Food Research International 44 (2011) 1408–1413

Contents lists available at ScienceDirect

Food Research International

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

Antioxidant and antiradical properties of cranberry juice and extracts

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ARTICLE INFO

Article history:
Received 8 September 2010
Received in revised form 26 January 2011
Accepted 12 February 2011

Keywords:
Cranberry juice
Phenolic extracts
Free radical scavenging capacity
Lipid peroxidation inhibition capacity

ABSTRACT

The antioxidant capacities of cranberry juice and three extracts isolated from frozen cranberries containing anthocyanins, water-soluble and apolar phenolic compounds, were evaluated at pH 2.5 and 7, respectively. The free radical-scavenging (FRS) and the lipid peroxidation inhibition (LPI) activities of each samples, and extracts, were studied using the N,N-diethyl-p-phenylenediamine (DPD) decoloration test and the thiobarbituric acid reactive substances (TBARS) assay, respectively. The cranberry phenols displayed good free radical-scavenging properties, but were less efficient at inhibiting the peroxidation of lipids. Of all the samples tested, the water-soluble phenolic compounds showed the greatest free radical-scavenging (68.2 mmol TE/mg phenol) and antioxidant (13.4 mmol TE phenol) activities. The polarity of the phenols, the pH of the medium and the juice process had a great influence on the antioxidant activities. The phenols isolated from cranberries with an aqueous solvent have greater antioxidant properties than those extracted with an organic solvent mixture. The antioxidant activity of the cranberry samples adjusted at pH 2.5 was greater than those adjusted at pH 7. Compared to the cranberry extracts, the juice exhibited a much lower antioxidant activity, especially when compared with the extract containing water-soluble compounds which the extraction conditions were similar to those used to obtain the juice.

1. Introduction

Stresses, physical damage, viral infection, cytotoxic or carcinogenic compounds resulting from chemical or biological aggression may cause the peroxidation of cell membrane lipids and the liberation of toxic substances, such as free radicals (Aruoma, 1998). Oxidative stress by free radicals or reactive oxygen species (ROS) has been associated with aging and the development of numerous chronic diseases, including cancer, multiple sclerosis, Parkinson's disease, autoimmune disease and senile dementia. The peroxidation of membrane lipids appears to be involved in the processes causing cellular mutations and decay (Aruoma, 1998). The study of the relationship between the morbidity due to cancer and heart diseases and the consumption of fruits and vegetables indicates that polyphenols, present in large amount in fruits and vegetables, play a significant role (Heim, Tagliaferro, & Bobilya, 2002; Rice-Evans, 2001). Fruits, including berries, are one of the most important food sources of phenolic compounds in our diets (Macheix, Fleuriet, & Billot, 1990). The hydroxybenzoic and hydroxycinnamic acid derivatives, the anthocyanins, the flavonols, the catechins, and the hydrolyzable or condensed tannins, all exhibits a wide range of biological properties, including antioxidant, antimicrobial, anti-inflammatory, and vasodilatory actions (Mantley & Buslig, 1998). Fruit extracts have also shown a great antioxidant potential in several studies. Phenolic extracts obtained from berries (blackberries, red raspberries, sweet cherries, blueberries, and strawberries) were shown to inhibit the oxidation of low-density lipoproteins and of liposomes (Heinonen, Meyer, & Frankel, 1998). Berries have also shown a remarkably high scavenging activity toward chemically generated ROS (Wang & Jiao, 2000). Effective antioxidants are radical scavengers that interfere with the oxidation process by reacting with free radicals and chelating catalytic metals (Shahidi & Wanasundara, 1992).

Cranberry fruits are excellent raw materials for juice production as they contain significant amount of phenolic compounds which have antioxidant properties and other health benefits (Kahlon & Smith, 2007). Historically, cranberry juice has been consumed to prevent urinary tract infections. These and other health benefits, including reduced risks of cancer and cardiovascular disease, are believed to be due to the presence of various polyphenolic compounds, including anthocyanins, flavonols, and procyanidins, and the synergistic effects among them (Chu & Liu, 2005; Heinonen, 2007; Seeram, Adams, Hardy, & Heber, 2004). Considerable *in vitro* evidence has shown the antioxidant potential of cranberry phenolic compounds (Borges, Degeneve, Mullen, & Crozier, 2010; Narwojsz & Borowska, 2010). However, the great diversity of methods applied to study both the free radical-scavenging activity and the antioxidant activity, extraction procedures, oxidation models, and conditions, have resulted in great differences in the outcome and therefore interpretation and comparison of results are complicated (Heinonen, 2007). Still, cranberry phenolic

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doi:10.1016/j.foodres.2011.02.019

compounds have been shown to have free radical-scavenging properties against superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and singlet oxygen ($^1O_2$), and they can also inhibit lipid peroxidation, as well as protein and lipid oxidation in liposomes (Seeram & Heber, 2007; Wang & Jiao, 2000).

The aim of the present study was to evaluate the antioxidant properties of cranberry juice and extracts from cranberry fruits adjusted at pH 2.5 and 7.0. Because no single antioxidant assay can accurately reflect the antioxidant potency, two tests were used to measure the antioxidant properties: the N,N-diethyl-p-phenylenediamine (DPD) decoloration free radical-scavenging test and the thiobarbituric acid reactive substances (TBARS) lipid peroxidation inhibition assay.

2. Materials and methods

2.1. Raw material

Frozen cranberries (raw material) and clarified cranberry juice (final product) samples (*Vaccinium macrocarpon*) were provided by Atoka Cranberries Inc. (Manseau, QC, Canada) and were stored at −80 °C until used.

2.2. Extraction of phenolic compounds and sample preparation

The extraction conditions employed were as mild as possible to avoid oxidation, thermal degradation and other chemical and biochemical changes in the sample. The method consisted of the following steps: homogenization, maceration and evaporation. Extraction of phenolic compounds was done from frozen cranberries and achieved according to three methods using solvents of different graded polarity for the recovery of specific classes of phenolics which have different solubility. The extract 1 (E1) containing mostly water soluble phenolic compounds was recovered using water/methanol (85:15, v/v) (Seeram et al., 2004), whereas the extract 2 (E2) containing the most apolar phenolic compounds (flavonols, flavan-3-ols and proanthocyanidins) was recovered using acetone/methanol/water (40:40:20, v/v), a solvent combination modified from a method described by Neto et al. (2006). The extract 3 (E3) composed mainly of anthocyanins was recovered with methanol/water/acetic acid (85:15:0.5, v/v/v) as described by Wu and Prior (2005). Frozen cranberries were crushed at 4 °C for 40 s in a Waring commercial blender (Waring Laboratory, Torrington, CT) to obtain a fine puree immediately after crushing the fruit, extractions have been performed at 4 °C under agitation and a constant flow of nitrogen for 40 min by macerating of 300 g of the fruit powder with the extracting solvents. Three successive extractions in each extracting solvent were performed using the same procedure. The first extraction was done using 700 ml of solvent, but for the two last ones, 500 ml was used. The solvent containing the phenolic compounds was recuperated after each extraction and the solvents from the successive extractions were combined, then filtered on Whatman paper no. 4 (Fisher Scientific, Nepean, ON, Canada). The filtrate was concentrated by evaporating the solvent using the SpeedVac automatic evaporation system (Savant System, Holbrook, NY), then dry matter was determined by freeze-drying the extracts for 48 h with a Virtis Freeze mobile 12 EL (The Virtis Co., Gardiner, N.Y), and stored at −80 °C until used.

Prior to the experiment, the freeze-dried extracts were weighed and redissolved in their extracting solvent to a specified volume, then the cranberry juice (20 ml) and phenolic extracts were adjusted to pH 2.5 with 1 mol/L HCl or pH 7 with 1.25 mol/L NaOH.

2.3. Total phenol concentration

The total phenolic compound (TP) content in each cranberry extract or juice was determined by Folin–Ciocalteu method (Singleton & Rossi, 1965) with absorbance measured at 760 nm. The TP content of samples, adjusted at either pH 2.5 and 7.0, was then calculated using actual absorbance versus a gallic acid standard curve (10, 20, 40, 80, 100, and 500 μg/mL; $r^2 = 0.9886$) and expressed as grams of gallic acid equivalent (GAE) per gram of dry sample.

2.4. Determination of free radical-scavenging capacity

The free radical scavenging (FRS) capacity of phenolic compounds in cranberry extract or juice samples adjusted at natural fruit pH (pH 2.5) and neutral pH (pH 7.0) was analyzed using a procedure of DPD (N,N-diethyl-p-phenylenediamine; Sigma-Aldrich Ltd, Oakville, ON, Canada) colorimetric assay, modified by others (Caillet, Salmiéri, & Lacroix, 2006). Two hundred (200) microliter of sample (1.25 mg/mL) was added to a cell containing 3 mL of 0.15 M NaCl and submitted to electrolysis for 1 min (continuous current, 400 V, 10 mA DC) using a power supply (Bio-Rad, model 1000/500, Mississauga, ON, Canada). After electrolysis, an aliquot of 200 μL was added to 2 mL of DPD solution (25 mg/mL). The generated oxidative species (superoxide anion (O_2^-), singlet oxygen ($^1O_2$), and hydroxyl radicals (OH^-) and their by-products (hydrogen peroxide (H_2O_2) and hypochlorite ion (OCl^-)) react instantly with DPD, producing a red coloration that can be measured at 515 nm using a DMS 100 S spectrophotometer (Varian Canada Inc., Mississauga, ON, Canada). The FRS activity is equivalent to the capacity of phenolic compounds to inhibit the accumulation of oxidative species (able to oxidize DPD) and consequently the red coloration at 515 nm. The reaction was calibrated using the non-electrolyzed NaCl solution (no oxidative species, ascribed to 100% scavenging) and the electrolyzed NaCl solution (0% scavenging, in the absence of any antioxydants). The FRS percentage was calculated according to the following equation:

$$Scavenging\ (\%) = \left[1 - \left[\left(OD_{sample} - OD_{0\%}\right) / \left(OD_{100\%} - OD_{0\%}\right)\right]\right] \times 100,$$

where OD_{sample} represents the OD of the cranberry sample, $OD_{0\%}$, the OD of the cranberry sample in the non electrolyzed NaCl solution, and $OD_{100\%}$, the OD of the electrolyzed NaCl solution in the absence of the cranberry sample. The degree of discoloration indicates the FRS capacity of the sample. The FRS activity of a substance is usually ranked with the following scale: i) FRS activity ≥ 70%: elevated, ii) FRS activity between 40 and 70%: average, iii) FRS activity ≤ 40%: weak or absent.

The reaction was calibrated using the positive control Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma-Aldrich Ltd) whose FRS activity was 100%, and after taking the sample dry matter weight and TP concentration into consideration, the results were expressed in mmol Trolox® equivalent (TE)/mg of phenol.

2.5. Determination of the lipid peroxidation inhibition capacity

The lipid peroxidation inhibition (LPI) activity of cranberry extracts or juice at pH 2.5 and 7 was determined by the thiobarbituric acid reactive substances (TBARS) assay done using a microtechnique based on the non-enzymatic peroxidation of rat liver microsomes method modified by others (Caillet et al., 2007) where artificial membranes were used instead of rat liver microsomes, in order to obtain a more stable and reproducible system. This test measures by spectrophotometry the TBARS concentration produced during the peroxidation of liposomes exposed to iron ions in 20 mM phosphate buffer solution in presence of ascorbate. The antioxidant activity is equivalent to the LPI capacity.

2.5.1. Liposomes preparation

Liposomes were formed by an injection method, as described by Batzri and Korn (1973). Linoleic acid (Sigma-Aldrich, Oakville, ON, Canada) was dissolved in 95% ethanol. The mixture was injected into

phosphate buffer (20 mM, pH 7.4) in a proportion of 1:9 (v/v), using a hypodermic syringe fitted with a fine needle (G26).

2.5.2. Microplate preparation

Twenty five microliter of samples (1.25 mg/mL) and negative controls was added to a microplate (96 wells). The reaction mixture containing 4 mL of liposomes solution, 2.25 mL of phosphate buffer (20 mM, pH 7.4) and 0.25 mL of ascorbate solution (3.1 mg/mL) was prepared. Sixty-five microliter of reaction mixture was added a microplate using a multichannel pipette. Finally, 10 μL of $FeCl_3$ solution (Sigma-Aldrich, 4.3 mg/mL) was added to the wells. The microplate was then incubated at 37 °C for 15 min. One hundred fifty microliter of a fresh SDS solution (Sigma-Aldrich, 10% v/v) and of thiobarbituric acid (Sigma-Aldrich, 0.67% v/v) in a 1:2 ratio was added in the microplate. The colorimetric reaction was produced at 80 °C for 30 min. The presence of TBARS in the controls and samples was evaluated at 540 nm using a Microplate Autoreader (model EL 309, Biotek Instruments, Winooski, VT). A positive control was represented by the reaction mixture without the sample, and the optical density of the chromogene formed when the peroxidation was completed. The negative control contained only the phosphate buffer without liposomes. The reaction was calibrated with Trolox® (Sigma-Aldrich Ltd) whose LPI activity was 100%. The LPI capacity was calculated using the following equation:

$$AA(\%) = \left[OD_{(negative\ control)} - OD_{(sample)} \,/\, OD_{(negative\ control)} - OD_{(positive\ control)}\right] \times 100,$$

and after taking the sample dry matter weight and TP concentration in consideration, the LPI activity results were expressed in mmol TE/mg of phenol.

2.6. Statistical analysis of data

This experiment was done in replicate and three samples of each replicate were analyzed. Data were analyzed using SPSS for windows. Analyses of variance were performed by ANOVA procedures. Significant differences between means were determined by Ducan's multiple range test ($P \leq 0.05$).

3. Results

3.1. Antiradical activity

The free radical-scavenging (FRS) capacity of the cranberry juice and of the three cranberry extracts is presented in Table 1. When the results are reported in mmol TE of dry matter (DM), the extracts rich in apolar phenolic compounds (E2) and in anthocyanins (E3) have the highest free radical-scavenging activities (2.1 mmol TE/mg DM). Compared to the extracts, the cranberry juice had a low FRS (0.2 mmol TE DM). Although, the solvent mixture used to extract E1 (water/methanol, 85:15, v/v) resembled the aqueous juice extraction process, the FRS activity of E1 was nine times greater than that of the juice. The FRS activity of the extracts was significantly affected by the pH of the dissolving solvent, whereas that of the juice was unaffected. The FRS capacity of E2 and E3 adjusted at pH 2.5 was at least three times greater than that at pH 7. When comparing the FRS capacity results in mmol TE phenol, the extract rich in water-soluble phenolic compounds was the extract that exhibited the highest free radical-scavenging potential (68.2 and 27.57 mmol TE/mg phenol at pH 2.5 and 7.0, respectively).

3.2. Antioxidant activity

The lipid peroxidation inhibition (LPI) capacity of the cranberry juice and of the three cranberry extracts is presented in Table 2. Of all the samples tested, the anthocyanin-rich extract (E3) was the most efficient at inhibiting the lipid peroxidation (1 mmol TE DM). Compared to the other extracts, the LPI capacity of E3 was at least 2.5 times higher. The cranberry juice exhibited LPI activities 10 to 25 times lower than those of the extracts (0.04 mmol TE DM). Increasing the pH from 2.5 to 7.0 reduced greatly the LPI capacity of the juice and the extracts. When comparing the LPI capacity results in mmol TE phenol, the extract rich in water-soluble phenolic compounds was the extract that exhibited the highest antioxidant potential (13.45 and 6.76 mmol TE phenol at pH 2.5 and 7.0, respectively). The extract rich in apolar phenolics also showed significant LPI capacity, with 10.26 and 5.13 mmol TE phenol at pH 2.5 and 7.0, respectively. The LPI capacity of the anthocyanin-rich extract and of the juice was much lower. Finally, the LPI activities of the extracts and the juice were significantly reduced by pH neutralization.

Table 1
Radical-scavenging capacity of cranberry juice and three extracts from cranberry fruits.

Samples[a]	pH[b]	Free radical scavenging capacity[c,d]		
		mM TE/ml juice	mmol TE/mg dry matter	mmol TE/mg phenol
E1	2.5	–	1.99 ± 0.03bB	68.25 ± 1.00dB
	7	–	1.39 ± 0.01cA	27.57 ± 0.22dA
E2	2.5	–	2.12 ± 0.01 cB	13.39 ± 0.07bB
	7	–	0.60 ± 0.01bA	3.43 ± 0.03aA
E3	2.5	–	2.13 ± 0.03cB	17.09 ± 0.25cB
	7	–	0.63 ± 0.02bA	8.04 ± 0.21cA
Juice	2.5	3.82 ± 0.03 B	0.23 ± 0.007aA	8.31 ± 0.09aB
	7	3.69 ± 0.04 A	0.22 ± 0.002aA	6.74 ± 0.08bA

[a] E1, E2 and E3 are three extracts from cranberry fruits. E1: the most water-soluble phenolic compounds extracted with water/methanol (85/15, v/v); E2: the most apolar phenolic compounds extracted with acetone/methanol/water (40/40/20, v/v); E3: anthocyanins extracted with methanol/water/acetic acid (85/14.5/0.5, v/v/v).
[b] 2.5: natural fruit pH, and 7: physiological pH.
[c] TE: Trolox® equivalent.
[d] Values are means ± standard deviations. Within each column, means for a same pH bearing the same lowercase letter are not significantly different ($P > 0.05$). Within each column, means for a same sample bearing the same uppercase letter are not significantly different ($P > 0.05$).

4. Discussion

4.1. Free radical-scavenging activity versus lipid peroxidation inhibition activity

The data obtained confirmed the free radical-scavenging and antioxidant properties of cranberry extracts. Despite the fact that all three cranberry extracts contained a wide range of phenolic compounds (phenolic acids, anthocyanins, flavonoids and tannins), their FRS ability was greater than their LPI capacity (Côté et al., 2010; Neto et al., 2006; Wu & Prior, 2005). The method used to evaluate the LPI activity of phenols is based on the Fenton reaction and can detect non-enzymatic autoxidation (Caillet et al., 2007). In the presence of major cellular iron chelators ATP or citrate, quercetin which is predominant flavonoid in cranberries, is known to completely suppress Fe-promoted Fenton chemical reaction (Guo et al., 2007). However, the radical scavenging activity of quercetin only provides partial protection against the Fenton damage while its iron chelation ability completely inhibits the oxidative reaction, indicating that metal chelation may be a key to its antioxidant activity (Morel et al., 1993; Morel, Lescoat, Cillard, & Cillard, 1994). Moreover, at pH 7 quercetin appears to have more affinity with Fe^{2+} than with the well known Fe^{2+}−chelator ferrozine (Guo et al., 2007). This could explain the very low LPI capacity results obtained when the samples were adjusted at pH 7. The antioxidant activity of phenols can also differ depending on their molecular structure (Ruel & Couillard, 2007), and on the presence of a substituent on the flavonoid ring (Chen, Chan, Ho, Fung, & Wang, 1999). Flavonoids with an adjacent dihydroxy

Table 2
Lipid peroxidation inhibition capacity of cranberry juice and three extracts from cranberry fruits.

Samples[a]	pH[b]	Lipid peroxidation inhibition capacity[c,d]		
		mM TE/ml juice	mmol TE/mg dry matter	mmol TE phenol
E1	2.5	–	0.39 ± 0.007bB	13.45 ± 0.24dB
	7	–	0.34 ± 0.004bA	6.76 ± 0.08dA
E2	2.5	–	0.40 ± 0.006bB	2.33 ± 0.03bB
	7	–	0.33 ± 0.004bA	1.13 ± 0.02bA
E3	2.5	–	1.04 ± 0.017cB	10.26 ± 0.16cB
	7	–	0.63 ± 0.06cA	5.13 ± 0.08cA
Juice	2.5	0.70 ± 0.01B	0.04 ± 0.001aB	1.54 ± 0.03aB
	7	0.24 ± 0.004A	0.01 ± 0.001aA	0.71 ± 0.01aA

[a] E1, E2 and E3 are three extracts from cranberry fruits. E1: the most water-soluble phenolic compounds extracted with water/methanol (85/15, v/v); E2: the most apolar phenolic compounds extracted with acetone/methanol/water (40/40/20, v/v); E3: anthocyanins extracted with methanol/water/acetic acid (85/14.5/0.5, v/v/v).
[b] 2.5: natural fruit pH, and 7: physiological pH.
[c] TE: Trolox® equivalent.
[d] Values are means ± standard deviations. Within each column, means for a same pH bearing the same lowercase letter are not significantly different ($P>0.05$). Within each column, means for a same sample bearing the same uppercase letter are not significantly different ($P>0.05$).

substituent on the B ring, for example, have been shown to be more effective at scavenging free radicals. Several flavonoids were shown to quench ROS and inhibit *in vitro* oxidation of low-density lipoproteins and therefore reduce the risk of thrombosis (Frankel, German, Kinsella, Parks, & Kanner, 1993) and the key role flavonoids play as scavengers of free radicals was emphasized in several reports (Saint-Cricq de Gaulejac, Provost, & Vivas, 1999; Wang, Cao, & Prior, 1997). Zheng and Wang (2003) showed for example that the catechol unit of quercetin, cyaniding, catechins, and procyanidins of poanthocyanidins was an effective radical scavenger. Despite their apparent lack of effect in bulk lipids, anthocyanins exhibit good free radical-scavenging activities and are powerful antioxidants in lipid-containing hydrophilic environments such as toward oxidation of emulsified lipids, liposomes, and LDL (Heinonen, 2007). Indeed, anthocyanin isolates and anthocyanin-rich mixtures of bioflavonoids were shown to prevent lipid peroxidation (Acquaviva et al., 2003; Ramirez-Tortosa et al., 2001). The polymerized chains of catechin monomers that form procyanidin trimers usually have a lower ability to prevent radical damage in lipid system, than in aqueous phase (Plumb, De Pascual-Teresa, Santos-Buelga, Cheynier, & Williamson, 1998). Dimeric and oligomeric B-type procyanidins possess greater scavenging activity toward peroxyl radicals than catechins, which is probably due to the presence of interflavonoid linkage that increases the electron delocalization capacity of the phenyl radical (Ursini, Rapuzzi, Toniolo, Tubaro, & Bontempelli, 2001). In cranberry, anthocyanins are among the principal antioxidant constituents, although hydroxycinnamates such as chlorogenic acid, and hydrolyzable tannins and condensed tannins are also effective antioxidants. The contribution of other flavonoids, such as flavonols, to the antioxidant effect of cranberry is generally much less important (Heinonen, 2007). The anthocyanin antioxidant effect observed in cranberries appears to vary significantly across species and cultivars of cranberries. Cranberry extracts rich in these compounds reportedly inhibit oxidative processes including oxidation of low-density lipoproteins, oxidative damage to rat neurons during simulated ischemia, and oxidative damage to the vascular endothelium (Neto, 2007). The condensed tannins (proanthocyanidins) in cranberry were shown to be effective antioxidants in various food environments such as bulk oil, emulsions, liposomes, as well as toward oxidation of LDL. Indeed, the dimeric and trimeric proanthocyanidins isolated from cranberries inhibited effectively the oxidation of methyl linoleate emulsion, lactalbumin-containing liposomes, and LDL (Heinonen, 2007). Also, certain compounds identified by He and Lui (2006) in cranberry extract, such as quercetin, 3,5,7,3′,4′-pentahydroxyflavonol-3-O-β-D-glucopyranoside, 3,5,7,3′,4′-pentahydroxyflavonol-3-O-β-D-galactopyranoside, and 3,5,7,3′,4′-pentahydroxyflavonol-3-O-α-L-arabinofuranoside, showed potent antioxidant activities.

4.2. Polarity effect

The antioxidant and antiradical activities of E1, isolated from cranberries using an aqueous solvent mixture (85%), differed greatly from those of E2 and E3 obtained with organic solvent mixtures (80 or 85%). Indeed the cranberry water-soluble phenols were more potent antioxidant than the apolar phenolic compounds and anthocyanins, suggesting that the polarity play a determining role in the antioxidant and antiradical activities of phenolic compounds. The flavonoid polarity is greatly influenced by the presence of a carbonyl group at C_4 position, followed by hydroxyl groups at positions C_2 and C_3, and then by the presence of glycosides (Heim et al., 2002). Thus, the spatial arrangement of substituents can influence the antiradical and antioxidant activities more than the flavan backbone alone (Burda & Oleszek, 2001; Heim et al., 2002). The differences in antioxidant activity between polyhydroxylated and polymethoxylated flavonoids are most likely due to the differences in both hydrophobicity and molecular planarity (Heim et al., 2002). The FRS capacity is primarily attributed to the high reactivity of the hydroxyl substituents, and the hydroxyl configuration on the B-ring in particular plays an important role by donating a hydrogen and an electron to the hydroxyl, the peroxyl, and the peroxynitrite radicals, stabilizing them and giving rise to a relatively stable flavonoid radical (Sekher Pannala, Chan, O'Brien, & Rice-Evans, 2001). Finally the glycosylation can also influence on the polarity of the molecule; in general, the glycosylated compounds have a weaker antiradical activity than their aglycone equivalent (Rice-Evans, Miller, & Paganga, 1996).

4.3. pH effect

The presence and abundance of organic acids, mainly citric, malic, quinic, and benzoic acids, are responsible for cranberries characteristically low pH (Shui & Leong, 2002). These acids also aid in the stabilization and protection of anthocyanins, and help protect the much desired red color of cranberries. In theory, the pH should also affect the cranberry phenolics ability to inhibit lipid peroxidation and their free radical-scavenging capacity.

Significant differences in the antioxidant and radical scavenging activities were observed between the samples adjusted at pH 2.5 and pH 7. In general, the antioxidant power of cranberry extracts adjusted at pH 2.5 was greater than those adjusted at pH 7. Anthocyanins are known to exist in a variety of forms, and in relative proportions determined by pH. At a pH of approximately 3 or lower, anthocyanin is orange or red and exists as a flavylium cation. As the pH is raised, kinetic and thermodynamic competition occurs between the hydration reaction of the flavylium cation and the proton transfer reactions related to the acidic hydroxyl groups of the aglycone. While the first reaction gives a colorless carbinol pseudo-base, which can undergo ring opening to a chalcone pseudo-basem the latter reactions give rise to quinonoidal bases. Further deprotonation of the quinonoidal bases can take place at pHs between 6 and 7 with the formation of purplish, resonance-stabilized quinonoid anions (Brouillard, 1982). These forms may play an important role in the antioxidant action of anthocyanins (Dangles, Fargeix, & Dufoir, 2000). The completely conjugated structure of anthocyanins (flavylium cation) that allows electron delocalization resulting in very stable radical products, favors their antioxidant ability (Van Acker et al., 1996). The degree and position of hydroxylation and methoxylation in the B ring can also affect their stability and reactivity (Pereira, Donate, & Galembeck, 1997) and thereby also antioxidant actions (Satué-Gracia, Heinonen, & Frankel, 1997; Wang et al., 1997). Together these structural factors modulate the stability and polarity as well as the ability of anthocyanins to act as free radical scavengers and metal chelators. When the pH of the cranberry extract or juice was

adjusted to 7.0, it most certainly caused a shift in the equilibrium of the pH-dependent forms of anthocyanins, and favored the quinonoidal forms which contributed to the observed FRS and LPI activities. The formation of chalcones is very slow at neutral pH (Dangles et al., 2000), and therefore they were probably not involved in the FRS activity observed with E3 when using the DPD assay that has a very short incubation time. In TBARS test which utilizes a longer incubation time at elevated temperature can cause to some degree the formation of chalcones (Lapidot, Harel, Granit, & Kanner, 1998). Consequently, it is possible that LPI activity of the anthocyanin-rich extract adjusted at pH 7.0 was due to the activity of the quinonoidal and chalchone forms. In addition, quercetin is known to strongly bind to Fe^{2+} when at pH 7.0 (Guo et al., 2007), and could have contributed furthermore to the lower LPI capacity observed at that pH. In a liposome system, the antioxidant activity of caffeic and hydroxycinnamic acids is strongly dependent on the pH of the buffer solution: at pH 4 they behave as weak peroxidation inhibitors whereas at higher pHs their antioxidant activity can increase substantially, becoming comparable to or even better than that of Trolox (Amorati, Pedulli, Cabrini, Zambonin, & Landi, 2006). The increased antioxidant activity observed near their pK_a values, where one of their catecholic hydroxyl groups is ionized, can be attributed to the presence of the phenolate anion. However, it appears that caffeic and hydroxycinnamic acids did not improve the antioxidant properties of the cranberry at pH 7.0, probably because these acids are not predominant in the fruit (Pappas & Schaich, 2009).

4.4. Cranberry juice process effect

According to the obtained results, the cranberry juice process had an influence on the FRS and the LPI activities of cranberries. Indeed, the juice exhibited much lower activities compared to the cranberry fruit extracts, and to the extract rich in water-soluble phenolic compounds (E1), in particular, which extraction conditions resembled those used for the juice extraction. The conditions used in the juice process, such as fruit crushing and mash heating, as well as by the type of enzymatic preparation used for mash maceration, and juice pressing conditions, can have a significant effect on the concentrations of the cranberry bioactive compounds in the juice and on their final properties (Bagger-Jørgensen & Meyer, 2004; Borowska & Szajdek, 2009; Buchert et al., 2005; Landbo & Meyer, 2004; Narwojsz & Borowska, 2010; Szajdek, Borowska, & Czaplicki, 2009). Heat treatments were found to have the most destructive impact on the anthocyanin content and the FRS properties of cranberry juice (Narwojsz & Borowska, 2010). Enzymes can also contribute to the destruction of antioxidants, which can in turn decrease the antioxidant capacity of juices and cause undesirable changes in color and flavor (Skrede, Wrolstad, & Durst, 2000).

5. Conclusion

The results obtained in this study showed the antioxidant and antiradical activities of cranberry phenolic compounds. The extract rich in apolar phenolic compounds (E2) and the anthocyanin-rich cranberry extract (E3) exhibited the highest levels of FRS activity. When comparing the activity of the extracts per milligram of phenol however, the water-soluble phenolic compounds (E1) had the greatest FRS properties. The anthocyanin-rich cranberry extract (E3) was the most efficient at inhibiting the peroxidation of lipids, but again the E1 presented the most important activity when reporting the results mmol TE/mg phenol. The present work indicates that cranberry phenolic compounds are more effective at neutralizing free radicals than at inhibiting the peroxdation of lipids. Also, the polarity of the compounds, the pH of the dissolving solvent and the juice process had an influence on the LPI and the FRS capacities. The results showed that the phenols isolated from cranberries using an aqueous solvent mixture (85%) were more potent antioxidant than the those obtained with organic solvent mixtures (80 or 85%). The antioxidant power of cranberries extracts adjusted at pH 2.5 was greater than when those adjusted at pH 7. The juice exhibited much lower activities compared to the cranberry fruit extracts, and to the extract rich in water-soluble phenolic compounds (E1), in particular, which extraction conditions resembled those used for the juice extraction.

Acknowledgments

This research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and by Atoka Cranberries Inc. (Manseau, QC, Canada).

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