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Volatile, anthocyanidin, quality and sensory changes in rabbiteye blueberry from whole fruit through pilot plant juice processing

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Abstract

BACKGROUND: High antioxidant content and keen marketing have increased blueberry demand and increased local production which in turn mandates new uses for abundant harvests. Pilot scale processes were employed to investigate the anthocyanidin profiles, qualitative volatile compositions, and sensorial attributes in not-from-concentrate (NFC) 'Tifblue' rabbiteye blueberry juices.

RESULTS: Processing prior to pasteurization generally resulted in increased L^* and hue angle color, while a^*, b^*, and C^* decreased. After 4 months pasteurized storage, non-clarified juice (NCP) lost 73.8% of total volatiles compared with 70.9% in clarified juice (CJP). There was a total anthocyanidin decrease of 84.5% and 85.5% after 4 months storage in NCP and CJP, respectively. Storage itself resulted in only 14.2% and 7.2% anthocyanidin loss after pasteurization in NCP and CJP. Storage significantly affected nine flavor properties in juices; however, there were no significant differences in the blueberry, strawberry, purple grape, floral, sweet aroma, or sweet tastes between processed and stored juices.

CONCLUSIONS: NFC pasteurized blueberry juices maintained desirable flavors even though highly significant volatile and anthocyanidin losses occurred through processing. Maintenance of color and flavor indicate that NFC juices could have an advantage over more abusive methods often used in commercial juice operations.

Keywords: anthocyanidins; flavor; liquid chromatography; *Vaccinium ashei*; volatiles

INTRODUCTION

Diets rich in blueberries deliver anti-inflammatory, anticarcinogenic, and antimutagenic components that help protect the brain, cardiovascular and central nervous system, as well as reduce cancer, obesity and type 2 diabetes.[1–4] Recently, juice manufacturers, marketers and trade publications have taken advantage of the antioxidant status and health claims revolving around fruits termed superfruits and their perceived health benefits which, have increased demand. Subsequently, North American US blueberry production acreage has increased significantly over the past few years in response to high demand and prices. From 2002 to 2012 in the US there has been a 2.2-fold increase in cultivated and wild blueberry production and utilization from 113.8 million kg to 250.8 million kg.[5] More important than production, during this same period the US value of utilized cultivated blueberries increased over four-fold from US$194.6 million to $781.8 million.[6] Likewise, during this same period, the south-eastern US (Alabama, Arkansas, Florida, Georgia, Mississippi and North Carolina) has sustained a 3.6-fold increase in cultivated blueberry production and utilization from 17.1 million kg to 62.2 million kg.[5] In 2014, US cultivated yield and value for fresh and processed blueberries was 256.0 million kg, valued at $824.9 million.[7] The blueberry industry in Georgia has experienced phenomenal growth (with 41.7 million kg produced in 2014), and is on the verge of becoming the leading US producing state.[8]

Statistics by the US Department of Agriculture (USDA) indicate per capita fruit juice consumption spiked around 34.8 L year^{-1} in 1999, and have steadily declined to approximately 22.7 L year^{-1} in 2012.[9] However, around 2003, the USDA's Economic Research Service (ERS) began omitting data supplied by the Beverage Marketing Corporation (BMC, New York, NY, USA), which coincides with increased market shares for 100% juices containing 'superfruits' and the inclusion of fruit and vegetable blended juices and smoothies. According to the BMC, sales of 100% fruit juices doubled from 2003 to 2008 and comprised 5.2% of the fruit beverage market,[10] 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.[11]

Significant anthocyanin loss and/or polymerization can occur in processed northern highbush (NHB) blueberry juice and

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products,[12−17] and likewise, in rabbiteye (RAB).[18] Purported health promoting polyphenolic compounds in blueberry are susceptible to chemical polymerization and/or degradation during processing.[3,12−14] Enzymes, along with heat, can contribute to the breakdown of associated pectin, phenolic, and lipid components that are known to create bitter/astringent flavors and lead to precipitation and color instability during storage.[19] Consumer dissatisfaction with flavor seems common in shelf-stable and/or new to the market flash pasteurized juices and smoothies containing some 'superfruits'.[20,21] Few articles are published illustrating the volatile compounds in RAB blueberries[22−24] and studies are scant regarding relationships between flavor volatiles and bitter/astringent compounds, processing, and sensory attributes related to RAB blueberries.[18,25]

Few studies have focused on not-from-concentrate (NFC) blueberry juice using large-scale pilot plant equipment, hydraulic pressing, ultra-filtration and high temperature short time (HTST) pasteurization, with a focus on quantified anthocyanidins and flavor. In light of blueberry demand, over-production and flavor issues often encountered in high phenolic-containing fruits, we investigated value-added means by which to extend the 'harvest window' and market potential using frozen local RAB blueberries to produce NFC juices. We believe that certain qualitative attributes in an NFC juice might endure less physico-chemical abuses that are often encountered in industrial juice processes. Subsequently, the objectives of this study were to investigate the anthocyanidins, qualitative, and volatile compositions and sensorial attributes in ultra-filtered and non-filtered 'Tifblue' blueberry juices prepared in an industrial-like manner, and stored as pasteurized product for up to 4 months.

MATERIALS AND METHODS

Blueberries and juice mash production

Commercially ripe *Vaccinium ashei* cv. 'Tifblue' (RAB) were harvested by Blue River Farms, LLC (Mt Olive, MS, USA), sorted, graded, cleaned, washed and boxed (13.6 kg) for commercial blast freezing with forced air (−23 to −29 °C for 72 h) and stored at −20 °C (Nordic Cold Storage, Hattiesburg, MS, USA). Within 1 week of commercial harvest and freezing, fruit were shipped on dry ice to the USDA Southern Regional Research Center (SRRC), and stored at −20 °C until processed 4 days later.

Frozen 'absolute control' (FAC) blueberries (27 kg) were placed into a 37.9 L steam jacketed kettle (SJK; Groen-A Dover Industries Co. Byram, MS, USA) with steam delivered at ∼207 kPa to rapidly heat fruit under constant manual mixing with 91.4 cm long wooden stirring paddles (#360; American Metalcraft, Inc., Franklin Park, IL, USA) to eliminate contact burning. Mash temperature was monitored with probes ('K Milkshake'; ThermoWorks, Salt Lake City, UT, USA) and steam stopped when 95 °C was attained. Then mash was held 3 min, and crude mash (CM95) evacuated to large 37.9-L holding vessels which were room cooled (2 h) to 55 °C for the addition of pectinase enzyme. Rohapect® 10 L (AB Enzymes, Darmstadt, Germany) was added at 200 mL ton^{-1} with occasional stirring during 1 h cooling (to ∼45 °C). A negative alcohol precipitation test was used to assess pectin stability by adding 5 mL juice to 15 mL of HCl-acidified ethyl alcohol and observing the mixture for 5 min for gel formation.[26] The experiment was repeated three times.

Juice pressing

A single-layer hydraulic press (X-1; Goodnature, Orchard Park, NY, USA) was used on the mash at 12.4 MPa using medium-weave polyester mesh sacks (Goodnature, #2636), resulting in not-from-concentrate (NFC) non-clarified juice (NCJ) and press cake.

Juice ultra-filtration and pasteurization

A portion of the NCJ was ultra-filtered on a BRO/BUF pilot unit (Membrane Specialists, Hamilton, OH, USA) with a 200 000 molecular weight cut-off (0.2 µm) polyvinylidene fluoride (PVDF) membrane (XP-201; ITT PCI Membrane Systems, Zelienople, PA, USA), as previously described.[27] The filter area (0.864 m^2) is approximately half the linear distance (21.6 m) often encountered in commercial juice operations. The 5.59 kW, 1800 rpm pump motor was run at ambient (∼25 °C) with an approximate inlet and outlet pressure of 620.5 and 137.9 kPa, respectively, with an inlet and outlet flow rate of between 18.9 and 29.9 L min^{-1}. Retentate was recirculated through the system to equilibrate the membrane, with comparison of the initial juice color and °Brix of the filtered juice for roughly 20 min. After running in filtration mode with several liters (∼15 L) going to drain, clarified ultra-filtered (CUF) NFC blueberry juice was collected for sampling and pasteurization.

NCJ and CUF juice were pasteurized on a MicroThermics Electra UHT/HTST Lab-25EDH (Raleigh, NC, USA), resulting in non-clarified pasteurized (NCP) and clarified juice pasteurized (CJP). Conditions were 90 °C for 10 s at 1.2 L m^{-1}, followed by hot-filling into pre-sterilized 250- mL transparent media bottles (Corning, Tewksbury, MA, USA) in the MicroThermics clean fill hood at 85 °C. Bottles were capped, inverted for ∼10 s then chilled in an ice water bath (∼0 °C) for 5 to 10 min prior to storage in the dark at 4 °C for 0, 1, 2 and 4 months; resulting in NCP0, NCP1, NCP2, NCP4 and CJP0, CJP1, CJP2 and CJP4 samples.

Juice quality appraisals

Brix was measured using a refractometer (Atago Pocket PAL-1, Tokyo, Japan). pH and titratable acidity (TA) were measured with an automated titrator (Metrohm 836 Titrando, Riverview, FL, USA) with 10 mL of juice, diluted for TA in 50 mL of MilliQ H_2O, carried to an endpoint pH of 8.2 using 0.1 mol L^{-1} sodium hydroxide, expressed as % citric acid (wt/wt). Color was measured in 20 mL juice placed in a glass 20 mL Petri dish lid, placed atop the meter, covered with a black lid using a Konica Minolta CR400 Chroma Meter (Ramsey, NJ, USA) and analyzed with Konica SpectraMagic NX lite software.

GC-MS volatile analysis

Headspace volatile samples were prepared in triplicate from all processing stages and pasteurized sample bottles after freezing samples at −20 °C. Analysis was performed similar to a previous report[24] using 1-cm 50/30 µm stableflex divinylbenzene carboxen polydimethylsiloxane (DVB/Carboxen/PDMS; Supelco, Bellefonte, PA, USA) solid phase microextraction (SPME) fibers in 20-mL sample vials with 10 mL juice, saturating NaCl (2.2 g) and an internal standard (3-carene, CAS# 13466-78-9, 100 µg L^{-1} final concentration) was used with an autosampler (MPS2 XL; Gerstel, Inc., Baltimore, MD, USA) on a GC-MS (HP6890/5973, Agilent Technologies, Santa Clara, CA, USA) with a HP-5 cross-linked 5% phenyl methyl silicone column, 30 m × 0.25 mm × 0.25 µm film thickness (Agilent Technologies). Data were collected (HP ChemStation A.03.00 software; Agilent Technologies), searched against the Wiley 7th/NIST02 registry then identified by library search and confirmed by standards, GC retention time (RT), MS ion spectra and an in-house retention index (RI).[24] GC-MS grade (> 99.5% purity) standards were obtained from Aldrich (Madison, WI, USA), Aaper Alcohol and Chemicals (Shelbyville, KY, USA), Bedoukian (Danbury, CT, USA), Berje (Carteret, NJ, USA), Fluka (Madison, WI, USA), Fisher (Pittsburgh, PA, USA), Mallinckrodt (St Louis, MO, USA), Sigma (Madison, WI, USA) and SAFC (Madison, WI, USA).

Anthocyanidin sample preparation and UPLC analysis

Juice samples were freeze dried (Virtis Genesis 25ES; SP Scientific, Warminster, PA, USA) and hydrolyzed for five blueberry anthocyanidins using similar conditions of an ultra-performance liquid chromatography (UPLC-TUV) method,[28] as modified.[29] Hydrolysates were analyzed by UPLC (Waters Aquity; Waters Corporation, Milford, MA, USA) using an Acquity BEH C_{18} column (50 mm × 2.1 mm × 1.7 µm) and a tunable ultra-violet (TUV) detector at 525 nm, controlled by Empower software (Waters Corporation). Anthocyanidin standards (Chromadex, Santa Ana, CA, USA) were run in a logarithmic concentration gradient (0.001, 0.003, 0.010, 0.030 and 0.100 mg mL^{-1}; $r^2 = 0.995$) to report anthocyanidins (g kg^{-1}).

Sensory evaluation

A group of ten trained panelists with 1–20 years of experience used a universal intensity scale (0–15) for all foods using a validated blueberry lexicon.[30] After six practice sessions utilizing previously published references to describe blueberry aroma and flavor,[30] panelists evaluated the intensity of 22 flavor attributes, three taste attributes, and six mouth feeling factors in ~50 mL blueberry juice. A store-bought bottled blueberry juice (Just Blueberry; Knudsen & Sons, Inc. Chico, CA, USA) was presented first as a warm-up, followed by three randomly ordered experimental samples each day. After four months storage, three more practice sessions with references were performed prior to evaluating pasteurized samples in three sessions. Hand-pressed juice from frozen blueberries (FAC), prepared according to Bett-Garber and Lea[30] from the same fruit lot, were used as juice controls.

Statistics

There were nine experimental units per treatment, representing the three repeated SJK, press, UF batches and pasteurization with three true replicates each, as individual 250 mL bottles. For all parameters, analysis of variance was done with SAS Enterprise Guide, v 5.1 Proc Mixed procedure (SAS Inc., Cary, NC, USA). Mean comparison was accomplished with least square means with Tukey's adjustment at $P < 0.05$. Sensory scores were digitally entered (Compusense five 4.6-SP3, Guelph, Ontario, Canada) and analysis of variance (ANOVA) was accomplished with Procedure Mixed (SAS Enterprise Guide 5.1). UF processing and storage were the main sensory effects and LS Means analysis was utilized to compare means within an effect. A factorial ANOVA was used to compare the ultra-filtered (CJP) and non-filtered (NCP) with both storage times, and the interaction between filtered and non-filtered juice and storage at 0 and 4 months.

RESULTS AND DISCUSSION

The SJK temperature rose above the desired set-point (95 °C) during processing and maxed at 97.8 ± 0.1 °C, with the average high temperature of 96.5 ± 1.1 °C (Fig. 1). The system took 14.5 ± 0.1 min to stabilize and the total heating time was 17.5 ± 0.1 min. The blueberry juice extraction method attained 74.0 ± 0.9% free juice recovery, 13.1 ± 0.5% lost juice and 13.0 ± 0.6% press cake. Technically, total juice recovery could approach 87.0 ± 0.6% if 'lost juice' due to weighing every container/vessel, additional transfers and using new/clean press sacks did not occur. This matches the yields of 87.8% in individually quick-frozen (IQF) blueberries run through SJK at 75 °C prior to pressing[31] and 74–83% in frozen NHB berries processed at 95 °C in SJK followed by pectinase, pressing then centrifuge clarification and HTST pasteurization.[15] Comparable yields of 79–81% have also been reported with six NHB IQF berry varieties subjected to steam blanching, milling, depectinization, pressing then HTST pasteurization.[17]

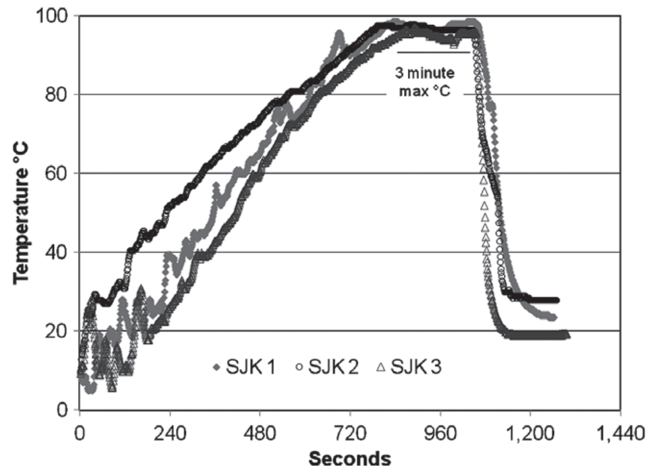

Figure 1. Temperature plots recorded during 'Tifblue' blueberry mash treatments in a steam jacketed kettle (SJK).

Juice quality appraisals

Prior to pasteurization, the initial stages of processing generally had significantly higher color levels (L^*, a^*, b^* and C^*), except for hue angle. For example, the FAC and CM95 were significantly different and greater than all other processing stages in a^*, b^* and chroma (C^*) (Table 1). The maximum temperature attained during processing (CM95, 95 °C) altered color significantly as compared to FAC control berries. Processing prior to pasteurization generally resulted in L^* and hue angle increases, and a^*, b^*, and C^* decreases. Color was occasionally significantly lower after pressing (NCJ) and ultra-filtration (CUF). However, pasteurization (NCP and CJP) generally resulted in the most marked and significant color loss, except hue angle. Albeit generally significantly lower than initial processing stages prior to pasteurization, there were irregular trends in color values for pasteurized samples through storage (4 months), regardless of filtration (Table 1).

During many berry and polyphenolics-rich juice operations, mash is heated then pressed or pressing is done hot followed by skin and seed removal, resulting in an unfiltered juice. In these steps, color and polymeric compound extraction are desirable. However, phenolic compounds interact with proteins to form complexes and clarification steps are commonly used to reduce haze, astringency, color change and sedimentation. Thus, various methods of filtration are used to reduce sedimentation, clarify juice and remove polymeric compounds which can affect overall color, turbidity and sedimentation.[26] Our color data reflect that possible polyphenolic polymerization appears to have minor perceptible color change in NFC juice after pasteurization and through storage. pH change and oxidation (browning) were minimal, likely due to

Table 1. Juice quality appraisals in 'Tifblue' rabbiteye blueberry at various processing stages

Treatment	L^*	a^*	b^*	C^*	Hue angle	°Brix	pH	TA	Brix:acid
FAC	19.3^{b}	5.6^{a}	1.3^{a}	5.8^{a}	12.8^{b}	12.7^{d}	3.1^{efg}	0.3^{g}	40.3^{a}
CM95	23.3^{a}	3.0^{b}	0.6^{b}	3.1^{b}	11.6^{b}	14.2^{bc}	3.3^{a}	0.4^{cde}	36.0^{cde}
NCJ	23.0^{a}	1.2^{d}	0.3^{c}	1.2^{d}	15.5^{ab}	14.1^{bc}	3.2^{b}	0.4^{cd}	33.9^{ef}
CUF	23.4^{a}	0.9^{de}	0.2^{cd}	0.9^{de}	14.7^{ab}	14.0^{c}	3.2^{cde}	0.4^{cde}	34.5^{def}
NCP0	16.5^{cd}	0.4^{f}	0.0^{e}	0.4^{g}	12.5^{b}	14.7^{a}	3.2^{cd}	0.4^{c}	33.3^{f}
NCP1	17.3^{c}	1.7^{c}	0.2^{cd}	1.7^{c}	8.2^{b}	14.7^{a}	3.1^{fg}	0.4^{de}	37.7^{abc}
NCP2	17.4^{c}	1.6^{c}	0.3^{c}	1.6^{c}	11.1^{b}	14.7^{a}	3.1^{g}	0.4^{ef}	39.1^{ab}
NCP4	15.1^{e}	1.0^{d}	0.2^{cd}	1.1^{d}	15.5^{ab}	14.5^{ab}	3.1^{g}	0.9^{a}	15.5^{g}
CJP0	16.1^{d}	0.9^{de}	0.0^{e}	0.9^{de}	3.4^{b}	14.0^{c}	3.2^{bc}	0.4^{ef}	38.9^{ab}
CJP1	17.0^{cd}	0.5^{f}	0.0^{e}	0.5^{fg}	3.1^{b}	13.9^{c}	3.1^{def}	0.4^{ef}	37.0^{bcd}
CJP2	17.2^{c}	0.5^{f}	0.1^{de}	0.6^{fg}	11.6^{b}	14.0^{c}	3.1^{defg}	0.4^{f}	39.3^{ab}
CJP4	16.5^{cd}	0.6^{ef}	0.3^{c}	0.7^{ef}	25.9^{a}	13.9^{c}	3.2^{b}*c*	0.9^{b}	15.3^{g}
Avg.	18.5	1.5	0.3	1.5	12.2	14.1	3.2	0.5	33.4
Max	23.4	5.6	1.3	5.8	25.9	14.7	3.3	0.9	40.3
Min	15.1	0.4	0.0	0.4	3.1	12.7	3.1	0.3	15.3

FAC = Frozen absolute control berries; CM95 = crude mash (liquid decant, non-cellular) after the 95 °C heat and 3-min hold; NCJ = non-clarified juice after the Goodnature press; CUF = clarified ultrafiltered juice; NCP = non-clarified, pasteurized not-from-concentrate (NFC) juice; CJP = clarified, pasteurized NFC juice and 0, 1, 2 and 4 indicate months of storage.
TA, titratable acidity, expressed as % citric acid (wt/wt).
LSM (least squares means, SAS ANOVA, $\alpha = 0.05$) with the same letter per column are not significantly different.

the fact that the pigment chromophore status and color is not altered through polymerization.[32]

The initial °Brix level in the 'Tifblue' fruit was 12.7 ± 0.5 (Table 1). This was within range[33,34] or slightly lower than commonly reported values[25,35] for RAB. Heat treatment and enzymatic liquefaction possessing increased markedly °Brix levels, with the unfiltered (NCP) juices having the significantly highest levels measured. Processing resulted in significant increases in °Brix (Table 1) but since this was an NFC product that did not involve concentrating and reconstituting, the °Brix of the 100% single strength juice was not adjusted down to the minimum US requirement (10°Brix).[36] There was no consistent trend observed with regard to pH changes from the initial fruit (3.12 ± 0.06), or as filtered versus non-filtered stored juices. Initial acid levels (0.32 ± 0.01) approximated those found in other 'Tifblue'.[25,34] Both pasteurized juices (NCP and CJP) had significantly higher TA after 4 months storage (Table 1). Likewise the sugar:acid ratio reflected a 4-months trend, as those values were significantly lower than all other stages. The ratio for control (40.3 ± 2.6) and processed fruits (33.3 – 38.9) was within the wide range (although often on the high side) for previously reported blueberries,[33,37] on par with other RAB[35] yet lower compared to another 'Tifblue' report.[25] However, higher and lower sugar:acid ratios have been reported in highbush blueberry types (RAB, southern highbush, SHB and NHB) and are occasionally not reliable predictors of consumer acceptance.[25,33,34,37]

Volatile changes during processing and storage

Twenty-one compounds [ethanol, ethyl acetate, ethyl 3-methylbutyrate, 3-methylbutyl acetate, (*Z*)-dehydroxylinalool oxide, (*E*)-dehydroxylinalool oxide, *p*-cymene, isocineole (1,4-cineole), limonene, eucalyptol (1,8-cineole), (*Z*)-linalool oxide, (*E*)-linalool oxide, linalool, 2-phenethyl alcohol, δ-2-carene, (*Z*)-geraniol (nerol), (*E*)-geraniol, vitispirane (tentative), (*E*)-β-damascenone and two unknowns] were integrated. Fifteen compounds and total volatiles were highest in FAC and significantly greater than all other treatments (Fig. 2). Heating caused ethyl acetate and phenylethyl alcohol (Table 2) and 3-methylbutyl acetate and vitispirane (data not shown) to increase compared to the FAC. Significant total volatile loss occurred in CM95 (66.1%) and CM95 was also significantly lower (5.0%) than NCJ, indicating additional losses through pressing. This is likely directly due to the fact that samples were collected while hot, and vapor pressure or flash point effects had driven off some volatiles. NCJ volatile recovery was significantly higher (7.3%) than CUF, indicating that processing through UF again stripped away compounds. Volatile loss generally continued through pasteurized storage, as the significantly lowest total volatile amounts were recovered after 2 and 4 months storage (Fig. 2). After pasteurization, not all compounds exhibited significant losses through 4 months storage (Table 2, not all data shown). In general, several compounds that had very large recoveries in FAC (e.g. ethyl 3-methylbutyrate, *p*-cymene, (*Z*)-linalool oxide, (*E*)-linalool oxide, δ-2-carene, (*E*)-geraniol and (*E*)-β-damascenone) had the most dramatic significant losses during mash, enzyme treatment and ultra-filtration but seldom had radical additional or markedly significant decreases with pasteurization and storage (Table 2, not all data shown). Volatile compounds which contribute to the blueberry aroma (ethyl acetate and linalool) decreased with processing and storage (Table 2). The change in relative amount of ethyl acetate, a sweet aroma associated with freshness, showed a major decrease in concentration with processing, with an overall 71.7% and 60.5% decrease through 4 months storage in NCP and CJP, respectively. On the other hand, linalool, a sweet floral aroma generally believed to be one of the key contributors to blueberry aroma, showed marked losses with processing and only a slight reduction in pasteurized storage.

Certain compounds such as (*E*)-2-hexenol (leaf, green, wine, fruit), (*E*)-2-hexenal, (*Z*)-3-hexenol (grassy) and α-terpineol (anise, mint, lilac, floral), previously designated as important in RAB,[22] were infrequently or not recovered. This could be due to berry freezing, thawing, or rapid loss during SJK processing. Linalool

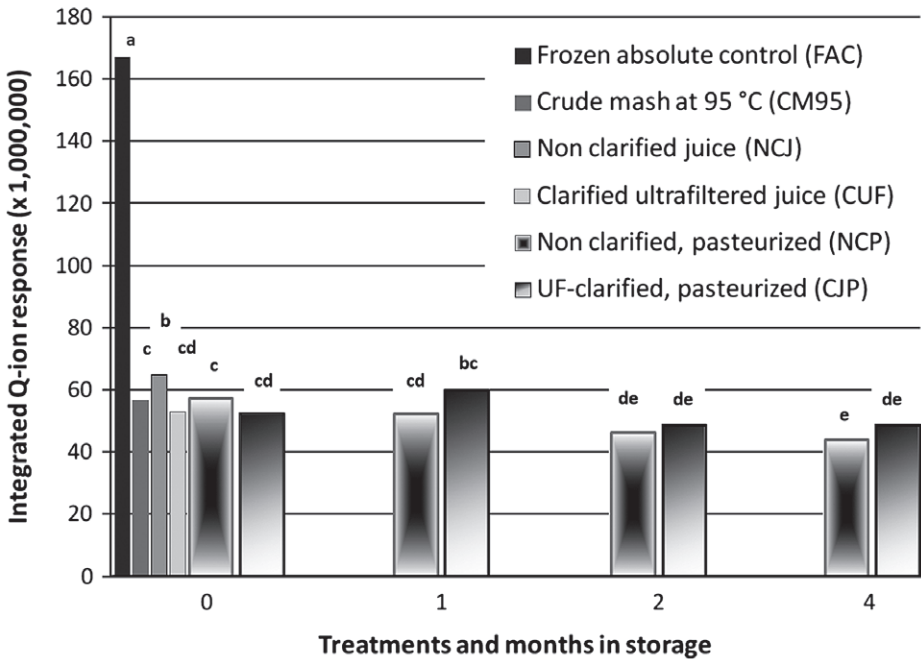

Figure 2. Total volatiles in pilot plant not-from-concentrate 'Tifblue' blueberry juice during processing, HTST pasteurization and storage. Least squares means ($\alpha = 0.05$) with the same letter are not significantly different.

Table 2. Select averaged compound responses (integrated on unique MS qualifying ions $\times 10^6$) in 'Tifblue' rabbiteye blueberry at various juice processing stages

Treatment	Ethanol	Ethyl acetate	Ethyl 3-methylbutyrate	Eucalyptol	(*E*)-Linalool oxide	Phenylethyl alcohol	Linalool	(*E*)-Geraniol
FAC	11.51^{a}	0.60^{e}	42.00^{a}	1.43^{a}	4.61^{a}	0.05^{f}	46.80^{a}	8.89^{a}
CM95	7.27^{b}	1.01^{a}	12.32^{b}	0.16^{bc}	0.80^{d}	0.13^{bcd}	16.13^{bc}	3.23^{b}
NCJ	7.45^{b}	1.10^{a}	12.37^{b}	0.21^{b}	0.95^{cd}	0.11^{cde}	16.97^{b}	3.10^{bc}
CUF	6.41^{cd}	0.78^{bc}	6.61^{de}	0.16^{bc}	0.88^{cd}	0.09^{e}	14.24^{bcd}	2.21^{ef}
NCP0	7.36^{b}	0.83^{b}	6.15^{def}	0.17^{bc}	1.17^{bcd}	0.12^{cde}	14.21^{bcd}	2.78^{bcde}
NCP1	6.09^{d}	0.72^{cd}	9.10^{c}	0.12^{cde}	1.25^{bc}	0.14^{bc}	12.14^{d}	2.46^{cdef}
NCP2	4.19^{f}	0.37^{f}	7.93^{cd}	0.13^{cde}	1.06^{cd}	0.11^{de}	11.83^{d}	1.94^{f}
NCP4	4.62^{f}	0.17^{g}	3.90^{gh}	0.07^{e}	0.86^{cd}	0.13^{bcd}	11.15^{d}	2.77^{bcde}
CJP0	6.53^{cd}	0.85^{b}	9.68^{c}	0.14^{cd}	0.86^{cd}	0.10^{de}	13.24^{cd}	2.33^{def}
CJP1	6.75^{c}	0.68^{de}	5.47^{efg}	0.15^{bc}	1.54^{b}	0.19^{a}	13.73^{cd}	3.49^{b}
CJP2	4.50^{f}	0.44^{f}	4.45^{fgh}	0.14^{cd}	1.08^{cd}	0.11^{de}	12.56^{d}	2.19^{ef}
CJP4	5.33^{e}	0.24^{g}	2.60^{h}	0.09^{de}	1.07^{cd}	0.15^{b}	11.45^{d}	3.05^{bcd}
Avg.	6.50	0.65	10.22	0.25	1.34	0.12	16.20	3.20
Max	11.51	1.10	42.00	1.43	4.61	0.19	46.80	8.89
Min	4.19	0.17	2.60	0.07	0.80	0.05	11.15	1.94

FAC = Frozen absolute control berries; CM95 = crude mash (liquid decant, non-cellular) after the 95 °C heat and 3-min hold; NCJ = non-clarified juice after the Goodnature press; CUF = clarified ultrafiltered juice; NCP = non-clarified, pasteurized not-from-concentrate (NFC) juice; CJP = clarified, pasteurized NFC juice and 0, 1, 2 and 4 indicate months of storage.
LSM (least squares means, SAS ANOVA, $\alpha = 0.05$) with the same letter per column are not significantly different.

oxides (flower) increased slightly during storage yet these changes were seldom significant (Table 2). Other compounds like eucalyptol (sweet, mint, herbal, eucalyptus, camphor) significantly decreased by 4 months in storage. Two recent reports have illustrated 11 or 33 possibly important consensus compounds in blueberries[24,37] but, only nine of those compounds were recovered herein. However, we are not considering probable method and berry type differences. (*E*)-geraniol (rose, geranium), another 'important' volatile, actually increased slightly by the end of storage after 4 months although the trends were not clear-cut. (*E*)-Geraniol is not always recovered in blueberry, even in the same SHB types.[38] Interestingly, a recent report has negatively associated linalool with liking in SHB,[39] even though GC-O and sensory work previously indicated linalool as important in NHB,[40] SHB[38] and RAB.[22] Regardless of which compounds are 'important', 61.1% and 68.4% of the total compounds were lost through initial stages of juice processing (FAC → NCJ and FAC → CUF), respectively. Pasteurization resulted in greater volatile losses in non-clarified juice (11.5%) compared to UF-clarified juice (0.8%) which is logical since clarification had already stripped some volatiles. Yet only

Table 3. Average UPLC anthocyanidin concentrations (g kg^{-1}) in 'Tifblue' rabbiteye blueberry at various juice processing stages

Treatment	Delphinidin	Cyanidin	Petunidin	Peonidin	Malvidin	Total
Press cake	0.557^{a}	0.777^{a}	0.565^{a}	0.160^{a}	0.597^{a}	2.656^{a}
FAC	0.137^{b}	0.147^{b}	0.259^{b}	0.041^{b}	0.266^{b}	0.851^{b}
CM95	0.119^{b}	0.114^{c}	0.103^{c}	0.027^{c}	0.121^{c}	0.484^{c}
NCJ	0.067^{c}	0.067^{d}	0.058^{cd}	0.016^{cd}	0.072^{d}	0.280^{d}
CUF	0.047^{cde}	0.049^{de}	0.043^{d}	0.012^{d}	0.057^{d}	0.209^{de}
NCP-0	0.053^{cd}	0.061^{d}	0.053^{d}	0.016^{cd}	0.070^{d}	0.253^{de}
NCP-1	0.054^{c}	0.046^{de}	0.038^{d}	0.011^{d}	0.049^{d}	0.198^{de}
NCP-2	0.041^{cde}	0.046^{de}	0.040^{d}	0.011^{d}	0.053^{d}	0.191^{de}
NCP-4	0.032^{de}	0.032^{e}	0.026^{d}	0.007^{d}	0.034^{d}	0.132^{e}
CJP-0	0.048^{cd}	0.043^{de}	0.037^{d}	0.010^{d}	0.046^{d}	0.184^{de}
CJP-1	0.036^{de}	0.039^{de}	0.033^{d}	0.010^{d}	0.044^{d}	0.161^{de}
CJP-2	0.031^{de}	0.034^{e}	0.029^{d}	0.008^{d}	0.038^{d}	0.140^{e}
CJP-4	0.027^{e}	0.030^{e}	0.026^{d}	0.007^{d}	0.033^{d}	0.124^{e}
Avg. (−cake)*	0.058	0.059	0.062	0.015	0.074	0.267
Max (−cake)	0.137	0.147	0.259	0.041	0.266	0.851
Min (−cake)	0.027	0.030	0.026	0.007	0.033	0.124

FAC = Frozen absolute control berries; CM95 = crude mash (liquid decant, non-cellular) after the 95 °C heat and 3-min hold; NCJ = non-clarified juice after the Goodnature press; CUF = clarified ultrafiltered juice; NCP = non-clarified, pasteurized not-from-concentrate (NFC) juice; CJP = clarified, pasteurized NFC juice and 0, 1, 2 and 4 indicate months of storage.
LSM (least squares means, SAS ANOVA, $\alpha = 0.05$) with the same letter per column are not significantly different.
*Press cake values are not considered in the average, maximum and minimum values reported

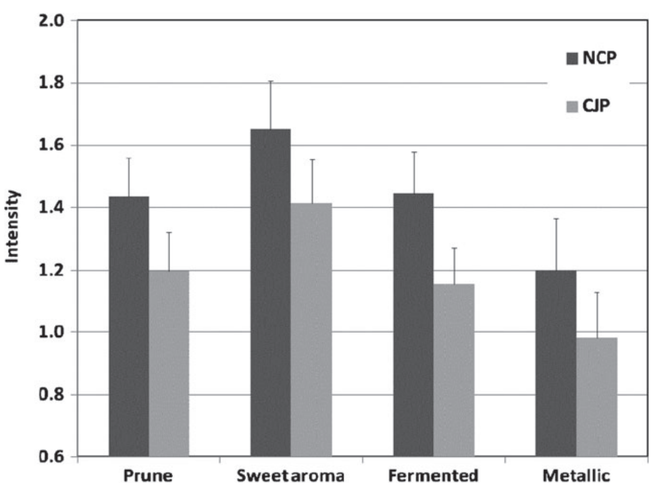

Figure 3. Sensory attribute means and standard errors for differences between pasteurized clarified juice (CJP) and non-clarified pasteurized (NCP) 'Tifblue' blueberry juices. Illustrated attribute means are significantly different (Pr > F of 0.05).

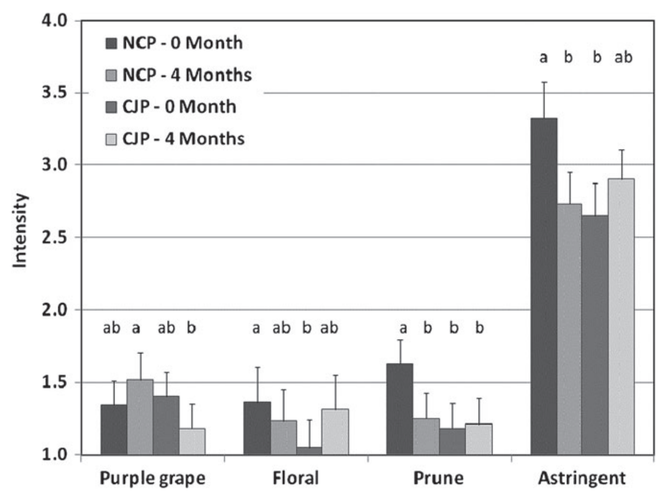

Figure 4. Sensory attribute differences and standard errors between day zero HTST pasteurized clarified juice (CJP) and non-clarified pasteurized (NCP), and 4-month stored 'Tifblue' blueberry juices. Mean significant differences (Pr > F of 0.05) are denoted by different letter designations.

an additional 12.7% and 2.5% was lost after 4 months storage in FAC → NCP and FAC → CJP, respectively. At the end of storage, NCP had lost 73.8% of total volatiles compared with 70.9% in CJP. However, these levels are still appreciable considering that we observed a large suite of compounds and higher relative levels of volatiles in freshly pressed 'Wonderful' pomegranate juices compared against commercial products.[27] Furthermore, a gently produced in-house concentrate and industrial 'Wonderful' concentrates contained fewer compounds,[27] at remarkably lower levels. In many processed berries, the stabilization of the product (e.g. heated mash, enzyme liquefaction and pasteurization) is required which unfortunately degrades intrinsic qualities, yet these losses are difficult to avoid.

Anthocyanidins

Petunidin and malvidin were the dominant anthocyanins in 'Tifblue' FAC (Table 3). However, through the initial heating, enzyme treatment and pressing, petunidin decreased substantially and malvidin was generally the dominant anthocyanidin throughout processing portions of the experiment. The dominant anthocyanin varies between various NHB and SHB varieties and processes.[15,41] Several reports have documented the concentration of phenolic compounds and anthocyanins normally found in all highbush blueberry types vary markedly based on method and berry type, and is variety-dependent.[42–44] Total anthocyanidins in FAC 'Tifblue' were 0.851 g kg^{-1} fresh weight (FW) (Table 3). Using the same UPLC methods reported herein, we observed a wide range

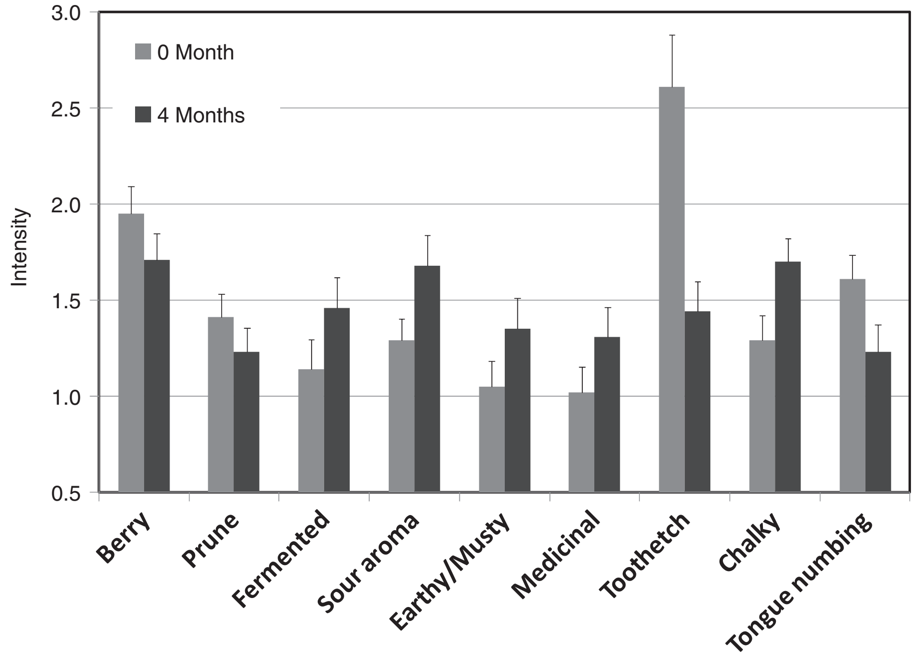

Figure 5. Combined sensory attribute means and standard errors of interaction effects between processing and storage in freshly HTST pasteurized and stored (4 °C) 'Tifblue' blueberry juices. Illustrated attribute means are significantly different (Pr > F of 0.05).

of total anthocyanidins in six different SHB and RAB from 0.86 to 2.72 g kg^{-1} FW (data not shown). 'Tifblue' total anthocyanins have been reported ranging from 2.31,[43] 2.10,[45] 1.16[44] and 1.09[18] g kg^{-1} FW. Our 'Tifblue' anthocyanidin results were generally lower than previous anthocyanin reports. However, in general, blueberry results reported from a spectrophotometric method are higher in the same sample than results from methods quantifying the five aglycone backbones.[15,41,44] The spectrophotometric anthocyanin method[32,46] versus detailed HPLC-MS analyses can deliver differing results from the same sample, but blueberry data tend to be closely correlated, indicating both approaches are reliable.[44,47]

Total anthocyanidin concentration decreased substantially with processing and storage in both NCP and CJP juices (Table 3). With few exceptions, this trend was also observed in individual anthocyanidins. There was a 67.1% and 75.5% total anthocyanidins decrease through initial stages of juice processing (FAC → NCJ and FAC → CUF) and clarified juices consistently contained fewer anthocyanidins than the non-clarified juice. Pasteurization resulted in greater anthocyanidin changes in CJP (11.6%) compared to NCP (9.8%) which, was contrary to volatile losses. Storage itself only resulted in 14.2% and 7.2% anthocyanidin loss for NCP-0 → NCP-4 and CJP-0 → CJP-4, respectively (Table 3). There was a linear decrease in total anthocyanidins from crude NFC juices through pasteurization and storage. For example, after 4 months storage: NCJ → NCP (y as g kg^{-1} $= -3.59x + 31.83$, $R^2 = 0.963$ and CUF → CJP ($y = -2.14x + 22.79$, $R^2 = 0.995$). In summary, there was a total anthocyanidin decrease of 84.5% and 85.5% after 4 months storage in NCP and CJP, respectively.

Several reports using NHB fruit to produce pasteurized juice are published; however, apparently only one report exists where RAB was utilized (with batch pasteurization).[18] Considering various processing regimes, somewhat similar to our methods, the range of anthocyanin losses varied from 27.5 to 58.8%,[12] 68.0%,[14] 76.2 to 79.1%,[18] 76.6 to 84.3%,[15] 77.4 to 88.9%[17] and 95.7%.[16] Low anthocyanins recovery in juices occur in lab and pilot experiments,[12,14,15] similar to our results, and remarkably low levels have also been found in commercial pomegranate juice.[48] Berry juice color change is generally thought to be a direct result of polymerization of lower molecular weight phenolics and/or inactivation of polyphenol oxidase as anthocyanins are 'lost' and polymeric compounds increase.[12,13,17] Reports indicate that processing and storage of blueberry juices leads to an increase in polymeric color as monomeric anthocyanins decreased during storage[12,31] and polymerized.[13,15,31,49] Meanwhile, ORAC values remained stable,[12] or increased.[31] Subsequently, as published findings indicate clearly that polymerization products remain in juices which, likely corresponded with retained color in stable pasteurized NCP and CJP juices, we simplified our color measurement using a color meter. Overall, marked anthocyanin 'losses' occur in processed blueberry juices and we did not prevent losses using heated SJK ('blanching') and UF with NFC juices.

Overall 'loss' of bioactive compounds in juice production is also a direct consequence of their abundance in either polymeric compounds or residual press cake.[3,12,14,15,50] The press cake (2.656 g kg^{-1}) contained over three-fold higher concentrations of total anthocyanidins compared to FAC, and press cake always contained the most significant concentration of individual anthocyanins compared to all processing treatments (Table 3). These results emanated from cake residual representing 13% of the total initial blueberry weight. This is a common finding in the aforementioned blueberry juice reports,[12,14,15] as pressing can lead to loss of anthocyanins as skins have the highest concentrations.[3,51] Obviously, results can vary based on moisture content of the press cake and extraction efficiency to get the compounds isolated from the cake. For example blueberry pomace can contain 25–50% of the procyanidins,[50] and up to >42% of the anthocyanins.[15]

Sensory effects in pasteurized juices

Ultra-filtration decreased the mean intensity values (including 0 and 4 months stored juice) for some typical processed blueberry juice flavors, as previously described[30] such as: prune,

Table 4. Sensory analysis of variance results (Pr > F) for processing effect (P) reflecting HTST clarified pasteurized (CPJ) versus non-clarified pasteurized (NCP), and storage (S) effect in stored 'Tifblue' blueberry juices

Flavor	Process	Storage	P x S
Blueberry	0.609	0.114	0.928
Berry	0.669	0.042	0.500
Strawberry	0.791	0.632	0.975
Cranberry	0.652	0.585	0.336
Purple grape	0.204	0.650	0.071§
Citrus	0.176	0.508	0.955
Floral	0.328	0.839	0.092§
Sweet aroma	0.093§	0.685	0.189
Molasses/dark corn syrup	0.232	0.389	0.676
Can tomato	0.184	0.516	0.194
Prune	0.024	0.058§	0.055§
Dried fruit	0.869	0.703	0.353
Fermented	0.040	0.048	0.883
Wine-like	0.424	0.306	0.965
Processed berry juice	0.511	0.905	0.203
Sour aroma	0.239	0.008***	0.618
Pungent aroma	0.419	0.819	0.922
Earthy/musty	0.259	0.017	0.855
Green/stems	0.112	0.715	0.536
Over-ripe fruit/straw-like	0.359	0.522	0.966
Tobacco	0.886	0.874	0.827
Medicinal	0.287	0.024	0.929
Sweet taste	0.247	0.723	0.450
Bitter taste	0.935	0.968	0.642
Sour taste	0.609	0.945	0.145
Astringent mouth feel	0.151	0.283	0.017
Metallic	0.093§	0.998	0.193
Toothetch	0.251	< 0.001***	0.948
Chalky	0.549	0.014	0.399
Tongue tingle	0.133	0.979	0.373
Tongue numbing	0.457	0.008***	0.194

***Means are significantly different at Pr > F of 0.05 to 0.01, and Pr > F <0.01, respectively.
§Means are markedly different, but not significantly different (Pr > F of 0.1).

fermented, metallic, and sweet aroma flavor (associated with fresh pressed juice flavor) also decreased (Fig. 3). In non-clarified pasteurized juice (NCP), purple grape flavor increased with storage, but decreased with storage in clarified pasteurized juice (CJP) (Fig. 4). Floral flavor and astringent mouthfeel decreased with storage in NCP juice, but increased with storage in the CJP (Fig. 4). Prune flavor decreased with storage in the NCP juice, but had only a slight increase with storage in the CJP (Fig. 4). For data including both CJP and NCP juice samples combined, berry and prune flavor significantly decreased during storage (Fig. 5). However, several other attributes which could lend to an inferior product, such as: molasses/dark corn syrup, can tomato, wine-like, processed berry juice, pungent aroma, green/stems, over-ripe fruit/straw-like, tobacco, bitter taste, sour taste, astringent mouth feel and tongue tingle, were not significantly different though storage (Table 4).

Storage at 4 °C for 4 months significantly affected nine flavor properties (berry, prune, fermented, sour aroma, earthy/musty, medicinal, toothetch, chalky and tongue numbing) in processed blueberry juice (Table 4). Four months storage caused a decrease in berry and prune flavors, toothetch, and tongue numbing (Fig. 5). Meanwhile, the attributes fermented, sour aroma, earthy/musty, medicinal, and chalky increased with storage. There was an interaction effect (Pr > 0.10) between processing (ultra-filtration CJP, and no clarification NCP), and storage effects in sensory attributes (purple grape, floral, prune) which significant affected astringent mouth feel attributes. Toothetch was interestingly very high on day 0 and decreased the most compared to all other sensory parameters after 4 months storage (Fig. 5).

Addition of sugars (glucose/fructose combinations) to a coarsely chopped partially deodorized tomato puree (acidic) generally weakened sour, citrus, and bitter taste descriptors, while adding acids (citric/malic combinations) decreased floral and sweet taste.[52] The dominant acid generally found in NHB is citric acid whereas RAB often contain higher amounts of succinic and malic acids.[53] Different proportions of organic acids affect sensory quality as the combination of citric and malic acids gives a sour taste, while succinic acid gives a bitter taste.[54] Lower molecular weight monomeric and polymeric phenolics (MW < 500) tend to be bitter whereas, higher molecular weight (MW > 500) polyphenolics are more likely to confer astringency.[55,56] Most previous blueberry sensory work has involved NHB varieties, and few sensory terms were assessed per study which, were insufficient to describe fully sensory changes through juice processing steps.[30] Perhaps the much sweeter NFC juices (approximately 4.0 to 4.5°Brix higher than reconstituted commercial US juices at 10°Brix) combined with anthocyanin decreases and a different acid profile (as compared to former NHB juice reports) may contribute to and/or explain how sensory dissatisfaction of the NFC juices herein was apparently masked. This assumption is made as there was high perception and significant differences in sour, tongue numbing, toothetch and interaction effects for astringent (Table 4). Yet, there were no significant differences in blueberry, strawberry, purple grape, floral, sweet aroma, and sweet taste between ultra-filtered and stored juices, indicating the juice maintained desirable flavors. On the contrary, some of the non-blueberry juice type flavors increased with storage, such as fermented, sour aroma, earthy/musty, and medicinal flavors. Yet, similar tendencies are common to pasteurized juice products.

Although this experiment did not formally compare NFC pilot plant pasteurized juices to commercial juices, analogies can be extrapolated based upon the sensory lexicon where commercial samples were compared against freshly pressed blueberry juices.[30] The attributes prune, tobacco and medicinal flavors tended to be higher in processed commercially bottled juices compared to hand-pressed (muslin cloth) juices.[30] Previously, many of these same panelists revealed that processed juices were significantly higher in cranberry, molasses/dark corn syrup, can tomato, fermented, processed berry juice, sour aroma and pungency aroma, whereas hand-pressed fresh juices were significantly lower than commercially bottled juices in wine-like, bitter taste, sour taste, astringent, metallic flavors, tongue numbing and throat burn attributes.[30] However, there were no significant differences found for most of these attributes in CJP and NCP blueberry juices (Table 4). Astringent was one of the highest scoring attributes (Fig. 4) yet this is a common occurrence in fruit beverages high in polyphenolics, and the intensity responses were actually higher than commercial samples previously reported.[30] However, there was no significant processing or storage effects for astringency (Table 4).

CONCLUSION

Based on color data, anthocyanidin change and the literature indicating increased polymeric color change during blueberry juice processing and/or storage, one presumes that our anthocyanidin loss was therefore most likely from transformation and polymerization (unpublished data, submitted as: Stein-Chisholm *et al.*, 'LC-MS-MS and UPLC-UV evaluation of anthocyanins and anthocyanidins in rabbiteye blueberry juice processing'). Most monomeric anthocyanins found in blueberry juice have a molecular weight just below 500. However, in NFC juices, there was not abnormal astringency problems observed in pasteurization or storage which is believed to occur with MW > 500 polyphenolics. The pilot plant methods and ultra-filtration herein resulted in a sweeter than normal commercial NFC juice with very minor sedimentation. Subsequently, anthocyanin loss/change was likely through polymerization, especially since the product's flavor was not unacceptable. Consumers and health advocates desire maximum antioxidant loads. Yet, the manufacturing process whereby juice stability and shelf-life are maintained cannot generally preserve volatiles or low molecular weight anthocyanins at markedly high levels. Nonetheless, in order to effectively compare an in-house NFC juice to a commercial counterpart, a complete study would be required to assess the same field-harvested fruit through NFC juice processing that was simultaneously used commercially to produce a concentrate which, would be reconstituted, pasteurized and bottled. However, achieving this would be extraordinarily difficult to accomplish. It remains a significant challenge to maintain low molecular weight anthocyanin and volatile compound concentrations, and hopefully bioavailability, in commercial-like NFC juice operations.

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