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ARTICLE

Jam Processing and Storage Effects on Blueberry Polyphenolics and Antioxidant Capacity†

Luke R. Howard,*,§ Chelsey Castrodale,§ Cindi Brownmiller,§ and Andy Mauromoustakos#

§Department of Food Science, University of Arkansas, 2650 North Young Avenue, Fayetteville, Arkansas 72704, and #Agricultural Statistics Lab, 104 Agricultural Annex, University of Arkansas, Fayetteville, Arkansas 72701

Fresh blueberries were processed into sugar and sugar-free jams and stored for 6 months at 4 and 25 °C. The jams were analyzed immediately after processing and over 6 months of storage for polyphenolic content, percent polymeric color, and antioxidant capacity. Processing resulted in losses of anthocyanins, procyanidins, chlorogenic acid, and ORAC in both jam types, but flavonols were well retained. Marked losses of anthocyanins and procyanidins occurred over 6 months of storage and were accompanied by increased polymeric color values. Chlorogenic acid levels also declined during storage, but flavonols and ORAC changed little. Jams stored at 4 °C retained higher levels of anthocyanins, procyanidins, and ORAC and had lower polymeric color values than jams stored at 25 °C. Sugar-free jams retained higher levels of anthocyanins and had lower polymeric color values than sugar jams late during storage. Blueberry jams should be refrigerated to better retain polyphenolics and antioxidant capacity.

KEYWORDS: Anthocyanins; blueberries; flavonols; jams; processing; procyanidins; storage

INTRODUCTION

Blueberries (*Vaccinium corymbosum* L.) contain an abundance of different types of polyphenolics, namely, anthocyanins (*1*), procyanidins (*2*), chlorogenic acid (*3*), and flavonols (*3*, *4*). These compounds are believed to confer protection against chronic diseases through a multitude of biological activities including antioxidant, anti-inflammatory, antihypertensive, and antiallergy properties, in addition to their ability to modulate enzymes, gene regulation, and cell signaling pathways (*5*, *6*).

Blueberries are commonly consumed fresh, but can also be consumed as shelf-stable processed products such as jams, jellies, juices, canned berries, and purees that are available year round. Berry jams are a popular product produced by both home canners and commercial processors. The number and type of unit operations used in different preservation methods can have a marked effect on retention of polyphenolics. Significant losses of anthocyanins and procyanidins were observed during processing of juices (*7*−*11*), berries canned in water or syrup (*10*, *11*), and purees (*10*, *11*). The polyphenolic losses incurred during juice processing are typically more severe than other preservation methods due to physical removal of the polyphenolic-rich seeds and skins following the pressing operation (*7*, *8*, *10*, *11*). In addition to physical removal, the retention of polyphenolics, especially anthocyanins during processing, is influenced by enzymes, temperature, oxygen, pH, metals, and sugars (*12*). Polyphenolics in processed blueberry products are also susceptible to losses during storage at ambient temperature, especially anthocyanins and procyanidins, which decrease markedly during storage of juices (*9*−*11*), berries canned in water or syrup (*10*, *11*), and purée (*10*, *11*). The losses of anthocyanins and procyanidins during storage of processed blueberry products were accompanied by increased polymeric color values, indicating formation of anthocyanin−procyanidin polymers (*10*, *11*). Anthocyanin−procyanidin polymers have been shown to form through direct condensation reactions (*13*) or reactions mediated by acetaldehyde (*14*) and furfural (*15*). Although the mechanisms responsible for polyphenolic losses during storage of processed blueberry products have not been elucidated, refrigerated storage has been shown to improve polyphenolic retention in juices (*9*).

Blueberry jam, a popular product produced by both consumers and commercial processors, is available in both sugar and sugar-free forms. Unfortunately, no information is available on how processing and storage of processed jams with different formulations influence the retention of blueberry polyphenolics. This study was undertaken to determine the stability of polyphenolics and antioxidant capacity in conventional sugar and sugar-free jams following processing and over 6 months storage at both ambient (25 °C) and refrigerated (4 °C) temperatures.

MATERIALS AND METHODS

Materials. Blueberries (cultivar unknown) were obtained from Sam's Club (Fayetteville, AR). The berries were stored at 4 °C for 1 day prior to processing.

Jam Processing. Ball jam jars and screw bands (Ball Corp., Broomfield, CO) were washed in a dishwasher before use. Lids were prepared by pouring boiling water over the lids and allowed to stand until use. Fresh

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*Author to whom correspondence should be addressed [telephone (479) 575-2978; fax (479) 575-6936; e-mail lukeh@uark.edu].

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blueberries were crushed using a stainless steel masher. Conventional sugar jams were prepared following the recipe on the Sure-Jell Premium Fruit Pectin package (Kraft Foods, Northfield, IL) for cooked blueberry jams. Crushed fruit (1040 g) and one box of Sure-Jell Premium Fruit Pectin (49 g) were added to a 6.7 L saucepot. The mixture was heated on high heat until it reached a full boil (101−103 °C), and 900 g of sugar was added and stirred thoroughly. The mixture was brought to a boil again (103−105 °C) and held for 1 min.

Sugar-free jams were prepared following the recipe on the Sure-Jell Premium Fruit Pectin No Sugar Needed package (Kraft Foods) for cooked no-sugar-added triple-berry jams. Crushed fruit (810 g), one box of Sure-Jell Premium Fruit Pectin No Sugar Needed (49 g), and 129 mL of water were added to a 6.7 L saucepot. The mixture was heated on high heat until it reached a full boil (101−103 °C). After 1 min, 15 g of granulated Splenda was added and stirred thoroughly. Both jam types were dispensed into 227 mL glass jars, immediately capped, and pasteurized in boiling water for 10 min. The weights of jams (g) before and after processing were recorded. Half of the sugar and sugar-free jams were stored in the dark at 25 °C and the other half stored in the dark at 4 °C.

Extraction of Anthocyanins, Chlorogenic Acid, and Flavonols. Jams were pureed using a common household blender (Black & Decker, Towson, MD). Polyphenolics were extracted using the method of Cho et al. (*3*) with modifications. Pureed jam (5 g) was homogenized with 20 mL of methanol/water/formic acid (60:37:3 v/v/v) using a Euro Turax T18 Tissuemizer (Tekmar-Dohrman Corp., Mason, OH). Fresh blueberries were pureed in a household blender (Black & Decker), and then 10 g of puree was homogenized with the Tissuemizer using the same solvent. The samples were filtered through Miracloth (Calbiochem, La Jolla, CA), filter cakes were isolated, and the extraction was repeated twice. The filtrates were pooled and adjusted to a final volume of 100 mL with extraction solvent.

HPLC Analysis of Anthocyanins, Chlorogenic Acid, and Flavonols. Anthocyanins and chlorogenic acid were analyzed by HPLC using a 250 × 4.6 mm i.d. Symmetry C_{18} column (Waters Corp., Milford, MA) as described by Cho et al. (*3*). Chlorogenic acid, flavonol, and anthocyanin peaks were monitored at 320, 360, and 520 nm, respectively, using a Waters model 996 photodiode array detector. The identification of anthocyanin glycosides was determined by HPLC-MS using the same column and conditions previously described (*3*). Individual anthocyanin monoglycosides and acylated anthocyanin derivatives were quantified as delphinidin, cyanidin, petunidin, peonidin, and malvidin glucoside equivalents using external calibration curves of a mixture of anthocyanin glucosides obtained from Polyphenols Laboratories AS (Sandnes, Norway). Total anthocyanins were calculated as the sum of individual anthocyanin monoglycosides and acylated anthocyanin derivatives. Chlorogenic acid was quantified using external calibration curves of an authentic standard obtained from Sigma-Aldrich (St. Louis, MO). Individual flavonol peaks were quantified as rutin equivalents using external calibration curves of an authentic standard obtained from Sigma-Aldrich. Total flavonols were calculated as the sum of individual flavonols. Results were expressed as milligrams of each respective polyphenolic per kilogram of original berry.

Extraction and Purification of Procyanidins. Jams were pureed using a common household blender (Black & Decker). Procyanidins were extracted using the method of Gu et al. (*16*) with modifications. Pureed jams (5 g) were homogenized with 20 mL of acetone/water/acetic acid (70:29.05:0.5 v/v/v) using a Euro Turax T18 Tissuemizer (Tekmar-Dohrman Corp.). Fresh blueberries were pureed in a household blender (Black & Decker), and then 10 g of berry puree was homogenized with the Tissuemizer using the same solvent. The samples were filtered through Miracloth (Calbiochem), filter cakes were isolated, and the extraction was repeated twice. The filtrates were pooled and adjusted to a final volume of 100 mL with extraction solvent. Samples were purified by solid phase extraction using Sephadex LH-20. After equilibration of 3 g (DW) of the Sephadex LH-20 with water overnight, the hydrated material was manually packed into 6 × 1.5 cm columns. Samples were prepared for purification by evaporating 25 mL of the crude extract under vacuum in a SpeedVac (SPE 1010, Thermo Savant, Holbrook, NY) at 25 °C to approximately 7.5 mL. The concentrated extract was loaded onto the LH-20 column, and the column was washed with 40 mL of 30% methanol in water to remove interfering compounds. Procyanidins were then eluted by washing the column with 80 mL of 70:30 acetone/water (v/v). The eluted procyanidin fraction was evaporated to dryness using a SpeedVac and reconstituted with 2 mL of acetone/water/acetic acid (70:29.5:0.5 v/v/v). The reconstituted samples were filtered through 0.45 μm PTFE syringe filters prior to HPLC analysis.

HPLC Analysis of Procyanidins. Procyanidins were analyzed by HPLC using a 250 × 4.6 mm i.d., 5 μm, Develosil diol column (Phenomenex, Torrance, CA) as described by Kelm et al. (*17*). The procyanidin peaks were monitored by fluorescence detection with excitation at 276 nm and emission at 316 nm using a Waters model 474 fluorescence detector. Individual procyanidins with degrees of polymerization from DP1 (monomer) through DP8 (octamer) were quantified using external calibration curves of a mixture of procyanidin standards isolated from cocoa that was obtained from Mars Inc. (Hackettstown, NJ). Total procyanidins were calculated as the sum of individual procyanidins with results expressed as milligrams per kilogram of original berry.

Polymeric Color Analysis. Percent polymeric color was determined using the spectrophotometric assay described by Giusti and Wrolstad (*18*).

Determination of Antioxidant Capacity. Antioxidant capacity was measured using the oxygen radical absorbance capacity ($ORAC_{FL}$) assay of Prior et al. (*19*). Extracts were diluted 400-fold in phosphate buffer (pH 7.0) prior to analysis. The assay was carried out using a FLUOstar microplate reader (BMG Labtechnologies, Durham, NC) with fluorescein as the fluorescent probe. Results were expressed as micromoles of Trolox equivalents per gram of original berry.

Determination of pH, Water Activity, and Total Soluble Solids. pH was measured using an Accument Basic pH-meter (Fisher Scientific, Bridgewater, NJ). Water activity was measured using an AquaLab CX2 water activity meter (Decagon Devices Inc., Pullman, WA). Total soluble solids (%) were measured using a Bausch & Lomb Abbe Mark II refractometer (Scientific Instrument, Keene, NH).

Calculations. Anthocyanin, procyanidin, chlorogenic acid, flavonol, and ORAC levels in jams were converted to original berry weight by taking into account the weight change (g) of jams before and after cooking and the weight (g) of blueberries used in each jam formulation.

Sampling. Four batches of each jam type were processed under identical conditions. Three jars each of sugar and sugar-free jams stored at 25 °C were sampled 1 day after processing, and three jars of each jam type stored at 4 and 25 °C were sampled after 2, 4, and 6 months of storage.

Statistical Analysis. The effects of processing on anthocyanins, procyanidins, chlorogenic acid, flavonols, percent polymeric color, and ORAC were determined by analysis of variance (ANOVA) using JMP software (SAS Institute Inc., Cary, NC). Significant differences ($p \leq 0.05$) between means were determined by Student's t test. ANOVA was also used to determine the effects of jam type, storage time, storage temperature, and all interactions on anthocyanins, procyanidins, chlorogenic acid, flavonols, percent polymeric color, and ORAC. Mean values were compared using Student's t test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Sugar and sugar-free jams were analyzed 1 day after processing for pH, water activity, and percent soluble solids. The sugar jams had pH, water activity, and percent soluble solids values of 2.47, 0.857, and 63, respectively, whereas the sugar-free jams had corresponding values of 3.12, 0.988, and 20, respectively. We suspect that the lower pH of the sugar jams was due to a higher concentration of acids in the Sure-Jell Premium Fruit Pectin mix used to prepare the jam.

Processing Effect on Polyphenolics, Percent Polymeric Color, and Antioxidant Capacity. Jams were analyzed 1 day after processing for anthocyanins, chlorogenic acid, flavonols, procyanidins, percent polymeric color, and ORAC, with the results presented in **Figure 1**. The levels of total anthocyanins were determined by summation of the concentrations of individual anthocyanins that were identified by HPLC and HPLC-MS. Nine peaks were identified, with two peaks reflecting coeluting compounds: peak 4 = petunidin 3-galactoside + cyanidin 3-arabinoside and peak 8 = malvidin 3-glucoside + peonidin 3-arabinoside (**Figure 2**). The total anthocyanin content of the unknown blueberry cultivar (5947.6 mg/kg of FW) was much higher than the

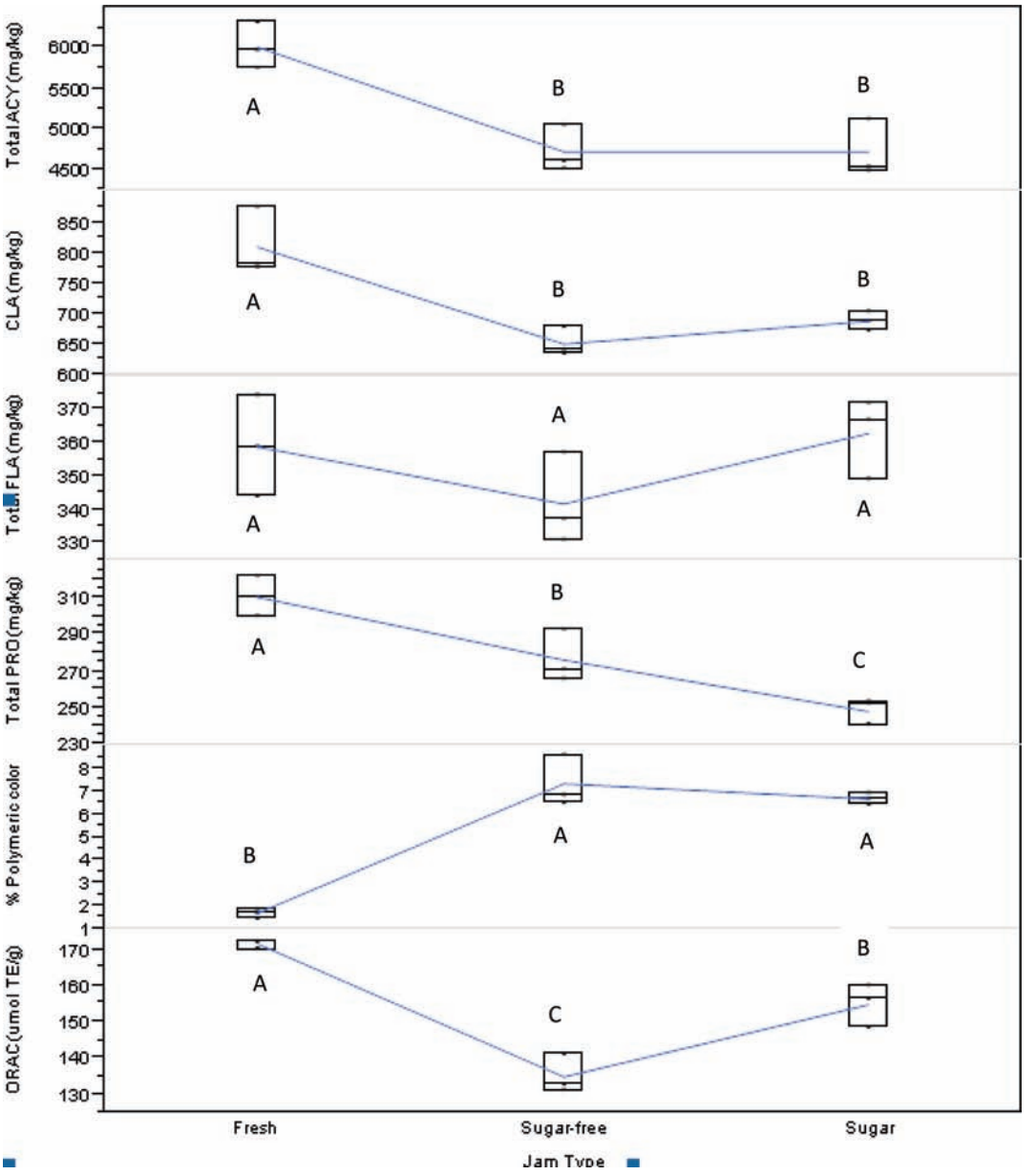

Figure 1. Processing effect on total anthocyanins (ACY), chlorogenic acid (CLA), total flavonols (FLA), total procyanidins (PRO), percent polymeric color, and ORAC in sugar-free and sugar blueberry jams. Boxes with similar letters are not significantly different ($p > 0.05$).

mean value of 3866 mg/kg of FW reported for seven cultivated blueberry cultivars (*1*). The levels of total anthocyanins in both sugar and sugar-free jams were similar after processing, with 79% retention compared to levels found in fresh berries. Percent retention of individual anthocyanin glycosides varied considerably, with galactosides generally being better retained than arabinosides (**Table 1**). Petunidin 3-glucoside, which was a minor constituent, showed the lowest retention. Our results are generally consistent with those of Ichiyanagi et al. (*20*), who reported that the acid-catalyzed hydrolysis rate of anthocyanins was affected more by the type of sugar attached rather than aglycon structure, with the rate of hydrolysis following the order arabinosides > galactosides > glucosides. Although the exact mechanism is unclear, it appears that the larger hexose sugar imparts greater resistance to hydrolysis than the smaller pentose sugar.

The fresh blueberries had a chlorogenic acid content of 810.3 mg/kg of FW, which falls within the range of values (363−1082 mg/kg of FW) previously reported for cultivated blueberries (*3*). Jam processing resulted in significant losses of chlorogenic acid, but chlorogenic acid levels were unaffected by jam type. Sugar-free and sugar jams retained 80 and 85% of the chlorogenic acid found in fresh berries, respectively.

Fresh berries had a total flavonol content of 363 mg/kg of FW, which was higher than the range of levels (172−328 mg/kg of FW) reported by Cho et al. (*3*, *4*). In contrast to other polyphenolics, levels of total flavonols in both jam types were stable in response to processing, with >94% retention compared to levels found in fresh berries. Flavonols in red raspberry (*21*) and strawberry jams (*22*) also showed high stability following processing.

Procyanidin monomers through octamers were detected and quantified by HPLC analysis (**Table 2**). The unknown blueberry cultivar contained 43.9, 67.5, 47.8, 38.5, 26.1, 40.3, 29.4, and 16.8 mg/kg of FW from monomers to octamers, respectively, which were similar to values previously reported for fresh cultivated blueberries (*2*, *11*). Sugar-free jams retained higher levels of total procyanidins (89%) than sugar jams (80%) after processing. The greater loss of procyanidins in sugar jams may be the result of greater binding to cell wall polysaccharides/proteins

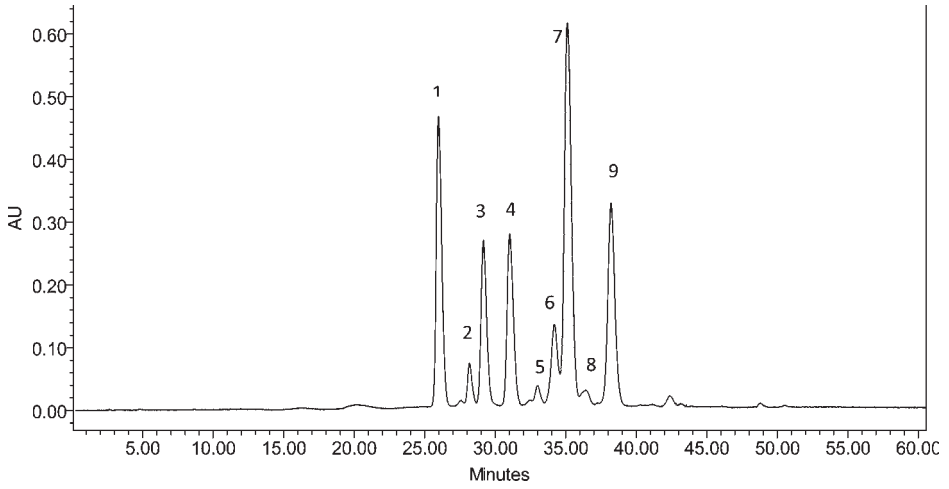

Figure 2. Representative HPLC chromatogram (510 nm) of anthocyanin glycosides in blueberry jams. Corresponding peak assignments: (1) delphinidin 3-galactoside (*m*/*z* total/aglycon 465/303); (2) cyanidin 3-galactoside (*m*/*z* total/aglycon 449/287); (3) delphinidin 3-arabinoside (*m*/*z* total/aglycon 435/303); (4) petunidin 3-galactoside (*m*/*z* total/aglycon 479/317) + cyanidin 3-arabinoside (*m*/*z* total/aglycon 419/287); (5) petunidin 3-glucoside (*m*/*z* total/aglycon 479/317); (6) peonidin 3-galactoside (*m*/*z* total/aglycon 463/301); (7) malvidin 3-galactoside (*m*/*z* total/aglycon 465/303); (8) malvidin 3-glucoside (*m*/*z* total/aglycon 493/331) + peonidin 3-arabinoside (*m*/*z* total/aglycon 433/301); (9) malvidin 3-arabinoside (*m*/*z* total/aglycon 463/331).

Table 1. Content of Anthocyanins in Fresh Blueberries and in Sugar and Sugar-free Jams 1 Day after Processing

	anthocyanins[a] (mg/kg of FW) in		
anthocyanin	fresh blueberries	sugar jam	sugar-free jam
delphinidin 3-galactoside	1126.9 ± 83.7 a	935.2 ± 39.9 ab (83%)	859.6 ± 34. 0b (76%)
cyanidin 3-galactoside	148.9 ± 9.2 a	145.9 ± 5.6 a (98%)	122.4 ± 4.4 b (82%)
delphinidin 3-arabinoside	703.8 ± 32.3 a	517.6 ± 29.6 b (73%)	514.9 ± 19.1 b (73%)
petunidin 3-galactoside + cyanidin 3-arabinoside	908.9 ± 29.1 a	694.6 ± 33.5 b (76%)	698.3 ± 21.5 b (77%)
petunidin 3-glucoside	75.5 ± 2.1 a	35.7 ± 4.4 b (47%)	46.2 ± 6.9 b (61%)
peonidin 3-galactoside	290.8 ± 5.8 a	230.5 ± 13.5 b (79%)	261.2 ± 9.8 ab (90%)
malvidin 3-galactoside	1688.6 ± 20.7 a	1382.4 ± 43.9 b (82%)	1366.6 ± 42.4 b (81%)
malvidin 3-glucoside + peonidin 3-arabinoside	121.1 ± 2.0 a	88.8 ± 2.2 b (73%)	108.2 ± 15.2 ab (89%)
malvidin 3-arabinoside	949.7 ± 14.7 a	682.7 ± 36.0 b (72%)	740.8 ± 23.6 b (78%)

[a] Values represent means ± standard error (n = 3). Means within rows with different letters are significantly different ($p \leq 0.05$). Values in parentheses represent percent retention compared to frozen berries.

Table 2. Content of Procyanidin Oligomers in Fresh Blueberries and in Sugar and Sugar-free Jams 1 Day after Processing

	procyanidin oligomers[a] (mg/kg of FW) in		
procyanidin	fresh blueberries	sugar jam	sugar-free jam
monomers	43.9 ± 0.5 a	45.5 ± 1.4 a (104%)	37.3 ± 0.8 b (85%)
dimers	67.5 ± 0.3 b	71.9 ± 1.3 b (107%)	85.7 ± 1.6 a (127%)
trimers	47.8 ± 10.1 a	37.3 ± 2.0 a (78%)	38.7 ± 1.5 a (81%)
tetramers	38.5 ± 5.9 a	32.7 ± 0.32 a (85%)	34.7 ± 1.12 a (90%)
pentamers	26.1 ± 4.5 a	16.6 ± 0.9 b (64%)	20.3 ± 1.5 ab (78%)
hexamers	40.3 ± 5.2 a	30.3 ± 4.3 a (75%)	32.3 ± 2.3 a (80%)
heptamers	29.4 ± 3.7 a	13.8 ± 1.5 b (47%)	18.3 ± 0.6 b (62%)
octamers	16.8 ± 1.9 a	ND (0%)	9.1 ± 0.3 b (54%)

[a] Values represent means ± standard error (n = 3). Means within rows with different letters are significantly different ($p \leq 0.05$). Values in parentheses represent percent retention compared to frozen berries.

in response to reduced water activity. The monomers and smaller oligomers showed greater retention than larger oligomers, a result consistent with those previously observed following pasteurization of blueberry juices, canned samples, and purees (*11*). The greater loss of larger oligomers was most likely due to binding of the large molecular weight procyanidins to cell wall polymers following cell disruption through either hydrogen bonding or hydrophobic interactions (*23*, *24*). The sugar-free jams had higher levels of dimers than fresh berries and sugar jams, which may be due to enhanced extraction or possible conversion of oligomers to dimers in response to thermal treatment. Procyanidins are known to undergo acid-catalyzed decomposition in wines at ambient temperature under a similar pH range (3.0−4.0) of the jams studied (*25*), and the reaction theoretically would proceed at a much higher rate due to the thermal treatment applied.

Percent polymeric color values increased from 1.6 in fresh berries to 6.7 and 7.3 in sugar and sugar-free jams, respectively, indicating anthocyanin polymers were formed in response to processing. The increased percent polymeric color values were consistent with losses of anthocyanins and procyanidins observed in both jam types during processing and corroborate previous studies reporting increased polymeric values in response to thermal processing of blueberry juices, purees, and canned blueberries (*8*, *10*). In addition to the formation of anthocyanin polymers, losses of anthocyanins, procyanidins, and chlorogenic acid incurred during processing may be associated with enzymatic and thermal degradation. Polyphenol oxidase and peroxidase have been shown to play a role in degradation of anthocyanins and catechins via oxidation of chlorogenic acid and catechin, and the generation of highly reactive quinones (*26*−*29*). Exposure of the berries to elevated temperatures during jam production and pasterurization most likely contributed to losses as well, because anthocyanin degradation is time and temperature dependent (*30*).

The $ORAC_{FL}$ value of fresh berries (171.2 μmol/g of FW) was much higher than the mean value previously reported for eight

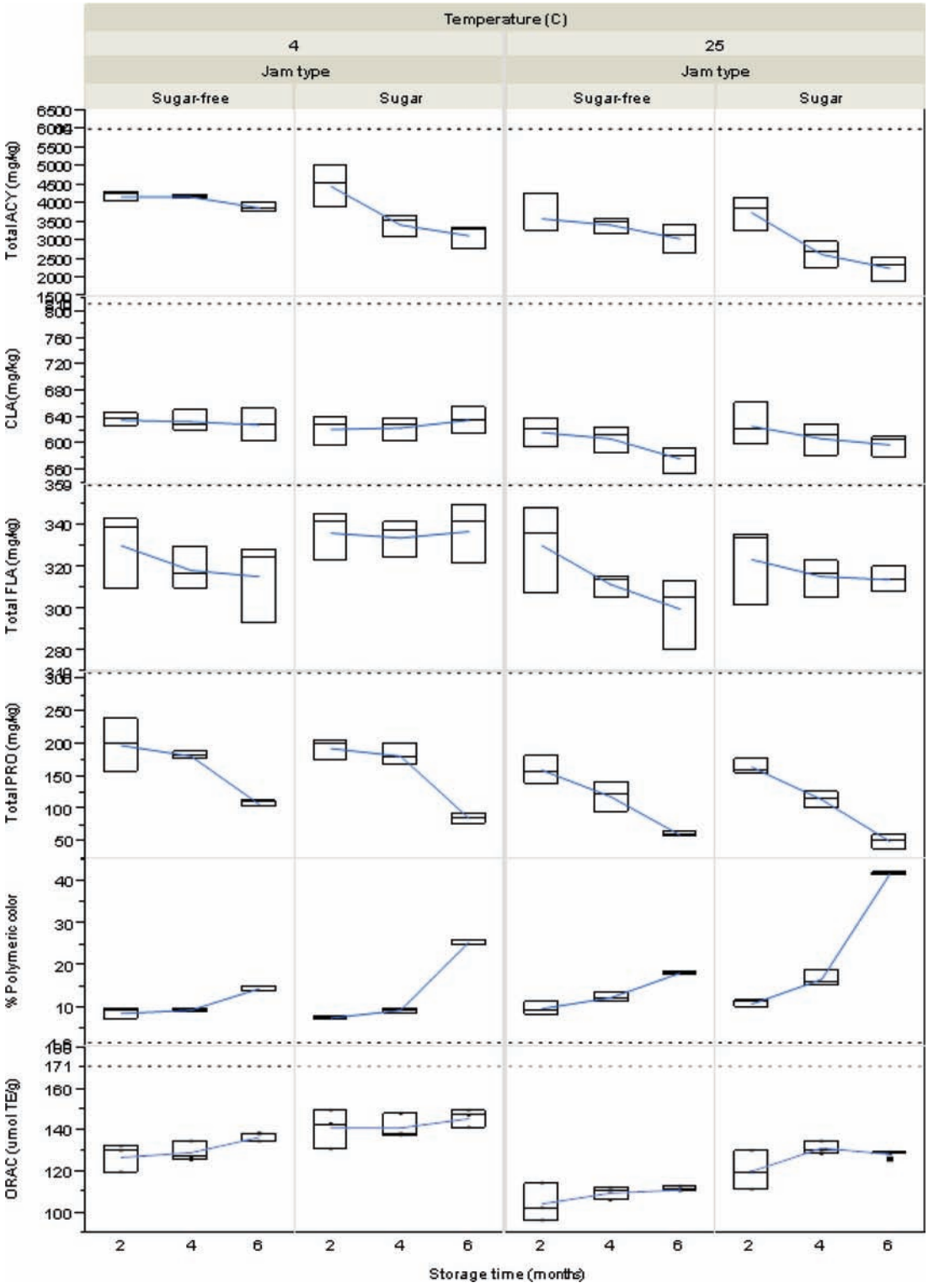

Figure 3. Storage effect on total anthocyanins (ACY), chlorogenic acid (CLA), total flavonols (FLA), total procyanidins (PRO), percent polymeric color, and ORAC in sugar-free and sugar blueberry jams stored at 4 and 25 °C. The dashed lines represent concentrations found in fresh blueberries.

cultivated blueberries (61.8 μmol of TE/g of FW) (*31*), which was consistent with the high level of anthocyanins detected in the fruit. $ORAC_{FL}$ values decreased following processing and were affected by jam type, with sugar and sugar-free jams retaining 90 and 79%, respectively, of the $ORAC_{FL}$ found in fresh berries. The higher $ORAC_{FL}$ of sugar jams, despite having levels of anthocyanins similar to those of sugar-free jams, was likely due to reductones formed via the Maillard reaction. Maillard reaction products (MRPs) are known to possess potent antioxidant capacity (*32*), and the high sugar content in the sugar jams provided favorable conditions for Maillard browning, following sucrose hydrolysis. Hydroxymethylfurfural (HMF), a MRP, was detected in sugar jams following processing (18 mg/kg of FW, data not shown), but was not detected in fresh berries or sugar-free jams (data not shown).

Storage Effects on Polyphenolics, Percent Polymeric Color, and Antioxidant Capacity. Changes in total anthocyanins, chlorogenic acid, total flavonols, total procyanidins, percent polymeric color,

Table 3. Content of Anthocyanins in Sugar and Sugar-free Blueberry Jams over 6 Months of Storage at 4 and 25 °C

	anthocyanins[a] (mg/kg of FW) after storage					
	2 months		4 months		6 months	
anthocyanin[b]	4 °C	25 °C	4 °C	25 °C	4 °C	25 °C
			Sugar Jam			
dpd 3-gal	839.9 ± 58.6 (74%)	692.6 ± 31.6 (61%)	733.5 ± 26.7 (65%)	555.0 ± 44.9 (49%)	680.6 ± 36.9 (60%)	498.4 ± 42.9 (44%)
cyd 3-gal	145.6 ± 7.6 (97%)	125.2 ± 12.9 (84%)	111.0 ± 6.3 (74%)	97.0 ± 10.5 (65%)	111.5 ± 8.5 (74%)	82.4 ± 6.7 (55%)
dpd 3-ara	502.7 ± 39.4 (71%)	460.5 ± 11.7 (65%)	387.7 ± 20.7 (55%)	319.7 ± 16.3 (45%)	358.7 ± 25.1 (51%)	265.9 ± 21.6 (38%)
ptd 3-gal + cyd 3-ara	646.0 ± 54.4 (71%)	530.8 ± 45.8 (58%)	485.1 ± 36.1 (53%)	386.8 ± 12.9 (43%)	425.0 ± 28.7 (47%)	328.9 ± 42.7 (36%)
ptd 3-glu	42.0 ± 22.6 (56%)	20.5 ± 11.3 (27%)	9.7 ± 4.0 (13%)	9.4 ± 5.5 (12%)	4.0 ± 5.6 (5%)	ND (0%)
pnd 3-gal	250.3 ± 12.7 (86%)	180.9 ± 18.7 (62%)	168.6 ± 10.6 (58%)	143.6 ± 17.7 (49%)	159.6 ± 9.4 (55%)	123.1 ± 7.2 (42%)
mvd 3-gal	1219.0 ± 78.6 (72%)	1020.6 ± 98.8 (60%)	1016.0 ± 43.2 (60%)	753.1 ± 53.7 (44%)	914.6 ± 51.7 (54%)	638.4 ± 50.6 (38%)
mvd 3-glu + pnd 3-ara	101.6 ± 6.5 (84%)	98.1 ± 23.2 (81%)	25.5 ± 3.2 (21%)	24.7 ± 0.8 (20%)	25.5 ± 2.7 (21%)	25.5 ± 2.2 (21%)
mvd 3-ara	670.2 ± 51.6 (71%)	575.0 ± 33.6 (61%)	489.5 ± 31.3 (51%)	339.3 ± 60.6 (36%)	448.1 ± 22.6 (47%)	293.5 ± 19.2 (31%)
			Sugar-free Jam			
dpd 3-gal	726.7 ± 27.0 (64%)	682.0 ± 67.7 (60%)	814.6 ± 22.1 (72%)	728.5 ± 36.6 (64%)	799.9 ± 11.4 (70%)	668.6 ± 49.1 (59%)
cyd 3-gal	121.9 ± 9.1 (82%)[c]	112.9 ± 10.9 (76%)	110.8 ± 1.2 (74%)	94.3 ± 2.7 (63%)	106.6 ± 2.9 (72%)	86.5 ± 2.8 (58%)
dpd 3-ara	456.7 ± 21.0 (65%)	402.8 ± 44.0 (57%)	452.8 ± 14.3 (64%)	383.4 ± 17.7 (54%)	425.7 ± 7.6 (60%)	359.3 ± 31.1 (51%)
ptd 3-gal + cyd 3-ara	604.9 ± 13.2 (67%)	554.6 ± 59.9 (60%)	583.2 ± 6.9 (64%)	552.2 ± 25.5 (61%)	554.8 ± 12.3 (61%)	449.3 ± 42.8 (49%)
ptd 3-glu	66.9 ± 5.1 (89%)	47.8 ± 10.9 (63%)	33.1 ± 0.9 (44%)	22.5 ± 2.8 (30%)	28.5 ± 2.1 (38%)	21.7 ± 7.4 (29%)
pnd 3-gal	256.3 ± 10.5 (88%)	188.0 ± 9.8 (65%)	222.0 ± 3.2 (76%)	165.3 ± 5.2 (57%)	186.3 ± 4.5 (64%)	173.6 ± 14.8 (59%)
mvd 3-gal	1155.0 ± 17.6 (68%)	990.3 ± 98.1 (59%)	1217.5 ± 25.9 (72%)	965.7 ± 27.8 (57%)	1123.7 ± 24.3 (66%)	846.9 ± 69.9 (50%)
mvd 3-glu + pnd 3-ara	93.6 ± 16.9 (77%)	97.4 ± 13.3 (80%)	100.4 ± 2.0 (83%)	32.3 ± 4.4 (26%)	89.3 ± 3.9 (74%)	15.7 ± 0.4 (13%)
mvd 3-ara	697.5 ± 19.6 (73%)	527.5 ± 29.8 (56%)	627.3 ± 13.8 (66%)	465.0 ± 9.5 (49%)	544.4 ± 14.2 (57%)	421.5 ± 32.3 (44%)

[a] Values represent means ± standard error (n = 3). Values in parentheses represent percent retention compared to fresh berries. [b] Cyd, cyanidin; dpd, delphinidin; mvd, malvidin; pnd, peonidin; ptd, petunidin; gal, galactoside; glu, glucoside; ara, arabinoside.

and ORAC in sugar and sugar-free jams over 6 months of storage at 4 and 25 °C are shown in **Figure 3**. The levels of total anthocyanins were affected by storage temperature, storage time, and jam type × storage time interaction ($p \leq 0.05$). Jams stored at 4 °C had on average 230−1086 mg/kg of FW higher total anthocyanin values than jams stored at 25 °C, with an overall mean difference of 658.3 ± 207.4 mg/kg of FW. Jams stored for 2 months had higher levels of total anthocyanins (4323.5 mg/kg of FW) than jams stored for 4 (3791.4 mg/kg of FW) and 6 (3496.4 mg/kg of FW) months. Sugar-free jams stored for 4 and 6 months had higher levels of total anthocyanins (4179.5 and 4161.8 mg/kg of FW, respectively) than sugar jams stored for 4 and 6 months (3421.1 and 3133.6 mg/kg of FW, respectively). The greater retention of anthocyanins in sugar-free jams during storage indicates that sugar was detrimental to anthocyanin stability. The sugar-free jams also had much lower polymeric color values than the sugar jams, suggesting that sugar promoted the formation of anthocyanin−procyanidin polymers. The effects of sugar on anthocyanin stability are controversial. Some studies report that sugar stabilizes anthocyanins (*33*, *34*), whereas other studies show reduced stability of anthocyanins in the presence of sugar (*35*−*37*). We suspect that the lower water activity and pH of the sugar jams resulted in a more favorable environment for the formation of anthocyanin−procyanidin polymers, which may be mediated by HMF or may occur by a direct condensation reaction.

The contents of individual anthocyanins in sugar-free and sugar jams over 6 months of storage at 4 and 25 °C are shown in **Table 3**. Similar to total anthocyanins, levels of individual anthocyanins were affected by storage temperature, storage time, and jam type × storage time interaction ($p \leq 0.05$). Storage of jams at 4 °C resulted in greater retention of all anthocyanins compared to jams stored at 25 °C. Levels of all anthocyanins declined from 2 to 6 months of storage. Differences in anthocyanin retention were influenced by anthocyanidin structure and the type of sugar attached. For the four anthocyanidins containing galactose, the average percent retention after 6 months of storage followed the order cyanidin (65%), delphinidin (59%), peonidin (55%), and malvidin (52%). For the two anthocyanidins containing arabinose, delphinidin showed greater retention (50%) than malvidin (45%). Our results are generally consistent with those of Tröst et al. (*38*), who found that cyanidin anthocyanins were the most stable during storage of blueberry−aronia nectar, followed by peonidin, petunidin, delphinidin, and malvidin, the only discrepancy being that we found delphinidin to be more stable than peonidin. In comparing similar anthocyanidins with different sugars attached, we observed that galactosides were more stable than arabinosides. The mean percent retentions of the galactosides and arabinosides of delphinidin following 6 months of storage were 59 and 50%, respectively, and the mean percent retentions of the galactosides and arabinosides of malvidin were 52 and 45%, respectively. Tröst et al. (*38*) also reported that anthocyanin galactosides in blueberry−aronia nectars were more stable over storage than arabinosides, but they found glucosides to be the most stable glycoside. We detected only one glucoside in our study, petunidin 3-glucoside, which showed the lowest mean percent retention (20%) after 6 months of storage, but was present at very low concentrations compared to the other anthocyanin glycosides. Although some of the apparent losses of individual anthocyanins during storage were actually due to the formation of anthocyanin−procyanidin polymers, further losses may result from hydrolytic reactions resulting in conversion of anthocyanin glycosides to chalcones, which spontaneously degrade into phenolic acids and aldehydes (*39*). We also cannot rule out the possibility that heat-stable forms of polyphenol oxidase or peroxidase played a role in the degradation of anthocyanins. Further research is needed to identify the mechanisms responsible for anthocyanin degradation during storage.

Levels of chlorogenic acid were affected by storage time, but were unaffected by jam type, storage temperature, or any interactions. Jams stored for 2 months had higher levels of chlorogenic acid (622.5 mg/kg of FW) than jams stored for 6 months

Table 4. Content of Procyanidin Oligomers in Sugar and Sugar-free Blueberry Jams over 6 Months of Storage at 4 and 25 °C

	procyanidin oligomers[a] (mg/kg of FW) after storage					
	2 months		4 months		6 months	
procyanidin	4 °C	25 °C	4 °C	25 °C	4 °C	25 °C
monomers	30.2 ± 2.1 (69%)	26.7 ± 1.5 (61%)	28.6 ± 1.9 (65%)	24.6 ± 1.9 (56%)	11.4 ± 0.6 (26%)	11.5 ± 2.2 (26%)
dimers	64.0 ± 4.0 (95%)	59.6 ± 2.4 (88%)	59.6 ± 2.3 (88%)	44.2 ± 2.7 (65%)	36.8 ± 2.7 (55%)	19.1 ± 2.5 (28%)
trimers	29.7 ± 2.2 (62%)	22.5 ± 1.2 (47%)	22.0 ± 0.9 (46%)	12.0 ± 0.9 (25%)	11.8 ± 0.5 (25%)	6.3 ± 0.2 (13%)
tetramers	21.9 ± 1.4 (57%)	16.9 ± 1.2 (44%)	21.6 ± 0.7 (57%)	11.4 ± 1.3 (30%)	11.6 ± 0.8 (29%)	5.4 ± 0.6 (14%)
pentamers	14.7 ± 0.6 (56%)	11.0 ± 1.8 (56%)	14.7 ± 0.9 (41%)	7.0 ± 1.1 (26%)	6.8 ± 1.3 (24%)	2.7 ± 0.7 (10%)
hexamers	18.5 ± 1.2 (46%)	13.9 ± 1.0 (34%)	20.7 ± 2.4 (51%)	11.1 ± 2.4 (28%)	7.3 ± 1.1 (18%)	5.5 ± 1.0 (14%)
heptamers	11.7 ± 0.9 (47%)	8.1 ± 1.2 (28%)	11.7 ± 0.9 (40%)	4.9 ± 0.5 (17%)	6.1 ± 0.8 (21%)	2.5 ± 0.9 (9%)
octamers	5.7 ± 0.5 (34%)	3.3 ± 1.2 (20%)	6.5 ± 1.2 (37%)	2.8 ± 0.9 (17%)	5.8 ± 1.0 (34%)	2.3 ± 1.2 (14%)

[a] Values represent means ± standard error ($n = 3$). Values in parentheses represent percent retention compared to fresh berries.

(587.5 mg/kg of FW), but the chlorogenic acid content of jams stored for 4 months (607.5 mg/kg of FW) was similar to that of jams stored for 2 and 6 months. After 6 months of storage, the jams retained 73% of the chlorogenic acid found in fresh berries.

Levels of total flavonols were unaffected by jam type, storage time, storage temperature, or any interactions ($p > 0.05$), demonstrating that the compounds were not only stable in response to processing but were also stable during long-term storage. After 2 months of storage, the total flavonol contents of samples varied little, ranging from a low of 323.2 mg/kg of FW in sugar jams stored at 25 °C to a high of 336.7 mg/kg of FW in sugar jams stored at 4 °C. The total flavonol values of samples after 6 months of storage ranged from a low of 299.8 mg/kg of FW in sugar-free jams stored at 25 °C to a high of 337.1 mg/kg of FW in sugar jams stored at 4 °C. After 6 months of storage, the jams retained 87% of the total flavonols found in fresh berries. Our results are consistent with those of Häkkinen et al. (*22*), who reported excellent stability of flavonols in strawberry jams over 9 months of storage at 5 or −20 °C.

The levels of total procyanidins were significantly affected by storage temperature and storage time ($p \leq 0.05$), but not by jam type or any interactions. Jams stored at 4 °C had on average 13−56 mg/kg of FW higher total procyanidin values than jams stored at 25 °C, with an overall mean difference of 34.4 ± 10.4 mg/kg of FW. Jams stored for 2 months had higher levels of total procyanidins (161.9 mg/kg of FW) than jams stored for 4 (117.4 mg/kg of FW) and 6 (55.8 mg/kg of FW) months. After 6 months of storage, jams stored at 4 °C retained 31% of total procyanidins found in fresh berries, whereas jams stored at 25 °C retained only 18%.

The contents of individual procyanidins in sugar-free and sugar jams over 6 months of storage at 4 and 25 °C are shown in **Table 4**. Similar to total procyanidins, levels of individual procyanidins were affected by storage temperature and storage time ($p \leq 0.05$). Jams stored at 4 °C contained higher levels of all procyanidin oligomers than jams stored at 25 °C, and all procyanidin oligomers declined markedly from 2 to 6 months of storage, with the exception of octamers, which were present in minor amounts after 2 months of storage. Generally, smaller oligomers were retained better than larger oligomers throughout storage, which reflects the changes observed following processing. The losses during storage were most likely due to polymerization reactions with anthocyanins and/or binding of procyanidins to proteins and cell wall polysaccharides.

Polymeric color values were affected by jam type, storage temperature, storage time, and all interactions ($p \leq 0.05$). Polymeric color values increased in both jams types during storage, with the largest increases occurring from 4 to 6 months. Sugar-free jams had lower polymeric color values than sugar jams, which was most evident in samples stored for 6 months. Storage of both jam types at 4 °C ameliorated the increase in polymeric color values observed in jams stored at 25 °C. Polymeric color values were inversely correlated with levels of total procyanidins ($r_{xy} = -0.75$) and total anthocyanins ($r_{xy} = -0.74$), suggesting that anthocyanin−procyanidin polymers were formed during storage.

ORAC values were affected by jam type and storage temperature ($p \leq 0.05$). Sugar jams had on average 3.9−23.7 μmol of TE/g of FW higher ORAC values than sugar-free jams, with an overall mean difference of 13.8 ± 4.8 μmol of TE/g of FW. The higher ORAC values observed for sugar jams were most likely due to higher levels of polymeric anthocyanins or the formation of MRPs. Jams stored at 4 °C had on average 14.9−28.8 μmol of TE/g of FW higher ORAC values than jams stored at 25 °C, with an overall mean difference of 21.9 ± 3.4 μmol of TE/g of FW. These findings were consistent with greater retention of anthocyanins and procyanidins in jams stored at 4 °C. ORAC values did not change over storage despite significant losses of anthocyanins and procyanidins, indicating that formation of polymeric compounds such as anthocyanin−procyanidin complexes or MRPs compensated for the loss of antioxidant capacity as a result of anthocyanin and procyanidin degradation.

In summary, processing of sugar and sugar-free jams resulted in losses of chlorogenic acid, ORAC, total anthocyanins, and total procyanidins, whereas polymeric color values increased and total flavonols remained constant. Further losses of anthocyanins and procyanidins occurred from 2 to 6 months of storage, which coincided with increased polymeric color values. In contrast, levels of chlorogenic acid, total flavonols, and ORAC changed little during storage. Jams stored at 4 °C retained higher levels of ORAC, anthocyanins, and procyanidins and had lower polymeric color values than jams stored at 25 °C. Sugar-free jams retained higher levels of anthocyanins than sugar jams late during storage and had much lower polymeric color values. Our results indicate that blueberry jams should be refrigerated to better preserve polyphenolics and antioxidant capacity.

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