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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, 5°C and 90-95% RH are recommended 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, O₃, 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, 1998, 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 °Brix 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 5°C 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 climatic 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 of 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 cryptogammic 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 5°C 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 5°C 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 5°C 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 (Irlés, 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 O₂ partial pressure and that of the CO₂ 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 O₂ and/or moderate CO₂ to restrain respiration and other vital processes of fruit, reducing weight loss, decay and CI. ME stored under 5 kPa O₂ + 0-5 kPa CO₂ at 5°C for 2 months followed by 6 days in air at 20°C, 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 O₂ + 10 kPa CO₂ inhibits CI in ME after 3 months at 5°C and 95% RH regarding fruits stored in air or in 15 kPa CO₂ enriched air (López-Rubira *et al.*, 2007). However 4-5 kPa O₂ + 12-14 kPa CO₂ after 80 days at 2 and at 5°C, 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 O₂ + 5-10 kPa CO₂ at 5°C was recommended (Hess-Pierce and Kader, 2003) or, as an option to avoid superficial scald, 2-4 kPa O₂ at 2-6 °C (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 33°C and 95% RH followed by 3 months at 2°C and 90-95% RH and a period of 8 days at 15°C and 70% RH lowered CI compared to non-cured fruits. Also the IW at 20°C of ME during 1 day every 6 to 14 days at 0, 2 or 5°C 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 15°C and 70% RH. The IW of 1 day at 20°C every week at 2°C 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 45°C before 90 days at 2°C and 90% RH, followed by 3 days at 20°C, 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 5°C under 5 kPa O₂ + 10 kPa CO₂ followed by 8 days at 20°C 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 2°C 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 25°C to ME before storage for 84 days at 2°C 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 20°C, delayed ripening, reduced CI, increased antioxidant activity and maintained the bioactive compounds and nutritive value after 84 days at 2°C plus 4 days at 20°C (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 Albaterra 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 °Brix 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 5°C, pre-washing with water at 5°C and pH 6-6.5 with 100-125 ppm free chlorine as antimicrobial, followed by sorting and drying by cold (5°C) forced air. Sound fruits are then transported by a conveyor belt to the proper and refrigerated (about 5°C) area, physically separated from the previous dirty area. Fruits are then cut with disinfected sharpened knives 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 (broken, 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 waded water at 5°C, 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 5°C, dried by cold (5°C) 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 O₂ + 5-10 kPa CO₂). 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 (0/1°C) room for picking nearby the shipping area at 0/1°C. The transport and distribution temperature must be 5°C 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 5°C did not control always microbial growth (López-Rubira *et al.*, 2005a). The peroxyacetic acid and the ozonated (0.4 ppm O₃) 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 m³). In MAP ME arils treated with 0.1 g l⁻¹ NaClO, after 14 days at 0°C a clean room ISO 5 reduced 1 log ufc g⁻¹ the initial counts of aerobes, mesophilic and psychrotrophic. However this scarce effect does not probably justify the industrial application, although the very low temperature could mask their effect (Conesa *et al.*, 2005). It could be of interest to continue the studies because the technique starts being implanted.

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