Food Chemistry 229 (2017) 553–564

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Not-from-concentrate pilot plant 'Wonderful' cultivar pomegranate juice changes: Volatiles

John C. Beaulieu [a,*], Javier M. Obando-Ulloa [b]

[a] Food Processing & Sensory Quality, United States Department of Agriculture, Agricultural Research Service, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70124, USA

[b] Technology Institute of Costa Rica (ITCR), San Carlos Headquarter, P.O. Box 223-21001, Alajuela, San Carlos, Ciudad Quesada, Costa Rica

ARTICLE INFO

Article history:
Received 20 October 2016
Received in revised form 17 February 2017
Accepted 23 February 2017
Available online 24 February 2017

Chemical compounds studied in this article:
2-Methyl-3-buten-2-ol
(*E*)-3-hexenol
1-Hexanol
1,4-Cineole
α-Terpinene
p-Cymene
limonene
4-Terpineo
α-Terpineol

Keywords:
Aroma
Flavor
Gas chromatography
Mass spectrometry
Pasteurization
Punica granatum
Solid phase microextraction

ABSTRACT

Pilot plant ultrafiltration was used to mimic the dominant U.S. commercial pomegranate juice extraction method (hydraulic pressing whole fruit), to deliver a not-from-concentrate (NFC) juice that was high-temperature short-time pasteurized and stored at 4 and 25 °C. Recovered were 46 compounds, of which 38 were routinely isolated and subjected to analysis of variance to assess these NFC juices. Herein, 18 of the 21 consensus pomegranate compounds were recovered. Ultrafiltration resulted in significant decreases for many compounds. Conversely, pasteurization resulted in compound increases. Highly significant decreases in 12 consensus compounds were observed during storage. Principal component analysis demonstrated clearly which compounds were tightly associated, and how storage samples behaved very similarly, independent of temperature. Based on these data and previous work we reported, this solid-phase microextraction (SPME) method delivered a robust 'Wonderful' volatile profile in NFC juices that is likely superior qualitatively and perhaps quantitatively to typical commercial offerings.

Published by Elsevier Ltd.

1. Introduction

Strong marketing and heightened consumer awareness of the health benefits of consuming phytonutrient-rich fruits have created the demand for and availability of juices and functional beverages. Several pomegranate-containing juices and beverages have appeared rapidly, driven by the consumer's awareness and demand for "superfruits." Superfruits are an easy mechanism by which to deliver proven nutritional benefit and antioxidants into the diet and have become popular in the juice and functional beverage category. Anti-inflammatory, anti-carcinogenic and anti-mutagenic characteristics of pomegranate-enriched diets, that help protect the brain, cardiovascular and central nervous system, and reduce type 2 diabetes, etc., have led to a remarkable surge in production and consumption of pomegranate products (Basu & Penugonda, 2009; Del Rio et al., 2013; Johanningsmeier & Harris, 2011).

The company who started the pomegranate juice niche in the U.S., POM Wonderful, LLC, increased sales from $12 million in 2003 to $91 million in 2006 (Cline, 2008). In 2012, California had an estimated 32,887 acres of pomegranates (USDA, 2016), and in 2011

Abbreviations: GC, gas chromatograph; GNP, Goodnature press; MS, mass spectrometry; PDMS, polydimethylsiloxane; NFC, not from concentrate; PJ, pomegranate juice; RI, retention index; RT, retention time; HTST, high-temperature short-time; SPME, solid phase microextraction.

* Corresponding author.

E-mail addresses: John.Beaulieu@ars.usda.gov (J.C. Beaulieu), jaobando@itcr.ac.cr (J.M. Obando-Ulloa).

http://dx.doi.org/10.1016/j.foodchem.2017.02.114
0308-8146/Published by Elsevier Ltd.

there was an estimated $115 million farm gate value (Pollock, 2011). According to the Beverage Marketing Corporation (BMC, New York, NY), sales of 100% fruit juices doubled from 2003 to 2008 and comprised 5.2% of the fruit beverage market (Anonymous, 2011), and Euromonitor predicts increasing global per capita consumption of fruit and vegetable juices from 10.5 L in 2012 to 12.4 L by 2016 (Rohan, 2013). In much of Europe, the recent trend has been slight decreases in overall 100% juice consumption. However, a health and wellness trend has stoked the 100% not-from-concentrate (NFC) juice market sector to experience marked increases (4.4%) in 2015, which are offsetting the overall category declines (AIJN, 2016). In the United States, NFC juices have an expected annual average growth of 5.3% through 2016 (Rohan, 2013), and, in terms of value, North America leads the way, trending toward higher price per liter as consumers demand more NFC juices (AIJN, 2016).

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 beverages with such sensory attributes (Drewnowski & Gomez-Carneros, 2000; Lesschaeve & Noble, 2005). Running parallel with supply and demand, highly colored, dark-pigmented superfruits and berries offering potential health benefits often have several constituents that can suffer chemical polymerization and/or degradation during processing, which might augment the already perceived bitterness or astringency (Lawless, Threlfall, Meullenet, & Howard, 2013). Consumer dissatisfaction of flavor seems especially true in several shelf-stable and/or new to the market flash-pasteurized juices and smoothies containing açaí, blackberry, black cherry, blackcurrant, wild blueberry, cranberry, tart cherry, mangosteen, maqui, and pomegranate (Lawless et al., 2013; Sabbe, Verbeke, Deliza, Matta, & Van Damme, 2009). Therefore, the long-term increased demand and full potential use of superfruits 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. For example, a product development survey indicated that demand for superfruit beverages may have already lost preference to traditional fruit flavors, and fusion/coupled flavors (Sloan, 2011). Although anecdotally, current world-wide pomegranate market offerings do not seem to support this supposition. A plausible research and development effort for currently produced superfruits and 100% juices could be improving the existing consumer quality attributes, while maintaining high phytonutrient status.

Most published pomegranate juice articles have not addressed flavor/sensory issues revolving around heating and chemical oxidation (e.g., possible Maillard or Strecker reactions). To the best of our knowledge, only one study to date has used stored commercial organic pomegranate juice in blends and this was presumably a pasteurized product (Vazquez-Araujo, Chambers, Adhikari, & Carbonell-Barrachina, 2010). Four articles have reported sensory and volatile information for fresh 'Wonderful' juice, yet the methods mainly involved a potentially oxidative extraction process using a centrifugal or homogenizing blender (Cadwallader, Tamamoto, & Sajuti, 2010; Vazquez-Araujo, Chambers, Adhikari, & Carbonell-Barrachina, 2011; Vazquez-Araujo, Koppel, Chambers, Adhikari, & Carbonell-Barrachina, 2011), or in one case, an exceedingly ill-defined volatile suite was reported (Mphahlele, Fawole, Mokwena, & Opara, 2016). Two additional reports illustrated volatile and sensory attributes in several pomegranate juices in Spanish cultivars but, these juices were blended in domestic blenders and no commercial heating or pasteurization was performed (Calin-Sanchez et al., 2011; Melgarejo et al., 2011). Recently, other reports assessing pomegranate juices wherein sampling protocols did not oxidize samples, have only assessed fresh juices which were from Turkish and Chinese cultivars (Guler & Gul, 2016; Yi et al., 2016). Other studies have evaluated commercial samples, yet these were unfortunately not the 'Wonderful' cultivar (Caleb et al., 2013; Carbonell-Barrachina et al., 2012). Publications also exist describing actual compounds responsible for typical Spanish, Iranian and Turkish pomegranate aroma and flavor from commercial and fresh-squeezed arils (Andreu-Sevilla, Mena, Marti, Garcia Viguera, & Carbonell-Barrachina, 2013; Carbonell-Barrachina et al., 2012; Guler & Gul, 2016). Furthermore, juices produced from concentrates often have significantly reduced volatile and aroma profiles, as demonstrated in 'Wonderful" (Beaulieu & Stein-Chisholm, 2016). In summary, it appears that volatile information regarding pasteurized NFC juices from one of the world's most prominent pomegranate cultivars, 'Wonderful', has not previously been reported.

We have reported recently that pilot-plant-produced juices made from freshly pressed high quality blueberries and pomegranates along with NFC juicing appear to deliver above average volatiles, which are often lacking in most concentrates and commercial juices (Beaulieu & Stein-Chisholm, 2016; Beaulieu et al., 2016). To the best of our knowledge, a unified publication that examines the volatiles in an industry-like NFC pomegranate juice created in a pilot plant has not been reported. Various commercial methods of filtration may be used to reduce cloud and sedimentation to clarify juice and remove polymeric compounds that can affect overall color, turbidity and sedimentation (Alper, Bahceci, & Acar, 2005; Bates, Morris, & Crandall, 2001). After in-depth consultation with juicing professionals, we opted for a streamlined system, similar to a large-scale pomegranate juice facility, where ultrafiltration was used. We mimicked the dominant U.S. commercial-like pomegranate juice extraction (hydraulic whole-pressing), without maceration or concentrating; then followed-up by HTST pasteurizing a truly NFC juice. Subsequently, the objective of this study was to investigate the volatile compositions in ultrafiltered pasteurized 'Wonderful' juices prepared in a pilot plant, and stored as pasteurized product for up to 3 months. This article will highlight the volatiles reported in this NFC pomegranate juice study. To the best of our knowledge, this is the first report of such juice processing and NFC pomegranate volatiles.

2. Materials and methods

2.1. Plant material

Commercially ripe 'Wonderful' pomegranate (*Punica granatum*) fruit was harvested mid-season, run through a packaging line and stored under proprietary controlled atmospheres (CA) at 8 °C by Paramount Farms in the San Joaquin Valley (Del Rey, CA). Fruit were stored in commercial boxes containing a shelf-life extending polymeric sack (Xtend Fresh Product Packaging Technology; StePac L.A. Ltd. Encinitas, CA) that creates a modified atmosphere similar to commercial CA, prior to overnight shipment to the SRRC. At the SRRC, boxes were opened, fruit inspected and/or culled and stored overnight utilizing a flow-through CA with 10 kPa CO_2 + 5 kPa O_2, balance N_2 at 8 °C.

2.2. Hydraulic pressing

Unblemished fruit were handled and pressed as previously reported (Beaulieu & Stein-Chisholm, 2016). Upon cutting, any whole fruit exhibiting brown heart were discarded and ~ 79 kg were pressed on a single-layer hydraulic press (X-1; Goodnature Products Inc., Orchard Park, NY) at 12.4 MPa (1800 psi) using a medium-weave polyester mesh press bag (#2636, Goodnature Products Inc.). Pressing was repeated with an additional ~79 kg

fruit, delivering repeated juices (GNP). No maceration, blending, concentrating, additives, preservatives or flavors were added to these fresh whole-pressed 100% 'Wonderful' juices, which served as controls.

2.3. Juice ultrafiltration

Filtration of freshly pressed, NFC GNP chilled (~5 °C) juice was performed on a BRO/BUF pilot unit (Membrane Specialists, Hamilton, OH) with an in-line membrane filtration module (PCI B-1 Module Series; Aquious PCI Membrane, Hamilton, OH) using a 200,000 molecular weight cut-off XP-201 polyvinylidene fluoride (PVDF) membrane (ITT PCI Membrane Systems, Zelienople, PA), as previously described (Beaulieu & Stein-Chisholm, 2016; Beaulieu et al., 2016). This membrane was chosen for pomegranate juice based upon consultation with a large commercial fruit juice company. The PVDF configuration accommodated a 24 30 L min^{-1} flow rate in a 25-mm tube with a water flux of 400 L m^2 h^{1}, and there was insignificant membrane fouling (data not shown) while clarifying whole-pressed pomegranate juice. Filtration with a total filter area of 0.864 m^2 was fed by a 5.59 kW pump, run with cool chilled water around the shroud at ~15 °C with an average inlet and outlet pressure range of 889 972 kPa (129 139 psi) and 186 193 kPa (27 28 psi), respectively. Each UF juice batch started with ~30 L juice and while evacuating the water volume out of the membrane and hosing, about 6 7 L of juice went to drain. Then, juice was recirculated 20–23 min from the 100 L hopper through the system until filtered permeate exiting the sampling port was comparable to the original color and brix. The system was considered equilibrated (original Brix was ~17.8 18.1 and permeate attained ~16.9 17.2 °Brix) only after running an additional ~ 8.5 L to drain, with an average outlet juice flow rate of ~57 L h^{1}. For the subsequent UF run, the hopper was emptied, juice drained, new juice added, and an additional 6 L was likewise run to drain to clear one batch and begin equilibrating the next batch. The ultrafiltered (UF) pomegranate juice was collected for crude juice analysis, pasteurization and subsequent storage. Due to rigid fruit culling procedures, a designed complete third repeated experiment was not possible. Therefore, the residual ultrafiltration (UF) hopper retentate from the 2 repeated experiments was combined and refiltered to deliver a third experimental juice used for pasteurization. Roughly 8 L were collected in the first and third UF runs while ~20 L were collected in the second UF run.

2.4. Juice pasteurization and storage

Three batches of UF juice were pasteurized on a MicroThermics Electra UHT/HTST (high-temperature short-time) Lab-25EDH (Raleigh, NC) at 90 °C for 10 s at 1.2 L m^{1}. Juice continued through a room-water recirculating cool chill prior to chilled-filling in a sanitized MicroThermics clean fill hood at ~20 °C into presterilized 250-mL media bottles (Corning, Tewksbury MA), cap twisting, inversion of the bottles for ~10 s and ice water bath chilling (~0 °C) for ~10 min (PJ0). Post pasteurization, 250-mL bottles (9 experimental units per treatment/time, representing the 3 UF batches, with 3 true replicates each) were held in the dark and stored for 1, 2 and 3 months refrigerated at 4 °C (4C-1, 4C-2 and 4C-3) and under "ambient" shelf conditions at ~25 °C (25C-1, 25C-2 and 25C-3). Cool-filling pasteurized juice into bottles is often considered to be inferior to hot-filling, and this was established as one replicate from both 25C-1 and 25C-2, and all 25C-3 samples molded, and those data were therefore not collected. Furthermore, three other samples data were not collected due to lost or corrupted runs/samples in one UF rep, and two PJ0 reps.

2.5. GC–MS volatile preparation and data analysis

Volatile samples were prepared in triplicate from each repeat and experimental unit, representing either $n = 18$ (GNP and UF), $n = 27$ (PJ0) or $n = 9$ (4C-1, 4C-2, 4C-3, 25C-1 and 25C-2) replicates per delivered average. Volatiles were assayed with 1-cm 50/30 μm Stableflex divinylbenzene/Carboxen/polydimethylsiloxane (DVB/Carboxen/PDMS; Supelco, Bellefonte, PA) solid phase microextraction (SPME). Sample vials (20-mL) with 10-mL juice, 2.2 g NaCl and internal standard (IS) 2-methylbutyl isovalerate (CAS# 2445-77-4) at 100 μg L^{-1} were equilibrated for 10 min by oscillation in a 35 °C MPS2 XL autosampler (Gerstel, Inc., Baltimore, MD), then headspace was exposed to SPME for 15 min at 35 °C. Vials were continuously swirled (100 rpm) during SPME adsorption and fibers were injected into an HP6890/5973 GC–MS (Agilent Technologies, Santa Clara, CA) with an HP-5 crosslinked 5% phenyl methyl silicone column, 30 m × 0.25 mm × 0.25 μm film thickness (Agilent Technologies), as previously reported (Beaulieu & Stein-Chisholm, 2016). Data were collected with HP ChemStation (Agilent Technologies) software (A.03.00) and also searched against the 7th edition Wiley7th/NIST02 registry of mass spectral data. Compounds were preliminarily identified by library search, then their identity was confirmed by standard comparisons, GC retention time (RT), MS ion spectra and an in-house retention index, RI (Table 1). High purity GC–MS grade standards were obtained from: Aldrich (Madison, WI), Aaper Alcohol and Chemicals (Shelbyville, KY), Bedoukian (Danbury, CT), Berje (Carteret, NJ), Fluka (Madison, WI), Fisher (Pittsburgh, PA), JT Baker (Phillipsburg, PA), Mallinckrodt (St. Louis, MO), S = Sigma (Sigma-Aldrich) Inc. (St. Louis, MO) and SA = Sigma-Aldrich Fine Chemicals (SAFC), Flavors and Fragrances Products (St. Louis, MO).

Compounds presented are expressed as the relative semi-quantified composition based on an integrated unique quantifying target ion response, divided by the integrated unique quantifying target ion response of the internal standard, multiplied by 100, per individual sample, averaged. Volatiles were also displayed as compound classes (Table 1) and comprised aldehydes (ALD), aromatics (ARO), alcohols (OLS), esters (EST), ketones (ONE), terpenes (ENE), terpenoids (OID) and total volatiles (VOL).

2.6. Statistics

The experimental design included two factors: pasteurized juice storage time (1 or 2 months) and two temperatures (4 and 25 °C). Initially, a 2-way ANOVA was utilized, whereby all data were split into two experiments as: 1) GNP, UF and PJ0 and 2) PJ0, 4C-1, 4C-2, 4C-3 (later removed), 25C-1 and 25C-2. If significant differences at $p < 0.05$ were identified, data were analyzed as a 2-factor linear model with temperature (T) and storage time (t) as the factors. A Dunnett's test at $p < 0.05$ was used to determine the differences between treatments and pasteurized ultrafiltered juice on day 0 (control, PJ0). To evaluate the effect of ultrafiltration and pasteurization, the control (PJ0) was also compared against crude pressed juice (GNP) and ultrafiltered juice (UF). The significant differences after ANOVA were established by a Tukey test at $p \leq 0.05$. This analysis did not illustrate marked differences between means for the treatment that separated out the two-way analysis (PJ0; Supplementary Tables 1 and 2) and therefore, a combined (1-way single factor design) analysis of the data was implemented that would allow visualization and graphing across the whole experiment. Raw data were subjected to a random effects ordinal regression analysis. In addition, a t-test at $p < 0.05$ was used to compare the aroma composition of pomegranate juice along storage (time and temperature) against the controls [crude press (GNP), ultra filtration (UF) and pasteurization (PJ)] on the aroma composition of pomegranate juice along storage (time and temperature). All anal-

Table 1
Volatile and semi-volatile compounds recovered with head space DVB-Carboxen-PDMS SPME in whole-pressed and pasteurized not-from-concentrate (NFC) 'Wonderful' pomegranate juice.

Compound[a]	CAS[b]	Class	RT	RI	ID	Std[c]	Attributes[d]
Ethanol	64-17-5	OLS	0.47	457	S	AA	Sweet[1], medical[4], ethereal[4], alcoholic[4]
2-Methyl-3-buten-2-ol	115-18-4	OLS	0.66	606	S	F	Herb[1,3,4],oily[3,4], earthy[3,4]
Ethyl acetate	141-78-6	EST	0.67	609	S	M	Pineapple[1,3,4], anise[3], ethereal[3,4], brandy[4], fruity[4], weedy[4], green[4], sweet[4], grape[4], cherry[4]
Hexanal	66-25-1	ALD	3.55	798	S	A	Fat[1,3,4], tallow[1], grass[1,3,4], fruity[3,4], apple[4], sweaty[4], woody[4]
Ethyl butyrate	105-54-4	EST	3.62	800	S	F	Apple[1,4], banana[3,4], pineapple[3,4], sweet[3,4], ethereal[3], fruity[4], cognac[4], buttery[4], rum[4], strawberry[4]
(E)-2-Hexenal	6728-26-3	ALD	5.39	851	S	A	Fat[1,4], green[1,2,4], citrus[2], orange[2], pungent[2], cheese[4]
(*Z*)-3-Hexen-1-ol[e]	928-96-1	OLS	5.39	851	S[f]	A	Grass[1]
(E)-3-Hexenol[f]	928-97-2	OLS	5.51	855	MS, T	–	Moss[1], fresh[1], green[2,4], bitter[2,4], earthy[2,4], fatty[2,4]
1-Hexanol	111-27-3	OLS	5.96	867	S	F	Resin[1], flower[1], green[1,4], woody[4], sweet[4], fruity[4], ethereal[4]
α-Pinene	80-56-8	ENE	7.98	927	S	A	Turpentine[1], pine[1,4], warm[4], resin[4], woody[4], camphor[4], sweet[4], earthy[4], spicy[4]
Benzaldehyde	100-52-7	ALD ARO	9.04	958	S	JT	Burnt sugar[1,4], almond[1,3,4], cherry[3,4], sweet[3,4], bitter[4], spicy[4], nutty[4]
β-Pinene	18172-67-3	ENE	9.43	970	S	A	Turpentine[1,4], resin[1,4], pine[1,4], woody[3,4], dry[4], hay[4], fresh[4]
6-Methyl-5-hepten-2-one	110-93-0	ONE	9.81	981	S	A	Oily[1,3], green[1,3,4], herbaceous[1], citrus[4], pear[4], fruity[4], lemongrass[4], apple[4], cheesy[4], banana[4], green bean[4]
β-Myrcene	123-35-3	ENE	9.91	984	S	S	Spicy[1,4], must[1], balsamic[1,4], anise[3], grape[3], fruity[3], peach[3], sweet[3], vanilla[3], green[3,4], wine[3], woody[3,4], metallic[4], plastic[4], rosy celery[4], carrot[4], citrus[4], mango[4], minty[4]
Ethyl hexanoate	123-66-0	EST	10.20	993	S	A	Apple[4] peel[1,3], fruit[1,4], banana[3,4], wine[3,4], pineapple[4], green[4], sweet[4], brandy[4], strawberry[4], waxy[4]
(*Z*)-3-Hexenyl acetate	3681-71-8	EST	10.44	1000	S	B	Green[1,2, 3,4], banana[1,3], sharp fruity[4], sweet[2], green banana[2,4]
1,4-Cineole (isoeucalyptol)	470-67-7	OID OLS	10.67	1008	S	F	Spice[1], herbal[4], cooling[4], pine[4], minty[4], camphor[4], terpene[4], green[4]
α-Terpinene	99-86-5	ENE	10.70	1009	S	A	Lemon[1,3,4], berry[3], sweet[3], woody[3,4], pepper[3], medicinal[3,4], camphoraceous[3,4], terpenic[4], citrus[4], sharp[4], thymol[4], spicy[4], lime[4], mint[4]
p-Cymene	99-87-6	ENE	10.96	1017	S	A	Citrus[1,3,4], solvent[1], gasoline[1,4], kerosene[4], fresh[4], woody[4], spice[4], cumin[4], oregano[4], cilantro[4], green pepper[4], rancid[4]
Limonene	5989-27-5	ENE	11.09	1021	S	Fi	Citrus[1,4], orange[1,4], lemon[1,3], sweet[3,4]
2-Ethyl-1-hexanol	104-76-7	OLS	11.11	1021	S	F	Oily[3,4], rose[3], sweet[3,4], citrus[4], fresh[4], floral[4]
1,8-Cineole (eucalyptol)	470-82-6	OID OLS	11.25	1026	S	A	Sweet[1], mint[1], herbal[4], eucalyptus[4], camphor[4]
γ-Terpinene	99-85-4	ENE	12.03	1050	S	A	Turpentine[1], gasoline[1] green[3], citrus[3]
1-Octanol	111-87-5	OLS	12.42	1063	MS	A	Orange[3,4], floral[3], rose[4], sharp[4], fatty[3,4], citrus[3,4], coconut[4], green[4], fruity[4], sweet[4]
α-Terpinolene	586-62-9	ENE	12.96	1080	S	F	Plastic[3], fresh[4], lime-pine top note[4], sweet-piney[4], oily[4], woody[4], citrus[4]
2-Nonanone	821-55-6	ONE	13.07	1083	S	A	Fresh[2], sweet[2], weedy[2], earthy[2], herbal[2,3,4], cheese[3,4], coconut[3], oily[3], fatty[3,4], floral[3,4], fruity[3,4], fishy[3], waxy[3], soapy[3], rose[4], tea[4], lavender[4], violet[4], creamy[4]
Methyl benzoate	93-58-3	EST	13.16	1086	S	F	Prune[1], lettuce[1], herb[1], sweet[1,4], fruity[3,4], fragrant[4], aromatic[4], floral[4], heavy sweet[4], berry[4], cherry[4], phenolic[4], ylang ylang (cananga)[4], tuberose[4], medicinal[4], balsamic[4], wintergreen[4], almond[4], cherry pit[4], camphoraceous[4], chemical[4],
Linalool	78-70-6	OID	13.29	1090	S	A	Flower[1,3,4], lavender[1,4], lemon[3], orange[3,4], citrus[3,4], sweet[3,4], woody[4], bergamot[4], rosewood[4], petitgrain[4], bois de rose[4], fruity[4], green[4], blueberry[4], rose[4]
Nonanal	124-19-6	ALD	13.42	1094	S	A	Citrus[1,3,4], fat[1,3,4], green[1,4], apple[3], coconut[3,4], grape[3], grapefruit[3], lemon[3,4], lime[3], melon[3,4], oily[3,4], orange[3,4], waxy[3,4], nutty[3,4], peach[3], rose[3,4], meaty[3], fishy[3], floral[4], orange peel[4], cucumber[4], raw potato[4]
Phenylethyl alcohol	60-12-8	OLS ARO	13.67	1102	S	B	Honey[1,3], spice[1], rose[1,3,4], lilac[1, green[2], herbaceous[2], woody[2], floral[3,4], bready[4], fresh[4]
Isoborneol	124-76-5	OID OLS	15.05	1151	MS, T	S	Camphor[1], must[1], balsamic[4], camphoreous[4], herbal[4], woody[4]
Borneol [f]	507-70-0	OID OLS	15.29	1160	S	S	Camphoreous[1,4], must[1], balsamic[4], pine[4], woody[4]
Ethyl benzoate	93-89-0	EST	15.42	1164	S	A	Camomile[1], flower/floral[1,3], celery[1], fruity[1,3,4], cherry[3,4], grape[3,4], anise[3], balsam[3], cranberry[3], plum[3], raspberry[3], strawberry[3], vanilla[3], spicy[3], minty[3], wine-like[3], banana[3], berry[3], dry[4], musty[4], sweet[4], medicinal[4], wintergreen[4]
4-Terpineol	562-74-3	OID	15.61	1171	S	SA	Turpentine[1], nutmeg[1], sweet[4], herbaceous[3,4], fresh[4], chocolate[3], grapefruit[3], lemon[3], lime[3], fruity[3], minty[3], woody[3,4], peppery[4], earthy[4], musty[1,4], spicy[4], clove[4]
α-Terpineol	10482-56-1	OID	15.94	1183	S	A	Oil[1], anise[1], mint[1], lilac[3,4], floral[4]

Table 1 (*continued*)

Compound[a]	CAS[b]	Class	RT	RI	ID	Std[c]	Attributes[d]
Decanal	112-31-2	ALD	16.29	1195	S, T	S	Orange/peel[1,4], soap[1], tallow[1], floral[3,4], citrus[3,4], sweet[3,4], fatty[4], waxy[4], green melon[4]
1-Decanol	112-30-1	OLS	17.72	1262	S, T	SA	Waxy[3,4], fruity[3], fatty[3,4], rose[3,4], oily[4], floral[4], orange[4], sweet[4], clean[4], watery[4], tart[4]
Phenethyl acetate (2-Phenylethyl acetate)	103-45-7	EST ARO	17.58	1256	S	S	Rose[1,3], honey[1,3], tobacco[1], fruity[3], sweet[3]
Eugenol	97-53-0	OLS	19.47	1358	S	S	Clove[1,3,4], honey[1], spicy[3,4], clove leaf[4], cinnamon[3], woody[4], phenolic[4], savory[4], ham[4], bacon[4], allspice[4]
(*E*)-β-Damascenone	23726-93-4	ONE ARO	19.81	1378	S	S, Be	Apple[1], rose[1], honey[1]
α-Cedrene [f]	68608-32-3	ENE	20.26	1405	S, T	Be	Woody[4], cedar[4], sweet[4], fresh[4],
β-Caryophyllene	87-44-5	ENE	20.38	1418	S	F	Woody[1,3,4], spice[1,3,4], clove[4], oily[4], citrus[4], pepper[4], camphoraceous[4], sweet[4], dry[4]
β-Cedrene	68608-32-3	ENE	20.42	1421	S, T	Be	–
Bergamotene	17699-05-7	ENE	20.54	1432	MS	–	Wood[1], warm[1], tea[1]
β-Bisabolene	495-61-4	ENE	21.39	1509	MS	–	Herbaceous[1], balsamic[1,4]
Italicene	94535-52-1	ENE	22.42	1643	MS	–	–

[a] Compounds indicated by bold and italics are considered consensus, as enumerated and expounded upon in the literature (Beaulieu & Stein-Chisholm, 2016; Mayuoni-Kirshinbaum & Porat, 2014).

[b] CAS = Chemical Abstract Service number. Class = Compound classes or groupings as further illustrated in Figures. RT = Retention Time on an HP-5 column (Agilent Technologies), calculated via triplicated averaged alkanes and in-house standards per (Beaulieu & Stein-Chisholm, 2016). RI = Retention Index which was comparable with NIST (http://webbook.nist.gov/chemistry/name-ser.html). Note that some RI's at the front end of the GC run are not highly congruent with the literature (some values high) because occasionally alkanes were run without column cryofocusing whereas all SPME samples were run with cryofocusing. ID = Identification used as confirmation of compounds per: MS = Library match; S = Standards; T = Tentative.

[c] Std, standard source, per Materials and Methods, from: A = Aldrich (Madison, WI), AA = Aaper Alcohol and Chemicals (Shelbyville, KY), B = Bedoukian (Danbury, CT), Be = Berje (Carteret, NJ), F = Fluka (Madison, WI), Fi = Fisher (Pittsburgh, PA), JT = JT Baker (Phillipsburg, PA), M = Mallinckrodt (St. Louis, MO), S = Sigma (Sigma-Aldrich) Inc. (St. Louis, MO) and SA = Sigma-Aldrich Fine Chemicals (SAFC), Flavors and Fragrances Products (St. Louis, MO). A = Aldrich; AA = Aaper Alcohol and Chemicals; AR = Avocado Research Chemicals; B = Bedoukian; Be = Berje; F = Fluka; Fi = Fisher; JT = JT Baker; M = Mallinckrodt; and S and SA = Sigma Aldrich SAFC, Flavors and Fragrances.

[d] Commercial flavor descriptors, or online according to: [1]Flavornet (http://www.flavornet.org/flavornet.html); [2]Bedoukian Research (http://www.bedoukian.com/); [3]Sigma Aldrich SAFC, Flavors and Fragrances (http://www.safcglobal.com/safc-supply-solutions/en-us/home/flavors-and-fragrances.html) and [4]The Good Scents Company (http://www.thegoodscentscompany.com/). All www url accessed 8-18-2016.

[e] (*E*)-3-Hexen-1-ol and (*Z*)-3-hexen-1-ol co-eluted due to high abundance and have been combined and reported as (*E*)-3-hexen-1-ol based upon the most confidently recovered and integrated 67 *m*/*z* ion.

[f] Impurity (*E*)-3-hexen-1-ol isomer found in authentic standard of (*Z*)-3-hexen-1-ol, CAS 928-96-1. Similarly, both α- and β-cedrene were recovered in the authentic standard (CAS 68608-32-3). Likewise, both borneol and isoborneol were recovered from the authentic standard (CAS 507-70-0).

ysis was performed on JMP Pro (Version 11 for Windows; SAS Institute, Cary, NC). Data were subjected to principal components analysis (PCA) to identify traits that best explained the variability among treatments and to group them according to their volatile profiles in JMP Pro. The PCA graphs were split into two sections (scoring plot above the loading plots) to facilitate visualization of the analysis. The scoring plot was performed with the mean component value obtained for each PC, while the loading plot was achieved with the eigenvectors greater than 0.20 for all parameters evaluated, to define the orientation of each PC axis.

3. Results and discussion

The pilot plant UF, with an in-line membrane filtration module consisting of a series of 18 filter elements of 18 mm internal diameter and 1.2 m in length, delivered approximately ½ the linear distance (21.6 m) often encountered in a commercial juice filtration operation. UF flow rates herein (56.8 L h ^{1}) were within manufacturer's range, albeit run slightly below the maximum 60 Hz motor speed at 48 49 Hz. Also, flow rates were much faster than previously reported (Beaulieu et al., 2016) on the same equipment with NFC blueberry juices which, had slower flow rates (18.9 29.9 L h ^{1}), likely due to pectin and running the motor at a lower speed. Since this was an NFC juice, we did not alter (dilute) the native initial Brix to achieve the mandated 100% single strength reconstituted pomegranate juice, 16 °Brix [per U.S. Code of Federal Regulation, CFR 101.30, Title 21, Part 101, (FDA., 2014)]. Brix of the initial GNP juice was 17.93 ± 0.05, and that of the pasteurized juice (PJ0) was 17.63 ± 0.31.

3.1. Overall volatile trends and consensus compounds

Previously, we searched 60 compounds and routinely recovered 36 compounds using SPME in freshly pressed 'Wonderful' pomegranate juices (arils-only and whole-pressed) with the same GC–MS methods and similar quantification (Beaulieu & Stein-Chisholm, 2016). Herein, due to anticipated pasteurization and storage effects, we expanded our list of searched compounds to 70. Throughout the juice processing and storage, 46 compounds were recovered and their chromatographic conditions and sensory attributes have been listed in Table 1. However, only 38 compounds were recovered routinely (which were subjected to ANOVA) and some compounds were found sporadically in only one through a few replicates per scattered treatment(s), and were therefore not analyzed by ANOVA. There was an almost saturating abundance of (*E*)-3-hexen-1-ol at very close RT's where both (*Z*)

and (*E*) isomers of 3-hexen-1-ol were recovered. Since these two compounds could not be resolved, only (*E*)-3-hexen-1-ol was reported based on the predominate ion (*m*/*z* 67). The identification for some compounds remains tentative because either a standard contained the "impurity" isomer, or a second confirmation (method) was not realized. Compounds falling into that category were: (*E*)-3-hexen-1-ol, isoborneol, decanal, 1-decanol, α-cedrene, and β-cedrene (Table 1).

Viewing compound classes, the following were routinely recovered, although not all were subjected to ANOVA, since occasionally there were insufficient replicates representing a given treatment. There were 5 ALD [hexanal, (*E*)-2-hexenal, benzaldehyde, nonanal, decanal], 8 OLS [ethanol, 2-methyl-3-buten-2-ol, (*Z*)-3-hexen-1-ol, (*E*)-3-hexen-1-ol, 1-hexanol, 2-ethyl-1-hexanol, phenethyl alcohol and 1-octanol], 5 EST [ethyl acetate, ethyl butyrate, ethyl hexanoate, (*Z*)-3-hexenyl acetate and ethyl benzoate], 13 ENE [α-pinene, β-pinene, β-myrcene, α-terpinene, p-cymene, limonene, γ-terpinene, α-terpinolene, α-cedrene, β-cedrene, β-bergamotene, β-caryophyllene and β-bisabolene], 5 OID [isoeucalyptol (1,4-cineole), eucalyptol (1,8-cineole), linalool, 4-terpineol and α-terpineol], 2 ARO [benzaldehyde and phenethyl alcohol] in a total of 38 volatile (VOL) compounds.

When comparing our results to the literature, we will purposely focus comparisons to articles where optimum quality 'Wonderful' fruit was used [e.g., no "second quality and over-ripe fruit" (Andreu-Sevilla et al., 2013)] and generally avoiding other cultivars (Yi et al., 2016) and mixed juices [e.g. (Andreu-Sevilla et al., 2013; Nuncio-Jauregui, Calin-Sanchez, Hernandez, & Carbonell-Barrachina, 2014)], where both juice production and volatile extractions were not abusive (e.g., no blenders, whirling and spinning centrifugal blending/pulping, concentrating, and where we believe uncertain MS methods were employed (per introductory literature citations). Based on these rigorous criteria, this implies we will attempt to focus comparison and discussion revolving around "endogenous volatiles" reported within the literature [e.g. (Beaulieu & Stein-Chisholm, 2016; Beaulieu et al., 2015; Caleb et al., 2013; Carbonell-Barrachina et al., 2012; Mayuoni-Kirshinbaum, Daus, & Porat, 2013; Mayuoni-Kirshinbaum & Porat, 2014; Mayuoni-Kirshinbaum, Tietel, Porat, & Ulrich, 2012)]. Understandably, not all of these selected works utilized solely 'Wonderful' pomegranates. We are not excluding the fact that several of the consensus compounds have also been reported by the authors, which we are purposely neglecting; for example, the consensus listing originated in (Mayuoni-Kirshinbaum & Porat, 2014) via summing (Calin-Sanchez et al., 2011; Mayuoni-Kirshinbaum et al., 2012, 2013; Melgarejo et al., 2011; Vazquez-Araujo, Chambers, Adhikari, & Carbonell-Barrachina, 2011; Vazquez-Araujo et al., 2010; Vazquez-Araujo, Koppel et al., 2011). However, several other compounds which are generally not recorded across the board in pomegranate are likewise reported by some of these authors. Due to the various differences between cultivar, horticultural aspects of growth conditions and harvest, juicing and processing regimes (e.g. arils only versus whole-pressed, juicing machines etc.), and various volatile extraction and identification methodologies, it is nearly impossible to make ubiquitous statements regarding what are the absolute critically important flavor and aroma compounds in a commodity, or specific fruit like pomegranate, and especially a single cultivar.

There are 21 consensus compounds considered to convey aroma/flavor impact importance in pomegranates (Beaulieu & Stein-Chisholm, 2016; Mayuoni-Kirshinbaum & Porat, 2014). These include 1-hexanol, (*Z*)-3-hexen-1-ol, 2-ethyl-1-hexanol, hexanal, (*E*)-2-hexenal, heptanal, octanal, nonanal, (*Z*)-3-hexenal, 6-methyl-5-hepten-2-one, α-pinene, β-pinene, α-terpinene, p-cymene, limonene, γ-terpinene, 4-terpineol, α-terpineol, α-bergamotene, β-caryophyllene and β-bisabolene. Herein, 18 of the 21 consensus compounds were recovered (Tables 1 and 2, italicized font). Indeed, the consensus compounds 1-hexanol, (*E*)-3-hexen-1-ol, α-terpineol, limonene and *p*-cymene were the highest recovered semi-quantitatively (Table 2). Only two compounds dominated the overall volatile profiles throughout the course of all juice processing and storage, 1-hexanol and (*E*)-3-hexen-1-ol (Fig. 1). Both these consensus compounds have been reported in other reports illustrating pasteurized pomegranate products (Beaulieu & Stein-Chisholm, 2016; Carbonell-Barrachina et al., 2012; Nuncio-Jauregui et al., 2014), yet other reports only indicate one or the other compound (Vazquez-Araujo et al., 2010; Vazquez-Araujo, Koppel et al., 2011).

3.2. Compounds that displayed inconsistent trends

There were several compounds that displayed inconsistent trends. For example, three compounds were confidently recovered and integrated from every fresh whole-pressed 'Wonderful' GNP replicate (Table 2). These compounds (expressed parenthetically by the integrated semi-quantified composition, as per *Materials and Methods*) were β-pinene (13.21), bergamotene (9.36) and β-bisabolene (0.93). Also, (*Z*)-3-hexenyl acetate (4.30) and β-caryophyllene (0.17) were recovered in roughly half the GNP samples. However, these five compounds were not found through any further processing stages. In the GNP juices, based on the total number of compounds integrated (32), these comprised relatively 13.2, 9.4, 0.3, 2.1 and 0.2%, respectively, and only β-pinene and bergamotene were somewhat high (8th and 12th, respectively) in the relative proportion ranking (Table 3). Aside from (*Z*)-3-hexenyl acetate, the other four compounds are reported as consensus compounds (Mayuoni-Kirshinbaum & Porat, 2014). All were previously isolated in several fresh extractions in several 'Wonderful' juices. However, with the exception of (*Z*)-3-hexenyl acetate, the other four compounds were not found in several commercial juices, nor an in-house concentrate, essence and reconstituted in-house juice (from our concentrate made with UF pasteurized juice concentrated mildly with a Rotavapor) prepared from this same 'Wonderful' NFC process (Beaulieu & Stein-Chisholm, 2016). These compounds were apparently destroyed or lost due to UF and never appeared due to pasteurization or throughout both temperatures during storage. Commercial juices contained β-pinene but it was a mixture of 'Mollar de Elche' and 'Wonderful (Nuncio-Jauregui et al., 2014) or a Spanish germplasm (C25) or 'Mollar de Elche' (Carbonell-Barrachina et al., 2012). Aside from one report emanating from an undesignated commercial pomegranate juice (Vazquez-Araujo et al., 2010), only 3-hexenyl acetate has been reported in other commercial samples. The other compounds were not recovered by these aforementioned authors. Commercial concentrates and an in-house concentrate have far fewer consensus compounds compared against a native 'Wonderful' fruit, and the essence (captured condensate while making the concentrate) and reconstituted 100% single strength juice (per CFR) also experience a tailing off and substantial reduction in their volatile loads (Beaulieu & Stein-Chisholm, 2016).

Three compounds were not recovered in GNP juices but appeared after UF. Decanal, (*E*)-β-damascenone and β-cedrene (Table 2, Supplementary Table 1) were very low regarding their relative recovery ranking (Table 3). However decanal disappeared after pasteurization (PJ0). Decanal has been reported previously in freshly-pressed arils (Mayuoni-Kirshinbaum et al., 2013) and commercial juices (Beaulieu & Stein-Chisholm, 2016). (*E*)-β-Damascenone was present throughout storage, yet it did not present significant trends (Table 2). UF juices contained the most significant amount of (*E*)-β-damascenone (0.76), which displayed no clear trend through storage at either temperature. (*E*)-β-Damascenone has not been isolated in the other reports we

Table 2
ANOVA results for semi-quantified volatile compounds (integrated ion response of selected qualifying ion divided by the internal standard qualifying ion, ×100 × 100, per replicate) through juice processing and storage stages in not-from-concentrate pasteurized 'Wonderful' pomegranate juice. Significant differences ($\alpha = 0.05$) exist across treatments with different letter designations per compound.

	GNP[a]	UF	PJ0	4C-1	4C-2	4C-3	25C-1	25C-2
Ethanol	13.0b	9.81b	41.9a	9.36b	11.1b	18.9ab	17.4ab	21.8ab
2-Methyl-3-buten-2-ol	12.8a	9.17ab	32.4a	5.40bc	2.80c	11.6a	14.0ab	7.90ab
Ethyl acetate	2.06a	0.76ab	1.77ab	0.87abc	0.31c	1.80bc	0.59bc	0.27abc
Ethyl butyrate	5.02a	2.49c	3.54b	0.27d	–[b]	0.32d		
Hexanal[c]	1.67a	1.01a	1.88a	0.23a	0.22a	0.38a	0.28a	
(E)-2-Hexenal	4.20a	2.09a	3.44a					
(E)-3-Hexen-1-ol	308ab	218b	377a	81.8cd	66.4cd	120c	66.0cd	43.6d
1-Hexanol	425b	313c	559a	127de	98.1de	171d	84.5de	31.4e
α-Pinene	1.48a	1.16a	0.98a					
Benzaldehyde						0.61b	1.23ab	1.72a
β-Pinene	13.21[d]							
6-Methyl-5-hepten-2-one	0.29ab	0.18abc	0.31a		0.10abc	0.08c		0.14bc
β-Myrcene	7.70a	2.24a	5.95a					
Ethyl hexanoate	23.9a	10.3b	15.6ab					
(Z)-3-Hexenyl acetate	4.30							
1,4-Cineole	1.29a	0.48c	0.81b	0.20d	0.14d	0.31cd	0.28cd	0.19cd
α-Terpinene	4.57b	4.72b	10.3a	1.00c	1.64c	1.31c	0.50c	0.53c
p-Cymene	25.9c	54.8b	109a	6.46d	8.00d	7.21d	2.45d	3.14d
2-Ethyl-1-hexanol				2.33c	1.94c	4.66a	3.50b	3.81b
Limonene	30.5b	195a	177a	0.91b	2.57b	0.44b	0.78b	1.42b
Eucalyptol	0.52a			0.10c	0.09c	0.22b	0.05c	
γ-Terpinene	7.51b	26.3b	69.2a	1.32b	1.83b	2.09b	0.93b	1.03b
1-Octanol	0.42ab	0.27bc	0.46a	0.09c	0.18c	0.26bc	0.31abc	0.24abc
α-Terpinolene	3.19b	4.22b	7.39a	0.45c	0.76c	0.69c	0.49c	0.43c
2-Nonanone	11.7a	3.60c	6.61b	0.77d	1.01d	1.30cd	0.62d	0.76cd
Linalool	3.89a	2.46a	5.02a	1.45a	1.23a	2.62a	1.80a	1.40a
Nonanal	1.06ab	0.82ab	2.27a	0.21b	0.35b	0.21b		
Phenylethyl alcohol							14.5a	12.4a
Ethyl benzoate	20.6a	5.89bc	9.81b	0.33c	0.72c	0.36c	1.79c	
4-Terpineol	7.28b	5.07c	9.60a	2.48de	2.21de	3.95cd	1.89e	1.23e
α-Terpineol	56.7a	33.7b	60.6a	15.0cd	14.6cd	23.8bc	12.9cd	8.91d
Decanal		0.72b	2.02a					
(*E*)-β-Damascenone		0.27bc	0.76a	0.15c	0.18bc	0.26bc		0.35b
α-Cedrene	2.34a	1.02c	1.38b				0.62c	
β-Cedrene		0.26b	0.43a				0.21b	
Bergamotene	9.36							
β-Caryophyllene	0.17							
β-Bisabolene	0.93							
ALD[e]	11.0a	5.00bc	9.59ab	0.29c	0.24c	1.05c	1.09c	3.24c
OLS	769b	554c	981a	226de	180de	326d	218de	122e
EST	54.4a	19.2c	30.7b	1.73d	1.30d	2.58d	2.21d	1.13d
ENE	107b	300a	301a	10.1c	14.8c	11.8c	5.21c	6.52c
OID	69.5a	41.5b	76.0a	19.2cd	18.2cd	30.8bc	16.7cd	11.7d
ONE	12.1a	4.13c	7.69b	0.92d	1.15d	1.62cd	0.29d	0.67d
ARO					0.10c	0.61bc	16.7ab	22.0a
VOL	1028b	915b	1739a	258c	216c	374c	243c	147c

[a] GNP = Goodnature hydraulically pressed whole-fruit fresh juice, UF = ultrafiltered juice, PJ0 = HTST (high temperature short time) day zero pasteurized juice, stored for 1, 2 and 3 months refrigerated at 4 °C (4C-1, 4C-2 and 4C-3) and 1 and 2 months at ~25 °C (25C-1 and 25C-2).
[b] Empty cells indicate no detection, or not enough replicates for ANOVA.
[c] Italicized bold font indicates consensus compounds.
[d] ANOVA not possible in cases where only one treatment displayed compound recovery.
[e] Compound classes represented by: ALD = aldehydes, OLS = alcohols, EST = esters, ENE = terpenes, OID = terpenoids, ONE = ketones, ARO = aromatics and VOL = total volatiles, per listing in *the Materials & Methods*, and Table 1.

deemed most congruent and native but we sporadically isolated this compound in some arils-only and whole-pressed 'Wonderful' (Beaulieu & Stein-Chisholm, 2016; Beaulieu et al., 2015).

Twenty-six compounds were identified in pomegranate juice after the hydraulic pressing, which were also identified after ultrafiltration (UF) and pasteurization (PJ). Among the common compounds in GNP, UF and PJ, 10 of these compounds were present in a higher content in GNP in comparison to UF and PJ. On the other hand, *p*-cymene, limonene and α-terpinolene increased their relative content by 314, 1188 and 178%, respectively in PJ0 samples in comparison to GNP, while, hexanal, (*E*)-3-hexen-1-ol, linalool, 4-terpineol, decanal, (*E*)-β-damascenone and β-cedrene were reduced in UF in comparison to PJ. In general, OLS, ENE, and VOL were present in a higher content in pomegranate juice after pasteurization (Supplementary Table 1).

Some compounds were isolated in the initial processing stages, as in the controls (GNF or PJ0) and UF but, after pasteurization throughout storage, they essentially disappeared (Tables 2 and 3). Such compounds were (*E*)-2-hexenal, α-pinene, β-myrcene and ethyl hexanoate. Even though these compounds are considered consensus (Mayuoni-Kirshinbaum & Porat, 2014), and some of them have been reported in South African and Spanish cultivars and fresh 'Wonderful' juices (Beaulieu & Stein-Chisholm, 2016; Beaulieu et al., 2015; Caleb et al., 2013; Carbonell-Barrachina et al., 2012; Mayuoni-Kirshinbaum & Porat, 2014; Mayuoni-Kirshinbaum et al., 2013), they essentially disappeared before, or due to, pasteurization. Furthermore, there were no 1-way ANOVA significant differences in the level of (*E*)-2-hexenal, α-pinene or β-myrcene recovered between GNP, UF and PJ0 (Table 2). Of these compounds, ethyl hexanoate contained the highest relative pro-

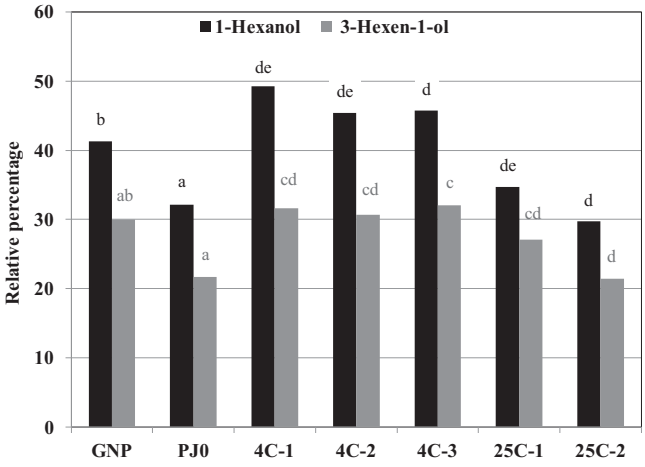

Fig. 1. Relative percentage of dominant volatile compounds in pilot plant 'Wonderful' pomegranate juices. Significant differences ($\alpha = 0.05$) across treatments with different letter designations, per compound, represent the ANOVA based on semi-quantified numerical results (not percentages) in Table 2. Treatment names are: GNP = Goodnature pressed juice, PJ0 = pasteurized juice, stored for 1, 2 and 3 months refrigerated at 4 °C (4C-1, 4C-2 and 4C-3) and 1 and 2 months at ~ 25 °C (25C-1 and 25C-2).

portion (6th most abundant) in GNP and decreased the most from GNP due to UF (Tables 2 and 3).

Other compounds were occasionally present in different stages of the initial processing from the controls (GNP or PJ0) or UF then were not recovered or sporadically recovered during storage. Ethyl butyrate was significantly greatest in GNP (Table 2, 5.02) but markedly declined through UF and PJ0, then almost absent during 4 °C storage and gone at ambient (25 °C) storage. Decanal only appeared in both UF (0.72) and PJ0 (2.02) then, like the above trends, it disappeared in all stored samples. Interestingly, decanal has been reported previously in commercial samples (Beaulieu & Stein-Chisholm, 2016; Nuncio-Jauregui et al., 2014). To the best of our knowledge, the only report for these two compounds in pure commercial 'Wonderful' samples were in our previous findings (Beaulieu & Stein-Chisholm, 2016). 6-Methyl-5-hepten-2-one was likewise recovered in GNP, UF and PJ0 yet it tailed off slightly and was not always recovered during storage at both temperatures (Table 2). 6-Methyl-5-hepten-2-one is considered consensus (Mayuoni-Kirshinbaum & Porat, 2014), and is commonly observed in 'Wonderful' (Mayuoni-Kirshinbaum & Porat, 2014; Mayuoni-Kirshinbaum et al., 2013) and commercial 'Wonderful' juices (Beaulieu & Stein-Chisholm, 2016), and likewise in most of these NFC stored pasteurized juices.

On the other hand, other compounds were not isolated in the initial stages in the controls (e.g. GNF or PJ0) but appeared after pasteurization throughout storage. Such compounds were: benzaldehyde (4C-3, 25C-1 and 25C-2), phenylethyl alcohol (25C-1 and 25C-2) and a consensus compound, 2-ethyl-1-hexanol which was only isolated confidently throughout storage. Few of these compounds were routinely recovered throughout pasteurization with significant differences (Table 2). However, these three compounds comprised a fair amount of the total relative abundance in juices that were stored at 25 °C (Table 3). Benzaldehyde and phenylethyl alcohol did not have pronounced trends or significance through storage. However, benzaldehyde appeared late in storage (4C-3) and at elevated temperature storage (25C-1 and 25C-2), and phenylethyl alcohol only occurred in roughly half the samples in both 25C-1 and 25C-2. Oddly, the consensus compound, 2-ethyl-1-hexanol, was absent in native samples (GNP) and did not appear until 1 month into storage (Table 2). In terms of relative composition per treatment (Table 3), 2-ethyl-1-hexanol was about the 7th 9th highest compound recovered throughout storage. Previously, we rarely found this compound in control 'Wonderful' juices, yet it was present in an in-house concentrate, reconstituted juice and commercial concentrate (Beaulieu & Stein-Chisholm, 2016). We and others have isolated this compound in concentrates, essence, and in commercial samples (Beaulieu & Stein-Chisholm, 2016; Nuncio-Jauregui et al., 2014), as well as in blended samples (Vazquez-Araujo, Koppel et al., 2011). Interestingly, 2-ethyl-1-hexanol has been listed as an artifact from hydrolyzed glued plastics (Uhde & Salthammer, 2007), yet it has been reported previously in fresh pomegranate juices (Vazquez-Araujo, Chambers et al., 2011; Mayuoni-Kirshinbaum & Porat, 2014; Mayuoni-Kirshinbaum et al., 2012). It remains unclear as to how and why this compound arises, was generally not isolated in our laboratory in many native samples but, comes on board after pasteurization and through concentrating and storage.

3.3. Compounds with significant differences through processing and pasteurized storage

There was a somewhat strange trend whereby many compounds had higher means for both GNP and PJ0 compared with juices immediately after UF, as illustrated in Table 2. However, UF can effectively strip compounds (Beaulieu et al., 2016). As a matter of fact, juice immediately following pasteurization (PJ0) often contained the highest significant amounts for several compounds, such as ethanol, ethyl acetate, (*E*)-3-hexen-1-ol, 1-hexanol, α-terpinene, *p*-cymene and (*E*)-β-damascenone (Table 2). Previously, elevated volatiles in pasteurized NFC blueberry juice were not observed using almost exactly similar pilot plant methods (Beaulieu et al., 2016). Pomegranates are not known for a highly diverse volatile profile (Beaulieu et al., 2015; Calin-Sanchez et al., 2011), and it has been noted that volatile losses occurring during juice manufacturing steps leading to commercial juices were dissimilar to that of fresh fruits (Beaulieu & Stein-Chisholm, 2016; Melgarejo et al., 2011). Although sensory was not formally performed herein, 4 trained scientists with years of aroma and flavor profiling experience determined these NFC juices to be of extremely high quality through 2 months storage. However, this speculative statement must be taken with caution because these juices were also sweeter than what is customarily marketed in the U.S. Nonetheless, a head-to-head comparison of commercial juices (non-concentrated) and those delivering an NFC juice would need to be performed using the identical fruit which is logistically and realistically very hard to accomplish.

Only a few compounds considered as consensus (hexanal, limonene and nonanal) did not have markedly significant differences or trends through storage (after PJ0). Similarly, although not consensus, the same observation held for ethanol and linalool. On the other hand, almost all consensus compounds displayed highly significant decreases during storage (Table 2). For example, this was true concerning (*E*)-3-hexen-1-ol, 1-hexanol, 6-methyl-5-hepten-2-one, α-terpinene, *p*-cymene, γ-terpinene, 4-terpineol and α-terpineol (Table 2). As an example, if one considers the top 5 or 6 dominant consensus compounds in GNP, UF and PJ0 [1-hexanol, (*E*)-3-hexen-1-ol, α-terpineol, limonene, *p*-cymene and γ-terpinene] on a relative percentage basis (Table 3), there were appreciable and highly significant reductions after UF observed across storage at both temperatures for all except α-terpineol (Table 2). The total semi-quantitative response confirmed this as well: PJ0 = 1739 versus 4C-1 = 258 and 25C-1 = 244 (Table 2). α-Terpineol has been considered as an oxidative breakdown product from limonene, and as limonene concentrations decrease, α-terpineol concentrations increase (Perez-Lopez, Saura, Lorente, & Carbonell-Barrachina, 2006). We cannot prove that this was the

Table 3
Relative percentage per compound of all compounds integrated and reported per treatment through ANOVA in not-from-concentrate 'Wonderful' pomegranate juices through pasteurized storage.

Rank	GNP[a]		UF		PJ0		4C-1	
	Rel%	Compound	Rel%	Compound	Rel%	Compound	Rel%	Compound
1	41.34	1-Hexanol	34.16	1-Hexanol	32.16	1-Hexanol	49.24	1-Hexanol
2	29.97	(*E*)-3-Hexen-1-ol	23.85	(*E*)-3-Hexen-1-ol	21.71	(*E*)-3-Hexen-1-ol	31.66	(*E*)-3-Hexen-1-ol
3	5.52	α-Terpineol	21.28	Limonene	10.18	Limonene	5.81	α-Terpineol
4	2.96	Limonene	5.99	*p*-Cymene	6.26	*p*-Cymene	3.62	Ethanol
5	2.52	*p*-Cymene	3.69	α-Terpineol	3.98	γ-Terpinene	2.50	*p*-Cymene
6	2.32	Ethyl hexanoate	2.88	γ-Terpinene	3.49	α-Terpineol	2.09	2-Methyl-3-buten-2-ol
7	2.00	Ethyl benzoate	1.13	Ethyl hexanoate	2.41	Ethanol	0.96	4-Terpineol
8	1.29	β-Pinene	1.07	Ethanol	1.87	2-Methyl-3-buten-2-ol	0.90	2-Ethyl-1-hexanol
9	1.26	Ethanol	1.00	2-Methyl-3-buten-2-ol	0.90	Ethyl hexanoate	0.56	Linalool
10	1.24	2-Methyl-3-buten-2-ol	0.64	Ethyl benzoate	0.59	α-Terpinene	0.51	γ-Terpinene
11	1.14	2-Nonanone	0.55	4-Terpineol	0.56	Ethyl benzoate	0.39	α-Terpinene
12	0.91	Bergamotene	0.52	α-Terpinene	0.55	4-Terpineol	0.35	Limonene
13	0.75	β-Myrcene	0.46	α-Terpinolene	0.43	α-Terpinolene	0.34	Ethyl acetate
14	0.73	γ-Terpinene	0.39	2-Nonanone	0.38	2-Nonanone	0.30	2-Nonanone
15	0.71	4-Terpineol	0.27	Ethyl butyrate	0.34	β-Myrcene	0.17	α-Terpinolene
16	0.49	Ethyl butyrate	0.27	Linalool	0.29	Linalool	0.13	Ethyl benzoate
17	0.44	α-Terpinene	0.24	β-Myrcene	0.20	Ethyl butyrate	0.11	Ethyl butyrate
18	0.42	(*Z*)-3-Hexenyl acetate	0.23	(*E*)-2-Hexenal	0.20	(*E*)-2-Hexenal	0.09	Hexanal
19	0.41	(*E*)-2-Hexenal	0.13	α-Pinene	0.13	Nonanal	0.08	Nonanal
20	0.38	Linalool	0.11	α-Cedrene	0.12	Decanal	0.08	1,4-Cineole
21	0.31	α-Terpinolene	0.11	Hexanal	0.11	Hexanal	0.06	(*E*)-β-Damascenone
22	0.23	α-Cedrene	0.09	Nonanal	0.10	Ethyl acetate	0.04	Eucalyptol
23	0.20	Ethyl acetate	0.08	Ethyl acetate	0.08	α-Cedrene	0.03	1-Octanol
24	0.16	Hexanal	0.08	Decanal	0.06	α-Pinene		
25	0.14	α-Pinene	0.05	1,4-Cineole	0.05	1,4-Cineole		
26	0.13	1,4-Cineole	0.03	(*E*)-β-Damascenone	0.04	(*E*)-β-Damascenone		
27	0.10	Nonanal	0.03	1-Octanol	0.03	1-Octanol		
28	0.09	β-Bisabolene	0.03	β-Cedrene	0.02	β-Cedrene		
29	0.05	Eucalyptol	0.02	6-Methyl-5-hepten-2-one	0.02	6-Methyl-5-hepten-2-one		
30	0.04	1-Octanol	–[b]					
31	0.03	6-Methyl-5-hepten-2-one						
32	0.02	β-caryophyllene						

Rank	4C-2		4C-3		25C-1		25C-2	
	Rel%	Compound	Rel%	Compound	Rel%	Compound	Rel%	Compound
1	45.38	1-Hexanol	45.71	1-Hexanol	34.69	1-Hexanol	29.77	(*E*)-3-Hexen-1-ol
2	30.71	(*E*)-3-Hexen-1-ol	32.05	(*E*)-3-Hexen-1-ol	27.10	(*E*)-3-Hexen-1-ol	21.42	1-Hexanol
3	6.76	α-Terpineol	6.36	α-Terpineol	7.16	Ethanol	14.88	Ethanol
4	5.12	Ethanol	5.05	Ethanol	5.94	Phenylethyl alcohol	8.48	Phenylethyl alcohol
5	3.70	*p*-Cymene	3.10	2-Methyl-3-buten-2-ol	5.75	2-Methyl-3-buten-2-ol	6.08	α-Terpineol
6	1.30	2-Methyl-3-buten-2-ol	1.93	*p*-Cymene	5.28	α-Terpineol	5.39	2-Methyl-3-buten-2-ol
7	1.19	Limonene	1.25	2-Ethyl-1-hexanol	1.44	2-Ethyl-1-hexanol	2.60	2-Ethyl-1-hexanol
8	1.02	4-Terpineol	1.06	4-Terpineol	1.01	*p*-Cymene	2.14	*p*-Cymene
9	0.90	2-Ethyl-1-hexanol	0.70	Linalool	0.78	4-Terpineol	1.17	Benzaldehyde
10	0.85	γ-Terpinene	0.56	γ-Terpinene	0.74	Linalool	0.97	Limonene
11	0.76	α-Terpinene	0.48	Ethyl acetate	0.74	Ethyl benzoate	0.95	Linalool
12	0.57	Linalool	0.35	α-Terpinene	0.50	Benzaldehyde	0.84	4-Terpineol
13	0.47	2-Nonanone	0.35	2-Nonanone	0.38	γ-Terpinene	0.70	γ-Terpinene
14	0.35	α-Terpinolene	0.18	α-Terpinolene	0.32	Limonene	0.52	2-Nonanone
15	0.33	Ethyl benzoate	0.16	Benzaldehyde	0.25	2-Nonanone	0.36	α-Terpinene
16	0.16	Nonanal	0.12	Limonene	0.25	α-Cedrene	0.29	α-Terpinolene
17	0.14	Ethyl acetate	0.10	Hexanal	0.24	Ethyl acetate	0.24	(*E*)-β-Damascenone
18	0.10	Hexanal	0.10	Ethyl benzoate	0.21	α-Terpinene	0.19	Ethyl acetate
19	0.08	(*E*)-β-Damascenone	0.09	Ethyl butyrate	0.20	α-Terpinolene	0.16	1-Octanol
20	0.08	1-Octanol	0.08	1,4-Cineole	0.13	1-Octanol	0.13	1,4-Cineole
21	0.07	1,4-Cineole	0.07	1-Octanol	0.12	1,4-Cineole	0.09	6-Methyl-5-hepten-2-one
22	0.04	6-Methyl-5-hepten-2-one	0.07	(*E*)-β-Damascenone	0.12	Hexanal		
23	0.04	Eucalyptol	0.06	Eucalyptol	0.09	β-Cedrene		
24			0.06	Nonanal	0.02	Eucalyptol		
25			0.02	6-Methyl-5-hepten-2-one				

[a] GNP = Goodnature hydraulically pressed whole-fruit fresh juice, UF = ultrafiltered juice, PJ0 = HTST (high temperature short time) day zero pasteurized juice, stored for 1, 2 and 3 months refrigerated at 4 °C (4C-1, 4C-2 and 4C-3) and 1 and 2 months at ~25 °C (25C-1 and 25C-2).
[b] Empty cells indicate no more compounds recovered per specific treatment.

case herein. However, it is interesting how limonene was in the top 4 compounds in UF and PJ0 and α-terpineol was in the top 6 yet, much lower in relative percentage (Table 3). Then, during storage, the relative loss of α-terpineol was not nearly as profound as that of limonene, and α-terpineol remained in the top 8 compounds while limonene dropped to as low as #16 (Table 3).

Similar to above, other non-consensus compounds followed the same trend, displaying (generally) significant decreases throughout storage. Such compounds were ethyl acetate, 1,4-cineole, eucalyptol, 1-octanol, α-terpinolene, 2-nonanone and ethyl benzoate. 2-Methyl-3-buten-2-ol and α-terpinolene, which were found throughout pasteurization and storage, also displayed significant

trends (Table 2). Without exception, this same trend of significant compound decreases through storage was observed for all classes of compounds (ALD, OLS, EST, ENE, OID, ONE and VOL), except for ARO (Table 2). In only a few instances were the means not significantly different between UF and scattered storage means per compound class, and usually UF means were significantly lower than both GNP and PJ0. Aromatic compounds (benzaldehyde and phenethyl alcohol) might be a direct indicator of increasing anaerobic conditions or the initiation of fermentation in the longer-term and higher temperature storage (25C-1 and 25C-2). Aside from the ARO trend, an interesting observation was that increasing storage temperature did not generally augment the observed significant loss of volatiles during storage, even though storage temperature had a significant effect on 17 aroma volatile compounds, while storage time and the interaction of temperature and storage time together affected 14 and 9 compounds, respectively (Supplementary Table 2).

Previously, albeit without sensory confirmation, we proposed based on commercial attributes ascribed that five compounds (ethanol, β-myrcene, 1,4-cineole, 2-nonanone and linalool) isolated in almost every 'Wonderful' fresh juice analyzed, might be worth consideration regarding their contribution to pomegranate juice flavor. We too suggested other compounds, such as (*Z*)-3-hexenyl acetate, 2-methyl-3-buten-2-ol, (*Z*)-3-hexenal and α-terpinolene, might be worth evaluating (Beaulieu & Stein-Chisholm, 2016). Herein, (*Z*)-3-hexenyl acetate and (*Z*)-3-hexenal were not recovered often enough to report, and all the other compounds except β-myrcene were retained in the pasteurized and stored NFC juices. Indeed, throughout pasteurization and storage in NFC juice, ethanol, 2-methyl-3-buten-2-ol, 1,4-cineole, α-terpinolene, 2-nonanone and linalool were recovered, and all displayed significant trends (Table 2 and Supplementary Table 2). As noted above, most compounds decreased markedly after pasteurization but they were present. Similarly, β-pinene and bergamotene in processed arils-only 'Wonderful' juices (not commercial) displayed a tailing off due to batch pasteurization and additionally due to making a concentrate (Koppel, Anderson, & Chambers, 2015).

In a study with commercial juices produced in the USA from concentrate, 56 compounds were isolated from four different concentrates, with 10 consensus compounds [(*Z*)-3-hexen-1-ol, 2-ethyl-1-hexanol, hexanal, β-damascenone, β-pinene, β-cymene, limonene, γ-terpinene, α-terpineol and β-caryophyllene] (Vazquez-Araujo, Koppel et al., 2011), whereas a 100% pressed pomegranate juice (USDA certified organic pomegranates grown in Florida) only delivered 10 compounds, of which 2 were consensus [hexanal and (*Z*)-3-hexen-1-ol] (Vazquez-Araujo et al., 2010). In commercial pasteurized Spanish juices, 18 compounds were recovered and 12 were consensus. These included α-pinene, β-pinene, α-terpinene, limonene, γ-terpinene, terpinen-4-ol, α-terpineol, (*Z*)-3-hexenal, hexanal, nonanal, (*Z*)-3-hexen-1-ol and 1-hexanol (Carbonell-Barrachina et al., 2012). Using Spanish commercial juice (VitalGrana, Spain) which was a 4:1 mixture of 'Mollar de Elche' and 'Wonderful', 39 compounds were reported and 16 were consensus. These included [(*Z*)-3-hexen-1-ol, 1-hexanol, 2-ethyl-1-hexanol, α-pinene, β-pinene, α-terpinene, *p*-cymene, limonene, γ-terpinene, (*E*)-α-bergamotene, hexanal, (*E*)-2-hexenal, heptanal, nonanal, terpinen-4-ol and α-terpineol] (Nuncio-Jauregui et al., 2014). When reviewing the limited data on commercial samples, it becomes apparent that wide variation is observed within each study regarding the quantity of compounds recovered per juice and, even more limiting, the number of consensus compounds. Subsequently, more recently, using commercial pomegranate juice marketed as 100% 'Wonderful', 31 compounds were found with SPME in 3 juices from the same company, yet only 12 of the same compounds were isolated in all three of those replicated juices, and only 16 similar compounds were the same between two different companies (Beaulieu & Stein-Chisholm, 2016). Furthermore, marked similarities between freshly pressed 'Wonderful' arils-only and whole-fruit pressed juices existed (34 compounds were the same) while incredible dissimilarities existed between those juices and several commercial samples, with 35 unique compounds recovered (Beaulieu & Stein-Chisholm, 2016). Some of the aforementioned commercial samples likewise had several compounds we seldom observe in native 'Wonderful'. Herein, we furthered this comparison with a less abusive NFC juice which contained 18 consensus compounds (Tables 1 and 2) and 13 of those compounds were retained in the pasteurized juice (PJ0) (Tables 2 and Supplementary Table 2). Three consensus compounds were completely lost through storage [(*E*)-2-hexenal, α-pinene and β-pinene), whereas only one consensus compound (2-ethyl-1-hexanol) emerged during storage (Table 2).

3.4. PCA cluster analyses

The first 3 PC's explained 85% of the total volatile variance. PC1, representing 53% of variation, was most affected by the total content of aldehydes (ALD) and volatiles (VOL) on its positive orientation (Fig. 2). PC2 (21% of the variation) was mainly defined by ethyl butyrate, α-pinene, ethyl hexanoate, 1,4-cineole, eucalyptol, β-bisabolene, β-pinene, (*Z*)-3-hexenyl acetate and bergamotene on its positive orientation (i.e. this PC was most influenced by the content of terpenes). On the other hand, PC3 (11% of the variation) was affected by 6-methyl-5-hepten-2-one, limonene, γ-terpinene,

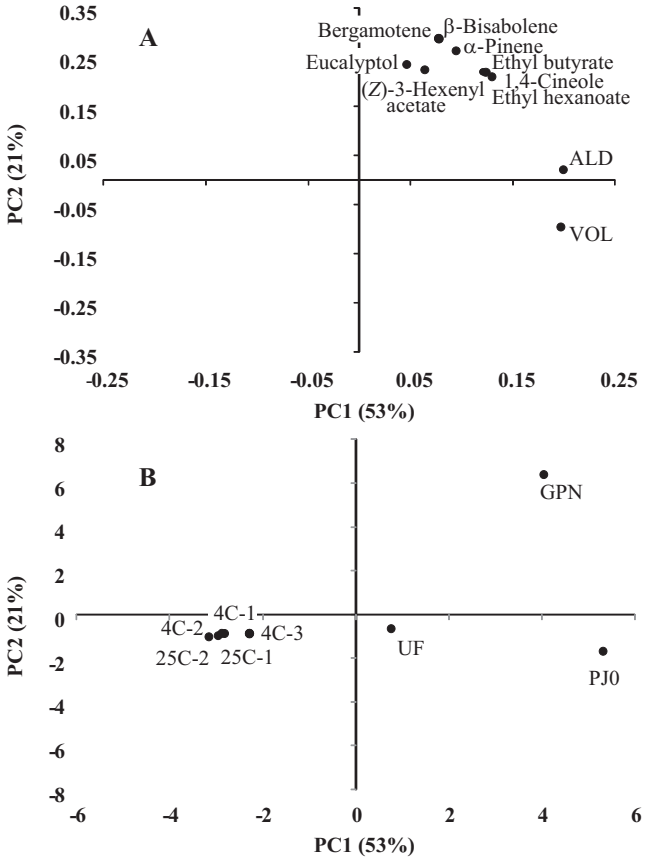

Fig. 2. Plot of the centroid scores (A) and loading plots (B) of the two main principal components (PC1 and PC2) of the volatile compounds and compound classes in 'Wonderful' not-from-concentrate pomegranate juices. Treatment names are: GNP = Goodnature pressed juice, UF = ultrafiltered juice, PJ = pasteurized juice, stored for 1, 2 and 3 months refrigerated at 4 °C (4C-1, 4C-2 and 4C-3) and 1 and 2 months at ~25 °C (25C-1 and 25C-2).

decanal, β-cedrene and ENE on the positive orientation, while 2-methyl-3-buten-2-ol defined the negative orientation (Fig. 3). This figure depicts the distribution of the samples according to their content of aroma volatile compounds on the space of the PC's. According to this plot, PJ and UF were clearly separated from the samples in storage by their content of decanal, β-cedrene, limonene, γ-terpinene and ENE (Fig. 3). In addition, GNP juice was separated from all the samples by its content of bergamotene, β-bisabolene, β-pinene, (*Z*)-3-hexenyl acetate and eucalyptol (Figs. 2 and 3). Both plots also demonstrate markedly that storage temperature (4 vs. 25 °C) appeared to have little effect on the overall samples, as all storage samples (4C-1, 4C-2, 4C-3, 25C-1 and 25C-2) had remarkably tight clustering (Figs. 1 and 2).

The PCA allowed an assessment of the major compounds that influence the aroma of pomegranate juice after different treatments. Accordingly, PC1 was mostly affected by the summed compound classes. However, it did not differentiate any sample during storage from the juice controls (GNP, UF and PJ0), while PC2 and PC3 were influenced by different single compounds, showing the effect of these compounds on the aroma of pomegranate juice (as previously stated).

4. Conclusion

Throughout this study evaluating volatile changes in NFC pasteurized juices, 13 of the consensus pomegranate compounds were isolated, of which 12 were routinely recovered (hexanal, (*E*)-3-hexen-1-ol, 1-hexanol, 6-methyl-5-hepten-2-one, α-terpinene, *p*-cymene, 2-ethyl-1-hexanol, limonene, γ-terpinene, nonanal, 4-terpineol and α-terpineol) using this SPME method. There were generally significant losses for almost all individual compounds and compound classes through storage. Additionally, other consensus compounds were isolated in initial processing steps but, were absent in storage. We have previously recovered several consensus and other flavor-related compounds and massive differences across endogenous native-pressed versus commercial 'Wonderful' juices and deduced that this SPME method with NFC delivered a robust 'Wonderful' volatile profile that is likely superior qualitatively and perhaps quantitatively, to a typical, unadulterated, commercial offering. It has been demonstrated that the interaction of temperature and length of storage plays an important role in the preservation of the aroma composition of NFC pomegranate juice. Further olfactometry, flavor profiling and sensory quantification would be required to substantiate if all the compounds reported herein indeed confer "superior" positive flavor attributes in processed NFC 'Wonderful' juices.

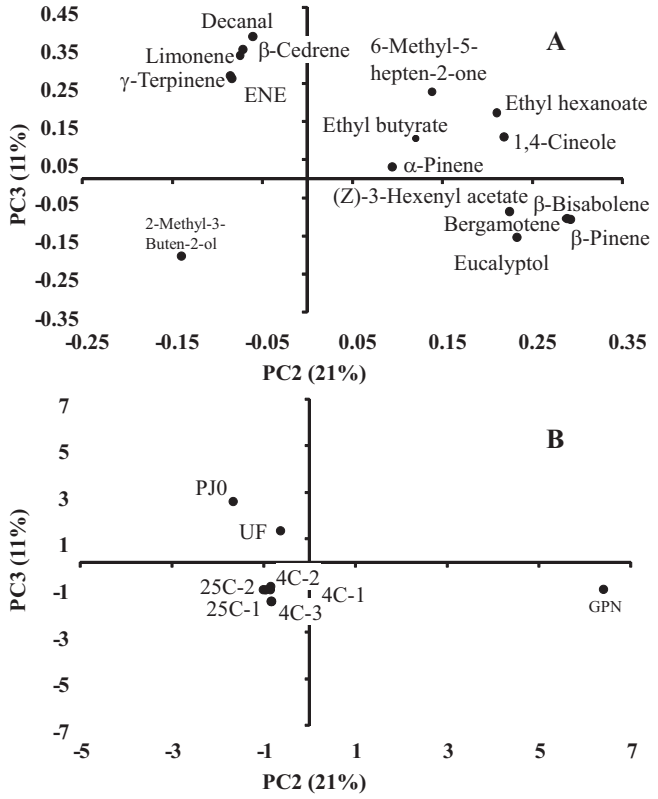

Fig. 3. Plot of the centroid scores (A) and loading plots (B) of the two main principal components (PC21 and PC3) of the volatile compounds and compound classes in 'Wonderful' not-from-concentrate pomegranate juices. Treatment names are: GNP = Goodnature pressed juice, UF = ultrafiltered juice, PJ = pasteurized juice, stored for 1, 2 and 3 months refrigerated at 4 °C (4C-1, 4C-2 and 4C-3) and 1 and 2 months at ~25 °C (25C-1 and 25C-2).

Acknowledgements

The authors express sincere gratitude to Dr. Debbie Boykin, USDA ARS SEA Statistician, for refresher training and guidance using SAS JMP Pro (Version 11). We also thank Dr. Karen L. Bett-Garber, Jeanne M. Lea, Michael A. Watson and especially Rebecca E. Stein-Chisholm for laboratory help.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.02.114.

References

AIJN. (2016). AIJN European Fruit Juice Association. 2016 Liquid Fruit Market Report. Brussels, Belgium, European Fruit Juice Association. 8-19-2016. Ref Type: Pamphlet.

Alper, N., Bahceci, K. S., & Acar, J. (2005). Influence of processing and pasteurization on color values and total phenolic compounds of pomegranate juice. *Journal of Food Processing and Preservation, 29*(5–6), 357–368.

Andreu-Sevilla, A. J., Mena, P., Marti, N., Garcia Viguera, C., & Carbonell-Barrachina, T. A. (2013). Volatile composition and descriptive sensory analysis of pomegranate juice and wine. *Food Research International, 54*(1), 246–254.

Anonymous (2011). Superpremium Juice in the U.S.: The Impact of Superfruits and Antioxidants. *Focus Report,* Beverage Marketing Corporation. (Accessed 10-09/2014). Retrieved from: http://www.beveragemarketing.com/SuperpremiumJuice.doc.

Basu, A., & Penugonda, K. (2009). Pomegranate juice. a heart-healthy fruit juice. *Nutrition Reviews, 67*(1), 49–56. http://dx.doi.org/10.1111/j.1753-4887.2008.00133.x.

Bates, R. P., Morris, J. R., & Crandall, P. G. (2001). Principles and practices of small- and medium-scale fruit juice processing. In *FAO agricultural services bulletin – 146.* Rome, Italy: FAO.

Beaulieu, J. C., Stein-Chisholm, R. E., Lloyd, S. W., Bett-Garber, K. L., Grimm, C. C., Watson, M. A., et al. (2016). Volatile, anthocyanidin, quality and sensory changes in rabbiteye blueberry from whole fruit through pilot plant juice processing. *Journal of the Science of Food and Agriculture, 97*(2), 469–478. http://dx.doi.org/10.1002/jsfa.7748.

Beaulieu, J. C., Lloyd, S. W., Preece, J. E., Moersfelder, J. W., Stein-Chisholm, R. E., & Obando-Ulloa, J. M. (2015). Physicochemical properties and aroma volatile profiles in a diverse collection of California-grown pomegranate (*Punica granatum* L.) germplasm. *Food Chemistry, 181*, 354–364. http://dx.doi.org/10.1016/j.foodchem.2015.02.026.

Beaulieu, J. C., & Stein-Chisholm, R. E. (2016). HS-GC-MS volatile compounds recovered in freshly pressed 'Wonderful' cultivar and commercial pomegranate juices. *Food Chemistry, 190*, 643–656. http://dx.doi.org/10.1016/j.foodchem.2015.06.005.

Cadwallader, K. R., Tamamoto, L. C., & Sajuti, S. C. (2010). Aroma components of fresh and stored pomegranate (*Punica granatum* L.) juice. In N. C. DaCosta & R. J. Cannon (Eds.), *Flavors in noncarbonated beverages* (103 ed.. *ACS Symposium Series American Chemical Society* (103 ed.), pp. 93–101). . http://dx.doi.org/10.1021/bk-2010-1036.ch007.

Caleb, O. J., Opara, U. L., Mahajan, P. V., Manley, M., Mokwena, L., & Tredoux, A. G. J. (2013). Effect of modified atmosphere packaging and storage temperature on volatile composition and postharvest life of minimally-processed pomegranate

arils (cvs. 'Acco' and 'Herskawitz'). *Postharvest Biology and Technology., 79*, 54–61.

Calin-Sanchez, A., Martinez, J. J., Vazquez-Araujo, L., Burlo, F., Melgarejo, P., & Carbonell-Barrachina, A. A. (2011). Volatile composition and sensory quality of Spanish pomegranates (Punica granatum L.). *Journal of the Science of Food and Agriculture, 91*(3), 586–592. http://dx.doi.org/10.1002/jsfa.4230/full.

Carbonell-Barrachina, A. A., Calin-Sanchez, A., Bagatar, B., Hernandez, F., Legua, P., Martinez-Font, R., et al. (2012). Potential of Spanish sour-sweet pomegranates (cultivar C25) for the juice industry. *Food Science and Technology International, 18*(2), 129–138.

Cline, H. (2008). *Loquaci Ranch site of first mechanical pomegranate harvester demonstration*. Western Farm Press.

Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P. E., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants & Redox Signaling, 18*(14), 1818–1892. http://dx.doi.org/10.1089/ars.2012.4581.

Drewnowski, A., & Gomez-Carneros, C. (2000). Bitter taste, phytonutrients, and the consumer: A review. *American Society for Nutrition, 72*(6), 1424–1435.

FDA. (2014). *CFR – Code of Federal Regulations Title 21; Food labeling; Specific Food Labeling Requirements*. (21CFR101.30. Percentage juice declaration for foods purporting to be beverages that contain fruit or vegetable juice.). Retrieved from: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=101.30.

Guler, Z., & Gul, E. (2016). Volatile organic compounds (Vocs) in the aril juices and seeds from selected five pomegranate (*Punica Granatum* L.) cultivars. *International Journal of Food Properties*. http://dx.doi.org/10.1080/10942912.2016.1155057.

Johanningsmeier, S. D., & Harris, G. K. (2011). Pomegranate as a functional food and nutraceutical source. *Annual Review of Food Science and Technology, 2*(1), 181–201. http://dx.doi.org/10.1146/annurev-food-030810-153709.

Koppel, K., Anderson, E. L., & Chambers, E. (2015). Influence of processing on pomegranate (*Punica granatum* L.) juice flavor and aroma. *Journal of the Science of Food and Agriculture, 95*(5), 1066–1071. http://dx.doi.org/10.1002/jsfa.6799.

Lawless, L. J. R., Threlfall, R. T., Meullenet, J.-F., & Howard, L. R. (2013). Applying a mixture design for consumer optimization of black cherry, Concord grape and pomegranate juice blends. *Journal of Sensory Studies, 28*(2), 102–112. http://dx.doi.org/10.1111/joss.12026.

Lesschaeve, I., & Noble, A. C. (2005). Polyphenols: factors influencing their sensory properties and their effects on food and beverage preferences. *The American Journal of Clinical Nutrition, 81*(1), 330S–335S.

Mayuoni-Kirshinbaum, L., & Porat, R. (2014). The flavor of pomegranate fruit: A review. *Journal of the Science of Food and Agriculture, 94*(1), 21–27. http://dx.doi.org/10.1002/jsfa.6311.

Mayuoni-Kirshinbaum, L., Daus, A., & Porat, R. (2013). Changes in sensory quality and aroma volatile composition during prolonged storage of 'Wonderful' pomegranate fruit. *International Journal of Food Science and Technology, 48*(8), 1569–1578.

Mayuoni-Kirshinbaum, L., Tietel, Z., Porat, R., & Ulrich, D. (2012). Identification of aroma-active compounds in 'wonderful' pomegranate fruit using solvent-assisted flavour evaporation and headspace solid-phase micro-extraction methods. *European Food Research and Technology, 235*(2), 277–283.

Melgarejo, P., Calin-Sanchez, A., Vazquez-Araujo, L., Hernandez, F., Martinez, J. J., Legua, P., et al. (2011). Volatile composition of pomegranates from 9 Spanish cultivars using headspace solid phase microextraction. *Journal of Food Science, 76*(1), S114–S120.

Mphahlele, R. R., Fawole, O. A., Mokwena, L. M., & Opara, U. L. (2016). Effect of extraction method on chemical, volatile composition and antioxidant properties of pomegranate juice. *South African Journal of Botany, 103*, 135–144.

Nuncio-Jauregui, N., Calin-Sanchez, A., Hernandez, F., & Carbonell-Barrachina, A. A. (2014). Pomegranate juice adulteration by addition of grape or peach juices. *Journal of the Science of Food and Agriculture, 94*(4), 646–655. http://dx.doi.org/10.1002/jsfa.6300/abstract.

Perez-Lopez, A. J., Saura, D., Lorente, J., & Carbonell-Barrachina, A. A. (2006). Limonene, linalool, α-terpineol, and terpinen-4-ol as quality control parameters in mandarin juice processing. *European Food Research and Technology, 222*(3–4), 281–285.

Pollock, D. (2011). *Pomegranate interest expands across California agriculture*. Western Farm Press.

Rohan, M. (2013). The markets for soft drinks and fruit juices change as a constant – Focusing on health. http://www.fdbusiness.com/2013/07/the-markets-for-soft-drinks-and-fruit-juices-change-as-a-constant-focusing-on-health/. Retrieved from: http://www.fdbusiness.com/?s=fruit+juices&.x=24&.y=5.

Sabbe, S., Verbeke, W., Deliza, R., Matta, V. M., & Van Damme, P. (2009). Consumer liking of fruit juices with different açaí (*Euterpe oleracea* Mart.) concentrations. *Journal of Food Science, 74*(5), S171–S176. http://dx.doi.org/10.1111/j.1750-3841.2009.01146.x.

Sloan, A. E. (2011). What's next in beverages. *Food Technology, 3*, 19.

Uhde, E., & Salthammer, T. (2007). Impact of reaction products from building materials and furnishings on indoor air quality – A review of recent advances in indoor chemistry. *Atmospheric Environment, 41*(15), 3111–3128.

USDA (2016). *USDA's national agricultural statistics service*. USDA National Agricultural Statistics Service, Pacific Regional Field Office (and the California Field Office), Quick Stats.

Vazquez-Araujo, L., Chambers, E., IV, Adhikari, K., & Carbonell-Barrachina, A. A. (2011). Physico-chemical and sensory properties of pomegranate juices with pomegranate albedo and carpellar membranes homogenate. *LWT Food Science and Technology, 44*(10), 2119–2125.

Vazquez-Araujo, L., Chambers, E., IV, Adhikari, K., & Carbonell-Barrachina, A. A. (2010). Sensory and physicochemical characterization of juices made with pomegranate and blueberries, blackberries, or raspberries. *Journal of Food Science, 75*(7), S398–S404. http://dx.doi.org/10.1111/j.1750-3841.2010.01779.x.

Vazquez-Araujo, L., Koppel, K., Chambers, E., IV, Adhikari, K., & Carbonell-Barrachina, A. A. (2011). Instrumental and sensory aroma profile of pomegranate juices from the USA: Differences between fresh and commercial juice. *Flavour and Fragrance Journal, 26*(2), 129–138.

Yi, Z., Feng, T., Zhuang, H., Ye, R., Li, M., & Liu, T. (2016). Comparison of different extraction methods in the analysis of volatile compounds in pomegranate juice. *Food Analytical Methods, 9*, 2364–2373. http://dx.doi.org/10.1007/s12161-016-0410-0.