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Morpho-pomological and chemical characterization of pomegranate (*Punica granatum* L.) genotypes in Apulia region, Southeastern Italy

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ABSTRACT

A recent increasing demand in Western countries for pomegranate products by consumers is especially supported for the nutritional and medicinal characteristics, due to the antioxidant properties of this fruit. Some studies have been published on the morphological and biochemical characteristics of pomegranate fruits in some Mediterranean countries, but little information is available about the genotypes present in Italy and in particular in Apulia (Southeastern region of Italy). This study (2008–2009) evaluated morpho-pomological and chemical parameters of eight pomegranate genotypes localized in private small orchards. Significant differences were observed among the pomegranate genotypes for many of the parameters investigated. In particular, fruit weight ranged from 168.9 g (SouMol) to 574.9 g (SouOst), °Brix from 14.7 (ComTri) to 18.0 (SouMol), titratable acidity from 5.4 (ComMol) to 25.0 (SouTri) g/L. SouMol showed the highest polyphenols (97.1 mg/L) and vitamin C (236.3 mg/L) contents. Oil content of the seeds was between 5.90% and 10.30%, no differences have been observed for the fatty acid composition with conjugated linoleic acid (CLA) isomers as the most abundant fraction (81.23%). Considering all the evaluated parameters, and especially those referring to the organoleptic characteristics and antioxidants content, it must be stated that the best genotypes worthy to be considered from agricultural and industrial points of view were AdeSgi for fresh market and SouOst for the juice industry.

1. Introduction

Pomegranate is generally cultivated in the Mediterranean basin, and in regions of Southern Asia, in India and in North and South America, where high temperatures allow a good fruit ripening. In particular, its successful adaptation to the Mediterranean climate has produced a wide diffusion in various countries thus originating several local genotypes along the centuries.

The edible portions of pomegranate fruit are the arils which constitute 45–60% of total fruit weight (Kulkarni and Aradhya, 2005; Sarkhosh et al., 2009; Tehranifar et al., 2010). Arils include two parts: the testa that is seed coat and is fleshy or pulpy. The testa is sweet, sweet–sour or sour, its color is white, pink or red and is rich in organic acids and phenolic compounds. The other portion of an aril is the seed consisting of the covering and the embryo inside. Embryos contain nutritive substances such as fatty acids, and about 10 fatty acids are found in the pomegranate seed oil (Sarkhosh et al., 2009). As for many fruit species, pomegranate varieties differ in their taste, ranging from sweet to sour (Holland et al., 2008) and this is related directly to the quality and quantity of the organic acids and sugars in the fruit. The desired pomegranate taste varies, however, in different countries and regions.

A recent increasing demand in Western countries for pomegranate products by consumers is especially supported for medicinal and nutritional properties (Lansky and Newman, 2007), due to the antioxidant properties of this fruit (Gil et al., 2000; Seeram et al., 2008) that contains anticarcinogenic (Bell and Hawthorne, 2008), antimicrobial (Reddy et al., 2007), antiviral (Kotwal, 2007) and antiatherosclerotic compounds even able to reduce blood pressure and LDL oxidation (Aviram et al., 2004). These activities are mainly attributed to the pomegranate's high levels of antioxidant activity and its high total polyphenols content (Gil et al., 2000; Tzulker et al., 2007). Pomegranate juice was shown to possess a 3-fold higher antioxidant activity than that of red wine or green tea (Gil et al., 2000), and 2-, 6- and 8-fold higher levels than those detected in grape/cranberry, grapefruit, and orange juice, respectively (Rosenblat and Aviram, 2006). However, most of the data on chemical properties and on the health beneficial compounds of pomegranate juice were derived from the globally important variety Wonderful (Gil et al., 2000; Seeram et al., 2008; Shwartz et al., 2009; Tehranifar et al., 2010) and from Turkish (Ozgen et al., 2008), Iranian (Akbarpour et al., 2010; Sarkhosh et al.,

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2009) Israelian (Borochov-Neori et al., 2009), Italian (Barone et al., 2001), Tunisian (Mars and Marrakchi, 1999) and Indian accessions (Kulkarni and Aradhya, 2005).

The pomegranate seed is usually a by-product which comes out after the process of juice extraction. It represents an important portion of the fruit weight, ranging from 40 to 100 g/kg of the fruit (Fadavi et al., 2006). The seed, for the presence of various compounds, could be a rich source of beneficial phytochemicals (Pande and Akoh, 2009). The presence of oil in pomegranate seeds has stimulated interest in their fatty acids composition, especially the content of unsaturated fatty acids (PUFA), including isomers of conjugated linoleic acid (CLA). CLA is a mixture of octadecatrienoic fatty acid isomers, which are positional and geometric (Kýralan et al., 2009). These compounds are recently becoming interesting because their role in preventing cardiovascular diseases, cancer, asthma and in reducing cholesterol levels (Kýralan et al., 2009; Melgarejo et al., 1995; Abbasi et al., 2008). Pomegranate seed oil might also benefit menopausal women as phytoestrogen medicaments (Lansky and Newman, 2007).

To capitalize on the potential benefits of pomegranate cultivation, more information is needed on the morpho-pomological and chemical characteristics of the different genotypes localized in various Mediterranean areas. 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 (Italy). In particular, in Italy data of 2009 indicate only 8 ha cultivated to pomegranate and no hectares result officially under cultivation in Apulia (ISTAT, 2010). In this region the main important crops are table grape, wine grape, olive and sweet cherry, but considering the pedo-climatic conditions even pomegranate could be an interesting and promising crop.

The objective of this paper was to study and compare morpho-pomological characteristics and the levels of antioxidants, total polyphenols, vitamin C, total lipids and fatty acids composition, in eight genotypes of pomegranate localized in apulian orchards in order to better characterize genotypes that may be used for cultivation or for breeding programs in the next future as a perspective of further development of pomegranate cultivation.

2. Materials and methods

2.1. Fruits collection

The collection of pomegranate fruits was conducted in the years 2008 and 2009, in central area of Apulia region (Southeastern Italy). Fruits were collected from adult trees (≅20-year-old) located in private small orchards. All the genotypes selected had a similar maturity date based on their internal and external color and the fruits were picked up when greenness disappeared from the surface and yellow or red color appeared. For both years harvesting time ranged from mid-end September to mid October. Four genotype were sour and four were considered sweet. The genotypes analyzed were: Sour Triggiano (SouTri), Sour Molfetta (SouMol), Sour Ninetta Ostuni (SouOst), Sour S. Giorgio (SouSgi), Common Triggiano (ComTri), Modugno Triggiano (ModTri), Common Molfetta (ComMol), A dente S. Giorgio (AdeSgi).

2.2. Fruits morpho-pomological and organoleptic characteristics

Morpho-pomological measurements of fruits (Mars and Marrakchi, 1999), arils and seeds characteristics (Martínez et al., 2006) and chemical analyses were carried out on samples of 15 mature fruits per genotype and per year selected at random throughout the external and internal canopy in the four cardinal directions (Sarkhosh et al., 2009). The following morpho-pomological traits were measured on the fruits: fruit weight (g), length (mm) and diameter (mm), sepal number (n.), calyx length (mm) and diameter (mm). The following arils characteristics were analyzed: maximum diameter (mm) and length (mm), aril weight (mg), juice volume (cm^3/100 g of arils), sugars content (°Brix), pH and total acidity (g/L) of juice and, on the woody portion of the seeds, weight (mg) and a woody portion index measured as seed weight/aril weight ratio × 100 (%). A maturity index (MI) was also adopted (°Brix/acidity) as previously proposed for some Spanish varieties (Martínez et al., 2006).

The fruits were collected in plastic bags and stored in a portable ice box to be carried in the laboratory, where they were characterized by morpho-pomological and organoleptic parameters, as described above. The peels and arils were separated from every fruit obtained for each pomegranate genotype. Juice was prepared by squeezing the arils through a metal sieve. The juice was then filtered and separated in two aliquots and one was immediately frozen at −20 °C for HPLC analysis. On the other aliquot of the fresh juice a digital refractometer was used to measure the °Brix value. A semi-automatic titrator was used for total titratable acidity determination. In particular, 5 mL juice was diluted to 50 mL distilled water and titrated with 0.1 N NaOH to pH 8.1. Titratable acidity was calculated as g citric acid/1000 mL juice. Measurements were replicated three times for each arils juice.

2.3. HPLC-PAD conditions and analysis

The HPLC system Ultimate 3000 (Dionex, Germering, Germany) was equipped with an photodiode array detector (PAD 3000), low pressure pump Ultimate 3000 pump, injector loop Rheodyne (Rheodyne, USA) of 20 μL, the column Acclaim C18 reverse (150 mm × 4.6 mm; 3 μm) and precolumn Acclaim C18 reverse (10 mm × 4.6 mm; 5 μm) and column oven. The HPLC was controlled and data were elaborated using Chromeleon Software *vs* 6.8 (Dionex, Germering, Germany). The gradient profile for the separations of gallic acid (GAL), 3,4-dihydrobenzene (DB), catechin (CATH) and chlorogenic acid (CHL) was as follows: starting with methanol/water/acetic acid (10/89/1, v/v/v) for 1 min, then linear gradient from 10% to 35% methanol in 13 min, maintained for 8 min at methanol/water/acetic acid (35/64/1, v/v/v) and equilibration for 5 min by methanol/water/acetic acid (10/89/1, v/v/v) mobile phase and flow rate of 1 mL/min. The analyses were performed at UV wavelengths of 272, 295, 280, 320 nm for GAL, DB, CATH and CHL, respectively, and scan mode range was 190–450 nm.

The gradient profile for the separations of CHL, caffeic acid (CAF), ferulic acid (FER), *p*-coumaric acid (*p*-COU), quercetin (QUE) was as follows: starting with methanol/water/acetic acid (40/59/1, v/v/v) for 10 min, then linear gradient from 40% to 80% methanol in 5 min, maintained for 2 min at methanol/water/acetic acid (80/19/1, v/v/v) and equilibration for 1 min by methanol/water/acetic acid (40/59/1, v/v/v) mobile phase and flow rate of 1 mL/min. The analyses were performed at UV wavelengths of 320, 320, 320, 310 and 375 nm for CHL, CAF, FER, *p*-COU, QUE, respectively, and scan mode range was 190–450 nm.

2.4. Total phenolic assay

Total phenolic contents (TPC) of the pomegranate juices were assayed according to Folin–Ciocalteu method. Briefly, stock solutions were prepared by dissolving 100 μL of juices in 1 mL of deionized water. 300 μL of stock solutions and blank were pipetted into separate test tubes and 300 μL of Folin–Ciocalteu reagent (Singleton and Rossi, 1965) were added to each of them. The mixture was mixed well and allowed to equilibrate. After 2 min, 2.4 mL