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Physicochemical properties and aroma volatile profiles in a diverse collection of California-grown pomegranate (*Punica granatum* L.) germplasm ☆

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ABSTRACT

Colorful antioxidant-rich fruits often convey astringency and sourness that juice consumers may not appreciate. We assessed properties in juices from a collection of California-grown pomegranate from the National Clonal Germplasm Repository. The goal was to evaluate overall differences in germplasm with quality traits classified as sweet, sweet–sour and sour. Previous relationships noted in sweet and sour cultivar attributes were observed. Wonderful generally clustered with sweet–sour and sour cultivars. Sweet low acid cultivars occasionally clustered closely with Wonderful which is hard to rationalize. The dominant compounds were 3-hexenol and 1-hexanol which allowed separation of Kara Gul, Hakubotan and Wonderful. Aldehyde and terpene content can be used to characterize cultivars. The study represents the first data on variation in juice qualities in different sweet, sweet–sour and sour cultivars, grown in California, compared with Wonderful. Data may help the juice industry better select raw juice materials in order to ultimately satisfy consumers.

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1. Introduction

Pomegranate (*Punica granatum* L.) is one of the oldest cultivated fruits, as well as richest in history and folklore (Teixeira da Silva et al., 2013). Pomegranates originated in Central Asia (Iran, Turkmenistan and northern India) and were later dispersed to the Mediterranean Basin, East Asia, North Africa, Europe and later, to North and South America (Stover & Mercure, 2007; Teixeira da Silva et al., 2013). Worldwide, there are thousands of accessions and more than 500 pomegranate cultivars with around 50 available commercially (Teixeira da Silva et al., 2013). In wild-type and local pomegranate germplasm, there is a high genetic diversity of morphological and quality/sensorial traits (Mayuoni-Kirshinbaum & Porat, 2014) that are sometimes not correlated with morphological and genetic traits (Melgarejo et al., 2009). Pomegranate has become popular due to current consumer awareness of the health benefits of consuming phytonutrient-rich fruits. Recent studies have shown pomegranate provides a rich and unique source of bioactive phytochemicals and consumption helps protect the brain, cardiovascular and central nervous system, reduce type 2 diabetes; generally based upon demonstrated anti-inflammatory, anti-carcinogenic and anti-mutagenic characteristics [see references within Del Rio et al., 2013]. Health-benefit properties and marketing campaigns have led to a surge in production and consumption of pomegranate products during the last two decades. The juice/beverage and functional food industry has rapidly advanced several pomegranate containing products driven by this consumer awareness of "superfruits." POM Wonderful, the company who started the pomegranate juice niche in the U.S., increased sales from $12 million in 2003 to $91 million in 2006 (Cline, 2008). Production of pomegranates in California in 2011

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was estimated to be 30,000 acres (Geisler, 2012). The European company Pomegreat and POM Wonderful have led to a increase in world trading and consumption of pomegranate (Rymon, 2011).

Some consumers find bitterness and astringency acceptable. However, a sizable portion of the population avoids bitter foods and will not be repeat purchasers of juices and functional foods with these sensory attributes. Consumer dissatisfaction of flavor seems especially true in several shelf-stable and/or new to the market flash pasteurized juices and smoothies. Such juices may contain açaí, aronia, blackberry, black cherry, black currant, wild blueberry, cranberry, tart cherry, mangosteen, maqui, and pomegranate [e.g. (Sabbe, Verbeke, Deliza, Matta, & Van Damme, 2009)]. The commonality among these products is their significant high levels of polyphenolic and terpenoid compounds. The association of highly colored superfruits and "off-flavors" are likely due to a combination of factors such as inherent bitterness, oxidation or transformations that occur during juice processing.

Although production and consumption of pomegranates have surged recently, there have been limited studies into the complicated organoleptic attributes of Wonderful juices (Carbonell-Barrachina et al., 2012; Hasnaoui, Mars, et al., 2011; Mena et al., 2011). Pomegrante cultivars are thought to have low aromatic intensities (Calin-Sanchez et al., 2011) and volatile changes that occur during juice manufacturing render commercial pomegranate juices to be dissimilar to that of fresh fruits (Melgarejo et al., 2011). Fruit taste is mainly determined by the balance between organic acids and sugar content and sensory assessment has been used to characterize and classify pomegranate genotypes (Hasnaoui, Jbir, et al., 2011; Melgarejo, Salazar, & Artés, 2000; Poyrazoglu, Gokmen, & Artik, 2002), even though environmental conditions significantly affect fruit and juice quality (Schwartz, Tzulker, et al., 2009). Consumer acceptance/preference is also complicated by polymorphic genetic diversity in the crop. Nonetheless, the long-term full potential use of Wonderful pomegranates and their expanding markets may not be sustained if flavor changes and/or possible off-flavors associated with processing, bitterness and astringency are not studied, understood, and ultimately resolved.

Subsequently, a plausible research and development effort for currently produced superfruits and 100% juices might be to improve quality attributes important to the consumer, while maintaining high phytonutrient status. We have not found a survey looking at the opposing or extreme differences in sweetness, sourness, astringency and even color types in pomegranate juices, including the U.S. dominant cultivar, Wonderful. Therefore, with the aid of the USDA, ARS National Clonal Germplasm Repository, we assessed differences in a small collection of California-grown pomegranate cultivars. The aim of this work was to evaluate the quality attributes and aroma profiles and view major chemical differences in selected cultivars of pomegranates from around the world that were grown in California, USA which, are considered "high" to "low" in organoleptic (sugar, acidity, sweetness, color, flavor etc.) characteristics.

2. Materials and methods

2.1. Plant material

Freshly harvested pomegranate (*P. granatum* L.) fruit were obtained from the USDA, ARS National Clonal Germplasm Repository (Davis, CA) in 2010 and 2011. Trained horticulturalists used historical dates, sensory information (multiple fruit, color, taste, texture of the aril), size and external color as harvest criteria. Accessions were grown at the Wolfskill Experimental Orchard in Winters, CA. Sin Pepe and Wonderful (DPun 81, control) trees were planted in the 1960's in a single row at 3 m spacing between trees. Haku-botan trees were planted around 1992; Fleischman in 1998; Salavatski and Nikitski ranni in 1999; and the other cultivars, around 2001 at 4×4 m spacing. Trees were irrigated weekly during the growing season with microjet sprinklers. Accessions (cultivars) were classified subjectively and empirically by the germplasm curators, pomegranate tasting days at the Wofskill farm, and per information available on the germplasm database (http://www.ars-grin.gov/npgs/acc/acc_queries.html; Table 1) and/or UC Davis Fruit and Nut Research Information pages (http://fruitandnuteducation.ucdavis.edu/education/fruitnutproduction/Pomegranate/Pomegranate_Cultivar_Table/). Mid-season (early November), optimum quality commercial-like ripe fruit were washed, dried, chilled overnight and individually wrapped and packaged into boxes that were over-night shipped to the Southern Regional Research Center, where photographic representations were captured, as illustrated in Supplementary Fig. S1. Ripe commercial Wonderful fruit (PW-1) was harvested, sorted, graded, waxed, and shipped immediately, or stored under proprietary controlled atmospheres (CA) at 8 °C by Paramount Farms in the San Joaquin Valley of CA. All fruit were repacked into, or already stored in commercial boxes containing a shelf-life extending polymeric sack (Xtend Fresh Product Packaging Technology, StePac L.A. Ltd. Encinitas, CA). Fruit were used immediately upon receipt or, after short-term (maximum 2 weeks) storage at the SRRC utilizing a flow-through controlled atmosphere (CA) with 10 Kpa CO_2 + 5 Kpa O_2, balance N_2 at 8 °C.

2.2. Pressing and juice production

Unblemished fruit exhibiting no symptoms of chilling injury, sun scald or surface browning were selected and washed in 100 ppm NaOCl followed by ice-chilled (~1 °C) deionized water and air drying. Fruit containing internal browning (brown heart) or damaged arils were discarded, and associated yellowish-whitish flavedo and membranes that were not the characteristic background color for a given cultivar were also removed. Whole fruit (generally 8) were pressed on an X-1 single-layer hydraulic press (Good Nature, X-1, Orchard Park, NY) at 124.1 MPa using a medium-weave polyester mesh press bag (#2636). Arils were also removed from commercial Wonderful fruit using an Arils Removal Tool (ART, Miriam Shoham, Ltd., Israel) and machine pressed (Good Nature) in a medium-weave polyester mesh sack. Free standing commercial Wonderful juice (PW-1) remaining in the ART was decanted and analyzed for anthocyanidins and organic acid content. Arils removed by ART were also floated in deionized water to remove all extraneous flavedo, membranes and juice, and then gently dried in cloth towels followed by pressing and analysis, per below.

2.3. Juice quality appraisals (Brix, color, pH and titratable acidity, TA and sensory)

Brix (total soluble solids) was measured with an Atago Pocket PAL-1 (Tokyo, Japan) refractometer. A few drops from a plastic pipette were placed onto the meter after zeroing with deionized water. Titratable acidity (TA) was measured by a Metrohm 836 Titrando (Riverview, FL) in 10 mL of juice in 50 mL of MiliQ H_2O, and expressed as percentage of citric acid. pH was measured by a Metrohm 836 Titrando in 10 mL juice. Color was measured with a Konica Minolta CR400 Chroma Meter (Ramsey, NJ) and analyzed with SpectraMagic NX lite software. Twenty milliliter juice was placed in a glass 20 mL Petri dish, atop the meter, covered with a black lid. Color was also measured on the peel surface around the equatorial region using 10 fruit per cultivar and 6 readings per fruit. Sensory appraisal was performed by 6 trained in-house panelists, using a one to four scale for none, slight, moderate and high perception, utilizing a published lexicon (Koppel & Chambers, 2010).