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Effect of Storage Conditions on the Biological Activity of Phenolic Compounds of Blueberry Extract Packed in Glass Bottles

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Recent research suggests that blueberries are rich in total polyphenols and total anthocyanins. Phenolic compounds are highly unstable and may be lost during processing, particularly when heat treatment is involved. There is no systematic study available providing information on the fate of phenolic compounds during storage and how that affects their biological activity. We provide a systematic evaluation of the changes observed in total polyphenols (TPP), total anthocyanins (TACY), Trolox equivalent antioxidant capacity (TEAC), phenolic acids, and individual anthocyanins of blueberry extract stored in glass bottles and the ability of blueberry extract to inhibit cell proliferation. The extract was stored at different temperatures (−20 ± 1, 6 ± 1, 23 ± 1, and 35 ± 1 °C). Two cultivars, Tifblue and Powderblue, were chosen for the study. The recoveries of TPP, TACY, and TEAC in blueberry extract after pressing and heating were ∼25, ∼29, and ∼69%, respectively, for both cultivars. The recovery of gallic acid, catechin, and quercetin was ∼25%. Ferulic acid was not detected in the final extract in both Tifblue and Powderblue cultivars. The recovery of peonidin, malvidin, and cyanidin glycosides was ∼20% in the final extract in both cultivars. Losses due to storage were less when compared with initial losses due to processing. At −20 °C, no statistically significant loss of TPP, TACY, and TEAC was observed up to 30 days ($P < 0.05$). At 6 °C storage, there was a significant loss observed from 15 to 30 days. Similar results were obtained at 23 and 35 °C ($P < 0.05$). There was retention of more than 40% of ellagic and quercetin after 60 days at 35 ± 1 °C. Anthocyanins were not detected after 60 days of storage at 35 ± 1 °C. Significant retention ($P < 0.05$) was obtained for malvidin (42.8 and 25.8%) and peonidin (74.0 and 79.5%) after 60 days of storage at 23 ± 1°C in glass bottles for Tifblue and Powderblue, respectively, when compared with other individual anthocyanins. A linear relationship was observed between TEAC values and total polyphenols or total anthocyanins. A cell viability assay was performed using HT-29 cancer cell lines and anthocyanins extracted from 30, 60, and 90 days of stored extract at 6 ± 1 and 23 ± 1 °C. A significant cell proliferation inhibition percentage was observed in 30 days, although this was reduced significantly after 30−90 days. These results suggest that heating and storage conditions significantly affect the phenolic compounds and their biological activities. Frozen and low temperature storage are suggested for blueberry extract in order to retain the bioactive components.

KEYWORDS: Anthocyanins; blueberries; cell proliferation; flavonoids; glass bottles; phenolic compounds; storage; TEAC

INTRODUCTION

Epidemiological evidence suggests that diets rich in fruits and vegetables are associated with a reduced risk of cancer (*1*), coronary heart disease (*2*, *3*), and stroke (*4*). Fruits and vegetables are excellent sources of phenolic compounds, including phenolic acids and flavonoids. Plants produce phytochemicals for protection from herbivores, parasites, and oxidative stress. More than 4000 of these compounds generally referred to as flavonoids have been identified in both higher and lower plants. Most of the phenolic compounds in plants occur as glycosides or as esters. **Figure 1** shows representative examples

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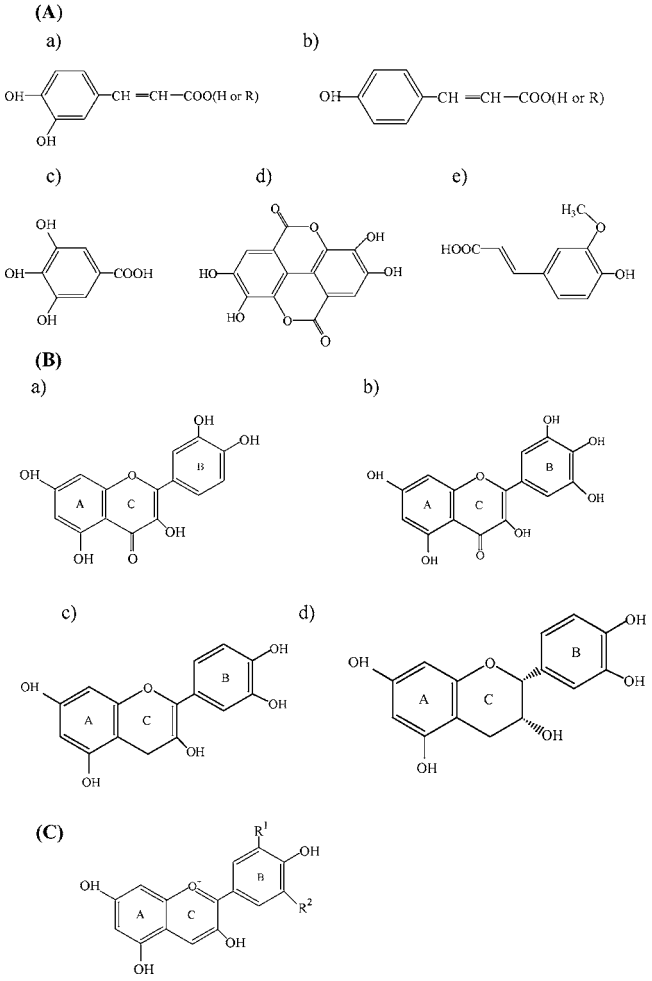

Figure 1. Structure of phenolic compounds. (**A**) Phenolic acids: a, caffeic acid (number of OH-2); b, *p*-coumaric acid (number of OH-1); c, gallic acid (number of OH = 3); d, ellagic acid (number of OH = 4); and e, ferulic acid (number of OH-1). (**B**) Flavonoids: a, quercetin (number of OH = 5); b, myricetin (number of OH = 6); c, (+)-catechin (number of OH = 5); and d, epicatechin (number of OH = 5). (**C**) Anthocyanins: i, pelargonidin (R^1 = H, R^2 = H); ii, cyanidin (R^1 = OH, R^2 = H); iii, peonidin (R^1 = OMe, R^2 = H); iv, delphinidin (R^1 = OH, R^2 = OH); v, petunidin (R^1 = OMe, R^2 = OH); and vi, malvidin (R^1 = OMe, R^2 = OMe).

of phenolic acids and flavonoid compounds estimated in the study (*5*, *6*). Blueberry (*Vaccinium corymbosum*) of the family Ericaceae is reported to have a high antioxidant activity as compared to other fruits and vegetables (*7*, *8*). This is highly correlated with the anthocyanins and total polyphenolic contents (*9*).

Polyphenolic compounds including anthocyanins are not completely stable. After harvest, these compounds undergo changes during processing and storage (*10*, *11*), which may alter their biological activity. Anthocyanins and polyphenolics are readily oxidized because of their antioxidant properties and are thus prone to degradation. The native enzyme polyphenol oxidase (PPO), which is present in blueberry, is responsible for the oxidation of polyphenolics to quinones, which produce brown pigments (*12*) and affect the color of the extract/juice/concentrate. Heating was shown to inhibit PPO activity (*12*). The significant deterioration of phenolic compounds in highbush blueberries when converted to juice has already been discussed in several reports (*13*, *14*). Oxygen, pH, and various storage conditions are shown to have marked effects on anthocyanin stability (*15*). Previous reports are available on pomegranate juice color and bioactive compounds during storage (*16*). Reports describing changes in antioxidant capacity or antiproliferation activity due to storage of the extract are the focused area of our research.

The objectives of the present work were (i) to study the effect of storage in glass bottles on phenolic compounds and their antioxidant capacities in blueberry extract and (ii) to extract anthocyanin fractions from blueberry extract stored in glass bottles under different temperature conditions and evaluate their effects on cell proliferation activity using the HT-29 colon cancer cell line. Tifblue and Powderblue were the cultivars used. These are the most common berries grown in Georgia and are gaining attention due to their high anthocyanin contents.

MATERIALS AND METHODS

Chemicals. Pure standards of gallic acid, *p*-hydroxybenzoic acid, (+)-catechin, caffeic acid, (−)-epicatechin, *p*-coumaric acid, ferulic acid, ellagic acid, quercetin, and kaempferol were purchased from Sigma (St. Louis, MO). Anthocyanin standards were purchased from Polyphenols Laboratories (AS) (Sandnes, Norway). These standards were Dp-Glc (delphinidin 3-*O*-*β*-glucopyranoside), Cy-Gal (cyanidin 3-*O*-*β*-galactopyranoside), Cy-Glc (cyanidin 3-*O*-*β*-glucopyranoside), Pt-Glc (petunidin 3-*O*-*β*-glucopyranoside), Pn-Gal (peonidin 3-*O*-*β*-galactopyranoside), Pn-Glc (peonidin 3-*O*-*β*-glucopyranoside), Mv-Glc (malvidin 3-*O*-*β*-glucopyranoside), and Pn-Ara (peonidin 3-*O*-α-arabinopyranoside). Folin−Ciocalteu reagent, dimethylsulfoxide (DMSO), and pectinase enzyme (Pectinex ultra SP-L, ≥26000 units/mL) were purchased from Sigma. Acetone, acetonitrile, methanol, *O*-phosphoric acid [85% purity, high-performance liquid chromatography (HPLC) grade], hydrochloric acid (analytical grade), sulfuric acid, formic acid, and water (HPLC grade) were purchased from Fisher Scientific (Norcross, GA). Glass bottles were purchased from Speciality Bottle Supply (Seattle, WA). MTT Cell Proliferation Assay kits were purchased from ATCC (Manassas, VA). The human colorectal adenocarcinoma HT-29 cell line was purchased from ATCC.

Sample Collection. Mature blueberries were harvested from the farms at the Experiment Station, University of Georgia, Tifton, Georgia, in 2005. The blueberry cultivars collected were Tifblue and Powderblue. Samples were frozen and stored at −40 °C until use.

Extract Preparation. Blueberry extract was prepared using a modified method reported by earlier workers for juice preparation (*11*). Frozen berries were thawed at 5 °C for 12 h. Berries were blanched using boiling water for 3 min. Blanched berries were milled in a household blender in three lots of 650 g each. Pectinase enzyme (2.2 mL) was added to 2100 g of crushed berries. The mix was stirred well manually and kept at room temperature for 1 h. The crushed berries were centrifuged at 9740*g* for 20 min at 10 °C. The extract (supernatant) was collected, and the residue was discarded. The extract (1.2 L) was gradually heated to 85 °C and was held at 85 °C for 2 min. The temperature was brought down to 75 °C using cold water (15 °C). The extract was filled in glass bottles, capped, and cooled to 30 °C using chilled water.

Storage Conditions. The extract was packed in glass bottles (30 mL) and kept at −20 ± 1, 5 ± 1, 23 ± 1, and 35 ± 1 °C, and the withdrawal time was set for 15, 30, 45, and 60 days. For the cell proliferation assay, extract samples were withdrawn after 30, 60, and 90 days of storage. All samples were analyzed in triplicate, and average results were reported.

Anthocyanin Extraction. The anthocyanin fraction from extract was obtained using a method reported by Yi et al. (*17*). After incubation, extract samples were applied to an activated Oasis HLB cartridge (Waters Corp., Milford, MA). The 15% methanol fraction contained the phenolic acids, and the acidified methanol (5% formic acid in methanol) eluted the anthocyanins. The fraction containing the anthocyanins was freeze-dried using a Unitop 600L freeze dryer (Virtis, Gardiner, NY). Extraction and hydrolysis for total polyphenols (TPP) and total anthocyanins (TACY) of blueberry fruit were done using the method reported by Sellappan et al. (*18*).

Table 1. Individual Phenolic Acids and Flavonoids in Frozen Blueberries[a]

	frozen blueberries							
	phenolic acid (mg/100 g fruit weight)					flavonoids (mg/100 g fruit weight)		
	gallic acid	caffeic acid	*p*-coumaric acid	ferulic acid	ellagic acid	catechin	quercetin	myricetin
Tifblue	5.5 ± 3.5	32.5 ± 4.9	29.5 ± 4.9	69.0 ± 11.3	11.0 ± 5.7	146.5 ± 10.6	8.5 ± 0.7	3.5 ± 2.1
Powderblue	31.0 ± 1.4	56.5 ± 0.7	65.5 ± 6.4	11.5 ± 0.7	36.0 ± 1.4	75.5 ± 6.4	6.5 ± 3.5	4.0 ± 1.4

[a] Each value is expressed as a mean ± SD; $n = 3$.

Total Soluble Solids (TSS) and pH. TSS and pH were measured using an Atago Abbey hand refractometer (0−32 °Brix) and a pH meter-340, respectively.

Estimation of TACY. The TACY content of the juice was estimated on a UV−visible spectrophotometer (Shimadzu UV-1601, Norcross, GA) by the pH-differential method using two buffer systems: potassium chloride buffer, pH 1.0 (0.025 M), and sodium acetate buffer, pH 4.5 (0.4 M). Samples were diluted in pH 1.0 and pH 4.5 buffers and then measured at 520 and 700 nm. The absorbance was calculated as $A = (A_{510nm} - A_{700nm})_{pH1.0} - (A_{510nm} - A_{700nm})_{pH4.5}$.

The monomeric anthocyanin pigment concentration in the extract was calculated as cyanidin-3-glucoside. The monomeric anthocyanin pigment (mg/L) $= A \times MW \times DF \times 1000/(\epsilon \times 1)$ where A = absorbance, MW = molecular weight (449.2), DF = dilution factor, and ϵ = molar absorptivity (26900). The final concentration of anthocyanins (mg/100 g of frozen fruit) was calculated based on the total volume of the extract and the weight of the sample.

Estimation of TPP. The TPPs were estimated colorimetrically using the Folin−Ciocalteu method (*19*). Extract samples were filtered through a 0.2 μm nylon syringe filter. A sample aliquot of 20 μL was added to 800 μL of water, 1 mL of 0.2 N Folin−Ciocalteu reagent, and 0.8 mL of saturated sodium carbonate solution (7.5%) and mixed well. The absorbance was measured at 765 nm with a Shimadzu UV−visible spectrophotometer after incubation for 30 min at room temperature. Quantification was based on the standard curve generated with 100, 200, 300, and 400 mg/L of gallic acid.

Assay of Antioxidant Capacity. The antioxidant capacity was performed on the Shimadzu UV−visible spectrophotometer in a kinetic mode based on the method of Re et al. (*20*). Briefly, $ABTS^{\bullet+}$ radical cation was produced by reacting 7 mM 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2.45 mM potassium persulfate after incubation at room temperature in the dark for 16 h. The $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.1 at 734 nm. The filtered sample was diluted with ethanol to give 20−80% inhibition of the blank absorbance with 20 μL of sample. A 980 μL aliquot of $ABTS^{\bullet+}$ solution (absorbance of 0.70 ± 0.1) was read at 734 nm for a minute; after exactly 1 min, 20 μL of the sample was added and mixed thoroughly. Absorbance was continuously taken at every 6 s up to 7 min. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a vitamin E analogue) standards of final concentrations of 0−15 μM in ethanol were prepared and assayed under the same conditions. The Trolox equivalent antioxidant capacity (TEAC) of the sample was calculated based on the inhibition exerted by the standard Trolox solution at 6 min.

Hydrolysis. Hydrolysis was done as reported by Yi et al. (*17*). For the phenolic acid and flavonoids analysis, fractions were dissolved in methanol containing 1.2 N HCl (40 mL of methanol + 10 mL of 6 N HCl). The samples were then placed in a water bath at 80 °C with continuous shaking at 200 rpm for 2 h, to hydrolyze phenolic glycosides to aglycones. Anthocyanin fractions were dissolved in 50% methanol solution containing 2 N HCl (50 mL of methanol + 33 mL of water + 17 mL of 37% HCl). Samples were placed in a water bath at 80 °C with shaking at 200 rpm for 2 h to hydrolyze anthocyanins.

HPLC Analysis. HPLC was performed with a Hewlett−Packard (Avondale, PA) model 1100 liquid chromatograph with quaternary pumps and a diode array UV−visible detector. For the analysis of phenolic acids and flavonoids in blueberry juices, procedures previously reported by our laboratory were used (*17*, *18*). A Beckman ultrasphere C18 ODS 4.6 mm × 250 mm column was used with the column temperature at 40 °C. The mobile phases were solvent A, methanol/acetic acid/water (10:2:88, v/v/v); solvent B, acetonitrile; and solvent C, water. The gradients were as follows: at 0 min, 100% solvent A; at 5 min, 90% solvent A and 10% solvent B; at 25 min, 30% solvent A and 70% solvent B; and at 30 min, 30% solvent A and 70% solvent B, with 5 min postrun with 100% solvent C. The flow rate was 1 mL/min. Phenolic acids were detected at wavelengths of 260, 313, and 360 nm. For the anthocyanin analysis, the mobile phase was as follows: solvent A, O-phosphoric acid/methanol/water (5:10:85, v/v/v); solvent B, acetonitrile. The flow rate was 0.5 mL/min. The gradient for the separation was a linear gradient of 100 to 50% solvent A and 0−50% solvent B over 25 min, followed by 5 min postrun with HPLC grade water. Anthocyanins were detected at 520 nm.

Cell Cultures. The human colorectal adenocarcinoma HT-29 cancer cells were cultured in ATCC McCoy's medium with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM nonessential amino acids, 1.0 mM sodium pyruvate (90%), and 10% fetal bovine serum. Cells were incubated under 37 °C with 5% CO_2. The medium was changed 2−3 times per week.

Cell Proliferation Assay. After digestion with trypsin−EDTA, uniform amounts (~2×10^4) of HT-29 cells in growth media were inoculated into each well of a 96 well flat-bottom plate. After 24 h of incubation at 37 °C in 5% CO_2, the growth medium was replaced with 100 μL of medium containing anthocyanin extract from juice stored under different storage conditions. On the basis of the results of earlier reports from our laboratory (*17*), concentrations of each extract were kept at 100 μg/mL. Control cultures received everything but the anthocyanin fractions, and blank wells contained 100 μL of growth medium and extract without cells. Anthocyanin fractions (water soluble) were directly dissolved in culture medium, and DMSO was added initially to the extracts/fractions to help dissolve the sample. The final DMSO content in the highest concentration of fraction treatment was 0.25%. Therefore, the control for these treatments also contained the same amount of DMSO. After 48 h of incubation, cell population growth was determined using the ATCC MTT cell proliferation assay at 570−655 nm with a Bio-Rad model 680 Microplate Reader (Hercules, CA). Briefly, a mitochondrial enzyme in living cells, succinate dehydrogenase, reduced the yellow tetrazolium salt (MTT) to insoluble purple formazan crystals. Therefore, the amount of formazan produced was proportional to the number of viable cells (*21*). To better explain the inhibitory results, the inhibition of cell population growth was calculated based on the following formula:

$$\text{inhibition percentage} = \frac{(\text{cell no. in control} - \text{cell no. in treatment})}{(\text{cell no. in control} - \text{original cell no. before the extract was added})} \times 100$$

Statistical Analysis. Statistical analysis was done with the SAS software package (*22*). One-way analysis of variance (ANOVA) was performed to determine the difference among stored samples. When F values for the ANOVA were significant, differences in means were determined using Duncan's multiple range tests as a procedure of means separation ($P < 0.05$).

RESULTS AND DISCUSSION

The contents of individual phenolic acids and flavonoids of frozen blueberries are given in **Table 1**, and individual anthocyanins are shown in **Table 2**. Catechin was the major flavonoid present in both cultivars: Tifblue (146.5 mg/100 g of berry)

Table 2. Individual Anthocyanins in Frozen Blueberries[a]

	frozen blueberries (mg/100 g of fruit weight)							
	Dp-Glc	Cy-Glc	Cy-Gal	Pn-Glc	Pn-gal	Pt-Glc	Pn-Ara	Mv-Glc
Tifblue	8.5 ± 0.7	5.5 ± 0.7	18.0 ± 2.8	6.0 ± 1.4	10.0 ± 1.4	6.5 ± 0.7	15.5 ± 0.7	39.0 ± 2.8
Powderblue	9.0 ± 0.0	4.5 ± 0.7	12.5 ± 2.1	5.5 ± 2.1	9.5 ± 2.1	5.5 ± 2.1	14.0 ± 1.4	38.0 ± 5.7

[a] Each value was expressed as a mean ± SD; $n = 3$.

Table 3. TPP, TACY, and TEAC Contents of Frozen Blueberry and Blueberry Extract after Pressing and Heating[a]

	Tifblue				Powderblue			
	frozen berries	extract after pressing	extract after heating	recovery (%)	frozen berries	extract after pressing	extract after heating	recovery (%)
TPP[b]	344.8 ± 4.7	87.3 ± 2.7	87.3 ± 1.8	25.3	383.4 ± 8.4	87.9 ± 0.7	90.7 ± 0.8	23.7
TACY[c]	114.5 ± 3.2	29.9 ± 0.3	33.6 ± 0.5	29.3	120.9 ± 3.7	33.4 ± 1.8	36.1 ± 0.3	29.9
TEAC[d]	26.1 ± 1.1	17.0 ± 0.9	17.2 ± 0.1	65.9	27.3 ± 1.2	17.5 ± 0.6	17.4 ± 0.3	63.7

[a] The recovery percent was calculated based on frozen berries concentration as 100%. Each value was expressed as a mean ± SD; $n = 3$. [b] TPP is the TPP (mg/100 g of frozen blueberry or extract). [c] TACY is the TACY mg/100 g of frozen blueberry or extract. [d] TEAC is the TEAC (μM/g whole frozen blueberry or extract).

Table 4. Phenolic Acids and Flavonoids in Frozen Blueberry and Blueberry Extract after Pressing and Heating[a]

	Tifblue				Powderblue			
phenolic acids and flavonoids	frozen berries	extract after pressing	extract after heating	recovery (%)	frozen berries	extract after pressing	extract after heating	recovery (%)
gallic acid	5.5 ± 3.5	1.8 ± 0.4	2.0 ± 0.2	35.5 ± 3.9	31 ± 1.4	27 ± 5.7	12.7 ± 1.1	41.0 ± 3.7
caffeic acid	32.5 ± 4.9	21.5 ± 1.8	4.4 ± 0.6	13.4 ± 2.0	56.5 ± 0.7	19.1 ± 1.2	5.4 ± 2.2	9.5 ± 3.9
p-coumaric acid	29.5 ± 4.9	6.0 ± 0.4	4.7 ± 0.5	15.8 ± 1.7	65.5 ± 6.4	8.5 ± 0.92	5.5 ± 0.9	8.3 ± 1.4
ferulic acid	69.0 ± 11.3	7.6 ± 0.4	ND	ND	11.5 ± 0.7	4.9 ± 0.3	ND	ND
ellagic acid	11.0 ± 5.7	5.9 ± 1.0	2.8 ± 0.1	24.4 ± 1.2	36 ± 1.4	17.2 ± 1.0	12.1 ± 1.3	33.5 ± 3.7
catechin	146.5 ± 10.6	79.6 ± 10.8	36.3 ± 0.4	24.8 ± 0.3	75.5 ± 6.4	68.2 ± 1.1	33.1 ± 4.5	43.8 ± 6.0
myrecitin	3.5 ± 2.1	0.6 ± 0.4	ND	ND	4.0 ± 1.3	0.5 ± 0.1	ND	ND
quercetin	8.5 ± 0.7	2.2 ± 0.4	2.9 ± 0.4	33.5 ± 4.2	6.5 ± 3.5	2.7 ± 0.6	2.3 ± 0.5	34.6 ± 7.6

[a] Abbreviations: ND, not detected. Phenolic acids and flavonoids are expressed in mg/100 g of frozen fruit and mg/100 mL of extract. The recovery percent was calculated based on frozen berries concentration as 100%. Each value was expressed as a mean ± SD; $n = 3$.

and Powderblue (75.5 mg/100 g of berry). Ferulic acid, caffeic acid, *p*-coumaric acid, and ellagic acid were the other predominant phenolic acids present in blueberry. These values were higher than those for fresh berries reported by Sellappan et al. (*18*). The major anthocyanidin found in Tifblue and Powderblue cultivers was malvidin followed by peonidin > cyanidin > delphinidin > petunidin (**Table 2**). A similar order was reported in highbush and lowbush varieties (*23*). The initial analysis of TPP, TACY, and TEAC of frozen blueberries and blueberry extract after pressing and heating is given in **Table 3**. As compared to the present study, our previous study showed 20% less TPP content and TEAC and 30% more TACY (*9*, *18*). These variations may be due to differences in blueberry cultivars, storage conditions, stage of maturity, and environmental factors such as light, temperature, agronomic practices, and various stresses. A single genotype of lowbush blueberries may differ in their anthocyanin contents by 30% between two seasons (*15*). The Powderblue cultivar exhibited a higher antioxidant capacity, TPP, and TACY than Tifblue. The initial analysis of individual phenolic acids and flavonoids and individual anthocyanins of frozen blueberries and blueberry extracts after pressing and heating of frozen blueberries is given in **Tables 4** and **5**, respectively. The recoveries of gallic acid, catechin, and quercetin were above 25% in the final extract after heating and removal of the residue. Ferulic acid was not detected in the final extract in both cultivars, Tifblue and Powderblue. The recovery of peonidin, malvidin, and cyanidin glycosides was ~20% in the final extract in both cultivars. These results suggest that most of the phenolic compounds are lost during removal of the residue and during heating. Similar results were reported by earlier workers during juice preparations (*11*, *13*, *14*).

Changes in TPP, TACY, and TEAC during Preparation of Extract. There was no significant change observed in TSS (11.8−12.5 °Brix) and pH (3.2−3.4) in the extracts obtained after pressing and heating. Similar results were observed in both cultivars (Tifblue and Powderblue). The recoveries of TPP, TACY and TEAC in blueberry extract after pressing and heating were ~25, ~29, and ~69%, respectively. The primary steps of preparation (thawing, crushing, depectinization, and pressing) may have contributed to the considerable loss. Similar results were reported by other investigators (*14*, *24*). This may be attributed to the oxidation of anthocyanins and polyphenols (*14*). Many researchers have suggested that native blueberry PPO oxidizes polyphenolics to quinones, which produce brown pigments (*12*). However, after heating, there was a slight increase in TPP in Powderblue. A slight increase in TACY was also observed in both cultivars (Tifblue and Powderblue). This might be due to greater extraction due to fruit skin permeability/concentration during heating or complete inactivation of PPO (*15*).

Effect of Storage Conditions on TPP. TPPs at different temperatures and time intervals are given in **Figure 2**. The cultivar type was not a significant predictor for the retention of TPP at all temperatures. However, the interaction term, time × temperature, was found to be a significant contributor to the degradation of TPP ($P < 0.05$). At −20 ± 1 °C, no statistically significant losses of TPP were observed until 30 days ($P <$

Table 5. Individual Anthocyanins in Frozen Blueberry and Blueberry Extract after Pressing and Heating[a]

	Tifblue				Powderblue			
anthocyanins	frozen berries	extract after pressing	extract after heating	recovery (%)	frozen berries	extract after pressing	extract after heating	recovery (%)
Dp-glc	8.5 ± 0.7	3.2 ± 0.1	ND	ND	9.0 ± 0.0	3.5 ± 0.6	ND	ND
Cy-glc	5.5 ± 0.7	6.8 ± 0.7	1.1 ± 0.2	19.9 ± 3.2	4.5 ± 0.7	8.3 ± 0.2	1.1 ± 0.2	25.1 ± 5.3
Cy-gal	18.0 ± 2.8	18.4 ± 1.0	1.0 ± 0.1	5.6 ± 0.8	12.5 ± 2.1	18.9 ± 0.1	1.0 ± 0.1	7.7 ± 0.8
Pn-glc	6.0 ± 1.4	3.6 ± 1.0	3.2 ± 0.1	52.7 ± 0.9	5.5 ± 2.3	2.8 ± 0.1	3.4 ± 0.1	62.1 ± 2.2
Pn-gal	10.0 ± 1.4	22.6 ± 0.8	1.1 ± 0.0	10.5 ± 0.1	9.5 ± 1.9	25.0 ± 0.7	1.2 ± 0.0	12.4 ± 0.3
Pt-glc	6.5 ± 0.7	20.9 ± 3.1	ND	ND	5.5 ± 2.0	24.0 ± 1.3	ND	ND
Pn-ara	15.5 ± 0.6	20.7 ± 5.0	8.7 ± 0.3	56.2 ± 1.8	14 ± 1.4	20.6 ± 1.2	8.1 ± 0.1	58.0 ± 0.8
Mv-glc	39.0 ± 2.8	38.8 ± 14.5	7.7 ± 0.0	19.7 ± 0.0	38 ± 5.7	33.3 ± 7.9	8.6 ± 0.2	22.7 ± 0.5

[a] Abbreviations: ND, not detected. Each anthocyanin is expressed in mg/100 g of frozen fruit and mg/100 mL of extract. The recovery percent was calculated based on frozen berries concentration as 100%. The recovery percent was calculated based on frozen berries concentration as 100%. Each value was expressed as a mean ± SD; $n = 3$.

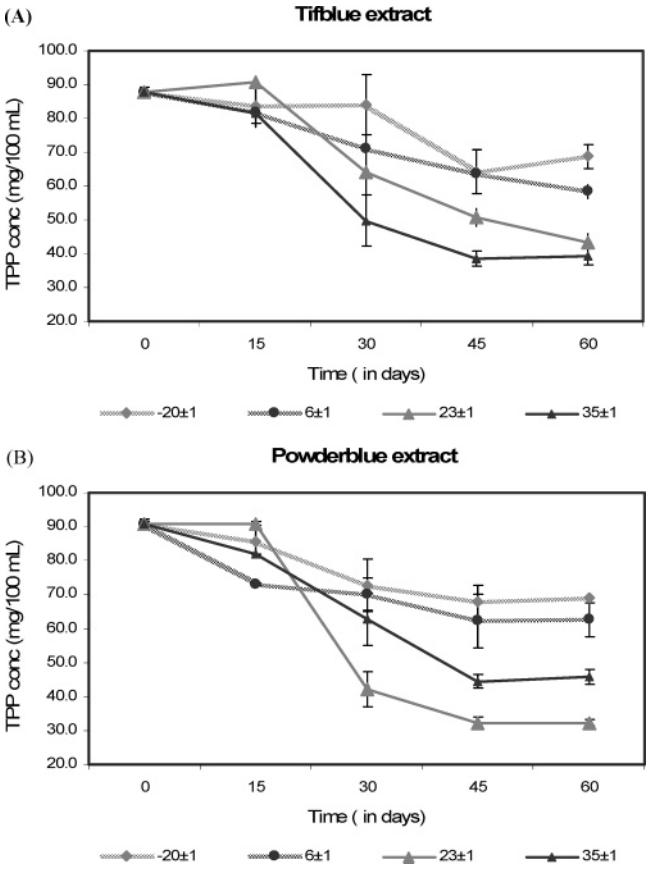

Figure 2. Changes in concentration of TPP in blueberry extract during storage at different temperatures. The *X*-axis represents time (in days). The *Y*-axis represents the concentration of TPP (mg/100 mL of extract). Samples were analyzed in 15 day intervals. Storage temperatures were at −20 ± 1, 6 ± 1, 23 ± 1, and 35 ± 1 °C. Cultivars were (**A**) Tifblue and (**B**) Powderblue.

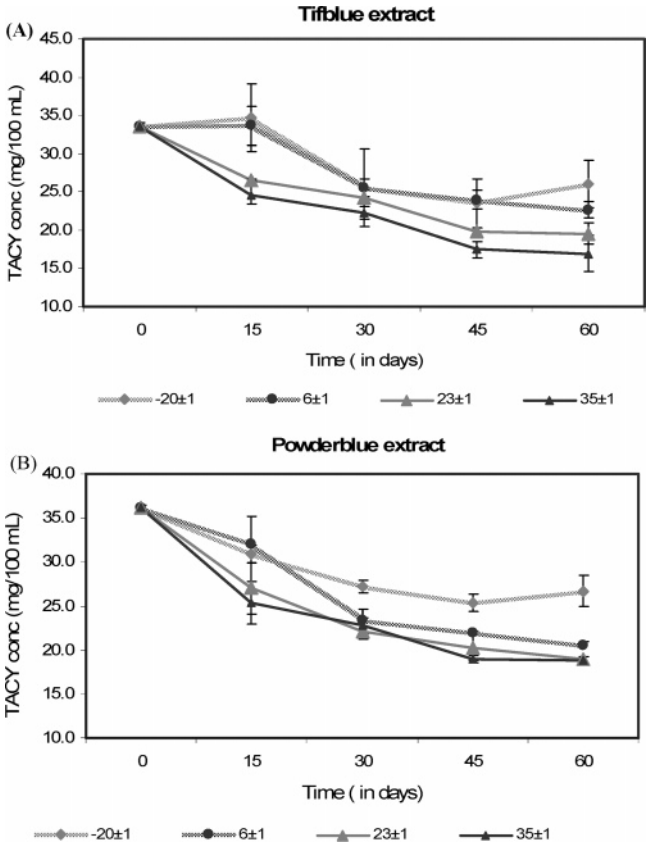

Figure 3. Changes in concentration of TACY in blueberry extract during storage at different temperatures. The *X*-axis represents time (in days). The *Y*-axis represents the concentration of TACY (mg/100 mL of extract). Samples were analyzed every 15 days. Samples were analyzed in 15 day intervals. Storage temperatures were at −20 ± 1, 6 ± 1, 23 ± 1, and 35 ± 1 °C. Cultivars were (**A**) Tifblue and (**B**) Powderblue.

0.05). A slight reduction was observed by 30 days, but thereafter, no significant degradation of TPP was observed. At 6 ± 1 °C, there were significant losses observed from 15 to 30 days and then the retention became constant. Similar results were observed at 23 ± 1 and 35 °C ($P < 0.05$). Earlier researchers reported no significant change in TPP for 12 months for frozen blueberry fruit (*25*), whereas high temperature and oxidative conditions were shown to significantly reduce TPPs of lowbush blueberries (*13*, *15*).

Effect of Storage Conditions on TACY. The TACY at different temperatures and time intervals is given in **Figure 3**. The effect of storage on individual anthocyanins will be discussed separately. All variables (time, temperature, and interaction term, time × temperature) were significant contributors ($P < 0.05$) to the degradation of TACY. Statistically significant ($P < 0.05$) differences were observed for the two cultivars in the degradation of TACY. Duncan's multiple range tests showed that the highest value (34.7 mg/100 mL of extract) of TACY was observed at −20 ± 1 °C after 15 days of storage. The lowest retention (17.0 mg/100 mL) was observed after 60 days at 35 ± 1 °C in extract obtained from the Tifblue cultivar. At all temperatures, significant differences in degradation were observed from 0 to 30 days ($P < 0.05$); thereafter, it became constant. As discussed above, PPO is involved in the degradation

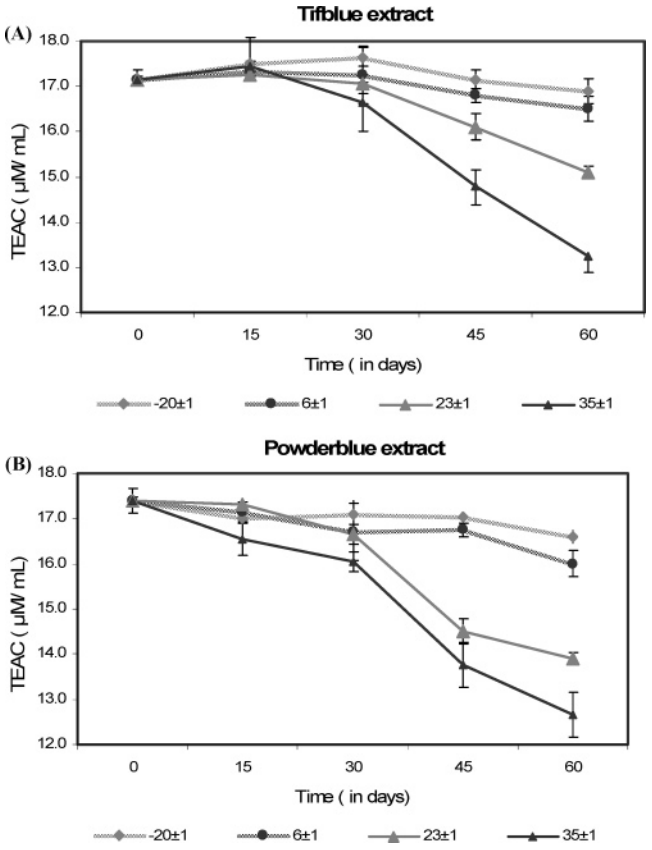

Figure 4. Changes in TEAC in blueberry extract during storage at different temperatures. The *X*-axis represents time (in days). The *Y*-axis represents TEAC in μM/mL of extract. Samples were analyzed every 15 days. Samples were analyzed in 15 day intervals. Storage temperatures were at -20 ± 1, 6 ± 1, 23 ± 1, and 35 ± 1 °C. Cultivars were (**A**) Tifblue and (**B**) Powderblue.

of anthocyanins. There was no significant difference observed between 45 and 60 days. The chemical structure is a main factor affecting the stability of the anthocyanins. The stability and structure of individual anthocyanins are discussed later in this paper.

Effect of Storage Conditions on TEAC. The TEAC at different temperatures and time intervals is given in **Figure 4**. Statistically significant ($P < 0.05$) differences were observed for the two cultivars with Tifblue having higher antioxidant activity than Powderblue. At -20 ± 1 °C, the storage time did not affect the antioxidant activity. However, at 35 ± 1 °C, there was significant loss of activity after 15 days, which continued through 60 days. **Table 6** gives the total retention of TPP, TACY, and TEAC after 60 days under all temperature conditions. Heat, storage time, and oxidation contribute to the loss of antioxidant activity (*13*). The antioxidant activity of phenolic acids depends on the number and position of hydroxyl (−OH) groups and methoxy ($-OCH_3$) subsitituents in the molecules. Hydroxylation and glycosylation modulate the antioxidant properties of flavonoids (*26*). Prolonged storage at high temperature may affect the hydroxylation and glycosylation of compounds and lead to gradual reduction in antioxidant activity (TEAC) as we observed. However, the rate of reduction was not similar to that of TPP and TACY, suggesting that there may be other compounds responsible, in part, to the antioxidant activity. A good example would be ascorbic acid, which is present in blueberry and other fruits.

Effect of Storage Conditions on Phenolic Acids and Flavonoids. The percent retention of phenolic acids and flavonoids at different temperatures after 60 days of storage is given in **Tables 7** (Tifblue) and **8** (Powderblue). Temperature and time affected the retention of phenolic acids and flavonoids ($P < 0.05$). Phenolic acids in Tifblue and Powderblue were gallic acid, caffeic acid, *p*-coumaric acid, and ellagic acid, and flavonoids were catechin and quercetin. At -20 ± 1 °C, the

Table 6. Percent Retention of TPP, TACY, and TEAC after 60 Days at Different Temperature Conditions[a]

	temperature (°C)							
	Tifblue				Powderblue			
	-20 ± 1	6 ± 1	23 ± 1	35 ± 0.5	-20 ± 1	6 ± 1	23 ± 1	35 ± 0.5
TPP[b]	78.3 ± 3.5 a	66.6 ± 2.2 b	49.4 ± 2.5 c	49.4 ± 2.5 c	75.9 ± 0.1 a	68.9 ± 4.1 b	35.6 ± 1.1 d	50.3 ± 2.3 c
TACY[c]	77.8 ± 3.1 a	67.5 ± 1.1 c	50.4 ± 1.3 e	50.7 ± 2.3 e	75.4 ± 1.1 b	68.5 ± 0.4 d	51.3 ± 0.6 e	31.5 ± 0.5 e
TEAC[d]	98.0 ± 0.3 a	95.7 ± 0.5 b	87.6 ± 0.1 e	76.6 ± 0.4 g	94.6 ± 0.4 c	91.2 ± 0.4 d	79.2 ± 0.6 c	79.2 ± 0.6 f

[a] The retention percent was calculated after 60 days at given temperature conditions. The percent was calculated based on the 0 day concentration as 100%. Each value was expressed as a mean ± SD; $n = 3$. [b] TPP is TPP (mg/100 mL of blueberry extract). [c] TACY is TACY mg/100 mL of blueberry extract). [d] TEAC is TEAC (μM/mL of blueberry extract).

Table 7. Retention of Blueberry Phenolic Acids and Flavonoids in Tifblue Blueberry Extract after 60 Days under Different Temperature Conditions

		after 60 days							
	0 day	-20 ± 1 °C		6 ± 1 °C		23 ± 1 °C		35 ± 1 °C	
	concn[a]	concn[a]	retention (%)	concn[a]	retention (%)	concn[a]	retention (%)	concn[a]	retention (%)
gallic acid	2.0 ± 0.2	0.5 ± 0.0	23.5 ± 1.4	1.2 ± 0.1	61.3 ± 2.5	1.1 ± 0.1	53.3 ± 2.5	1.2 ± 0.0	59.8 ± 1.7
caffeic acid	4.4 ± 0.6	1.7 ± 0.1	39.2 ± 2.4	0.6 ± 0.1	13.6 ± 3.2	0.7 ± 0.1	14.8 ± 1.6	0.9 ± 0.1	19.3 ± 1.6
p-coumaric acid	4.7 ± 0.5	1.3 ± 0.1	27.6 ± 1.7	0.3 ± 0.1	6.4 ± 2.1	0.6 ± 0.1	12.8 ± 3.0	0.4 ± 0.1	8.5 ± 3.0
ferulic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND
ellagic acid	2.8 ± 0.1	2.5 ± 0.1	87.5 ± 2.5	1.5 ± 0.4	53.6 ± 1.5	1.4 ± 0.2	48.2 ± 7.6	1.6 ± 0.1	57.0 ± 5.1
catechin	36.3 ± 0.4	5.6 ± 0.4	15.4 ± 1.2	5.9 ± 0.6	16.1 ± 1.8	3.7 ± 0.3	10.2 ± 0.9	5.4 ± 0.3	14.9 ± 0.8
myrecitin	ND	ND	ND	ND	ND	ND	ND	ND	ND
quercetin	2.9 ± 0.4	2.6 ± 0.3	89.7 ± 9.8	2.1 ± 0.1	70.1 ± 2.4	2.2 ± 0.2	74.1 ± 7.3	1.8 ± 0.3	61.2 ± 11.0

[a] Concn, concentration of phenolic acids and flavonoids expressed in mg/100 mL of extract; ND, not detected. The percent was calculated based on the 0 day concentration as 100%. Each value was expressed as a mean ± SD; $n = 3$.

Table 8. Retention of Blueberry Phenolic Acids and Flavonoids in Powderblue Blueberry Extract after 60 Days under Different Temperature Conditions

		after 60 days							
	0 day	−20 ± 1 °C		6 ± 1 °C		23 ± 1 °C		35 ± 1 °C	
	concn[a]	concn[a]	retention (%)	concn[a]	retention (%)	concn[a]	retention (%)	concn[a]	retention (%)
gallic acid	12.7 ± 1.1	5.0 ± 1.6	18.9 ± 1.1	7.3 ± 0.3	57.7 ± 2.3	8.0 ± 1.3	63.0 ± 1.0	7.0 ± 0.1	54.7 ± 0.6
caffeic acid	5.3 ± 2.2	1.8 ± 0.4	34.0 ± 8.0	0.8 ± 0.1	14.2 ± 1.3	0.8 ± 0.1	15.1 ± 2.7	0.7 ± 0.1	12.3 ± 1.3
p-coumaric acid	5.5 ± 0.9	1.3 ± 0.1	23.4 ± 0.9	0.3 ± 0.0	6.3 ± 0.3	0.4 ± 0.0	8.0 ± 0.8	0.7 ± 0.1	12.0 ± 1.3
ferulic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND
ellagic acid	12.1 ± 1.3	6.0 ± 0.1	49.6 ± 0.6	6.5 ± 0.7	54.2 ± 5.9	5.6 ± 0.5	46.3 ± 4.1	7.2 ± 0.4	60.0 ± 3.5
catechin	33.1 ± 4.5	6.3 ± 0.4	17.4 ± 1.0	3.7 ± 0.3	11.8 ± 0.0	7.8 ± 0.2	23.4 ± 0.6	4.0 ± 0.2	11.9 ± 0.6
myrecitin	ND	ND	ND	ND	ND	ND	ND	ND	ND
quercetin	2.3 ± 0.5	2.3 ± 0.4	97.8 ± 1.5	1.1 ± 0.3	47.8 ± 1.2	1.6 ± 0.4	67.4 ± 1.5	1.3 ± 0.5	54.4 ± 2.1

[a] Concn, concentration of phenolic acids and flavonoids expressed in mg/100 mL of extract; ND, not detected. The percent was calculated based on the 0 day concentration as 100%. Each value was expressed as a mean ± SD; $n = 3$.

Table 9. Retention of Individual Anthocyanins in Tifblue Blueberry Extract after 60 Days under Different Temperature Conditions

		after 60 days							
	0 day	−20 ± 1 °C		6 ± 1 °C		23 ± 1 °C		35 ± 1 °C	
	concn[a]	concn[a]	retention (%)	concn[a]	retention (%)	concn[a]	retention (%)	concn[a]	retention (%)
Dp-glc	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cy-glc	1.1 ± 0.2	0.8 ± 0.1	73.1 ± 2.3	0.5 ± 0.0	42.3 ± 3.2	0.2 ± 0.0	17.7 ± 1.9	ND	ND
Cy-gal	1.0 ± 0.1	0.5 ± 0.0	44.5 ± 3.5	0.4 ± 0.0	37.2 ± 0.1	0.1 ± 0.0	15.0 ± 5.8	ND	ND
Pn-glc	3.2 ± 0.1	1.3 ± 0.2	39.7 ± 8.0	0.7 ± 0.1	20.2 ± 2.4	0.5 ± 0.0	15.5 ± 0.2	ND	ND
Pn-gal	1.1 ± 0.0	0.9 ± 0.0	78.6 ± 1.9	0.7 ± 0.1	64.6 ± 3.9	0.5 ± 0.1	41.4 ± 5.6	ND	ND
Pt-glc	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pn-ara	8.7 ± 0.3	4.1 ± 0.1	46.6 ± 1.0	2.1 ± 0.0	24.3 ± 0.2	1.5 ± 0.1	17.1 ± 1.5	0.1 ± 0.0	17.8 ± 3.1
Mv-glc	7.7 ± 0.0	7.2 ± 0.9	93.7 ± 1.7	4.3 ± 0.1	55.3 ± 0.8	3.3 ± 0.1	42.8 ± 9.1	ND	ND

[a] Concn, concentration of anthocyanins expressed in mg/100 g of frozen fruit and mg/100 mL of extract; ND, not detected. The percent was calculated based on the 0 day concentration as 100%. Each value was expressed as a mean ± SD; $n = 3$.

Table 10. Retention of Individual Anthocyanins in Powderblue Blueberry Extract after 60 Days under Different Temperature Conditions

		after 60 days							
	0 day	−20 ± 1 °C		6 ± 1 °C		23 ± 1 °C		35 ± 1 °C	
	concn[a]	concn[a]	retention (%)	concn[a]	retention (%)	concn[a]	retention (%)	concn[a]	retention (%)
Dp-glc	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cy-glc	1.1 ± 0.2	0.8 ± 0.0	70.0 ± 1.3	0.6 ± 0.0	56.9 ± 3.3	0.2 ± 0.0	17.7 ± 0.6	ND	ND
Cy-gal	1.0 ± 0.1	0.6 ± 0.0	74.5 ± 4.9	0.8 ± 0.0	59.5 ± 2.1	0.3 ± 0.0	27.5 ± 0.7	ND	ND
Pn-glc	3.4 ± 0.1	1.2 ± 0.0	36.3 ± 0.2	1.1 ± 0.1	31.2 ± 1.7	1.4 ± 0.0	42.9 ± 1.3	ND	ND
Pn-gal	1.2 ± 0.0	0.8 ± 0.0	69.4 ± 4.4	0.6 ± 0.0	47.1 ± 1.8	0.2 ± 0.0	16.7 ± 0.0	ND	ND
Pt-glc	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pn-ara	8.1 ± 0.1	2.7 ± 0.2	33.7 ± 2.6	2 ± 0.0	24.6 ± 0.2	1.6 ± 0.1	19.9 ± 0.5	1.6 ± 0.3	19.1 ± 3.3
Mv-glc	8.6 ± 0.2	4.0 ± 0.2	45.9 ± 2.5	6.1 ± 0.1	70.5 ± 1.0	2.2 ± 0.2	25.8 ± 1.4	ND	ND

[a] Concn, concentration of anthocyanin expressed in mg/100 g of frozen fruit and mg/100 mL of extract; ND, not detected. The percent was calculated based on the 0 day concentration as 100%. Each value was expressed as a mean ± SD; $n = 3$.

retention of quercetin was 89.7 and 97.8% for Tifblue and Powderblue, respectively. However, at 35 ± 1 °C, these values reduced to 61.2 and 54.4%, respectively. There are reports available indicating that there was no degradation of quercetin in whole fruits at 5 °C for 9 months (*27*). The retention of ellagic acid varied from 87.5 to 48.2% in Tifblue and from 46.3 to 60.0% in Powderblue at all temperature conditions. The rate of degradation of phenolic acid and flavonoids at room temperature (23 ± 1 °C) is in the following order: quercetin > gallic acid > ellagic acid > catechin > caffeic acid > *p*-coumaric acid. Phenolic acids with higher hydroxyl group attachment may have contributed to the stability. A significant loss of caffeic may have occurred because caffeic acid is a good substrate for blueberry PPO (*12*).

Effect of Storage Conditions on Individual Anthocyanins. The percent retention of individual anthocyanins at different temperatures after 60 days of storage is given in **Tables 9** (Tifblue) and **10** (Powderblue). Anthocyanins found in Tifblue and Powderblue were delphinidin, cyanidin, petunidin, peonidin, and malvidin glycosides. Temperature had a significant effect on retention of anthocyanins. The proportion of Mv-Gly was highest in the extract obtained from both cultivars. The highest retention of Mv-Gly was observed when the extract was stored at −20 and 6 °C for Tifblue and Powderblue, respectively. Delphinidin glycoside was not detected in the extract. It was found most unstable. This was attributed to the methoxylation of the molecule, which increases the stability of anthocyanins (*25*). The stability of Mv-Gly and Pn-Gly increased due to the single hydroxyl group on the phenolic ring, which makes them the least reactive anthocyanins and the least affected by PPO inactivation (*11*). There was a little change observed in peonidin-3-galactoside. A substantial decrease was observed in Cy-Gly.

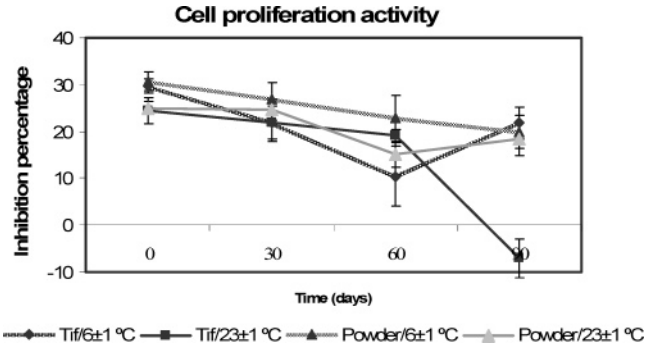

Figure 5. Cell viability after treatment with anthocyanin fractions from extract stored under different temperature conditions. The *X*-axis represents time (in days). The *Y*-axis represents inhibition percentage of cells. Samples were analyzed every 30 days. Storage temperatures were at 6 ± 1 and 23 ± 1 °C. The packaging material was glass bottles. Cultivars were Tifblue and Powderblue. Abbreviations: Tif, Tifblue; Powder, Powderblue.

These results were in agreement with earlier studies (*14*). No anthocyanins were detected at 35 ± 1 °C after 45 days. An increase in glycosidic substitution, acylation, and methoxylation tends to improve the stability of anthocyanins. Methoxylation also increases the stability of anthocyanins (*26*).

Effect of Storage Conditions on Cell Proliferation. Inhibition of cell proliferation at different time intervals and temperatures is given in **Figure 5**. The initial inhibition percentages were 48.8 and 43.1% with anthocyanins from Tifblue and Powderblue, respectively. This decreased slightly in 30 days. However, there were significant decreases after 30−90 days in both cultivars (Tifblue and Powderblue). We observed a slight decrease in inhibition percentage in cells treated with an anthocyanin fraction from extract stored at different time intervals. Interestingly, there was some cell growth (+7%) with anthocyanins from Tifblue extract stored for 90 days at 23 ± 1 °C. The low antiproliferation activity may be due to low or insignificant levels of delphinidin, petunidin, and cyanidin glycosides in the extract. Several researchers reported marked inhibitory effects of anthocyanins and extracts from wild bush blueberries (*28*, *29*). The effects of anthocyanin on HepG2 cell viability have been reported. The estimated IC_{50} values of cyanidin, delphinidin, and malvidin were 18.4, 10.8, and 50.4 μM (equivalent to 5, 3, and 17 μg/mL), respectively (*30*). This suggests that after initial preparatory steps, heating and storage have led to significant reduction in inhibition percentages.

Correlation between TPP, TACY, and TEAC. The correlation between TEAC and TPP or TACY of blueberry extract from different time intervals stored at 23 ± 1 °C is presented in **Figure 6**. The average values of TEAC showed positive correlation with average values of TACY and TPP. A linear relationship was observed between TEAC and TPP or TACY. In Tifblue, the correlation coefficient, r^2, was 0.66 for TPP and 0.56 for TACY while in Powderblue r^2 was 0.65 for TPP and 0.52 for TACY. These values indicate that the antioxidant capacity is moderately related to TPP and TACY. Fresh blueberries showed strong positive correlation between TEAC and TPP (*18*).

This study showed that prolonged storage of blueberry extract at room temperature significantly reduces the phenolic compounds and their biological activities. No difference in TPP, TACY, and TEAC was observed between the two different cultivars. The retention of phenolic acids and anthocyanins was least influenced by cold storage and frozen conditions.

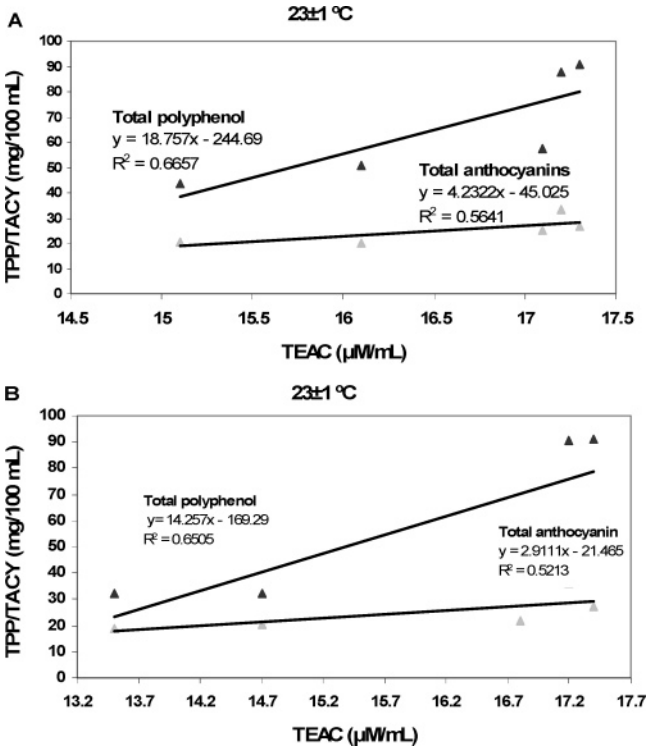

Figure 6. Correlation between TPP and TACY (*y*-axis) to the TEAC value. The *Y*-axis represents TPP and anthocyanins (mg/100 mL of extract). The *X*-axis represents TEAC (μM/mL). Average values were used for plots at different time intervals. (**A**) Tifblue and (**B**) Powderblue at 23 ± 1 °C.

LITERATURE CITED

(1) Steinmetz, K. A.; Potter, J. D. Vegetables, fruit and cancer 1. Epidemiology. *Cancer Cause Control.* **1991**, *2*, 325−57.

(2) Hertog, M. G. L.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B.; Kromhout, D. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen elderly study. *Lancet* **1993**, *342*, 1007−1011.

(3) Knekt, P.; Jarvinen, R.; Reunanen, A.; Maateka, J. Flavonoids intake and coronary mortality in Finland: A cohort study. *Br. Med. J.* **1996**, *312*, 478−481.

(4) Keli, S. O.; Hertog, M. G. L.; Feskens, E. J. M.; Kromhout, D. Dietary flavonoids, antioxidant vitamins and incidence of stroke: The Zutphen study. *Arch. Intern. Med.* **1996**, *156*, 637−642.

(5) Harborne, J. B.; Grayer, R. J. The anthocyanins. In *The Flavonoids*; Harborne, J. B., Ed.; Chapman and Hall: London, 1988; pp 1−20.

(6) Cai, Y. Z.; Sun, M.; Xing, J.; Luo, Q.; Corke, H. Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. *Life Sci.* **2006**, *78*, 2872−2888.

(7) Kalt, W.; Ryan, D. A. J.; Duy, J. C.; Prior, R. L.; Ehlenfeldt, M. K.; Vander Kloet, S. P. Interspecific variation in anthocyanins phenolics and antioxidant capacity among genotypes of highbush and lowbush blueberries (*Vaccinium* section *cyanococcus* spp.). *J. Agric. Food Chem.* **2001**, *49*, 4761−4767.

(8) Wu, X. L.; Gu, L. W.; Holden, J.; Haytowitz, D. B.; Gebhardt, S. E.; Beecher, G.; Prior, R. L. Development of a database for total antioxidant capacity in foods: A preliminary study. *J. Food Compos. Anal.* **2004**, *17*, 407−422.

(9) Prior, R. L.; Cao, G. H.; Martin, A.; Sofic, E.; McEwen, J.; O'Brien, C.; Lischner, N.; Ehlenfeldt, M.; Kalt, W.; Krewer, G.; Mainland, C. M. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *J. Agric. Food Chem.* **1998**, *46*, 2686−2693.

(10) Kader, F.; Irmouli, M.; Nicolas, J. P.; Metche, M. Involvement of blueberry peroxidase in the mechanisms of anthocyanin degradation in blueberry juice. *J. Food Sci*. **2002**, *67*, 910−915.
(11) Rossi, M.; Giussani, E.; Morelli, R.; Lo, Scalzo, R.; Nani, R. C.; Torreggiani, D. Effect of fruit blanching on phenolics and radical scavenging activity of highbush blueberry juice. *Food Res. Int*. **2003**, *36*, 999−1005.
(12) Kader, F.; Rovel, B.; Girardin, M.; Metche, M. Mechanism of browning in fresh highbush blueberry fruit (*Vaccinium corymbosum* L). Role of blueberry polyphenol oxidase, chlorogenic acid and anthocyanins. *J. Sci. Food Agric*. **1997**, *74*, 31−34.
(13) Schmidt, B. M.; Eerdman, J. W.; Lila, M. A. Effects of food processing on blueberry antiproliferation and antioxidant activity. *J. Food Sci*. **2005**, *70*, 389−394.
(14) Skrede, G.; Wrolstad, R. E.; Durst, R. W. Changes in anthocyanins and polyphenolics during juice processing of highbush blueberries (*Vaccinium corymbosum* L.). *J. Food Sci*. **2000**, *65*, 357−364.
(15) Kalt, W.; McDonald, J. E.; Donner, H. Anthocyanins, phenolics and antioxidant capacity of processed lowbush blueberry products *J. Food Sci*. **2000**, *65*, 390−393.
(16) Pérez-Vicente, A.; Serrano, P.; Abellán, P.; García-Viguera, C. Influence of packaging material on pomegranate juice colour and bioactive compounds during storage. *J. Sci. Food Agric*. **2004**, *84*, 639−644.
(17) Yi, W.; Akoh, C. C.; Fischer, J.; Krewer, G. Effects of phenolic compounds in blueberries and muscadine grapes on HepG2 cell viability and apoptosis. *Food. Res. Int.* **2006**, *39*, 628−638.
(18) Sellappan, S.; Akoh, C. C.; Krewer, G. Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. *J. Agric. Food Chem*. **2002**, *50*, 2432−2438.
(19) Singleton, V. L.; Rossi, J. A., Jr. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.
(20) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231−1237.
(21) Mosmann, T. Rapid colorimetric assay for cellular growth and survival−Application to proliferation and cyto-toxicity assays. *J. Immunol. Methods* **1983**, *65*, 55−63.
(22) SAS Institute. *SAS Statistics Software*, release 8.2; SAS Institute: Cary, NC, 2000.
(23) Gao, L.; Mazza, G. Quantitation and distribution of simple and acylated anthocyanins and other phenolics in blueberries. *J. Food Sci*. **1994**, *59*, 1057−1059.
(24) Lee, J.; Durst, R. W.; Wrolstad, R. E. Impact of juice processing on blueberry anthocyanins and polyphenolics: Comparison of two pretreatments. *J. Food Sci*. **2002**, *67*, 1660−1667.
(25) Zheng, Y.; Wang, C. Y.; Wang, S. Y.; Zheng, W. Effect of high-oxygen atmosphere on blueberry phenolics, anthocyanins and antioxidant capacity. *J. Agric. Food Chem*. **2003**, *51*, 7162−7169.
(26) Rice-Evans, C. A.; Miller, N. J.; Pagana, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med*. **1996**, *20*, 933−956.
(27) Häkkinen, S. H.; Kärenlampi, O.; Mykkänen, H. M.; Heinonen, I. M.; Törrönen, A. R. Ellagic acid content in berries: Influence of domestic processing and storage. *Eur. Food Res. Technol.* **2000**, *212*, 75−80.
(28) Hagiwara, A.; Yoshino, H.; Ichihara, T.; Kawabe, M.; Tamano, S.; Aoki, H.; Koda, T.; Nakamura, M.; Imaida, K.; Ito, N; Shirai, T. Prevention by natural food anthocyanins, purple sweet potato color and red cabbage color of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-associated colorectal carcinogenesis in rats initiated with 1,2-dimethylhydrazine. *J. Toxicol. Sci.* **2002**, *27*, 57−68.
(29) Kraft, T. F. B.; Schmidt, B. M.; Yousef, G. G.; Knight, C. T. G.; Cuendet, M.; Kang, Y. H.; Pezzuto, J. M.; Seigler, D. S.; Lila, M. A. Chemopreventive potential of wild lowbush blueberry fruits in multiple stages of carcinogenesis. *J. Food Sci*. **2005**, *70*, S159−S166.
(30) Hsu, T. C.; Young, M. R.; Cmarik, J.; Colburn, N. H. Activator protein 1 (AP-1)- and nuclear factor κB (NF-κB)-dependent transcriptional events in carcinogenesis. *Free Radical Biol. Med.* **2000**, *28*, 1338−1348.

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