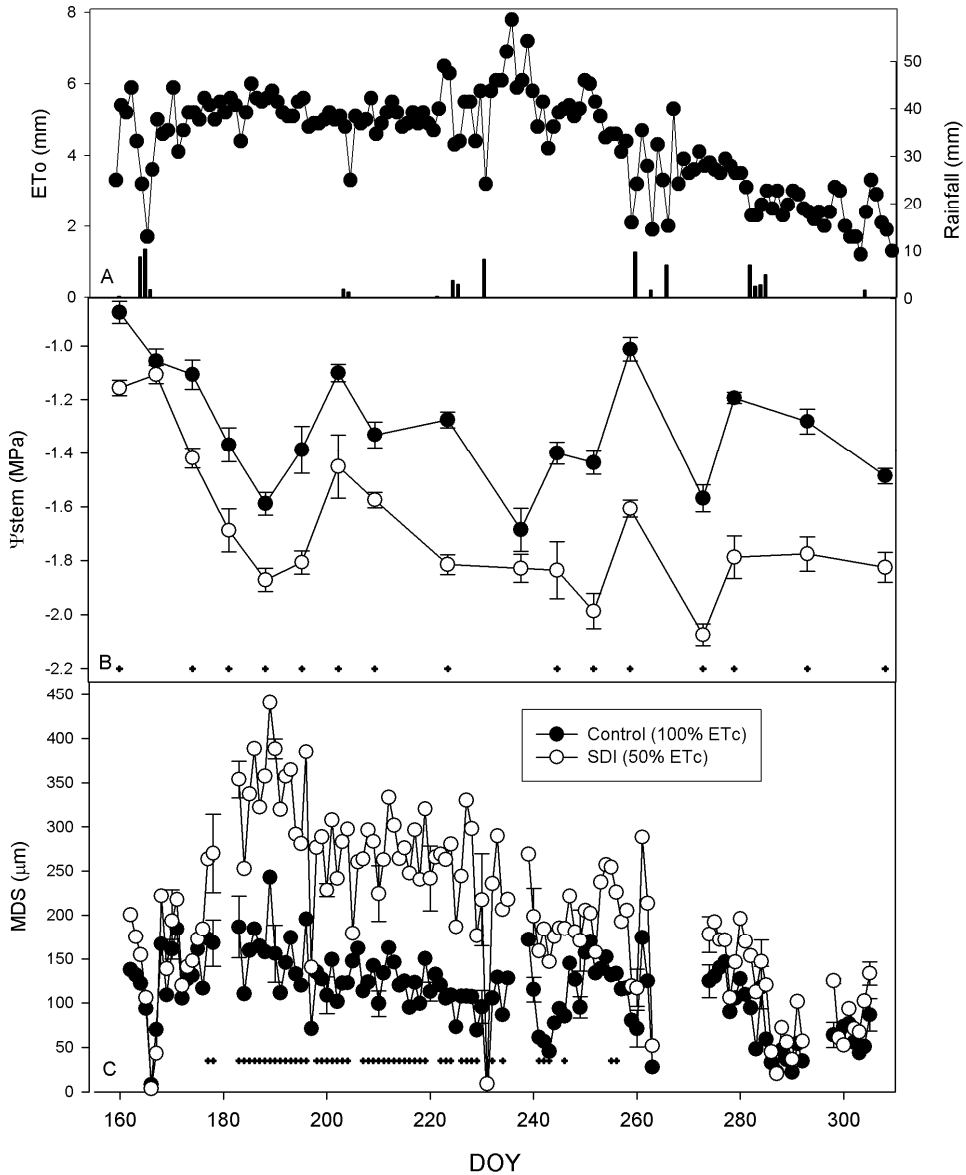
Fig. 1. Seasonal pattern of A) daily reference evapotranspiration (ETo) and rainfall, B) midday stem water potential (Ψstem), C) maximum diurnal trunk shrinkage (MDS). The error bars indicate the standard error. + denotes statistically significant differences among treatments at P<0.05. DOY, day of the year.

At the beginning of the experiment, the RDI trees had surprisingly higher Ψstem than the control trees (Fig. 2A), but similar MDS (Fig. 2B). Only two weeks after restrictions started, the RDI trees water status became significantly lower than the control. A decrease in Ψstem was associated with a progressive increase in MDS. However statistically significant differences

among treatments could be detected earlier for Ψstem than for MDS (Fig. 2) On the other hand, once the water stress cycle was ended, the RDI trees very quickly recovered to values similar to the control ones (Fig. 2).
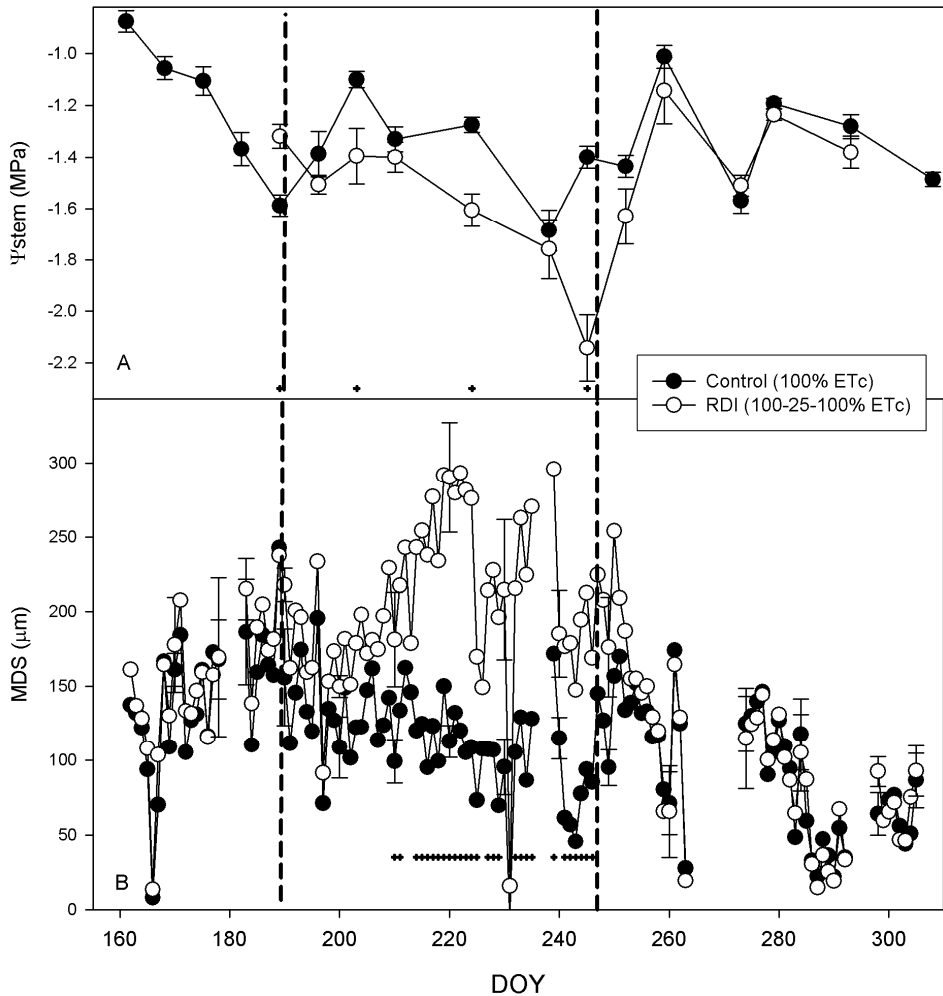
Fig. 2. Seasonal pattern of A) midday stem water potential (Ψstem) and B) maximum diurnal trunk shrinkage (MDS). The error bars indicate the standard error. + denotes statistically significant differences among treatments at $P<0.05$. The vertical dotted lines enclosed the period when RDI trees where irrigated at 25% of the control regime. DOY, day of the year.

Taking into consideration SDI and control trees, in general, the signal intensities (stress/control) was almost double for MDS than for Ψstem (Fig. 3). In fact, maximum signal values obtained for Ψstem were around 1.5; while for MDS they reached up to 3.2 (Fig. 3). However, by the end of the experiment, under lower evaporative demand and with some rainfall events (Fig. 1A) the MDS signal intensity decreased to similar Ψstem signal intensity values (Fig. 3).

Considering MDS and Ψstem values obtained in the RDI and control trees, there was a progressive increase in the MDS signal during the restriction period (Fig. 3). Immediately after restriction ended signal values returned to values close to 1.
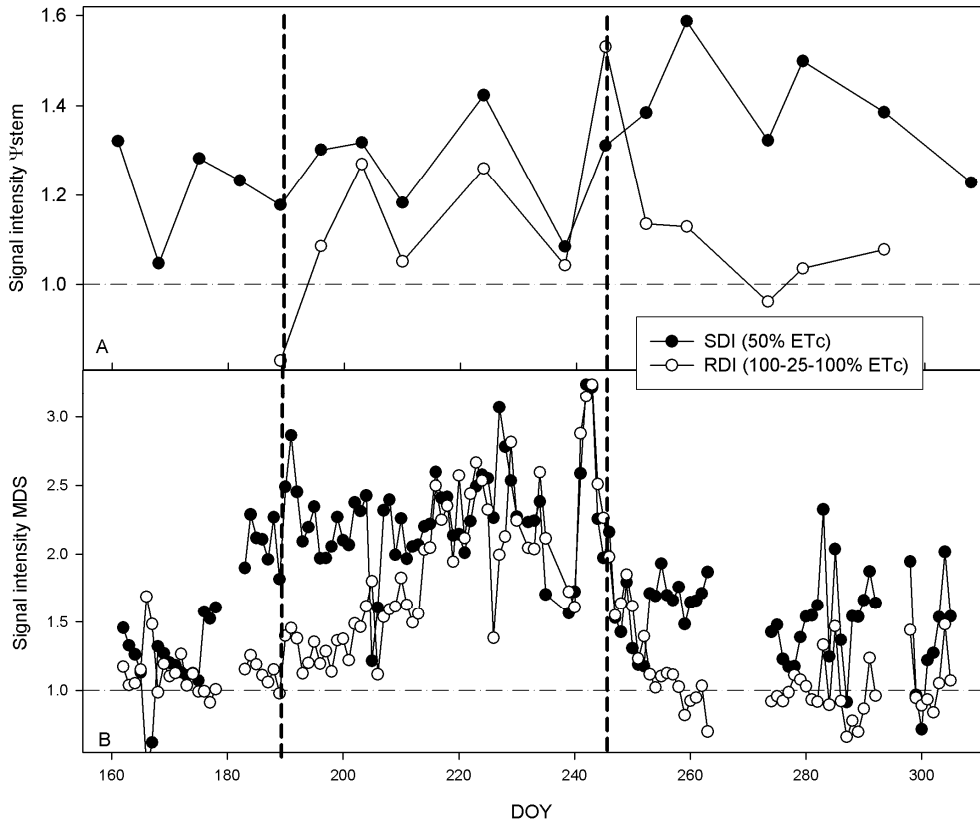
Fig. 3. Seasonal pattern of the signal intensity (stress:control) for midday stem water potential (Ψstem) and maximum diurnal trunk shrinkage (MDS). The vertical dotted lines enclosed the period when RDI trees where irrigated at 25% of the Control regime. DOY, day of the year. The SDI trees were always irrigated at 50% of the Control water applications. Data shown are average of 12 and 6 determinations per treatment for Ψstem and MDS, respectively.

The results presented showed that MDS can be considered as a sensitive indicator of Pomegranate plant water status. As water was restricted, trunk shrinkage appeared to be a good indicator of the onset of plant water stress. However, it is important to note that statistically significant differences between irrigation regimes could be detected earlier for Ψstem than for MDS. This is probably due to the high MDS tree-to-tree variability that precluded to detect with statistical significance small differences between treatments. Contrarily, previous reports in plum (Intrigliolo and Castel 2004) and peach (Remorini and Massai 2003) trees showed how MDS was able to detect differences in plant water status between trees under different irrigation regimes earlier than Ψstem.

Indeed, the good sensitivity of MDS as a water stress indicator makes it a suitable parameter for scheduling irrigation in this crop. Data reported in Fig. 1C, clearly show that in control trees

MDS was always lower than 150-175 μm. These values could be adopted as a threshold for detecting the onset of mild water stress.

Pooling data from the entire experiment the relationship between MDS and Ψstem was weak (r^2=0.30**). When data were separated according to different periods (from DOY 168 to DOY 224 and from DOY 225 onwards) there was a statistically significant difference (P<0.05) in the slopes of the MDS-Ψstem relationships (Fig. 6). Within each single period, the relationship was tighter than with the entire data set, particularly for data corresponding with the last part of the season.
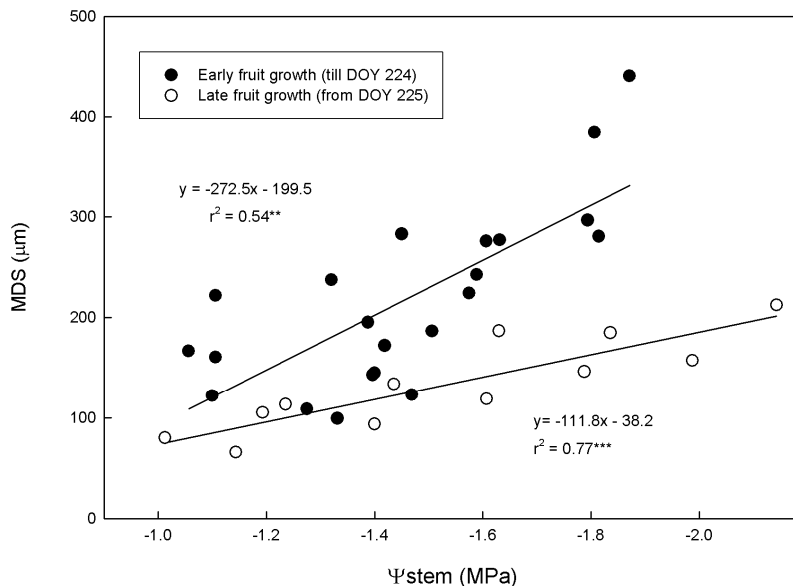
Fig. 4. Relationship between maximum diurnal trunk shrinkage (MDS) and midday stem water potential (Ψstem). Data are average treatment of 12 and 6 determinations for Ψstem and MDS, respectively. Values are separated according to two periods: from day of the year (DOY) 168 to DOY 224 and from DOY 225 onwards. * and ** indicates statistically significance at P<0.001 and P<0.01, respectively. DOY, day of the year.**

This suggest that there was not a single unique relationship between MDS and Ψstem valid for the whole period. This behavior is similar to other cases, such plum (Intrigliolo and Castel 2006) where the relationship between MDS and Ψstem changed in concordance with some changes in the fruit growth pattern or fruit removal. In the present experiment, fruit were present during all the course of the experiment, but fruit growth rates were higher till DOY 224 than later on (results not-shown). It is also possible that the different relationship obtained between MDS and Ψstem during the seasons is due to changes in tissue elasticity. It is generally accepted that tissue age affects its elasticity, older tissues being less elastic (higher resistance to shrinkage; Tyree and Jarvis 1982). Therefore, the lower MDS for a given Ψstem value obtained late in the season may be due to less elastic, older tissues.

IV – Conclusions

Results presented indicate that MDS could be used in commercial orchards for detecting plant water stress; however, a large number of trees need to be monitored and occasional determinations of plant water status need to be carried out in order to complement the on-line continuous monitoring plant water status obtained by MDS. This is particularly important considering the change in the relationship between MDS and Ψstem reported.

Acknowledgements

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Inhibition of carob moth damage using *Ferula assafoetida* essential oil in pomegranate orchards of Iran

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Abstract. The carob moth, *Ectomyelois ceratoniae* is a polyphagus insect pest worldwide. In Iran it is the key pest of pomegranate and alternates to conventional insecticides are being developed. The essential oil of *Ferula assafoetida* was tested in four pomegranate orchards. In each garden 10 trees were treated with three concentrations of oil. The number of rotten fruits/tree that fell to the ground were counted and removed every 2 weeks during the season. Furthermore, the percentage of rotten fruits/ tree at the harvest time was calculated. All three concentrations of the essential oil significantly reduced fruit infestation by carob moth (P<0.001). However, the 1:1 dose of the oil caused fruits to be scorched. The reduction in infestation may be due to: (i) a direct repellent effect of the oil on the adult carob moth, or (ii) a disruption of mating by volatile compounds emitted by the essential oil.

Keywords. Repellency – Reproductive behavior – *Ectomyelois ceratoniae* – Medicinal plants – Clevenger.

I – Introduction

The indiscriminate use of conventional insecticides has given rise to many well-known problems, as human and domesticated animals health, and contamination of environment. These undesired effects have stimulated the search for biologically based alternatives. So, natural compounds from plants are attractive alternatives because botanicals reputedly pose little threat to the environment or to human health (Isman, 2006). Extracts or essential oils of medicinal plants have a broad spectrum of bioactivity (Baskar *et al.*, 2009; Cosimi *et al.*, 2009; Tunç and Şahinkaya, 1998) due to the presence of different ingredients which work through several mode of action.

Ferula assafoetida is a medicinal plant origin of Iran and the neighbor countries. Its essential oil is a candidate (Peyrovi *et al.*, 2011) for integrated management of the carob moth, *Ectomyelois ceratoniae* (Lep.: Pyralidae) the key pest in pomegranate orchards of Iran. In this work different concentrations of the oil were used in natural conditions of the pomegranate orchards to see if it reduces carob moth damage during the growing season 2008-2009 in Yazd province, Iran.

II – Materials and methods

1. Experimental sites

The experimental sites were located in four cities (Taft, Yazd, Meybod, Ardakan) at Yazd province, Iran. The experiment was done in one pomegranate orchard on an area of about 1 ha/site.

2. Essential oil

Ferula assafoetida gum was obtained from its herbaceous plants in Taft Mountains, Yazd, Iran. The essential oil was derived by Clevenger apparatus using hydro distillation method.

3. Field experiments

The experiments were done from June to October 2008. The oil was diluted by ethanol as solvent. Three concentrations: 1:1 (oil: solvent), 1:3, and 1:5 were prepared and sprayed on the canopy of the plants (5 ml/plant) every two weeks. Considering our laboratory studies that the solvent, ethanol in our conditions due to high volatility has not effect on repellency of the pest so, four treatments were applied (3 oil concentrations, and control without oil). In each garden 10 trees were treated with each concentration. The number of rotten fruits/tree that fell to the ground were counted and removed every 2 weeks during the growing season. Furthermore, the percentage of rotten fruits/tree at the harvested time (about early October) was calculated.

III – Results and discussion

All three concentrations of the essential oil of *F. assafoetida* significantly (P<0.001) reduced fruit infestation by carob moth, *Ectomyelois ceratoniae*. There was no significant differences between the experimental sites (P>0.05). However the highest dose, 1:1, of the oil caused the fruits to be scorched. The monoterpenes comprise the major components of essential oils and phytotoxicity of them was shown on some plants such as maize (Lee *et al.*, 1997).

The rotten fruits in treated plots that fell to the ground during the growing season were significantly lower (P<0.001) than the control. The percentage of infected fruits by the pest larvae in treated gardens were also significantly lower (P<0.001) than the control in the end of season.

The reduction in pomegranate fruits infestation may be due to the direct repellent effect of the oil on the adult carob moth, or a disruption of reproductive behavior of the adult carob moth by volatile compounds emitted by the essential oil, or combination of the two effects. Reproductive behavior of the adult insects mediated by some host plant volatile and the sex pheromone. So, calling and searching behavior belong the biological characteristics of the insects may be disrupting by air pollution and/or the non-host plant volatile compounds such as some medicinal plants (Bisseleua *et al.*, 2008; Kumar *et al.*, 2008)

Our study revealed some essential oils can be used in integrated pest management programs as the safe compounds for human health but the effect of the oils on insect natural enemies must be well studied. It has been shown that there are some negative effects on biological and behavioral characteristics of some insect natural enemies, *Telenomus busseolae* (Hym. Scelionidae) and *Trichogramma* spp. (Hym.: Trichogrammatidae) (Bayram *et al.*, 2010; Poorjavad *et al.*, 2011). So, we suggest the essential oils may be used with the insect natural enemies in IPM programs, not simultaneously but periodically. Complementary studies under laboratory and field conditions are needed to determine how may be used the live biological agents and the botanical insecticides such as essential oils in combination in IPM programs.

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Effect of chemicals on control of fruit cracking in pomegranate (*Punica granatum* L.) var. Ganesh

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Abstract. During drought period, strengthened tissue develops in xylem and phloem and loss their ability to divide and enlarge. If after a dry spell (April-May) water supply is increased the meristematic tissue quickly resumes growth but the strengthened tissue does not, and owing to differential growth rate, tissues ruptures appear. Hypertrophy of lenticels may be caused or promoted greatly by retarded transpiration accompanied by plentiful water supply to the regions of hypertrophy. Heavy summer rains (April-May) causes fruit cracking if the plants were previously under severe water stress, because the skin get hardened and the arils are filled-up with water. If 25% available soil moisture is maintained during summer, then fruit cracking will be less. Calcium is a cell binding material, and spraying of calcium chloride (1 kg/100 l water) or calcium ammonium nitrate (2 kg/100 l of water) reduces fruit cracking. Dry heat accomplished by dry hot wind at the time of fruit ripening in pomegranate was the main cause of cracking: during the rapid flesh growth, temperatures higher than 38°C combined with less than 60% humidity favoured cracking. Sharp fluctuation in day and night temperatures coupled with heavy irrigation after dry spell also cause cracking. Nutrients like boron, zinc, calcium, copper, molybdenum manganese and potash are involve in physiological processes during fruit growth period, and theirs deficiencies cause cracking. Boron and copper help to increase the growth rate by stimulating enzymatic action in the peripheral tissue which otherwise could not be due to their inherent deficiency in the area. Boron application may probably help in translocation of sugars and synthesis of cell wall.

I – Introduction

Pomegranate (*Punica granatum* L.) belonging to the family Punicaceae is one of the most favourable fruits of tropical and sub tropical regions. The fruit is a native of Iran and is extensively cultivated in Mediterranean regions since ages, especially in Spain, Morocco, Egypt and Afghanistan. It is also grown to some extent in Burma, China, Japan, United States of America, Russia, Bulgaria and Southern Italy.

In India it is cultivated in States like Maharashtra, Gujarat, Rajasthan, Tamil Nadu, Uttar Pradesh, Haryana, Andhra Pradesh and Karnataka. Maharashtra, accounts for maximum area (about 4500 ha), particularly in Ahmednagar, Solapur, Satara, Sangali, Pune Wardha districts. In Karnataka, pomegranate is cultivated on an area of 3000 ha with an annual production of 13536 tonnes (Anon 1990). In Karnataka it is grown mainly in Bijapur, Bagalkot, Tumkur, Kolar, Belgaum, Dharwad and Bangalore.

The versatile adaptability, hardy nature, drought resistance, low cost of maintenance, steady yield and good keeping quality, and the therapeutic value of pomegranate are the main features of its spread at a wide scale. The cultivars grown in India are Alandi, Dholka, Kabul, Kandhari and Ganesh. Ganesh is a prolific bearer with medium size fruits with soft seeds, pinkish red arils, sweet juice and agreeable taste.

Pomegranate losses due to fruit cracking are quit high. The fruit have this problem due to improper water management and deficiency of micronutrients.

II – Materials and methods

The following treatments were imposed: Control, Boric acid 0.2%, Boric acid 0.4%, Ferrous sulphate 0.5%, Ferrous sulphate 1%, Calcium chloride 0.5%, and Calcium chloride 1% as foliar spray. Observations recorded were: percentage of cracked fruits, mean healthy fruit weight, yield of healthy fruits per plant and mineral content of cracked fruits.

III – Results and discussion

The data on percentage of cracked fruit, mean weight of fruit and yield of healthy fruits per plant, mineral content of cracked fruits are presented here (Tables 1 and 2).

Table 1. Effect of chemicals on control of fruit cracking in Ganesh pomegranate

Treatments	Percentage of cracked fruits			Mean fruit weight of healthy fruits (g)			Yield of healthy fruits/plant (kg)		
Control	25.45	31.43	28.44	223.83	225.00	224.17	20.69	19.31	20.00
Boric acid 0.2%	3.14	3.51	3.33	213.33	221.67	217.50	34.33	33.68	34.05
Boric acid 0.4%	6.89	7.23	7.06	231.67	228.33	230.00	26.85	26.24	26.54
Ferrous sulphate 0.5%	8.32	8.49	8.40	190.00	200.00	195.00	23.45	25.46	24.46
Ferrous sulphate 1.0%	6.57	7.67	7.12	201.67	208.33	205.00	28.66	29.34	29.00
Calcium chloride 0.5%	5.46	5.55	5.51	198.33	203.33	200.83	30.14	31.14	30.64
Calcium chloride 1.0%	5.33	5.65	5.19	200.00	208.33	204.17	31.23	32.93	32.08
SEm±	0.44	1.48	0.91	5.51	4.91	3.54	1.73	0.83	0.84
CD at 5%	1.33	4.54	2.69	16.92	15.08	10.48	5.31	2.55	2.48

Table 2: Effect of chemicals on mineral content of cracked fruits in Ganesh pomegranate

Treatments	P (% DW)	K (% DW)	Ca (% DW)	Mg (% DW)	S (% DW)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	Mo (ppm)	B (ppm)
Control	0.040	1.57	1.22	0.13	0.08	135.37	3.16	13.50	6.67	5.70	0.82
Boric acid 0.2%	0.130	1.54	1.11	0.19	0.04	57.67	4.33	8.67	6.00	8.17	6.00
Boric acid 0.4%	0.040	1.42	0.61	0.13	0.09	47.67	3.33	17.00	6.00	1.55	10.00
Ferrous sulphate 0.5%	0.061	2.02	2.55	0.14	0.11	67.67	5.00	16.67	7.17	5.17	11.08
Ferrous sulphate 1.0%	0.080	1.86	1.81	0.14	0.14	78.50	4.50	15.50	2.67	26.00	8.67
Calcium chloride 0.5%	0.060	2.31	3.35	0.14	0.04	83.70	8.50	16.00	2.17	0.95	11.67
Calcium chloride 1.0%	0.120	1.94	1.62	0.16	0.03	59.67	8.50	11.17	4.75	5.50	12.83

Two year pooled data of percentage of cracked fruits show that all the tried chemicals reduced the percentage of cracked fruits. Pre-harvest spray of 0.2% boric acid resulted in the lowest percentage of cracked fruits (3.33%) against 28.44% in control. This is followed by 5.51% in calcium chloride spray. Among the concentration of boric acid, the lower concentration 0.2% was better than the higher. The spray of ferrous sulphate resulted in fruit cracking in a range of 7.12 to 8.4%. Skok (1958) observed that boron in general improved translocation of sugars and synthesis of cell wall material. Bramlage and Thompson (1963) have also reported that boron

increased methyl esterase activity in apple. Misra and Khan (1981) reported that the role of boron application may probably due to translocation of sugars and synthesis of cell wall material and increase in methyl esterase activity which was enhanced due to application of 2,4,5 T in litchi cv. Rose scented. The maximum reduction was noted with 0.4% boric acid. The direct application could meet the requirement of Boron.

Effect of chemicals on fruit cracking, healthy fruit weight, yield per plant and mineral content in Ganesh pomegranate shows that pre-harvest spray of boric acid, did not affect the mean fruit weight of healthy fruits. However, the other treatments significantly reduced the mean fruit weight. The two year pooled data show that there was a significant increase in the yield. The highest yield (34.05 kg/plant) was recorded in the trees sprayed with 0.2% boric acid, followed by 32.08 kg/ha with 1% calcium chloride spray as against 20 kg per plant in the control treatment. The data shows that boron content of cracked fruits shown an increase due to chemical spray. The highest content was noted in 1% calcium chloride spray followed by 0.5% calcium chloride and 0.5% ferrous sulphate respectively. Even in boric acid treatment, boron content was higher than in the untreated trees. So was the case with the content of phosphorus and manganese. Sharma (1983), Sharma and Ray (1987) and Sharma and Dhillon (1987) reported that cracked litchi fruits contain higher nitrogen, potash and phosphorous, but lower calcium, zinc than the normal fruits. There was no difference in pH and TSS in cracked fruits and normal fruits.

With regards to phosphorus, potash, calcium, sulphur, zinc, the contents were higher in all cracked fruits of treated trees, except with the boric acid treatment. Sulphur content also increased in all treatments except in 1% calcium chloride. The iron content decreased in all treatments. Copper and molybdenum content also decreased with the exception of treatments with 0.5% ferrous sulphate and 0.2% boric acid.

Randhawa *et al.* (1958), reported that cracking occurs after heavy rainfalls, followed by a period of drought. Nutrients like potassium, calcium, zinc, copper, molybdenum and manganese are involved in some physiological processes during the fruit growth period, and their deficiency results in fruit cracking. Misra and Khan (1981) found that spraying of 0.4% boric acid at pit hardening gave the maximum reduction of cracked litchi fruits. Bohlmann (1962) also opined that application of boron in boron deficient area also check the cracking of apple fruit, the physiological role of boron is due to synthesis of pectic substance in plants. Agrios (1967) suggested that drought, nutrient deficiency and virus are possible cause for net like and ring like cracking in pear fruits. The spray of Bordeux mixture, calcium chloride and NAA would control fruit cracking in pear.

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Effects of different periods and levels of water deficit on physiological, productive and quality parameters of pomegranate cv. Wonderful fruits

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Abstract. Pomegranate is a crop tolerant to water deficit and is suitable in areas of water scarcity. This work presents results from a three year study on different levels of water deficit applied at the beginning, end or during the growth period of the pomegranate cv. Wonderful fruit under full production and young plants raised in 1 m^3 containers. Over this period the following parameters were measured: gas exchange, chlorophyll fluorescence, soil and xylem water potential, solar radiation intercept, osmotic adjustment and fruit and shoot growth. Fruit weight, yield and size were determined at harvest. The results help determining controlled water stress thresholds and periods for the production of fresh fruit.

I – Introduction

Pomegranates are known for their ability to withstand long drought periods and for their relatively low water requirements for producing optimal crops, as compared to other fruit trees. This is one of the main reasons why pomegranates have been planted in semi-arid and arid zones of Chile where irrigation water is scarce and droughts are frequent. A strategy for saving water in fruit production under such conditions is controlled deficit irrigation (CDI) which consists of reducing irrigation under its optimal level during periods in which such restrictions do not affect the amount and quality of the harvested fruits.

In this study we aimed at studying the effect of different levels and periods of water deficit on the yield and quality of a commercial cv Wonderful pomegranate orchard in central Chile (trial 1) and the effect of drought on container grown two year old Wonderful pomegranate plants (trial 2).

II – Materials and methods

Trial 1: measurements were performed during the 2009-2010 and the 2010-2011 seasons on a commercial cv. Wonderful pomegranate orchard which was planted in 2004 in a 3 x 5 m frame in the Chacabuco province (Metropolitan Region: 33º04'S 70º45'W) on a sandy loam. In both seasons the farmer's irrigation was used as reference control treatment. During the 2009-2010 season four irrigation treatments were implemented from the beginning of February till the end of harvest (may 2010): daily irrigation (T1), and irrigation each 3rd (T2: control treatment), 6th (T3) and 9th (T4) day. In the 2010-2011 season the irrigation frequency was kept constant (each 3rd day) and we applied either a moderate deficit irrigation (TM), by reducing from 12 to 8 drippers per plant, or a sever deficit irrigation (TS), by reducing from 12 to 5 drippers per plant. These treatments were applied from fruit set to the final fruit growth stage (period 1) or from then to harvest (period 2) or throughout both periods. Shoot growth, gas exchange, as well as yield and fruit size were measured on four replicate plats per treatment during both seasons.

Trial 2: eight cv. Wonderful pomegranates were grown for two years in 1 m^3 containers in a 1:1:1 mixture of sand, soil and organic soil. Plants were drip irrigated in order to keep adequate water availability in the soil and a drought treatment was implemented on half of the plants by

withdrawing irrigation for 42 days during the summer (no rain fell during that period). Thereafter, irrigation was reestablished to the level of un-stressed plants. Before, during and after the 42 day water withdrawal measurements of chlorophyll fluorescence, gas exchange and leaf shedding were performed on stressed and un-stressed plants.

III – Results and discussion

As shown on Fig. 1, increasing the irrigation frequency from the commercial standard of once every 3rd day to daily irrigation significantly increased shoot growth but did not significantly affect fruit growth. Both, shoot and fruit growth, were not reduced by halving the commercial irrigation frequency but severely decreased when applying a third of this frequency (Fig. 1). Yield, on the other hand, was similar between daily and commercial frequency but was reduced with the less frequent irrigation treatments (Fig. 2). Results of the second season of trial 1 showed that most treatments significantly reduced individual fruit weight except for the moderate water deficits applied during the second period and during the whole period, which had lower averages but where not significantly different from the control treatment (Fig. 3). Similarly, all water deficit intensities and periods reduced yield as compared to the control treatment; the lowest reduction in yield was achieved with the moderate water stress during the second period (Fig. 4).
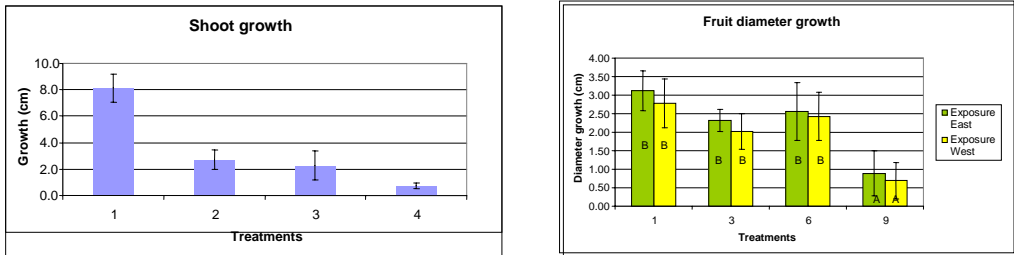
Fig. 1. **Shoot growth and fruit diameter growth as affected by irrigation frequency: daily (1) and each 3rd (2), 6th (3) and 9th (4) day.**
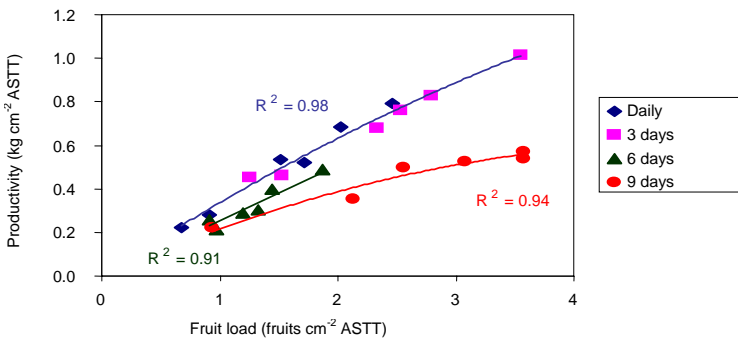
Fig. 2. **Relationship between yield and fruit load as affected by irrigation frequency: daily (1) and each 3rd (2), 6th (3) and 9th (4) day.**

Regarding trial 2, the drought treatment progressively reduced its photosynthesis and stomatal conductance as compared to non stressed plants; both variables completely recovered after reestablishing irrigation (Fig. 5). The plants submitted to total irrigation withdrawal lost most of

their leaves but no significant differences in variable to maximal chlorophyll fluorescence (Fv/Fm) was observed (data not shown).
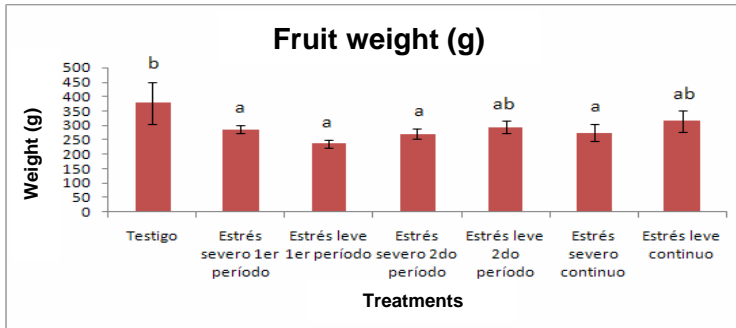
Fig. 3. Effect of different water deficit intensity and periods on individual fruit weight.
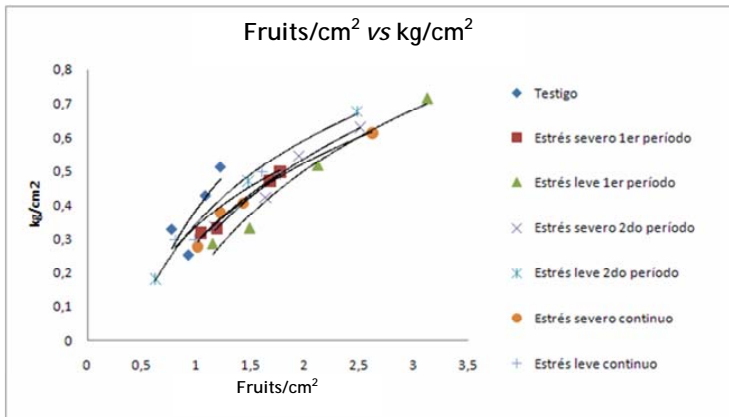
Fig. 4.   Relationship between yield and fruit load as affected different water deficit intensity and periods.

Taken together, these results indicate that, although pomegranates can withstand drought by reducing their transpiration through a reduction of their stomatal conductance and leaf area, lowering irrigation beyond commercial recommendations affects yield and fruit size. If deficit irrigation should be applied as a strategy for saving water, less harm to yield and fruit size will be caused when applying such deficit late in the season, between the last fruit growing stage and harvest.
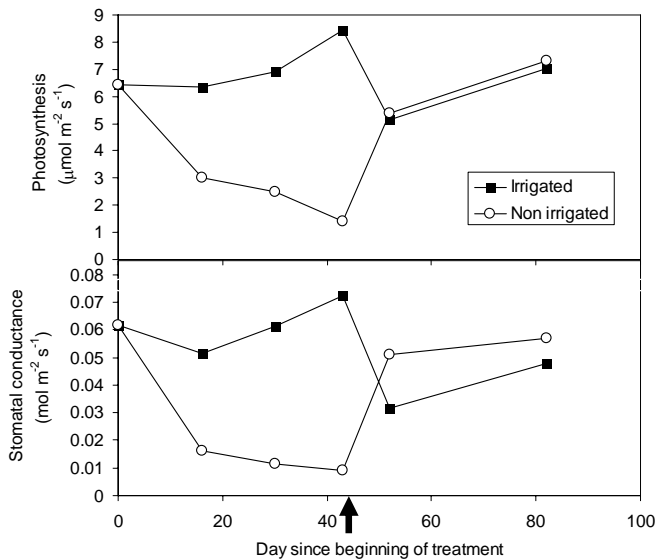
Fig. 5. Photosynthesis and stomatal conductance as affected by irrigation withdrawal (day 1) and reestablishment (day 42: indicated by black arrow).

Development of an irrigation scheduling recommendation for pomegranate trees (*Punica granatum*)

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Abstract. Pomegranate culture in Spain is concentrated in the south east, where water available is scarce. There is a need for providing growers with some irrigation recommendations for improving water use efficiency. For this purpose, information from indicators of the continuous soil-plant-atmosphere is being used in a commercial drip irrigated pomegranate orchard, for delivering to growers a weekly irrigation recommendation, taking into account the reference evapotranspiration, plant water status determined via stem water potential measurements and the soil water available to plant obtained from capacitance probes that measured soil water content at different depths. Watering recommendations are calculated in order to fulfil crop water requirements and also avoid water percolation. The average annual water application has been around 425 mm. The information obtained has been transferred by means of an experimental crop coefficient (Kc) integrated in a dissemination tool for calculating water crops requirements. Experimental Kc values increased from 0.32 in March to 0.74 in July, decreasing to 0.42 in November.

Keywords. Soil capacitance probes – Crop coefficient – Stem water potential.

I – Introduction

Pomegranate trees are considered as a culture crop tolerant to soil water deficit and high soil salt levels (Holland *et al.* 2009). Because of this, in Spain, its culture is concentrated in the south east, where fresh water available for agriculture is very scarce. However, very little is known about *Punica granatum* orchard water management. Currently, the most widespread methods for irrigation scheduling is based on the combination of a parameter that depends on the weather, the reference evapotranspiration (ETo), together with a parameter specific for each culture, the crop coefficient (Kc) as suggested by Allen *et al.* (1998). However, for pomegranate trees, Kc are not listed in the FAO water use book by Allen *et al.* (1998). Because of this, in the Alicante and Murcia area growers normally schedule irrigation of pomegranate trees as it is done for citrus trees. However, there are obvious differences between both cultures; thus irrigation management can and should be different.

The adequacy of an irrigation scheduling procedure to the orchard soil characteristics and the actual plant water needs can be tested by checking the plant and soil water status. Regarding the measurement of soil water content, the multi-sensor capacitance probes are one of the most commonly used devices because of their effectiveness (Starr and Paltineanu, 1998a,b). For determining plant water status, stem water potential is nowadays the benchmark indicator (McCutchan and Shackel, 1992).

This manuscript summarizes the research conducted by the Valencian Institute for Agricultural Research for transferring to growers an irrigation scheduling recommendation for pomegranate trees.

II – Materials and methods

The experiment was performed in a commercial mature pomegranate tree orchard (*Punica granatum*, L cv. 'Mollar de Elche') at Elche, Alicante, Spain, (38ºN, elevation 97 m). The soil was sandy-loam with an effective depth over 120 cm. The irrigation water had a moderate risk of salinization with an average electrical conductivity, EC at 25 ºC of 2.63 dS m^{-1} and an average Cl^- and Na concentration of 43.5 and 326.3 mg/l^{-1}, respectively. Trees were planted in 2000 at a spacing of 5 x 4 m and average tree shaded area was 56% of the soil allotted per tree. Trees received 100, 40 and 80 kg ha^{-1} $year^{-1}$ of N, P_2O_5 and K_2O, respectively. Agricultural practices followed were those common for the area.

Weather was recorded at an automated weather station near the orchard. Meteorological variables measured included, solar radiation, air temperature, air humidity, wind speed and direction and rainfall. This weather station belongs to the Spanish national weather station net for irrigation recommendations.

Plant water status was determined by occasional determinations of midday stem water potential (Ψstem, McCutchan and Shackel, 1992) carried out by means of a pressure chamber (Soil Moisture Equip. Corp. mod. 5100A). Two mature leaves per tree, from the north face near the trunk, were enclosed in plastic bags covered with silver foil at least two hours prior to measurements, which were between 1112:30 and 1314:00 h solar time GMT. Measurements of Ψstem were carried out approximately every week from May to October in a total of 4 trees.

Soil water content (SWC) was measured by using two multi-sensor capacitance probes C-Probe (Agrilink Inc., Adelaida, Australia). Each probe had four sensors located at 10, 30, 50 and 70 cm depth. Probes were installed inside a PVC tube located at 10-15 distance from the emitter guarantying a tight contact between the soil and the probe. Data were obtained every 15 minutes and could be visualized using the manufacturer software addVANTAGE Pro 5.1.

In 2009, as a starting point and in the absence of a specific guideline for pomegranate water needs, irrigation scheduling was applied as the farmer's own standard. However, recommendations were given to the grower in order to maintain SWC in the first 0.6 m of soil between field capacity and a theoretical refill point, fixed in 85% of the field capacity. In addition, plant water status determinations were also used to maintain crop water status at appropriate levels. In 2010, taking into account the water applications made in the previous season, we obtained some tentative experimental crop coefficients that were used as a baseline for irrigation scheduling. In addition irrigation dose and frequency were finally adjusted taking into account the seasonal trends of soil water content, plant water status and the weather forecast prediction for the following week This was reflected in a recommendation report, which was supplied to farmers for their knowledge and application in the plot. In 2011, the updated crop coefficients were used after taking into account results obtained in 2010. Again the information from plant and soil water status was considered.

III – Results and discussion

By the end of the 2009 season, irrigation applied was 434 mm (Fig.1). The reference evapotranspiration was 1130 mm while total yearly rainfall was 349 mm. The seasonal (March to November) rainfall was 256 mm. Ψstem varied along the season from –0.85 MPa registered by the end of October (week 44) down to –1.65 MPa registered at the beginning of the summer (week 26, Fig. 2).
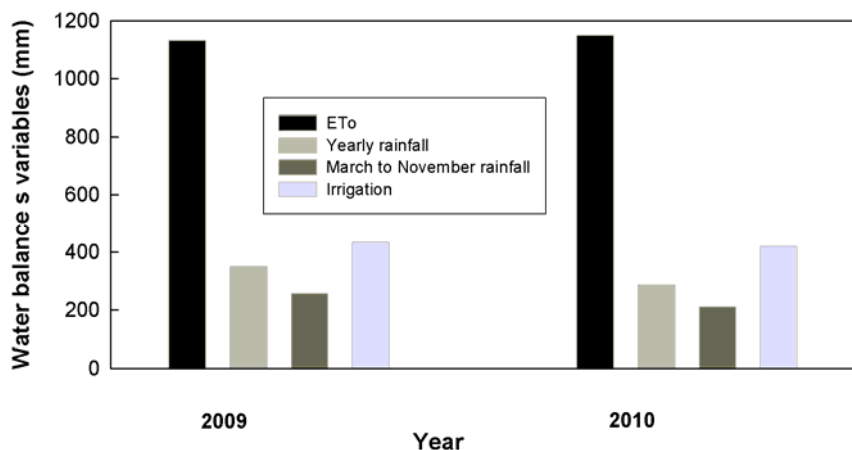
Fig. 1. Reference evapotranspiration (ETo), yearly rainfall and seasonal rainfall during the 2009 and 2010 growing seasons.

During 2010, irrigation applied was 420 mm, ETo 1149 mm, while rainfall was equal to 287 mm, of which 211 occurred during the March to November period (Fig. 1). Ψstem varied between –0.68 MPa registered at the beginning of the fall (week 39) and -1.68 MPa at the end of august, week 35 (Fig. 2).
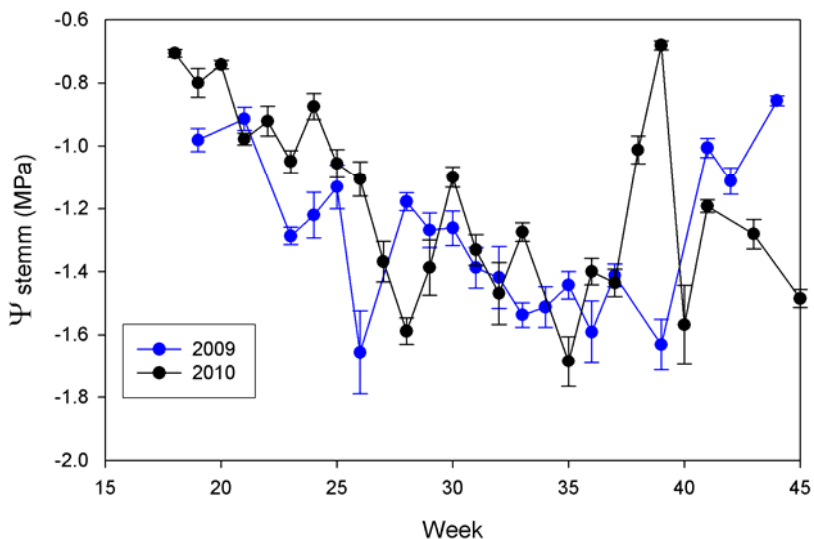
Fig. 2. Seasonal variation of midday stem water potential (Ψstem) during the 2009 and 2010 seasons.

In 2010, soil water content (SWC) was maintained during all the season between field capacity and the refill point without any drainage (Fig. 3). This suggest that the irrigation recommendations given to the grower can be considered as adequate.
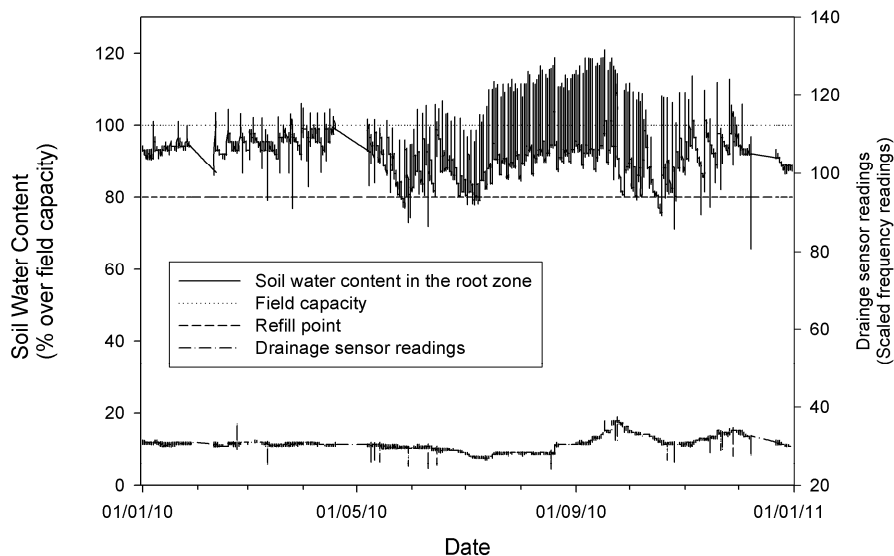
Fig. 3.   Seasonal trend of soil water content for the 2010 season. Values are the average of two capacitance probes.

In the 2011 irrigation campaign, using the updated experimental crop coefficient, at 25 august irrigation applications were of 392 mm. From March to August 2011 precipitation was 92 mm. Stem water potential readings varied between –0.57 MPa registered by midday April (week 17) to -1.33 MPa registered by the end of may (week 22, Fig.4). Soil water content, similarly to what occurred the previous season, was kept always within the established range and drainage rarely occurred (Fig. 4).
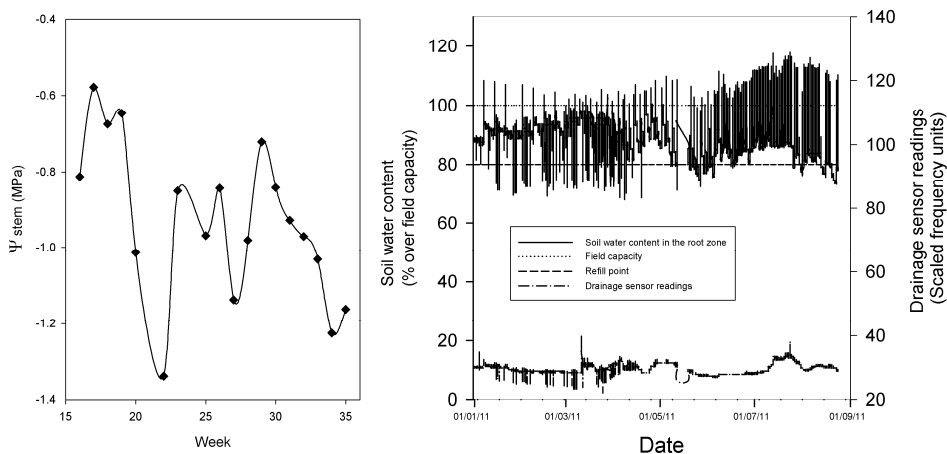
Fig. 4.   Seasonal variations during 2011 of midday stem water potential (Ψstem) and soil water content. For Ψstem and soil water content values shown are averages of 8 and two determinations.

Results obtained during the two experimental seasons have allowed to derive some tentative crop coefficient seasonal trends (Fig. 5). These crop coefficient patterns have been transferred to growers by means of an user friendly software that allows calculating the irrigation volumes to schedule in order to fulfil the estimated tree crop water needs.
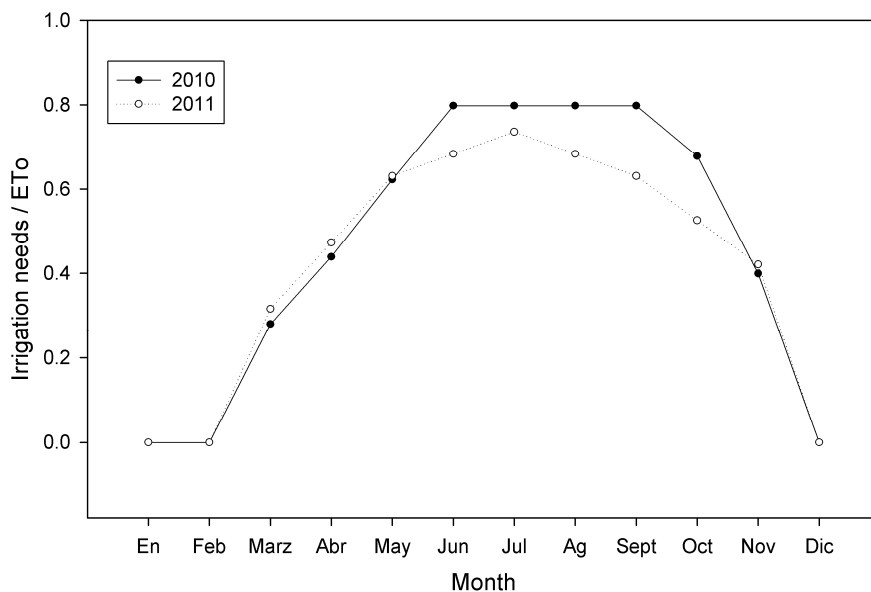
Fig. 5. Seasonal trends of the experimental crop coefficient used during the 2010 and 2011 seasons.

IV – Conclusions

By analysing together the information obtained from the reference evapotranspiration, plant water status and seasonal trends of soil water content, it was possible to derive an irrigation scheduling recommendations to carry out an efficient irrigation management for pomegranate trees. However, in order to finally apply the results obtained at a commercial scale, is important to transfer this information to the grower by means of simple procedures and tools.

Acknowledgements

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La culture du grenadier dans la région du Tadla (Maroc)

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Abstract. The main objective of this study is to contribute to a better knowledge of the pomegranate sector in the region of Tadla (Morocco), through a technico-economical analysis of the various stages of development and marketing of the fruits. The expected outputs of this diagnosis is a better knowledge of the cultivation methods, the comprehension of their reasoning and the identification of the problems limiting the development of the sector and the productivity of the orchards. This study will help in defining research topics that will be taken into consideration by the regional development agencies and nongovernmental organization in their plans of action.

Keywords. Pomegranate – *Punica granatum* L. – Tadla – Morocco.

I – Introduction

Etant donné le nombre limité des études qui ont été fait sur le grenadier et pour mieux orienter nos activités de recherche, un diagnostic portant sur les conditions de production et de commercialisation s'est avéré primordial. Ce diagnostic avait comme objectifs la connaissance des pratiques culturales, la compréhension de leur raisonnement et l'identification des problèmes limitant le développement du secteur et la productivité des vergers. Ceci dans le but de répondre aux attentes des producteurs et des différents partenaires régionaux (ORMVAT, ONGS, etc.) en matière de recherche pour une intensification raisonnée de la culture.

II – Matériels et méthode

Les informations concernant la culture du grenadier dans la plaine du Tadla, ont été collectées moyennant une enquête auprès de 30 arboriculteurs choisis aléatoirement dans la zone de Béni-Amir. Des observations et des entretiens informels sur le terrain avec des agents du Centre de Développement Agricole et quelques arboriculteurs ont permis de compléter les informations collectées.

III – Résultats et discussion

1. Installation des vergers

La multiplication du grenadier se fait par bouturage pour 86% des producteurs enquêtés ce qui permet d'obtenir très rapidement des sujets ayant tous les caractères du pied mère. Les boutures ligneuses (40 à 50 cm) sont prélevées par les producteurs entre novembre et décembre pour être plantées soit directement où après enracinement. Dans le premier cas, quatre boutures sont généralement enterrées par trou de plantation en laissant deux yeux au-dessus du sol. Dans le deuxième cas de vergers, la plantation à lieu entre février et mars.

Les densités de plantation pratiquées sont très variables. On rencontre généralement la densité de 100 pieds/ha dans les anciens vergers installés à l'époque du protectorat. Cependant, les

nouveaux vergers crées dans la région adoptent des hautes densités allant de 250 plants/ha à 1100 plants/ha.

2. Travail du sol

Comprend un sous-solage réalisé avant l'installation du verger. Des labours annuels sont réalisés généralement entre janvier et mars, par covercrope, à l'araire ou manuellement à la sape. Cette opération vise à éliminer les mauvaises herbes, d'aérer le sol, enfouir le fumier, confectionner les seguias et cuvettes d'irrigation. Dans les vergers contenant la luzerne et/ou céréales en intercalaire, la seule intervention réalisée est la confection des cuvettes au tour des arbres.

3. Irrigation

L'irrigation gravitaire couvre 98% de la superficie plantée par le grenadier tandis que l'irrigation localisée ne représente qu'environ 2%. Cependant, 42% de la superficie est irriguée par pompage soit directement d'Oum Erbia où à partir des puits, le reste (58%) étant lié au réseau hydro-agricole du périmètre irrigué.

La fréquence des apports varie d'un verger à l'autre, selon le stade phénologique de la plante et la source des eaux utilisées. Dans le cas des vergers irrigués par pompage, les apports d'eau sont apportés d'une façon régulière tous les 8 à 15 jours dès le mois de mars jusqu'à la récolte.

Cependant, pour les vergers desservis par le réseau hydroagricole, la fréquence d'apports dépend uniquement des programmes de lâchers d'eau. En général, les pratiques les plus courantes font états de 8 à 9 apports par campagne avec un intervalle de 20 jours en moyenne entre deux lâchers d'eau.

4. Taille

Dès les premières années, une taille de formation est pratiquée par une minorité des producteurs (12%) afin de constituer des charpentes fortes. En l'absence des éléments de base sur la conduite technique de la taille de fructification du grenadier, la taille pratiquée par 93% des producteurs enquêtés se limite à l'élimination des gourmands, du bois chevauchant et mort à l'intérieur de la frondaison. La période de taille se prolonge de décembre à janvier.

5. Fertilisation

Elle est pratiquée par des apports d'engrais chimique et/ou du fumier. Les apports des engrais de synthèse sont pratiqués par 66% des producteurs enquêtés sous forme du complexe 14-28-14. Ces apports sont effectués manuellement à partir de février en apportant entre 200 et 500 kg/ha. Les vergers équipés de système d'irrigation localisé utilisent plus d'engrais chimique et moins de fumier organique. Cependant, 84% des producteurs enquêtés procèdent à partir du mois de décembre à l'épandage des quantités comprises entre 20 et 30 tonnes de fumier par ha.

Le bilan des unités fertilisantes apportées par les producteurs de la région est donné par le Tableau 1.

6. Traitements phytosanitaires

La protection phytosanitaire est limitée pour 72% des arboriculteurs contre les pucerons à la floraison et la cératite vers la maturité. Le nombre de traitements effectués sur les grenaderaies questionnées peut atteindre 6 applications durant le cycle végétatif de la culture. Ainsi, à partir du mois d'avril, 2 traitements en moyenne sont appliqués contre les populations du puceron *Aphis punicae* en employant essentiellement deux matières actives (Endosulfan et

Pyrimicarbe). A l'approche de la maturité (juillet-septembre), de 2 à 5 traitements chimiques sont employés en vue de limiter les dégâts causés par la cératite. Le produit le plus utilisé est à base de Malathion+Diméthoate. Le volume de bouillie utilisé ne dépasse pas 170 l/ha pour 84% des producteurs enquêtés puisque le pulvérisateur à dos reste l'outil de traitement pour plus de 92% des vergers.

Le désherbage chimique est pratiqué par 12% des producteurs enquêtés et il est dirigé essentiellement contre la morelle jaune en employant des produits à base de glyphosate.

Tableau 1. Bilan des unités fertilisantes apportées dans le périmètre irrigué du Tadla

Type d'irrigation	Type de fumure	N (kg/Ha)	P_2O_5 (kg/ha)	K_2O (kg/ha)
	Fumure organique	182-219	73-109	219-328
Irrigation gravitaire	Fumure minérale	16	24	8
	Fumure organique	105-126	42-63	126-189
Irrigation localisée	Fumure minérale	48	75	27

7. Récolte, rendements et commercialisation

Les vergers de grenadiers arrivent à maturation généralement à la fin du mois de septembre et la récolte s'étale jusqu'au mois de décembre. Le rendement moyen du grenadier dans le périmètre n'a pas cessé de s'améliorer durant ces dernières années en passant de 9,29 t/ha (1991) à 24,80 t/ha (2005). Le rendement potentiel peut atteindre 50 t/ha dans le Tadla.

Le mode de vente dominant et celui sur pieds (85%) et les prix suivent la loi de l'offre et de la demande (1,80 à 3 dhs/kg). La production de la région s'écoule essentiellement sur le marché local, son unique utilisation est la consommation en frais et ce par manque de l'exportation ou des unités technologiques qui peuvent dans une certaine mesure valoriser la grenade.

IV – Conclusion

Le diagnostic portant sur la culture du granadier dans le Tadla, révèle les principales opportinités et contraintes inhérentes à cette filière. Ainsi, la zone d'étude offre toutes les conditions naturelles et édaphiques se traduisant par une productivité optimale de la culture.
La diversité du profil variétal en clones marocains peformants est un atout incontestable dans la mesure où il permet l'élargissement de la période productive qui s'étale de septembre à décembre d'une part. D'autre part, la zone est considérée comme une banque de gènes pour toutes les autres régions du Maroc.

Outre, les niveaux de productivité réalisés dans la zone d'étude (25,12 t/ha) qui dépassent de loin la moyenne nationale (9,4 t/ha), ce qui témoigne du degré du savoir faire des producteurs locaux et l'importance accordée à cette espèce. Néanmoins, malgré ces opportinutés, la filière du grenade est exposée à de nombreuses contraintes qui limitent la rentabilité des vergers et qui se présentent ainsi : (i) niveau de rendement réalisé faible comparé aux potentialités de la région ; (ii) faible maîtrise de la protection phytosanitaire ; (iii) dominance de l'irrigation gravitaire ; (iv) circuit de commercialisation non organisé ; (v) dominance de la vente sur pieds ; et enfin (vi), manque d'actions de valorisation de la production.

Remerciements

Contrôle intégré d'*Aphis punicae* Passerini en grenadier dans la région du Tadla (Maroc)

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Abstract. The work to identify pests associated with the pomegranate in Tadla generated a list of the main species encountered during the study period. Among these pests, the aphid *Aphis punicae* is the most important and destructive. This pest could cause heavy yield losses, reaching in some cases 24 tons/ha in the absence of chemical intervention. Plant protection systems on the basis of the threshold of intervention can lead to avoid a few systematic treatments applied by farmers in the region against these pests.

Keywords. Pomegranate – *Punica granatum* L. – Pest – *Aphis punicae* – Tadla – Morocco.

I – Introduction

Au Tadla, le grenadier est sujet annuellement à des attaques du puceron *A. punicae*. Cette espèce manifeste une période d'infestation en avril et pouvant s'étaler jusqu'à fin juin sur les jeunes pousses, les feuilles, les boutons floraux et même sur les jeunes fruits (Fakhour, 2006). En Tunisie, une seconde infestation s'observe en septembre, à l'approche de la maturité des fruits, sur les jeunes pousses et les feuilles. Elle contribue à l'affaiblissement de l'arbre, à l'altération de la qualité et la quantité de production (Ben Halima-Kamel et Ben Hamouda, 2005). En Israël, *A. punicae* est responsable d'une inhibition de la croissance observée sur jeunes plantations de grenadier (Blumenfeld *et al.*, 2000).

Ainsi, 72% des producteurs de grenadier dans le Tadla interviennent à partir du mois d'avril moyennant 2 traitements en moyenne par campagne contre les populations d'*A. punicae* en employant essentiellement deux matières actives (Endosulfan et Pyrimicarbe). Le volume de bouillie utilisé ne dépasse pas 170 l/ha pour 84% des producteurs enquêtés puisque le pulvérisateur à dos reste l'outil de traitements pour plus de 92% des vergers (Fakhour, 2006).

En Espagne, les traitements aphicides représentent 8,68% de l'ensemble des charges liées à la culture. Selon Juan *et al.*, (2000), deux traitements chimiques en moyenne sont effectués. Le premier traitement est effectué durant la deuxième quinzaine du mois d'avril (apparition des premiers boutons floraux) alors que le deuxième est appliqué la première quinzaine de mai (nouaison de la première vague de floraison).

II – Matériels et méthode

Dans le but d'améliorer les pratiques de la lutte contre *A. punicae*, 3 grenaderaies ont été choisies dans le Tadla (G1, G2 et G3). Le premier verger G1 ne bénéficie d'aucune application chimique est représente le témoin. La stratégie de lutte contre *A. punicae* adoptée dans le cas du G2 et celle prévue par le Projet des Normes Techniques pour la Production Intégrée des Grenades à Valence et qui consiste en l'application des traitements chimiques à l'aide de l'endosulfan ou de pyrimicarbe une fois le seuil d'intervention est dépassé (Toledo et Albujer, 2000) : (i) Avant l'apparition des boutons floraux : 20 à 40% de pousses occupées ; (ii) Après l'apparition des boutons floraux : 10 à 20% de boutons occupés par pousse. Par contre, dans le

verger G3, deux applications aphicides ont été appliqués systématiquement selon les pratiques courantes des producteurs en fin avril et fin mai successivement.

Afin d'établir une méthode d'échantillonnage, un pré-échantillonnage a été effectué dans le but d'évaluer l'état d'infestation du verger par *A. punicae*. Ainsi, l'effet du facteur strate et celui de l'orientation sur la distribution verticale et horizontale du ravageur au sein du plant de grenadier a été testé. L'analyse de la variance à deux critères de classification a révélé que :

(i) L'effet du facteur strate est significatif (p=0,0023). La strate haute paraît la plus contaminée par *A. punicae*.

(ii) L'effet du facteur orientation n'est pas significatif (p=0,4253).

Sur la base de ces résultats, nous nous sommes alors limités à prélever deux rameaux par plant à raison d'un rameau par strate sur un total de 10 arbres pour l'évaluation de l'infestation sur le grenadier. Lorsque le seuil est atteint, un traitement par le pyrimicarbe est appliqué avec un volume de bouillie de 600 litres/ha.

III – Résultats et discussion

D'après la Fig. 1, il ressort que l'évolution de l'infestation des pousses et des boutons floraux par *A. punicae* est caractérisée par des fluctuations importantes. Ces fluctuations sont dues principalement au traitement aphicide appliqué, à l'action des hyménoptères parasitoïdes et aux conditions climatiques représentées essentiellement par la température.

Avant l'apparition des boutons floraux (1ère quinzaine d'avril), le taux moyen des pousses infestées a atteint 14,3% dans les deux vergers. Par la suite, les populations de puceron ont continué à croître d'une façon très rapide amenant le taux d'occupation des boutons par pousse au environ de 26% et 35% 30,2% vers la fin du mois d'avril pour les trois vergers G1, G2 et G3 respectivement. Dans le verger G2, le taux a été ramené à 2,8% au début mai suite à l'application d'un aphicide le 27 avril avec le pyrimicarbe. Par la suite, les populations d'*A. punicae* ont pu se reconstituer ce qui a porté le taux d'infestation à 12% le 22 mai. Parallèlement et à partir de la fin du moi d'avril, le taux d'infestation enregistré dans le verger non traité G1 croît régulièrement pour atteindre 43% à la mi-mai. A partir de la troisième décade de mai, le taux d'infestation décroît rapidement dans les deux vergers (G1 et G2) d'étude suite à une augmentation considérable de la température et à l'action des parasitoides autochtones. Dans le verger G3, un deuxième traitement aphicide est appliqué le 23 mai.
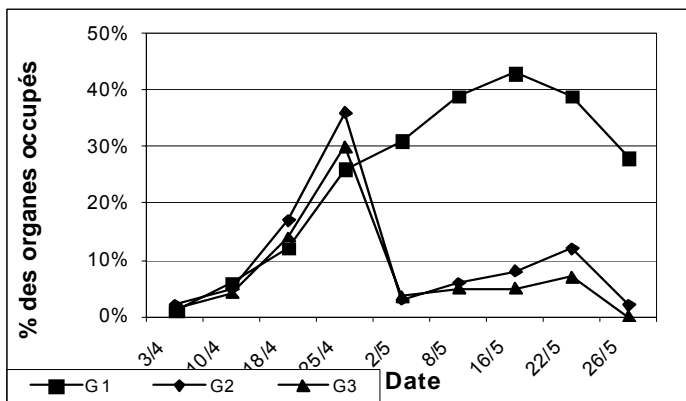
Fig. 1. Evolution de l'infestation du grenadier par *A. punicae*.

L'analyse des rendements obtenus dans les trois grenaderaies d'études, montre une différence significative entre les vergers traités et celui témoin. Ainsi et en absence d'intervention chimique contre les pucerons, les pertes de rendement avoisine les 24 tonnes/ha. Le rendement obtenu par le producteur dépasse celui du verger pilote d'environ 2 tonnes mais ne diffèrent pas significativement (Fig. 2).
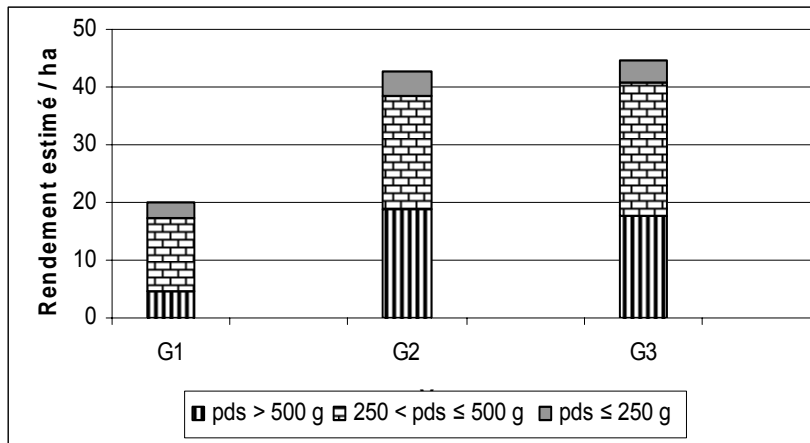
Fig. 2. Rendement estimé dans les trois vergers étudiés.

La comparaison des calibres obtenus montre une différence significative du nombre de fruits à moyen calibre (entre 250 et 500 g) entre le G2 et G3, soit une moyenne de 105 contre 125 fruit/arbre respectivement. Ce résultat peut être expliqué par l'effet bénéfique du 2ème traitement appliqué par le producteur au début de la 2ème vague de floraison.

IV –Conclusion

Les essais conduits ont montrés que l'application du seuil d'intervention adopté en Espagne a permis une protection correcte des deux premières vagues de floraison du grenadier contre les pucerons. L'utilisation des systèmes d'avertissements phytosanitaires basés sur le seuil d'intervention peut conduire à supprimer quelques traitements systématiques appliqués par les producteurs de la région contre les pucerons.

Remerciement

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Effets de quelques pulvérisations foliaires sur l'éclatement des grenades sous les conditions du Tadla (Maroc)

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Abstract. Pomegranate cv Sefri occupies more than 70% of the total acreage dedicated to this fruit specie in Tadla plain (Morocco). However, fruit splitting does not allow a suitable economical valorization of the pomegranate production in this area. This work aimed at studying the effect of three foliar products namely Fertibore, Ferticalcium and Cutisan on yield parameters and fruit cracking of Sefri pomegranates. Four treatments, namely control (untreated trees), Fertibore 15% (200 cm^3/100 l), Ferticalcium 11.5% (200 cm^3/100 l) and Cutisan (2 kg/ha) were compared according to a completely randomized block design. Highest fruit weight was recorded under Ferticalcium treatment while fruit bark weight and thickness was improved by the three products. Total aril mass and arils width were improved by the three products. Fruit juice volume was improved by Cutisan and Fertibore while total soluble solid was not affected. The three products reduced pomegranates splitting.

Keywords. Pomegranate – Tadla – Calcium – Boron – Kaolin – Production – Splitting.

I – Introduction

La superficie dédiée au grenadier dans le Tadla (Maroc) est estimée à plus de 1400 ha. Elle représente près de 25% de la superficie consacrée à cette culture au niveau national et assure près de 45% de la production marocaine en grenades. Plus de 70% des plantations de grenadier de cette région sont constituées de la variété Sefri. Cette variété est sensible à l'éclatement du moins dans les conditions de culture de cette région. Lequel phénomène se trouve aggravé par plusieurs facteurs notamment la non maîtrise de l'irrigation, la pratique de cultures intercalaires à forte demande en eau d'irrigation et fixatrices de l'azote atmosphérique telle que la luzerne, les fortes chaleurs estivales et/ou automnales et la teneur en sels relativement élevée dans l'eau d'irrigation. Plusieurs procédures ont été testées par différentes équipes de chercheurs en vue de réduire l'incidence de ce phénomène (El-Masry, 1995 ; Singh et al., 1990). L'objectif de ce travail est de contribuer à la recherche de solutions susceptibles d'améliorer la qualité des grenades produites dans la région tout en réduisant l'incidence de l'éclatement des fruits à travers l'application de quelques pulvérisations foliaires.

II – Matériel et méthodes

L'essai est réalisé sur des arbres de grenadier adultes de la variété Sefri localisés dans un verger de la plaine du Tadla (Maroc) (Latitude 32°29,643' N, Longitude 6°25,506' et Altitude 485 m). Les grenadiers sont conduits en vase libre selon une densité de plantation de 4 m sur 4 m et des techniques de conduite conventionnelles. Le système d'irrigation utilisé est le goutte à goutte avec deux rampes par ligne de plantation et 10 goutteurs par grenadier. Le protocole expérimental est choisi de façon à comparer l'effet de quatre traitements notamment le témoin T0, Tk, Tca et Tb. Les arbres du témoin (T0) sont pulvérisés à l'eau douce. Ceux du Tk ont été

traités au Cutisan selon la dose de 2 kg/ha. Tca consiste à pulvériser les grenadiers au Ferticalcium 11,5% à la dose de 200 cm^3/100 l alors que Tb a porté sur l'application du Fertibore 15% selon une dose de 200 cm^3/100 l. Ces applications ont été apportées le 14 juillet 2009 et répétées une deuxième fois le 24 juillet 2009. Les mesures ont porté sur les paramètres de production notamment le nombre de fruit par arbre, le poids et le diamètre du fruit, le nombre de carpelles par fruit, le poids et l'épaisseur de l'écorce, le poids total des arilles dans le fruit, le poids, la largeur et la longueur de l'arille, le poids la largeur et la longueur du bois de l'arille, le pourcentage en chair, la teneur en jus, la teneur en sucres totaux et l'acidité totale du jus et l'incidence de l'éclatement des grenades.

III – Résultats et discussions

1. Effet des pulvérisations foliaires sur les paramètres quantitatifs et qualitatifs de la grenade

Les données relatives à l'effet des pulvérisations foliaires sur les paramètres quantitatifs et qualitatifs de la grenade sont récapitulées dans le Tableau 1.

Tableau 1. Effet des pulvérisations foliaires sur les paramètres quantitatifs et qualitatifs de la grenade

Paramètre	T0	Tk	Tca	Tb
Nombre de fruits par arbre	67,75a	60,00a	46,97b	49,69b
Poids du fruit (g/fruit)	352,71c	436,04b	597,09a	450,53b
Diamètre du fruit à la maturité (cm)	76,72b	96,48a	100,87a	94,05a
Nombre de carpelles par fruit	6,08a	6,00a	6,00a	6,05a
Poids de l'écorce (g/fruit)	132,79b	177,70a	186,41a	171,86a
Epaisseur de l'écorce (mm)	2,74c	3,48a	3,95a	3,31b
Poids des arilles (g/fruit)	255,36c	262,26c	353,79a	314,01b
Incidence de l'éclatement (%)	8,58a	5,58b	5,50b	5,86b

Dans une même ligne, les chiffres suivis d'une même lettre ne sont pas significativement différents (Newman et Keuls, 5%).

A part le nombre de carpelles par fruit qui n'a pas été affecté par le produit, tous les autres paramètres quantitatifs et qualitatifs étudiés semblent varier d'une façon significative avec le type de pulvérisation apporté. En effet, l'application du Ferticalcium ou Fertibore ont réduit le nombre de fruits par arbre. Cela semble plutôt dû aux dates d'application qui étaient relativement tardives. Cependant, le poids et le diamètre du fruit ont été améliorés par l'application de l'une des trois spécialités surtout par le Ferticalcium. Ces applications ont probablement amélioré la division cellulaire au niveau de l'écorce car le poids et l'épaisseur de cette dernière ont été relativement élevés lorsque les grenadiers ont été pulvérisés par l'une des trois produits testés. Quant à l'éclatement des grenades, il paraît clair que les trois types de produits testés permettent de réduire sensiblement l'incidence de ce phénomène. Ferticalcium a permis une réduction de l'éclatement d'environ 36% par rapport au témoin.

2. Effet des pulvérisations foliaires sur les paramètres quantitatifs et qualitatifs de l'arille

Le Tableau 2 résume les résultats de l'effet des pulvérisations foliaires sur les paramètres quantitatifs et qualitatifs de l'arille.

Tableau 2. Effet des pulvérisations foliaires sur les paramètres quantitatifs et qualitatifs de l'arille

Paramètre	T0	Tk	Tca	Tb
Poids de l'arille (g)	0,49b	0,49b	0,63a	0,50b
Largeur de l'arille (mm)	6,64c	7,24b	8,19a	7,26b
Longueur de l'arille (mm)	12,05a	12,08a	12,41a	12,08a
Poids du bois de l'arille (g)	0,053b	0,061a	0,065a	0,060ab
Largeur du bois de l'arille (mm)	2,52b	2,58b	3,55a	2,75b
Longueur du bois de l'arille (mm)	7,94a	7,88a	7,63a	7,69a
Importance de la chair (%)	89a	87a	89a	87a

Dans une même ligne, les chiffres suivis d'une même lettre ou même groupe de lettres ne sont pas significativement différents (Newman et Keuls, 5%).

La largeur et la longueur du bois de l'arille ainsi que l'importance de la chair dans l'arille n'ont pas été affectées par le type de pulvérisation alors que le poids et la largeur tant de l'arille que de son bois ont varié avec la spécialité appliquée. En effet, Tca a induit les arilles les plus lourds et larges. Cette supériorité de Tca se retrouve au niveau du poids et de la largeur du bois de l'arille. Ainsi, Tca semble engendrer des fruits à la fois plus gros et plus riches en bois.

3. Effet des pulvérisations foliaires sur les paramètres qualitatifs du jus

Le Tableau 3 résume les résultats de l'effet des pulvérisations foliaires sur la teneur en jus et le °Brix et l'acidité totale du jus.

Tableau 3. Effet des pulvérisations foliaires sur la teneur en jus (ml/100g arille) et la teneur en sucres totaux (°Brix) et l'acidité totale (g/l) du jus

Paramètre	T0	Tk	Tca	Tb
Teneur en jus (%)	62,99b	65,75a	63,00b	65,22a
Teneur en sucres (°Brix)	13,23a	16,33a	14,06a	14,30a
Acidité totale (g/l)	1,14a	1,08b	1,17a	1,06b

Dans une même ligne, les chiffres suivis d'une même lettre ne sont pas significativement différents (Newman et Keuls, 5%).

Le Cutisan et le Fertibore ont engendré des améliorations respectives de la teneur des grenades en jus d'environ 4,6 et et 4,4% par rapport aux arbres témoins. Cette amélioration de la teneur en jus a été accompagnée par une réduction concomitante de l'acidité totale du jus.

IV – Conclusion

L'effet des pulvérisations des trois spécialités testées semble dépendre du paramètre de production étudié. Le Ferticalcium améliore le poids et le diamètre du fruit et le poids des arilles mais enrichit les arilles en bois tandis que le Cutisan améliore l'accrochage des fruits et la teneur en jus des arilles. Quant au Fertibore, il permet une amélioration nette du diamètre du fruit et le poids de son écorce et de la teneur en jus des arilles. L'utilisation de ces produits pour réduire l'incidence de l'éclatement des grenades semble prometteuse. Cependant, ces pulvérisations pourraient être plus avantageuses si elles sont appliquées à des stades de croissance de la grenade beaucoup plus appropriés.

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Besoins en eau du grenadier cultivé sous les conditions de la plaine du Tadla (Maroc)

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Abstract. Acreage dedicated to pomegranates in Tadla plain (Morocco) is estimated in more than 1400 ha. Pomegranate orchards are mainly irrigated by submersion. Therefore, water consumption is very high. This study aimed at estimating water requirements under the conditions of this area and evaluating the effects of different drip irrigation strategies on tree developpement and yield parameters. Five drip irrigation strategies were compared namely 60% ETc, 80% ETc, 100% ETc, 120% ETc and the own farm drip irrigation strategy. Daily mean ETc was calculated by using the Penman-Monteith formula for a period of 11 years. Results suggested that drip irrigation based on 120% ETc conditions constitutes the optimal drip irrigation strategy when vegetative growth, fruit yield parameters and water consumption are considered.

Keywords. Pomegranate – Tadla – Water requirements – Production – Fruit quality parameters.

I – Introduction

La culture du grenadier dans le Tadla porte sur plus de 1400 ha. Elle représente près de 25% de la superficie consacrée à cette culture au niveau national et assure près de 45% de la production marocaine en grenades. La méthode d'irrigation la plus adoptée dans cette région du Maroc est le gravitaire ; ce qui engendre une consommation importante en eau d'irrigation au moment où cette ressource naturelle devient de plus en plus rare et par conséquent chère. En effet, Diallo (2007) a démontré que le coût d'irrigation constitue 41% des charges totales consenties par les producteurs de cette région pour entretenir un hectare de grenadier. En plus, Segmani (2008) a mentionné que les irrigations copieuses ainsi que les à-coups d'irrigation renforcent l'éclatement des grenades. L'objectif de ce travail est de chiffrer la consommation en eau dans différentes stratégies d'irrigation du grenadier et d'évaluer l'impact de ces stratégies sur les composantes du rendement en grenades.

II – Matériel et méthodes

L'essai a eu lieu dans un verger de grenadier situé dans la plaine du Tadla (Latitude : 32°29.643' N, Longitude 6°25.506' et Altitude 485 m) et sur des grenadiers adultes de la variété Sefri conduits en vase libre selon une densité de plantation de 4 m sur 4 m et recevant des techniques de conduite conventionnelles. Le système d'irrigation utilisé est le goutte à goutte avec deux rampes par ligne de plantation et 10 goutteurs par grenadier. Le protocole expérimental vise la comparaison de cinq stratégies d'irrigation notamment T1, T2, T3 et T4 désignant respectivement 60%, 80%, 100% et 120% de l'évaporation de la culture (ETc) et T5 assimilée la stratégie telle que adoptée par le producteur. La stratégie T5 consiste en des apports copieux en eau d'irrigation généralement supérieurs à ceux apportés selon T1, T2, T3 ou T4. ETc est calculée en se basant sur l'évaporation de référence (ET_0) moyenne journalière d'une période de 11 ans (1997-2007) moyennant la méthode de Penman-Monteith (FAO) corrigée par le coefficient cultural du grenadier (Kc). La dose brute d'irrigation est calculée en

tenant compte des pluies effectives (Peff) reçues au cours de la période de l'essai. La dose nette d'irrigation est par la suite calculée en tenant compte du débit moyen du goutteur (Dmoy) et de l'efficience du système d'irrigation (Eff). Une même irrigation (irrigation de démarrage) a été appliquée entre le 15 avril et le 13 mai 2008 en apportant une dose journalière de 6,25 mm/j alors que les irrigations ont été suspendues entre le 15 et le 25 mai 2008 pour des raisons de sensibilité de la floraison aux fortes irrigations. Les différentes stratégies d'irrigation testées dans ce travail ont été appliquées entre le 26 mai et le 20 septembre 2008. Les mesures ont porté sur la quantification des quantités globales d'eau d'irrigation apportées à travers chacune des cinq stratégies et l'effet de ces dernières sur le développement végétatif et les paramètres quantitatifs et qualitatifs de production.

III – Résultats et discussions

1. Besoins nets en eau d'irrigation

Les quantités d'eau apportée entre le 26 mai et le 20 septembre 2008 à travers les cinq stratégies étudiées sont résumées dans le Tableau 1.

Tableau 1. Doses nettes d'irrigations (m^3/ha) apportées au cours de la période de l'essai

Décade	Stratégie d'irrigation (m^3/ha)				
	T1	T2	T3	T4	T5
Mai-03	234	313	391	469	625
Juin-01	248	331	414	497	750
Juin-02	245	327	408	490	750
Juin-03	245	327	408	490	750
Juillet-01	250	334	417	501	750
Juillet-02	262	350	437	524	750
Juillet-03	261	348	435	522	750
Aout-01	267	355	444	533	625
Aout-02	261	348	435	521	625
Aout-03	253	337	421	506	625
Sept-01	248	330	413	496	625
Sept-02	220	294	367	440	625
Total	2994	3992	4990	5989	8250

La quantité globale d'eau d'irrigation apportée par le producteur (T5) est de loin beaucoup plus élevée par rapport à celles appliquées selon les quatre autres stratégies (T1, T2, T3 ou T4). En outre, cette supériorité est valable pour toutes les décades des mois pendant lesquels l'étude est réalisée. En effet, la consommation en eau selon la stratégie T5 est supérieure de respectivement 65 et 38% par rapport à la stratégie T3 et T4. La stratégie T5 engendrerait par conséquent des dépenses supplémentaires probablement non justifiés sur le plan aussi bien économique que qualitatif.

2. Effet de la stratégie d'irrigation sur l'accroissement de la végétation

Les données relatives à l'impact des stratégies d'irrigation sur le développement végétatif de l'arbre sont données dans le Tableau 2.

Les stratégies T4 et T5 ont induit des développements végétatifs similaires mais plus importants que celui engendré par T3. Quant aux traitements T2 et T1, ils semblent gêner la végétation et par conséquent compromettre le développement de nouveaux supports de production pour l'année suivante. Ainsi, il ne serait pas conseillé de réduire les apports de plus de 20% par rapports aux besoins de la culture.

Tableau 2. Effet de la stratégie d'irrigation sur l'accroissement de la végétation (cm)

T1	T2	T3	T4	T5
3,50c	3,64c	6,14b	8,89a	9,49a

Les chiffres suivis d'une même lettre ne sont pas significativement différents (Newman et Keuls, 5%).

3. Effet de la stratégie d'irrigation sur les paramètres quantitatifs de production

Les résultats relatifs à l'effet de la stratégie d'irrigation sur les principaux paramètres quantitatifs de production sont résumés dans le Tableau 3.

Tableau 3. Effet de la stratégie d'irrigation sur les paramètres quantitatifs de production

Paramètre	T1	T2	T3	T4	T5
Nombre de fruits par arbre	246a	214a	208a	244	271a
Poids du fruit (g/fruit)	205,94b	249,21b	259,00b	323,81a	329,68a
Diamètre du fruit à la maturité (cm)	7,68b	8,06b	8,14b	8,15b	8,98a
Poids de l'écorce (g/fruit)	62,29bc	73,02b	48,55c	68,68b	85,48a
Epaisseur de l'écorce (mm)	2,45bc	2,78b	2,15c	3,36a	3,43a
Poids des arilles (g/fruit)	124,33d	155,33c	183,36b	219,27a	198,77ab

Dans une même ligne, les chiffres suivis d'une même lettre ou même groupe de lettres ne sont pas significativement différents (Newman et Keuls, 5%).

A part le nombre de fruits par arbre qui n'a pas été affecté par la stratégie d'irrigation, tous les autres paramètres quantitatifs de production étudiés semblent varier d'une façon significative avec le régime hydrique. En effet, le poids du fruit a été amélioré de 57% en passant de T1 à T4 alors qu'aucune amélioration significative de ce paramètre n'a été enregistrée en passant de T4 à T5. Quant au de diamètre du fruit, les chiffres les plus intéressants sont enregistrés sous le régime d'irrigation pratiquée par le producteur. Cependant, cette amélioration du poids et diamètre du fruit est accompagnée par une amélioration concomitante du poids et de l'épaisseur de l'écorce du fruit. La stratégie T4 s'est révélée comme étant la plus adéquate sur le plan poids des arilles.

4. Effet de la stratégie d'irrigation sur les paramètres qualitatifs de production

Le Tableau 4 résume les résultats de l'effet de la stratégie d'irrigation sur les paramètres qualitatifs de production.

Parmi les caractéristiques qualitatives de la grenade étudiées, seuls le poids de l'arille, la teneur en jus et l'acidité totale du jus ont montré des tendances claires avec l'augmentation des apports d'eau d'irrigation. En effet, le poids de l'arille et la teneur en jus ont augmenté alors que l'acidité a diminué significativement avec l'augmentation de la dose d'irrigation. Le poids de

l'arille le plus important est enregistré sous le régime T5 alors que la teneur en jus ne s'est pas améliorée significativement lorsqu'on passe de T2 à T5. Quant à l'acidité totale du jus, elle n'a pas diminué davantage quant on passe à une stratégie d'irrigation plus confortable que celle envisagée dans T2. En revanche, la teneur en sucres n'a pas varié avec le régime d'irrigation.

Tableau 4. Effet de la stratégie d'irrigation sur les paramètres qualitatifs de production

Paramètre	T1	T2	T3	T4	T5
Poids de l'arille (g)	0,33b	0,35b	0,32c	0,34b	0,38a
Longueur de l'arille (cm)	1,41a	1,12b	1,09b	1,21ab	1,21ab
Diamètre de l'arille (cm)	0,30a	0,30a	0,27b	0,30a	0,30a
Poids du bois de l'arille (g)	0,027ab	0,029a	0,023c	0,022c	0,027ab
Longueur du bois de l'arille (cm)	0,72a	0,72a	0,67b	0,71a	0,73a
Largeur du bois de l'arille (cm)	0,30a	0,30a	0,27b	0,30a	0,30a
Teneur en jus (%)	78,43b	80,37a	81,58a	81,72a	82,63a
Teneur en sucres (°Brix)	14,90a	14,94a	14,53a	13,82a	14,42a
Acidité totale (g/l)	2,78a	2,52ab	2,33b	2,27b	2,20b

Dans une même ligne, les chiffres suivis d'une même lettre ou même groupe de lettres ne sont pas significativement différents (Newman et Keuls, 5%).

IV – Conclusion

Des irrigations copieuses au-delà de 120% ETc ou telles que pratiquées par le producteur propriétaire de ce verger objet de l'étude ne sont pas justifiées si l'on tient compte des résultats de ce travail. En effet, l'absence d'une amélioration additionnelle du poids du fruit lorsqu'on dépasse 120% ETc et une indifférence du nombre de fruits par arbre à au régime d'irrigation laissent présager que des rendements plus importants que ceux obtenus sous les conditions de T4 ne pourraient pas être enregistrés. En plus, la plupart des paramètres tant de végétation que de production ne sont pas améliorés lorsqu'on adopte des stratégies d'irrigation beaucoup plus confortables que celle de 120% ETc.

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Pomegranate fruit ripening: nutritional and bioactive compounds

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Abstract. Pomegranate fruit undergoes several biochemical changes during growth and ripening on tree leading to the fruit to reach an optimal quality at full ripening stage. These changes are increase in sugar concentration (glucose and fructose), diminution in total acidity (citric and malic acids) and increase in anthocyanin concentration. In addition an increase occurs in antioxidant activity which reaches values higher than those found in other fruits of the Mediterranean diet. However, important differences are found among cultivars in these parameters responsible for organoleptic, nutritive and functional properties of pomegranate fruit and thus, cultivar is an important factor determining fruit quality. In addition, pomegranate is a perishable fruit having limited storage possibilities, due to the occurrence of several alterations due to mechanical damage during harvesting and packaging or during storage, such as dehydration, over-ripening (leading to flavor alterations and decreases of antioxidant properties), decay incidence and chilling injury (CI). CI damage appears when fruits are transferred to 20°C after storage at temperatures lower than 5°C and are manifested as pitting, with purple color, desiccation and skin browning, which can reach the carpelar membranes and arils. Then, proper harvesting, handling and storage conditions should be chosen to preserve pomegranate fruit quality attributes from tree to table.

Keywords. Fruit rippening – Quality – Sugars – Organic acids – Phenols – Antioxidant – Harvest time.

I – Introduction

Pomegranate fruit (*Punica granatum* L.) is one of the oldest of edible fruit, originated in the north of Turkish and cultivated extensively in Mediterranean countries including Spain (Ward, 2003). The fruit is originated from an infer ovary and contains the arils or seeds, which are the edible part, contributing to 55-60 % of the whole fruit, while the skin supposes the 40-45%. Skin color ranges from light yellow to deep red as well as the color of the arils, depending on the cultivar, although, in general, no correlation exists between skin and arils color. Chemical composition of pomegranate arils changes as fruit ripens on tree, so it is important to know the most appropriated harvest date to have fully ripe fruits with high quality attributes, which last from 4.5 to 6 months after full bloom, depending on cultivar and agronomic and environmental conditions (Kader, 2006).

Quality is a subjective term that includes the fruit characteristics most appreciated by consumers, which can be divided into three blocks: organoleptic, nutritional and functional. Sensory or organoleptic quality is appreciated by the senses and includes color, flavor, aroma, firmness and appearance (absence of defects or damage and uniform size and color). The nutritional quality is determined by fruit components that serve as nutrients such as carbohydrates, lipids, proteins, organic acids and minerals. Finally, the functional quality is due to the contribution of bioactive compounds with beneficial health effects, such as phenols, carotenoids and vitamins (Gil *et al.*, 2000; Mertens-Talcott *et al.*, 2006, Lansky and Newman, 2007; Aviram *et al.*, 2008).

II – Quality changes during fruit ripening on tree

During ripening of pomegranate an accumulation of sugars and a decrease in total acidity occurs in arils (Kulkarni and Aradhya, 2005). The major sugars are fructose and glucose, with concentrations at harvest between 3 and 8%, depending on cultivar the range with concentrations of soluble solids of 10 to 18% (Melgarejo *et al.*, 2000; Poyrazoğlu *et al.*, 2002; Fadavi *et al.*, 2005; Ozgen *et al.*, 2008; Mirdehghan *et al.*, 2006). The organic acid composition is different depending on the type of grenade. Thus, in the acidic group varieties, which have a total acid value of 2-2.5%, the citric acid is the majority, while sweet varieties, with an acidity of 0.2 to 0, 4%, have similar amounts of citric acid and malic acid or a higher concentration of the latter (Hernández *et al.*, 1999; Melgarejo et., 2000; Poyrazoğlu *et al.*, 2002; Mirdehghan *et al.*, 2006; Ozgen *et al.*, 2008). Special attention should be paid to ascorbic acid, for his role as vitamin C and its antioxidant properties, whose concentration decreases during the early stages of fruit development and remains more or less stable in the final stages of maturation, with values between 10 and 36 mg/100 g, depending on the variety (Aradhya and Kulkarni, 2005; Sayyari *et al.*, 2010).

The colour of the arils increases during ripening due to the accumulation of anthocyanins, the pigments responsible for pink-red colour. The major anthocyanin in acid varieties is cyanidin 3,5-diglucoside, followed by cyanidin 3-glucoside and delphinidin 3,5-diglucoside, whereas in sweet varieties the major is cyanidin 3-glucoside and delphinidin 3-glucoside and pelargonidin 3-glucoside are found in minor concentration (Miguel *et al.*, 2004, Kulkarni and Aradhya, 2005; D'Aqquino *et al.*, 2010). However, the concentration of anthocyanins in the mature fruit depends on cultivar, with values between 10 and 220 mg/100 g, leading to arils having from light pink to dark red colour (Mirdehghan *et al.*, 2006; Ozgen *et al.*, 2008; Sayyari *et al.*, 2010, 2011).

During fruit development the concentration of total phenols decreases sharply in the early stages, this decrease being slow at the end of development, reaching concentrations in the ripe fruit also different depending on cultivar, ranging from 90 to 210 mg/100 g (Kulkarni and Aradhya, 2005; Mirdehghan *et al.*, 2006; Ozgen *et al.*, 2008; Sayyari *et al.*, 2011). The main phenolic compouns in pomegranate arils are the phenolic acids gallic, chlorogenic, caffeic, ferulic and *o*-and *p*-coumaric acids, as well as catechin and quercetin (Poyrazoğlu et la., 2002). In addition, it is interesting to note that in the skin the phenol content is much higher than in the arils, for what pomegranate skin could be an important source of antioxidants (Li *et al.*, 2006).

Antioxidant activity also decreases during the early stages of fruit development, but then increases again, reaching the highest levels in the state of commercial maturity (Kulkarni and Aradhya, 2005). In a study performed with different varieties of pomegranate from the Germplasm Bank of Orihuela Polytechnic School, it has been found that pomegranate juice has high antioxidant activity, higher than other fruits typical of the Mediterranean diet. Figure 1 shows the values corresponding to the antioxidant activity of hydrophilic compounds (H-TAA) in these varieties, which were significantly different among cultivars (Fig. 1). The antioxidant activity in the liposoluble fraction (L-TAA) was much lower than the H-TAA (data not shown), indicating that the antioxidant capacity of these fruits is mainly due to hydrophilic compounds, such as phenolic compounds, anthocyanins and ascorbic acid. In fact, a high correlation was found between H-TAA and total phenolic concentration, although other studies have shown that ascorbic acid is a water-soluble compound that also contributes significantly to the antioxidant capacity of this fruit (Mirdehghan *et al.*, 2006, 2007). By other hand, L-TAA was correlated with the content on carotenoids, which are the main lipo-soluble compounds with antioxidant potential in this fruit.

Given the high organoleptic, nutritive and functional properties of pomegranate fruits, it can be concluded that pomegranate has a high potential for commercialization and new markets. However, it should be harvested at maturity stage, since if harvesting is performed too soon fruits have low quality attributes because they have not developed their color, aroma and flavor. On the contrary, if harvesting is done too late, high quality fruits could be achieve, although they

deteriorate faster. Then, fruits should be harvested at full ripe stage, with high quality attributes and stored in appropriate conditions and after specific treatments to avoid the appearance of decay, chilling injury and other disorders that depreciate its quality, as will be commented by other researchers in this Symposium.
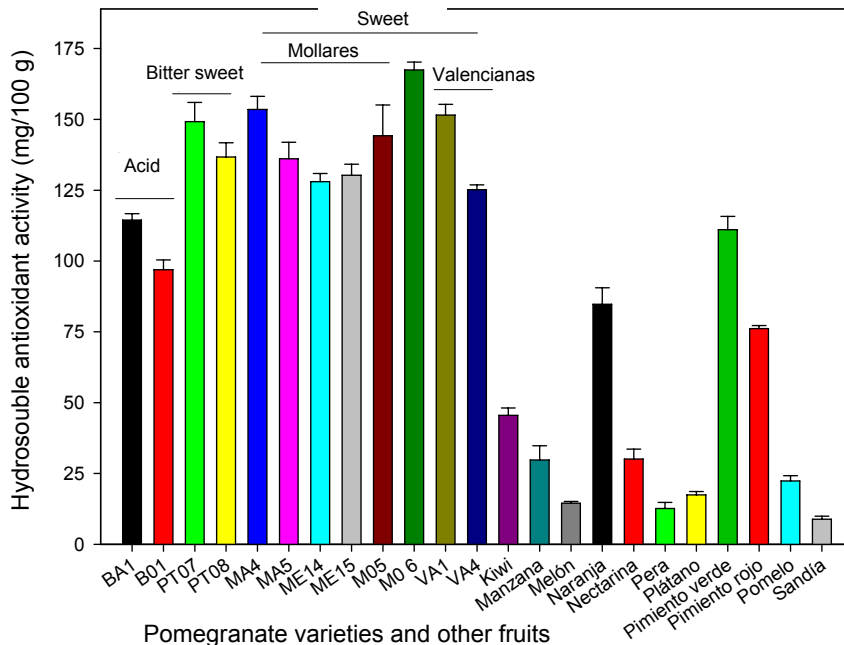
Fig. 1. Hydrophilic antioxidant activity (AAT-H) in different varieties of pomegranates and other fruits of the Mediterranean diet. Data are the mean ± SE of determinations made in five fruits.

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Advances in postharvest and refrigeration techniques in whole and minimally processed pomegranate

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Abstract. The varietal selection of pomegranates will improve its sales and, growing in fertile soils, will accumulate more anthocyanins than in poor and saline ones. Pomegranate fruit is quite sensitive to mechanical damages and chilling injuries (CI). To store pomegranates up to 3 months, 5ºC and 90-95% RH are recommend because these conditions will limit fungal development, weight loss and CI. Controlled or modified atmospheres, alone or in combination with UV-C light, improve its overall quality. Several curing, intermittent warming and sustainable agro-chemicals (salicylic, acetyl salicylic or oxalic acids, methyl jasmonate or methyl salicylate), before storage at 2°C of 'Mollar de Elche' *cv*, reduce CI and nutrient losses. The minimal processing of the arils generates high added value. In this work the different steps of minimal processing as well as some viable innovative techniques like the use of 'clean room technology' are referred. Several sustainable disinfection alternatives to chlorine (UV-C, O_3, or peroxyacetic acid), and particular conditions of packaging and distribution to optimize the arils shelf life are recommended. About all these aspects regarding Spanish *cvs*, relevant contributions are reviewed.

Keywords. *Punica granatum* – Selection – Overall quality – Damages – Fungal attacks – Controlled atmosphere – Mild warming – Agrochemicals – Processing – Sustainable disinfection – Packaging.

I – Interest of chilling storage of pomegranate

Although the excellent sensory and nutritional attributes of the Spanish pomegranates, some factors still limit their production, consumption and exportation. The most relevant are the seasonality and concentration of its production, the heterogeneous quality of the fruits into the market (consumers are confused and reduce repeat purchase) and the difficulty of peeling and extracting the eatable part (arils). Some advances for overcoming challenges in its cultivation and postharvest steps are afterward discussed, reviewing some significant Spanish contributions.

For optimizing the overall quality as well as for extending their marketing period, shippers must put into practice the adequate techniques. In particular the *cvs.* must be suitable, the optimum ripening stage at harvest should be defined and good agricultural, production, handling and minimal processing practices have to be implemented. In addition, a chilled storage under high relative humidity (RH) combined with thermal and gaseous coadjutants, and proper packaging, shipping and commercial distribution should be applied (Artés, 1992, 1993 and 2007; Artés and Tomás-Barberán, 2001; Artés *et al.*, 1996, 1998acde, 1999, 2000; López-Rubira *et al.*, 2007).

The usual heterogeneity of pomegranate fruits in the markets is due to the diversity of commercialized clones under the same denomination, as occurs with 'Mollar de Elche' (ME), as well as to the inconsistent quality (mainly in external and arils color, and in total soluble solids content – TSS). Also, as the harvesting period is relatively short in Spain, from the end of August until the beginning of November, for extending the presence in the markets earlier and later clones are needed, as well as some sweet-sour ones with increased demand (like Wonderful type), and if possible with soft seeds. Some progress have been reached in

identifying, typifying and selecting the Spanish pomegranate for helping its diversification and commercialization (Melgarejo, 1993; Melgarejo and Martínez, 1992; Hernández, 1999; Hernández *et al.*, 1999; Melgarejo *et al.*, 2001; Legua, 2002; Valdés *et al.*, 2007; Martínez *et al.*, 2010). However, the commercial offer of Spanish pomegranate must be still greatly improved.

Pomegranate fruit is very sensitive to mechanical injuries produced in the tree and during its handling and commercial distribution. These injuries open the ways for the installation and future early development of fungal attacks. It is also sensitive to weight loss and CI, like superficial scald, pitting, husk and carpel membrane browning (membranosis) and arils discoloration, with concomitant increased susceptibility to fungal attacks (Artés, 1992, 1993, 2007). Suitable handling and chilling treatments could mitigate the considerable economic losses caused by these disorders. As the demand and prices are usually increased in Christmas and New Year time in practically all markets, it is of a great economic interest the refrigerated storage. For this purpose it is also quite important to have suitable late clones (Artés, 1992, 1993; Artés *et al.*, 1998ab, 2010). Chilling also allows satisfying the increased demand of several Asian countries, by mean of refrigerated maritime transport of more than one month in-transit period. Some Spanish companies import pomegranate fruits from the South-East of Asia as well as from South-America, by using also long term refrigerated maritime transport (Artés, 2007).

Pomegranate fruit is very rich in bioactive compounds of a great interest, like flavonoids (including anthocyanin pigments). These compounds are secondary metabolites biosynthesized and accumulated in the vacuolar juice of mature epidermal cells of the husk and in arils throughout ripening. Color is very probably the first quality attribute and maturity criteria to be considered for consumer's acceptance. The anthocyanins confer to husk and arils their typical color from clear-rose to red-violet-purple, contributing to flavor (Du *et al.*, 1975; Shulman *et al.*, 1984; Gil *et al.*, 1995; Hernández *et al.*, 1999; Melgarejo *et al.*, 2001; Artés *et al.*, 2002; López-Rubira *et al.*, 2004). In addition pomegranate has a high antioxidant and anticarcinogenic potential (Aviram *et al.*, 2000; Noda *et al.*, 2002; Lansky and Newman, 2007).

In the above described situation the cultivation and international demand of pomegranate fruits are in moderate expansion, although in Spain they are only maintained. For increasing the pomegranate demand, better cultivation and postharvest techniques should be implemented. This will generate more add value, increasing the general economic benefits of its commercialization.

II – Measures for optimizing pomegranate fruits quality

1. Improvement of the intrinsic quality of pomegranate fruits

For reaching pomegranate fruits of good quality and to stimulate its consumption, good agricultural and handling practices are crucial. Among these are the follows (Artés, 2007): continue hand power training; avoiding mechanical damages and CI; controlling fruits at the reception step in the packinghouses; cleaning and disinfecting fruits (if needed), equipment, installations and water processing; correct employ of pre and postharvest agrochemicals; efficient use of handling and refrigeration equipment; keeping hygienic practices, and implementing good HCCP and traceability systems.

The most appreciated quality attributes of pomegranate fruit are size, caliber, husk and arils red color, typical flavor, free from defects and soft seeds (Artés, 1992; Melgarejo, 1993). The fruit intrinsic quality is reached by right cultivation and handling practices. In this way cultivation of pomegranate tree in poor and saline soils produce fruits with lower anthocyanin accumulation than fruits from more fertile soils (Gil *et al.,* 1995). The anthocyanin content in aril juice from red colored ME fruits picked from the external zone of the tree was 40% lower than that of more yellowish colored fruits picked in the interior zone (Gil *et al.,* 1995). In preliminary essays ME

fruits cultivated under deficit irrigation system reached more intense red color and better sensory acceptance than conventionally irrigated fruits (Peña *et al.*, 2011).

Pomegranate fruit has a low respiratory intensity with a non-climacteric behavior. Consequently it must be harvested once good size and flavor are reached. Fruit and arils weight and pigmentation increased throughout ripening in the tree, while titratable acidity (TA), TSS and total sugars remain quite constant during the last weeks (Shulman *et al.*, 1984; Gil *et al.*, 1995, 1996b; Hernández *et al.*, 1999; Melgarejo *et al.*, 2001; Legua, 2002). In the 'Mollar' population *cvs.* between the 26 and 32 weeks after full bloom, TSS and TA did not change (about 17 ºBrix and 0.25 g citric acid/100 ml), the average fruit weight increased from 230 to 490 g, that of the arils from 0.30 to 0.42 g, total anthocyanin from 5 to 389 mg/l and arils yield changed from 65.9 to 57.2%. All this changes indicate that the optimal harvest date of 'Mollar' *cvs.* must be around 30 to 32 weeks after full bloom (Gil *et al.,* 1996b).

The ratio TSS/TA is a good maturity index for sweet *cvs* (Artés, 1992; Artés *et al.*, 1996) being considered sweet these clones showing values between 32 and 98 at harvest (Melgarejo, 1993). In ME the TSS/TA ratio should be at least 60 (Artés, 1992, 1993; Artés *et al.*, 1998a). 'Mollar de Elche' (ME) fruit firmness measured by its resistance to compression in the equatorial zone should be higher than 60 N at harvest and the minimum for commercialization is 35 N (Artés *et al.*, 2005). The red color intensity of the arils juice increased or was kept after 6 weeks at 2 and 5ºC in ME *cv* (Gil *et al.*, 1996b) and in Wonderful *cv* (Ben-Arie *et al.*, 1984; Kader *et al.*, 1984).

2. Mechanical damages and browning

When pomegranates are still in the tree, fruits suffer mechanical damages due to wind (abrasions, bruising) and other climactic agents, spines (punctures), insects, etc., and injuries continue at picking (gossip cutting could produce scratches, punctures and cuts). Throughout handling until consumption fruits also suffer shocks, scraps and compressions which lower its commercial quality. The most serious damages are the degradation of epidermal and subjacent tissues due to breaking of cellular membranes and fluid leakage inducing browning caused by the oxidation of phenolics compounds located in the vacuole catalyzed by the polyphenol oxidase enzyme located in the cytoplasm. In addition injuries facilitate the fungal growth causing decay. Although pomegranate husk seems resistant to mechanical injuries, their high polyphenols content made fruit very susceptible to superficial and internal browning and the critical points of impacts during harvesting, handling, and distribution should be analyzed and minimized. The husk damage depends basically of two factors: the impact acceleration (ms^{-2}) and the change of velocity (ms^{-1}). Both factors could be determined along the run in the confectioning line by mean o an electronic sphere ('electronic fruit'). This device has a triaxial accelerometer which collects all impacts received. The speed of the impacts is calculated and depend of the energy absorption characteristics of the impact surface (conveyor belt, lateral walls, gauge fall, packing bottom, others fruits, etc.). The combination of both parameters inform about if an impact is able or not to damage the fruit and if the packing line must be or not protected (Techmark, 2002). Although the propensity to injuries, the impacts don't produce an immediate fruit damage, except if they are very severe, but some days later at high temperatures. A speed change lower than 1.3 ms^{-1} and an acceleration 50 fold higher than gravity induce damages to ME fruits. The most critical points in the packing line are those of transfer, mainly dumping, gauging and bulk packaging. The correction of these points avoids substantial losses. Also it was found that lowering fruit temperature before handling, the extension and severity of damages are lowered (Artés *et al.*, 2005).

3. Microbial diseases

The most important factors for favouring cryptogrammic diseases in pomegranate fruits are wounding, enzymatic process and microbial contamination. Once quoted the first two, it must be

cited that the natural microbiota is formed by fungal genera and, in a lesser extent, mould and bacteria. It could be distinguish two groups of pathogenic fungus: those which accede by the injuries, germinating the spores in a few days after infection, and those latent, which penetrate by the natural opening of the fruit and wait in the epidermis the favourable conditions for infecting. The sources of contamination are in the farm and mainly in the packinghouse (packages, pallets, bins and cold rooms). Due to this, in each packinghouse disinfection programmes should be implemented.

The most frequents fungal genera are *Botrytis* sp., *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp. and *Alternaria* sp. Good agricultural, handling and chilling practices commonly are enough for fungal control, avoiding the use of fungicides, not authorized in Spain for harvested pomegranate and rejected by consumers (Artés, 2007; Artés *et al.*, 1998abcd; Palou, 2007, 2010).

4. Physiological disorders

Pomegranate is sensitive to CI showing moderate to severe symptoms when after about 5 weeks at 5ºC or less, fruits are transferred to ambient warm temperatures for marketing issues. The above cited scald browning and membranosis (although this last is not strictly a CI) are originated when chilling damaged the vacuole membrane (Artés, 1993; 1998c). For avoiding CI storage temperature could be increased, but the practical difficult is the increased risks of fungal attacks, weight loss and browning.

III – Recommendations for chilling storage

1. Conventional storage

In addition to intrinsic fruit quality which limits its potential storage life, some factors are crucial for extending shelf life: temperature (the most important), RH and atmosphere composition. In this way it's useful the air-forced precooling before handling and/or cold storage (Artés, 1992; Artés and Tomás-Barberán, 2001). The recommended conditions for 3 months of storage of ME and 'Mollar de Orihuela' *cvs.* are 5ºC to reduce decay and practically all CI, and 90-95% RH to lower browning and loss of weight, appearance and flavour (Artés, 1987; Artés *et al.*, 1998abcd). For the storage up to 2 months of 'Wonderful' *cv.* also 5ºC and 90-95% RH have been recommended (Elyatem and Kader, 1984; Kader *et al.*, 1984).

2. Packaging in perforated polymers

The use of perforated plastics films for packaging pomegranate creates and maintains within the packages a high RH, which reduce weight loss and kept the good appearance of fruits. At the same time, attractive sales units for consumers are generated (Irles, 2007). For a right application of these films the optimal perforation density of each kind of package, according to commercial distribution conditions, should be determined.

3. Controlled atmosphere storage and modified atmosphere packaging

The initial quality could be better kept if the atmosphere surround the fruits under storage or transport at optimal chilling temperature has a lower O_2 partial pressure and that of the CO_2 is moderately higher than those of the air. This technique is called controlled atmosphere (CA) when generated into gastight cold rooms or containers, and modified atmosphere packaging (MAP) if reached within hermetically sealed plastic packages. This is based on the effect of low O_2 and/or moderate CO_2 to restrain respiration and other vital processes of fruit, reducing weight loss, decay and CI. ME stored under 5 kPa O_2 + 0-5 kPa CO_2 at 5ºC for 2 months followed by 6 days in air at 20ºC, kept very well the initial quality, drastically reduced CI and

weight loss, and lowered decay compared to fruits in air (Artés *et al.*, 1996). A CA of 5 kPa O_2 + 10 kPa CO_2 inhibits CI in ME after 3 months at 5ºC and 95% RH regarding fruits stored in air or in 15 kPa CO_2 enriched air (López-Rubira *et al.*, 2007). However 4-5 kPa O_2 + 12-14 kPa CO_2 after 80 days at 2 and at 5ºC, induced off-flavour and low chromaticity due to probable fermentative processes (Artés *et al.*, 1998d). For storing 5 months the Wonderful *cv.* 3-5 kPa O_2 + 5-10 kPa CO_2 at 5ºC was recommended (Hess-Pierce and Kader, 2003) or, as an option to avoid superficial scald, 2-4 kPa O_2 at 2-6 ºC (Ben-Arie and Or, 1986).

The main benefit of MAP against CA is that it can be modified passively. In addition, MAP is cheaper, flexible (from individual fruit to pallets), and applicable to small scale during the storage in conventional cold rooms, chilled transport, and retail sale, keeping the gas composition and high RH effects without hardly any specific equipment (Artés *et al.*, 2006).

4. Thermal conditioning or curing and intermittent warming (IW)

Curing ME during 3 days in air at 33ºC and 95% RH followed by 3 months at 2ºC and 90-95% RH and a period of 8 days at 15ºC and 70% RH lowered CI compared to non-cured fruits. Also the IW at 20ºC of ME during 1 day every 6 to 14 days at 0, 2 or 5ºC and 90-95% RH, lowered CI and weight loss without stimulating fungal attacks, compared to fruits constantly kept at these temperatures for 80 to 90 days, followed of 8 days at 15ºC and 70% RH. The IW of 1 day at 20ºC every week at 2ºC was the most effective, but the implementation at industrial scale is difficult. Curing and mainly IW, facilitate fruits with external and internal red color and appearance, anthocyanins content, and flavour higher than control (Artés *et al.*, 1998cde, 1999, 2000).

Dipping ME for 4 min in water at 45ºC before 90 days at 2ºC and 90% RH, followed by 3 days at 20ºC, increased the content of sugars, organic acids, vitamin C, phenolics, anthocyanins and antioxidant activity compared to control and levels at harvest (Mirdehghan *et al.*, 2006).

5. UV-C radiation

The non-ionizing UV-C radiation in the range of 200-280 nm wavelength of light spectrum has germicidal effect on microorganism, alters DNA of microorganisms and prevents both DNA transcription and translation, avoiding replication. It's a sustainable simple technique, of low cost of installation and maintenance, for disinfecting fruit and vegetables before conventional chilling or CA/MAP storage (Yaun, 2002; Allende and Artés, 2003). In whole ME treated with 9 kJ m^{-2} or 23 kJ m^{-2} UV-C before 21 weeks of storage at 5ºC under 5 kPa O_2 + 10 kPa CO_2 followed by 8 days at 20ºC and 75% RH, weight losses were lower and sensory scores higher than in control, with no decay or severe CI and without differences between both UV-C doses neither with the single CA treatment (López-Rubira *et al.*, 2007).

6. Sustainable agrochemicals

In 'Malas Saveh' *cv.* dipped for 10 min in 2 mM salicylic acid and stored 3 months at 2ºC and 85% RH, CI, the electrolyte leakage and loss of vitamin C were reduced compared to control (Sayyari *et al.*, 2009). The 6 M oxalic acid applied for 10 min at 25ºC to ME before storage for 84 days at 2ºC and 90% RH kept the visual quality, and reduced CI and phenolics compound loss, while vitamin C, total anthocyanins and antioxidant activity increased (Sayyari *et al.*, 2010). The 1M salicylic acid applied to ME for 10 min in a water dip at 20ºC, delayed ripening, reduced CI, increased antioxidant activity and maintained the bioactive compounds and nutritive value after 84 days at 2ºC plus 4 days at 20ºC (Sayyari *et al.*, 2011).

IV – Recent advances in minimal processing of pomegranate fruits

The arils have a great interest as a minimally processed product (MPP) because it facilitates its

consumption overcoming the difficulty of extraction by consumers. Also MPP are being increasingly demanded due to the extended habits for healthy foods as well as for the scarce time able of consumers for preparing meals. For keeping sensory and microbial quality the MPP should be kept chilled under MAP (Artés et al., 1995). This processing generates great added value, reaching their cost at the retail sale level 20 fold that of the raw material cost.

For having success with MP arils the *cv.*, ripening stage and hygiene are crucial because will determine both final quality and yield. The most commonly used *cvs.* for processing arils in Spain are ME and Wonderful (from Albatera and Elche, or imported from Israel, Peru and Chile) and, in a lesser extent 'Bhagwa' and 'Ghanesh', from India. The early 'Valenciana' *cv.* has not much interest because its light-pink color and hard seeds. Regarding ripening stage at harvest for this purpose in ME the above cited recommendations are useful (Gil *et al.,* 1996b, Artés *et al.,* 1998a, 2005) while in 'Wonderful' an intense red color, at least 14 ºBrix and a maximum of 0.4 g citric acid/kg have been recommended (Alberola, 2010).

For extracting arils, it is suitable to precool the fruit until 5ºC, pre-washing with water at 5ºC and pH 6-6.5 with 100-125 ppm free chlorine as antimicrobial, followed by sorting and drying by cold (5ºC) forced air. Sounds fruits are then transported by a conveyor belt to the proper and refrigerated (about 5ºC) area, physically separated from the previous dirty area. Fruits are then cut with disinfected sharpened knifes and arils are manually or mechanically extracted (by injecting cold air) taking care of avoiding damages. Immediately the carpel membranes and the teguments of insertion of arils are eliminated. Arils are then manually sorted by color and defects (breaked, decayed or discolored) or by using automatic sorting devices. Sound arils are washed (to eliminate juice and decontaminate) and disinfected for 1-2 min in waved water at 5ºC, with 50-150 ppm free chlorine and pH 6-6.5 (adjusted with citric or ascorbic acids, which are also anti-browning agents at about 5 g l^{-1}). The estimated water consumption is 2-3 l kg^{-1} and, for reusing it, accurate chlorine and pH levels must be assured. Arils are then rinsed in non-chlorinated water at 5ºC, dried by cold (5ºC) forced air, weighted and MAP packed, by using right polymers (polyethylene, polypropylene or ethyl-vinyl-acetate, among others), of right thickness in order to passively generate the intended gas composition (usually 5-7 kPa O_2 + 5-10 kPa CO_2). This atmosphere could be also actively generated in MAP conditions. All packages pass a weight and metals control and to an overall quality control in the factory (sensory, gastight test, and gas composition of packages), and microbial in a homologate laboratory for assuring legal levels (EU Regulation 1441, 2007). The packages are palletized and conducted to a cold (0/1ºC) room for picking nearby the shipping area at 0/1ºC. The transport and distribution temperature must be 5ºC or lower, and the shelf life is commonly of 7-10 days for ME and 10-14 days for 'Wonderful' (Artés *et al.*, 1995, 2007, 2009; Gil *et al.*, 1996a; Sepúlveda *et al.*, 1998; Villaescusa *et al.*, 2000; Artés, 2007; Artés and Artés-Hernández, 2005; Artés-Hernández *et al.*, 2009; Alberola, 2010).

Due to the risks of chlorine for the health of the people working in the factory as well as for particularly sensitive consumers, some alternatives for arils disinfection are being studied. In ME arils 0.6 to 13.6 kJ UV-C m^{-2} reduced initial mesophilic, psychrotrophic, lactic acid bacteria, *Enterobacteriaceae* and moulds counts, even if after 15 days at 5ºC did not control always microbial growth (López-Rubira *et al.,* 2005a). The peroxiacetic acid and the ozonated (0.4 ppm O_3) water could be an industrial alternative to chlorine in ME arils (López-Rubira *et al.,* 2005b).

MAP of arils could be accomplished in aseptic conditions avoiding recontamination and microbial growth by mean of 'clean rooms' (ambient with a contamination level specified by the contained number of particles of a certain size per m^3). In MAP ME arils treated with 0.1 g l^{-1} NaClO, after 14 days at 0ºC a clean room ISO 5 reduced 1 log ufc g^{-1} the initial counts of aerobes, mesophilic and psycrotrophic. However this scarce effect does not probably justify the industrial application, although the very low temperature could mask their effect (Consea *et al.*, 2005). It could be of interest to continue the studies because the technique starts being implanted.

Acknowledgements

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UV-C light and mild hot water for keeping overall quality of fresh-cut pomegranate arils

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Abstract. Manually extracted pomegranate arils (cv. 'Mollar de Elche'), chlorine disinfected (100 ppm), rinsed and drained arils (Control) were exposed to either 4.54 kJ m^{-2} ultraviolet-C light (UV-C) or immersed for 30 s in water at 55ºC (HW), packaged in polypropylene (PP) baskets (100 g), sealed on the top and then stored up to 14 days at 5ºC. The respiration rate (RR), atmosphere composition, titratable acidity (TA), total soluble solids content (TSS), microbial growth and sensory quality were monitored. Passive MAP composition at the steady state was the same (16 kPa O_2 + 5 kPa CO_2) independently of the treatment. RR tended to slightly increase during storage being in general slightly lower for the Control (between 1.5 and 1.7 ml CO_2 kg^{-1} h^{-1}). Significant changes were detected for TA which decreased from the initial value (0.19 g citric acid/100 ml) to 0.09, 0.08 and 0.1 g citric acid/100 ml for control, HW and UV-C respectively at the end of storage. From the initial TSS of 15.8ºB, a slight reduction in HW treatment was found. No noticeable changes in TSS for control and UV-C treatment were found. *Mesophilic* counts strongly decreased by HW and in a lesser extent by both UV-C and chlorinated water. The same trend was found for molds and yeasts. No *Enterobacteriaceae* growth was observed in any treatment. Control as well as all HW treated arils were sensory scored as poor, being qualified as unmarketable after 14 days. In contrast arils from UV-C kept well enough their sensory quality and were considered as good, being this treatment reliable for keeping overall quality and safety.

Keywords. *Punica granatum* L. – Ultraviolet – Hot water – Minimal processing – Modified atmosphere packaging – Respiration – Quality – Safety.

I – Introduction

Pomegranate is mainly consumed fresh and although their excellent healthy properties and quality attributes, difficulties in peeling to obtain the arils have limited its consumption. Production of minimally fresh processed or fresh-cut (FC) arils ready-to-eat may increase the consumption of pomegranates (Artés *et al.*, 1995). There is an increasing consumer demand to eliminate or reduce the use of agrochemicals on fresh products for extending their shelf life. One method has been the use of heat treatments for reducing postharvest decay in fruit and vegetables (Chan and Tian, 2005). However, no information is available on the use of mild hot water (HW) treatments on pomegranate arils. Another approach is the use of germicidal UV light at 200-280 nm (UV-C) for surface disinfection of FC commodities due to the lack of a residual compound on the surface of the produce and their low cost for the industry (Allende and Artés, 2003).

The objective of the current work was to evaluate the efficacy of HW and UV-C treatment in order to prolong the shelf-life and maintain the overall quality of FC pomegranate arils. From the best of our knowledge this kind of strategy on pomegranate arils is firstly reported here.

II – Material and methods

Plant material. 'Mollar de Elche' fruits were supplied by Cambayas S.C. (Elche, Alicante), and after harvesting were transported 70 km to the Pilot Plant of Postharvest and Refrigeration Group in the Technical University of Cartagena. Arils were manually extracted in a disinfected cold room at 8ºC, washed in 100 µl l^{-1} NaOCl, acidified with citric acid (5%), and rinsed.

Treatments conditions. The treatments were as follows: arils left in a water bath set at 55ºC for 30s (HW); arils exposed to 4.54 kJ/m^2 UV-C (UV-C); arils without any treatment as Control. PP trays (about 100g arils each) were sealed on the top with a bioriented PP film and then stored at 5ºC and 95% RH up to 14 days. Analyses were made on days 0 (processing), 4, 8, 12 and 14.

Respiration rates and gas composition changes. For each treatment and day 3 replicates (100 g each) were placed within 750 ml glass jars at 5ºC for 14 days. The respiratory CO_2 and changes in O_2 and CO_2 levels within packages were monitored during shelf life by mean of a gas chromatograph, equipped with a thermal conductivity detector and Poropack-N 80/100 column.

Microbial analysis. Microbial analysis was performed as described in Aguayo *et al.* (2007). Mesophilic, *Enterobacteriaceae*, yeast and mould counts were reported as log cfu g^{-1}.

Total soluble solids content and titratable acidity. In arils juice TSS (°Brix) were determined by a refractometer at 20°C and TA (g of citric acid 100 ml^{-1}) in an automatic titrator by titrating with 0.1N NaOH of 10 ml diluted with 50 ml water, reaching pH 8.1.

Sensory evaluation. Ten members of an experienced sensory panel rated the arils, using a 5-point scale (1, dislike extremely 5, like extremely). Aroma, taste, firmness, visual appearance, color, browning and dehydration were evaluated (López-Rubira *et al.*, 2005).

III – Results

Gas composition and respiration rate. As expected the O_2 levels decreased and those of CO_2 increased within all packages during storage (Fig. 1). The RR was almost constant during shelf life in Control and treated arils, according to pomegranate is non-climacteric (Gil *et al.*, 1995). The increase in RR found at day 14 seems due to microbial growth, without differences between Control and UV-C and HW arils (Fig. 2.A). HW and UV-C didn't provoke any respiratory stress on FC arils in agreement with López-Rubira *et al.* (2005).
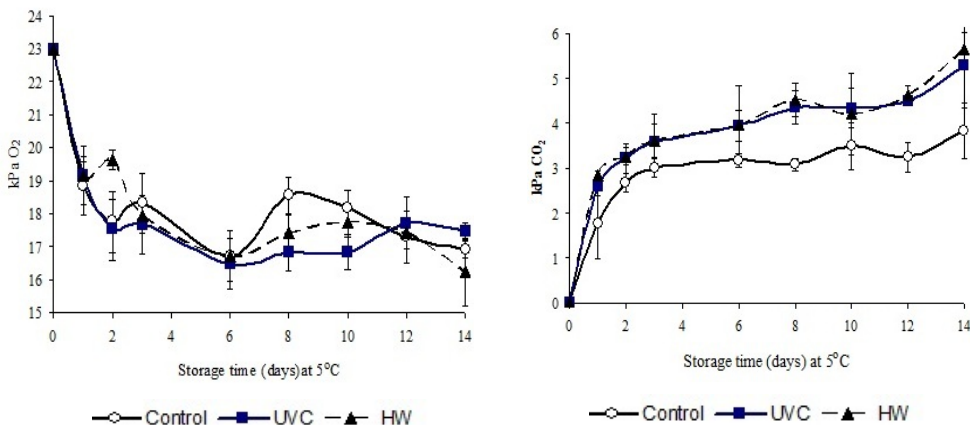
Fig. 1. Gas composition within packages of minimally processed arils during shelf life. Data represent means of three replicates (n=3 ± SD).
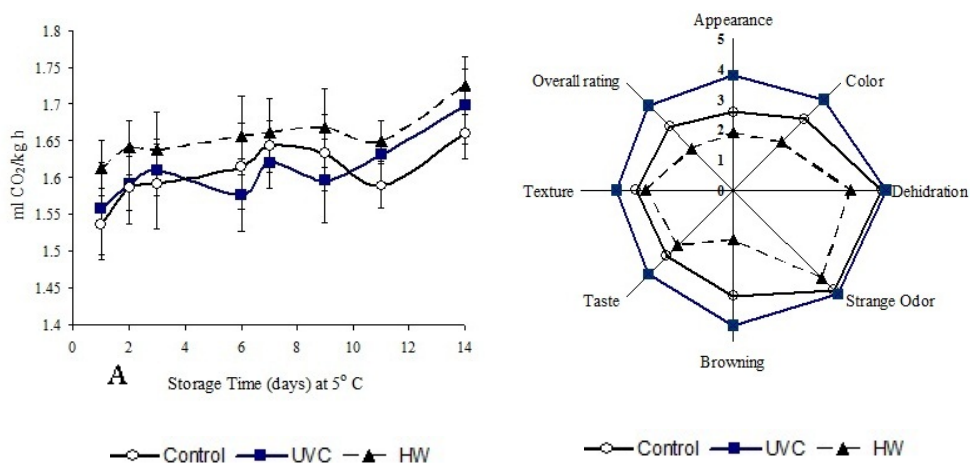
Fig. 2. **(A) Respiration rate (ml CO$_2$/kg h) throughout 14 days at 5ºC in air of minimally fresh processed arils. Data represent means of three replicates (n=3 ± SD). (B) Sensory scores of fresh-cut arils stored up to 14 days at 5°C. Data represent means of 10 replicates (n = 10).**

Microbial analysis. Throughout shelf life no *Enterobacteriaceae* growth (log cfu g^{-1}) on FC arils was found. Mesophilic counts yeast and mould were influenced by HW and UV-C (Table 1). Detrimental effect of the UV-C light and heat on the microorganism could directly retarded fungal spore germination and indirectly, can activate defense responses in fruit (Pan *et al.*, 2004).

Table 1. **Shelf life Microbiological accounts in pomegranate arils under control, ultraviolet-C (UV-C) and hot water (HW) treatments. Values are mean log cfu g^{-1} (n=5 ± SD)**

Bacteria	Days	Control	UVC	HW
Mesophilic (10Log cfu/g)	Initial	4.15b	4.15b	4.15b
	0	3.77±0.06d	3.25±0.046fg	2i
	7	3.9±0.04c	3.21±0.052fg	2i
	14	4.31±0.07a	3.49±0.08e	2.13±0.02h
Fungi	Days	Control	UVC	HW
Mould (10Log cfu/g)	Initial	3.17a	3.17a	3.17a
	0	2.85±0.06b	2.69±0.05b	2e
	7	3.2±0.09a	2.76±0.06b	2e
	14	3.28±0.14a	2.73±0.09b	2.24±0.05d
Yeast (10Log cfu/g)	Initial	3.85b	3.85b	3.85b
	0	3.11±0.03d	2k	2k
	7	3.81±0.03b	2.13±0.07j	2.21±0.04j
	14	4.03±0.06a	2.82±0.03g	2.93±0.01ef

Values within rows followed by different letters are significantly different according to Duncan's multiple range test at p ≤ 0.05.

Total soluble solids content and titratable acidity. In comparison to initial values, at the end of the storage, practically no changes in TSS were detected in FC arils (except a slight reduction in HW treatments as well as slight increase in UV-C) (Table 2). However TA decreased in all treatments in a similar level (Table 2). This could be due to organic acids were predominant

substrates for respiratory activity of arils. These results confirm those of studies showing no remarkable TSS and TA changes in the same 'Mollar de Elche' cv (Gil *et al.*, 1995).

Table 2. **Total soluble solids content (TSS, ºBrix), total titratable acidity (TA, g citric acid/100 ml) during shelf life for pomegranate arils under control, ultraviolet-C (UV-C) and hot water (HW) treatments. Values are mean (n=3) ± standard deviation**

	Day	Control	UVC	HW
TSS (ºBrix)	0	15.8±0.05bc	15.8±0.02bc	15.8±0.04bc
	4	15.7±0.05c	15.8±0.05bc	15.2±0.03hij
	8	15.8±0.01bc	16±0.05a	15.1±0.05jk
	11	15.7±0.4c	15.6±0.03cd	15±0.04kl
	14	15.9±0.02ab	16±0.02a	15.3±0.05ghi
TA (g Citric acid/100 ml)	0	0.19a	0.19a	0.19a
	4	0.1±0.01cdefg	0.11±0.008bcdef	0.09fg
	8	0.09efg	0.09±0.003efg	0.11±0.01cdefg
	11	0.1defg	0.1±0.005defg	0.1±0.003cdefg
	14	0.09±0.006efg	0.1±0.003defg	0.08±0.003g

Values within rows followed by different letters are significantly different according to Duncan's multiple range test at p ≤ 0.05.

Sensory evaluation. External visual appearance aroma, taste, firmness, visual appearance, colour, browning and dehydration, of UVC after 14 days of MAP storage at 5ºC were still acceptable for consumption. However, according to sensory test control arils as well as all HW treated arils were scored as poor, and consequently were unmarketable (Fig. 2B).

IV – Conclusions

UV-C treated arils showed lower mesophilic, yeast and mould counts and higher marketability than Control and HW. This treatment did not modify the SSC and TA, two key factors associated to fruit flavour and consumer's acceptance. Therefore, the use of UV-C seems to be justified for improving the shelf life of FC pomegranate arils in the current studied conditions.

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Preharvest foliar application of methyl jasmonate, salicylic acid and potassium sulfate on improving the quality of pomegranate fruit

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Abstract. Recently, a pomegranate physiological disorder called 'aril browning' or 'aril paleness' in which a part or all of the arils show discoloration, affect the quality of fruit and such fruits are not suitable for consumption. The present research design to evaluate the effects of jasmonates, salicilates and potassium nutrient on reducing this disorders and improving fruit quality. Ten treatments including salicylic acid (0.3, 0.6 and 0.9 mM), methyl jasmonate (0.5, 1 and 2 mM) potassium sulfate (0.5, 1 and 1.5%) and distilled water (control), were sprayed on pomegranate tree 10 and 45 days after full bloom and after harvest. Different parameters related to quality of fruit were measured. The obtained results indicated that salicylic acid could improve the total acidity, TSS, chroma and hue angel of skin and b* index of arils. Although the phenolic content of arils increased in all treatment compare to control, the differences were not significant. The highest antioxidant activity was observed in the 1.5% potassium sulfate treatment and the differences with the control were significant.

Keywords. Aril and skin color – Internal breakdown – Physiological disorder – Antioxidants.

I – Introduction

Pomegranate (*Punica granatum* L.) belongs to the Punicaceae family and is one of the oldest known edible fruits. It is sometimes called Chinese apple (Mars, 1994). Pomegranates are mainly grown for fresh consumption of arils (botanic exact term is seed) or juice, although in various countries they are produced for the food and beverage industry as flavouring and coloring agents (Gil *et al.*, 2000).

The edible part of the fruit contains considerable amounts of acids, sugars, vitamins, polysaccharides, polyphenols, and important minerals (Al-Maiman and Ahmad, 2002; Mirdehghan and Rahemi, 2007). Pomegranate has been of recent interest for its nutritional, chemical and antioxidant characteristics. Very recently, the incidence of a physiological disorder called 'aril browning' or 'aril blackening' (internal breakdown of arils) has threatened the popularity of pomegranate fruit. This physiological disorder was reported for the first time in Ferdows region of Iran in the year of 2001. Affected arils are soft, light creamy-brown to dark blackish-brown, deformed, acidic and possess unacceptable off-flavour and are unsuitable for consumption. The extent of damaged arils could vary from a few to all in a fruit. Fruits having this disorder do not show any external signs and often possess good eye appeal. Defective arils are detected only after cutting the fruits open, posing a serious challenge to quality control in export. Sometimes consumers hesitate to buy this fruit because of the hidden brown arils (Jalikop,*et al.*, 2010).

Shivashankara *et al.* (2004) attributed browning of arils in pomegranate to the oxidative damage of membranes leading to higher activities of certain enzymes like polyphenol oxidase and peroxidase. Similarly, enzymatic browning in litchi, pears and apples is ascribed to polyphenol oxidase and peroxidase (Murata *et al.*, 2000).

Potassium is an essential macronutrient in pomegranate and its concentration in peel and aril of pomegranate fruits was the highest compared to other macronutrients (Al-Maiman and Ahmad, 2002; Mirdehghan and Rahemi, 2007). It is also known as the quality nutrient because of its important effects on quality factors (Lester *et al.*, 2006). Soares, *et al.* (2005) reported that potassium soil application increased significantly antioxidant activity and reduced oxidative damage.

He, *et al.* (2002) have shown that exogenous salicylic acid (SA) can regulate the activities of antioxidant enzymes and increase plant tolerance to abiotic stress (He *et al.*, 2002). Shi, *et al.* (2006) also reported that pretreatment by a foliar spray of 1 mM SA may have a signaling function in the induction of heat tolerance in cucumber seedling as indicated by an increase in H_2O_2 concentration. Low concentrations of reactive oxygen species (ROS), especially H_2O_2 are known to act as signal molecules initiating several protective resistance mechanisms against pathogens, chilling and heat stress.

There are some evidences indicating that methyl jasmonate (MejA) can affect the antioxidant system in plant cells (Wang, 1999). The role of MeJA in protecting plants from various stresses has been reported, for example, amelioration of chilling injury and water stress in rice (Lee *et al.*, 1996), tomato (Ding *et al.*, 2001) and strawberry (Wang, 1999). It also has been reported that MeJA mitigated the ROS effects in strawberry under water stress and in maize seedlings subjected to paraquat (Norastehnia and Nojavan-Asghari, 2006).

Many studies have shown a correlation between resistance to environmental stress and the efficiency of the antioxidative systems. The aim of this work was to evaluate the influence of foliar application of salicylic acid, methyl jasmonate and potassium sulfate during the fruit growth and maturation on fruit quality of pomegranate.

II – Material and methods

Plant material and treatments. Experiment was carried out in a commercial orchard of pomegranate cv. Malas Yazdi at Agricultural Research Center of Yazd, with 10 treatments and 4 replications. The treatments included: salicylic acid (0.3, 0.6 and 0.9 mM), methyl jasmonate (0.5, 1 and 2 mM), potassium sulfate (0.5, 1 and 1.5 mM) and distilled water (control) applied on the base of factorial with completely randomized block design. Trees were sprayed two times at 2010/4/29 and 2010/6/3 dates (2[ed] and 7th week after full bloom respectively). Fruits were harvested on 2010/9/12 date at the commercially maturity stage, and were transferred to postharvest labratory in Vali-e-Asr university of Rafsanjan.

Quality characteristics assessments. Total soluble solids (TSS) was measured from the fruit juice, using a hand refractometer and results were expressed as °Brix (PAL-1 ATAGO, Japan). Total acidity (TA) was determined by titration of 5 ml of juice with 0.2 M of NaoH and the results were calculated as a percentage of citric acid. The pH of juice was measured using a pH meter (Inolab pH 720). Peel and aril color were determined using a colorimeter (Minolta CR400) and results were expressed as L*(lightness), a*, b*, hue angle and chroma.

For phenolics measurement, 200 µl of supernatant (after 5 gr of arils were extracted with 10 ml potassium buffer at pH 8.7) was mixed with 300 µl of potassium buffer, 2.5 ml of 0.2 N Folin-Ciacalteu and 2.5 ml of 7.5% sodium bicarbonate. The mixture was allowed to stand for 5 min at 50°C, before the absorbance was measured at 760nm using a spectrophotometer (Uv/Vis T80). The final result expressed as mg galic acid /100 gfw. The antioxidant activity was measured based on ATBS (diamonium salt), described by Serrano *et al.* (2005) and the absorbance was measured at 730nm.

Statistical analysis. Obtained data were analyzed by SAS software, and mean values were compared at the level of 5% according to Duncan multiple test.

III – Results and discution

1. Total phenolics and antioxidant activity

Although SA, MejA and potassium sulfate increased the total phenolics, this was not significant compared to control (Fig. 1). It was reported that total phenolic increased by different treatment include: SA in 'Caracara Novel' orange (Huang *et al.*, 2008), potassium foliar application during fruit growth and development in pomegranate (Tehranifar and Mahmoodi Tabar, 2009) and grape (Delgado *et al.*, 2004) and postharvest application of methyl jasmonate in white guava. However, pre-harvest treatment of methyl jasmonate has no effect on total phenolic of white guava (Gonzalez-Aguilar 2004), that is in agreement with our results. Ghasemnezhad and Javaherdashti (2008) expressed that MejA could enhance the total phenolics and therefore induce the defense mechanism of raspberry. SA and MejA could stimulate the phenylalanine ammonia lyase activity, an enzyme involved in the synthesis of phenolics and flavonoids through the phenylpropanoid pathway, (Chen *et al.*, 2006), and in consequence increase the amount of phenolic compounds (Yao and Tian, 2005).
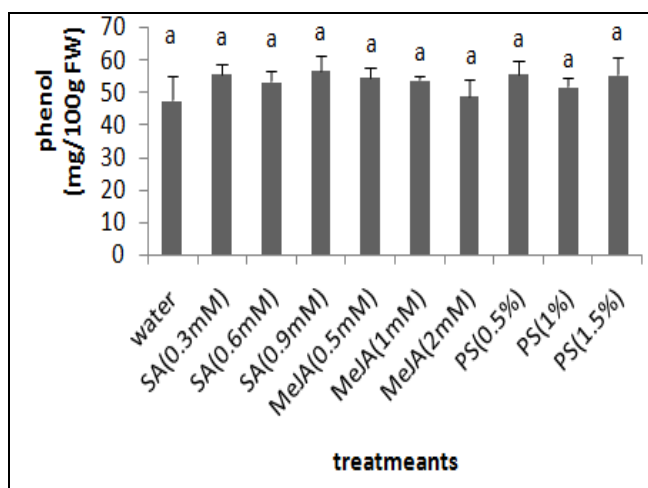
Fig. 1.   The effect of SA (0.3, 0.6 and 0.9 mM), MejA (0.5, 1 and 2 mM) and potassium sulfate (0.5, 1 and 1.5%) on phenolics of fruit.

The antioxidant activity of arils was influenced by the treatment. 1.5% of potassium sulfate and 0.5 mM MejA significantly increased the antioxidant activity of pomegranate arils at harvest, but the increment was not significant in other concentration and SA treatments (Fig. 2).

Treatment with signalling molecules like SA or MejA may induce H_2O_2 production, which in turn may induce the synthesis or activate various transcription factors and are associated with the induction of different antioxidant enzymes (Agarwal *et al.*, 2005). However, SA and MejA response may vary with organisms, concentration of phytohormones and light intensity (Raman and Ravi, 2011). It was reported that preharvest application of MejA increase the antioxidant activity of raspberry fruit (Wang and Zheng, 2005), and also potassium increased the antioxidant activity in pomegranate fruit (Tehranifar amd Mahmoodi Tabar, 2009) and pineapple (Soares *et al.*, 2005).

Wang and Lin (2000) found that there is a positive correlation between total phenolic and

anthocyanin with antioxidant activity. Likewise in our experiment a correlation (0.59) was found between antioxidant activity and total phenolics.
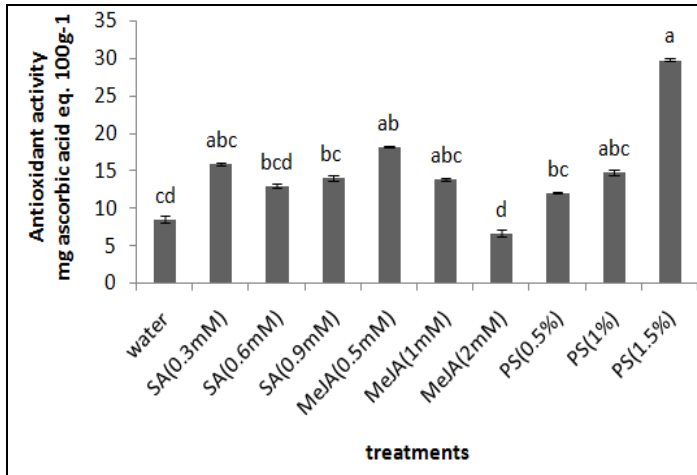
Fig. 2. The effect of SA (0.3, 0.6 and 0.9 mM), MejA (0.5, 1 and 2 mM) and potassium sulfate (0.5, 1 and 1.5%) on antioxidant activity of fruit.

2. Total soluble solids, acidity and pH

The influence of treatments on TSS, titrable acidity and pH are shown in Table 1. Although the TSS and titrable acidity of fruit juice were not influenced by most of the treatments, 0.3 and 0.9 mM of SA decreased the TSS of fruit juice significantly comparing to control. 1% and 1.5% potassium sulfate significantly increased the pH of fruit juice compared to other treatments. Delgado *et al.* (2006) showed that application of potassium may decrease the amount of tartaric acid in grape and in consequence may increase the pH. Sayyari *et al.* (2009) and Ding *et al.* (2007) showed that the amount of acidity and TSS were not influenced by SA treatment in pomegranate and mango respectively. Besides these results Wang (1998) found that MejA application may reduce the glucose, fructose, and sucrose levels of radishes.

3. Color indices

There was not any significant differences between all treatment on L* and b* value of arils and peel color of pomegranate fruit (Tables 2 and 3). Also the hue angle and chroma of arils was not influenced by all the treatments. A higher value of a* and hue angle in fruit peel was observed in 0.3 SA compared to other treatments, although the differences were not significant. Contrary to our results, Rudell and Fellman (2005) expressed that MejA treatment could enhance the peel red color and reduce peel hue angle of 'Fuji' apple. Different factors influence the color of fruit such as environmental condition, species and cultivar, etc. In fact it has been observed that fruit response to MejA treatment could be ascribed to differences in the fruit developmental stage (Fan *et al.*, 1997).

Table 1. Effect of SA (0.3, 0.6 and 0.9 mM), MejA (0.5, 1 and 2 mM) and potassium sulfate (0.5, 1 and 1.5%) on TSS, total acidity and pH of fruit juice

		TSS	Total acidity	pH
Water (control)		13.625a	0.8615a	3.785b
Salicylic acid	0.3 mM	13.6a	0.7477a	3.81b
	0.6 mM	12.685bc	1.0256a	3.7025b
	0.9 mM	13.25abc	0.8123a	3.83b
	0.5 mM	12.575c	0.6585a	3.82b
Methyl jasmonate	1 mM	13.5ab	0.9631a	3.8825ab
	2 mM	13.2abc	0.8123a	3.95ab
	0.5%	13.2abc	0.84a	3.9475ab
Potassium sulfate	1%	13.275abc	0.8287a	4.2124a
	1.5%	13.35abc	0.7477a	4.2025a
CV		4.03	26.48	5.62

Table 2. Effect of treatments SA (0.3, 0.6 and 0.9mM), MejA (0.5, 1 and 2mM) and potassium sulfate (0.5, 1 and 1.5 %) on aril colors

		L*	a*	b*	Hue angle	Chroma
Water (control)		29.93a	1.475a	13.775ab	6.07a	13.864a
Salicylic acid	0.3 mM	34.053a	2.14a	15.8152a	7.78a	15.968a
	0.6 mM	31.7a	4.438a	14.37ab	15.324a	15.587a
	0.9 mM	34.108a	1.735a	15.415ab	6.524a	15.517a
	0.5 mM	35.093a	1.745a	14.5425ab	6.724a	14.674a
Methyl jasmonate	1 mM	30.495a	1.855a	14.2725ab	7.406a	14.394a
	2 mM	34.825a	2.933a	13.895ab	11.996a	14.404a
	0.5%	33.313a	1.74a	13.6475b	7.24a	13.763a
Potassium sulfate	1%	33.485a	1.668a	14.8475ab	6.47a	14.197a
	1.5%	29.288a	1.948a	14.3425ab	7.909a	14.49a
CV		16.66	12.65	8.62	10.2	11.53

Table 3. Effect of treatments SA (0.3, 0.6 and 0.9 mM), MejA (0.5, 1 and 2 mM) and potassium sulfate (0.5, 1 and 1.5%) on peel colors

		a*	L*	b*	Hue angle	Chroma
Water (control)		19.933abc	63.123a	35.765a	29.168abc	41.004ab
Salicylic acid	0.3 mM	22.38a	61.273a	35.053a	32.641a	41.695ab
	0.6 mM	18.935bc	63.743a	37.855a	26.663bc	42.475a
	0.9 mM	18.318bc	61.82a	34.475a	27.978abc	39.074b
	0.5 mM	17.448bc	62.03a	37.695a	24.841c	41.45ab
Methyl jasmonate	1 mM	20.135ab	62.02a	35.795a	29.328abc	41.089ab
	2 mM	20.785ab	62.103a	35.253a	30.582ab	41.085ab
	0.5%	18.718bc	61.883a	34.45a	28.549abc	39.226b
Potassium sulfate	1%	16.703c	61.763a	36.275a	24.667c	40.016ab
	1.5%	18.22bc	62.988a	35.87a	26.969bc	40.25ab
CV		10.84	2.71	6.01	12.14	4.25

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New insights on the postharvest technologies to maintain the overall quality of pomegranate fruits

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Abstract. Pomegranate is a subtropical fruit and its arils contain a high concentration of sugars, organic acids, vitamins, polysaccharides, and essential minerals. In recent years its antioxidant properties and its effect against degenerative diseases have been claimed, which are attributed to their polyphenol content. Pomegranate, as other tropical and subtropical fruits are sensitive to low temperatures and develop a complex of physiological disorders known as "chilling injury". The incidence of this physiopathy limits the application of cold storage, the most widely strategy employed for marketing the horticultural produce. Therefore the impact of chilling injury on the agro-food industry has serious economic consequences. In the present work, we provide the different postharvest technologies of an environmentally friendly nature assayed in research or applied in the agro-food industry with the aim of inhibiting or delaying the emergence of chilling injury in sensitive fruits such as pomegranate and the effects of these technologies on the content of bioactive compounds with antioxidant properties.

Keywords. Pomegranate – Chilling injury – Natural volatiles – Dip treatments.

I – Introduction

Pomegranate as other tropical and subtropical fruits is sensitive to low temperature of storage and thus develops different physiological disorders that are known as chilling injury which limits the application of cold storage and has serious economic consequences for the agro-food business. In the present work several environmentally friendly postharvest technologies, assayed in research or applied in the agro-food industry, with the aim of inhibiting or delaying the emergence of chilling injury in sensitive fruits such as pomegranate and effects of this technology on nutritional quality of sensitive fruits is provided.

II – Materials and methods

Pomegranates (*Punica granatum* L. cv. Mollar de Elche) were picked, randomized and divided into different lots for the following treatments: salicylic acid (2 mM), acetyl salicylic acid (1 mM), oxalic acid (6 mM), methyl salicylate (1 mM) and methyl jasmonate (1 mM) and control (no treatments). These concentrations were based on previous experiments. The pomegranates were stored at 2°C for 3 months and analytical determinations (chilling injury incidence, quality and bioactive compounds content as well as antioxidant activity) on monthly basis (Sayyari *et al.*, 2009; Sayyari *et al.*, 2010; Sayyari *et al.*, 2011a; Sayyari *et al.*, 2011b).

III – Results and discussion

As expected, chilling injury symptoms increased during storage but they were affected by treatment, since after 3 months control fruit exhibited significantly higher chilling injury symptoms than that observed for pomegranates treated with salicylic acid, acetyl salicylic acid,

oxalic acid, methyl salicylate and methyl jasmonate (Fig. 1). The observed chilling injury symptoms were husk pitting (disperse spots on the whole surface), browning and desiccation being responsible for extensive postharvest losses and limiting the fruit storability. It is known that chilling injury is characterized by membrane disruption resulting in cellular decompartmentation and loss of tissue integrity accompanied by skin browning, this effect being attributed to the inhibition of polyphenoloxidase and peroxidase activities. Thus, treatments were able to reduce these symptoms and in turn to increase the shelf life and marketability period. It has been proposed that oxalic acid could act as antisenescence agent by enhancing fruit tolerance to low-temperature stress, the effect being attributed to the ability of oxalic acid to inhibit O^{2-} accumulation, delay H_2O_2 decrease and induce higher reducing status of ascorbate-gluthatione. The efficacy of acetyl salicylic acid on reducing chilling symptoms could be also attributed to salicylic acid, since acetyl salicylic acid is converted to salicylic acid in vegetable tissues, and the exogenous salicylic acid application was effective in reducing chilling injury in pomegranate by decreasing electrolyte leakage and PAL activity (Sayyari *et al.*, 2009). The mechanism of action by which methyl salicylate and methyl jasmonate increased the fruit tolerance to chilling injury has not been clearly elucidated, although in early report this effect was associated with increase in polyamines and ABA (Wang and Buta, 1994). These results were further confirmed since treatments with polyamines (putrescine or spermidine) or heat shocks alleviated chilling injury by increasing the polyamine endogenous concentration and maintaining the unsaturated/saturated fatty acid ratio which could account for the high membrane integrity and fluidity (Mirdehghan *et al.*, 2007a; 2007b). Methyl salicylate could induce some defence-mechanism responses that indirectly provide protection against chilling damage, rather than the compound itself producing a direct effect.
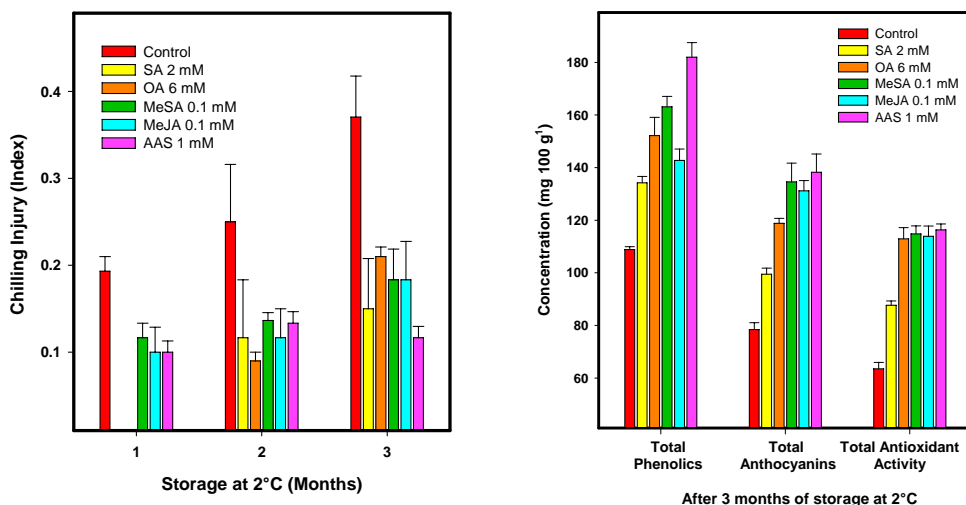
Fig. 1. **Chilling injury symptoms, bioactive compounds and antioxidant activity of control and treated pomegranates.**

Along prolonged storage, control pomegranates showed significant reduction in the content of total phenolics and total antioxidant activity in hydrophilic fraction, while total anthocyanins increased. The application of salicylic acid, acetyl salicylic acid, oxalic acid, methyl salicylate and methyl jasmonate maintained higher concentration of the bioactive compounds (total phenolics and total anthocyanins) and higher H-TAA after 3 months of cold storage (Fig. 1), although final concentrations were affected by treatment. The increase in anthocyanin

concentration has been associated with the advancement of the ripening process during postharvest storage. Exogenous oxalic acid could act as elicitor of anthocyanin synthesis. The enhancement of total phenolics including anthocyanins was correlated to higher levels of phenylalanineammonialyase (PAL) activity, the key enzyme in the first step of the phenylpropaind pathway directly involved in the biosynthesis of phenolic compounds. There is no scientific literature about the role of salicylic acid or acetyl salicylic acid modulating the content of bioactive compounds and/or antioxidant activity in fruit, although some evidences exist when salicylic acid was used as treatment. In this sense, in salicylic acid-treated sweet cherry the phenylalanine ammonia-lyase (PAL) activity increased during storage, which is one of the enzyme involved in the biosynthesis of phenolics and could be responsible for the higher phenolic concentration found in acetyl salicylic acid-treated pomegranates, as has been reported for grapes. In conclusion, the data presented here unequivocally suggest for the first time that salicylic acid, acetyl salicylic acid, oxalic acid, methyl salicylate and methyl jasmonate reduced the chilling injury symptoms in pomegranate and increased the antioxidant potential by enhancing or maintaining bioactive compounds such as total phenolics and total anthocyanins.

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Effects of calcium chloride dip and 1-methylcyclopropene on quality changes in arils from stored pomegranates

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Abstract. The objective of this study was to determine the effect of $CaCl_2$ dips, methylcyclopropene (1-MCP), and the combination of both on quality of extracted arils of 'Wonderful' cv. pomegranates. Arils were extracted from whole fruit stored 10 weeks at 7.5ºC in air. Treatments consisted of washing arils in chlorinate water (7 ºC, 100 ppm of NaOCl) (control), dipping arils into $CaCl_2$ (10 g l^{-1}) for 2 min, treating arils with 1-MCP (1 μl l^{-1}) during 24 h at 5ºC, and the combination of $CaCl_2$ dip and 1-MCP. All treatments were packaged in jars and kept in humidified air flow for up to 12 days at 5ºC. The use of $CaCl_2$ or 1-MCP helped to keep a better overall quality and firmness at the end of storage (reduced C_2H_4 and respiration rates, respectively). All treatments scored above the limit of marketability, indicating the viability of long term storage of whole pomegranate for fresh-cut produce.

Keywords. Ethylene – Fresh-cut – Quality.

I – Introduction

The fresh-cut industry needs products which can be marketed during several months. Studies on pomegranate reported it is possible to market pomegranate from early October until March (Hess-Pierce and Kader, 2003) when stored at 7.5 ºC in 5 kPa O_2 + 15 kPa CO_2. However, the arils from these whole fruits usually lose firmness with long periods of storage. In addition, when fresh-cut fruits are mixed with climacteric fruit in the same tray, ethylene (C_2H_4) emission from the climacteric fruit may accelerate the ripening process and reduce the shelf-life of this tray. Treatments like calcium dips can help maintain firmness and visual quality resulting in a longer shelf life of the fresh-cut products as melon (Aguayo *et al.*, 2008) and apple slices (Aguayo *et al.*, 2010). Methylcyclopropene (1-MCP) can also retard flesh softening. Blankenship and Dole (2003) demonstrated 1-MCP can inhibit C_2H_4 action by blocking its receptor for extended periods. However, the effect of 1-MCP on fresh-cut fruits is variable. For example, the application of this compound in fresh-cut apples decreased the ethylene production, respiration, softening, color change and synthesis of aroma compounds (Jian and Joyce, 2002; Bai *et al.*, 2004; Calderón-López *et al.*, 2005). Vilas-Boas and Kader (2007) found different responses in firmness, color and CO_2 and C_2H_4 production, depending on the timing of 1-MCP application to kiwifruit, persimmon, and mango before (whole fruits) and after cutting (slices). In addition, exposure to 1-MCP has a synergistic effect when combined with $CaCl_2$ (1% for 2 min) plus controlled atmosphere (3 kPa O_2 + 10 kPa CO_2). This combined treatment slowed down softening, deterioration rate, and microbial growth of strawberries wedges (Aguayo *et al.*, 2006).

The aim of this study was to determine the potential of using calcium dips ($CaCl_2$) or 1-MCP (1 μl l^{-1} for 24h at 5 ºC) for extending the shelf life of arils pomegranate when whole fruit is stored with long periods. The combined effects of $CaCl_2$ dips with 1-MCP were also investigated.

II – Materials and methods

1. Material, fresh-cut preparation and treatments

'Wonderful' cv. pomegranates were stored for 10 weeks at 7.5 ºC in air until they were minimally processed. The whole fruit were sorted to eliminate any defects or decay. The arils were gently removed by hand, collected in a container and washed in cool (7ºC) chlorinated water (100 ppm NaOCl) (control). For calcium dip treatment, 10 g l^{-1} of $CaCl_2$ (99% purity, Merck) was added to the water and arils were dipped during 2 min. After the dip, arils were drained in a colander and dried with cheesecloth. For 1-MCP treatments, arils washed in chlorinated water was kept in glass jars inside a container with $1\mu l\ l^{-1}$ of 1-MCP during 24 h at 5ºC. The combination of $CaCl_2$ plus 1-MCP was also studied. Finally, 100 g of arils pomegranate from each treatment were packaged in glass jar (0.5 l) and kept in humidified air flow (725-900 ml h^{-1}) up to 12 days at 5ºC. For each treatment, three replicates were used.

2. Evaluations

Respiration rate and C_2H_4 emission. The respiration rate and C_2H_4 emission of arils were measured daily by taking 10 ml gas samples from the headspace of each jar through a pipe silicone using a plastic syringe. The CO_2 concentrations were determined in an infrared gas chromatograph (CG) (Model PIR-2000 R, Horiba Instruments, Irvine, CA) equipped with a thermal conductivity detector, and C_2H_4 emission was measured with a CG (model 211 Carle Instruments, Anaheim, CA) equipped with a flame ionization detector.

Firmness. A deformation test was used based on the resistance of each piece to a pressure applied by a Texture analyzer instrument (Texture Technologies Corp., Stable Micro System, NY). A 25 mm diameter steel cylindrical plate deformed each aril (1 mm depth) at a speed of 2 mm s^{-1}. The firmness of 54 pieces from each treatment was measured.

Sensorial quality. Five panelists were given approximately 5 g of fresh arils. After eating, they were asked about overall quality. This parameter was rated on a scale of 1 (very poor) to 9 (excellent), with the limit of marketability being at 5 and the limit of usability at 3.

Soluble solids content (SSC). Soluble solids content (SSC) of the juice was measured with an Abbé Refractometer, model 10450 (American Optical, Buffalo, NY) and expressed as %.

3. Statistical analysis

The experiment followed a completely randomized design (n = 3). To evaluate firmness, overall quality and SSC, the mean and standard error was calculated and analysis of variance (ANOVA) and least significant difference test ($P \leq 0.05$) to compare means within each sampling date.

III – Results and discussion

In all treatments, a uniformly low respiration rate (1-2 ml CO_2 kg^{-1} h^{-1}) was found (Fig. 1). This low respiration rate could be to a long period under cool storage of the whole fruits (10 weeks). A wound response was detected in the control treatment arils, as indicated by the increase in CO_2 (5 ml CO_2 kg^{-1} h^{-1}) and C_2H_4 (350-150 nl C_2H_4 kg^{-1} h^{-1}, Fig. 2). This stress was observed during the first days, probably due to the peeling operation. At the end of storage, arils treated with 1-MCP had a slightly reduced respiration rate. In all treatments, C_2H_4 emissions were decreasing as time storage increased (Fig. 2).
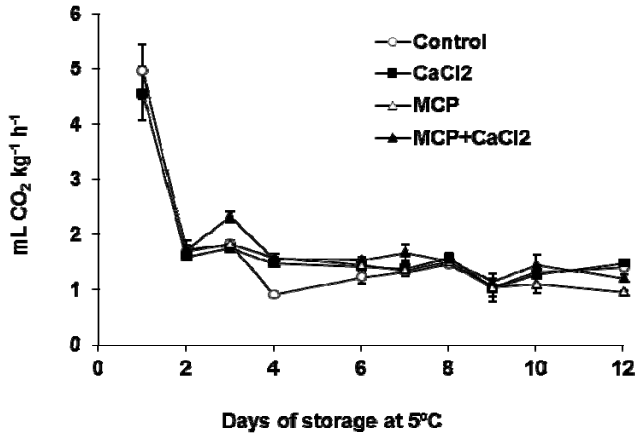
Fig. 1. Respiration rate of arils pomegranate 'Wonderful' in air up to 12 days at 5^oC. Mean (n = 3) ± S.E.
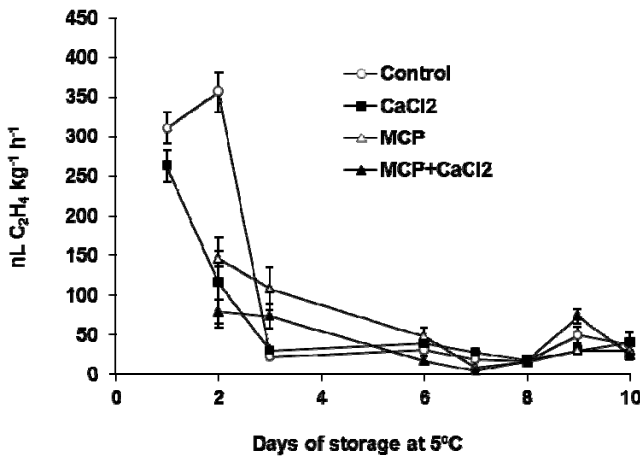
Fig. 2. C_2H_4 emission of arils pomegranate 'Wonderful' in air up to 10 days at 5^oC. Mean (n = 3) ± S.E.

During the peeled stress, arils dipped in $CaCl_2$ showed a lower C_2H_4 emission than control (350 nl C_2H_4 kg^{-1} h^{-1} vs 125 nl C_2H_4 kg^{-1} h^{-1}). Arils from 1-MCP, $CaCl_2$ and 1-MCP + $CaCl_2$ treatments kept a better firmness than control arils (Fig. 3).

However, this behavior was found only at the end of storage. Overall quality decreased at the end of storage. At day 12, arils treated with $CaCl_2$ or 1-MCP kept the best overall quality (Table 1) although all treatments were qualified above the limit of marketability. In SSC, no differences were found in any treatment (data not shown), with levels ranging from 16.6 to 17.2 °Brix. It is well known that firmness and resistance to softening can be increased by the addition of Ca, due to stabilization of the membrane systems and formation of Ca-pectates increasing the rigidity of middle lamella and cell wall and retarding the polygalacturonase activity (Poovaiah, 1986). The use of $CaCl_2$ (1%) dips reduced the initial stress from peeled arils (as indicated by C_2H_4 results).The delay of senescence often depends on the state of calcium in the tissue and

when this level is increased various senescence parameters are altered (Poovaiah, 1986). Arils treated with 1-MCP ($1\mu\lambda$ l^{-1} 24 h at 5ºC) reduced lightly the respiration rate during the last days. In this study, arils treated with $CaCl_2$ or 1-MCP kept the best overall quality and the firmness at the end of storage. These results are in agreement to other researchers who (treating with 1-MCP) found a lightly reduced respiration rate, retarded softening and provided a better overall quality in fresh-cut apples (Jian and Joyce, 2002; Bai *et al.*, 2004; Calderón-López *et al.*, 2005).
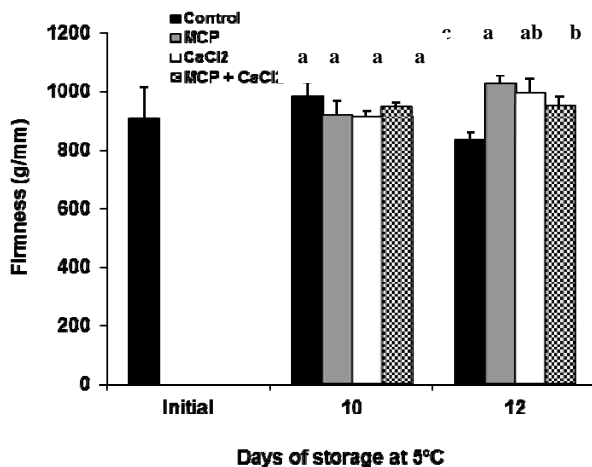
Fig. 3. **Firmness of arils pomegranate 'Wonderful' in air up to 12 days at 5ºC. Mean (n = 3) ± S.E.**

Table 1. **Effect of 1-MCP and calcium treatment on overall quality of arils pomegranate 'Wonderful' in air up to 12 days at 5ºC**

Treatment	LSD mean
Control	6.7 b
CaCl$_2$	7.6 a
1-MCP	7.2 a
CaCl$_2$ + 1-MCP	6.7 b

IV – Conclusions

CaCl$_2$ dips and 1-MCP treatments improved the firmness and market acceptability of pomegranate arils at the end of the storage life. The use of CaCl$_2$ dips is recommended rather than 1-MCP, as a lower cost-effective method of extending storage life in arils pomegranate.

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Harvest maturity and postharvest storage condition effects on pomegranate fruit quality

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Abstract. Little is known of the postharvest quality and storage potential of locally grown pomegranate cultivars in Georgia, U.S.A. Seven cultivars were harvested at two maturity stages (early and late) and stored either in regular air (RA; 5 °C, 90% to 95% R.H.) or in controlled atmosphere (CA; 3% O2, 5% CO2, 5 °C, 90% to 95% R.H.) for three months. Fruit were evaluated for physical and physiochemical attributes immediately after removal and after 7 days of keeping the fruit at 21°C. Late harvested fruits had significantly increased total soluble solids (TSS) and anthocyanins content compared to early harvested fruit. CA storage significantly reduced fruit disease compared to RA conditions. Acids degradation was significantly reduced in CA as compared to RA. Juice/weight of 50 arils was increased in CA which is an important consideration for fruit destined for juice production.

Keywords. Quality – Harvest maturity – Storage – Controlled atmosphere – Total soluble solids – Anthocyanins – Acid.

I – Introduction

Pomegranate (*Punica granatum* L.*)*, a species of Punicaceae family, is a fruit plant native to the region extending from Iran to the Himalayas. It is believed to be among the first fruits cultivated by humans since about 4000 BC. Within the past few thousand years it has spread all over the world, from Asia to Europe and then to North America. Presently cultivated in the tropical and subtropical regions of the world, it is a plant able to thrive in many different climates and soil conditions and can tolerate drought and salt stress. The production of this fruit has been increasing worldwide in response to increased consumption due to its health benefits (Basu and Penugonda, 2009).

Pomegranate juice has been reported to have antioxidant and antitumor activities (Singh *et al.*, 2002). Various alkaloids, flavonoids, polyphenolic compounds and hydrolyzable tannins, such as punicalin, pedunculagin, punicalagin and ellagic acid esters of glucose are present in the juice of the whole fruit. The juice has shown potential anti-atherogenic properties *in vivo* (Fuhrman *et al.*, 2005). Various organic acids are present in its juice, usually straight chain fatty acids, such as malic acid and citric acid (Neuhofer, 1990; Badria, 2002). Unsaturated fatty acids present in the juice also play an additional role in the antioxidant activity (Melgarejo *et al.*, 1995). The resulting antioxidant activity of the pomegranate juice is due to the contribution of all the potential compounds present in it.

The objectives of this work were:

(i) To study the changes in the physical properties of pomegranate cultivars in different storage conditions.

(ii) To determine the effect of harvest maturity and storage conditions on the physiochemical properties of juice.

II – Materials and methods

Pomegranate fruit were harvested from Ponder Farm in Ty Ty, Georgia, U.S. Seven cultivars ('Afganski', 'Crab', 'Cranberry, 'Entek-habi-saveh', 'Kaj-acik-anor', 'Nikitski ranni', 'Salavatski') were used for this study. Fruit were harvested at two maturity stages, early and late. At early maturity the fruit is unripe and has not reached the marketable quality. Fruit were brought to the Vidalia Onion Research Laboratory, University of Georgia, Tifton Campus, for analysis. After sorting, fruit were randomly divided into two groups and stored for three months in two storage conditions: regular air storage (RA; 5°C, 90%-95% R.H.) or controlled air storage (CA; 3% O_2; 5% CO_2; 90-95% R.H.). After removal from storage, fruit were analyzed immediately and seven days after removal.

Fruit were evaluated for physical quality attributes as weight, color, skin smoothness, disease incidence and physiochemical attributes as total soluble solids, titratable acidity and anthocyanins content. Fruits were weighed and graded for skin smoothness on scale from 1 to 5. Occurrence of sunscald, cracks, bruises and diseases (*Cercospora* and fungal infections) were graded on a scale from 1 to 3. Twenty five arils from two fruit per replication were squeezed in a cheese cloth to collect juice. Titratable acids were measured with an automatic titrator model DL-15 (Mettler Toledo, Switzerland) by using approximately 0.5 g of juice diluted with 25 ml of water. It was titrated to pH 8.2 using 0.1 M NaOH solution. Total soluble sugars were measured with a refractometer (Brixstix digital handheld refractometer, Livermore, CA). A colorimeter (CR-400, Konica Minolta, Ramsey, NJ; 8mm aperture, D65 illuminant) was used to record the fruit skin color, as L*, a*, b*, Chroma, and Hue. L* describes the degree of darkness or lightness with L=0, corresponding to black and L=100 as white; b* refers to the colors in the range yellow to blue; and a* refers to colors ranging from red-purple to blue-green. Color data were transformed using an arc-sine transformation. ANOVA procedure from the SAS Enterprise (SAS Institute, Cary, SC) was used to carry out the statistical analysis.

III – Results and discussion

The harvest time of pomegranate is very important in relation to the physical and physiochemical properties. With advancing maturity, there is increase in content of total anthocyanins, from early 847.40 ppm (s.e. 46.231) to late 1358ppm (s.e.78.3270). TSS and total titrable acids (early 2.7795%; S.E. 0.106 to late 2.92383%; S.E. 0.14479) content also increases with maturity in the juice (Fig. 1). Postharvest storage conditions affect pomegranate fruit quality and thus its marketability (Fig. 2). CA storage significantly reduced the incidence of the fruit disease caused by *Cercospora punica spp.* (CA average 1.50962, S.E. 0.0482 and RA 1.62019; S.E. 0.0439). The major effect of CA storage is on the acid content of fruit. Acids present in the juice degrade to a larger extent in fruit stored in RA (2.24103, S.E. 0.09806) as compared to CA (3.29362, S.E. 0.11343). Acids degradation leads to a change in flavor to a sweetened taste of the fruit. More information is required in order to establish proper harvest maturity.

IV – Conclusions

Controlled atmosphere storage was beneficial in maintaining pomegranate fruit quality in comparison to regular air storage by decreasing both fruit decay and the rate of degradation of juice constituents. Fruit maturity at harvest played an important role in determining fruit quality. Fruit harvested unripe had reduced total soluble solids, reduced acidity and reduced phytonutrient (anthocyanins) concentration.
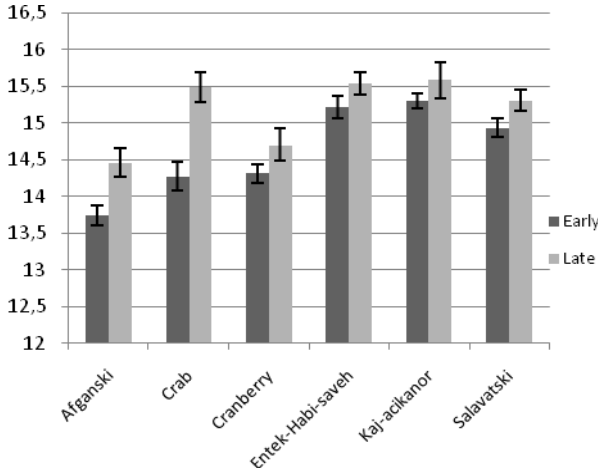
Fig. 1. Total soluble solids (%) in pomegranate fruit of various cultivars at two maturity stages (*P*<0.05).
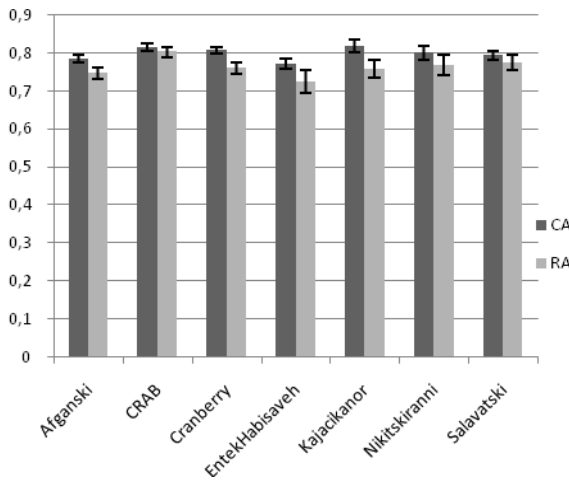
Fig. 2. Effect of storage conditions (CA and RA) on pomegranate juice/weight (g/g) of 50 arils (P<0.05).

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Changes in some free sugars and phenolic contents of pomegranate fruits (*Punica granatum* L.) in three development stages

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Abstract. In this study, some free sugars and phenolic contents at three development stages (immature, unripe and full ripe) of pomegranate cv. Hicaznar fruits grown in Antalya, Turkey were determined. While total sugar contents showed a regular increment, total phenol content decreased from the immature fruit to full ripe fruit. The predominant sugars quantified were fructose and glucose, while sucrose was minor sugar in all development stages. Fructose contents in immature fruits to mature fruits varied from 3.53 to 5.78 g per 100g fresh weight basis, while glucose contents varied from 3.44 to 6.37 g per 100g fresh weight. On the other hand, sucrose contents varied from 0.21 to 0.41 g per 100g fresh weight. On the contrary to sugars, the lowest levels of total phenolics and flavanoids were determined in full ripe fruits. Results indicate that both free sugars and phenolic contents of pomegranate were strongly influenced by development stages.

Keywords. Pomegranate fruit – Sugar – Phenolic – HPLC.

I – Introduction

Pomegranate is grown mainly in Mediterranean and Asian countries. Among these countries, India, Iran, China and Turkey are main producers. Other important producers are Spain, Tunisia, Morocco, Afghanistan, China, Pakistan, Azerbaijan, Armenia, Cyprus, Egypt, Israel and Saudi Arabia (Ercisli *et al.*, 2007; Gozlekci *et al.*, 2011). The number of pomegranate orchards in Turkey has been increasing rapidly in recent years because of high profit earnings. The cultivar 'Hicaznar' is the most important cultivar for export to European countries (Gozlekci *et al.*, 2011).

Pomegranate fruit is rich in polyphenols including ellagitannins, gallotannins, ellagic acids, gallagic acids, catechins, anthocyanins, ferulic acids, and quercetins. Phenolic compounds and flavonoids are unique category of plant phytochemicals especially for their vast potential in health-benefiting properties. They represent the most abundant and the most widely represented class of plant natural products (Fraga, 2010). Many studies focused on the physico-chemical characteristics of pomegranate fruits at maturity stage. We aimed to compare physico-chemical characteristics of pomegranate on three development stages.

II – Material and methods

Pomegranate cv. 'Hicaznar' sampled at three development stages: 1st stage; immature fruit (May 15), 2nd stage; un-ripe fruit (July 15); 3rd stage; full ripe fruit (October 15). A total of 30 fruits were used for physical measurements (Table 1). Pomegranate juice was obtained from pomegranate arils by a hand press, and homogenized for total phenolic, total flavonoid and sugar extractions. Total soluble solid, pH and acidity were determined directly on juice (AOAC, 1995). Total phenolics were estimated in Folin-Ciocalteu reagent according to Singleton *et al.*,

(1999). Total flavonoids were estimated according to Miliauskas *et al.* (2004). All determinations were carried out in quadruplicate and the mean values were used. Sugars in the samples were determined by HPLC analysis (Miron and Schaffer, 1991).

Table 1. Physical properties of pomegranate fruits at development stages (mean±SE)

Fruit characteristics	Fruit development stages		
	Immature	**Un-ripe**	**Full ripe**
Fruit weight (g)	12.51±2.27 [c]	128.21±2.27[b]	418.62±2.12[a]
Fruit diameter (mm)	27.76±0.66[c]	64.31±0.66[b]	95.18±0.62[a]
Fruit length (mm)	52.75±1.52[c]	80.41±1.52[b]	106.38±1.42[a]
Shape index (FD/FL)	0.52 ±0.02[c]	0.80±0.02[b]	0.90±0.01[a]
Calyx length (mm)	24.05±1.16[ns]	24.14±1.16	25.74±1.08
100-aril weight (g)	0.28±0.33[a]	7.56±0.33[b]	32.08±0.31[c]
Aril yield (%)	13.22±1.05[c]	46.09±1.05[b]	56.72±0.99[a]
Juice yield (%)	8.23±0.85[c]	26.97±0.85[b]	49.37±0.80[a]
Seeds in aril (%)	35.81±2.14[a]	41.48±2.14[a]	16.48±2.01[b]
Skin thickness (mm)	5.13±0.13[a]	4.78±0.13[a]	4.12±0.12[b]
Skin color parameters			
a*	21.90±1.58[c]	-8.71±1.58[b]	39.88±1.48[a]
b*	20.08±1.15[c]	36.41±1.15[a]	27.01±1.08[b]
Lightness (L*)	38.50±1.43[c]	54.51±1.43[b]	59.21±1.34[a]
Chroma (C*)	29.83±1.64[c]	37.48±1.64[b]	48.27±1.53[a]
Hue angle (H[o])	42.11±1.62[a]	-76.58±1.62[c]	34.41±1.52[b]
Aril color parameters			
a*	-0.66±0.21[c]	2.55±0.21[b]	5.97±0.20[a]
b*	13.95±0.35[a]	11.74±0.35[b]	2.04±0.33[c]
Lightness (L*)	61.08±0.70[a]	48.37±0.70[b]	31.22±0.66[c]
Chroma (C*)	13.97±0.39[a]	12.02±0.39[b]	6.31±0.36[c]
Hue angle (H[o])	-87.22±0.64[c]	77.65±0.64[a]	18.89±0.60[b]

Means in each row followed by different letters are significantly different (P≤ 0.05); ± standard error.

All the statistical analyses were carried out using SPSS statistical software (SPSS 19.0, 2010) and results were expressed as mean ± standard error. ANOVA was performed by ANOVA procedures. Significant differences among the means were determined by 'Duncan's multiple range test' at a level of p<0.05.

III – Results and discussion

Table 1 shows physical properties of pomegranate fruits at different development stages and demonstrated significant differences on most characteristics (*P*<0.05). Average fruit mass of 'Hicaznar' ranged from 12.51g (immature fruit) to 418.62 g (full ripe fruit). The fruit length and diameter values were determined between 52.75 mm and 106.38 mm and 27.76 mm and 95.25 mm in immature and full ripe stages, respectively. Fruit shape (expressed as diameter/length ratio), of immature fruits were oblong and full ripe fruits were oblate. There were significant differences (P<0.05) in aril ratio and juice yield among the development stages. Full ripe fruits had the highest aril and juice yield (56.72% and 49.37%) (Table1). In previous studies, a wide variation in fruit mass of pomegranate cultivars varying between 150 and 568 g were reported, (Ercan *et al.*, 1992; Yilmaz *et al.*, 1992; Tehranifar *et al.*, 2010). Fruit length and width of full ripe

pomegranate cultivars grown in different countries ranged from 61 to 91 mm and 36 to 104 mm, respectively (Yilmaz *et al.*, 1992; Amaros *et al.*, 1998; Barone *et al.*, 2001). Fruit skin and aril colors of pomegranate fruits at different stages of development are shown in Table 2. Skin color of fruits varied from green to dark red color during fruit development. Skin and aril colors expressed as luminosity and chroma were ≈42.11 and 29.83 for immature fruit and ≈34.41 and 48.27 for full ripe fruits, respectively. Red aril color is one of the most important sensory characteristics of pomegranate pulp and juice (Celik and Ercisli, 2009).

As shown in Table 2, significant statistical differences (P<0.05) among all studied chemical parameters in different fruit development stages were observed. Soluble solid content values showed an increase during fruit development stages which is the highest (16.08%) in full ripe fruits. Titratable acidity was significantly lower in full ripe fruits (1.64%) compared to unripe fruits (3.98%). Dark red aril color, high SSC and relatively high acidity of pomegranate arils are considered to be a good choice for both fresh fruit and juice markets (Ozgen *et al.*, 2008).

Table 2. Chemical properties of pomegranate juice at development stages (mean±SE)

Juice characteristics	Fruit development stages		
	Immature	Un-ripe	Full ripe
Titratable acidity (%)	1.16±0.08[c]	3.98±0.08[a]	1.64±0.08[b]
pH	4.60±0.07[a]	2.37±0.07[c]	3.19±0.06[b]
Soluble solid content (%)	5.79±0.11[c]	9.03±0.11[b]	16.08±0.10[a]
T. phenolics (mg GAE/ L)	2920.0±0.16[a]	2340.0±0.16[b]	986.7±0.16[c]
T. flavonoids (mg GAE/ L)	800.0±0.03[a]	330.0±0.03[b]	580.0±0.03[c]
Fructose (g/100 ml)	3.54±0.29[c]	5.04±0.29[ab]	5.78±0.29[a]
Glucose (g/100 ml)	3.44±0.25[c]	5.00±0.25[b]	6.37±0.25[a]
Sucrose (g/100 ml)	0.21±0.18[c]	0.32±0.18[b]	0.41±0.18[a]
T. sugars (g/100 ml)	7.19±0.60[c]	10.40±0.60[b]	12.56±9.60[a]

The values shown are mean ± standard error of three replications. Significant differences among the means were determined by Duncan's multiple range test (*P*≤ 0.05) within parameters in each line; means with the same letter do not differ significantly.

There were significant differences in total phenolics and total flavonoid contents in juice obtained from different fruit development stages at *P*<0.05 (Table 2). Pomegranates showed a rapid and significant depletion in total phenolics during the fruit development from initial stage to full ripe stage. Total phenolics and total flavonoids ranged from 986.7 (full ripe fruit) to 2920.0 mg GAE/L (immature fruit) and 330.0 (un-ripe fruit) to 800.0 mg GAE/L (immature fruit) in juice. Karadeniz *et al.* (2004) reported total phenolics (2408 mg kg^{-1}) and total flavonoids (459 mg kg^{-1}) in pomegranate.

Fructose concentration varied from 3.54% (immature fruit) to 5.78% (full ripe fruit), while glucose values were between 3.44% (immature fruit) and 6.37% (full ripe fruit) in juice (Table 2). Ozgen *et al.* (2008) reported an average of 6.4% and 6.8%, and Al-Maiman and Ahmad (2002) of 6.66% and 7.72% fructose and glucose contents. Glucose and fructose were the dominant sugars in juice at fruit development stages. Glucose, fructose, sucrose and total sugar values increased with the advance in maturity. The increase in total and individual sugars in fruit juice can be due to hydrolysis of starch into simple sugars as it was similarly reported by Biale (1960).

IV – Conclusion

Physical and chemical properties of the pomegranate cultivars indicated that the developmental

stage of fruit for 'Hicaznar' is an important factor in determining fruit quality. The study provided important data for physical and compositional changes of the fruits at different development stages of pomegranate, emphasizing that pomegranate fruit can be a good source of nutrients at different maturation stages.

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Effects of salicylic acid, jasmonic acid and calcium chloride treatments on reduction of chilling injury in pomegranate fruit

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Abstract. The aim of this experiment was to investigate the effects of salicylic acid, jasmonic acid and calcium chloride on reduction of chilling injury in pomegranate fruits cvs. ˈMalas Yazdiˈ and ˈMalas Ashkezarˈ. Salicylic acid (1 and 2 mM from source of acetyl salicylic acid), jasmonic acid (0.3 and 0.4 mM from source of n-propyl dihydrojasmonate), calcium (1 and 2 % from source of calcium chloride) and control (distilled water) were used as treatment. After the treatments fruits were stored in cold storage at 1.5 ^{o}C±0.5 and 85±5% relative humidity for 2 months. The results revealed that treatments with salicylic acid (SA), jasmonic acid (JA) and calcium chloride ($CaCl_2$) significantly reduced the percentage of chilling injury of pomegranate fruits. The lowest chilling injury was observed in 0.4 mM of JA-treated fruits and the highest one was for untreated fruits. Treatments of fruits with 0.3 mM JA and 2 mM SA increased the electrolyte leakage of fruits but it was not significantly affected by other treatments. It was also showed that treatments of fruits with SA, JA and $CaCl_2$ had no significant effect on pH, ripening index, but the total soluble solids of fruit juices was increased. The results evidenced that pomegranate fruits treated with 2 mM SA had the lowest ascorbic acid, but it was not significant difference between other treatments and untreated fruits. In conclusion, cultivar of ˈMalas Ashkezarˈ exhibited higher tolerance to cold storage compared to ˈMalas Yazdiˈ, therefore cultivar of ˈMalas Ashkezarˈ recommended to keep in cold storage for long time.

Keywords. Arils – Phenolic compounds – Antioxidants – Browning.

I – Introduction

Pomegranate (*Punica granatum* L.) belonging to the family punicaceae is one of the favorite table fruits of tropical and subtropical regions. The edible part of the fruit is called arils, which contain around 80% juice and 20% seed. The fresh juice contains 85.4% moisture and considerable amounts of total soluble solids, total sugars, reducing sugars, anthocyanins, phenolics, ascorbic acid and proteins (El-Nemr *et al.*, 1990) and has also been reported to be a rich source of antioxidants (Gil *et al.*, 2000; Kulkarni *et al.*, 2004).

To reduce the occurrence of chilling injury (CI) in pomegranate several techniques have been applied, including intermittent warming (Artes *et al.*, 2000; Mirdehghan *et al.*, 2007b), polyamine (Mirdehghan *et al.*, 2007a), salicylic acid (Sayyari *et al.*, 2009).

Salicylic acid (SA) is known for its induction of plant defense against biotic and abiotic stress and is reported to increase chilling tolerance in peach fruit (Wang *et al.*, 2006), tomato fruit (Ding *et al.*, 2002) and sweet peppers (Fung *et al.*, 2004. Exogenous SA treatment may also induce the expression of pathogenesis-related (PR) protein (Malamy *et al.*, 1990) and establish systemic acquired resistance (SAR) (Gaffney *et al.*, 1993).

In recent research, methyl jasmonate (MeJA) has been applied to reduce the development of chilling injury symptoms in a number of horticultural crops, including Zucchini squash (Wang and Buta, 1994), mango (Gonzalez-Aguilar *et al.*, 2000), avocado, grapefruit and peppers (Meir *et al.*, 1996). Reduction in chilling injury by MeJA might be due to enhanced antioxidant enzyme activity and a higher unsaturated/saturated fatty acid ratio (Cao *et al.*, 2009).

The postharvest application of calcium to some horticultural commodities has been demonstrated to reduce the incidence of chilling-induced disorders. Application of calcium significantly reduces the severity of chilling injury in avocados (Chaplin and Scott, 1980), peaches (Wade, 1981) and tomatoes (Moline and Teasdale, 1981). Lester and Grusak (1999) have shown that calcium application in plums was effective in terms of membrane functionality and integrity maintenance, with lower losses of phospholipids and proteins and reduced ion leakage. The aim of this study was to determine the effects of SA, JA and $CaCl_2$ on reducing CI in pomegranates stored at $1.5 \pm 0.5^{\circ}C$ followed 3 days at $20^{\circ}C$.

II – Material and methods

1. Plant material and treatment

Pomegranate fruits cvs. ˙Malas Yazdi, and ˙Malas Ashkezar, at ripe stage were harvested on October 14, 2008 from the Agricultural Research Center of Yazd province. Fruits were immediately transported to the laboratory. Pomegranates were sorted based on size and the absence of physical injuries or sunburn, then were randomly divided into 7 lots of 128 fruits for the following treatments: lot 1 and 2 were immersed into solution of 1 mM and 2 mM ASA, pH 3.5 for 5 min. lot 3 and 4 were immersed into the solution of 0.3 mM and 0.4 mM PDJ at 25ºC for 15 min. lot 5 and lot 6 were dipped in 1% and 2% $CaCl_2$ solution containing tween-20 (2ml l⁻¹) for 5 min. The last lot of fruits was dipped for 5 min in distilled water, which served as the control. After immersion, the fruits were air-dried and then stored at $1.5 \pm 0.5ºC$ and $85 \pm 5\%$ relative humidity (RH) for 63 days. Fruit samples were taken after immersion (0) and at 21-day intervals during storage and further stored at 20ºC for 3 days.

2. Chilling injury and electrolyte leakage

CI index was evaluated according to external skin browning, as follows: 0 (no symptom); 1 (20% of the browning lesion); 2 (40% of the browning lesion); 3 (60% of the browning lesion); 4 (80% of the browning lesion); 5 (100% of the browning lesion). The severity of CI was calculated by the following formula: CI= Total No × 100/ 5 × N. of fruits

The rate of electrolyte leakage was determined as described by McCollum and McDonald (1991). For each husk, six discs (10 mm) of peel tissue were cut with a cork borer. Conductivity after incubation in 25 ml of 0.4 M Manitol was measured with a conductivity meter after 4h of incubation under constant shaking. After reading was taken, the vials were autoclaved at 121ºC for 20 min, held overnight and conductivity was measured again for total electrolytes. The rate of electrolyte leakage was expressed as a percentage of total: (initial/total) × 100.

3. Total soluble solids, titratable acidity and ascorbic acid

Total soluble solids concentrations (TSS) were measured with refractometer and expressed as ºBrix. Titratable acidity (TA) was assayed by titration with 0.2 N NaoH to pH 8.2 and expressed as gram of citric acid equivalent per 100 g⁻¹ fresh weight. Ascorbic acid content was determined by titration with Iodine and expressed as (mg⁻¹ 100g of fresh fruits).

4. Antioxidant activity and total phenolic compounds

The arils of each replicate were combined and frozen in liquid N_2, were milled to obtain homogeneous samples, and were stored at -20ºC until analysis. For each sample, 5 g of arils was homogenized in 10 ml of 50 mM phosphate buffer pH 7.8 and then centrifuged at 4800 rpm for 15 min at 4ºC. The supernatant was used for total antioxidant activity and total phenolic compounds quantification in duplicate, as previously described (Serrano et al., 2005). For TAA, L-ascorbic acid was used for calibration curve, and the results were expressed as mg ascorbic

acid equivalent 100 g^{-1} fw (fresh weight). The total phenolic compounds were quantified using the Folin-Ciocalteu reagent and results were expressed as mg gallic acid equivalent 100 g^{-1} fw.

5. Statistical analysis

The experimental design was factorial complete randomized design (CRR) with four replicates. Data for the analytical determinations were subjected to analysis of variance (ANOVA). Mean comparisons were performed by Duncan's Multiple Rang Test. Differences at $p < 0.05$ were considered as significant. All analyses were performed with MSTATC.

III – Results and discussion

1. Chilling injury symptoms and electrolyte leakage

In pomegranate fruits CI, manifested as skin browning, increased during storage but was affected by treatment. The highest chilling injury was observed for control fruit at 63 days, and the lowest one was for 0.4 mM JA and 1 mM SA at 21 days (Fig. 1). These results were agreement with Ding *et al.* (2001) who reported that MeSA and MeJA treatments reduced chilling injury in tomato fruit. The effect of MeSA and MeJA on alleviating chilling injury cold storage may be attributed to its ability to induce the accumulation of heat shock protein (HSP) (Ding *et al.*, 2001) and antioxidant systems (Wang *et al.*, 2006; Evans *et al.*, 1991).
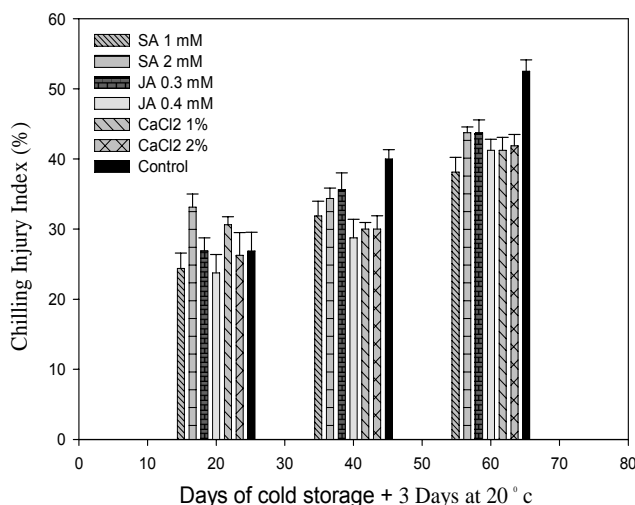
Fig.1. Chilling injury index of control and treated pomegranates after several periods of cold storage +3 days at 20ºC (shelf-life). Data are means +S.E.

With respect to electrolyte leakage (EL), there were no significant differences between treated fruits and control, whereas EL in fruits treated with 0.3 mM JA and 2 mM SA was higher than that of control (Fig. 2). Results in Fig. 2. Were showed that EL gradual increase within 42 days, followed by a decrease from 42 to 63 days. contrary to our findings Meng *et al.* (2009) who had observed that MeJA treatment in peach fruit could reduce cell membrane electrolyte leakage by

maintaining membrane integrity. Also Sayyari *et al.* (2009) have reported that SA treatment were effective in reducing electrolyte leakage in pomegranate.
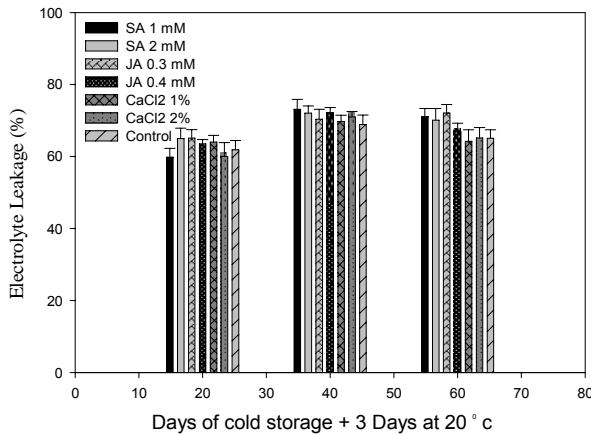
Fig. 2. **Electrolyte leakage of control and treated pomegranates after several periods of cold storage +3 days at 20 ⁰C (shelf-life). Data are means +S.E.**

2. Total soluble solids, total acidity and ascorbic acid

The influence of all treatment on total soluble solids (TAA) is shown in Table 1. SA applied at 1 mM had significantly higher TSS than non-treated fruits. These results are in line with Serivastava and Dowidy (2000) who reported that treatment with SA increased TSS in banana fruits.

Table 1. **Changes in total soluble solids (TSS, ⁰Brix), total acidity (TA, g 100 ml^{-1}), ascorbic acid (AA, mg 100 ml^{-1}) and total phenolic compounds (mg eq. gallic acid 100 g^{-1}) in control and treated pomegranate during storage**

	TSS	TA	AA	Total phenolic compounds
SA 1 mM	13.27 a	0.88 a	15.62 ab	65.69 a
SA 2 mM	13.00 ab	0.90 a	14.01 b	62.79 a
JA 0.3 mM	12.92 ab	0.89 a	15.68 ab	59.44 a
JA 0.4 mM	12.83 ab	0.90 a	15.75 ab	63.13 a
CaCl$_2$ 1%	13.09 ab	0.92 a	15.75 ab	62.78 a
CaCl$_2$ 2%	13.13 ab	0.89 a	16.48 a	62.62 a
Control	12.75 b	0.90 a	15.57 ab	62.71 a

For each parameter, similar letter within rows are not significantly at p< 0.05 level.

The results showed that total acidity (TA) were not influenced by any treatments (Table 1). Similar observation has been reported by Ding *et al.* (2007) and Biten Court De Souza *et al.* (1999) who had described that TA were not affected by SA or MeJA or CaCl$_2$, respectively. Results evidenced that pomegranate fruits treated with 2 mM SA had the lowest ascorbic acid (AA), but it was not significant difference between other treatments and untreated fruits (Table

1). Our results are consistent with the finding of González-Aguilar *et al.* (2004) who reported that ascorbic acid was not affected by the MJ treatment.

3. Total phenolic compounds and total antioxidant activity

Total phenolic compounds were not influenced by all treatments (Table 1). These results are agreement with González-Aguilar *et al.* (2004) who had described that Total phenolics were not affected by the MJ treatment. While, Rudell *et al.* (2002) reported that treatments of MJ in apple Fuji induced the accumulation of chlorogenic acid. The explanation for this behavior can be due the fact that we measured changes in total phenols. According to previous reports punicalagin has been described as the major compound in pomegranate arils contributing to total antioxidant activity (TAA) (Kulkarni *et al.*, 2004). Therefore, further research is necessary to assay amount of punicalagin separately.

Total antioxidant activity (TAA) increased at the midpoint of cold storage, then gradually decreased (Fig. 3). The results revealed that treatments with 1, 2 mM SA and 0.3 mM JA decreased TAA, but it was not significantly affected by other treatments. Contrary to our findings Huang *et al.* (2008) reported that application of SA could increase antioxidant enzyme activity and thus delay membrane lipid peroxidation. Therefore, they suggested that pretreatment with SA in combination with low temperature may be a useful strategy for prolonging orange postharvest life and maintaining nutritional conditions during storage. In pomegranate cultivars, anthocyanin, ascorbic acid and phenolics are responsible for the TAA, alone or in combination (Kulkarni *et al.*, 2004). In our experiment the content of total phenolics did not change during storage, and the amount of ascorbic acid reduced throughout storage period. While, TAA increased with prolonging storage time that may be due to increased of anthocyanin or punicalagin as the major phenolic compound that contributes to TAA.
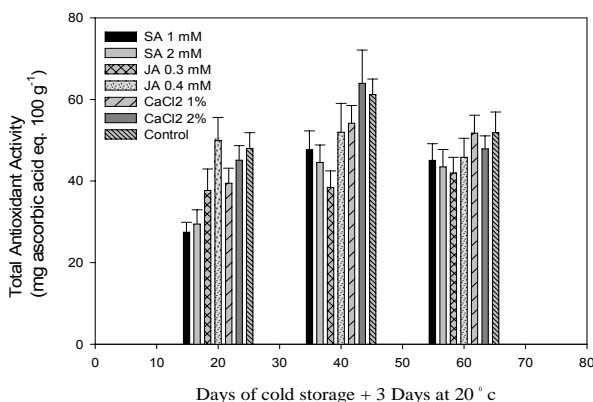
Fig. 3.   Total antioxidant activity in arils during cold storage +3 days at 20ºC (SL) of control and treated pomegranates.

Based on the data it was concluded that treatment with 0.4 mM JA was the most effective for reducing CI. The reduction in chilling injury by JA may be due to enhanced antioxidant enzyme activity (Cao *et al.*, 2009). However, further research is necessary to carry out with different postharvest applications (pressure-infiltration and vaccum infiltration) and concentrations for reducing CI in pomegranate fruits and maintaining antioxidant activity and nutritional conditions during storage.

In conclusion, cultivar of ˙Malas Ashkezar˙ had higher total soluble solids, total acidity, ascorbic acid, phenolic compounds and total antioxidant activity than that of ˙Malas Yazdi˙. Moreover, cultivar of ˙Malas Ashkezar˙ exhibited higher tolerance to cold storage compared to˙Malas Yazdi˙, therefore cultivar˙Malas Ashkezar is recommended to keep in cold storage for long time.

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Acetyl salicylic acid alleviates chilling injury and maintains nutritive and bioactive compounds during cold storage

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Abstract. Pomegranates are highly perishable and to extend storability the refrigeration is therefore necessary, but this fruit is susceptible to chilling injury (CI) if stored longer at temperatures lower than 5ºC. Acetyl salicylic acid (ASA) is present in vegetable tissues, its concentration being below 0.2 mg kg^{-1} in some fruits. In this experiment the effect of ASA treatments at three concentrations (0.1, 0.5 and 1.0 mM) on pomegranate quality and nutritive and bioactive compounds after storage for 14-84 days at 2ºC plus 4 days at 20ºC was assayed. Control fruit exhibited more CI symptoms (manifested by pitting and browning) than treated fruit during storage, which were accompanied by increased softening, ion leakage and respiration rate. The ASA treatments were also effective in maintaining higher contents of nutritive (sugars and organic acids) and bioactive compounds (acid, total phenolics and anthocyanins), ascorbic acid and total antioxidant activity. These results suggest that ASA could have potential postharvest application to reduce CI, maintain quality and improve the health benefits of pomegranate fruit consumption by increasing their antioxidant capacity.

Keywords. Pomegranate – Chilling injury – Acetyl salicylic acid – Quality – Bioactive compounds.

I – Introduction

The juice of pomegranate arils contains high concentration of sugars, organic acids, vitamins, polysaccharides, and essential minerals, as well as bioactive compounds with antioxidant activity and health beneficial effects (Mertens-Talcott *et al.*, 2006). Pomegranate is a highly perishable fruit and then to extend shelf life refrigeration is therefore necessary, but the fruits are susceptible to chilling injury (CI) with symptoms such as browning of the husk, pitting, husk-scald, loss of firmness, and higher sensitivity to decay (Mirdehghan, *et al.*, 2007a; 2007b). Acetyl salicylic acid (ASA) is a closed analogue of salicylic acid (SA) and has been shown to be effective on reducing CI in loquat (Cai *et al.*, 2006). Thus, the main objective of this paper was to study the effect of ASA on pomegranate fruit quality attributes during storage under chilling conditions, as well as their role on the content of nutritive (sugars and organic acids) and bioactive compounds (polyphenol and anthocyanins) and the antioxidant capacity determined in both hydrophilic (H-TAA) and lipophilic (L-TAA) fractions, separately.

II – Materials and methods

Pomegranates (*Punica granatum* L. cv. Mollar de Elche) were harvested when fully mature from a commercial plot in Elche (Alicante, Spain) and 195 homogeneous fruits were selected, from which 15 were used to determine the characteristics at harvest, and the remained 180 fruits were randomized and divided into 4 lots for the following treatments: control (no treatment) and acetyl salicylic acid (ASA) at 0.1, 0.5 and 1.0 mM concentration. Treatments were performed by dipping the pomegranates in 20-L solution for 10 min before storage at 2°C, in permanent

darkness and with relative humidity of 90%. Every 2 weeks 1 lot from each replicate and treatment was transferred to a chamber at 20°C for 4 days for analytical determinations. Chilling injury (CI), fruit firmness and skin colour were assayed in the whole fruit. Then, each husk was carefully cut at the equatorial zone with sharpened knives, the skin was used to determine ion leakage according to Mirdehghan *et al.*. (2007b), and arils were manually extracted. The arils of each replicate, obtained from equatorial fruit zones, were combined and frozen in liquid N_2, milled and stored at -20°C, in which total soluble solids (TSS) and total acidity (TA) were determined as reported by Mirdehghan *et al.* (2007b), and total phenolics, total anthocyanisns and total antioxidant activity according to Mirdehghan *et al.* (2007c).

III – Results and discussion

CI increased during storage although scores were always significantly higher in control than in treated pomegranates the 1 mM ASA doses being the most effective in reducing CI symptoms, which included husk pitting, browning and desiccation being responsible for extensive postharvest losses and limiting the fruit storability. Generally, CI leads to damage of the cell membranes that can be measured by the ion leakage, which was significantly higher in the skin of control than in treated fruit, ≈65 and ≈55%, respectively at the last sampling date (data not shown). These results show a role of ASA on maintaining membrane integrity, as has been reported for loquat fruit (Cai *et al.*, 2006). In addition, the application of ASA was able to reduce softening and acidity losses (data not shown) that occurred in control fruit during postharvest storage as consequence of the advance of the ripening process (Mirdehghan *et al.*, 2007a; Sayyari *et al.*, 2011), showing an effect on delaying the ripening process, according to previous reports in climacteric fruit such as kiwifruit (Zhang *et al.*, 2003), banana (Srivastava and Dwivedi, 2000), and sugar apple (Mo *et al.*, 2008), SA reduced the ripening process during storage by suppressing and/or delaying ethylene production and respiration rate, and the related parameters such as firmness, TA and TSS.

The content of total phenolic compounds at harvest was 261.19±6.97 mg 100 g^{-1} and diminished throughout storage in control fruit with final concentration of 234.10±2.59 mg 100 g^{-1} (Fig. 1), while no significant changes were observed in ASA-treated pomegranates for any of the applied dose with values ≈270 mg 100 g^{-1} at the end of the experiment. With respect to total anthocyanins, the levels at harvest (59.03±9.52 mg 100 g^{-1}) significantly increased along storage, the increase being higher in ASA-treated fruit than in control pomegranates with final levels of ≈110 and ≈130 mg 100 g^{-1} for control and treated fruits, respectively (Fig. 1). For H-TAA (Fig. 1), a significant reduction was observed in control fruit over storage, from levels at harvest of 85.08±4.96 to 46.06±0.82 mg 100 g^{-1} at the end of the experiment, while significant lower decreases were found in treated fruits. On the contrary, the levels at harvest of L-TAA were lower and did not change along storage irrespective of treatments. Taking into account the change in H-TAA, it could be confirmed that both total phenolics and ascorbic acid are the main compounds contributing to the antioxidant capacity of the pomegranate arils, in agreement with previous reports (Gil *et al.*, 2000; Mirdehghan *et al.*, 2006, 2007c).

In conclusion, the data presented here unequivocally suggest that ASA reduced CI symptoms in pomegranates, delayed postharvest ripening processes and increased antioxidant potential by enhancing or maintaining bioactive compounds such as total phenolics, total anthocyanins and ascorbic acid. Hence, the application of ASA treatments could be considered as a natural postharvest tool to extend the commercialization and marketability of pomegranate, even at low temperatures which usually induce CI occurrence. In future, determination of endogenous SA after ASA treatment would answer the question of whether these effects are due to ASA per se or its conversion to SA.
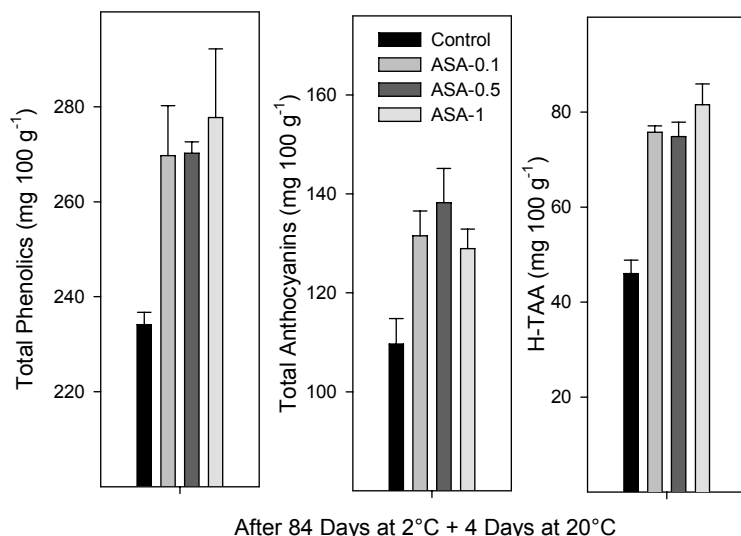
Fig. 1. Total phenolics, total anthocyanins and total antioxidant activity in the hidrophylic fraction of pomegranate arils treated with acetyl salicylic acid after 84 days of cold storage plus 4 days at 20ºC.

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Effect of oxalic acid treatment on maintaining pomegranate fruit quality and antioxidant potential

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Abstract. The aim of this work was to study the effect of pre-storage oxalic acid treatment at three concentrations (2, 4, and 6mM) on pomegranate CI and fruit quality after long-term storage at 2ºC. The CI symptoms were significantly reduced by oxalic acid treatment, especially for the 6 mM concentration. In addition, control pomegranates showed a significant reduction in the content of total phenolics and ascorbic acid as well as in total antioxidant activity (TAA), in both hydrophilic (H-TAA) and lipophilic (L-TAA) fractions after long term storage. However, the application of oxalic acid led to lower losses of total phenolics and significant increase in both ascorbic acid content and H-TAA, whereas L-TAA remained unaffected. Thus, oxalic acid could be a natural promising postharvest treatment to alleviate CI and increase antioxidant potential of pomegranate, by enhancing or maintaining the bioactive compounds.

Keywords. Fruit quality – Organic acids – Phenolic compounds – Antioxidant activity.

I – Introduction

During postharvest storage of pomegranate important quality loss occurs due to several physiological and enzymatic disorders, such as desiccation, browning symptoms in both peel and arils, and losses of firmness, aril color, vitamin C and acidity, leading to reduction of acceptability in terms of freshness, juiciness and taste (Kader, 2006). Refrigeration is effective to prolong the storability of pomegranate, but the fruit are susceptible to chilling injury (CI). Oxalic acid is a natural organic anion which is found ubiquitously in plant species and plays different roles in controlling fruit tissue browning, inducing systemic resistance, retarding fruit ripening and controlling decay. In addition, the endogenous concentration of oxalic acid in many plant foods has been considered as a natural antioxidant by suppressing in vitro lipid peroxidation (Kayashima and Katayama, 2002). Thus, the aim of this work was to study the effect of pre-storage oxalic acid treatment at 3 concentrations on pomegranate fruit quality and on the contents of ascorbic acid, total phenolic compounds, and anthocyanin profile, and total antioxidant activity after prolonged postharvest storage.

II – Materials and methods

Fully mature pomegranate fruits (*Punica granatum* L. cv. Mollar de Elche) were picked in a commercial orchard in Elche (Alicante), 75 homogeneous fruits in size and color were selected and sorted at random in lots of 5 fruits. Three lots were used to analyze fruit properties at harvest (day 0) and the remained lots were randomized and divided into 4 groups for the following treatments in triplicate (each replicate contained 5 individual fruits): control (distilled water at 25°C) and oxalic acid treatment at 3 concentrations (2, 4 and 6 mM). Oxalic acid was purchased from Sigma (Sigma Aldrich, Madrid, Spain 97% purity). Fruits were dipped in 20-L

solution for 10 minutes. Following treatments, fruits were placed on Kraft paper and allowed to dry for 20 hours at 20°C. Then, they were transferred to a temperature-controlled chamber at 2°C, in permanent darkness and with relative humidity of 90%. After 84 days, fruits were sampled for the following determinations: electrolyte leakage (EL), total soluble solids (TSS), total acidity (TA), fruit firmness, weight loss, respiration rate, chilling injury index (CI), total antioxidant activity, in both hydrophilic (H-TAA) and lipophilic fractions (L-TAA), total phenolic, total anthocyanin and ascorbic acid, according to Mirdehghan *et al.* (2006; 2007a; b).

III – Results and discussion

Control pomegranates exhibited CI symptoms manifested as browning, pitting and dehydration of the husk surface, reaching a CI index value of 1.64±0.24, that is CI symptoms affected from 25-50% of the fruit surface. In addition, increases in respiration rate, weight loss and electrolyte leakage (EL) were observed after 84 days of cold storage. However, these changes were significantly (P<0.05) reduced in oxalic acid-treated fruits, the efficacy being higher for the 6 mM oxalic acid applied dose (data not shown). This effect could be attributed to the inhibition of polyphenoloxidase and peroxidase activites, and to its role as antisenescente agent. Along prolonged storage, control pomegranates showed significant reduction in the content of total phenolics, ascorbic acid and TAA, in both hydrophilic (H-TAA) and lipophilic (L-TAA) fractions (Table 1). The application of oxalic acid led to lower losses of total phenolics and to a significant increase in ascorbic acid and TAA after 84 days of cold storage, while L-TAA remained without significant changes. A significant increase in total anthocyanins was also observed during storage, which was higher in OA treated fruits, specialy at 6 mM dose (data not shown). The increase in anthocyanin concentration in control fruits is in agreement with previous reports (Artés *et al.*, 2000; Mirdehghan et al, 2006), which was associated with the advancement of the ripening process during postharvest storage. Although no information is available on the role of oxalic acid on anthocyanin biosynthesis, exogenous oxalic acid could act as elicitor of anthocyanin synthesis. Five individual anthocyanins were identified HPLC-DAD analysis: cyanidin-3-glucoside (Cy-3-Gluc), delphinidin-3-glucoside (Dp-3-Gluc), pelargonidin-3-glucoside (Pg-3-Gluc), cyanidin-3,5-diglucoside (Cy-3,5-Digluc), and pelargonidin-3,5-diglucoside (Pg-3,5-Digluc), the predominant being Cy-3-Gluc, according to previous rapports in other cultivars, such as 'Assaria' (Miguel *et al.*, 2004) and Primosole (D'Aquino *et al.*, 2010). Contrarily, in the sour cultivars, total anthocyanins decreased during cold storage and the predominant anthocyanin was Cy-3,5-Digluc followed by Dp-3,5-Digluc and Cy-3-Gluc (Alighourchi *et al.*, 2008).

Table 1.   **Bioactive compounds (total phenolics, total anthocyanins and ascorbic acid) and antioxidant activity (hydrophilic H-TAA and lipophilic L-TAA) in pomegranate arils at harvest (day 0) and after 84 days of storage in control and oxalic acid treated fruits. mg 100 g^{-1}**

Parameter	Day 0	Day 84			
		Control	**Oxalic 2 mM**	**Oxalic 4 mM**	**Oxalic 6 mM**
Phenolics	252.4 a	167.6 b	192.8 c	212.5 d	182.1 c
Anthocyanins	59.0 a	109.5 b	107.1 b	122.5 c	135.9 d
Ascorbic acid	36.6 a	33.8 b	39.9 c	40.4 c	44.3 d
H-TAA	81.9 a	73.3 b	122.8 c	116.5 c	112.9 c
L-TAA	15.9 a	12.6 b	16.6 a	17.6 a	16.9 a

For each parameter means followed by different letter are significantly different at P<0.05 from day 0 to day 84 and among treatments.

During postharvest storage of fruit and vegetables, loss of health-beneficial compounds has been reported. However, the application of oxalic acid induced beneficial effects in terms of maintaining or increasing the pomegranate potential antioxidant activity during postharvest storage. The mechanism by which oxalic acid increased the bioactive compounds and antioxidant properties is not well known, although oxalic acid has been reported as a natural antioxidant by suppressing lipid peroxidation in vitro in a concentration-dependent manner and reducing the ascorbic acid oxidation (Kayashima and Katayama, 2002). Thus, oxalic acid treatment could be considered as a natural postharvest tool to reduce CI and maintain pomegranate fruit quality and its health beneficial effects.

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Reduction of chilling injury and maintenance of fruit quality after pre-storage salicylic acid application on Iranian pomegranates

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Abstract. Pomegranate is one of the most popular fruit in Iran being consumed as fresh arils. However, pomegranates are highly perishable fruit due to weight losses, decay and mainly chilling injury symptoms (CI) when stored below 5°C. With the aim to reduce CI in pomegranate (*Punica granatum* cv. Malas Saveh), the fruits were treated with salicylic acid (SA) at different concentrations (0.7, 1.4 or 2.0 mM), then stored at the temperature of 2°C for 3 months (to stimulate low temperature damage). SA treatments, especially at 2 mM concentration, were highly effective in reducing CI and electrolyte leakage in the husk of pomegranate, as well as the ascorbic acid loss compared with that observed in control fruit. In addition, no significant changes were observed in total soluble solids and total acidity, during storage for any treatments, with the only exception of total acidity in 1.4 mM SA-treated, which was rather high. Results found here showed that PAL activity increased during prolonged cold storage although the SA-treatment reduced those increases.

Keywords. Salicylic acid – Pomegranate – Electrolyte leakage – Chilling injury.

I – Introduction

Salicylic acid (SA) was considered as a plant hormone at early 1990's (Raskin, 1992) by its role in regulating some aspects of disease resistance in plant organs and tissues. More recently, the involvement of SA as a signal molecule in systemic acquired resistance associated with the production of pathogenesis-related proteins has been extensively proved (Beckers and Spoel, 2006). In chilling injury (CI)-sensitive fruit, such as peach, pre-treatment with SA reduced CI (Wang *et al.*, 2006). Thus, taking into account that pomegranate is also a CI sensitive fruits, the aim of this paper was to store pomegranate fruit at 2°C to induce CI and evaluate the effects of several SA concentrations (0.7, 1.4 or 2.0 mM) on alleviating this physiological disorder causing by low temperature.

II – Materials and methods

Pomegranates (*Punica granatum* cv. Malas Saveh) were harvested at commercial maturity stage from Pomegranate Research Center located at Saveh (Iran) and 180 fruit were selected and divided into 4 lots of 45 pomegranates for the following treatments in triplicate (15 fruit per replicate): control (0) and salicylic acid (SA) at 0.7, 1.4 or 2.0 mM by dipping them in a fresh 25-liter solution during 10 minutes. Another batch of 15 fruit was used to determine the parameters at d 0. For each treatment and replicate, fruit were. Following treatments, fruit were allowed to completely dry at room temperature before storage at 2°C and 85% RH for 3 months. After 1, 2 or 3 months, 5 fruit from each treatment and replicate were taken out at random from the cold chamber and further stored 3 d at 20°C (shelf-life, SL). The following analytical determinations were made: Chilling injury index (CI), rate of electrolyte leakage (EL), total soluble solids (TSS),

total acidity (TA), ascorbic acid (AA) and PAL activity according to Mirdehghan *et al.* (2007b) and Qin *et al.* (2003).

III – Results and discussion

CI increased during storage but it was affected by treatment, since after 2 months control fruit exhibited significantly higher CI symptoms than that observed for SA-treated pomegranates, although after 3 months only the highest SA concentration was still effective in reducing CI (Fig. 1). Similar results were reported in peaches, in which the alleviation of CI was achieved at 1 mM but failed at 0.7 mM or lower SA concentration (Wang *et al.*, 2006). EL increased after 3 months of cold storage, and it was significantly lower in 1.4 and 2.0 mM SA-treated fruit than in control or SA at 0.7 mM ones (Fig. 1). A positive but relatively low relationship was obtained between CI and EL (r^2=0.696) which would indicate that SA might partially maintain membrane integrity. Similarly, pre-storage heat or polyamine treatments in pomegranates alleviated CI paralleled to the reduced EL (Mirdehghan *et al.*, 2007a; 2007b).

After 90 Days at 2°C + 3 Days at 20°C
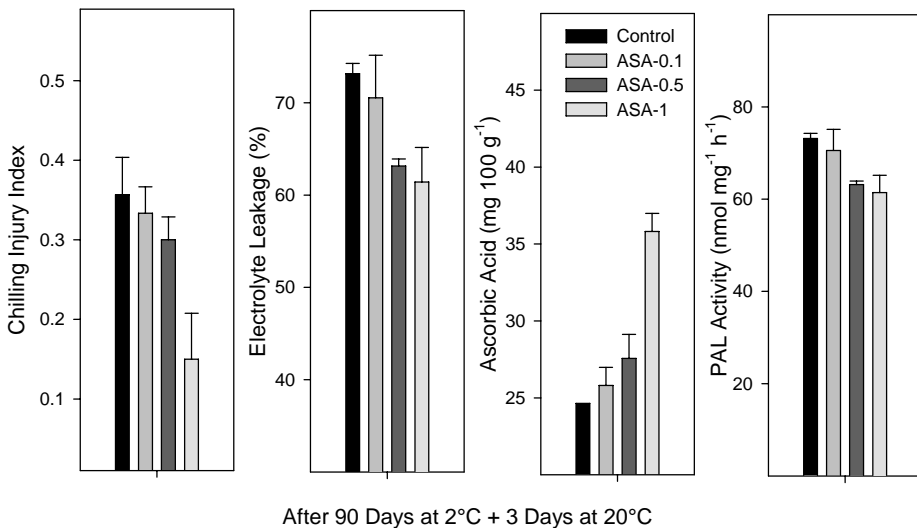
Fig. 1. **Chilling injury index, electrolite leakage, ascorbic acid and phenilamonioliase (PAL) activity in pomegranate arils after 90 days of cold storage plus 3 days at 20ºC.**

No significant changes were observed in TSS and TA during storage for any treatments, with the only exception of TA in 1.4 mM SA-treated, which was significantly higher (Fig. 1). Accordingly, SA applied at 2 mM did not modify TSS or TA of mango fruit stored at chilling temperatures (Ding *et al.*, 2007). The initial levels of AA significantly decreased in control arils during storage, this decrease being lower in SA-treated fruit and even AA remained unchanged in those pomegranates treated with the 2 mM dose (Fig. 1). Similarly, SA treatment at 1 or 2 mM also maintained AA at the end of storage of peaches and oranges, respectively (Wang *et al.*, 2006; Huang *et al.*, 2008).

PAL activity also increased during prolonged cold storage although the SA-treatment reduced that increases (Fig. 1). Results agree with previous report in loquat fruit treated with ASA (Cai *et al.*, 2006) or mangosteen stored at low O_2 (Dangcham *et al.* 2008). Accordingly, Nguyen *et al.* (2004) reported that modified atmosphere packaging reduced CI and PAL activity in banana,

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and heat treatment prevented both, CI and the increase in PAL activity, that normally occurs in 'Fortune' mandarin stored at low temperature (Sánchez-Ballesta *et al.*, 2000).

In conclusion, in this paper the first evidences about the role of SA treatment on reducing CI in pomegranates are addressed. From the assayed concentrations, 2 mM was the most effective for reducing CI, EL and maintenance of the AA levels. However, more in depth experiments should be carried out to understand the mechanism by which SA improves the quality of pomegranate stored at chilling temperatures.

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Polyamines applied by immersion or pressure maintain 'Mollar de Elche' pomegranate quality stored at chilling temperatures

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Abstract. Pomegranate fruits (*Punica granatum* L. cv. 'Mollar de Elche') were treated with putrescine (Put) or spermdine (Spd) at 1 mM under pressure-infiltration or immersion prior cold storage at 2°C. Non-treated fruit developed rapidly chilling injury (CI), with main symptoms being skin browning, increased electrolyte leakage and weight loss. During storage losses of firmness and colour and increases in °Brix/acidity ratio and respiration rate were observed. All these changes were significantly delayed by polyamine treatments, with effectiveness being found similar either by pressure-infiltration or by immersion. The reduction of CI severity was correlated to the increased levels of free endogenous Put and Spd in the skin, which could induce acclimation of pomegranate to cold temperature and also a mechanism of protection to CI, with a net increase of the shelf-life. In conclusion, in this paper a novel technology based on the exogenous application of Put or Spd under pressure-infiltration or immersion could induce acclimation of pomegranate to low temperature, and in turn protect the fruit to CI by increasing the levels of endogenous Put and Spd.

Keywords. Browning – Chilling injury – Firmness – Putrescine – Spermidine.

I – Introduction

Storage at low temperature is necessary to avoid excessive desiccation and decay and to prolong pomegranate storability. However, pomegranates stored below 5°C develop chilling injury (CI), the most common symptoms being surface pitting, husk scald and skin browning. Increases in polyamine concentration (putrescine, Put; spermidine, Spd; spermine, Spm) have been reported in fruits stored under CI conditions, although whether this polyamine enhancement is a result of the cold stress or a protective mechanism against CI is still unclear (Valero and Serrano, 2010). Thus, the aim of this paper was to study the role of exogenous Put or Spd application, either by pressure-infiltration or immersion, on pomegranate fruit quality stored at chilling temperatures.

II – Materials and methods

Mature pomegranates (*Punica granatum* L. cv. Mollar de Elche) were picked, randomized and divided into six lots of 125 fruit for the following treatments in 5 replicates (each replicate contained 25 individual fruit). Half lots were treated by pressure (in 20 l of solution, containing Tween-20 (2 g l^{-1}), and applying a pressure of 0.05 bar for 4 min) with 1 mM Put, 1 mM Spd or with distilled water, which served as the control. The other half was treated with the same concentration of polyamines and water by dipping at 25°C for 4 min. Following treatments, fruit were placed on Kraft paper and allowed to dry before storage the next day at 2°C (considered as day 0) in a temperature-controlled chamber, in permanent darkness and with a relative humidity of 90%. After 0, 30 and 60 days, 25 fruit for each treatment (5 from each replicate) were sampled and further stored at 20°C for 3 days (shelf-life, SL). External browning, weight

loss, fruit firmness, respiration rate and colour were measured in intact fruit, electrolyte leakage and polyamines were analysed in the skins, and soluble solid content (SSC), and titratable acidity were determined from the arils as described by Mirdehghan *et al.* (2007).

III – Results and discussion

In pomegranate fruit occurrence of CI developed from the first sampling date, as shown by the results of skin browning and electrolyte leakage (Fig. 1). However, these CI symptoms were significantly decreased by Put and Spd treatments, the efficacy of these treatments being similar whether applied by pressure-infiltration or immersion. This effect could be attributed to the role of polyamines on inducing cold acclimation, which would lead to maintenance of membrane fluidity at low temperatures, and could be responsible for reduced electrolyte leakage and skin browning, and thus for decreasing the severity of CI symptoms. Accordingly, during senescence of melons, polyamine treatment resulted in less membrane peroxidation and higher retention of chlorophyll (Lester, 2000). During storage of control pomegranates, decreases in fruit firmness and colour L* and increases in weight loss, respiration rate and ratio SSC/acidity (maturity index) were found. These changes, which are associated with acceleration of ripening, were significantly delayed after exogenous application of Put or Spd either by pressure or immersion (data not shown). Thus Put and Spd treatments, due to their anti-senescence properties, were able to retard the maturation process of pomegranates, as has been observed in a wide range of climacteric and non-climacteric fruit (Valero and Serrano, 2010).
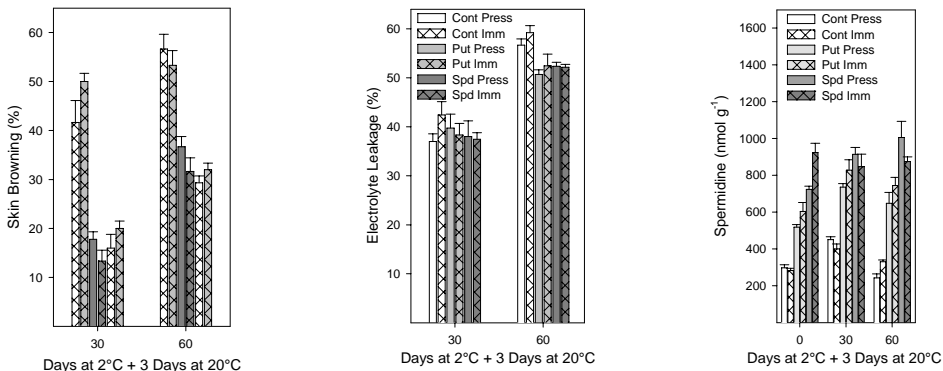
Fig. 1.   Sking browning, electrolyte leakage and spermidine concentration along storage in control and treated pomegranates. Data are de mean ± SE.

Endogenous Put showed slight but non-significant increases while greater increases in Spd occurred (Fig. 1). This polyamine response supports the proposal that accumulation of Put or Spd in tissues seems to be a general response of fruit to CI (Martínez-Romero *et al.*, 2003). Skin browning was negatively correlated with endogenous Put concentration in Put or Spd-treated fruit under pressure, with the same correlation being found for endogenous Spd concentration when Put or Spd were applied under immersion. These results suggest an activation of the polyamine biosynthesis pathway. Part of the exogenous Put appears as free endogenous Put and part is likely to have transformed to Spd using decarboxylate S–adenosylmethione as an aminopropyl donor catalysed by Spd-synthase, while the conversion of Spd to Spm did not occur, since no significant increases in Spm were shown. Finally, the increased levels of endogenous Put and Spd after polyamine treatments could be responsible for the lower rate of softening, lower increase in SSC/acidity and weight loss and in turn a net

increase in the shelf-life, since acceleration of ripening process and senescence has been associated with decreases in the content of endogenous polyamines (Valero *et al.*, 2002).

In conclusion, the exogenous application of Put or Spd under pressure-infiltration or immersion could induce acclimation of pomegranate to low temperature, and in turn protect the fruit from CI by increasing the levels of endogenous Put and Spd, since the normal levels would not be high enough to induce this adaptation to cold storage. In addition, the polyamine treatment retarded the maturation process by reducing softening and the increase of the ratio SSC/acidity, as well as the loss of weight. Thus storability and shelf-life could be extended in pomegranate stored at low temperatures that usually develop CI.

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Chilling injury is reduced and the content of bioactive compounds enhanced by methyl salicylate treatment

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Abstract. Methyl salicylate (MeSa) is a volatile plant compound having a role in plant defense-mechanism as signal molecule. Some evidences have shown that MeSA could indirectly provide protection against chilling damage (CI). Thus, given than pomegranate fruits are sensitive to CI, the effect of MeSa treatments (at 0.01 and 0.1 mM) on reducing the incidence of CI was analyzed. Control pomegranates exhibited CI symptoms manifested by pitting and browning when fruits were stored at 20ºC after cold storage, the severity being enhanced as storage time advanced. The CI symptoms, as well as softening and increase in electrolyte leakage (EL), were significantly reduced by MeSa treatments, without significant differences among 0.01 and 0.1 mM doses. In addition, total phenolic and anhocyanin concentration and antioxidant activity were higher in arils from MeSa treated fruits than in those from controls. In conclusion in this paper we report the positive effect of MeSa on reducing CI of pomegranate fruits stored at chilling temperatures, which was accompanied by reduced softening and EL, showing a protective role on cell membranes and for increases in bioactive compounds and their related antioxidant activity have been found.

Keywords. *Punica granatum* L. – Quality – Anthocyanins – Phenolics – Antioxidant activity.

I – Introduction

Methyl salicylate (MeSa) is a volatile plant compound synthesised from salicylic acid having a role in the defense plant mechanism, including wounding, pathogens/insects, mechanical, drought and chilling injury (CI), among others (Hayat and Ahmad, 2007). As a result of CI damage, dysfunction of cell membrane occurs, affecting its permeability and being considered the primary molecular leading to the development of CI, which is strongly influenced by its lipid composition (Mirdehghan *et al.*, 2007a). Recent research has shown that MeSa treatment increased resistance of tomato (Fung *et al.*, 2006) and peach fruit (Han *et al.*, 2006) to low temperature stress due to the enhancement of antioxidant enzymes, which protects fruit cell membranes from lipid peroxidative injury. Thus, the main objective of this paper was to study the effect of MeSa on pomegranate fruit quality attributes, bioactive compounds and the antioxidant capacity during storage under CI conditions.

II – Materials and methods

Pomegranates (*Punica granatum* L. cv. Mollar de Elche) were picked at mature stage (Melgarejo *et al.*, 1997), treated in triplicate with methyl jasmonate (MeSa) at 0.1 and 0.01 mM concentration for 16 h in 120-l container and stored at 2°C, in permanent darkness and with relative humidity of 90%. Sampling schedule was as follows: every 2 weeks 1 lot (5 fruits) from each replicate and treatment was transferred to a chamber at 20°C for 4 days and the following analytical determinations were performed: Chilling injury (CI), electrolite leakage (EL), fruit

firmness, total soluble solids (TSS), total acidity (TA), total phenolics, total anthocyanins and total antioxidant activity as described by Mirdehghan *et al.* (2007a,b).

III – Results and discussion

During postharvest of pomegranate fruits CI appeared after 14 days of storage manifested by pitting, browning and desiccation, the severity being enhanced as did storage time (Fig. 1). A similar behaviour was observed in EL, that is, increases along storage. However, the application of MeSa led to significantly lower CI and EL index (2-3 fold) than in control fruits, without significant differences among concentrations used (Fig. 1).
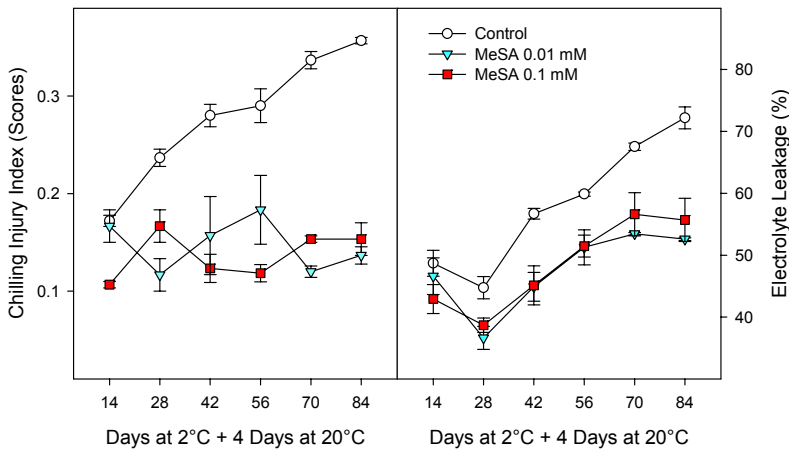
Fig. 1. **Chilling injury and electrolyte leakage during storage of control and methylsalicylate treated pomegranates. Data are the mean ± SE.**

In general, CI primarily occurs in cell membrane and then the membrane damage initiates a cascade of secondary reactions finally leading to disruption of cellular and sub-cellular structures Mirdehghan *et al.* (2007a). In tomato, MeSA could induce some defence-mechanism responses that indirectly provide protection against chilling damage, rather than the compound itself producing a direct effect. Specifically, MeSA could inhibit catalase activity and in turn increase the amount of cellular H_2O_2, which would increase the expression of pathogenesis-protein genes, although accumulation of heat shock proteins (Ding *et al.*, 2002) or expression of alternative oxidase (Fung *et al.*, 2006) could not be discharged. Fruit firmness decreased gradually along storage, the softening process being retarded by MeSa treatments (Fig. 2), according to previous results in mango (Han *et al.*, 2006). The mechanism by which MeSa may affect the cell wall structure and maintain fruit firmness is not clear yet, and no research has been carried out in pomegranate specifically. TSS at harvest was 16.1±0.1 °Brix and increased during storage in both control and treated arils, reaching final values of ≈17.5 °Brix, while the values of TA at harvest (0.30±0.01 g 100 g^{-1} FW) decreased along storage, the final levels being ≈0.24 g 100 g^{-1}, but without significant differences among treatments (data not shown). These changes are the result of the normal ripening process that occurs in these non-climacteric fruits, as previously reported (Mirdehghan *et al.*, 2007a; Sayyari *et al.*, 2009), which was delayed by the use of MeSa.

The content of total phenolics and total anthocyanin in the arils increased along storage in both

control and treated fruits, although concentrations were significantly higher in treated pomegranates than in control ones (data not shown). Total antioxidant activity due to hydrophilic compounds (H-TAA) decreased in control fruits after 56 days of storage, while in treated pomegranate an increase was found until the end of the experiment (Fig. 2).

Phenolics including anthocyanins are phytochemical compounds with antioxidant activity that impart beneficial health effects together with C and E plant vitamins (Valero and Serrano, 2010). 'Mollar de Elche' pomegranate cultivar have higher concentrations of ascorbic acid and total phenolics than 'Taifi', 'Wonderful' and 'Ganesh' cultivars (Gil *et al.*, 2000; Al-Maiman and Ahmad, 2002; Mirdehghan *et al.*, 2006). Thus, MeSa treatment could increase the health benefit of pomegranate fruit consumption, since concentration of bioactive compounds and antioxidant potential was higher, after long temp storage, in treated than in control pomegranates. In addition, application of MeSa treatments were effective on reducing CI and maintain quality attributes during storage.
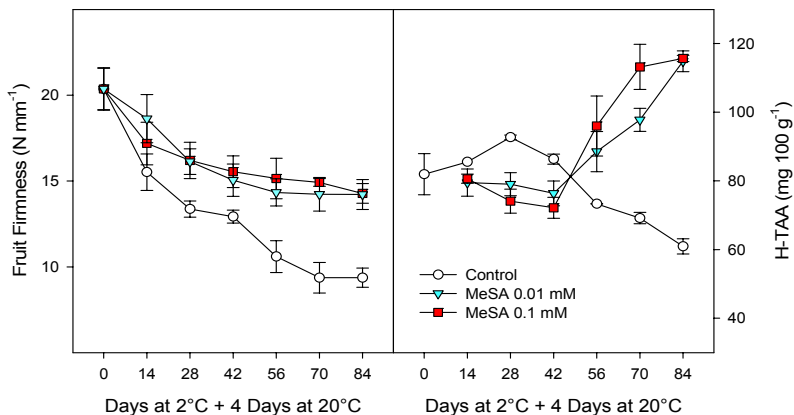
Fig. 2. **Fruit firmness and hydrophilic total antioxidant activity (H-TAA) during storage of control and methylsalicylate treated pomegranates. Data are the mean ± SE.**

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Methyl jasmonate treatment reduces chilling injury and improves antioxidant activity

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Abstract. Methyl jasmonate (MeJa) is an endogenous plant growth substance that regulates many aspects of plant development and growth, including chilling injury (CI) stress. Then, taking into account that pomegranate fruits are susceptible to CI, the main objective of this paper was to study the effect of MeJa on pomegranate fruit quality attributes during storage under chilling conditions. Pomegranates were treated after harvest with MeJa at two concentrations (0.01 and 0.1 mM), and then stored under chilling temperature for 84 days. Control fruits exhibited CI symptoms, manifested by pitting and browning, the severity being enhanced as storage time advanced, which were accompanied by softening and increase in electrolyte leakage (EL). The CI symptoms were significantly reduced by MeJa treatments, and total phenolic and anthocyanin concentration increased. Total antioxidant activity decreased in control arils, while increases occurred in arils from MeJa treated fruits. Results suggest that MeJa treatment has potential postharvest applications to reduce CI, maintain quality and improving the health benefits of pomegranate fruit consumption by increasing their content in bioactive compounds and antioxidant capacity.

Keywords. *Punica granatum* L. – Quality – Anthocyanins – Phenolics – Antioxidant activity.

I – Introduction

Methyl jasmonate (MeJa) is an endogenous plant growth substance that regulates many aspects of plant development and has been described as a signal molecule in plant stress responses, both biotic and abiotic types, including wounding, pathogens/insects, mechanical, drought and chilling injury (CI), among others (Creelman and Mullet, 1995). Recent research has shown that MeJa treatment reduced the development of CI symptoms in a wide range of fruits, such as guava (González-Aguilar *et al.*, 2004) and loquat (Cao *et al.*, 2009). Pomegranate arils contain high concentration of sugars, organic acids, vitamins, polysaccharides, and essential minerals as well as high content of antioxidant compounds with effect against degenerative diseases (Mertens-Talcott *et al.*, 2006). Thus, the main objective of this paper was to study the effect of MeJa on pomegranate fruit quality attributes during storage under chilling conditions, as well as its role on the content of bioactive compounds (polyphenol and anthocyanins) and antioxidant capacity.

II – Materials and methods

Pomegranates (*Punica granatum* L. cv. Mollar de Elche) were picked at mature stage (Melgarejo *et al.*, 1997), treated in triplicate with methyl jasmonate (MeJa) at 0.1 and 0.01 mM concentration for 16 h in 120-l container and stored at 2°C, in permanent darkness and with relative humidity of 90%. Sampling schedule was as follows: every 2 weeks 1 lot (5 fruits) from each replicate and treatment was transferred to a chamber at 20°C for 4 days and the following analytical determinations were performed: Chilling injury (CI), electrolite leakage (EL), fruit firmness, total soluble solids (TSS), total acidity (TA), total phenolics, total anthocyanins and total antioxidant activity as described by Mirdehghan *et al.* (2007a,b).

III – Results and discussion

The application of MeJa led to significantly lower CI index (2-3 fold) and EL than control fruits along storage period, without significant differences among concentrations used (data not shown). The increase in the degree of unsaturation of membrane lipids have been described as a mechanism of acclimation to low temperatures, which could be responsible for the lower EL and CI symptoms found in treated fruits. On the other hand, MeJA could also decrease incidence of CI by enhancing the activities of superoxide dismutase, catalase and ascrobate-peroxidase and lowering the activity of lipoxygensase, as has been proposed in loquat fruit (Cao *et al.*, 2009). Fruit firmness at harvest was 20.36 ± 1.22 N mm^{-1} and decreased during storage, reaching final levels of 9.37 ± 0.55 N mm^{-1} at the last sampling date in control pomegranates and significantly higher, ≈12 N mm^{-1}, in MeJa treated ones, independently of the applied dose (data not shown). The mechanism by which Meja may affect the cell wall structure and thus maintain fruit firmness is not clear yet, and no research has been carried out in pomegranate specifically, although it has been postulated that MeJa reduces pectinmethylesterase (PME) activity, decreasing de-esterification of pectin (Meng *et al.*, 2009), and thus maintaining fruit texture. TSS increased along storage, while decreased occurred in TA as a result of the normal ripening process that occurs in these non-climacteric fruits (Mirdehghan *et al.*, 2007a; Sayyari *et al.*, 2009), and both were delayed by MeJa.

The content of total phenolics in the arils increased for the initial levels of 233 ± 8 to 284 ± 3 mg 100 g^{-1} at the end of the experiment in control fruits, this increase being higher in MeJA treated pomegranates, in a manner dose dependent (Fig. 1). Total anthocyanins also increased along storage in both control and treated fruits (Fig. 1), although for this case without significant differences among treatments and applied doses. Accordingly, early reports on 'Mollar de Elche' pomegranate revealed that during postharvest storage an increase of total phenolics and total anthocyainins occurred (Mirdehghan *et al.*, 2007a,b). However, the application of MeJa leads to higher increases of these phytochemicals along storage and in turn to higher H-TAA as compared with arils from control fruits. For comparative purposes, literature about the effect of MeJa on the bioactive compounds and antioxidant activity in fruits is limited, and only a few examples are found in small berries, blackberries, bayberries, strawberries and raspberries (Wang *et al.*, 2008; Chanjirakul *et al.*, 2006).
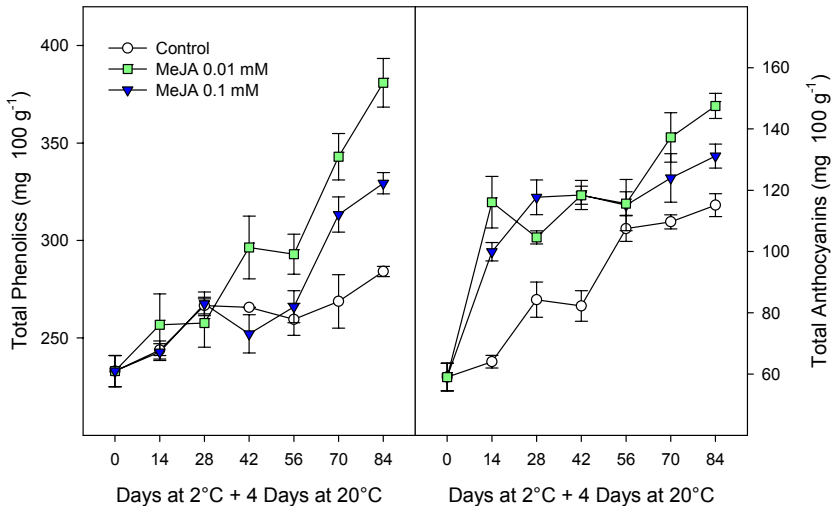
Fig. 1. **Total phenolics and anthocyanin concentration in arils furng storage as affected by methyl jasmonate (MeJa) treatment. Data are the mean ± SE.**

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Reduction of chilling injury of pomegranate by heat treatment before cold storage

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Abstract. Pomegranates are highly perishable fruit and sensitive to chilling injury (CI) when stored below 5ºC, manifested as skin browning, surface pitting, higher susceptibility to decay and reduction in both internal and external fruit quality. Thus, the aim of this experiment was to study the effect of hot water dip at 45 ºC for 4 min on reducing CI in pomegranates after storage at 2ºC for 15-90 days plus 3 days at 20ºC. Pomegranates developed CI, manifested as increases in skin browning and electrolyte leakage, which were highly correlated. The severity of damage in control fruit was related to loss of fatty acids with a concomitant reduction in the ratio of unsaturated/saturated fatty acids during storage. These CI symptoms were slightly, but significantly reduced in heat-treated pomegranates. In addition, the heat treatment induced increases in free putrescine and spermidine during storage. These higher polyamine levels as well as the maintenance of the unsaturated/saturated fatty acid ratio during storage could account for the maintenance of membrane integrity and fluidity and the reduction of CI.

Keywords. Browning – Electrolyte leakage – Fatty acids – Firmness – Putrescine – Spermidine.

I – Introduction

Pomegranates are highly perishable fruit due to problems of desiccation and especially chilling injury (CI) symptoms when stored below 5°C, manifested as skin browning, surface pitting and higher susceptibility to decay. These symptoms can reach the arils, leading to a reduction in both internal and external fruit quality (Sayyari *et al.*, 2009; 2011). Intermittent warming has been tested with satisfactory results in maintaining pomegranate quality during storage, in terms of retention of anthocyanin and titratable acidity, reduction of decay and alleviation of chilling injury (Fallik, 2004). Since heat treatments, which showed beneficial effects in alleviating chilling injury, were accompanied by increases in polyamines, a particular role for endogenous polyamines in increasing fruit tolerance to cold stress has been proposed (González-Aguilar *et al.*, 2000; Xu *et al.*, 2005). However, no information is available about the use of heat treatments (temperature over 35°C during short periods. Thus, the aim of this work was to study the effect of prestorage heat treatments on reducing CI in pomegranates evaluating browning, electrolyte leakage fatty acid composition of the skin. In addition, the mediation of polyamines in the reduction of CI caused by heat treatments before cold storage will be discussed.

II – Materials and methods

Pomegranates (*Punica granatum* L. cv. Mollar de Elche) were picked when fully mature according to commercial practice and randomized and divided into two lots of 175 fruits for the following treatments in quintuplicate (each replicate contained 35 individual fruits): control (distilled water at 25°C for 4 minutes) and heat treatment (hot water dip at 45°C for 4 minutes). Following treatments, fruits were placed on Kraft paper and allowed to dry before storage the next day at 2°C (considered as day 0) in a temperature-controlled chamber, in permanent

darkness and with relative humidity of 90%. After 15, 30, 45, 60, 75 and 90 days, 25 fruits for each treatment (5 from each replicate) were sampled and further stored at 20°C for 3 days (shelf life, SL). Fruit firmness, external browning and colour were measured in intact fruit. Then, each husk was carefully cut at the equatorial zone and peels were manually extracted. Some of the peel tissue of each replicate was used for electrolyte analysis, and the remaining was combined and frozen in liquid N_2, milled and stored at -20°C until analytical determinations of polyamines and fatty acid composition were made, as described in Valero *et al.* (1990), Mirdeghan *et al.* (2007) and Sayyari *et al.* (2010; 2011).

III – Results and discussion

All pomegranate husks developed CI from the first sampling date, which increased with storage time, as could be observed by skin browning (Fig. 1a) and ion leakage (Fig. 1b). However, the occurrence was significantly reduced in heat-treated fruit. The increased skin browning in control fruit was also observed by a reduction in Hue angle, which was retarded in heat-treated fruit. In addition, browning was positively correlated with electrolyte leakage, both in control (y=1.17 x – 24.65, r^2=0.80) and heat-treated fruit (y=1.09 x – 27.82, r^2=0.80), and negatively correlated to Hue angle (y=-2.31 x + 145, r^2=0.88 and y=-1.95 x + 127, r^2=0.73, for control and heat-treated fruit, respectively). However, no symptoms of decay were observed during storage, neither control nor heat-treated pomegranates.
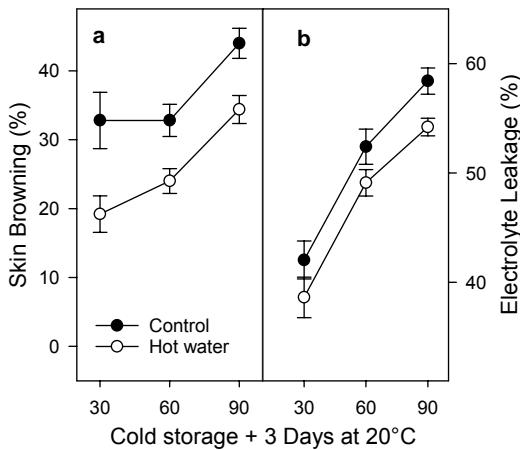
Fig. 1.   Skin browning and electrolyte leakage during storage of control and treated pomegranates. Data are the mean±SE.

In pomegranate skin 10 fatty acids were identified and quantified, five saturated (C10, C12, C14, C16 and C18), two mono-unsaturated (C16:1, C18:1), and three poly-unsaturated (C18:2 cis, C18:2 trans, and C18:3). Among the saturated, palmitic fatty acid (C16) was predominant (≈ 33%), while linolenic acid (C18:3) was the major unsaturated fatty acid (≈25%). During storage, all fatty acid significantly decreased in control fruit (Fig. 2), with losses of 53% in saturated fatty acids, and 70 and 76% for mono-unsaturated and poly-unsaturated fatty acids, respectively. However, the concentrations of all fatty acids in heat-treated pomegranates remained significantly higher than in control fruit over storage, and did not show significant losses from day 0 to day 90. Thus, the ratio of unsaturated/saturated fatty acids decreased in control fruit from an initial value of 1.27±0.15 to 0.72±0.06 at the end of the experiment, while no significant changes and higher ratios were found in heat-treated fruit for all sampling dates. It is interesting

to point out that in control fruit, the decrease in the unsaturated/saturated fatty acid ratio was highly correlated with the increase in electrolyte leakage (y= -0.02 x +1.79 r^2= 0.859).
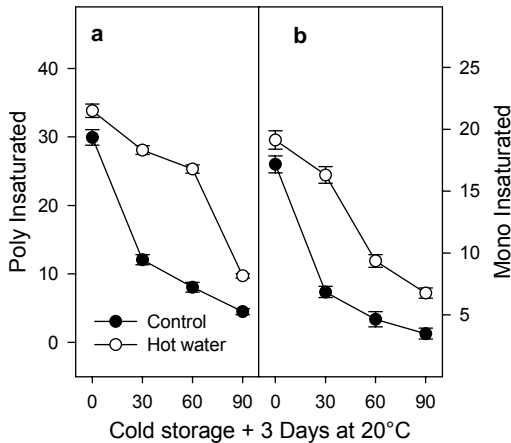
Fig. 2. **Poly and mono-insaturated fatty acids (mg 100 g^{-1}) during storage of control and treated pomegranates. Data are the mean±SE.**

This increase in the degree of unsaturation of membrane lipids has been described as a mechanism of acclimation to low temperatures (Campos *et al.*, 2003), which would lead to maintenance of membrane fluidity at low temperature of storage, and could be responsible of the lower electrolyte leakage and skin browning. Thus, our results show that control pomegranates were not able to develop this adaptation mechanism and thus CI occurred in greater extent, corroborated by the high relationship found between the decrease of unsaturated/saturated fatty acids and the increase in electrolyte leakage during cold storage. However, heat-treatment could induce this response by maintenance of unsaturated fatty acids during cold storage, and thus the severity of chilling injury symptoms was reduced.

The application of heat treatment led to an increase in putrescine concentration during storage compared to control fruit, for which a reduction of this polyamine was observed. However, the main change was shown for spermidine, which increased during storage, for both control and treated fruit, although their levels were always higher in heat-treated than in control pomegranates. This effect could be a defense mechanism against this stress involving protection of cell membrane lipids, and could be responsible for the lower electrolyte leakage and browning found in heat-treated pomegranates. In addition, the higher polyamine concentration could account for the greater firmness retention in heat-treated fruit, since exogenous polyamine treatments have been shown to reduce softening of a wide range of fruit through reduction in hydrolytic cell-wall enzymes or rigidification of cell-wall by cross-linking to pectic substances (Valero and Serrano, 2010).

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Influence of the temperature in the evolution of the coordinates of color in pomegranate

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Abstract. The colour quality of many fresh and processed fruits may influence consumer acceptance. Pomegranate acceptability depends on a combination of quality attributes related to physical-chemical and mechanical properties such as rind colour, sugar content, acidity, flavour, etc. This studyrealized at 2009 season, was undertaken to study the evolution of colour parameters with the environmental temperature. The results confirmed a strong influence between the colorimetric coordinates (L^*, a^*, b^*, C^* and h^*_{ab}), measured during fruit development, maturation and the maximum, mean and minimum temperatures.

Keywords. Colour – L^*, a^*, b^* – Pomegranate – Temperature.

I – Introduction

Spain produces about 40,000 tons of pomegranate (*Punica granatum* L.) per year. Although, knowledge about the importance of pomegranate in human nutrition has increased tremendously in recent years, the external color of the fruit has not yet been studied in detail. Pomegranate fruit maturity status is commonly assessed based on external (skin) colour, juice color and acidity of juice (Cristosto *et al.*, 2000). Some researches have studied the correlation between the parameters of the skin color (L^*, a^*, b^*, C, h_{ab}^*) and acidity, total soluble solids, citric acid, anthocyanins (Dafny-Yalin, *et al.*, 2010), but as far as we know no experiments have previously reported the correlation between the parameters of the skin color and the environmental temperature not even the evolution of the parameters of color.

II – Materials and methods

Plant material. The pomegranate accession chosen for this study was the ME-15 accession ("Mollar de Elche"). The cultivar was selected according to four main criteria: namely that he was sweet, a soft seed, produced large fruit size and was good yielders. The cultivar ME-15 was selected from the population variety "Mollar de Elche" (ME), which is one the most highly valued in the world, thanks to its extraordinary flavor and high antioxidant, vitamin and mineral content. The selected plant material belong to the principal pomegranate germoplasm bank of the EU, which is located at the experimental field station of Miguel Hernández University in the province of Alicante, Spain (02º03´50´´E, 38º03´50´´N, and 25 masl).

Experimental design. Skin color was measured from two trees, arranged at random inside the bank of germoplasma and twelve fruits per tree (3 fruits for orientation), located to half a height inside the tree. Six measurements were taken per fruit along the equatorial axis, given a total of 144 measurements. The measures of color were realized every seven days, the first measure being realized between the first and second week of June (phenological stage I) and the last one during the third week of October (phenological stage L) (Melgarejo *et al.*, 1996). These measures were realized during the year 2009.

Determination fruit colour. Colour measurements were performed using a Minolta colorimeter (CR-300, Minolta Ramsey, N.J. USA). Color was assessed according to the Commission International del´Eclairage (CIE) and expressed as L*, a*, b*, C* and h*$_{ab}$ color values. L* defines (brightness or lightness; 0 = black, 100 = white), a* (-a* = greenness, +a* = redness), b* (-b* = blueness, +b* = yellowness) and C* defines saturation. Hue angle (h*$_{ab}$) was calculated as hue angle arctang (b*/a*).

Statistical analysis. Statistical analyses were performed using SPSS 16.0 for Windows. Descriptive statistics were used to process and analyse all collected data.

III – Results and discussion

Evolution of L colour parameter.* The coordinate L* increased constantly from phenological stage I (young fruit), reaching a maximum during the second week of September (77.19 in 2009,) when top temperatures were recorded (minimum, mean and maximum values of 20.96ºC, 27.27ºC and 33.59ºC, respectively) (Fig. 1). Afterwards, the L* values dropped constantly until harvest in late October as previously reported by Shwartz *et al.* (2009).
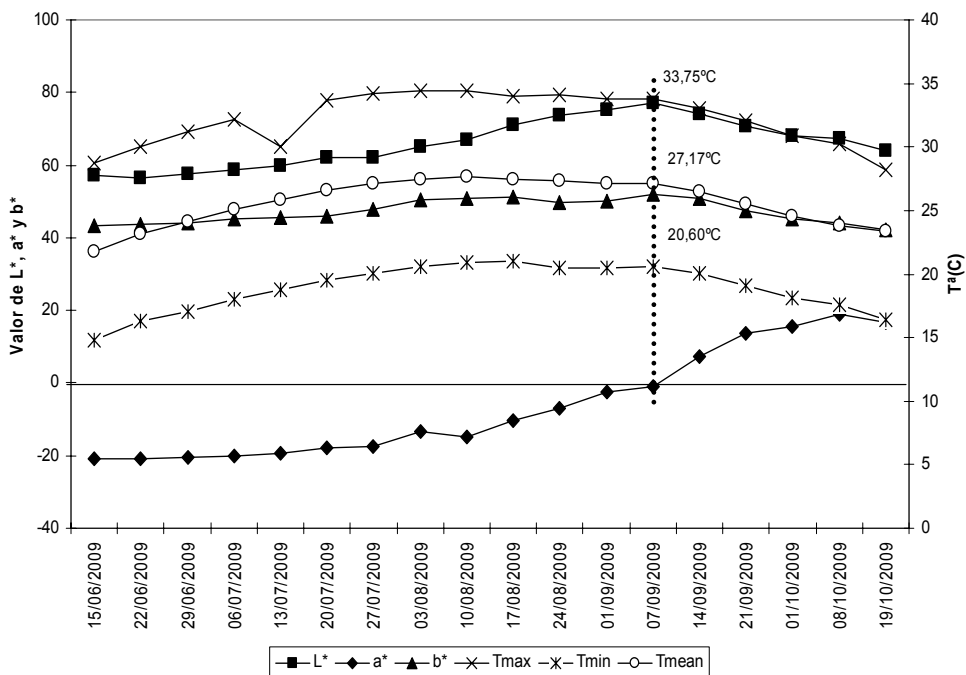
Fig. 1. Colour coordinates evolution of pomegranate rind and temperature response curve for 2009.

Evolution of the colorimetric coordinate a.* The colorimetric coordinate a* showed negative values (green) until the second week of September (Table 1), when rind colour turned from green to red (positive values) and coinciding with top temperatures records and the maximum value of L* (Fig. 1). From then onwards, while coordinate a* gradually increased (redness), the L* values constantly decreased; the highest values of a* were reached between the first and second week of October (Table 1). During this period the green colour of pomegranate rind was increasingly replaced by the red one. Similar results were found for 'Mollar' pomegranate accessions (Gil *et al.*, 1995) and for two Israelian cultivars (Shwartz *et al.*, 2009).

Table 1. Colour coordinates of pomegranate rind and temperatures for 2009

Dates	L*	a*	b*	C*	hue	Tmax	Tmin	Tmean
15/06/2009	57.2	-20.78	43.2	47.94	115.69	28.79	14.75	21.77
22/06/2009	56.63	-20.93	43.74	48.49	115.57	30.08	16.27	23.18
29/06/2009	57.54	-20.67	44.24	48.83	115.04	31.23	17.01	24.12
06/07/2009	58.56	-20.28	45.36	49.69	114.09	32.22	18.03	25.13
13/07/2009	59.7	-19.44	45.56	49.53	113.11	29.98	18.77	25.86
20/07/2009	62.12	-17.77	46.09	49.4	111.08	33.69	19.47	26.58
27/07/2009	62.14	-17.44	47.8	50.88	110.04	34.25	20.04	27.15
03/08/2009	64.92	-13.47	46.65	48.56	106.11	34.45	20.54	27.5
10/08/2009	67.1	-14.74	50.48	52.59	106.28	34.41	20.92	27.67
17/08/2009	71.27	-10.48	51.78	52.83	101.44	33.95	20.99	27.47
24/08/2009	73.57	-6.84	49.88	50.35	97.81	34.11	20.49	27.3
01/09/2009	75.17	-2.34	51.17	51.22	92.62	33.74	20.46	27.1
07/09/2009	77.19	-0.94	50.1	50.11	91.07	33.75	20.6	27.17
14/09/2009	73.99	7.17	50.72	51.22	81.95	33.05	20.03	26.54
21/09/2009	70.71	13.58	47.43	49.34	74.02	32.03	19.08	25.55
01/10/2009	67.33	15.53	45.16	47.76	71.02	30.86	18.17	24.51
08/10/2009	67.96	19.03	44.1	48.03	66.66	30.2	17.56	23.84
19/10/2009	64.09	16.7	42.19	45.37	68.4	28.22	16.46	23.34

Evolution of the colorimetric coordinate b*. The colorimetric coordinate b* exhibited high and positive values during the periods of fruit development and ripening (Fig.1). The b* values of pomegranate rind significantly fell from the second week of September onward, indicating that blue pigments were replacing the yellow colour during fruit maturation. The results for coordinate b* completely agreed with those found by Shwartz *et al.* (2009).

IV – Conclusion

The maximum values of L* and b* occurred between the first and second week of September, when fruit rind colour turned from green to red (a*≈0), and temperature records were 33.75ºC, 27.17ºC and 20.60ºC (the maximum, mean and minimum values, respectively). From then onwards to fruit ripening (first half of October), redness gradually increased (a*>0) while b* decreased as the lightness of pomegranate rind colour progressively dropped.

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Session 5
Industralization, derived products
and sensorial quality

Sensory quality of pomegranate products

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Abstract. Pomegranate has become very popular in the last years because of its healthy properties (high antioxidant activity and phenolic compounds content). However, do consumers really know how a fresh pomegranate fruit/juice tastes? In our opinion the answer is clear: no, they do not. Thus, our first task is to explain consumers the main characteristics describing fresh pomegranate fruits; then, they will able to assess whether the products they are buying/consuming come from natural sources. It is evident that the *appearance* of pomegranate fruits is very appealing and this is the main reason why intense garnet arils are always shown in advertisement. Pomegranate products can be manufactured using fruits from sweet, sour-sweet or sour cultivars, determining the relative intensities of the *basic tastes*. However, pomegranate has a big drawback, the low intensity of volatile compounds (*aroma*). The arils can be described by their basic tastes but not by their aroma; this makes very difficult to judge whether a pomegranate product is natural. In contrast, the rind has strong intensities of attributes such as astringency and toothetch mouthfeel. Without these notes a product will not have any healthy activity because polyphenols are mainly located in the rind. If a pomegranate juice can be described as having high intensities of candy-like and sweet overall, it can be a high success among consumers but it cannot be considered as a natural pomegranate product, and besides it will not have any healthy activity.

Keywords. Consumer studies – Descriptive analysis – Lexicon – New products – Sensory evaluation.

I – Introduction

Until a couple of years ago and despite the huge popularity and well-documented health benefits of pomegranate fruits, pomegranate based products and even pomegranate co-products, little research on the descriptive sensory attributes of pomegranate products was found, apart from that conducted by POM Wonderful, LLC (Los Angeles, CA, USA) on pomegranate juices (Koppel and Chambers IV, 2010).

Initially, researchers were only focused in studying the consumers' opinion on pomegranate products. For instance, consumer acceptability was important for the studies of Hayaloglu and Vardin (2001) and Riaz and Elahi (1992) in the research of fruit punch with watermelon/pomegranate juice mixtures and carbonated pomegranate drink, respectively.

Data showing differences in morphological, chemical and biochemical characteristics among pomegranate cultivars suggests that pomegranates may also vary in their sensory properties (appearance, flavor and texture). However most of the sensory research has been focused on pomegranate based products and not on fresh fruits, perhaps because fresh fruits were locally marketed and consumers in the producing areas were well-aware on how high-quality fruits looked like. In this way, Vardin and Fenercioglu (2003) studied the clarification of *pomegranate juices* and evaluated attributes like color, turbidity, overall appearance, bitterness and overall quality. López-Rubira *et al.* (2005) studied the aroma, taste, firmness, visual appearance, color, browning and dehydration of *pomegranates arils* during their shelf life. Singh and Sethi (2003) conducted a screening of pomegranate genotypes for the preparation of high quality anardana (*dried pomegranate seeds* original from India) and evaluated mouthfeel, color and flavor. Later,

Martinez *et al.* (2006) characterized the seeds of five new pomegranate varieties using sensory attributes such as seed hardness, visual color, taste, and overall quality appreciation. These authors tried to correlate their sensory data with physico-chemical parameters and to recommend a commercial destination for each of their varieties. Pomegranate varieties were classified into three categories based on the maturity index (MI) of the seeds: (i) sweet: MI = 31-98, (ii) sour-sweet: MI = 17-24, and (iii) sour: MI = 5-7. Finally varieties PTO2 and CRO1 were very interesting for the juice industry because of their high juice contents, while varieties ME14 and ME15 were more appropriate for the fresh fruit market because of the large fruit size and excellent seed organoleptic characteristics.

Until the work by Koppel and Chambers IV (2010), none of the research studies previously described included or use a detailed lexicon for the classification of the flavor attributes of pomegranate juices. A similar situation is still true and valid for the appearance, texture and flavor of fresh pomegranate fruits or the texture of other products.

Nowadays it seems crucial to "fully" characterize, using appropriate lexicons, fresh fruits from different pomegranate varieties and clones to obtain high quality products and to classify varieties and clones according to their suitability to manufacture different products, such as juices, dried arils, jams, etc. To properly characterize and classify commercially pomegranate varieties and clones there is an urgent need of properly develop appearance, flavor and texture lexicons and consumers studies linking products attributes with high consumers' liking and acceptability.

Here are some comments on the main attributes that can be used to describe the appearance, flavor and texture of pomegranate products; the attributes list can be completed by readers according to their experience with different products and their main commercial targets. Researchers participating in the session "Industrialization, Products and Sensory Quality" will give further details on the sensory characterization of different pomegranate based products.

II – Appearance

The appearance of pomegranate arils is very appealing for consumers (Fig. 1) and are always included in the labels of all pomegrante products, eventhoug in some cases, the percentage of pomegranate in the products is extremely low (sometimes just 1%). However, it is well-known that the intense garnet color of pomegranate arils will dissapear during pasteurization of juices and the addition of natural or artificial coloring substances will be required. Fresh fruits are marketed mainly according to their size and external color. Some of the main deffects are low intensity of color, excessive presence of spots or cracks, etc. Regarding the inner appearance of fruits important attributes could be: amount of carpelar membranes and rind compared to seeds, color intensity, ratio between whole seed/woody portion, dehydration degree, etc.

Fig. 1. Appearance of fresh pomegranate fruits, seeds and juices.

III – Flavor

Up to date, flavor is the only complex sensory property that can be fully described using the lexicon developed by Koppel and Chambers IV for pomegranate juices. The attributes included in this lexicon can be divided into three main categories: (i) aromatic notes: apple, beet, berry, brown spice, brown sweet, carrot, candy-like, cranberry, cherry, fermented, floral, sweet overall, vinegar, wine-like, woody, (ii) basic tastes: sweet, sour and bitter, and (iii) chemical feeling factors: astringent, toothetch, metallic, chalky, tongue tingle, tongue numbing, and throat burn.

IV – Texture

Probably this property is the less studied in pomegranate products. However, attributes such as firmness, hardness, elasticity, crunchiness, sound, moisture release, mouth drying, tooth-packing, pulpiness, particulates, etc. could also be of interest.

Using their flavor lexicon Koppel and Chambers IV (2010) were able to evaluate 34 pomegranate juices marketed in the USA and including concentrated products, products from concentrate, pasteurized and freshly squeezed products. Juices were clustered into five categories characterized by: (i) berry, dark fruity, toothetch mouthfeel and sweet overall, (ii) grape, cranberry, wine-like and of red or purple color, (iii) fermented and toothetch mouthfeel, (iv) brown color and musty/earthy, and (v) candy-like and sweet overall. According to these authors "it is clear that pomegranate juices are sweet, sour, bitter, astringent, and have toothetch. Although the flavors can be complex, the major components are grape, cranberry, berry, fruity-dark, musty/earthy and beet". Other aromatics can be added to this lexicon such as "citrus notes" and it can be discussed whether "candy-like" juices could come from authentic pomegranates or from artificial flavorings but there is no doubt that this is the only way to properly describe pomegranate products.

Finally it must be mentioned that publications from both the Universidad Miguel Hernández (Calín-Sánchez *et al.*, 2011; Carbonell-Barrachina *et al.*, 2011; Melgarejo *et al.*, 2011) and Kansas State University (Vázquez-Araújo *et al.*, 2011a,b,c) have tried to characterize using both instrumental [chemical composition (polyphenols, punicalagins, sugars, organic acids, volatile compounds), biochemical activity (antioxidant capacity)] and sensory (descriptive and affective tests) tools fresh pomegranate fruits (from different clones/genotypes and countries), juices (mixtures with other berries and fruits, with the addition of dried and milled rind) and dried products (arils and rind).

V – Conclusions

Even though a lot of work is been done on sensory characterization and consumers' behavior on pomegranate fruits, pomegranate based products and pomegranate products in the last three years, many more studies are needed to corrected allocate each variety to a proper commercial sector (fresh fruit, juice, jam, etc.) and to have all the information needed to easily explain consumers how to: (i) distinguish true/authentic products from imitations/low quality products and (ii) fully appreciate and enjoy the uniqueness of authentic pomegranate products.

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Convective and microwave drying influence on the chemical composition, functional properties and sensory quality of pomegranate arils and rind

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Abstract. The objective of this study was to evaluate the application of: (i) convective drying (50, 60 or 70ºC), (ii) vacuum-microwave drying (240, 360 or 480 W) and (iii) a combined method of convective pre-drying and vacuum-microwave finishing drying in the processing of dehydrated pomegranate arils and rind. The parameters under study included sugars and organic acids contents, total antioxidant activity and total polyphenols content. All parameters were calculated on a dry matter basis and analysis of data showed drying let to a reduction in all the parameters under study; however, the behavior of arils and rind were different. Vacuum-microwave drying at 240 W was the best drying treatment of arils, while rind required softer conditions of convective drying (50ºC) and vacuum-microwave drying (240 W). Convective pre-drying and vacuum-microwave finishing drying was not a proper treatment for pomegranate drying.

Keywords. Drying – Arils – Rind – Quality – DPPH.

I – Introduction

Pomegranate (*Punica granatum* L.) is mainly cultivated in Iran, Afghanistan, India, USA and Mediterranean countries. Spain is the greatest European producer. There is a huge amount of pomegranate fruits (PG) which quality is not good enough to be consumed as a fresh fruit due to small damages or defects that mainly affect fruit appearance but not jeopardize their global sensory quality. Due to the different market requirements and the huge popularity of the health benefits of pomegranate, there are many scientific manuscripts where researchers have developed or characterized PG based products and co-products, such as juices (Calín-Sánchez *et al.*, 2010; Mena *et al.*, 2011), jams (Melgarejo *et al.*, 2010), wines (Zhuang *et al.*, 2011) and dried arils (Singh *et al.*, 2007).

Pomegranate rind is a richer source of antioxidants compared with arils (Li *et al.*, 2006) and could be use as a nutraceutical supplement (Espín *et al.*, 2007), as well as condiment for food and drinks (Navarro *et al.*, 2011). Dried arils and rind seemed to be a proper PG based product and co-product, respectively; however, up to this time there is no research literature on the influence of different drying methods on the quality of PG arils and rind. Therefore, the main objective of this study was to determine the influence of different drying methods: (i) convective drying (CD), (ii) vacuum microwave drying (VMD) and (iii) combined drying (CPD-WMFD) on the chemical composition, functional properties and sensory quality of dried PG arils and rind.

II – Material and methods

1. Plant material and processing of samples

Fresh pomegranate fruits (*Punica granatum* L. cv. *Mollar de Elche*) were picked on October 30, 2010 in a commercial orchard in Elche (Alicante, Spain). Fifty fruits were randomly harvested at commercial ripening and 20 homogenous fruits were finally selected. In all the samples under study, arils were manually separated and rinds, including peel and carpelar membranes, were separately submitted to the treatments. The initial moisture contents of the arils and rind were 80.4% and 76.3%, respectively. PG samples of 60 g were subjected to three different drying protocols: (i) CD was operated at three different temperatures: 50, 60 or 70°C with an air velocity of 0.8 m s^{-1}; (ii) VMD was operated at three different power levels: 240, 360 or 480 W and a pressure ranging from 4 to 6 kPa; and (iii) CPD-VMFD consisted of convective pre-drying (CPD) at temperature 60 °C for 90 or 150 min, followed by VM finish-drying (VMFD) with a microwave wattage of 360 W.

2. Extraction and determination of sugars and organic acids of fresh and dried arils

Fresh and dried samples were extracted with ultra pure water and phosphoric acid 0.1%, homogenized and then centrifuged. One milliliter of the centrifuged supernatant was filtered and injected into a HPLC. Organic acids and sugars were quantified using a diode-array detector and a refractive index detector, respectively.

3. Antioxidant capacity (AOC) and total polyphenols (TP)

AOC using the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was evaluated in both arils and rind. Samples were homogenized and then centrifuged. 10 µl of the supernatant and 40 µl of MeOH were added to 950 µl of a 0.094 mM DPPH solution. The absorbance at 515 nm was measured after 50 min of the reaction. The TP content was quantified in both arils and rind using Folin-Ciocalteu reagent. Samples were homogenized, centrifuged and then the absorbance was measured at 760 nm.

4. Sensory evaluation with a trained panel

Sensory evaluation with a trained panel was used to describe fresh and dried PG arils. Seven panelists were trained in descriptive evaluation of pomegranate and pomegranate based products. Panelists were asked to evaluate the intensity of the following attributes: color, fresh PG odor and aroma, burnt odor and aroma, caramel odor and aroma, sourness, sweetness, bitterness, adhesiveness and solubility. The individual products were scored for the intensity of the different attributes on a scale of 0 to 10, where; 0 = non perceptible intensity and; 10 = extremely high intensity.

III – Results and discussion

1. Effect of drying treatments on sugars and organic acids

Fructose and glucose were the main sugars found in the dried pomegranate arils. In the current study, fructose values were higher than glucose contents (Table 1), as previous reported by different researchers (Tezcan *et al.*, 2009; Melgarejo *et al.*, 2000). According to the data, soft conditions of CD provided the best result regarding fructose and glucose contents, followed by 150 min CPD-VMFD, while medium and high VMD conditions seemed to be the best VMD

options. It can be observed that as the temperature increased during the CD, the sugars contents decreased; however, intensive treatments (short times and high VMD powers) led to high sugar contents.

Table 1. **Sugars and organic acids contents (g/100 g of DW) in pomegranate arils as affected by drying treatments**

Samples	Sugars		Organic acids	
	Glucose	Fructose	Citric	Malic
Fresh	27.1 ± 0.1 a	46.3 ± 0.2 a	0.52 ± 0.09 a	2.49 ± 0.02 a
CD 50 ºC	15.7 ± 1.4 b	27.8 ± 2.6 b	0.03 ± 0.01 cd	0.18 ± 0.01 b
CD 60 ºC	14.2 ± 0.2 c	24.2 ± 0.3 c	0.03 ± 0.01 bcd	0.20 ± 0.01 b
CD 70 ºC	14.1 ± 0.4 c	24.2 ± 0.6 c	0.03 ± 0.01bc	0.20 ± 0.01 b
VM 240 W	14.4 ± 0.2 bc	24.8 ± 0.3 c	0.03 ± 0.01 cd	0.30 ± 0.01 d
VM 360 W	14.8 ± 0.1 bc	25.7 ± 0.1 bc	0.02 ± 0.01 d	0.42 ± 0.02 c
VM 480 W	14.6 ± 0.1 bc	25.3 ± 0.6 bc	0.02 ± 0.01 d	0.40 ± 0.01 c
90 min CPD-VMFD	14.7 ± 0.2 bc	25.2 ± 0.4 bc	0.03 ± 0.01 cd	0.36 ± 0.01 c
150 min CPD-VMFD	15.0 ± 0.1 bc	25.7 ± 0.2 bc	0.03 ± 0.01 bcd	0.32 ± 0.01 d
ANOVA	***	***	***	***

Malic and citric acids were the main organic acids found in the dried pomegranate arils. Malic acid content was higher than that of citric acid (Table 1), as previously showed in sweet pomegranates by Mirdehghan *et al.* (2007). Results showed that high temperatures of CD and high VMD power seemed to be the best drying option for malic acid content; however the softest conditions of both CD and WM show the highest values of citric acid. Regarding the contents of organic acids, VMD and CPD-VMFD were better treatments than CD. Too high temperatures and too long drying times often cause poor color, flavor and nutritional value (Hu *et al.*, 2006). Figiel (2009) reported that during drying of garlic, the highest temperature was obtained for the highest VMD power. Finally, it can be concluded that pomegranate sugars were more stable with softer drying conditions (application of low temperatures during long times). A different situation was observed in the case of organic acids, where the data showed that shorter times but higher temperatures led to higher contents.

2. Changes in AOC and TP after processing of pomegranate arils and rind

To quantify the AOC and TP of fresh and dried pomegranate arils and rind, the DPPH and Folin-Ciocalteu method and reagent were respectively used. Among the eight treatments, the highest AOC and TP values were observed for VMD. Lower AOC and TP were obtained for dehydrated samples compared with the fresh ones (Table 2). Pomegranate rind showed higher values of both AOC and TP than pomegranate arils; this statement agrees with previous studies, such as that of Li *et al.* (2006). Pomegranate rind showed higher values of AOC and TP for soft conditions of both CD and VMD. This behavior could be the result of high temperatures decreasing the antioxidant activities of the products being dried, independently of the time required for the process. The CPD-VMFD did not improve the AOC and TP compared with CD and VMD. Pomegranate arils showed better results with low temperatures and long times in CD; however, when VMD was applied, higher power leading to high temperatures and low times was recommended. The first trend (CD) was similar to that already described for the rind, while the second one (VMD) could be explained due to Maillard reactions producing compounds with high antioxidant capacity (Yilmaz *et al.*, 2005; Manzocco *et al.*, 2001).

Table 2. Effect of drying method on AOC and TP of pomegranate arils and rind

Samples	Rind		Arils	
	TP (mg eq gallic acid/g DW)	TAA (mg eq Trolox/100 g DW)	TP (mg eq gallic acid/g DW)	TAA (mg eq Trolox/100 g DW)
Fresh	125 ± 1a	4511 ± 14e	7.57 ±0.07a	120 ± 2a
CD 50 ºC	108 ± 1d	2575 ± 13bc	2.05 ±0.05d	70.0 ± 1bc
CD 60 ºC	105 ± 1e	2447 ± 24d	1.99 ±0.07de	65.6 ± 0.3cde
CD 70 ºC	72.8 ± 0.1f	1255 ± 1e	2.01 ±0.04d	68.1 ± 2.3bed
VM 240 W	111 ± 1c	2535 ± 39c	1.57 ±0.01f	56.5 ± 1.5f
VM 360 W	69.2 ± 0.1g	1295 ± 7e	2.38 ±0.02c	59.6 ± 0.7ef
VM 480 W	57.3 ± 0.4i	1295 ± 7e	2.51 ±0.03b	73.9 ± 5b
90 min CPD-VMFD	63.4 ± 0.6h	1294 ± 14e	2.00 ±0.04de	69.9 ± 4.6bc
150 min CPD-VMFD	69.9 ± 0.1g	1256 ± 14e	1.87 ±0.03e	72.2 ± 1.7bc
ANOVA	***	***	***	***

3. Sensory analysis of fresh and dried pomegranate arils

In general, the drying type significantly affected the intensities of the dried aril attributes. Attributes such as color, fresh PG odor and aroma, sourness and solubility significantly decreased after the drying process compared with the fresh sample, while the rest of attributes, burnt odor and aroma, caramel odor and aroma, sweetness, bitterness and adhesiveness increased during the dehydration of the fresh fruits. The color changes were linked with the Maillard reactions, which modify the color becoming darker and with a higher intensity of the brown color. VMD at 240 W was scored with the maximum values for fresh PG odor and aroma (3.1 and 5.6, respectively) after the fresh sample; besides these attributes took lower values as the temperature increased. Sourness decreased too during the drying process and the increase in the sweetness may hide sour notes. Solubility obtained lower scores in dried products compared to fresh samples and this fact could be linked with lower cohesiveness of particles in the dried items. Caramel notes were clearly influenced by the temperature. Burnt odor and aroma were evaluated and the following treatments led to unacceptable (too high) values: VMD at 480 W and both CPD-VMFD treatments. Bitterness scores were positively correlated with burnt notes. The sweetness/organic acids ratio was always over 100, explaining the high sweetness scores obtained for all dried products.

IV – Conclusions

Dried pomegranate arils and rind could be good options to commercialize pomegranate fruits without generating huge amounts of wastes. Dried PG arils were a delicious and sweet product due to high but equilibrated sugars and organic acids contents; besides, this product had significant antioxidant capacity. VM at 240 W was the best drying treatment for PG arils. Dried PG rind is a suitable nutraceutical and food condiment, but in this case soft drying conditions were recommended (CD at 50ºC or VMD at 240 W). CPD-VMFD was not an appropriate treatment neither for PG arils nor rind.

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The pomegranate industry in China – Current status and future challenges

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Abstract. The pomegranate, one of the oldest fruits known to man, was originally thought to be a native to China. Actually, it was brought to China through the Silk Road during the Tang Dynasty (600-900 BCE). The current production is estimated as 1,600,000 tones and the planted area of 110,000 Ha. Although the pomegranate adopts itself to a variety of climatic conditions and pomegranate can be found in many parts of China , the major orchards in China are in the provinces of Sichuan, Chongqing, Shandong, Shaanxi and Henan. Other provinces where pomegranate is produced (although on a smaller scale) include the provinces of Xinjiang, Hebei, Guangdong and Yunnan. There are over 200 different varieties of pomegranate in China, which vary from province to province, with a range of weights, from 300-1200gr, a scale of colors from light green to deep red and a range of skin thicknesses and kernels hardness (from soft to hard kernels). They also vary in their sugar content and other ingredients in the arils and the rind. The vast size of China constitutes, both, its potential strength, but also the source of its difficulties, which is reflected also in the Chinese pomegranate industry. Although China is one of the world's biggest producer of pomegranate (the biggest, according to some sources), its methods of cultivation resembles a gardening art rather than modern agriculture. Although some significant changes were introduced in recent years, agriculture in China is still characterized by labor intensiveness and small scale and it is a far cry from modern horticultural methods. The current situation is characterized by rather primitive methods of irrigation, lack of adequate facilities for sorting, sizing, packing, transportation and storage especially, cold and CA storage, which results in a relatively low quality of the product and inconsistency in the quality. The fruit is marketed, almost exclusively, in local markets because the current quality can't meet the competitive market economy requirements. China's rapid growth and structural changes, while resolving many problems, have also given rise to new challenges. Nevertheless, China has the capacity to meet these challenges. With the proper guidelines, including training, technology transfer and acquisition of know-how and modern equipment its potential can be realized.

Keywords. China – Pomegranate – Arils – Processing – Arils extraction.

I – Introduction

The pomegranate, one of the oldest fruits known to man, was originally thought to be a native to China. Actually, it was brought to China through the Silk Road during the Tang Dynasty (600-900 BCE). The current production is estimated as 1,600,000 tones and the planted area of 110,000 ha. Because the pomegranate adopts itself to a variety of climatic and soil conditions, it can be found in many parts of China. However, this also accounts for the poor production and low fruit quality in some regions. The major orchards in China are in the provinces of Sichuan, Chongqing, Shandong, Shaanxi and Henan. Other provinces where pomegranate is produced (although on a smaller scale) include the provinces of Xinjiang, Hebei, Guangdong, Anhui, Ningxia and Yunnan. There are over 200 different varieties of pomegranate in China, which vary from province to province, with a range of weights, from 300-1200 g, a scale of colors from light green to deep red and a range of skin thicknesses and kernels hardness (from soft to hard kernels). They also vary in their sugar content and other ingredients in the arils and the peel.

The vast size of China constitutes, both, its potential strength, but also the source of the difficulties in its agriculture, which is reflected also in the Chinese pomegranate industry. Although China is one of the world's biggest and one of the oldest producer of pomegranate, its impact on world's market is very small and it's bound to remain as such, unless major changes are introduced.

II – Current status

For hundreds of years, The Chinese methods of orchards cultivation resemble a gardening art rather than modern agriculture. Although some significant changes were introduced in recent years, fruit growing in China is still characterized by labor intensiveness and small scale and it is a far cry from modern horticultural methods. The household is a basic unit for production, where typically, 2.63 laborers average per household, cultivating 11.*7 mu* (~0.8 Ha) of land. Farmers have to bear the cost of production, including seeds (or cuttings), fertilizers, tools, and other investments in farmland.

Until the late 1970's, farmers were tied down, forced into collectives and made to deliver their rural "surplus" to the government. Then, village by village, farming families started to bribe their way out of the collective. They undertook to grow their share of the grain quota privately, and put the rest of their efforts into chasing other sources of income. The "household responsibility system", meaning decollectivization and the introduction of markets for rural products, was formally endorsed by the central government in 1978. It went on to become the cornerstone of reforms in agriculture and arguably in the whole economy.

It is interesting to note that this reform did not happen gradually. It spread across the land like bush-fire, not because the central government said it should, but because it chose not to stop the spontaneous entrepreneurialism of its farmers. What the government was actually doing was to rush constantly to catch up with the country's natural inclination.

However, the reforms and structural changes didn't change much the farming methods and they still follow, for the most part, the old tradition methods of hundreds of years. Irrigation methods are based, almost exclusively, on flood or furrow irrigation, with very small use of sprinkler and drip irrigation methods. While most of the farmers are well aware of the need for proper fertilization to obtain optimal yield, the cost on one hand, and an inadequate quality assurance of the locally produced fertilizers and seeds on the other hand, result in a relatively low yield and low quality of the product. Moreover, the issue of contaminated product is quite serious in some places where fertilization is still being performed with human secretion.

While grain production remains top priority in the country's planning, the reform and open policy have greatly emancipated the production forces and promoted an all-around development of the rural economy. Hence, the interest in fresh fruit production, and even flowers, has been greatly aroused. Once the strict government focus on "grains only" policy has been slackened and especially when it became evident that fresh produce can bring much more income, agricultural production became much more diverse. In addition, with the expansion of market economy, the export of agricultural products, both fresh and processed, is becoming more and more important and Chinese agricultural products reach, not only to the markets in the proximity of the country, but also those in Europe and even America.

However, the competitive market economy entails, not only improvement in production, but also the use of proper postharvest techniques to reduce spoilage and obtain the highest quality required on the world's markets.

The current situation is characterized by lack of adequate facilities for sorting, sizing, packing, transportation and storage, which result in a relatively low quality of the product. It is estimated that more than 40% of the total production of fresh produce is wasted in China because of poor postharvest treatments. In addition, inconsistency in the quality and variation between farmers

and also within a given shipment of one producer, also result in inferior quality yielding low profit. Quality standards, as commonly accepted in world's markets, are still only in the planning, let alone differentiation between domestic and export markets. TQM (Total Quality Management) procedures, while already being introduced gradually in the industry, are still alien to the agricultural sector. The result is that fruit is marketed, almost exclusively, in local markets because the current quality can't meet the competitive market economy requirements.

The current status reflects both, the major progress and development in Chinese agriculture, but also point out the many issues and problems that still need to be solved. Its potential, based on its geography, size, diversity and human resources, is very big. However, it needs a much greater "leap forward", before this potential can be realized.

III – Major constraints and challenges

Agriculture is regarded as a fundamental sector of Chinese economy. However, While China is a country with ~100 million hectares of cultivated land, the conditions for agriculture are far from being ideal. Major constraints, both objective and subjective, present a formidable challenge for the most needed process of modernizing the agriculture in China.

China is a water-deficient country and water shortage has seriously retarded socioeconomic development. Water runoff is below the world average, only about a third is exploitable and most of this is geographically concentrated. The area south of the Yangtze River has 7.5 times more water per square kilometer than the area north of the river. In the south 450 million people – a third of the population – live under threat of flood; in the north 300 counties and 479 cities are short of water. Uneven precipitation and water distribution make the situation even worse and accounts for only 60 percent of exploitable water being actually used. In agriculture alone the shortage of water is estimated to be about 30 billion cubic meters. This shortage will double if China increases irrigated farmland as planned. Groundwater is being tapped to make up the difference, but evidence abounds of over-exploitation.

Despite the shortage of water, its use continues to be wasteful. Most irrigation and drainage systems are badly run and maintained because of fragmented responsibility among levels of government, no direct participation by farmers in decision making, inadequate budget, and water charges that are too low to cover maintenance costs.

The arable land, although large in absolute terms, represent only 7% of the world's total cultivated land, while it is required to support a population of 1.3 billion people, about 22% of the world's total.

Moreover, China is losing arable land due to urbanization. Each year 660,000 hectares of arable land are lost to urban sprawl and other non-agricultural uses. Urbanization has reduced arable land by 0.2% in the past decade. In 1994, for example, a net area of 5.7 million *mu* (~ 400,000 ha) of cultivated land was lost. Another factor, which accounts for the reduction of land available for cultivation is environmental degradation in all its forms. For example, some 3.7 million square kilometers of land – an area larger than Western Europe – suffer from water and wind erosion. Each year about 0.5 centimeter of topsoil is eroded from the 13 million hectares of mountains and rolling hills in the North China Plain, where some 250 million people live. Further loss of irrigated land is the result of out-of-date water conservation facilities. About one-third of China's water reservoirs do not work properly due to serious silt build-up; only 30% of irrigation projects for over ten-thousands *mu* each work in China, resulting in a loss of an irrigated area of more than three million *mu* every year. In addition, the ongoing process of desertification further reduces the potential available land for a viable agriculture. In the battle with the encroaching desert, the latter still has the upper hand.

Natural disasters, such as floods, draughts and earthquakes, and the declining ability to fight them also contribute to worsening the conditions for agricultural development by further reducing the availability of land and adversely affecting the livelihood of the farmers.

Environmental deterioration, which has been accelerated by the urbanization process, is reaching a nightmarish level of pollution which affects all aspects of life in China and also poses a threat to agricultural development. Water pollution in particular has, not only impact on health, but it affects directly agricultural output.

China's rapid growth and structural changes, while resolving many problems, have also given rise to new challenges. These, if unmet, could undermine the sustainability of agricultural development, including the development and modernization process of the pomegranate industry. Nevertheless, It is argued that China has the capacity to meet these challenges. With the proper guidelines, including training, technology transfer and acquisition of know-how and modern equipment its potential can be realized.

IV – Conclusions

Pomegranate is one of the important and oldest horticulture crop in China and can be found in many parts of the country. China is also one of the major producers (if not the biggest) of pomegranate in the world. However, faced with many constraints, both natural and structural, the current pomegranate is characterized by relatively low yield and poor fruit quality, its fruit is sold only in the domestic market and its contribution to the economy is marginal.

Nevertheless, there is a good chance that with its potential strength and based on the impressive record of its unprecedented economic growth, China has the capacity to meet the challenges that threaten to imperil its fruit industry, including pomegranate. With the proper guidelines, including training, technology transfer and acquisition of modern know-how, coupled with reforms and further structural changes and also providing the necessary financial support, the modernization process of its pomegranate can be realized.

Acceptance characteristics of pomegranate juice for four countries:
Spain, United States, Estonia, and Thailand

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Abstract. Five 100% pomegranate juices, representing various flavor profiles, were evaluated by at least 100 consumers in each country, Thailand, Estonia, Spain, and the USA, to determine which products were liked most by consumers in each country. Flavor of the juices was evaluated by a trained descriptive panel. The results suggest that Estonian, United States, and Thai consumers liked pomegranate juice samples that were sweet, sour, and had other fruity flavor notes, whereas, Spanish consumers liked the sample with fruity, musty/earthy, and vegetative flavors. One sample that was high in sweet aromatics, but also was described by consumers as having "artificial taste" or tasting like a "fruit flavored drink" was liked by some consumers in all countries. The sample that was high in astringency and had fermented and metallic flavors was not liked in any country. Clustering of the consumers showed some split opinions for several samples.

Keywords. Pomegranate – Sensory – Acceptability – Juice – Flavour.

I – Introduction

Studies on pomegranate juice have shown that flavors vary, but can be grouped into five major flavor categories (Koppel and Chambers, 2010). Overall, the flavor of pomgranate juice is sweet, sour, musty/earthy, fuity, and astringent, but individual groups are higher in certain flavors. One group was higher in dark fruity flavors and sourness, another higher in fruity, grape-like flavors with low sourness, a third group high in musty/earthy flavor and chalkyness with low sourness, a fourth one was high in astringency and fermented notes with high sourness, and the last group exhibiting higher sweetness and candy-like flavors. Vázquez-Araújo *et al.*, (2011) studied commercial and fresh pomegranate juice flavor and aroma and found that volatile composition was higher in fresh juice suggesting that volatiles, and potentially flavor, was being lost during processing. Calin-Sanchez *et al.*, (2011) found that the presence of certain monoterpenes was related to high acceptance of pomegranate juice by Spanish consumers.

This study was conducted to compare sensory characteristics of juice to consumer acceptance of five different pomegranate juices, in four different countries: Thailand, Estonia, Spain, and the United States of America (US)

II – Materials and methods

1. Samples

Samples, which represented the five flavor clusters (called, A,B,C,D,and E in this paper) reported by Koppel and Chambers (2010), were acquired from Estonia, Spain, the USA, and Thailand. Samplles were shipped from their purchase location to other countries. Sample B was originally purchased in the US, but later a similar substitute sample was purchased in Thailand for testing in that market because of shipping issues.

2. Descriptive sensory analysis

Flavor of the sample was measured by six highly trained panelists uisng a 0-15 intensity scale. The same procedure was used in descriptive profiling as described by Koppel and Chambers (2010).

3. Consumer study

Consumer acceptance was measured using a hedoic scale in Estonia, US, Spain, and Thailand. Approximately 100 consumers in each country rated each sample. The consumers had an age range of 18-65 with a ratio of 60:40 women and men. Samples were served chilled at 5-7°C in a randomized order.

4. Analysis

Significant differences (p=0.05) were found between countries for samples and also between juices for a country. The consumers were clustered using K-means clustering according to flavor liking scores. Consumer cluster flavor likings were mapped with descriptive sensory analysis data added as supplemental variables using Principal Component Analysis (PCA).

III – Results

As expected, Sample A was higher in sour, astringent and dark-fruity attributes; sample B had cranberry, grape, fruity and berry aromatics; sample C had some musty/earthy and beet notes and there was a chalky mouthfeel present; sample D was sour, astringent, bitter, but also carried fermented and metallic notes, and sample E was high in sweet overall, with cherry and candy-like notes present in addition to the sweet taste.

According to clustering results only one juice (D) caused negative liking scores for all consumer clusters (Fig. 1). Although mean scores showed some disliking in Estonia and US for sample A, it was actually liked by a cluster of consumers in those countries; the same applied for sample B in Spain and sample C in Thailand. Although the mean score of sample C showed liking in Estonia and Spain, and sample E in Estonia, Thailand, and US, there was also a cluster of consumers in those countries who disliked these juices. One of the Thai consumer clusters liked samples C and B with musty and berry flavors and the other cluster liked samples B and E with cranberry, fruity, and candy-like flavors high in sweet aromatics. The two Spanish clusters both liked sample E; however, only one of the clusters liked two additional juices (B and C), while the other cluster found all other juices unpleasant. The flavor liking of Estonian and US consumer clusters were very similar with the exception of one juice. Sample C was liked by one cluster in Estonia, although it was not liked by either of the clusters in the US. Clustering results combined with descriptive data showed toothetch, fermented, sour, bitter, astringent, or metallic flavors were not liked in any country (Fig. 1).
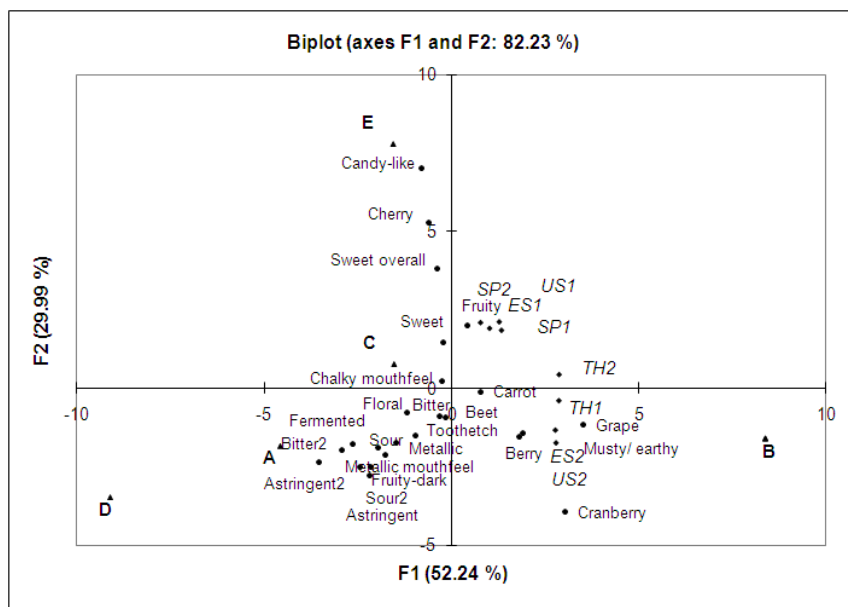
Fig. 1. Consumer clusters flavor liking PCA, descriptive data used as supplementary data. US1, US2 – US clusters 1 and 2; TH1, TH2 – Thailand clusters 1 and 2, ES1, ES2 – Estonian clusters 1 and 2; SP1, SP2 – Spanish clusters 1 and 2.

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Potential of Spanish sour-sweet pomegranates for juice industry

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Abstract. Commercial juices of sweet pomegranates and fresh juices of sour-sweet pomegranates were analysed for organic acids, sugars, antioxidant activity, volatile composition, sensory profile and consumer liking. Organic acids and sugars were analysed by HPLC, while volatiles were extracted using hydrodistillation and analysed by GC-MS and GC-FID. Malic acid was predominant in sweet juices while citric acid predominated in sour-sweet samples. Fructose and glucose were found as the predominant sugars in all juices. The high potential of sour-sweet pomegranate fruits for the juice industry was supported by (i) the high values of positive attributes, such as colour and fresh pomegranate flavour and (ii) the high overall liking of consumers.

Keywords. Antioxidant activity – Sensory evaluation – Volatile compounds.

I – Introduction

Pomegranate, *Punica granatum* L., is one of the oldest known edible fruits. Pomegranate (PG) arils can be consumed fresh; however, there is a huge amount of fruits which quality is not good enough to be consumed in fresh. For this reason, it is necessary to find a commercial application for the fruits which cannot be commercialised in fresh. The low quality of fruit appearance is not only due to the fact that PG tree produces at different ripening stages, but also produces low quality fruits as the ripening happens later (Melgarejo and Salazar, 2003). Besides, several physiopathies lead to deteriorate fruit appearance. PG fruit is considered a functional product of great benefit as it contains several groups of substances that are useful in disease risk reduction (Çam *et al.*, 2009). The beneficial health qualities of PG have been attributed to the exceptionally high antioxidant capacity of the fruit juice (Gil *et al.*, 2000). Commercial PG juices have shown an antioxidant capacity three times higher than red wine and green tea (Gil *et al.*, 2000). Therefore, it can be considered that juices are a great option for the PG fruits with low appearance but high organoleptic quality. The main aim of this study was to evaluate the potential of sour-sweet PG fruits, cultivar C25, in the juice industry compared with juices currently marketed in Spain. Parameters under study were organic acids, sugars, antioxidant capacity, sensory profile and overall liking of a consumer panel.

II – Material and methods

1. Plant material

Two freshly squeezed and two commercial PG juices were studied. Commercial juices, from sweet cultivar (*Mollar de Elche*), were obtained (from a private company, Granadas de Elche SLU). Fresh juices were prepared by manually extracting the arils from the fruits (removing all carpellar membranes) and using a pilot-plant press. Fruits from a sour-sweet PG cultivar (C25) were obtained from an experimental farm of UMH facilities (Table 1).

Table 1. PG juices under study

Category	Cultivar	Nomenclature	Abbreviation
Commercial	Mollar de Elche	Grana vida 100% PG juice	PPCJ
Commercial	Mollar de Elche	Grana Ké PG nectar	CPN
Fresh	C25	100% PG juice	c-SSPJ
Fresh	C25	100% PG juice + 5% sucrose	s-SSPJ

2. Analysis of organic acids and sugars

Fresh and dried samples were extracted with ultra pure water and phosphoric acid 0.1%, homogenized and then centrifuged. One milliliter of the centrifuged supernatant was filtered and injected into a HPLC. Organic acids and sugars were quantified using a diode-array detector and a refractive index detector, respectively.

3. Antioxidant capacity

Total antioxidant activity (TAA) was quantified in both hydrophilic (H-TAA) and lipophilic (L-TAA) compounds in the same extraction. Juices were homogenized and then centrifuged. TAA was determined using the enzymatic system composed of the chromophore 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), horse radish peroxidase enzyme (HRP) and its oxidant substrate (hydrogen peroxide). The decrease in absorbance was measured at 730 nm after adding the juice extracts.

4. Sensory evaluation with expert and consumer panels

Sensory evaluation with a trained panel was used to describe PG juices. 8 panellists were trained in descriptive evaluation of juices, including PG juices. Panellists were asked to evaluate the intensity of the following attributes: color, fresh PG odor, caramel odor, sweetness, sourness, fresh PG flavor, caramel flavour and astringency. The individual products were scored for the intensity of the different attributes on a scale of 0 to 10, where; 0 = Non perceptible intensity and; 10 = extremely high intensity. A sensory evaluation with a consumer panel was carried out with 50 consumers with the main requirement for their recruitment of being consumers of fruit juices at least twice a week. Consumers were asked about the overall liking of the samples. A hedonic 11 point scale was used.

III – Results and discussion

1. Organic acid and sugar contents

The organic acids profile could be an important source of information about the sensory properties of PG juices because each acid grants different organoleptic attributes to the final product. Malic and citric acid were the main organic acids in all juices under study. These results agree with previous data reported in the literature (Melgarejo *et al.*, 2000; Ozgen *et al.*, 2008).

The malic acid content was slightly higher than the citric acid content in the commercial samples because of the PG variety used, *Mollar de Elche*. The composition of organic acid found in the commercial samples agreed with those from previous studies carried out by Mirdehghan *et al.* (2007) in fruits from the Indian cultivar *Malas Yazdi*. On the other hand in fresh sour-sweet PG juices, citric acid predominated over malic acid as reported previously by Melgarejo *et al.* (2000) for several Spanish sour-sweet and sour cultivars. Three other organic acids were also detected

but occurred at much lower concentrations: oxalic, tartaric and ascorbic acid (Table 2). Finally, the total concentration of organic acids was significantly ($p<0.01$) lower in the commercial nectar than in all other juices under study.

Table 2: Organic acids and sugars of PG juices (g/100 ml)

Juices	Oxalic	Citric	Tartaric	Malic	Ascorbic
PPCJ	0.05 ± 0.02 a	0.68 ± 0.11 c	0.02 ± 0.01 b	0.72 ± 0.10 c	0.15 ± 0.03 b
CPN	0.03 ± 0.02 a	0.33 ± 0.03 d	0.08 ± 0.01 b	0.61 ± 0.04 c	0.10 ± 0.02 b
c-SSPJ	0.10 ± 0.04 a	1.54 ± 0.10 b	0.17 ± 0.04 a	1.24 ± 0.16 a	0.23 ± 0.02 a
s-SSPJ	0.13 ± 0.01 a	1.89 ± 0.01 a	0.16 ± 0.01 a	0.96 ± 0.02 b	0.22 ± 0.01 a
ANOVA	N.S.	***	**	***	**
Juices	**Fructose**	**Glucose**	**Sucrose**		
PPCJ	8.58 ± 0.29 c	6.54 ± 0.22 c	0 c		
CPN	2.52 ± 0.02 d	2.42 ± 0.02 d	0 c		
c-SSPJ	11.1 ± 0.03 b	9.05 ± 0.02 b	1.15 ± 0.01 b		
s-SSPJ	17.6 ± 0.07 a	13.8 ± 0.01 a	1.60 ± 0.01 a		
ANOVA	***	***	***		

Fructose and glucose were the main sugars found in the PG juices under study (Table 2). In the current study, fructose values were higher than glucose contents, as previous studies reported (Melgarejo *et al.*, 2000; Tezcan *et al.*, 2009); although differences were not always statistically significant ($p>0.05$). However, other studies have reported that glucose was slightly higher than fructose (Miguel *et al.*, 2004; Ozgen *et al.*, 2008). These differences could be related to, for example, fruit cultivar, climatic conditions and irrigation management, among other factors.

Sugar analysis also revealed the presence of sucrose in low but significant levels in juices from sour-sweet fruits; no sucrose was detected in the commercial juices. Besides, as shown in Table 2, a significant portion of the added sucrose to the juice s-SSPH was hydrolyzed and converted into fructose and glucose; therefore, increasing the contents of these two sugars in s-SSPJ compared to the control samples (c-SSPJ).

Finally, the total concentration of sugars was significantly ($p<0.001$) lower in the commercial nectar than in all other juices under study. However, the label of the PG nectar indicates that sweetener was added to improve the sweetness of the product.

2. Antioxidant capacity (AOC) of PG juices

The total antioxidant capacity was measured separately as H-TAA and L-TAA fractions by ABTS analytic method. H-TAA (mean of 3.02 ± 0.11 TEAC for all juices under study) was significantly higher ($p<0.001$) than L-TAA (mean of 0.58 ± 0.03 TEAC), showing that the major contributors to antioxidant activity of PG juices were hydrophilic compounds. The highest value of AOC in the hydrophilic extract was obtained in the control sour-sweet juice, 3.24 ± 0.09 TEAC; even though there were statistically significant differences ($p<0.05$) among the antioxidant activities of the four PG juices under study, the differences only represented a maximum value of 14 % (0.55 TEAC). In the present study, H-TAA represented about 84 % of the total antioxidant activity of PG juices. The initial total AOC of freshly squeezed juices from Mollar de Elche fruits was 4.1 TEAC. It is interesting to point out that even though commercial juices had a thermal treatment (98 ºC for 20 s) the antioxidant activity was only slightly reduced down to 3.52 TEAC.

3. Sensory evaluation

The descriptive sensory analysis of juices showed that commercial samples were characterised by significantly higher (p<0.001) intensities of caramel odour and flavour than freshly squeezed juices; these caramel notes could be originated during the soft pasteurisation of juices. Besides, commercial juices from *Mollar de Elche* fruits were characterised by high intensities of sweetness, astringency and low levels of sourness. Sensory results from the trained panel proved that fresh juices prepared using Spanish sour-sweet PG fruits (c-SSPJ) led very good sensory results and that these juices were characterized by *high intensities* of colour (8.8, intense garnet colour), fresh PG odour (5.8), fresh PG flavour (7.0) and sourness (7.0), *medium intensity* of sweetness (3.6) and astringency (3.3) and *low scores* of caramel odour (1.0) and caramel flavour (1.3). The addition of sucrose to the juice of sour-sweet fruits resulted in higher intensities of sweetness but at the same time in lower intensities of colour and fresh PG odour and flavour, finally leading to slightly lower but not statistically significant overall liking by consumers. The *overall liking* of the consumer panel regarding sour-sweet fresh PG juices (c-SSPJ) was 7.6 in a 0 to 10 scale. Fresh PG juice with sucrose addition (s-SSPJ) was the sample with the second overall liking score (6.8). In general, Spanish and USA consumers are not willing to consume juices from sour or sour-sweet varieties (Vázquez-Araújo *et al.*, 2011); however, fresh juices from selected sour-sweet cultivars of high quality (such as C25) have proved to be useful for the manufacturing of commercial PG juices. The level of sourness of C25 fruits was useful in improving the typical freshness, colour, odour and flavour of PG juices but it was low enough not to cause rejection by consumers.

IV – Conclusions

Spanish sour-sweet PGs have proved to be useful for juice manufacturing because they presented high levels of antioxidant activity, high intensities of positive sensory attributes and high overall liking by consumers. The sourness of the sour-sweet PG juices could also be useful in reducing the sometimes excessive sweetness of the most cultivated sweet Spanish PG cultivar, *Mollar de Elche*.

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Authentication of pomegranate juices using their volatile compositions

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Abstract. Pomegranate juice has gained much popularity mainly because of its health benefits; however, are you completely sure that when you pick up a bottle of juice from a store you are really getting a real pomegranate juice with all its antioxidants and full flavor? This is important especially because usually you are paying a high price for this juice. Pure pomegranate juices and blends (grape, apple, orange, etc.) with other fruits from around the world are under analysis using GC-MS to find a "volatile fingerprint" that allow us to discriminate which products really contain what it is promised in their labels and which ones are just promising much more than they are claiming. Artificial pomegranate aromas being used in the food industry are also being analyzed to find out which are the compounds being intentionally added to juices to pretend they have the unique flavor of fresh pomegranates.

Keywords. Artificial flavorings – Certification – GC-MS – Quality control – Volatile fingerprint.

I – Introduction

Spain is the main European pomegranate producer, and its production is mainly located in the Valencia Community, especially Elche and its surroundings. The edible part of pomegranate, arils, is consumed fresh and also used in the preparation of fresh juices, canned beverages, jellies, jams and for flavoring and coloring drinks (Melgarejo *et al.*, 2011).

Due to the low aromatic intensity of fresh pomegranates and the losses originated during juice manufacturing, most of the time the flavors of commercial pomegranate juices are not similar to that of fresh fruits. Besides and even though pomegranate products are getting more and more popular every day, only a limited number of consumers have tried fruits directly from the trees and know the real flavor of fresh pomegranate or pomegranate juice.

Up to the last few couple of years the literature references dealing with the volatile composition of pomegranates were very limited; however, during the last two years Miguel Hernández University, UMH (Spain) and Kansas State University, KSU (USA) have widely studied this topic and as a result a significant number of manuscripts have been published (Calín-Sánchez *et al.*, 2011; Carbonell-Barrachina *et al.*, 2011; Melgarejo *et al.*, 2011; Vázquez-Araújo *et al.*, 2011a,b,c). Consequently the volatile composition of freshly squeezed, commercial pomegranate juices and juices mixing pomegranate with other fruits or berries is well-known and can be compared to that of other more abundant and less expensive juices, such as grape, pineapple, etc.

Because of the high popularity of pomegranate some juice companies might include pomegranate in the labeling of their products without using real pomegranate fruits in them. Therefore, some claims are reaching different Quality Control Organisms around the world

about potential fraud of juices claiming to be mainly from pomegranate but only using artificial flavorings, e.g. SPF Economie, PME, Classes Moyennes et Energie (Direction Générale Qualité et Sécurité, Government of Belgium). The general aim of this study was to create a database of volatile compounds being in "true" pomegranate juices in its diverse formats (freshly squeezed, commercial, etc.) and comparing these volatile profiles with those of potentially fraudulent juices.

II – Materials and methods

Fruits were prepared or purchased and stored at 4-5 ºC until GC analyses.

1. Extraction procedure of volatile aroma compounds

Headspace solid phase micro-extraction (HS-SPME) was the method under study. After several preliminary tests to optimize the extraction system, 15 ml of the "problem" juices were hermetically placed in a 30 ml vial with a polypropylene cap and a PTFE/silicone septa; the volume ratio juice to headspace was approximately 1:1. A magnetic stirring bar was added and the vial was placed in a water bath with temperature control and stirring. Vials were equilibrated during 5 minutes at 45ºC in the bath and after this equilibration time, a 50/30 µm DVB/CAR/PDMS fiber was exposed to the sample headspace for 90 minutes at 45ºC. The fiber was chosen for its high capacity of trapping fruits volatile compounds. Extraction experiments were run in duplicate.

After sampling, desorption of the volatile compounds from the fiber coating was carried out in the injection port of the GC-MS during 4 min in splitless mode. The injector temperature was 200ºC for CG-MS.

2. Chromatographic analyses

The isolation, quantification and identification of the volatile compounds were performed on a gas chromatograph, Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector GC-MS QP-5050A. The GC-MS system was equipped with a TRACSIL Meta.X5 column, 95% dimethyl-polysiloxane and 5% diphenyl-polysiloxane (Teknokroma S. Coop. C. Ltd, Barcelona, Spain; 30 m x 0.25 mm x 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 ml min^{-1} in a split ratio of 1:20 and the following program: (i) 40ºC for 5 min; (ii) rate of 3.0ºC min^{-1} from 40 to 200ºC and hold for 1 min; (iii) rate of 15ºC min^{-1} from 200 to 280ºC and hold for 10 min. Detector was held at 300ºC.

Compounds were identified by using 3 different analytical methods: (i) Kovats indices (KI), (ii) GC-MS retention times (authentic chemicals), and (iii) mass spectra (authentic chemicals and NIST05 spectral library collection; NIST 2010). Identification was considered tentative when it was based on only mass spectral data.

III – Results and discussion

A complete database of volatile compounds isolated and identified in different types of pomegranate juices has been created at UMH based on manuscripts by UMH and KSU. When potentially fraudulent juices reach our facilities juices are analyzed and complete lists of compounds together with their relative abundances are prepared. For instance, a case study showed that the most abundant compounds in a "problem" juice were: nonanal, octanoic acid, α-terpineol, hexyl acetate, furfural, linalool oxide and ethyl hexanoate. This volatile profile was very close to the profiles described by Melgarejo *et al.* (2011), with non-significant differences

being probably related to the different cultivars and geographical origin of the pomegranates used to manufacture the juices.

Even though some compounds typically found in artificial pomegranate flavorings were also found in the "problem" juice (e.g. butyl acetate, isoamyl acetate, benzaldehyde, etc.), the concentrations at which these compounds were found seemed to imply that they came from natural sources.

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Optimization of pomegranate jam preservation conditions

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Abstract. One of the most important parameters to which consumers are sensitive when selecting jams is the color. Anthocyanin and colour development of pomegranate jams made from 'Mollar' cultivar were analysed during five months. Different temperatures (5ºC and 25ºC) and light exposures (daylight and darkness) were tested during storage. Also the influence of pectin on jam preparation was evaluated. The results concluded that high methoxy pectins yielded better pomegranate jams because of their high a* values (34% higher than low methoxy ones). Optimal storage conditions were achieved at 5ºC and no light exposure at all.

Keywords. Pomegranate – Jam – Anthocyanins – Color – Storage.

I – Introduction

Pomegranate fruits are rich in anthocyanins, pigments responsable for external and seed color development, being this a quality attribute. The following anthocyanins were identified in pomegranates: delphinidin 3-glucoside and 3,5-diglucoside, cyanidin 3-glucoside and 3,5-diglucoside, and pelargonidin 3-glucoside and 3,5-diglucoside (Gil *et al.*, 1995). Anthocyanins have a crucial role in the colour quality of many fresh and processed fruits. They are a good source of natural antioxidants, however, they are quite unstable during processing and storage. Temperature and time of processing (Markakis, 1982; Martí *et al.*, 2001) and storage (García-Viguera *et al.*, 1999) were found to exert a great influence on anthocyanin stability. Loss of anthocyanins have been attributed to many factors such as pH and acidity, phenolic compounds, sugars and sugar degradation products, oxygen, ascorbic acid, fruit maturity and thawing time (Markakis *et al.*, 1957; Withy *et al.*, 1993; García-Viguera *et al.*, 1998).

The effect of the pectin type on the jam colour has not been extensively studied. Although it has been suggested that pectin has a role in the colour degradation of the jam products (Lewis *et al.*, 1995). Kopjar *et al.* (2007) investigated the influence of different pectins and their concentration on the colour and texture of raspberry jams and they concluded that different pectins and their concentrations affect the colour and texture.

The objective of the present work was to evaluate anthocyanin content of pomegranate jam and its color development during storage under temperatures and light regimes. Also the influence of pectin on jam preparation was evaluated.

II – Material and methods

The pomegranate cultivar *Mollar* was used on this study since it is the largest cultivated in Spain. Fully ripe pomegranate fruits were harvested at the germplasm bank located at the Higher Polytechnic Agricultural College (Orihuela, Alicante, Spain) during the first half of October.

1. Jam preparation

Pomegranate jam was obtained according to a typical commercial protocol. Low and high methoxy pectins were used for jam production (Danisco Cultor España, S.A. (Grindsted® Pectin LA 210 and Grindsted® Pectin RS 400, respectively). Different types of jam were prepared:

(i) High methoxy pectin jam (HM) 350 g kg^{-1}of edible seeds, plus 1.65 g kg^{-1} of pectin, 3 g kg^{-1} of citric acid, 0.5 g kg^{-1} of ascorbic acid and 1 g kg^{-1} of sorbic acid. The final sucrose concentration was 65º Brix.

(ii) Low methoxy pectin jam (LM) recipe was 350 g kg^{-1}of edible seeds, plus 7 g kg^{-1} of pectin, 4,5 g kg^{-1} of citric acid, 0.5 g kg^{-1} of ascorbic acid and 1 g kg^{-1} of sorbic acid. The final sucrose concentration was 65º Brix.

2. HPLC anthocyanins analysis

Anthocyanins were extracted according to García-Viguera *et al.* (1997). An 1100 *Hewlett-Packard* High Performance Liquid Chromatograph (HPLC) was used for anthocyanin identification and quantification as described by García-Viguera *et al.* (1999).

3. Color measurements

A *Minolta* CR-300 color spectrophotometer was used for this study. Analyses were performed by reflection on a 2.5 mm thick sample placed over a white surface. L a* b* values were calculated using illuminant D65 (8 mm diameter measuring area) and a 10º observer.

4. Storage of pomegranate jam

Pomegranate jam samples were stored at different temperatures (5ºC and 25ºC) and light exposures (daylight and darkness). Both types of pomegranate jam (high and low methoxy pectin jam) were analysed after the preparation and after 30, 60, 90, 120 and 150 days of storage.

5. Statistical analysis

All data were subjected to statistical analyses. Analyses multifactorial ANOVA were performed along with the least significant difference tests (LSD) to detect any statistically significant differences ($p \leq 0.05$).

III – Results and discussion

When storage is done at 25ºC, anthocyanin total content went down quickly in both low and high methoxy pectin jams (Fig. 1). Regarding those jams stored at 5ºC, there were a 32% pigment degradation in HM ones and a 14% reduction in LM jams after 150 days. These results completely agreed with those obtained by García-Viguera (1999).

According to Markakis (1982), anthocyanin content could be negatively correlated to light exposure. Likewise, García-Viguera (1999) found no significant effect of light exposure on strawberry jam pigment composition. And these results could be definitively due to the protective effect of high amounts of sugar (Wrolstad *et al.*, 1990). All anthocyanin pigments showed a reduction over time. This statement completely agrees with pomegranate marmalade results obtained by Zafrilla *et al.* (1998).

Samples with high methoxy pectin had lower values of anthocyanins than samples with low methoxy pectin at 5ºC. Similar results were reported by Kopjar *et al.* (2007) on raspberry jam at 4ºC. This could be explained by interactions of anthocyanins and pectin. Regardless of

ingredients and storage conditions, pomegranate jams yielded the same predominant pigments. The most abundant ones were cyanidin 3-glucoside and 3,5-diglucoside. Degradation of them was quicker at 25ºC than at 5ºC storage (Figures 2 A and B).
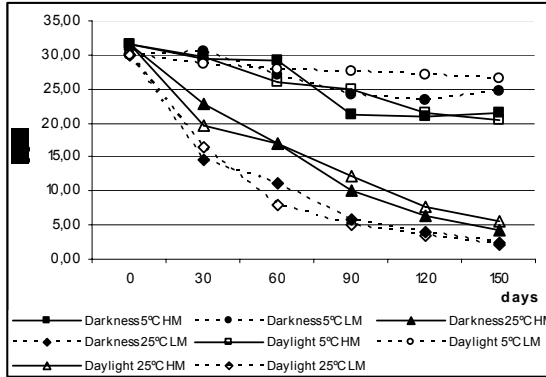

Fig. 1. Anthocyanin content evolution during storage conditions.
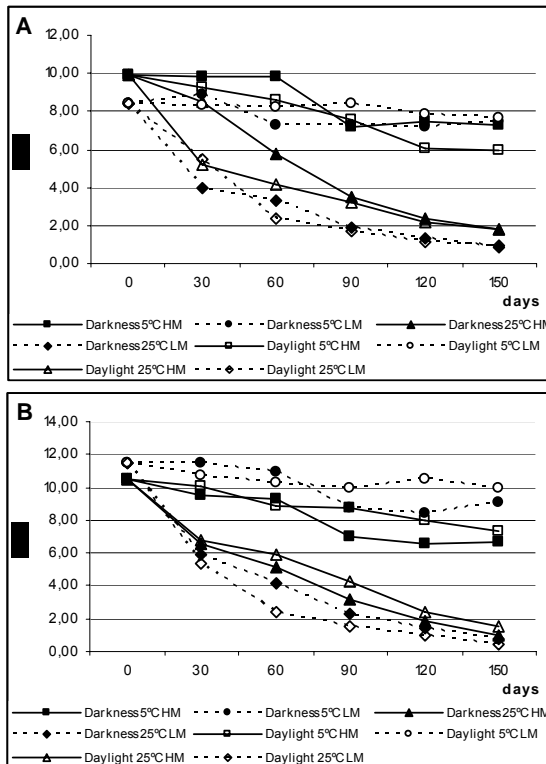

Fig. 2. Cyanidin 3,5-diglucoside (A) and cyanidin 3-glucoside (B) content development.

Regarding temperature effect on pomegranate jam color during storage conditions, there was a continuous decrease on a* values for both storage temperatures (Table 1).

Table 1. Temperature effect on pomegranate jam colour during storage

Storage (days)	a* ± SD	
	5ºC	25ºC
0	25,25 ± 6,40 a	22,71 ± 5,24 a
30	21,09 ± 1,82 b	19,00 ± 4,50 ab
60	17,75 ± 1,73 c	16,68 ± 6,47 bc
90	16,42 ± 3,10 cd	14,02 ± 3,48 cd
120	14,64 ± 2,25 de	13,40 ± 3,98 cd
150	12,84 ± 3,08 e	11,30 ± 3,90 d

The light conditions did not significantly affect pomegranate jam color (a* values). However, when considering the a* parameter evolution over time, there were reductions of 47% and 51% in daylight and dark conditions respectively after 150 days storage (Table 2).

Table 2. Light exposure effect on pomegranate jam colour during storage

Storage (days)	a* ± SD	
	Daylight	Darkness
0	25,83 ± 5,87 a	25,14 ± 5,74 a
30	19,62 ± 4,57 ab	20,48 ± 2,16 b
60	18,07 ± 5,95 bc	16,37 ± 2,91 c
90	16,20 ± 3,34 bc	14,24 ± 3,40 cd
120	14,47 ± 3,56 cd	13,57 ± 2,93 cd
150	12,03 ± 3,26 d	12,11 ± 3,91 d

High methoxy pectin jams (HM) yielded higher a* values (about 34% more) than low methoxy pectin ones (LM). Statistically significant differences were detected between HM and LM jams (Table 3). Even though high methoxy pectins yielded pomegranate jams with 34% more color than low methoxy pectins, there was an a* value reduction of 50% in both cases after a 5 months storage. The type of pectin as well as storage temperatures definitively affect pomegranate jam colour (Table 4).

Table 3. Pectin effect on pomegranate jam colour during storage

Storage (days)	a* ± SD	
	LM	HM
0	18,92 ± 2,10 a	29,05 ± 3,39 a
30	17,54 ± 3,01 a	22,56 ± 1,77 b
60	14,62 ± 3,18 b	19,82 ± 4,53 c
90	12,48 ± 2,03 c	17,97 ± 2,07 cd
120	11,66 ± 2,03 c	16,39 ± 2,32 de
150	9,33 ± 1,62 d	14,81 ± 2,64 e

Table 4. Mean a* values based on temperature, light conditions and pectins

a* ± SD					
Temperature		**Light conditions**		**Pectin**	
5ºC	18,00 ± 5,33 a	**Daylight**	17,20 ± 5,64 a	**LM**	14,09 ± 4,07 a
25ºC	16,18 ± 5,94 b	**Darkness**	16,98 ± 5,79 a	**HM**	20,09 ± 5,52 b

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Évolution et stabilité d'un jus fonctionnel à base de grenade et de kaki

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Résumé. La conception de ce produit, "gran&GO!", résulte de la combinaison de plusieurs développements technologiques innovants : la production de jus clarifié de grenade (*Punica granatum* L.) de diverses cultivars, le développement d'un jus industriel clarifié de kaki (*Diospyros kaki* L.) non astringent, l'utilisation, en tant qu'acidulant, d'un jus de citron (*Citrus limón* (L.) Burm.) clarifié par filtration tangentielle pour compenser la sensation sucrée, astringente et légèrement amère des premiers jus cités précédemment. De plus, des nanoparticules de flavonoïdes d'agrumes ont été employées pour éviter la détérioration de la vitamine C (dernière technologie en expérimentation pour augmenter la solubilité des extraits végétales). L'objectif de cette étude est de déterminer l'évolution et la stabilité des paramètres physico-chimiques et des propriétés nutritionnelles durant l'essai de stockage accéléré de 7 jours à 45°C. Avec la finalité d'estimer la vie utile d'un jus fonctionnel à base de grenade et de kaki.

Mots-clés. Jus clarifié – Grenade – Kaki – Fonctionnel.

I – Introduction

La combinaison des jus de fruits typiques de la Méditerranée se justifie par la grande quantité de tonnes de déchets originaires du marché des produits frais, qui diminuent parfois la rentabilité du processus de transformation. Le profit intégral de ces fruits, ainsi que sa transformation en jus et autres produits apportent une solution à quelques cultures de grande importance économique pour les zones de production.

Avec le développement de ce produit fonctionnel de fruits nous avons un grand apport de composés bioactifs pour l'organisme, en réduisant les risques d'apparition de maladies. Cela peut être la base de nouveaux aliments fonctionnels pour la prévention de différents problèmes de santé d'établir la relation étroite qui existe entre un état physiologique de stress oxydatif et l'apparition de différents problèmes de santé (cardiovasculaire, métabolisme de glucose et de lipides, activité neuronale, anxiété, etc.). Dans ce sens, les fruits que contient le combiné étudié (grenade, kaki et citron) sont une source naturelle de composés bioactifs avec des propriétés bénéfiques pour la santé. L'un des fruits qu'il faut remarquer est la grenade, ayant quelques composants très intéressants, les tanins hydrolysables de type élagitanin (isomères de la punicalagine et d'acide élagique), puisque de nombreuses propriétés bénéfiques pour la santé ont été décrites pour ceux-ci : une activité anticarcinogénique et antitumorale (Clifford et Scalbert, 2000; Sartippour et al., 2008). L'un des autres fruits est le kaki qui possède des substances phénoliques (un polyphénol) comme les proantocianidines, qui sont des tanins condensés de haut poids moléculaire qui attribuent l'astringence à ce fruit et des propriétés de

capture des radicaux libres, ce qui donne à ce fruit une activité antioxydante (Chunmei Li, 2010). Pour tout cela, l'objet d'étude est d'observer l'évolution et la stabilité des composés bioactifs dans des jus de grenade, de kaki et dans le combiné "gran&GO". Afin d'estimer la vie utile du produit et afin de pouvoir prédire le temps de conservation de ses propriétés organoleptiques et alimentaires durant un essai de stockage accéléré.

II – Matériel et méthodes

Détermination du contenu en vitamine C : on utilise la méthode officielle 43064 de l'Association of Official Analylical Chemist (AOAC, 1984).

Détermination de la composition poliphénolique par HPLC. Les composés phénoliques ont été analysés dans la fraction soluble des différents échantillons de jus (Pérez-Vicente *et al.*, 2004).

III – Résultats et discussion

Sur le Fig. 1, on observe l'évolution du contenu en vitamine C de différents jus. On peut observer comment dans la grenade et dans gran&GO! le contenu en vitamine C diminue au fur et à mesure du temps de stockage, cependant, le mélange de jus gran&GO! présente une plus grande résistance à la détérioration de la vitamine C. À 7 jours, on détecte 78,1% de perte contre 90,4% de perte dans le jus de grenade. Cet effet protecteur pourrait être attribué à l'action des flavonoïdes citriques qui protégeraient la vitamine C de sa destruction (Mena, 2008), ou bien à la présence d'un composé de nature phénolique originaire du jus de kaki dans le mélange gran&GO! Dans le jus de kaki, le contenu de vitamine C peut être pratiquement considéré comme méprisable durant l'essai réalisé puisqu'une perte se produit au moment même de la pasteurisation.
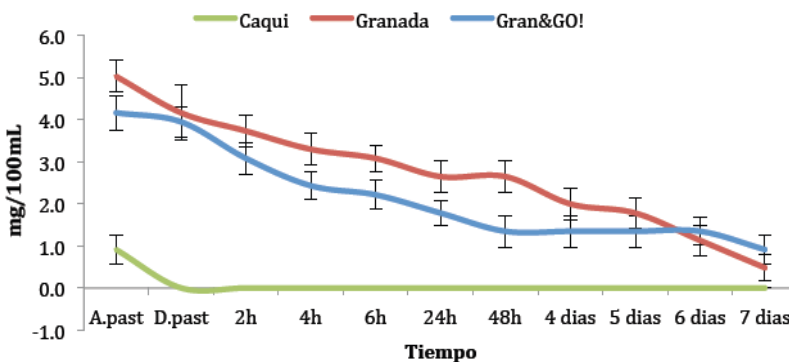
Fig. 1. Évolution du contenu en Vitamine C des différents jus durant la période de stockage.

Les composés prédominants du jus de grenade au commencement de l'expérience, depuis l'instant précédent la pasteurisation jusqu'à 6 heures, ne subissent pas de dégradation significative, comme on peut l'observer sur la Fig. 2. De plus on observe qu'à partir de 24 heures se produit une perte d'à peu près 50 % des anthocyanes dans la grenade et plus de 60 % dans le cas de gran*GO! (Fig. 3). Ce serait ce point qui marquerait la vie utile du produit.

Dans la Fig. 4 on observe le contenu en punicalagines du jus de grenade et du mélange gran&GO! On peut observer une diminution des punicalagines durant la période d'étude, probablement dû à la température et au temps de stockage comme d'autres études l'ont déjà démontré (González-Molina *et al.*, 2009). Certains auteurs (González-Molina *et al.*, 2009) ont

relié la présence de jus de citron, comme réducteur de l'effet de la dégradation des punicalagines, puisque l'hydrolyse de celles-ci est favorisé à de plus grands pH; cependant, la concentration de jus de citron dans notre jus est très réduite; il serait donc intéressant de répéter l'étude avec de plus grandes concentrations.
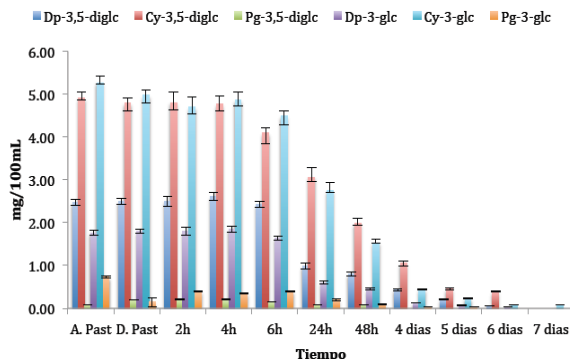
Fig. 2. Profil d'anthocyanes dans le jus de grenade en fonction de la période de stockage.
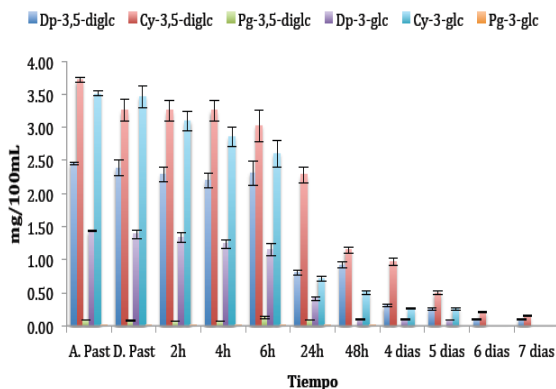
Fig. 3. Profil d'anthocyanes dans le jus gran&GO ! en fonction de la période de stockage.
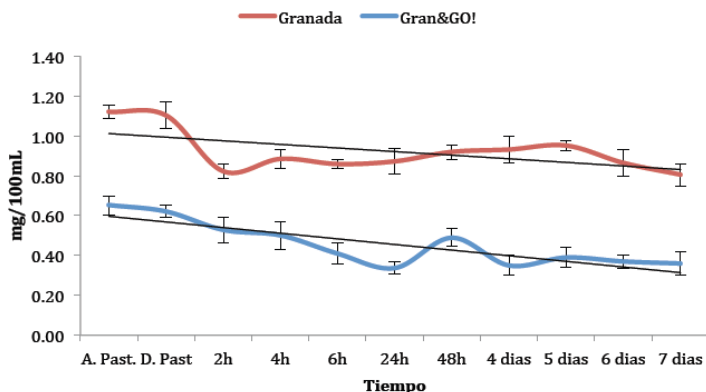
Fig. 4. Évolution du contenu en punicalagine des différents jus durant la période de stockage.

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Evolution of sugars and organic acids patterns during the elaboration of pomegranate varietal wines

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Abstract. Pomegranate is usually earmarked for arils fresh consumption and also used by food industry for the elaboration of juices and jams. Pomegranate wine constitutes a new alternative for the use by producers of second qualities and over-ripe fruits of this typical Mediterranean crop, with the subsequent environmental benefits. Hence, with the aim of developing new pomegranate-derived drinks, the evolution of sugars and organic acids patterns from different varieties throughout the winemaking stages was assessed. Noticeable changes were occurred for these compounds during wine elaboration, especially along fermentation period. In addition, a significant role of the cultivar used in the organic acids profile was noted.

Keywords. *Punica granatum* – Wine-making – Fermentation.

I – Introduction

Pomegranate fruits are usually earmarked for arils fresh consumption and are also used by food industry for the elaboration of juices, jams, and various other processed products. Nevertheless, large quantities of deteriorate second qualities and over-ripe pomegranate fruits are often not available in optimal conditions for consumers and, hence, are wasted as by-products. Therefore, with the aim of minimizing production losses and generating more profits with along a sustainable use of wastes, new uses and methods for pomegranate processing should be developed. In this sense, pomegranate wine elaboration may result in a promising alternative to employ underused fruits. Actually, many studies on fruit wine production have been performed in recent years as well as their possible therapeutic properties have been assessed. Likewise, some pomegranate fermented products have demonstrated to possess multiple beneficial implications on health management (Schubert *et al.*, 1999; Sezer *et al.*, 2007).

Taking into account the great diversity existing among the sensorial and phytochemical properties of pomegranate cultivars (Mena *et al.*, 2011), differences in the final quality of pomegranate varietal wines should be expected. Furthermore, it is important to note that changes in the general composition of food products may determine consumer acceptance and preference. Organic acids affect to the organoleptical properties of musts and wines (Kelebek *et al.*, 2009). Likewise, despite some works concerning pomegranate fermented juices have been performed, there is no substantial information on pomegranate wine composition, apart from studies covering all stages of the pomegranate winemaking process. Consequently, the purpose of this work was to produce and investigate the promising prospects of pomegranate wines as novel quality drinks. Research was focused on changes occurring to compounds with a technical relevance such as sugars and organic acids during the winemaking of different pomegranate varietal juices.

II – Material and methods

Second quality pomegranate fruits from cv. *Wonderful* and *Mollar de Elche*, harvested in Alicante region (SE Spain) during the 2010 season, were provided by "Cambayas Coop. V." (Elche, Alicante, Spain). Pomegranates were cut in halves and juices of each cultivar were obtained by pressure with a laboratory pilot press. *Wonderful* and *Mollar de Elche* freshly prepared juices were also mixed using the same proportion (v/v) of each one to study the behaviour of the blended juice, labelled as *Coupage*.

In general, the process of producing pomegranate wine followed the production procedures of grape wine. Juices were kept in vessels affixed with an airlock where 60 mg/l of potassium metabisulfite and 200 mg/l of a fermentation activator containing ammonium phosphate were added. Next, fermentation was started after yeasting (300 mg/l), and the temperature was kept at 22 °C ± 1 during the fermentation process, considered complete when the total soluble solids content was stable for 3 days more after day 6. Once fermentation was finished, the wines were clarified and racked for one day at 4ºC. Then, the wines were transferred to new vessels, 60 mg/l of potassium metabisulfite were added, and they were stored to stabilize them for 10 days in darkness at room temperature (Duarte *et al.*, 2010). All samples were done in triplicate. Samples were taken from each vessel at sampling time and stored frozen (-20ºC) until analyzed. Organic acids and sugar were determined simultaneously as described by Mena *et al.* (2011).

III – Results and discussion

Pomegranate juices presented glucose and fructose as major sugar components, being the fructose concentration higher than the glucose one in all the varietal juices (Fig. 1). The content of these carbohydrates was drastically reduced throughout the fermentation time (Fig. 1, stage α) and degradation kinetics for both sugars were similar. Nonetheless, while glucose was almost disappeared (0.02-0.06 g/100ml, for *Wonderful* and *Mollar de Elche* wines, respectively), fructose residues remained after the end of the winemaking process (0.20-0.29 g/100ml, for *Mollar de Elche* and *Wonderful*, respectively), as it happens to other fruit wines (Kelebek *et al.*, 2009; Kim *et al.*, 2008). Moreover, it is important to note that there were not differences among varietal wines assayed with regard to the sugar conversion rate.
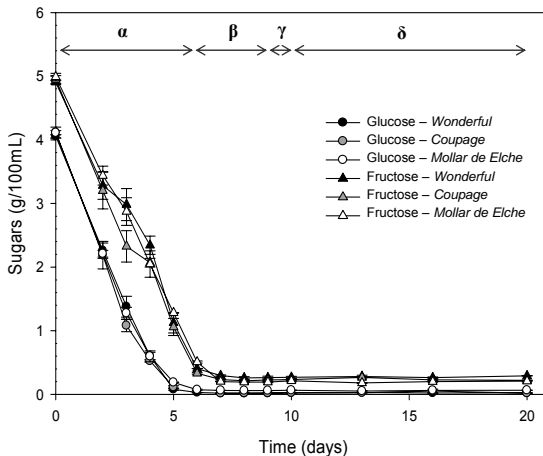

Fig. 1. Evolution of sugars in wines made from different pomegranate varieties during the winemaking. Winemaking stages: α, alcoholic fermentation; β, end of the alcoholic fermentation; γ, racking and clarification; δ, stabilization. Error bars are presented as SEM.

Both organic acids profile and concentration of *Wonderful* and *Mollar de Elche* juices were according to previous report (Mena *et al.*, 2011). *Coupage* juice presented intermediate values as it was expected (Fig. 2). Concerning pomegranate wines, changes in organic acids pattern were happened during winemaking procedures. Citric acid showed similar values in both varietal juices and wines, remaining almost constant, despite fluctuations in their concentrations were found throughout the different stages of wine elaboration (Fig. 2-A). Thus, citric acid values of pomegranate wines were close to the values of the juices and were 1.29, 0.64, and 0.28 g/100ml for *Wonderful*, *Coupage*, and *Mollar de Elche* wines, respectively. On the contrary, malic acid underwent noticeable changes during the winemaking process. Amounts of all varietal juices were around 0.30 g/100ml; however, malic acid was depleted rapidly until 0.10 mg/100ml within the fermentation period (Fig. 2-B, stage α) and, then, it was kept for all the samples during the rest of elaboration procedures (Fig. 2-B, stages β, γ, and δ). With respect to pomegranate minor organic acids, negligible variations took place for tartaric acid (Fig. 2-C) although a considerable increase was noted for acetic acid in some varietal wines (Fig. 2-D). In fact, pomegranate juices lacked of acetic acid and its occurrence was registered at the fermentation period. Moreover, great augments of acetic acid were found in *Wonderful* and *Coupage* fermented juices (0.008 and 0.005 g/100ml, respectively) whereas a slight one was found in *Mollar de Elche* one (Fig. 2-D, stage α). Later, a reduction in the content was done for all the varieties followed by the maintenance of the acetic acid levels in *Wonderful* and *Coupage* wines after wine racking and clarification since acetic acid in *Mollar de Elche* wines was disappeared previously (Fig. 2-D, stages β, γ, and δ).

On the whole, while citric and tartaric acids remained almost constant, malic acid was decreased owing to compositional variations during fruit wine elaboration (Kelebek *et al.*, 2009; Kim *et al.*, 2008). As a result of the malic acid degradation, the predominance of malic acid over citric acid in *Mollar de Elche* juice was reverted in *Mollar de Elche* wine but not in *Wonderful* and *Coupage* ones where citric was always the major organic acid. Likewise, regarding acetic acid formation, a significant role of the cultivar used on winemaking was noted.

IV – Conclusions

The elaboration of pomegranate wine from different varieties has been proved as a new and suitable way of industrialization for second qualities and over-ripe fruits. Noticeable changes in both sugars and organic acids patterns were described. Moreover, a significant role of the cultivar used together with a marked influence of winemaking procedures was noted.

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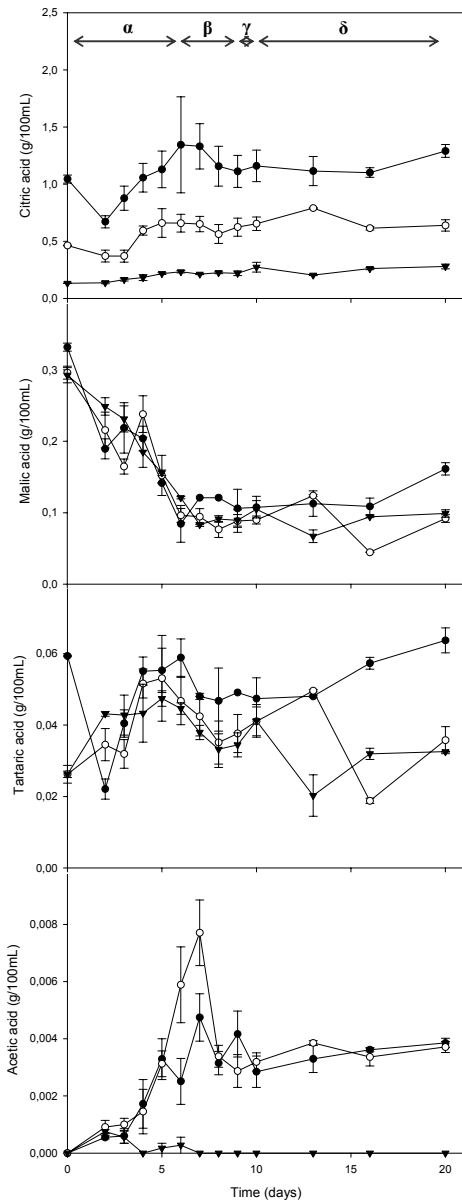
Fig. 2. Evolution of organic acids in wines made from different pomegranate varieties during the winemaking. (A) Citric acid, (B) Malic acid, (C) Tartaric acid, (D) Acetic acid. *Wonderful* (●), *Coupage* (○), and *Mollar de Elche* (▼). See winemaking stages in Fig. 1. Error bars are presented as SEM.

Influence of pasteurization treatment and storage in the red colour and microbiological stability of pomegranate juice

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Abstract. The combination of time and temperature of pasteurization treatments and storage conditions are critical in the degradation of the organoleptic and microbiological stability of juice. Therefore, the influence of pasteurization (high and low temperature) and storage conditions (refrigerated and room temperature) on the variation of the red color and microbiological stability of processed juice was evaluated. Both pasteurization treatments were effective in reducing microbial load and keeping the juice stable over time. However, storage temperatures had been decisive, because the juices stored at 25°C had lost 70-80% of its original red color.

Keywords. Pasteurization – Temperature –Stability – Colour –Pomegranate.

I – Introduction

Red fruits like pomegranate show a high content of bioactive compounds, which has placed them in the first line of functional juice market (Fadavi *et al.*, 2006). An important step in the processing of pomegranate juice is the pasteurization treatment. Pomegranate juice has a pH below 4 and thermal processing allow us to obtain a microbiologically stable product with excellent organoleptic and nutritional properties. The red color of pomegranate juice has been defined as an important quality parameter. Sepúlveda *et al.* (2010) suggested that the main responsible of red color of pomegranate juice is a group of polyphenolic compounds called anthocyanins. Also, they reported that these compounds have a maximum of absorbance at 520 nm. The measurements of this absorbance values can be related with the intensity of red color of the juices. Among the main factors affecting the stability of anthocyanins are included the processing and storage temperature. Therefore, the objective of this work was finding the combination of pasteurization temperature and time treatment to obtain a product organoleptically and microbiologically stable over time.

II – Materials and methods

1. Juice extraction

Ten kilograms of pomegranate variety "Mollar Elche" from Elche Experimental Farm were used to obtain the juice. Juice was obtained by pressing of arils inside a nylon mesh with a laboratory pilot press (Zumonat C-40; Somatic AMD, Valencia, Spain). The resulting cloudy juice

contained 10% pulp. For obtaining clarified juice, the cloudy juice was centrifuged at 4000 rpm 10 minutes.

2. Juice pasteurization and storage

Both cloudy and clarified juices were subjected to pasteurization treatments at high and low temperature (HT and LT) for specified times in a semi-tubular pasteurizer 25 l / h (Mipaser Prototype). The juices were stored at room (25°C) and refrigeration temperature (5ºC) for 45 and 120 days, respectively.

3. Red color measurement and microbiological analysis of juice

The red color of pomegranate juice was determined before and after pasteurization and during storage by measuring the absorbance at 520 nm with a plate reader Spectrostar Omega (BMG LabTech; GmbH Offenburg Germany). Fresh juices were tested for total mesophilic aerobic plate count (APC) using the spread-plate technique. Samples were serially diluted in peptone water and then 100 µl volumes of appropriate dilutions were spread-plated onto plate count agar (PCA; Scharlau Chemie, S.A.). Analyses of samples after pasteurization and storage was performed by direct seeding of 100 µl of juice in PCA plates.

III – Results and discussion

1. Changes in red color of pasteurized and stored pomegranate juice

Figure 1 shows the changes in the red color of pasteurized cloudy and clarified pomegranate juices stored at 25°C and 5ºC.
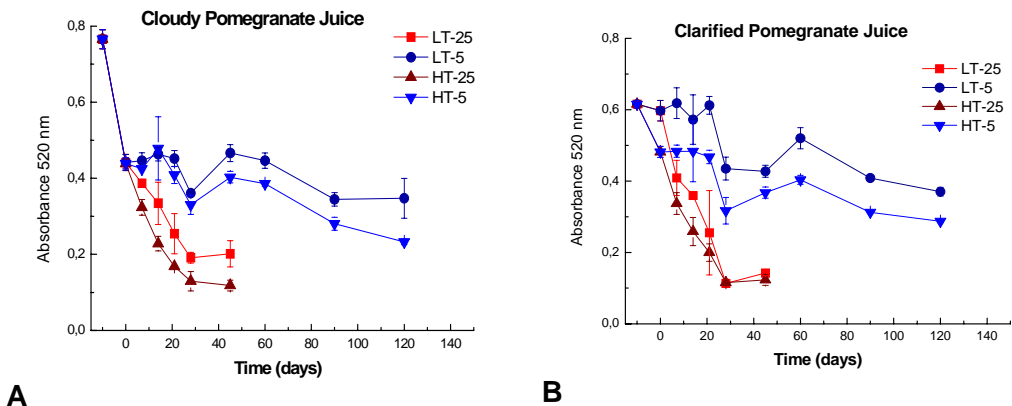
Fig. 1. Measurement of red color loss of pomegranate juices after pasteurization treatment (0 days) and its evolution throughout storage. A, cloudy and B, clarified juices.

The measuring of absorbance at 520 nm indicates that in clarified juice (Fig. 1B) the loss of red color due to the low-temperature pasteurization treatment was 4% (0 days of storage) while the loss produced by the high temperature treatment reached values of 20%. However, in cloudy juice (Fig. 1A) the loss of red color due to pasteurization treatments was similar in both conditions reaching values of 40%. In Fig. 1 we can observe that the losses of red color were similar in both juices, noting that the juices stored at 5°C had a lower loss of red color than those stored at 25°C. The cloudy juice pasteurized at high temperature and stored at 25°C for

45 days lost 80% of red color with respect to the baseline. On the contrary, the clarified juice pasteurized at low temperature and stored at 5ºC for 120 days lost 33% only.

2. Microbiological analysis of pomegranate juice pasteurized and stored

APCs (log cfu/ml) of cloudy and clarified pomegranate juices before and after pasteurization treatments and storage are shown in Table 1.

Table 1. Total mesophilic aerobic plate count (APC, log cfu/ml) of cloudy and clarified pomegranate juices before and after pasteurization treatments and storage

	Initial microbiology	Storage 25ºC 45 days	Storage 5ºC 120 days
Cloudy	5.50 ± 0.57		
LT	1.00 ± 0.00	3.20 ± 0.02	1.00 ± 0.00
HT	1.00 ± 0.00	1.30 ± 0.01	1.15 ± 0.21
Clarified	5.20 ± 0.00		
LT	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
HT	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00

The microbiological analysis of the pasteurized juices indicated that both pasteurization treatments were sufficiently effective to decrease the APCs to a level below the detection limit, producing 5.2 and 5.5 log reductions in cloudy and clarified juices, respectively. Microbial count in LT-treated pomegranate juice increased to 3.2 log cfu/ml after 45 days storage at 25ºC. In contrast, microbial growth was negligible in HT-treated juice under the same storage conditions. After 120 days storage at 5ºC, microbial growth was null or negligible in LT- and HT-treated pomegranate juices.

IV – Conclusion

The pasteurization treatment and storage temperature played an important role in the maintenance of red color in cloudy and clarified pomegranate juice. Results show that a low temperature of pasteurization combined with a refrigerated storage at 5ºC produces a minor loss of red compounds and microbial stability. In this way, we can obtain a processed product microbiologically safe and stable over 120 days of storage.

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Probiotic and antioxidant properties of synbiotic yoghurt supplemented with Australian-grown '*Wonderful*' pomegranate (*Punica granatum* L.) juice

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Abstract. Physicochemical properties of the Australian-grown '*Wonderful*' pomegranate juice (WPJ) including total phenolic compounds (TPC) and antioxidant activity (AA) were determined and compared with four brands of imported pomegranate juices (IPJs). The TPC in WPJ was found to be $2,400 \pm 200$ mg/l GAE while in the IPJs ranged from 1,000 to 2,800 mg/l GAE. The AA in WPJ was 11.0 ± 1.0 mM/l TEAC and ranged from 5.5 to 14.5 mM/l TEAC in IPJs. A synbiotic yoghurt supplemented with different levels of IPJ was developed. Maximum single strength PJ supplementation level with no adverse effects on yoghurt attributes and probiotics viability was found to be 20% in heat-treated milk resulting in a TPC level of 731 ± 69 mg/l GAE in synbiotic yoghurt.

Keywords. Pomegranate – Antioxidant activity – Total polyphenol content – Synbiotic yoghurt.

I – Introduction

Pomegranate (*Punica granatum* L.) is one of the oldest edible fruits widely grown in many tropical and subtropical countries (Fadavi *et al.*, 2005). Over 1,000 cultivars of *Punica granatum* exist, originating from the Middle East, extending westward throughout the Mediterranean, and eastward to China and India, and onto the American South-West, California and Mexico (Cam *et al.*, 2009; Lansky and Newman, 2007). Pomegranate's use in Australia has been primarily as a backyard ornamental tree (Lye, 2008). While no formal statistics are available in Australia, it is estimated that nearly 250 ha are currently grown with a similar area projected for new plantation, with expected increase of over 1,000 ha in the next 5-10 years (Eccles, 2009).

Over the last two decades consumers have become more aware of the relationship between food intake and good health, especially from natural foods such as fruits and vegetables. Pomegranate fruit which is renowned for its health benefits has become very popular worldwide over the last few years (Eccles, 2009). According to Tezcan *et al.* (2009), clinical research studies suggest that pomegranate juice (PJ) can reduce the level of oxidized LDL (low-density lipoprotein) cholesterol, and increase the activity of serum high-density lipoprotein (HDL)-associated paraoxonase 1 (Aviram *et al.*, 2000 and 2004; Aviram and Dornfeld, 2001; Kaplan *et al.*, 2001). The PJ also helps keep the prostate specific antigen (PSA) levels stable in men and even slows its rise (Pantuck *et al.*, 2006), is helpful against heart disease (Aviram *et al.*, 2008; Sumner *et al.*, 2005), Alzheimer's disease (Singh *et al.*, 2008), and some types of cancer such

Abbreviations: PJ, Pomegranate Juice; WPJ, Pomegranate juice from '*Wonderful*' variety; LPJ, Juice extracted from large size '*Wonderful*' pomegranates; SPJ, Juice extracted from small size '*Wonderful*' pomegranates; IPJ, Imported pomegranate juice.

as prostate and colon cancer (Adams *et al.*, 2006; Adhami and Mukhtar, 2007; Khan *et al.*, 2007; Malik and Mukhtar, 2006; Seeram *et al.*, 2007). It is reported that PJ can improve the sperm quality (Turk *et al.*, 2008) and erectile dysfunction in male patients as well (Forest *et al.*, 2007).

Studies have shown that pomegranate is a good source of flavonoids (flavonols, flavanols and anthocyanins) and hydrolysable tannins (HTs) (Hernandez *et al.*, 1999; Martin *et al.*, 2009; Seeram *et al.*, 2006). Hydrolysable tannins consist of gallotannins (hydrolysed to gallic acid and glucose), and ellagitannins (hydrolysed to ellagic acid and glucose). Each ellagic acid consists of 2 gallic acids, so, the monomeric part of this phenolic fraction is gallic acid (Alighourchi *et al.*, 2008; Gil *et al.*, 2000; Gonzalez-Molina *et al.*, 2009; Kulkarni *et al.*, 2004, 2007; Seeram *et al.*, 2005; Tanaka *et al.*, 1985, 1986a, b). Chemical analyses have shown that HTs as the predominant type of polyphenols in PJ (Ben Nasr *et al.*, 1996; Gil *et al.*, 2000; Seeram *et al.*, 2006) are responsible for over 92% of its antioxidant activity (AA) (Seeram *et al.*, 2006), which is higher than red wine, green tea, cranberry, grapefruit and orange juices (3, 3, 2, 6 and 8 folds higher, respectively) (Azadzoi *et al.*, 2005; Gil *et al.*, 2000; Rosenblat and Aviram, 2006; Tzulker *et al.*, 2007).

Due to increased consumer awareness of PJ antioxidant properties, the consumption of pomegranate has increased around the world. Currently pomegranate is mainly grown in the Northern Hemisphere. Australia has a diverse climate and there are areas that are very suitable for pomegranate production, which offers a counter-seasonal opportunity for Australian growers and exporters (Lye, 2008). There is an important body of work in the literature on the properties of pomegranate from different countries (Alighourchi *et al.*, 2008; Al-Said *et al.*, 2009; Fadavi *et al.*, 2005; Ozgen *et al.*, 2008; Pande and Akoh, 2009; Poyrazoglu *et al.*, 2002; Shwartz *et al.*, 2009; Tzulker *et al.*, 2007), but practically no published work on Australian PJ (Eccles, 2009). This study was therefore undertaken to characterise the juice extracted from the locally-grown pomegranate ('*Wonderful'* variety) and to evaluate the impact of processing on its TPC and AA levels, and to use the PJ in value-adding exercises to establish the maximum achievable level of supplementation in synbiotic yoghurt and its effect on probiotic activities.

II – Materials and methods

1. Materials

Pomegranate fruit and juice. Two different sizes of fresh '*Wonderful'* pomegranates with average sizes of 238 ± 10 and 573 ± 21 g were selected for the study. The fruits were supplied by a grower in Robinvale (between Mildura and Swan Hill in the North-west of Victoria, Australia, 34° 35' S latitude and 142° 46' E longitude) during the harvest season in April, 2010. Four brands of imported PJs (IPJ) coded as TT, TB, UP, IT were purchased from the local market. Samples TT and IT were sold in tetra pack cartons; the TB was marketed in 1000 ml bottles and for UP was sold in 236 ml PET (polyethylene terephthalate) bottles. Labels on all products claimed 100% juice with no added ingredients.

Chemicals and reagents. All chemicals used were analytical grade and sourced from Sigma-Aldrich Pty. Ltd. (NSW, Australia). These included Gallic acid, Folin-Ciocalteu reagent (F-C), ABTS (2, 2' -azinobis-(3 ethylbenzothiazoline-6-sulphonic acid) diammonium salt), potassium persulphate and trolox ® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid).

Yoghurt ingredients. Homogenised and pasteurised low-fat milk (1.3% fat, REV, Pramilet, Australia) and low-heat skim milk powder (LHSMP, 0.9% milk fat, 96% total solids, Bola Foods Ltd, Melbourne, and Vic, Australia) were used to produce yoghurt. The freeze-dried (FD) probiotic culture selected for this project was ABT-5-Probio-Tec [TM] (Chr. Hansen Pty. Ltd., Bayswater, Vic. Australia), a mixture of *Lactobacillus acidophilus (LA-5), Bifidobacterium*

bifidum (BB-12) and *Streptococcus thermophilus (ST)*. The culture was kept at -22 °C until required for yoghurt preparation as direct vat set (DVS).

2. Methods

Extraction of pomegranate juice. Arils of large and small pomegranate fruits were manually separated for juice extraction using an electrical juicer (Sunbeam, model IE-AD, Italy) in two stages (the pulp from first extraction stage was passed through the juicer again for further juice extraction). The fresh juices thus extracted from the large and small fruits (LPJ and SPJ) were pooled separately and stored in a blast freezer at -28 °C. The physicochemical and phytochemical properties of the thawed samples were determined and compared with those of IPJs. Six different processes were employed to extract PJ from small size pomegranates coded PJ1 to PJ6. PJs were extracted from manually separated arils either with an electrical juicer (PJ1) in two stages to improve yield (the pulp from the first stage was re-extracted) or by manually operated screw press (M-Press) (PJ2). For PJs 3 and 4 the outer leathery skin of pomegranates were peeled of, the fruit was then segmented and PJs were extracted from these segments (arils still inside the white pith) with electrical juicer either in single stage (PJ3), or in two stages followed by manual pressing of the residual pulp (PJ4). For PJs 5 and 6, chopped unpeeled fruits were processed in electrical juicer either in single stage (PJ5) or in two stages followed by manual pressing (PJ6). The fresh juices extracted from each step were pooled separately and used for yield calculation. Each stream was then divided into two lots, the first lot was frozen and stored in blast freezer at –28 °C (PJ1 – PJ6) and the second lot was heat treated as follows before storage at refrigerated temperature.

Heat treatment of pomegranate juice. Different streams of juices (coded PJ1P to PJ6P) were pasteurized at 90ºC for 15 sec, immediately cooled in an ice bath to below 10°C aseptically transferred into 450 ml glass bottles and tightly sealed and stored at 4ºC.

Determination of total phenolic compounds
Total phenolic compounds were determined by Folin-Ciocalteu (FC) colorimetry method which is based on chemical reduction of a mixture of tungsten and molybdenum oxides (Singleton and Rossi, 1965). This method relies on the transfer of electrons in alkaline medium from phenolic compounds to a mixture of phosphomolybdic and phosphotungstic acids to form blue complexes readable by a spectrophotometer (Ainsworth and Gillespie, 2007). Frozen juices were thawed first, then 20 µL of diluted (1:10 with Milli Q water) sample (PJ or yoghurt or gallic acid standard solution) was mixed with 1.58 ml Milli Q water in a 2-ml plastic cuvette. A blank was prepared using only Milli Q water. Aliquots of 100 µL FC reagent were added to each cuvette and mixed by pipetting for *ca.* 8 min at RT (20 – 25 ºC). Then, 300 µL of 20% sodium carbonate solution was added to all cuvettes (except those containing yoghurt) and allowed to stand for 2 h at room temperature (RT) before reading the absorbance at 765 nm in a UV/VIS Spectrometer equipped with UV Winlab software (Lambda 35, Perkin Elmer, MA, USA). The mixture of yoghurt and reagents was centrifuged (after 90 min standing at RT) at 18,500 *g* for 30 min at 4 ºC (5810R, Eppendorf Centrifuge, Hamburg, Germany), and the supernatant was used for absorbance reading as above. The results were expressed as mg gallic acid equivalent (GAE) in 1 L of sample by comparison with standard curve, which was obtained from different concentrations of gallic acid (50 to 1000 mg/l).

Determination of antioxidant activity (AA). Total antioxidant activity of PJ was measured spectrophotometrically based on the generation of ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) di-ammonium salt) radical cation (Gil *et al.*, 2000; Miller *et al.*, 1996; Rice-Evans & Miller, 1994 and 1995; Rice-Evans *et al.*, 1995 and 1997; Salah *et al.*, 1995; Wolfenden *et al.*, 1982). Aliquots of 7 mM ABTS and 2.45 mM potassium persulphate aqueous solutions were mixed and kept in dark at RT for approximately 24 h until the oxidation of ABTS was complete and the absorbance stabilised. The solution containing the generated blue/green ABTS[.+] chromophore was diluted with Milli Q water to an absorbance of 0.70 (±0.020) at 734 nm (Rice-Evans *et al.*, 1994; Robert *et al.*, 1999; Whitehead *et al.*, 1995; Zhou *et al.*, 2007). An

aliquot of 200 µl of diluted PJ (1:50 with Milli Q water), or Trolox ® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a water-soluble synthetic vitamin E analogue calibration standard ranging from 50 µM/l to 1 mM/l), or Milli Q water (blank) was mixed with 2.0 ml ABTS$^{\bullet+}$ in a plastic cuvette. The mixture was allowed to stand at RT for 10 min with continuous stirring before the absorbance was measured by UV/VIS spectrometer (Lambda 35, Perkin Elmer) at 734 nm. Absorbance values were taken to the standard curve prepared with synthetic antioxidant Trolox and results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC) (Gill *et al.*, 2000). The TEAC is equal to the millimolar concentration of a Trolox solution having the antioxidant capacity equivalent to a 1.0 mM solution of the substance under investigation (Antolovich *et al.*, 2002).

Colour measurement. Aliquots of 25 ml of experimental and commercial PJs were transferred into disposable plastic petri dishes, covered and the colour parameters were determine using a Chroma Meter CR-400 (Konica Minolta, Sensing, INC, Japan) according to Shwartz *et al.* (2009) and expressed in dimensions of L*, a*, b*, C and H°. The mean values of triplicate readings were reported for each sample. Values of L*- indicate darkness and L*+ indicate lightness of sample colour, while a*- indicates green colour and a*+ indicates red colour. The b*+ indicates a more yellow colour and b*- indicates blue colour. The chroma (C) value is calculated as $C = (a^{*2} + b^{*2})^{1/2}$ and indicates the colour intensity or saturation. Hue angle H° is a parameter that is effective in evaluating visual colour appearance and is calculated as $H° = \tan^{-1}$ (b*/a*) (Solomon *et al.*, 2006). The colour index was calculated from (180 - H°)/(L* + C) (Shwartz *et al.*, 2009 & Tzulker *et al.*, 2007).

Yoghurt preparation. Plain yoghurt was made according to Paseephol *et al.* (2008). Briefly, the low-fat milk (2.1.3) was standardised with LHSMP to a total solid content of 16% and heat-treated at 90°C for 10 min, then cooled to 43°C and inoculated with freeze-dried ABT-5-Probio-Tec^{TM} culture at a level recommended by the supplier (50U/250L). After gentle stirring to distribute the culture evenly, the inoculated milk samples were aseptically transferred into 100-ml plastic containers, tightly sealed and incubated at 43°C. At pH 4.7 the samples were transferred to a cold room at 4 °C. The commercial IPJ sample IT was selected for preliminary supplementation trials to produce synbiotic yoghurt. Four supplementation levels of 9, 13, 17 and 20% were trialled before or after heat treatment while keeping solids content constant at 16%

Physico-chemical analyses. Total soluble solids of fresh PJ was determined according to AOAC (2002) refractive index method with a Shibuya hand-held refractometer (Japan) and reported as degree Brix (ºB). Total solids of milk and yoghurt samples were determined using oven method according to Australian Standard (AS 2300.1.1-2008). pH values of all samples were measured using a pH-meter (HI 8424, Hanna instruments, USA). Titratable acidity (TA) of PJ samples was determined potentiometrically using 0.1M NaOH to the end point of pH 8.1 according the AOAC (2000) and reported as % citric acid (g per 100 ml). pH and TA of yoghurt and milk samples were determined according to Australian Standards (AS 2300.1.6-2010 and AS 2300.2.10-2008).

Statistical analyses. All tests were conducted in triplicate and the mean values ± standard deviation (SD) are reported. Statistical analyses were performed by applying one-way analysis of variance (ANOVA) to determine the significance of the 95% confidence interval and correlation coefficient using Minitab software (Version 14, Minitab Inc., State College, PA, USA).

III – Results and discussion

1. Physicochemical properties of pomegranate juices

The average weight of pomegranates was found to range between 238 ± 10 g for small fruits and 573 ± 21 g for the large ones. The yield of arils from small pomegranates was 61.44 ±

2.11% but only 45.58 ± 2.71% from the large fruits that appeared larger and more red than the small fruits arils. Juice yield from arils was comparable at 74.18 ± 3.19% and 76.54 ± 2.38% for small and large fruits respectively, however, on whole fruit basis the yield was expectedly lower, i.e. 45.58 ± 1.96% from small fruits and 34.89 ± 1.15% from larger fruits that had thicker skin.

Soluble solids in LPJ were significantly (P<0.05) higher (16.8 ± 0.2 ºB) than that of SPJ (15.2 ± 0.2 ºB). In comparison, only one of the IPJs showed high soluble solids content (16.1± 01 ºB) while others were significantly (P<0.05) lower and ranged from 13.3 to 14.5 ºB (Table 1). The SPJ was slightly more acidic than the LPJ (1.58 ± 0.07% vs. 1.15 ± 0.09%). The acidity of SPJ was closer (P<0.05) to that of IPJ coded TB. Likewise, the acidity of the LPJ and the IPJ coded IT was not significantly different (P<0.05) (Table 1).

Table 1. Chemical properties, total phenolic compounds and antioxidant activity of pomegranate juice samples

Samples	Soluble solids (º B)	pH	TA (% citric acid)	TPC GAE mg/l)	AA (TEAC mM/l)
SPJ [1]	15.2 ± 0.2[bc]	3.00 ± 0.02[de]	1.58 ± 0.07[ab]	2460 ± 164[ab]	11.06 ± 0.91[bc]
LPJ [2]	16.8 ± 0.2[a]	3.25 ± 0.01[b]	1.15 ± 0.09[cd]	2545 ± 97[ab]	11.36 ± 0.94[ab]
IPJ (TT)	14.1 ± 0.1[cd]	3.34 ± 0.01[a]	1.01 ± 0.06[de]	1923 ± 177[bc]	9.59 ± 0.93[bc]
IPJ (TB)	14.5 ± 0.1[c]	3.02 ± 0.01[d]	1.61 ± 0.08[ab]	1293 ± 113[cd]	6.91 ± 0.97[cd]
IPJ (UP)	16.1 ± 0.1[ab]	3.32 ± 0.01[ab]	1.09 ± 0.05[de]	2630 ± 245[ab]	13.43 ± 0.99[ab]
IPJ (IT)	13.3 ± 0.1[d]	3.15 ± 0.01[c]	1.27 ± 0.07[cd]	1193 ± 171[d]	6.48 ± 0.90[bc]

[1]Juice produced from small size fruit's arils by electrical juicer in two stages; [2]Juice produced from large size fruit's arils by electrical juicer in two stages. IPJ: Imported pomegranate juices. The data represent the means ± SD of triplicate analyses from each trial. ANOVA was used to determine the significance of differences at a confidence level of 0.05 as identified by different letters in the same column.

The colour evaluation results are presented in Table 2. The values of L*, a*, C, H and colour index were not significantly different (P<0.05) between SPJ and LPJ, however, SPJ showed higher b* value (yellowness) than LPJ. Compared to the fresh juices (SPJ & LPJ), the colour values were significantly different (P<0.05) for commercial products (IPJs) that were all produced from concentrated PJ. The commercial products showed significantly higher (P<0.05) L* values (i.e. brighter), a* value (more red except for IT) and b* value (yellower) than the fresh juices. The IPJ coded IT showed a significantly higher (P<0.05) L* and b* values but the lowest a* value (Table 2). According to H° formula [H° = $\tan^{-1}$ (b*/a*)] any increase in the redness of a sample (a*+) or drop in its yellowness (b*+) results in low H° value. The H° values of IPJs were significantly higher (P<0.05) than SPJ an LPJ, while their colour index values were significantly lower (P<0.05) than SPJ and LPJ (Table 2). These results were correlated with visual appearances of samples and indicated that SPJ and LPJ's colour were more appealing and the commercial products showed inferior colour compared to fresh juices (Shwartz *et al.*, 2009; Tzulker *et al.*, 2007).

2. Effects of extraction method on pomegranate juice properties

The yields of PJ1, 2, 3 and 5 were not significantly (P<0.05) different. Considering the yields of PJ1 and 2 (produced from same raw materials), double extraction with electrical juicer and manual pressing methods had the same effects on the final yields. But when these two methods were used in combination, it increased the yield of final product significantly (P<0.05). Thus, PJ4 had the highest yield followed by PJ6 (Table 3).

Table 2. Colour evaluation of pomegranate juice samples

Samples	L*	a*	b*	c	Hº	Colour index
SPJ[1]	17.50 ± 0.08[e]	15.83 ± 0.70[cd]	7.89 ± 0.46[d]	17.69 ± 0.83[de]	26.50 ± 0.36[e]	4.36 ± 0.10[b]
LPJ[2]	17.45 ± 0.32[e]	15.43 ± 1.50[cd]	6.24 ± 0.58[ed]	16.64 ± 1.60[de]	22.05 ± 0.44[e]	4.64 ± 0.25[b]
IPJ (TT)	26.67 ± 0.18[d]	20.38 ± 0.96[bc]	21.33 ± 0.80[ab]	29.36 ± 1.48[bc]	46.32 ± 0.30[c]	2.39 ± 0.07[d]
IPJ (TB)	27.16 ± 0.55[dc]	23.22 ± 2.06[b]	17.86 ± 1.90[b]	29.29 ± 2.79[bc]	37.54 ± 0.54[d]	2.53 ± 0.16[d]
IPJ (UP)	25.37 ± 0.99[d]	29.07 ± 1.22[ab]	19.56 ± 1.20[ab]	35.04 ± 1.68[ab]	33.92 ± 0.52[d]	2.42 ± 0.10[d]
IPJ (IT)	43.18 ± 0.89[a]	6.67 ± 0.02[ed]	23.46 ± 0.74[a]	24.40 ± 0.72[cd]	74.11 ± 0.42[ab]	1.57 ± 0.04[e]

[1]Juice produced from small size fruit's arils by electrical juicer in two stages; [2]Juice produced from large size fruit's arils by electrical juicer in two stages. IPJ: Imported pomegranate juices. The data represent the means ± SD of triplicate analyses from each trial. ANOVA was used to determine the significance of differences at a confidence level of 0.05 as identified by different letters in the same column.

Table 3. Yield and chemical properties of pomegranate juices extracted with different methods and the effect of heat treatment on TPC and AA of these juice sample

Samples	Yield1	Soluble solids (° B)	pH	TA (% citric acid)
PJ1	45.58 ± 1.96[bc]	15.2 ± 0.2[d]	3.00 ± 0.02[de]	1.58 ± 0.07[abc]
PJ2	45.04 ± 2.06[bc]	15.2 ± 0.2[d]	3.00 ± 0.01[de]	1.64 ± 0.10[ab]
PJ3	45.23 ± 2.07[bc]	15.9 ± 0.1[c]	3.08 ± 0.01[bc]	1.32 ± 0.07[cd]
PJ4	56.87 ± 1.64[a]	16.1 ± 0.1[bc]	3.10 ± 0.01[b]	1.42 ± 0.09[bcd]
PJ5	40.04 ± 2.04[bc]	16.4 ± 0.2[bc]	3.10 ± 0.01[ab]	1.36 ± 0.07[cd]
PJ6	49.78 ± 2.12[cd]	16.9 ± 0.1[ab]	3.10 ± 0.02[b]	1.38 ± 0.09[cd]

Samples	TPC (GAE mg/l)		AA (TEAC mM/l)	
	BHT[2]	AHT[3]	BHT[2]	AHT[3]
PJ1	2460 ± 164[de]	2293 ± 248[e]	11.06 ± 0.91[d]	10.24 ± 0.81[d]
PJ2	2071 ± 62[e]	2064 ± 123[e]	9.16 ± 0.97[d]	9.03 ± 0.95[d]
PJ3	5760 ± 609[cd]	5297 ± 746[d]	23.13 ± 2.88[c]	21.36 ± 2.63[c]
PJ4	7293 ± 605[c]	6930 ± 555[c]	30.25 ± 2.10[bc]	27.70 ± 1.40[bc]
PJ5	11545 ± 503[ab]	10456 ± 472[b]	50.65 ± 1.60[a]	48.54 ± 2.14[a]
PJ6	12516 ± 167[ab]	11619 ± 394[ab]	56.91 ± 2.79[a]	53.20 ± 2.03[a]

[1]Yield calculated on whole fruit base; [2]Before heat treatment; [3]After heat treatment (90 °C for 15 sec). Data shown represents the means ± SD of triplicate analyses from each trial. ANOVA was used to determine the significance of differences at a confidence level of 0.05 as identified by different letters in the same column.

Raw materials and extraction methods affected soluble solids content of each sample. Accordingly, soluble solids in PJ1 and 2 (15.2 ± 0.2) produced from arils with the same yields and extraction methods were not significantly ($P<0.05$) different.

Based on raw materials used and extraction method employed, soluble solids in PJ5 was significantly ($P<0.05$) higher (16.4 ± 0.2) than that of PJ3 (15.9 ± 0.1), and PJ6 showed significantly ($P<0.05$) higher soluble solids (16.9 ± 0.1) than PJ4 (16.1 ± 0.1), while all other samples showed similar soluble solids content. The higher soluble solids content in PJ4 and 6 resulted from the extraction methods used (Table 3).

The titratable acidity of PJ1 (1.58 ± 0.07) was close ($P<0.05$) to PJ2 (1.64 ± 0.10) and both were slightly more acidic than other samples that were not significantly different ($P<0.05$) (Table 3) in acidity.

Difference in raw materials and extraction methods affected the colour parameters of extracted juices. The correlations between these parameters are reported in Table 4. No significant

differences ($P<0.05$) were found between the L* values of PJ1 and 5, PJ2 and 3, and PJ4 and 6; between the a* values of PJ1 and 3, PJ5 and 6; and between the b* values of PJ4 and 5. PJ1 showed the highest H° value (26.50 ± 0.36) that declined as the extraction method became more extensive, the lowest value found in PJ6 (14.13 ± 0.96). The colour index was not affected by the type of raw materials used, and in contrast with H ° values, the highest and lowest colour index was observed in PJ6 (4.68 ± 0.19) and PJ1 (4.36 ± 0.10) respectively.

Table 4. Colour evaluation of pomegranate juices extracted with different methods

Samples	L*	a*	b*	c	H°	Colour index
PJ1	17.50 ± 0.08[cd]	15.83 ± 0.70[bc]	7.89 ± 0.46[a]	17.69 ± 0.83[abc]	26.50 ± 0.36[a]	4.36 ± 0.10[bcd]
PJ2	18.50 ± 0.15[ab]	15.44 ± 1.92[bcd]	7.47 ± 0.21[ab]	16.97 ± 2.08[abc]	24.54 ± 0.37[ab]	4.39 ± 0.27[bcd]
PJ3	18.42 ± 0.32[ab]	15.97 ± 0.77[bc]	5.86 ± 0.23[bc]	17.01 ± 0.79[abc]	20.17 ± 0.34[bc]	4.51 ± 0.14[bc]
PJ4	18.09 ± 0.04[bc]	13.37 ± 1.74[cd]	4.76 ± 0.52[cd]	17.20 ± 1.34[abc]	19.61 ± 0.41[c]	4.55 ± 0.18[bc]
PJ5	17.75 ± 0.20[cd]	17.45 ± 1.14[abc]	4.86 ± 0.35[cd]	18.12 ± 1.19[ab]	15.56 ± 0.48[d]	4.59 ± 0.15[abc]
PJ6	17.88 ± 0.03[bc]	17.06 ± 1.68[abc]	4.28 ± 0.12[d]	17.59 ± 1.65[abc]	14.13 ± 0.96[de]	4.68 ± 0.19[abc]

The data represent the means ± SD of triplicate analyses from each trial. ANOVA was used to determine the significance of differences at a confidence level of 0.05 as identified by different letters in the same column.

3. Phytochemical content of pomegranate juice

The health benefits attributed to pomegranate fruit consumption are related, at least in part, to their antioxidant activity (AA) (Shwartz *et al.*, 2009; Vardin & Fenercioglu, 2003). The AA of SPJ, LPJ and IPJs were determined using ABTS method (section 2.2.3) and the results were expressed as mM/l TEAC. The AA of SPJ and LPJ were 11.06 and 11.36 mM/l TEAC, while IPJs (coded UP, TT, TB and IT) showed 13.43, 9.59, 6.91 and 6.48 mM/l TEAC, respectively (Table 1).

In pomegranates like many other fruits and vegetables, the level of AA can be attributed to the level of TPC (Gil *et al.*, 2000; Shwartz *et al.*, 2009; Solomon *et al.*, 2006; Tzulker *et al.*, 2007). Therefore, TPC in these juices were also measured by F C method (section 2.2.2) and the results were expressed as mg/l GAE (Table 1).

Freshly extracted juices SPJ and LPJ contained 2,460 and 2,545 mg/l GAE, respectively; while the IPJs (UP, TT, TB and IT) contained 2,630, 1,923, 1,293 and 1,193 mg/l GAE, respectively (Table 1). Gil *et al.* (2000) suggested that the industrial extraction process either increases the amount or enhances the activity of the antioxidants. Industrially, the whole fruit is pressed hydrostatically which results in the extraction of a large amounts of polyphenols from the peels (Tzulker *et al.*, 2007). The juice is then filtered, concentrated, stored and sold to juice packaging companies where it is diluted with water and packed. It is this level of dilution that determines the TPC and the AA of the juice. Among the IPJs tested in this project, only sample coded UP showed a comparable TPC level to fresh juice obtained from arils while other samples failed in this regard. These results are in agreement with the TSS of samples tested although TSS alone could not be a good guide to AA activity, since some IPJs may have added sugar to adjust their TSS to an acceptable level.

These results confirm that the AA of different samples was directly related to the levels of TPC in those samples. Thus, any process that increases the level of TPC in a sample will result in an increased antioxidant activity.

4. Effects of fruit parts and extraction procedures on phytochemical content of pomegranate juice

To improve the TPC level in PJ samples different parts of pomegranate fruits were used and combined with different extraction procedures (PJ1 to PJ6). In juices extracted from arils, the TPC level in PJ1 (2460 ± 164 mg/l GAE) was marginally higher than PJ2 (2071 ± 62 mg/l GAE) due to the extraction method employed but the AA of both samples were not statistically different (P<0.05) (Table 2). The TPC level and AA of PJs from peeled fruits were higher than those from arils. On the other hand, due to intensity of extraction method PJ4 showed significantly (P<0.05) higher TPC and AA (7293 ± 605 mg/l GAE and 30.25 ± 2.10 mM/l TEAC) than PJ3 (5760 ± 609 mg/l GAE and 23.13 ± 2.88 mM/l TEAC) (Table 2).

Upon using unpeeled whole fruits for juice extraction the TPC level and AA of PJs 5 and 6 were further increased compared to all other samples (Table 2), however, changing the extraction method from PJ5 to PJ6 did not significantly (P<0.05) affect the TPC content (11545 ± 503 $vs.$ 12516 ± 167 mg/l GAE) or the antioxidant activity (50.65 ± 1.60 $vs.$ 56.91 ± 2.79 mM/l TEAC). The TPC level in PJ1P, 3P and 5P were marginally lower than PJ1, 3 and 5 respectively, while in other samples the TPC level remained unchanged (P<0.05) after heat treatment (90 ºC for 15 sec) (Table 2). Results obtained revealed that by changing the extraction methods the TPC level in PJ4 and 6 increased, however, heat treatment did not significantly (P<0.05) affect the TPC levels in these samples (PJ4P and 6P). Similarly, heat treatment did not adversely affect the AA of all samples (Table 2).

5. Symbiotic yoghurt supplemented with pomegranate juice

A synbiotic product was developed by incorporating PJ in the formulation of probiotic yoghurt. The IPJ coded IT was used in the preliminary supplementation trials. Different supplementation levels (9, 13, 17 and 20%) were trialled before and after heat treatment of milk at 90°C for 10 min. Supplementation with PJ before heat treatment was limited to 9%, beyond which the milk curdled, whereas after heat treatment up to 20% PJ could be added without any adverse effect. It appears that heat treatment of standardised milk increased its stability and the buffering capacity of milk proteins. The supplemented milk samples were inoculated with probiotic culture and incubated at 43°C. The activity of the cultures was monitored by measuring the pH (Fig. 1) and enumeration technique. Upon reaching pH 4.7 the incubation time was recorded and yoghurt samples were transferred to refrigerated storage at 4°C. Comparing the yoghurt setting times it was noted that PJ supplementation up to 20% did not adversely affect the cultures activity or yoghurt attributes.

Changes in viable counts of ST-01, LA-5 and BB-12 in the control and PJC- supplemented yoghurts from day 1 to the end of the shelf life of 28 days is presented in Table 5.

The number of ST-B01 in control sample decreased marginally in day 7 but did not significantly (P<0.05) change during the subsequent storage period. In the PJ-supplemented sample the population of ST-B01 declined by $ca.$ 0.2 log cycles in day 1 and remained stable for the next 3 weeks, and then dropped by $ca.$ 0.4 log cycles in the last week of the 4-week storage period, resulting in a viability of 98.8% in the control and 94.4% in the PJ-supplemented sample. The initial count of LA-5 (6.72 log CFU/g in control and 6.65 log CFU/g in supplemented sample) demonstrated a steady but slow decline reflecting a small effect of the PJ supplementation. By the end of storage, the population of LA-5 in control yoghurt dropped to 6.27 log CFU/g, i.e. a viability of 93%, while the PJ-supplemented yoghurt showed 6.03 log CFU/g i.e. 90% viability. A steady decline in the numbers of BB-12 was also observed at the end of the storage period, form the initial counts of 6.58 log CFU/g to 6.28 log CFU/g in control and from 6.47 log CFU/g to 6.06 log CFU/g in PJ-supplemented sample, corresponding to a viability of 95.5% and 93.7% respectively.
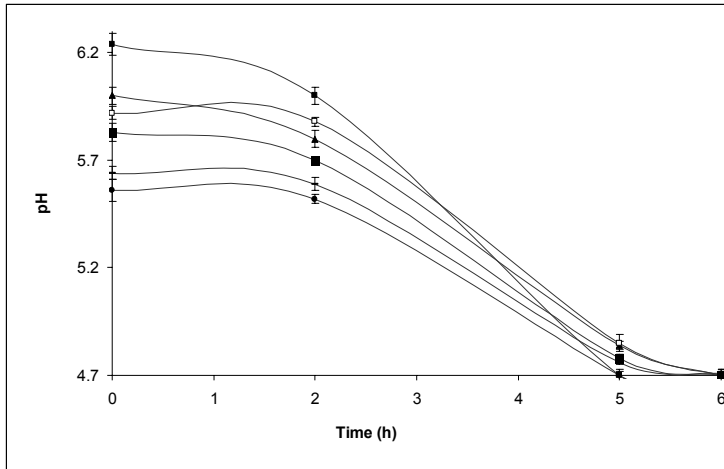
Fig. 1. pH range in yoghurts supplemented with PJ. ▪ Plain yoghurt; ■ +13% PJ after heat treatment; ▲ +9% PJ before heat treatment; — +17% PJ after heat treatment; □ +9% PJ after heat treatment; ● +20% PJ after heat treatment.

Table 5. Variations in the viable counts of ST-B01, LA-5 and BB-12 in control and Synbiotic (PJC-supplemented) yoghurts during storage at 4°C

Culture	Period (day)	Control yoghurt (log CFU/g)	Synbiotic yoghurt (log CFU/g)
ST-B01	1	7.47 ± 0.16^{abc}	7.20 ± 0.13^{ab}
	7	7.36 ± 0.12^{bcd}	7.17 ± 0.26^{ab}
	14	7.26 ± 0.07^{cde}	7.21 ± 0.16^{ab}
	21	7.42 ± 0.14^{bcd}	7.20 ± 0.13^{ab}
	28	7.38 ± 0.08^{bcd}	6.80 ± 0.15^{cd}
	% Viability	98.83^{AB}	94.48^{DE}
LA-5	1	6.72 ± 0.16^{ab}	6.65 ± 0.17^{ab}
	7	6.35 ± 0.09^{cd}	6.36 ± 0.13^{bcd}
	14	6.43 ± 0.16^{cd}	6.42 ± 0.12^{bc}
	21	6.30 ± 0.11^{cde}	6.20 ± 0.16^{cd}
	28	6.27 ± 0.10^{de}	6.03 ± 0.17^{de}
	% Viability	93.23^{AB}	90.61^{CD}
BB-12	1	6.58 ± 0.21^{abc}	6.47 ± 0.17^{abc}
	7	6.49 ± 0.15^{abc}	6.38 ± 0.14^{bc}
	14	6.41 ± 0.15^{bcd}	6.29 ± 0.10^{bcd}
	21	6.32 ± 0.09^{bcd}	6.23 ± 0.06^{cd}
	28	6.28 ± 0.10^{cd}	6.06 ± 0.10^{de}
	% Viability	95.52^{ABCD}	93.70^{BCDE}

Data represent the means ± SD of duplicate experiments with duplicate tests. ANOVA was used to determine the significance of differences at a confidence level of 0.05 as identified by different letters (lower case in the same column in each section and upper case in the same row).
% Viability = (CFU/g after 4 weeks storage/initial CFU/g) × 100.

These observations were consistent with the findings of Akalin *et al.* (2004), Dave and Shah (1996), Medina and Jordano (1994), Ozer *et al.* (2005) and Paseephol and Sherkat (2009) who reported higher stability of ST-B01 than LA-5 and BB-12 in probiotic yoghurts during storage time.

The TPC content of synbiotic yoghurts containing 9, 13, 17 and 20% PJ was found to be 583, 637, 688 and 731 mg/l GAE, respectively against a background TPC in plain yoghurt (control) of 514 mg/l GAE (Fig. 2).
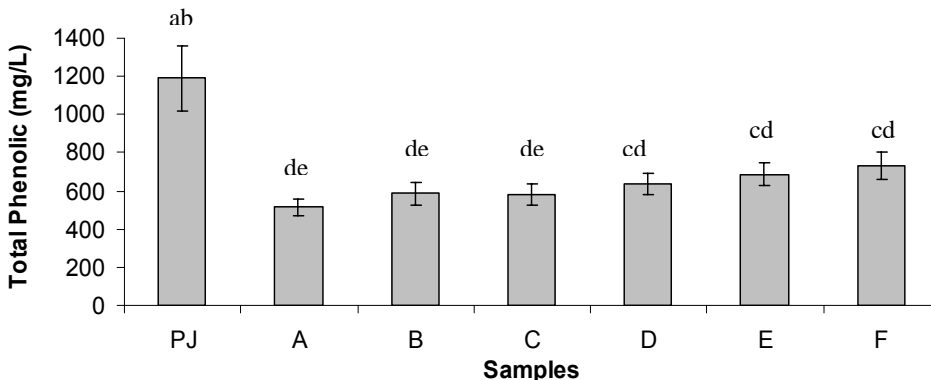

Fig. 2. Total phenolic compounds (mg/l GAE) in IPJ (coded IT) and in yoghurts supplemented with IPJ (IT). The data represent the means ± SD of triplicate analyses from each trial. ANOVA was used to determine the significance of differences at a confidence level of 0.05 as identified by different letters. A: Plain yoghurt; B: +9% PJ before heat treatment; C: +9% PJ after heat treatment; D: +13% PJ after heat treatment; E: +17% PJ after heat treatment; F: +20% PJ after heat treatment.

IV – Conclusions

The physicochemical and phytochemical properties of the Australian '*Wonderful*' pomegranates grown in Robinvale (Victoria) were determined and compared with 4 different brands of imported commercial pomegranate juices. The larger fruits seemed to have lower yield of juice, that was slightly less sour and sweeter (higher TSS), and contained over 3.3% more TPC and nearly 2.7% higher AA than juice from smaller fruits. Freshly extracted Juices showed higher TPC and AA than three of imported commercial PJs except the sample coded UP which had comparable levels of TPC and AA.

Up to six fold increase in TPC level could be achieved in PJs using different extraction methods and various parts of the fruit. While, strong astringency of these juices could limit their applications for direct or combined consumption, they may however find uses in nutraceuticals as AA supplements. Pasteurisation (90ºC for 15 sec) did not significantly ($P<0.05$) affect the AA of the extracted PJs, although TPC levels of PJ1, PJ3 and PJ5 slightly declined after heat treatment.

In the production of synbiotic yoghurt up to 20% single strength PJ could be added to heat-treated milk, and no apparent antagonism was observed between the cultures viability and the PJ phytochemicals. Considering the recommended polyphenols intake of *ca.* 1 g/day (Baghurst, 2006) this synbiotic yoghurt offers a pleasant and effective route to increasing the antioxidant intake in our daily diet.

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Session 6
Pomegranate and health

Usefulness of pomegranate in prostate cancer

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Abstract. Pomegranate is the fruit of the *Punica Granatum* tree, which grows in Mediterranean countries. Its therapeutic properties have been used to treat different conditions (cardiovascular, neurological, diabetes and cancer) for hundreds of years. Numerous *in vitro* and *in vivo* studies have recently shown the antioxidant, anti-inflammatory and anti-tumoral properties of pomegranate. Antiproliferative, proapoptotic, antiangiogenic and inhibition of tumor invasion have been proven in several experimental models of urological tumors such as in prostate cancer. Pomegranate juice has been shown to delay PSA duplication time in patients with biochemical cancer recurrence after initial treatment. Many phase II studies are currently in progress to demonstrate the effectiveness of pomegranate juice in several diseases and in prostate cancer.

I – Introduction

The pomegranate, fruit of the *Punica granatum* tree, is native to the Himalayas in northern India, and Iran. Since antiquity its cultivation has spread to the Mediterranean countries, India, China, Japan and Russia, as well as areas of the United States and Afghanistan. The pomegranate's medicinal properties have been known for thousands of years, mention being made in the Old Testament of the Bible, the Jewish Torah, and the Talmud of Babylon. It was used in the ceremonies and mythology of the Ancient Egyptians, Greeks and Romans. Ayurvedic medicine regards the pomegranate as a pharmacy in its own right, using it against parasites, diarrhoea, diabetes, and to cure ulcers. In South America, pomegranate bark, peel and petals are chewed to treat dysentery, and mouth and gum problems[1].

II – Phytochemical constituents of the pomegranate

Phytochemicals are plant secondary metabolites having health-giving benefits, although they are not considered essential nutrients. Generally, phytochemicals are produced by the plant as part of its defence mechanism against external dangers, such as ultraviolet radiation, pathogens, etc[2]. Diets rich in phytochemicals are associated with a reduced risk of developing illnesses such as certain types of cancer, and inflammatory, cardiovascular or neurodegenerative diseases. Although the greatest source of the pomegranate's phytochemicals are found in the fruit, other parts of the tree, such as leaves and seeds also contain them. More than 100 phytochemical compounds have been isolated in the pomegranate. Those detected most frequently are the polyphenols, which include: a- *flavonoids* such as the anthocyanins and anthocyanidins (cyanidin, delphinidin, pelargonidin); b- *flavonols* such as luteolin, quercetin and kaempferol; c- *hydrolysable tannins* such as the ellagitannins, punicalagins and gallotannins. Hydrolysable tannins are responsible for 92% of the antioxidant activity of pomegranate juice and the punicalagins are responsible for half of this antioxidant effect[3]. Pomegranates also contain catechins such as those found in green tea, and steroids such as estradiol, estriol, estrone, testosterone and ursolic acid. The oil obtained from pomegranate seeds contains fatty acids, the most common of these being punicic acid (>60%). The structural variations between the polyphenols extracted from the various components –the fruit, juice or other parts of the pomegranate or the tree– are large.

1. The pharmacokinetics of pomegranate juice

Ellagitannins are hydrolysed rapidly in the body, becoming ellagic acid, of which no trace is found in the circulatory system after five hours[4]. Once absorbed, ellagic acid is metabolised by enzymes, such as glucuronosyltransferase and sulphotransferase, which increase its excretion and detoxification by increasing its water solubility. Intestinal microflora transform ellagic acid into two principal metabolites, urolithin A and B, which can remain in urine for up to three to four days after ingesting pomegranate juice; this may explain the beneficial effects of its chronic administration[5,6]. González-Sarrias *et al.* have shown the presence of urolithin A and traces of urolithin B in the prostate of men who had previously received pomegranate juice or walnuts for three days before surgery[7].

2. Antioxidant effects of pomegranate

Recent research suggests that oxygen-dependent free radicals are the first step in physio-pathological mechanisms of chronic illness and the aging process[8]. The increase of nitric oxide (NO) and nitric oxide synthase (NOS), associated with an excess in O_2 production, produces the formation of high levels of peroxynitrite (ONOO-)[9]. This compound causes direct toxic effects, such as lipid peroxidation, protein oxidation and DNA damage, as well as the induction of various transcription factors, including the nuclear factor kappa B (NF-κB) and the activator protein-1 (AP-1), which lead to cytokine-induced chronic inflammation. As a result of the latter mechanism, nitro-oxidative stress is transformed into an inflammatory process as these cytokines spread the inflammatory message via blood circulation, thus causing continuing cell damage (for example, endothelial cell dysfunction)[10]. DNA exposure to ONOO- or NO plus O_2 causes breaks in the chains[11]. Furthermore, the ONOO- renders inactive various enzymes that are important in repairing damaged DNA. Due to all these effects, ONOO- induces apoptosis if oxidation is moderate, or cell necrosis if oxidative stress is severe[12]. The antioxidant activity of pomegranate juice is three times higher than red wine or green tea[13]. Consuming 250 ml of pomegranate juice for four weeks has been proven to eradicate free radicals from the body, and significantly increase plasma antioxidant capacity in older people when compared to those consuming apple juice[14]. Rosenblat and Aviram proved that pomegranate juice contains a total higher concentration of polyphenols (5 mmol/l) and a greater antioxidant activity than other fruit juices (kiwi, apple, grape, orange, pineapple, pear, peach), which contain 1.3 – 4 mmol/l of total polyphenols[15]. These properties have a potential use as a complement in anti-aging treatment in both sexes.

3. Anti-tumour effects of pomegranate on prostate cancer

A. Antiproliferative effects and proapoptosis

Several studies have shown that different parts of the pomegranate (arils, pericarp, seeds etc.), fresh or fermented, have antiproliferative effects. Albrect *et al*[16] showed *in vitro* that extracts derived from pomegranates inhibited the proliferation of several prostate cancer cell lines - hormone-sensitive (LNCaP) as well as hormone refractory (PC-3 and DU 145). However, normal prostate cells are not affected. Malik *et al*[17] assessed the antiproliferative effect and proapoptosis of pomegranate extract in aggressive hormone-refractory prostate cancer cells (PC-3), and observed a dose-dependent inhibition in cell-growth and apoptosis induction. This effect came about due to the decrease in expression of the anti-apoptotic Bcl-2 gene protein and the increase in expression of the pro-apoptotic Bax gene protein. In one *in vivo* experiment in which athymic mice were implanted with hormone-sensitive prostate cancer cells, tumour growth was observed to be slower in animals that had been administered with pomegranate extract as their sole source of liquid compared to those drinking only water. Furthermore, the animals receiving pomegranate extract showed a significant reduction (up to 85%) in PSA production[23]. Seeram *et al*[18] obtained similar results from pomegranate juice in respect to growth inhibition of prostate cancer cells *in vitro* and *in vivo*. They also observed that the

urolithins (ellagic acid metabolites) were localised in the prostate, inhibiting growth of both hormone-sensitive and hormone-refractory cancer cells. Recently, Koyama *et al* have shown that pomegranate juice induces apoptosis in prostate cancer cells by inhibition of IGF[19]. These results suggest that consuming pomegranate may delay the growth of prostate cancer, which could lengthen and improve patients' quality of life.

B. Effects on the nuclear kappa B (NF-κB)

NF-kB forms part of a family of transcription factors and is activated as a response to various stimuli: cytokines, carcinogens, chemotherapies, endotoxins, chemical or physical stress, radiation, hypoxia, and inflammation. Activated NF-κB is found in several tumours and it has been shown to regulate the expression of more than 200 genes with different functions that participate in regulating the immune system, carcinogenesis, cell proliferation and adhesion, anti-apoptosis, angiogenesis, invasion and metastasis[20]. NF-kB activity is regulated by an inhibiting protein that binds onto it, retaining it in the cytoplasm. When the NF-kB route is activated, the inhibiting protein degrades by phosphorylation, releasing NF-kB, which translocates to the nucleus where it acts as a transcription factor[21]. Prostate cancer is one of the tumours in which NF-κB activation has been shown to be present and represents an independent risk factor of tumour recurrence after radical prostatectomy[22,23]. Rettig *et al* have demonstrated *in vitro* that pomegranate juice, as well as pomegranate extract, inhibit NF-κB and cell viability in prostate cancer cells. In one *in vivo* model, pomegranate was seen to delay the appearance of hormone resistant prostate cancer[24]. NF-κB inhibition is a necessary mechanism to obtain the maximum pro-apoptotic effect from pomegranate juice.

C. Effects on angiogenesis

Hypoxia is the principal mechanism in the progression of more than 70% of tumours via the activation of angiogenesis, an essential factor for a tumour to be able to grow more than 200 micras[25]. However, in contrast to what happens with the vascularisation of normal tissue, the tumoural microvessels formed through angiogenesis are very disorganised. Thus greater hypoxia is produced with the subsequent activation of transcription factors associated with cellular hypoxia, such as hypoxia-inducible factor 1-α and 1-β (HIF-1α and HIF-1β); these in turn activate different genes related to angiogenesis, leading to greater progression and invasion[26]. Tumour-induced angiogenesis is regulated by factors produced by macrophages, neutrophils, and by the tumoural cells themselves as the vascular endothelial growth factor (VEGF). In prostate cancer, for example, it has been shown that androgens, which play an important role in tumour aetiology and progression, activate the expression of HIF-1α and VEGF[27]. Toi *et al* analysed the anti-angiogenesis potential of pomegranate seed oil or fermented pomegranate juice on estrogen-sensitive (MCF-7) or estrogen-resistant (MB-MDA-231) breast cancer cells, observing a significant reduction[28]. Sartippour *et al* carried out *in vitro* studies on the effect of pomegranate peel extract (standardised to 37% ellagitannins and 3.5% free ellagic acid) on hormone-sensitive prostate cancer cells (LNCaP) and human umbilical vein endothelial cells[29]. Pomegranate extract inhibited the proliferation of the endothelial cells under both nomoxic and hypoxic conditions, and inhibited the proliferation of LNCaP cells under hypoxic conditions. Under hypoxic conditions, a reduction was also observed in the concentration of HIF-1α protein and VEGF in both cell groups. In an *in vivo* experiment, human prostate cancer cells (LAPC4) were implanted into mice with severe combined immunodeficiency (SCID); the animals then received either pomegranate extract or a liquid serving as control by mouth five days a week for four weeks. The pomegranate extract the animals received was the equivalent of a human intake of 320 ml of pomegranate juice. After four weeks, tumour volume was observed to be significantly smaller (199 ± 37 mm^3 compared to 1179 ± 106 mm^3) in those animals that had received the pomegranate extract. Furthermore, VEGF concentration was significantly higher in those animals receiving the control liquid, while HIF-1α staining and blood vessel density were reduced significantly in those animals receiving pomegranate extract[29].

D. Effects on tumoural invasion

For tumours to be able to infiltrate surrounding tissue, tumour cells need to secrete proteolytic enzymes, such as metalloproteinases, in order to digest the extracellular matrix. Pomegranate extract has been proven to be effective in inhibiting metalloproteinase expression by inhibition of NF-κB in human chondrocytes[30]. In another study, several constituents of pomegranate (ellagic acid, caffeic acid, luteolin and punicic acid) were examined *in vitro* for their potential inhibiting effect on human hormone-refractory prostate cancer cell (PC-3) invasion through an artificial membrane[31]. Although each of the substances separately significantly inhibited invasion, when used together, a supra-additive effect was seen. Albrecht *et al* had similar results with the same type of prostate cancer cell[16].

III – Prostate cancer epidemiology

If non melanoma skin cancer are excluded, prostate cancer in Spain is the third tumour in frecuency alter lung and colorrectal cancer. In Spain one in six males will develop prostate cancer during his live. The probability to develop prostate cancer increases with the age, since one each ten cases are diagnosed in men above 65 years old. In relation with mortality, in Spain, prostate cancer is the third tumour after lung and colorectal cancer. Since 1998 prostate cancer has decreased in some regions like Madrid, Cataluña, Valencia and Baleares, while in others regions the incidence of prostate cancer is increasing. In Europe, prostate cancer causes 3% of all deaths in males. Scandinavian countries, Belgium, Nedherland and some regions of France have a high mortality meanwhile Bulgary, Hungary, Romania and Mediterranean countries mortality due to prostate cancer is below the mean. Mortality in Spain is between the lowest as Italy and Greece. Nowsaday less than 5% of the patients with prostate cancer present metastasis in the moment of diagnosis in front of 50% in the 80´decade.

1. Prostatic specific antigen (PSA)

Prostate-specific antigen (PSA) is a protein produced specifically by cells of the prostate gland. It is normal for men to have a low level of PSA in their blood; however, prostatic diseases like prostate cancer, benign prostatic hyperplasia o prostatitis can increase a man's PSA level. It is a very useful tumour marker. In patients with prostate cancer treated with radical prostatectomy serum level of PSA should be undetectable (<0,04 ng/ml). Increase of PSA level after surgery or radiotherapy means tumour recurrence.

2. Biochemical recurrence

If recurrent prostate cancer is detected by a rise in PSA levels after curative treatment, it is referred to as a "biochemical recurrence". In patients treated by radical prostatectomy the increase of PSA above 0,20 ng/ml is considered as biochemical recurrence. If it is observed before two years of surgery is considered a bad prognosis. In 37% of the patients with biochemical recurrence metastasis will be seen before 8 years and they will die in 5 more years. The likelihood of developing recurrent prostate cancer after curative treatment is correlated to various risk factors, such as the grade of prostate cancer (Gleason score), PSA level prior to treatment, and the stage of disease prior to treatment. Patients with low-grade cancer (Gleason score ≤ 6), PSA < 10, and tumors that are not palpable by digital rectal examination are at the lowest risk of recurrence.

3. Treatment of biochemical recurrence

Biochemical recurrence alter radical surgery can be treated with external radiotherapy or androgen deprivation. Radiotherapy should be started when PSA reach 0,50 ng/ml. La radioterapia externa debe iniciarse cuando el PSA alcanza el valor de 0,50 ng/ml ya que las

posibilidades de curación del cáncer son mayores que si se espera que el PSA alcance valores superiores. Patients treated with external beam radiotherapy as a primary treatment of prostate cancer only can receive hormonal therapy in case of recurrence. Any treatment than delay biochemical recurrence will avoid progression of cancer

4. PSA double time (PSADT)

Doubling time is defined as the duration for PSA levels in the blood to increase by 100 percent. A longer PSADT is associated with a longer time to metastasis, to prostate cancer-specific death, and to death from all causes. Furthermore, as the PSADT increases, so do the time to metastasis and the time to prostate cancer-specific death. Men with a PSADT of < 3 months are at high risk for adverse clinical outcomes. Men with a PSADT of > 15 months have an extremely low risk of death from prostate cancer. For men with a PSADT between 3 and 15 months, other clinical factors may have a larger role in determining risk.

IV – Clinical applications of pomegranate juice in prostate cancer

All parts of the pomegranate have been used to treat a variety of illnesses for over a thousand years. However, it was not until the early 90s that the first experimental and clinical trials began[1]. Pantuck *et al*[32] undertook a phase II clinical trial with 46 men with prostate cancer who had been treated by surgery, radiotherapy or criotherapy, and whose PSA levels had increased. The inclusion criteria were a Gleason score of ≤ 7 and PSA between 0.2 and 5 ng/ml. Treatment consisted of 240ml of pomegranate juice a day until the illness progressed. None of the patients had metastasis nor had they received hormonal treatment. Follow-up was carried out every three months and PSA levels determined. The aim of the investigation was to study the variation in PSA figures, such as doubling time. Concurrently an *in vitro* study of cell proliferation was undertaken in which patients' serum was incubated with a culture of hormone-sensitive prostate cancer cells (LNCaP). Of the 46 patients, 16 (35%) showed a reduction in PSA values. In four cases PSA dropped by more than 50%. PSA doubling time (PSADT) increased significantly, from an average of 15 months at the beginning of the study up to 54 months (p<0.001). In the *in vitro* study, after nine months a 12% reduction in prostate cancer cell proliferation was observed, and a 17% increase in apoptosis. Results from the patients who had continued the treatment with pomegranate juice were presented at the 2008 Annual Congress of the American Society of Clinical Oncology (ASCO), the findings showing that PSADT increased at 68 months[33]. These results suggest that pomegranate juice is effective in delaying the progression of prostate cancer in patients whose initial therapies had been unsuccessful. Carducci et al presented at 2011 Annual Meeting of ASCO the results of a randomized, multi-center, double blind, Phase II clinical trial that compared two different doses of pomegranate capsules (POMx) in men with a rising PSA after primary treatment who wished to delay starting androgen deprivation therapy (ADT)[34]. The trial randomized men who had a rising PSA but no metastases. The men received either high-dose (3 mg/d) or low-dose (1 mg/d) of POMx. The men were stratified based on their baseline PSADT values and their Gleason scores; however, there were no restrictions on PSADT and no upper limit PSA scores. The end points were until disease progression or for 18 months. PSA scores were recorded every 3 months. This study was designed to detect a 6-month increase in PSADT from baseline. The results of the study, as presented in the study abstract, are as follows: a- 104 men were enrolled and treated for up to 6, 12, and 18 months (92, 70, and 36 percent of men respectively); b- the men had a median age of 74.5 years, and a median Gleason score of 7; c- the average (median) PSADT was 11.9 months at baseline with a range of 1.6 to 54.6 months compared to18.5months with a range of 2 to 1.523 months after treatment (p < 0.001); d- there was no significant treatment difference in effect on PSADT between the two dose groups (p = 0.920); e- declining PSA levels were observed in 13/104 men (13 percent) during the study; f- there were no significant changes in serum testosterone levels in either group; g- mild to moderate diarrhea was seen in 8/104 men (7.7 percent). This study concludes that

pomegranate extract has some clinically positive effect on the PSADT. It also shows that the lower dosage of POMx is equally as effective as the higher dosage. The authors concluded that "POMx treatment significantly increased the PSADT by over 6 months in both treatment arms, with no effect on testosterone."

1. Our experience with pomegranate on prostate cancer

Progressive increase of PSA is the natural evolution when biochemical recurrence is diagnosed in patients treated initiually with radical prostatectomy. The objective of pomegranate treatment is to stop or delay cancer progression.

We are treating with pomegranate 30 patients with prostate cancer. Ten of the 30 cases are patients with increase of PSA after radical prostatectomy (between >0.04 and 0.50 ng/ml). Surgery had been performed between 1 and 132 months before (mean 35 months). Pomegranate treatment range from 1 to 30 months (mean 13 months). During follow up, PSA was measure each 3-6 months. Mean age was 68 years old (54-73 years). Three cases were followed ≥ 24 months, 6 cases 12 months, 7 cases 6 months and 5 cases 3 months. At 3 months PSA decrease in 3/5 cases (from 0.27 to 0.21, from 0.20 to 0.17 and from 0.09 to 0.08 ng/ml). In one case there was no changes and in one case PSA increases (from 0.20 to 0.31 ng/ml). At 6 months PSA decrease in 3 patients (from 0.31 to 0.15, from 0.21 to 0.16 and from 0.24 to 0.16 ng/ml), and in 4 patients there were no changes. At 12 months PSA decrease in one patient (from 0.20 to 0.04 ng/ml), in 4 patients there were no changes and in one case increase (from 0.11 to 0.16 ng/ml) but without reaching the level of biochemical recurrence (0.20 ng/ml). At 24 months, in one patient there was no change and in two cases PSA increases from 0.04 to 0.20 and from 0.16 to 0.20 ng/ml). Treatment with pomegranate was well tolerated without severe adverse effects. At the time of final review any patient treated with pomegranate have required treatment with radiotherapy because PSA is below 0.50 ng/ml.

2. Clinical cases

A. Case 1. Delay disease progressión

70 years old male. Initial PSA 11 ng/ml. Radical prostatectomy on May 2007 for adenocarcinoma Gleason 3+4, stage pT2c. Start pomegranate on June 2009. 2009.
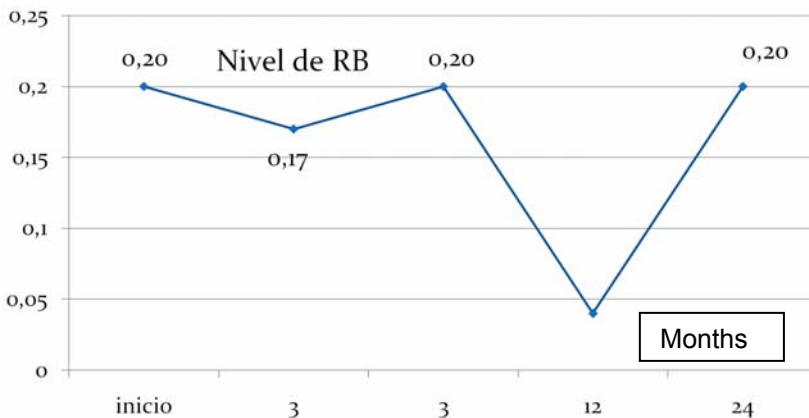

Fig. 1. PSA variation after 24 months of treatment with pomegranate.

B. Case 2. Delay of biochemical recurrence

67 years old male. Initial PSA was 6,08. Radical prostatectomy was performed on October 2007 for Gleason 3+4 adenocarcinoma. Pomegranate was started on October 2008.
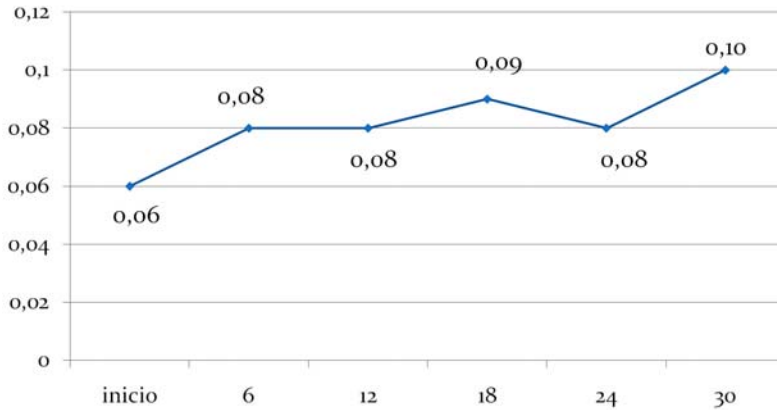
Fig. 2.   Variation of PSA after 30 months of treatment with pomegranate. Biochemical recurrence was delayed.

VARIACION DE PSA
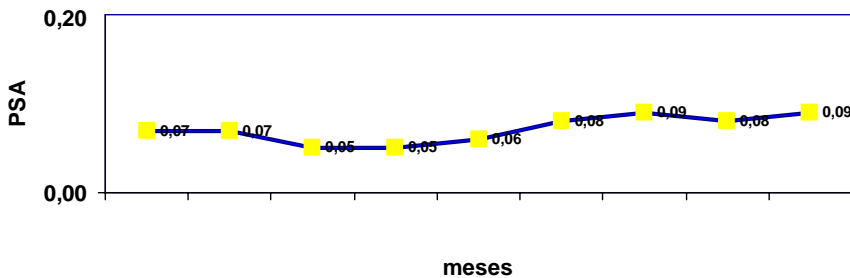
Fig. 3. Delay of hormonal treatment after failure of two treatments for préstate cancer.

C. Case 3. Delay to start hormonal treatment

73 years old male. Initial PSA was 4,16 ng/ml treated with external beam radiotherapy in 2000, hormonal treatment until September 2003. PSA increase from 2003 and 2006. High intensity focalizad ultrasound (HIFU) was performed on March 2007. PSA was undetected until March 2009 and later it increased. Pomegranate juice was started at this time.

VARIACION PSA

Fig. 3. PSA variations after 24 months of treatment with pomegranate. Hormonal treatment have been delayed during 2 years.

3. Ongoing clinical trials using pomegranate juice on prostate cancer

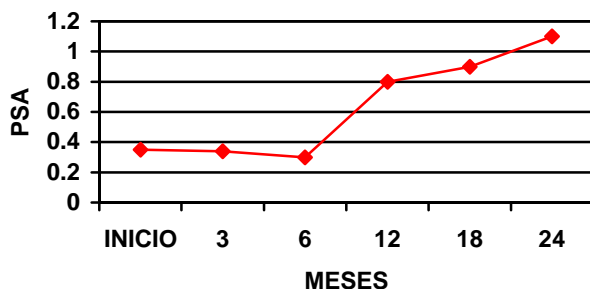Nine of the investigations are related to prostate cancer, six of which are recruiting participants with high PSA levels after the failure of the initial treatment with radical prostatectomy or radiotherapy. In another study patients are treated with pomegranate juice before undergoing radical surgery. Another trial treats patients with localised prostate cancer who have not yet received any treatment, and the final study evaluates supplementing prostate cancer patients' diets with phytochemicals and polyunsaturated fatty acids.

V – Conclusions

The properties of the pomegranate have been known for more than a thousand years, however, it has only been in the last couple of decades that the number of *in vitro* and *in vivo* trials analysing its various components (especially the juice) and their effect on different pathologies has increased. Likewise, over the last few years several multi-centre clinical trials have been designed and are currently in progress; when their results have been analysed, they will be able to offer us a great deal of information about the therapeutic effects of pomegranate. For the time being, its potent antioxidant activity, similar to or greater than green tea, has been proven; it could thus be used as an adjuvant in anti-aging treatments. In oncology, its antiproliferative, pro-apoptotic and angiogenesis effects have been widely studied in animal models and are pending confirmation from human studies. The capacity of pomegranate to regulate plasma levels of glucose, cholesterol and triglycerides, and to reduce blood pressure opens an ample therapeutic potential for patients with diabetes and cardiovascular disease. The possible use of pomegranate juice in other fields, such as neurology and contagious diseases, needs further research.

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Influence of pasteurization treatment and storage in antioxidant activity of pomegranate juice

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Abstract. The combination of time and temperature of pasteurization treatments and storage conditions are important factors in the degradation of the organoleptic and nutritional properties and bioactive compounds of pomegranate juice. Therefore, the influence of pasteurization (high and low temperature) and storage conditions (refrigerated and room temperature) on the variation of the antioxidant capacity of processed juice was evaluated. The analysis of the results indicates that cloudy juice does not suffers loss of antioxidant activity due to pasteurization. However, in the clarified juice the antioxidant activity loss due to the pasteurization treatments was between 20-25%. None of the juices studied showed loss of antioxidant capacity due to storage time.

I – Introduction

The antioxidant capacity of pomegranate juice is three times higher than those of red wine and green tea. It has been amply demonstrated that the antioxidant properties of pomegranate juice are mainly due to a group of compounds called polyphenols. The content of these compounds in pomegranate juice is limited to 0.2-1% depending on the variety. The major polyphenols described in the pomegranate are anthocyanins (as cyanidin-3-glucoside, cyanidin-3,5-diglucoside, and delphindin-3-glucoside), catechins, ellagitannins, gallic acid and ellagic acid (Gil *et al.,* 2000) even as anomers punicalagin (Seeram *et al.*, 2004). The compounds to which is attributed the high antioxidant capacity of pomegranate are the punicalagins group, followed by hydrolyzable tannins, anthocyanins and ellagic acid (Gil *et al.,* 2000). These compounds may also be affected by the pasteurization treatment and storage, reducing the quality of pomegranate juice.

II – Materials and methods

1. Juice extraction

Ten kilograms of pomegranate variety "Mollar Elche" from Elche Experimental Farm were used to obtain the juice. Juice was obtained by pressing of arils inside a nylon mesh with a laboratory pilot press (Zumonat C-40; Somatic AMD, Valencia, Spain). The resulting cloudy juice contained 10% pulp. For obtaining clarified juice, the cloudy juice was centrifuged at 4000 rpm for 10 minutes.

2. Juice pasteurization and storage

Both cloudy and clarified juices were subjected to pasteurization treatments at high and low temperature (HT and LT) for specified times in a semi-tubular pasteurizer 25 l / h (Mipaser Prototype). The juices were stored at room (25°C) and refrigeration temperature (5°C) for 45 and 120 days, respectively.

3. Antioxidant capacity determination in Trolox equivalent (TEAC)

Antioxidant capacity of pomegranate juice was determined according to the method of Re *et al.*, 1999. The radical cation was prepared by the reaction between a 7 mM solution of ABTS (2.2 '-azinobis (3-ethylbenzothiazoline-6-sulfonate, Sigma-Aldrich Corp. St. Louis, MO, USA) in water mixed with a 2.45 mM solution of potassium persulfate. The misture was incubated 24 h in dark at room temperature. Then this solution was diluted with water to reach an absorbance of 0.7 ± 0.02 at 734 nm, measured in a plate reader Spectrostar Omega (BMG LabTech GmbH, Offenburg Germany). To determine the antioxidant capacity of pomegranate juice, 200 µl of the ABTS[+] dissolution were mixed with 20 µl of juice and after 3 minutes the absorbance was measured at 734 nm, obtaining the value of the decrease in absorbance. This determination was carried out with a 1:50 dilution of juice. Trolox was used as standard and results were expressed in mmol Trolox equivalents per liter of juice.

III – Results and discussion

1. Antioxidant capacity of pomegranate juice

Figure 1 shows the changes in the antioxidant capacity of pasteurized cloudy and clarified pomegranate juices stored at 25°C and 5°C.
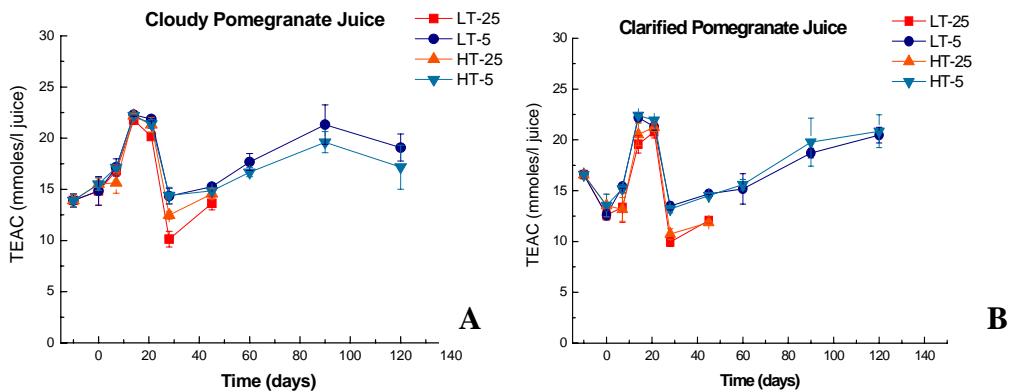
Fig. 1. Measurement of antioxidant capacity of pasteurized pomegranate juices stored at 25°C and 5°C. A, cloudy and B, clarified juices.

The analysis of the results indicates that in clarified juice (Fig. 1B), the loss of antioxidant capacity due to pasteurization treatment at HT and LT reached values between 20-25%. However, in cloudy juice (Fig. 1A) there was no loss in antioxidant capacity, increasing a 5% in both pasteurization treatments.

In Fig. 1, we observe how it behaves the antioxidant activity of juices due to the storage. Both

juices show an increase of the antioxidant capacity until day 14 after which it begins to decrease until day 28. This behavior is independent of pasteurization treatment and juice type. From day 28 the antioxidant capacity increases until the end of the storage period (45 days in samples stored at 25°C and up to 120 days in samples stored at 5°C). Hence, the juices have not a loss of antioxidant activity along the storage time, regardless of the juice type and pasteurization treatment. This may be due to the presence of polyphenol oxidation compounds with a high antioxidant activity (Gil *et al.*, 2000).

Conclusion

The treatment of pasteurization and storage temperature do not appear to influence decisively in the antioxidant capacity of pomegranate juice. Actually we are working in the identification and quantification of the major bioactive compounds occurring in the pomegranate juice (anthocyanins, phenolic acids, punicalagins, etc) to determine the relationship between changes in the profiles of these compounds and antioxidant capacity.

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Main pomegranate phytochemicals and their contribution to the antioxidant potencies of pomegranate juice

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Abstract. This research aims to screen the main phytochemicals and their antioxidant capacities from juice of some pomegranate cultivars from southern Tunisian. Total polyphenols, flavonoids, anthocyanins and hydrolysable tannins were determined by using classical spectrophotometric methods. Free radical scavenging activities were determined by DPPH and ABTS tests. Antioxidant capacities were determined by FRAP and ORAC methods. Results showed that the antioxidant potency of pomegranate extracts was correlated with their phenolic contents. In particular the highest correlations were reported between DPPH (as IC_{50}) and respectively total flavonoids (r =-0.960), anthocyanins (r = -0.866) and total polyphenols (r=-0.788). Therefore both total flavonoids and total anthocyanins showed high correlation to FRAP and ORAC assays. All of these findings confirm the differential contributions of pomegranate phytochemicals to the antioxidant potencies of pomegranate juice. The richness of pomegranate juice with antioxidants might encourage for the development of antioxidant function dietary food.

Keyword. *Punica granatum* L. – Juice phytochemicals – Antioxidant capacities – Correlation.

I – Introduction

Pomegranate has an abundance of antioxidants gaining a lot of attention in the past few years as a super fruit for the heart. The consumption of pomegranate fruit has been associated with a reduced risk of chronic human illness such as certain types of cancers, inflammation, and cardiovascular and neurodegenerative diseases (Aviram *et al.,* 2002). Recent studies on pomegranate antioxidants bring attention to the richness of pomegranate tree and particularly peel, juice and seed oil with natural antioxidants. Therefore, it can explain the interest of traditional medicine practitioners on pomegranate fruit and why they considered pomegranate as a medicinal plant (Seeram *et al.,* 2006).

A natural source of phenolic compounds, pomegranate is loaded with antioxidants such as tannin, polyphenol, flavonoid and vitamins C. Other antioxidants in pomegranate include tocopherols and anthocyanins were demonstrated to have protective and therapeutic qualities. The bioactivities of this phytochemicals are mainly due to their redox properties which make them act as reducing agents, hydrogen donors, singlet oxygen quenchers and also may have a metallic chelating potential (Seeram *et al.,* 2006).

The aim of this research was to quantify Main phytochemicals in pomegranate juice and to compare the efficiency of ABTS, DPPH, FRAP, and ORAC assays to estimate antioxidant activities of pomegranate juice and their correlations with the contents of total polyphenols, flavonoids, anthocyanins and hydrolysable tannins.

II – Materials and methods

1. Plant material

Six Pomegranate cultivars were used in this work: Chetoui (CH), Gabsi2 (GB2), Gabsi3 (GB3), Garsi (GR), Mezzi (MZ) and Zehri (ZH). All cultivars were grown in the same collection at Gabès oasis (south-East of Tunisia). Fully mature fruits were randomly picked from each tree and stored at room temperature for a few days until used.

2. Screening of natural phenolic compounds

Pomegranate juice extract was prepared following the method described previously by Elfalleh *et al.* (2011). The total phenolic coumpounds, flavonoids, anthocyanins and hydrolysable tannins were measured. Total polyphenols were estimated using the Folin-Ciocalteu method (Elfalleh *et al.* 2009). Total flavonoids were measured spectrophotometrically, based on the formation of a flavonoid–aluminium complex with maximum absorbance at 430 nm (Elfalleh *et al.,* 2009). Total anthocyanin content of pomegranate juice was determined by a pH differential method (Çam *et al.,* 2009). Hydrolyzable tannins were determined by modified method of Çam and Hişil, (2010).

3. Determination of antioxidant activities

The free radical scavenging activity was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)) assays (Elfalleh *et al.,* 2009). Antioxidant activity in the samples was determined using Ferric Reducing Antioxidant Power (FRAP) and Oxygen radical absorbance capacity (ORAC) reported by (Elfalleh *et al.,* 2011). This four methods were used to evaluate the antioxidant potencies of pomegranate juice. Results from ABTS, FRAP, and ORAC tests were expressed as Trolox equivalent antioxidant capacity (TEAC) and The DPPH value was expressed as IC_{50} values.

4. Statistical and chemometric methods

All tests were carried out in triplicate and the results were presented as means ± SD. Differences at $p < 0.05$ were considered statistically significant. An analysis of variance (ANOVA) was used to compare cultivars.

III – Results and discussion

Total polyphenols, flavonoids, anthocyanins, and hydrolysable tannins of six pomegranates cultivars are shown in Table 1. Total polyphenols in pomegranate juice were about 10.75 ± 2.20 GAE mg/ml. The total flavonoids in juice were about 5.25 ± 0.59 mg rutin/ml. The highest anthocyanin content was in CH cultivar (48.27 ± 5.01 CGE mg/l) and the lowest value was 28.15 ± 5.12 CGE mg/l of juice in GB3 cultivar. Quantitatively our results are in agreement with those reported by Hasnaoui *et al.* (2011) and lower than those reported by Gil *et al.* (2000) (306.0 mg/l). The anthocyanin fingerprints among pomegranate cultivars were quite different and known to be affected by several parameters such as harvest maturity, storage temperature and relative humidity (Miguel *et al.,* 2004; Shin *et al.,* 2008).

In juice, hydrolyzable tannins ranged from 1.97 ± 0.48 mg TAE/ml to 3.38 ± 0.67 mg TAE/ml with an average of 2.69 ± 0.58 mg/ml. Hydrolyzable tannin content depends on solvent and extraction method (Cam and Hısıl 2010). Gil *et al.* (2000) reported lower total hydrolyzable tannins in juice from fresh arils (0.539 mg/ml).

In previous studies we reported higher phenolic contents in pomegranate peel compared to juice (Elfalleh *et al.*, 2009, Elfalleh *et al.,* 2011). It implies that pomegranate peel can be subjected to phenolic compounds extraction for additional benefits. In particular, it makes commercialization of under-used cultivars after processing possible.

Table 1. Total polyphenols, flavonoids, anthocyanins and hydrolysable tannins from pomegranate juice[†]

Cultivar	Total Polyphenols	Total Flavonoids	Total Anthocyanins	Hydrolysable Tannins
CH	10.93 ± 0.59 B	5.75 ± 0.22A	48.27 ± 5.01 A	3.38 ± 0.67 A
GB2	13.70 ± 1.62 A	5.52 ± 0.48A	38.06 ± 6.72 A.B	2.60 ± 0.09 A.B
GB3	6.89 ± 1.97 C	4.11 ± 0.87B	28.15 ± 5.12 B	2.40 ± 0.21 A.B
GR	11.26 ± 0.65 B	5.63 ± 0.32A	43.29 ± 3.19 A	2.97 ± 0.16 A.B
MZ	10.59 ± 0.58 B	5.30 ± 0.29A	41.62 ± 6.53 A.B	2.82 ± 0.79 A.B
ZH	11.15 ± 0.63 B	5.21 ± 0.08A	35.79 ± 4.65 A.B	1.97 ± 0.48 B
Mean ± SD (n=6)	10.75 ± 2.20	5.25 ± 0.59	39.19 ± 7.69	2.69 ± 0.58

[*]Different letters in the same column of cultivar respectively, indicate significant difference (P <0.05) analyzed by Duncan's multiple range test.
[†]Total Polyphenols expressed as grams of gallic acid equivalents (GAE) per L of juice. Total Flavonoids expressed as grams of rutin equivalents (RE) per L of juice. Total Anthocyanins expressed as milligrams of Cyanidin 3-glucoside equivalents (CGE) per of L juice and Hydrolysable Tannins expressed as grams of tannic acid equivalents (TAE) per L of juice.

Because of the multiple reaction characteristics and mechanisms involved in the estimation of the total antioxidants, no single method could accurately reflect all antioxidants in a mixed system due to the complex nature of phytochemicals (Chu *et al.*, 2000). In the present study, four methods were used to evaluate the antioxidant capacities of pomegranate juice (Table2).

The free radical scavenging activity determined by DPPH was expressed as IC50 value which ranged from 15.98 ± 0.60 to 23.98 ± 1.60 µl of juice/ml DPPH. The ABTS values ranged from 4.67 ± 0.38 TEAC mM/l of juice in GB2 cultivar to 8.57 ± 1.16 TEAC mM/l of juice in GR, which enclose the highest TEAC activity.

Table 2. Total polyphenols, flavonoids, anthocyanins and hydrolysable tannins from pomegranate juice

Cultivar	DPPH (IC$_{50}$ µL/ml)	ABTS (TEAC mmol/l)	FRAP (TEAC mmol/l)	ORAC (TEAC mmol/l)
CH	15.98 ± 0.60C	6.13 ± 1.10BC	7.86 ± 0.95 A	7.59 ± 2.26 A
GB2	18.08 ± 1.00B	4.67 ± 0.38C	7.31 ± 1.27 A	6.50 ± 0.14 A
GB3	23.98 ± 1.60A	4.73 ± 0.40C	6.44 ± 0.01 A	5.94 ± 1.29 A
GR	18.08 ± 0.30B	8.57 ± 1.10A	8.65 ± 1.87 A	8.18 ± 2.38 A
MZ	19.38 ± 1.20B	5.67 ± 0.80BC	6.67 ± 0.83 A	7.00 ± 2.05 A
ZH	18.08 ± 1.00B	7.07 ± 0.57B	6.36 ± 0.79 A	6.62 ± 0.09 A
Mean ± SD (n=6)	18.93 ± 2.70	6.14 ± 0.33	7.24 ± 1.24	7.00 ± 1.52

[*]Superscript letters with different letters in the same column of cultivar respectively, indicate significant difference (P <0.05) analyzed by Duncan's multiple range test.

In pomegranate juice FRAP radical scavenging activities ranged from 6.36 mmol/l in ZH cultivar to 8.65 mmol/l in GR cultivar, with an average of 7.24 ±1.24 mmol/l. Our finding were similar to

results reported in the FRAP assay by Ozgen *et al.* (2008), in six pomegranate cultivars from southern Turkey with an average FRAP value of 7.35 ± 0.21 mmol/l.

ORAC activity ranged from 5.94 mmol/l in GB3 to 8.18 mmol/l in GR cultivar, with an average of 7.24±1.24 mmol/l. In all antioxidant assays, the capacities of CH and GR cultivars were slightly higher than other cultivars. However, no significant differences between cultivars were reported with the Duncan test (Table 2).

Correlations coefficients of total polyphenols, flavonoids, anthocyanins, and hydrolysable tannins to DPPH, ABTS, FRAP and ORAC assays were shown in Table 3. DPPH highly correlated with total flavonoids (r=-0.960), total anthocyanins (r=-0.866), and total polyphenols (r=-0.788). Therefore both total flavonoids and total anthocyanins showed high correlation to FRAP and ORAC assays. Results clearly confirm that this phytochemicals predominantly contribute to the antioxidant potencies of pomegranate juice.

Nevertheless, not all phytochemicals showed high correlation to antioxidant assays. This fact can mainly be explained by the uncertainty surrounding the chemistry of antioxidant compounds and that is why both phenolic content and antioxidant activity information must be discussed when evaluating the antioxidant potential of extracts. The fact that there is a rather poor correlation between juice phenolic content and the FRAP and ORAC antioxidant activities may mean that other components, such as ascorbic acid need to be investigated for their contribution to the antioxidant activity.

Table 3. Correlation coefficients of phenolic compounds to antioxidant capacities of pomegranate Juice

	Total polyphenols	Total flavonoids	Total anthocyanins	Hydrolysable tannins
DPPH	-0.788*	-0.960**	-0.866**	-0.254
ABTS	0.170	0.466	0.441	0.074
FRAP	0.390	0.662*	0.693*	-0.256
ORAC	0.358	0.763*	0.850**	0.189

*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.

IV – Conclusion

However the studied phytochemicals contribute widely to the antioxidant potencies of juice alone they cannot explain the mechanism involved in the antioxidant activity. This phytochemicals are only one facet of the DPPH, ABTS, FRAP and ORAC antioxidant capacities of pomegranate juices. Again, this showed that the constituents of a real matrix solution did not chemically interact with selected pure antioxidants, and that the antioxidant capacities were additive.

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Effect of drying process on phenolic compounds and antioxidant capacity of pomegranate (*Punica granatum* L.) fry flowers

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Abstract. The pomegranate (*Punica granatum* L.) is an important medicinal plant. It has been reported that phenolic compounds contained in the flowers have antioxidant potential. The objective was to determine the concentration of total phenolics and antioxidant capacity of three selections of pomegranate flower dried under different conditions. The highest phenolic content and antioxidant capacity was observed in the orange colored flowers dried at room temperature. Compared to red and dark red flowers, the dark red showed higher anthocyanins. The room temperature and oven drying processes preserved a higher content of phenols compared to sun drying. The results showed that orange pomegranate flowers dried at room temperature could be used for the preparation of antioxidant beverages.

Keywords. Pomegranate flower – Phenolic compounds – Antioxidant capacity – Drying process.

I – Introduction

Pomegranate (*Punica granatum* L.) flowers have a wide variety of bioactive compounds of therapeutic value and have been used in India's traditional medicine to treat diabetes (Lansky and Newman, 2007). Studies related to pomegranate flower extract have shown hypoglycemic activity, reduction of total cholesterol, tryglicerides and LDL-C in serum and also a potent antioxidant effect (Huang *et al.*, 2005; Bagri *et al.*, 2009). Currently in Mexico, the Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias (INIFAP) is involved in the genetic improvement of pomegranate, and their goal is to enhance the quality of the products and productivity of the pomegranate orchards in the benefit of small farmers. In this study flowers from different genotypes developed by INIFAP were used. These genotypes are characterized by its profuse flowering and deep red color, suggesting they could present high antioxidant capacity.

II – Materials and methods

1. Plant material

The pomegranate flowers were harvested during April 2008 at the Bajio Research Station (INIFAP) located in Celaya, Gto, México. The selections evaluated are characterized by abundant flowering of orange, red and dark red color. They were collected on a piece of white non-woven fabric of polypropylene (Agribon®), placed underneath the tree canopy. Thereafter they were subjected to three drying methods; sun drying (25 °C, 48 h), room temperature (20-22°C, 48 h) and convective oven (50 ºC, 48 h).

2. Preparation of pomegranate flowers (PGF) aqueous extract

One gram of ground pomegranate dry flowers was boiled in 100 ml of distilled water during 10 min, the aqueous extract was stirred for 1h and filtered through a Whatman filter and restored to a 1% (w/v) volume.

3. Determination of phenols and antioxidant capacity

The determination of total phenols was performed by the spectrophotometric method of Folin-Ciocalteu (Singleton *et al.*, 1999). Anthocyanins were determined by the method of Abdel-Aal and Hucl (Abdel-Aal and Hucl, 1999). The capacity of the PGF to scavenge a free radical was determined by the method of van der Berg *et al.* (van der Berg *et al.*, 1999).

4. Statistical analysis

The statistical analysis of data was performed by one-way ANOVA, and the differences among treatments were calculated by comparison of means using the Tukey-Kramer test. The level of statistical significance was taken at $P < 0.05$. All data are presented as mean ± SE.

III – Results and discussion

1. Effect of drying method on the concentration of phenolic compounds of pomegranate flower extracts

Maximum total phenol content was obtained in the orange flowers, dried at room temperature. This method [DH1] preserved the largest amount of these compounds, compared to the red and dark red colored flowers. Sun and oven dried processes did not show significant statistical differences ($P <0.05$) on total phenol content (Fig. 1). These results suggest that the content of phenolic compounds decreases at higher temperatures, similar results have been reported by other authors (Rozek *et al.*, 2008).
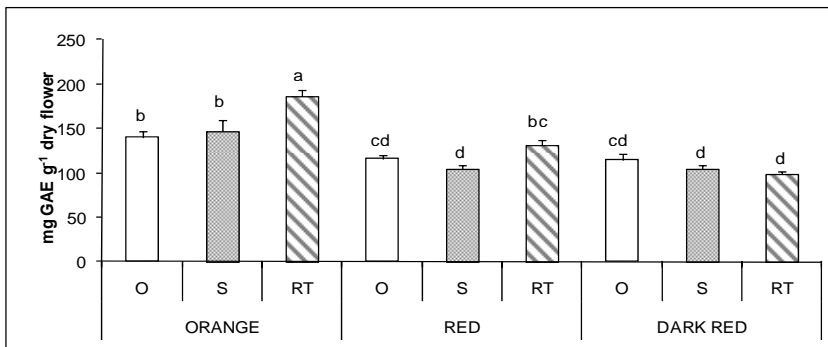

Fig. 1.   **Effect of drying method on the phenolic compounds in the aqueous extract of pomegranate flower selections. Different letters indicate significant statistical differences (p<0.05). (O) Oven (S) Sun (RT) Room temperature.**

2. Effect of drying method on the concentration of anthocyanins of pomegranate flower extracts

In this study we found that dark red flowers dried in the oven and at room temperature have the highest concentration of anthocyanins, followed by red flowers, then orange flowers (Fig. 2). It has been suggested that the degradation of anthocyanins increases with the temperature (Gil *et al.*, 2000), however, in the oven drying process and at room temperature the flowers contained the same anthocyanins concentration. One explanation for this event is that, the hydrolyzation of the pyrilium ring resulted in production of chalkons, which are responsible for brown color developed in food containing anthocyanin (Laleh *et al.*, 2006).
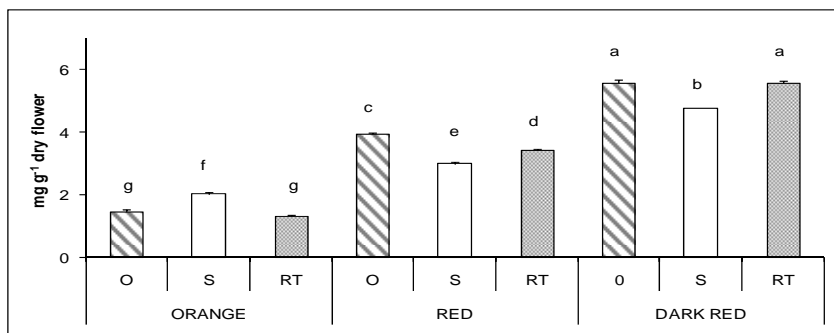

Fig. 2. Effect of drying processes on the anthocyanin content in the aqueous extract of pomegranate flower selections. Different letters indicate significant statistical differences (p<0.05). (O) Oven (S) Sun (RT) Room temperature.

3. Effect of drying processes on the antioxidant capacity of pomegranate flower aqueous extracts

The ABTS assay measures the antioxidant activity of hydrophilic and hydrophobic bioactive compounds (Re *et al.*, 1999). This study showed that the aqueous extract from orange flowers has the highest antioxidant capacity, followed by red and dark red flowers. Additionally, the room temperature drying process preserved the *in vitro* antioxidant potential of orange colored flowers. A different effect is observed for dark red flowers, which a sun drying process maintained the highest antioxidant potential. This result suggests that the antioxidant activity shown by each selection is due to the total phenol content.

IV – Conclusions

Our findings prove that the aqueous extract from orange flowers dried at room temperature preserved both, a highest phenolic content and in vitro antioxidant capacity, which suggests that this selection could be used for the preparation of beverages with antioxidant potential.
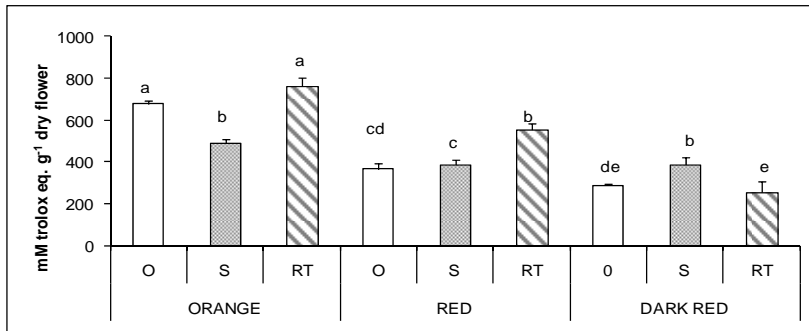
Fig. 3. **Effect of drying processes on the antioxidant activity of aqueous extract of pomegranate flower selections. Different letters indicate significant statistical differences (p<0.05). (O) Oven (S) Sun (RT) Room Temperature.**

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Physico-chemical and antioxidant properties of pomegranate genotypes in Greece

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Abstract. Fruit quality attributes and antioxidants contents were compared among 11 Greek pomegranate genotypes and the foreign cultivars Wonderful, Akko and Hicaznar, established in two collections in the northern Greece. The heaviest fruit were harvested by Wonderful (474 g), 11021 (400 g), Hicaznar (316 g), and T9 and T10 (mean 366 g). Kallisti had the greatest aril weight (0.472 ±0.015 g), whereas Wonderful had the lowest aril weight (0.260 ±0.025 g). The greatest edible portion percentage (62.3%) and juice percentage (44.7%) were found in the genotype 11019. Total soluble solid content ranged between 15.7% (Kallisti) and 18.3% (11019), and total acid content ranged between 4.8 (Hicaznar) and 22.4 g L^{-1} (Wonderful). The genotypes T9 and T10 contained the highest total phenol and ascorbate equivalent antioxidant capacity, which were comparable with Hicaznar and Wonderful. Total anthocyanin contents were lower in the Greek, compared with the foreign cultivars. In conclusion Kallisti is a worthy genotype in respect to having relatively great aril weight, fruit size and being soft seeded, and therefore it should be considered for fresh consumption. The genotype 11019 could be worthy for an industrial point due to its high juice content.

Keywords. Antioxidant capacity – Anthocyanins – Fruit quality – Phenols.

I – Introduction

The pomegranate cultivation has only recently been intensified in Greece; from being a minor fruit crop (51 hectares in 1994 and 200 hectares in 2007, Hellenic Statistical Authority), in the last four years the cultivated areas may have reached up to 1200 hectares (personal communications). A massive introduction of foreign pomegranate cultivars took place (e.g. mainly Wonderful), without prior experimentation on their suitability under the different Greek microclimate conditions. Nevertheless, local pomegranate genotypes with interesting market characteristics are abundant (Drogoudi *et al.*, 2005). A comparative study on fruit quality characteristics in local and newly imported cultivars would be useful for the selection of promising new genotypes.

II – Materials and methods

The experiment was performed in fruit collected from the pomegranate Greek genotypes 11029 (Kallisti), 11005, 11019 and 11021 established in a collection orchard at the Pomology Institute in Naoussa, and T1, T2, T4, T5, T7, T9, and T10 genotypes established in a collection orchard at T.E.I.Th. in Sindos Thessaloniki. Fruit from nearby commercial orchard of cultivars Akko, Hicaznar and Wonderful were also used. Twelve fruit from each genotype were transferred in the laboratory, weighed, peeled carefully, and juiced by pressing the arils in a four layer cheese-cloth. Fruit fresh weight, aril weight, edible portion and juice percentage were measured. Soluble solid content (SSC) was measured using a digital refractometer (model PR-1, Atago, Japan), and titratable acidity (TA) was measured by titration to pH 8.2 with 0.1 N NaOH and expressed as citric acid content (g l^{-1}). A portion (50 ml) of extracted juice was kept at -20°C until further analysis.

Total phenolic content of the pomegranate juice was assayed according to Folin-Ciocalteu method (Singleton and Rossi, 1965). Gallic acid was used as a standard and the results were expressed as mg gallic acid equivalent 100ml^{-1} juice. Total ascorbate equivalent capacity (AEAC) was determined using the stable 1,1- diphenyl-2-picryl hydrazyl (DPPH) free radical (Blois, 1958), which has an intense violet colour, but turns colourless as unpaired electrons are sequestered by antioxidants. Ascorbic acid used as a standard and the results were expressed as mg ascorbic acid equivalent 100ml^{-1} juice.

The total anthocyanin content was estimated by pH differential method using two buffer systems: potassium chloride buffer, pH 1.0 (25mM) and sodium acetate buffer, pH 4.5 (0.4M) (Cheng and Breen, 1991) and at two wavelengths of 510 nm and 700 nm. The samples were diluted ten times in 80% ethanol (1% HCl). The total anthocyanins content was calculated as follows: total anthocyanins = [(A×MW×DF×100)/MA], where A = (A$_{510}$ −A$_{700}$) pH1.0 −(A$_{510}$ −A$_{700}$) pH4.5; MW: molecular weight (449.2); DF: dilution factor; MA: molar absorptive coefficient of cyaniding-3-glucoside (26.900). Results were expressed as mg cyaniding-3-glucoside 100 ml^{-1} of juice.

Data were subject to ANOVA and then significant differences between individual means were determined using the Duncan's multiple range test at the 5% level, using the statistical software SPSS 12.0 (SPSS Inc., Chicago, USA). Correlations analyses were also performed.

III – Results

The heaviest fruit were harvested by Wonderful (474 g), 11021 (400 g), Hicaznar (316 g), and T9 and T10 (mean 366 g), and the lightest by Akko, T5 and T7 (264.0 g) (Table 1). The greatest aril weight (0.472 ±0.015 g) was measured in Kallisti, whereas Wonderful had the lowest aril weight (0.260 ±0.025 g) (data not shown). The greatest edible portion percentage (62.3 %) and juice percentage (44.7 %) were found in 11019 accession (Table 1). The highest and the lowest SSC were recorded in genotype 11019 (18.3%) and cultivar Kallisti (15.7%) respectively, whereas titratable acidity ranged between 4.8 (Hicaz) and 22.4 g l^{-1} (Wonderful).

Table 1   Mean fruit fresh weight (FW, g), % edible portion, % juice, soluble solid content (SSC, %), titrable acidity (TA, g l^{-1}), total phenolics (TPhs, mg gallic acid equivalent 100ml^{-1}), total anthocyanins (TAnth, mg cyaniding-3-glucoside 100 ml^{-1}) and ascorbate equivalent antioxidant capacity (AEAC, mg ascorbic acid 100ml^{-1}) in different pomegranate cultivars and accessions

	FW	% edible	% juice	SSC	TA	TPhs	TAnth	AEAC
Kallisti	332.0 ab	58.7 bc	41.3 ef	15.7 a	6.1 abc	46.8 a	5.2 a	60.2 a
11005	298.1 ab	52.3 abc	32.4 cd	17.1 cd	9.5 de	77.4 c	13.9 a	122.3 def
11019	296.9 ab	62.3 c	44.7 f	18.3 e	7.9 cd	54.0 a	8.2 a	72.4 ab
11021	400.3 cd	45.9 ab	31.3 bc	16.5 abc	10.4 e	62.5 b	4.9 a	89.6 bc
T1	302.2 ab	42.9 a	21.3 a	15.9 ab	5.0 ab	82.1 c	10.2 a	127.8 ef
T2	275.3 ab	47.7 ab	27.1 ab	16.2 abc	7.2 bc	80.3 c	10.3 a	126.1 ef
T4	308.0 ab	48.5 ab	25.3 ab	18.0 de	5.0 ab	81.9 c	9.8 a	123.4 def
T5	257.4 a	50.7 abc	27.9 ab	16.7 abc	4.5 a	80.7 c	11.7 a	118.3 de
T7	252.0 a	46.7 ab	24.4 a	16.4 abc	5.4 ab	80.2 c	9.7 a	119.4 def
T9	366.9 bc	40.4 a	20.6 a	16.5 abc	6.1 abc	84.0 cd	8.0 a	138.6 f
T10	364.5 bc	45.6 ab	25.5 a	16.0 ab	6.0 abc	84.3 cd	9.1 a	139.4 f
Akko	273.6 a	53.6 abc	37.9 de	16.9 bc	6.2 abc	83.3 cd	41.3 b	106.0 cd
Hicaznar	315.8 abc	47.2 ab	31.2 cd	16.5 abc	4.8 a	92.1 d	83.6 d	124.5 ef
Wonderful	474.2 cd	53.0 abc	35.0 cd	17.1 cd	22.4 f	88.3 cd	59.1 c	124.8 def

Means in each column followed by different letters are significantly different at $P < 0.05$.

Cultivar Hicaznar had the greatest total phenol (92.1 mg gallic acid equivalent 100ml^{-1}) and anthocyanin (91.2 mg 100ml^{-1}) contents, which were up to 2 and 18.6 times greater, respectively, compared to the rest studied genotypes. Significant differences among the studied pomegranates were also found in the AEAC ranging from 60.15 ±3.5 to 139.4 ±16.6 mg ascorbic acid equivalent 100ml^{-1}. The greatest and the lowest antioxidant activity were detected in Wonderful and Kallisti cultivars, respectively. AEAC was positively correlated with total phenols (r^2=0.846), but not with total anthocyanin content. Similar values for all measured traits were also previously reported for 11029 (Kallisti), 11005, 11019 and 11021 (Drogoudi et al., 2005).

Important variation was found in all parameters measured, suggesting that genotype is an important factor determining fruit quality attributes in pomegranate. Kallisti is a worthy genotype in respect to having relatively great aril weight, fruit size and being soft seeded, and therefore it should be considered for fresh consumption. The genotype 11019 could be worthy for an industrial point due to its high juice content. Hicaznar, Wonderful and the genotypes T9 and T10 were superior in respect to containing relatively high antioxidant contents.

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