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Influence of osmotic concentration, continuous high frequency ultrasound and dehydration on antioxidants, colour and chemical properties of rabbiteye blueberries

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Abstract

Rabbiteye blueberries (*Vaccinium ashei* Reade) were osmoconcentrated in a sucrose solution for 12 h, and for 3 h, with and without high frequency ultrasound (CHFU). Treated and untreated samples were air-dehydrated (70 °C, 10 h). Osmoconcentration decreased titratable acidity and induced a high loss of anthocyanins and phenolics. Approximately 60% of anthocyanins and phenolics were lost during osmoconcentration for 12 h. Air-dehydration further decreased anthocyanins and phenolics, with a higher negative influence on anthocyanins. Dehydration, after osmotic concentration, produced the largest colour differences in comparison to the control. High frequency ultrasound had a negative influence on anthocyanins and phenolics. Antioxidant activity was lowest in osmoconcentrated and dehydrated berries. Combination of high temperature, high sugar concentration and oxygen availability had the largest negative influence on colour and antioxidant properties (anthocyanins and phenolics) of dehydrated rabbiteye blueberries.

Keywords: Anthocyanins; Antioxidants; Blueberries; Dehydration; Phenolics; Ultrasound

1. Introduction

Mississippi ranks seventh in the nation among blueberry-producing states (MDAC, 2002) with rabbiteye (*Vaccinium ashei* Reade) and southern highbush (*Vaccinium corymbosum* L introgressed with *Vaccinium darrowi* Camp) being the major varieties grown. In the year 2003, Mississippi growers produced 1.5 million kg (3.3 million pounds) of which equal amounts were marketed as fresh and as frozen (Braswell pers. comm., 2003).

Numerous beneficial effects are ascribed to consumption of both wild and domestic blueberries, including reduction of coronary heart disease, treatment of urinary tract disorders, and anticarcinogen activity (Kalt & Dufour, 1997). Recent studies also show beneficial effects afforded by blueberry polyphenolics in retarding brain aging (Joseph et al., 1999; Youdim et al., 2000). Many of these biological properties are believed to be associated with the antioxidant activity of anthocyanin pigments, flavonoids, and other phenolic compounds (Skrede, Wrolstad, & Dust, 2000). Of all fruits and vegetables tested to date, blueberries are ranked highest in their antioxidant activity (Prior et al., 1998). These and many other reports have sparked considerable interest in blueberries by consumers, who demand confirmation that blueberries can contribute to the nutritional quality of their diet (Kalt, McDonald, & Donner, 2000).

Blueberries have a brief harvest season and can be stored under refrigerated conditions for only six weeks after harvesting (Kim & Toledo, 1987). Thus, blueberries are prime candidates for further preservation methods by dehydration, canning or freezing. Dehydration of blueberries can be used to extend the shelf life at room temperature, hence reducing storage and transportation costs. Food processors have found that dried cultivated

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blueberries can provide an added visual and taste appeal to cereals, confections and bakery goods (Feng & Tang, 1999).

Efforts have been made to develop appropriate drying methods that will result in high quality dehydrated berries. The techniques explored include explosion puffing (Sullivan, Craig, Dekazos, Leiby, & Konstance, 1982), fluidized bed-drying (Kim & Toledo, 1987), microwave drying (Feng & Tang, 1999; Yang & Atallah, 1985), high temperature fluidized bed-drying (Kim & Toledo, 1987), osmotic dehydration (Kim & Toledo, 1987; Nsonzi & Ramaswamy, 1998a; Ramaswamy & Nsonzi, 1998; Yang, Wills, & Yang, 1987) freeze-drying (Loong, Juming, & Jianshan, 1995; Yang et al., 1987; Yang & Atallah, 1985) and convection air drying (Yang & Atallah, 1985).

Osmotic concentration, prior to dehydration, has a protective effect on the structure of the dried material, making it more flexible and less dense (Feng & Tang, 1999; Kim & Toledo, 1987; Lenart, 1996). Reduced loss of fresh fruit flavour, increased sugar content, and removal of some fruit acids makes osmotically concentrated products more acceptable.

Continuous high frequency ultrasound (CHFU) can be used to enhance mass transfer during osmoconcentration. At high concentrations of sugar, ultrasound accelerates the rate of water movement out of the tissue and may result in significantly shorter times of osmoconcentration (Floros & Liang, 1994). Ultrasound can cause fast and complete degassing, initiate various reactions by generating free chemical ions (radicals), enhance polymerization/depolymerization reactions, improve diffusion rates and many other effects (Floros & Liang, 1994).

Water removal leads to a serious loss of the nutritive and sensory properties of the food. Because of the possible beneficial roles of phytonutrients present in blueberries, it is critical to measure their changes during processing to better assess the nutritional value of the processed products. Thus, the objectives of this study were to evaluate the effects of dehydration, osmoconcentration, and ultrasound on the anthocyanins, phenolics, antioxidants, colour and some chemical properties of rabbiteye blueberries.

2. Materials and methods

2.1. Rabbiteye blueberries (*Vaccinium ashei Reade*)

Fresh "cull" rabbiteye blueberries (*Vaccinium ashei* Reade) were obtained from a local commercial grower in South Mississippi in the summer of 2001. Cull blueberries were obtained as byproducts at the blueberry packinghouse after incoming berries were washed, sorted and graded. The cull berries were transported on ice within 4 h and placed in a refrigerator (2 °C) until graded and frozen (~2 d). Cull blueberries were further hand-graded in the laboratory by size and ripeness (~13% loss). Graded berries were individually frozen in a freezing chamber at −18 °C, and placed in plastic freezing Ziploc® freezer bags (S.C. Johnson & Son, Inc. Racine, WI) prior to further use. Berries were thawed overnight at 4 °C prior to treatment.

2.2. Treatments

2.2.1. Osmotic concentration

Osmotic concentration was carried out under three different conditions. The first experiment (O12) consisted of thawed blueberries that were placed in a plastic container and immersed in a 55 °Brix sucrose (commercially available) solution for 12 h at room temperature (21 °C). The ratio of sucrose (osmotic) solution to blueberries was maintained at 3:1 (w/w) in order to minimize changes in the sucrose concentration. The container with blueberries and osmotic solution was placed on an Orbital Shaker at medium speed (Bellco, Vineland, NJ) for 12 h, to maximize contact surface between blueberries and solution. The second experiment (O3 + U) consisted of thawed blueberries that were subjected to a continuous high-frequency ultrasound (CHFU) pretreatment to increase rate of solute diffusion. Blueberries were immersed in a 55 °Brix osmotic solution (1:3 w/w) for three hours at room temperature (21 °C) in an ultrasonic chamber (Meinhardt, Leipzig, Germany) connected to an ultrasonic K80 generator (Meinhardt, Leipzig, Germany) with 100 W maximum power output. Frequency was held constant at 850 kHz at intensity setting 3, (580 mV) (Chamul, 2000). The ultrasonic chamber had a useful inner diameter of 70 mm, 364 ml useful volume, 75 mm oscillator diameter and 33 cm ultrasonic area. Temperature was maintained at 21 °C (room temperature) by constantly circulating ice-cold water (1 °C) through a wall of the ultrasonic chamber.

The third experiment (O3) consisted of thawed blueberries that were placed in a 55 °Brix osmotic solution (1:3 w/w) for 3 h at room temperature (21 °C) in a plastic container. This treatment served as the control for the blueberries concentrated in the ultrasonic bath, to study the influence of CHFU on different parameters.

After each treatment, the blueberries were removed from the osmotic solution, quickly rinsed with distilled water (30 s) to remove adhering sucrose, and blotted dry with paper tissue to remove all excess water.

2.2.2. Dehydration process

Dehydration of the blueberries was performed using air-dehydration. Fresh and osmotically concentrated blueberries were placed on an oven shelf that was covered with a synthetic net with openings small enough to prevent the berries from falling through the shelf. The shelf was placed in the middle of a forced air oven ISOTEMP 300 Series Model 318F (Fisher Scientific Co., Fair Lawn, NJ). In this manner, hot air was allowed to circulate around all sides of the blueberries without any obstacles. Temperature was held constant at 70 °C and the drying

time (determined by preliminary runs) was 10 h. After dehydration, berries were placed in a desiccator overnight to cool down and for moisture to equilibrate. The berries were then placed in plastic Ziploc® bags (S.C. Johnson & Son, Inc.) and kept in a desiccator until they were utilized for further analysis. A diagram of treatments is presented in Fig. 1.

2.3. Moisture and dry matter content

Moisture and dry matter content were measured according to AOAC official method 934.06 (AOAC, 1999). Approximately 2 g of duplicate samples were used and placed in a vacuum oven Model 5851 (National Appliance Company, Winchester VA) at 70 °C and 94.82 kPa (28 in Hg). Drying time was 24 h for thawed and osmotically concentrated samples, and 6 h for dried samples. Moisture content was calculated on a wet weight basis.

2.4. Water activity

Water activity (A_w) was measured using a water activity meter Model TH200 (Novasina Pfäffikon, Switzerland).

2.5. Titratable acidity

Titratable acidity was determined according to the AOAC official method 942.15 (AOAC, 1999) and the results were expressed as both the percentage of citric acid in the products and in the dried matter.

2.6. Colour

A Labscan Model 6000 0/°45 Spectrocolorimeter (Hunter Associates Laboratory, Reston, VA) was utilized to measure the colours of the different treatments. From each treatment, 20 g of blueberries were blended for 5 min, using a commercial blender (Dynamic Co. of America, New Hartford, CT). The blended sample was placed in a 59 mm diameter and 38 mm deep glass cup (Hunter Associates Laboratory). The bottom part of the glass was placed on a 25 mm d port. Hunter colour was measured as 'L' (brightness); 'a' (redness+, greenness−); 'b' (yellowness+, blueness−). Three readings were taken on the same sample after rotating the cup 180°. Hue angle ($\tan^{-1} b/a$), chroma or saturation index ($SI = (a^2 + b^2)^{1/2}$), and total colour differences $\Delta E = [\Delta L^2 + \Delta a^2 + \Delta b^2]^{1/2}$ were calcu-

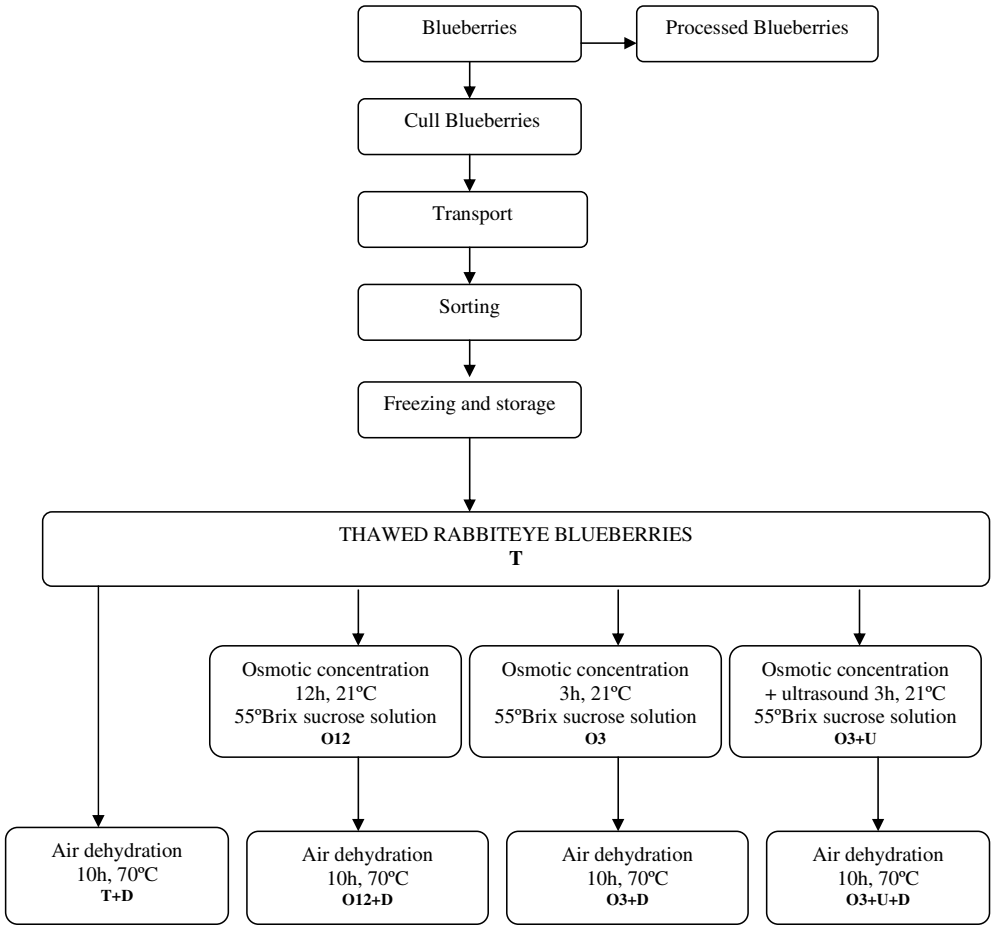

Fig. 1. Block diagram of treatments performed.

lated (Hunter Associates Laboratory). The ΔE represents the distance, in three-dimensional CIE colour space, between the point representing the thawed berries and the point for the treated berries.

2.7. Extraction of anthocyanins and phenolics

Fifty grammes of blueberries were added to 80 ml of methanol containing 4% acetic acid (Fisher Scientific Co.) and homogenized for 2 min using a Brinkmann homogenizer (Polytron, Switzerland). After the recovery of the homogenate, 20 ml of extraction solvent was used to wash the homogenate and pooled with the first homogenate. The pooled homogenate was kept at 4 °C for 12 h, and then centrifuged at 4000*g* for 15 min at 10 °C, using a Sorvall® RC 5B Plus Centrifuge (Sorvall Products, L.P. Newton, CT). The pellet was washed with 50 ml of methanol containing 4% acetic acid and centrifuged again under the same conditions. Resulting supernatants were combined and kept frozen (−18 °C) until used (⩽60 days).

Dried blueberries were homogenized using a commercial blender Model 31BL92 for 5 min at the high setting (Dynamic Co. of America, New Hartford, CT). Five gramme aliquots were mixed with 20 ml of solvent (methanol containing 4% acetic acid) and kept at 4 °C for 12 h with occasional vigorous shaking prior to centrifugation. The samples were centrifuged at 4000*g* for 15 min at 10 °C, using a Sorvall® RC 5B Plus Centrifuge (Sorvall Products, L.P). The pellet was washed with methanol containing 4% acetic acid and centrifuged again under the same conditions. Resulting supernatants were combined. When not used immediately, extracts were frozen in a dark vial and kept frozen (−18 °C) prior to use (⩽60 days).

2.8. Total phenolics

Total phenolics in blueberry extract were determined with the Folin–Ciocalteu (Fisher Scientific Co.) reagent (Singleton & Rossi, 1965), using gallic acid as the standard. The absorbance was determined at room temperature (21 °C) at $\lambda = 765$ nm using a Lambda 3B UV/Vis spectrophotometer (Perkin–Elmer Co.). Results were expressed as gallic acid equivalents per 100 g of product and per 100 g of dry matter.

2.9. Total anthocyanins

Total anthocyanins were determined utilizing the pH differential method (Wrolstad, 1976). Absorbance was measured in a Lambda 3B UV/Vis spectrophotometer (Perkin Elmer Co., Norwalk, CT) at $\lambda = 510$ nm and $\lambda = 700$ nm in buffers pH 1.0 and 4.5, using $A = (A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.5}}$ with the molar extinction coefficient of cyanidin-3-glucoside (29,600). Extracts were diluted in such a manner that the sample in buffer, pH 1, had an absorbance of less than 1. The dilution strength was the same for both buffers. Results were expressed as milligrammes of cyanidin-3-glucoside equivalents per 100 g of product and per 100 g of dry matter.

2.10. Polymeric colour

Polymerized coloured anthocyanin–tannin complexes are resistant to bleaching by bisulfite, whereas the bleaching reaction of monomeric anthocyanins will readily go to completion. Polymeric colour was determined using the method described by Wrolstad (1976). The same extracts and dilution strength were used as described previously. Two cuvettes, with 2.8 ml of the diluted sample, were used for each sample. In the first cuvette, 0.2 ml of freshly made 20% potassium metabisulfite was added (Fisher Scientific Co.) and 0.2 ml of distilled water in the other. Samples were left to equilibrate at room temperature (21 °C) for 15 min. Absorbance, for all samples, was measured at $\lambda = 420$, 510 and 700 nm (to correct for haze) against the blank cell, filled with distilled water, using a Lambda 3B UV/Vis spectrophotometer (Perkin–Elmer Co.) Colour density of the control sample (treated with water) was calculated using the following formula:

$$\text{CD} = [(A_{420} - A_{700}) + (A_{510} - A_{700})] \times \text{DF}.$$

Polymeric colour of the bisulfite-bleached sample was calculated using the following formula:

$$\text{PC} = [(A_{420} - A_{700}) + (A_{510} - A_{700})] \times \text{DF}.$$

Percent polymeric colour was calculated using the following formula:

$$\text{Percent polymeric colour} = (\text{polymeric colour/colour density}) \times 100.$$

Results were expressed as percentage polymeric colour.

2.11. Antioxidant activity

The antioxidant activities of the extracts were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Chemical Co, St. Louis, MO) as a free radical (Brand-Williams, Cuvelier, & Berset, 1995). All extracts were made to a concentration of 1 g dm/1000 ml, and 0.4 ml aliquots were added to 3.6 ml of 0.025 g/l DPPH solution in methanol. The decrease in absorbance was determined at $\lambda = 515$ nm at 0, 1, 3 min and every 5 min after for a period of 38 min, using a Lambda 3B UV/Vis spectrophotometer (Perkin–Elmer Co.). The concentration of DPPH in the reaction medium was calculated from the calibration curve with the equation $[\text{DPPH}] = 38.897(\text{Abs}_{515}) + 0.0903$ with $r^2 = 0.99$. The percentage of remaining DPPH ($\%\text{DPPH}_{\text{REM}}$) was determined with the formula: $\%\text{DPPH}^{\bullet}_{\text{REM}} = [\text{DPPH}^{\bullet}]_T/[\text{DPPH}^{\bullet}]_{T=0}$ (Sanches-Moreno, Larrauri, & Saura-Calixto, 1998). Results were expressed graphically by plotting time against the percentage of DPPH remaining (Fig. 2).

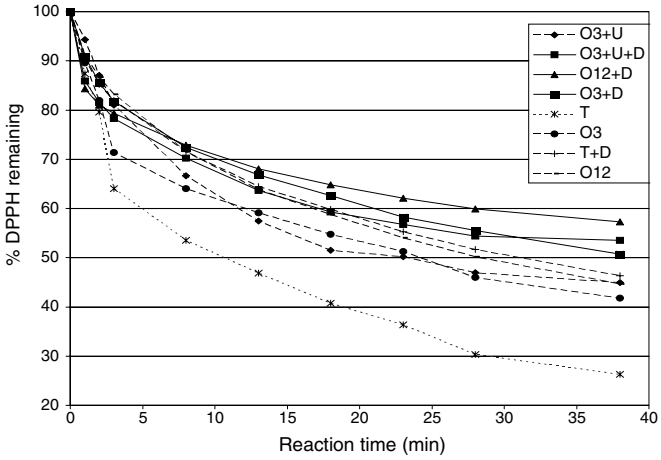

Fig. 2. Spectrophotometric recordings of the disappearance of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in the presence of 1 g dm/1000 ml of differently treated blueberries.

2.12. Statistical analysis

The experimental data (eight treatments) were arranged in a completely randomized design (CRD) with three replications. Proc GLM (SAS, 1999) was utilized to analyze data with $\alpha = 0.05$ and Fisher's protected least significant difference (LSD) was used to separate means when significant differences ($p < 0.05$) occurred (Kuehl, 2000).

3. Results and discussion

3.1. Titratable acidity and pH

Only the CHFU treatment (O3 + U) had a significant influence on pH (Table 1) compared to the non-treated berries (T). Values for titratable acidity (TA) in thawed berries were consistent with those reported by Marroquin (1994) and by Prior et al. (1998). All osmotic treatments significantly reduced the TA of the berries (Table 1). During osmotic concentration, elution of low-molecular weight substances (saccharides, organic acids, vitamins, mineral salts) occurred. This elution, although quantitatively insignificant in the mass exchange, can have a significant influence on the final nutritive values and sensory properties of foods (Lenart, 1996). The TA was significantly decreased when the time of osmotic concentration was increased from three to 12 h (Table 1). There were no significant differences between TA of berries osmotically concentrated for 12 h (O12) and treatment with CHFU (O3 + U). This suggested that a long osmotic treatment can be reduced with CHFU and have similar results.

3.2. Colour

Significant differences were found between treatments regarding all colour values (L, a, b, SI, Hue, and ΔE) (Table 2). In general, osmotic concentration did not have a significant influence on 'L', 'a', Hue, SI and ΔE values.

Table 1
Moisture content (wet basis), water activity, pH and titratable acidity for osmotically concentrated and dehydrated blueberries

Treatment[a]	Moisture (%)	A_w	pH	Titratable (%)	Acidity (%)/dm[e]
Control (T)	86.30a	0.97a	3.16a	0.67a	4.97a
O12	74.50c	0.93a	3.01ba	0.41b	1.59c
O3	81.70b	0.95a	3.08ba	0.58ab	3.15b
O3 + U	77.40bc	0.94a	2.93b	0.54ab	2.44bc
T + D	10.10d	0.40b	–[b]	–	–
O12 + D	13.60d	0.44b	–	–	–
O3 + D	9.40d	0.38b	–	–	–
O3 + U + D	11.10d	0.44b	–	–	–
LSD[c]	4.41	0.08	0.16	0.18	1.43
CV[d]	3.39	7.10	2.81	17.71	25.01

abcd – Means within the column followed by the same letter are not significantly different ($p > 0.05$).

[a] Treatments: T, thawed berries without pretreatment; O12, osmotically concentrated berries for 12 h; O3, osmotically concentrated berries for 3 h; O3 + U, osmotically concentrated berries for 3 h with CHFU; T + D, thawed berries without pretreatment and dried; O12 + D, osmotically concentrated berries for 12 h and dried; O3 + D, osmotically concentrated berries for 3 h and dried, O3 + U + D, osmotically concentrated berries for 3 h with CHFU and dried.

[b] Data not available.

[c] $LSD_{0.05}$, least significant difference.

[d] CV, coefficient of variation.

[e] TA/dry matter.

Table 2
'Hunter' colour values of osmotically concentrated and air-dehydrated blueberries

Treatment[a]	L	a	b	HUE $(\tan^{-1} b/a)$	SI $(a^2 + b^2)^{1/2}$	ΔE
Control (T)	7.73c	7.37a	1.66a	12.64a	7.55a	–
O12	6.80c	6.83ab	1.21b	11.09a	7.33a	1.20c
O3	6.98c	6.93ab	1.43ab	11.59a	7.07ab	1.05c
O3 + U	7.19c	7.58a	1.66a	12.41a	7.77a	0.71c
T + D	13.66ab	6.41ab	1.16b	10.26a	6.51ab	6.22b
O12 + D	12.40b	2.09d	0.06d	1.89c	2.09d	7.27a
O3 + D	13.95a	4.49c	0.39dc	6.52b	5.41bc	6.65ab
O3 + U + D	13.83a	5.38bc	0.62c	4.21cb	4.49c	7.05a
LSD[b]	1.26	1.67	0.43	2.75	1.73	0.71
CV[c]	7.07	16.4	24.03	17.99	16.59	10.91

abcd – Means within the column followed by the same letter are not significantly different ($p > 0.05$).

[a] Treatments: T, thawed berries without pretreatment; O12, osmotically concentrated berries for 12 h; O3, osmotically concentrated berries for 3 h; O3 + U, osmotically concentrated berries for 3 h with CHFU; T + D, thawed berries without pretreatment and dried; O12 + D, osmotically concentrated berries for 12 h and dried; O3 + D, osmotically concentrated berries for 3 h and dried, O3 + U + D, osmotically concentrated berries for 3 h with CHFU and dried.

[b] $LSD_{0.05}$, least significant difference.

[c] CV, coefficient of variation.

A significant increase in 'L' values was observed between concentrated and air-dehydrated samples (Table 2), making the products "lighter" and indicating a fading of the typical dark colour of blueberries. Within the dried samples, a significant increase was found between samples that were concentrated for 12 h and then dried (O12 + D) and

other concentrated and dried samples (O3 + D and O3 + U + D). Although a long osmotic concentration did not influence lightness of the concentrated samples, it had an influence on the dehydrated sample that was concentrated for the longer time. It preserved more typical blueberry lightness when compared to other treatments.

A significant decrease in '*a*' values (Table 2) was observed in samples that were osmotically pretreated and then air-dehydrated (O12 + D and O3 + D). This decrease in '*a*' values can be attributed to the heat-degradation of colour compounds (anthocyanins) that occurs during dehydration. Nsonzi and Ramaswamy (1998b) reported that with heat treatment, reddish anthocyanins are converted to a colourless carbinol base, such that the bluish-brown "co-pigments" dominate the colour of the blueberries. This reasoning may also explain the significantly lower '*b*' values of the samples that are concentrated and then dried.

The lowest '*a*' and '*b*' values were observed in samples that were concentrated for 12 h and dried (Table 2). This may suggest influence of the high sugar concentration on decrease of these values and changing of the colour. Hue values were significantly different for both osmotically concentrated and dried samples (O12 + D, O3 + D and O3 + U), suggesting an influence of the higher sugar concentration and dehydration on colour changes of the blueberries. Chroma (SI) of the osmotically concentrated and dried sample (O12 + D) was significantly lower (2.09 vs. 7.55 for control) than those of all other treatments. The largest differences (ΔE), compared to the control samples, were found in those that were osmotically concentrated and then dried. This suggests that a combination of high temperature, high sugar concentration, and oxygen availability had the largest influence on colour change of the blueberries. However, these colour changes were subtle to the eye (visual observation), indicating no major changes in the appearance of the blueberries.

3.3. Anthocyanins

Air-dehydration at 70 °C for 10 h resulted in dried samples averaging 12% moisture and a water activity of 0.4 (Table 1). Anthocyanin concentration, expressed in mg per 100 g (wet basis) of product, cannot be used to determine the degradation rate of anthocyanins that occurs during the different processes because of the different moisture contents of the samples. However, it is useful in comparing results with other authors, as well as from a nutritional standpoint (Table 3). Total anthocyanins in 100 g of thawed blueberries (136 mg) were comparable to results reported by Prior et al. (1998) of 124 mg/100 g for rabbiteye blueberries, but differ considerably from the results reported by Moyer, Hummer, Finn, Frei, and Wrolstad (2002). They reported a mean value of 406 mg/100 g, which was higher than in any other blueberry variety. Environmental factors which influence formation of anthocyanins (e.g., light, temperature, agronomic practices, and various stresses) may have contributed to the differences between the results in these studies. Single genotypes of blueberries were reported to differ in their anthocyanin contents by 30% between seasons (Kalt & McDonald, 1996).

Table 3
Total anthocyanin contents, total phenolics and percentages of polymeric colour in blueberries, as affected by osmotic concentration, ultrasound and air-dehydration

Treatment[a]	Anthocyanins		Phenolics		Polymeric colour (%)
	mg/100 g	mg/100 g dm	mg/100 g	mg/100 g dm	
Control (T)	136cde	1000a	550d	4038a	12a
O12	102f	402d	420d	1665d	22a
O3	146bcd	804b	586d	3215ab	16a
O3 + U	129def	583c	494d	2271dc	15a
T + D	285a	318d	2621a	2907bc	77b
O12 + D	108fe	126e	1396c	1618d	65b
O3 + D	173b	195e	1615c	1813d	60b
O3 + U + D	163cb	180e	2002b	2209dc	71b
LSD[b]	30	104	287	851	19
CV[c]	11	13	14	20	25

abcdef – Means within column followed by the same letter are not significantly different ($p > 0.05$).

[a] Treatments: T, thawed berries without pretreatment; O12, osmotically concentrated berries for 12 h; O3, osmotically concentrated berries for 3 h; O3 + U, osmotically concentrated berries for 3 h with CHFU; T + D, thawed berries without pretreatment and dried; O12 + D, osmotically concentrated berries for 12 h and dried; O3 + D, osmotically concentrated berries for 3 h and dried, O3 + U + D, osmotically concentrated berries for 3 h with CHFU and dried.

[b] $LSD_{0.05}$, least significant difference.

[c] CV, coefficient of variation.

Storage of blueberries in the fresh state, prior to freezing, anthocyanins extraction and determination, can have a great influence on final results, since an increase in anthocyanin content during storage was previously reported (Kalt, Forney, Martin, & Prior, 1999). From a nutritional standpoint, consuming 100 g of dehydrated product would be equal to or more advantageous than eating 100 g of fresh product, since dried products contain the same or higher amounts of anthocyanins, depending upon pretreatment. Significant differences (Table 3) in total anthocyanins (calculated in 100 g of dried matter) were found between all three osmotic concentration treatments (O12, O3 and O3 + U), meaning that time of concentration and high frequency ultrasound negatively influenced anthocyanin content in osmoconcentrated berries. Treatments O3, O3 + U and O12 decreased anthocyanin contents by 20%, 42% and 59%, respectively, when compared to the thawed (T) sample. Loss of 6% anthocyanins was previously reported by Forni, Polesello, and Torreggiani (1993) during osmoconcentration of cherries for 2 h.

Removal of the waxy layer and ruptures caused by freezing were photographed using cold storage scanning electron microscopy (Allan-Wojtas, Goff, Stark, & Carbyn, 1999). Since blueberries used in this study were frozen and then thawed before application of the treatments, high loss of anthocyanins during osmotic concentration could be mostly attributed to the leakage of anthocyanins through the cuticle and skin ruptures. This is because

anthocyanins are naturally concentrated in the epidermal and sub epidermal layers of blueberries. Adding sugar solution increased the pH value of solution and berries, increasing the percentage of anthocyanins in the colourless carbinol base form that is very unstable, making the pigment more susceptible to degradation by oxygen. High frequency ultrasound, with its phenomenon of cavitation, may additionally rupture the surface of the berries, causing even more leakage and loss of pigments.

Air-dehydration decreased the anthocyanin content by 69%, compared to both fresh and osmotically treated berries. Similar results were reported by Forni et al. (1993) where 50% of anthocyanins were lost during thermal treatment of cherries. Kwok, Hu, Durance, and Kitts (2004) reported approximately 85% loss of anthocyanins during air-dehydration of Saskatoon berries at 75 °C. Lohachoompol, Srzednicki, and Craske (2004) reported 49% loss of anthocyanins in pretreated blueberries dehydrated in a cabinet dryer for 5.5 h, with gradual decrease of temperature from 90 to 50 °C. Temperature elevation (70 °C) caused anthocyanin degradation since they began to degrade at temperatures greater than 63 °C, but more importantly, it enhanced the negative influence of high sugar concentration. At high temperatures, production of furfural and 5-hydroxymethyl furfural increases, and this can degrade the pigment molecule and enhance the negative influence of oxygen. Debicki-Pospišil, Lovrić, Trinajstić, and Sabljić (1983) concluded that cyanidin degradation in the presence of aldehydes can not be limited to one singular pathway, and they proposed three different ones. They also reported that aldehyde effect on pigment degradation at 70 °C was considerably diminished, but still proceeded, when the reaction was taking place in an inert nitrogen atmosphere. This suggests that oxygen appears to accelerate the degradation, but was not a necessary prerequisite for the degradation to occur. The combination of long dehydration time, availability of oxygen, high temperature, and high sugar concentration contributed to the loss of anthocyanins.

The percent polymeric colour is an index of anthocyanin degradation (Lee & Wrolstad, 2004). Increase in percent polymeric colour was apparent but not statistically significant in the osmotically concentrated samples, with a tendency to increase in the O12 treatment (Table 3). Differences in percent polymeric colour between osmotically concentrated and air-dried samples were significant. Percentage of polymerization increased by more than 40% in the air-dried samples.

3.4. Phenolics

The range of reported phenolics for rabbiteye blueberries is very wide, due to the variations between different environmental conditions, time of harvest, and extraction procedures. Total phenolics of 340 ± 14.6 mg, and 875 ± 80 in 100 g of fresh product, for rabbiteye blueberries were reported by Prior et al. (1998) and Moyer et al. (2002), respectively. A value of 551 mg/100 g of product reported in this research (Table 3) is between these reported values. Total phenolics of 4180 mg/100 g of dry matter, reported by Velioglu, Mazza, Gao, and Oomah (1998) is similar to the values reported in this research, of 4038 mg/100 g of dry matter.

Different osmotic concentration treatments induced loss of phenolics by migration into the osmotic solution. The highest significant decrease in the total phenolics was observed in the sample that was osmotically treated for 12 h (O12). More than one half of the total phenolics present in thawed samples was lost (58.8%). That is almost identical to the percentage of anthocyanins lost in the same process. Decreasing osmotic concentration time to 3 h (O3) decreased the loss of total phenolics to only 20% in comparison to the thawed ones (Table 3). Introduction of ultrasound may have accelerated formation of free radicals and increased the level of polymerization of phenolics that resulted in a 43.7% loss of total phenolics. The cavitation phenomenon close to the outer epidermal layer of the berries can cause ruptures and increase leaching of material from the berries. Total percentage phenolics lost during osmotic concentration was almost identical to the percentage of anthocyanins lost during the same treatments.

For the osmotic concentration process, a strong significant correlation between total phenolics and anthocyanins was found ($r = 0.90$). Kalt et al. (1999) reported strong correlation between anthocyanins and total phenolics ($r = 0.91$) during storage. Moyer et al. (2002) found a significant correlation ($r = 0.93$) among different varieties of *Vaccinium*, and Connor, Luby, Hancock, Berkheimer, and Hanson (2002) demonstrated a significant correlation ($r = 0.87$) between *Vaccinium* cultivars during cold-temperature storage. Drying of blueberries did not have as much influence on total phenolics as it did on the anthocyanins. A significant decrease in total phenolics, was found in the thawed sample that was dried (T + D) and the sample that was treated for 3 h (O3) and then dried (O3 + D) in comparison to T and O3, respectively. Since these were the samples with the highest moisture contents at the start of drying, the decrease in total phenolics during this process can mainly be attributed to the leaching of total phenolics through the skin of berries and just partly due to the higher temperature and presence of oxygen. Kalt et al. (2000) also reported that anthocyanins had a higher susceptibility to elevated temperature and oxygen than did phenolics (by more than half). Due to the different behaviour of anthocyanins and total phenolics during drying, the correlation between these two measurements in the dehydrated samples ($r = 0.77$) was less than that reported previously for the osmotically concentrated samples ($r = 0.90$), but nevertheless significant.

3.5. Antioxidant activity

The evaluation of different reaction kinetics of blueberry extracts depends on the nature of the antioxidants being

tested. Anthocyanins and phenolics, major contributors to antioxidant activity in berries, have different patterns of behaviour in response to temperature, oxygen and other processing factors. Faster reaction of blueberry extract antioxidants with DPPH and less percentage of DPPH remaining after a given time, indicate higher antioxidant activity. Three different types of behaviour can be observed (Fig. 2) according to the processing steps applied. Extracts of thawed blueberries (T) reacted rapidly with DPPH, reducing concentration of DPPH to 30% after 38 min of reaction time. The second type of behaviour includes all three osmoconcentrated blueberry extracts, and dehydrated berries without pretreatment (T + D), showing a 50% reduction in DPPH over the same period. The third kind of behaviour includes osmoconcentrated and air-dehydrated samples that left more than 50% of DPPH remaining during the same time frame. Air-dehydration seems to further reduce antioxidant activity, following the same pattern as the concentration step, but with larger reduction. Peterson (2001) showed that heat-processing reduced antioxidant capacity, but did not destroy it. Osmotically concentrated samples (for 12 h) and then air-dried (O12 + D) demonstrated the weakest antioxidant activity. Although total phenolics contribute greatly to antioxidant activity, it seems that the decrease in antioxidant activity follows the same pattern as anthocyanins reduction in the treated samples. Kalt et al. (2001) also concluded that anthocyanins may make a greater contribution to antioxidant activity than do other phenolics. Amakura, Umino, Tsuji, and Tonogai (2000) revealed a correlation between total phenolics and DPPH activity in blueberry jam ($r = 0.57$), and concluded that phenolic contribution to DPPH activity is mostly due to the selected phenolics (such as anthocyanins) among the total phenolics. Since blueberry extract is very complex, it is difficult to distinguish which compounds contribute the most to antioxidant activity.

4. Conclusions

Longer osmotic concentration time resulted in higher loss of phytonutrients, mainly due to the leaching into sucrose solution and negative influence of oxygen. The continuous high frequency ultrasound (CHFU) increased loss of phytonutrients. Decreased time of osmotic concentration, processing in reduced oxygen environment or vacuum, and reuse of the sucrose solution could make this process more practical from nutritional and economical standpoints.

Air-dehydration, at 70 °C for 10 h, resulted in good quality, shelf-stable samples regardless of pretreatment, since it produced sufficiently low moisture contents (≈12%) and water activity (≈0.4). Although osmotic concentration and air-dehydration influenced loss of phytonutrients (anthocyanins and total phenolics), their total amounts, were equal and even higher than those in fresh products, due to the concentration effect. This suggests that air-dehydration, with or without pretreatment, can be used to produce good quality dehydrated blueberries with high nutritional value. Since cull blueberries are low cost products, the low cost of air-dehydration in comparison to other dehydration methods can make this process economically feasible for small and medium entrepreneurs.

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