Free Radical-Scavenging Properties and Antioxidant Activity of Fractions from Cranberry Products

Stéphane Caillet¹, Guillaume Lorenzo¹, Jacinthe Côté¹, Jean-François Sylvain², Monique Lacroix¹*

¹Research Laboratories in Sciences Applied to Food, Nutraceutical and Functional Food Institute, INRS-Institut Armand-Frappier, Université du Québec, Laval, Canada; ²Atoka Cranberries Inc., Manseau, Canada.

Email: *Monique.lacroix@iaf.inrs.ca

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ABSTRACT

Lipid peroxidation inhibition capacity and antiradical activity were evaluated in HPLC fractions of different polarity obtained from two cranberry juices and three extracts isolated from frozen cranberries and pomace containing anthocyanins, water-soluble and apolar phenolic compounds, respectively. Compounds with close polarities were collected to obtain between three and four fractions from each juice or extract. The cranberry phenols are good free radical-scavengers, but they were less efficient at inhibiting the lipid peroxidation. Of all the samples tested, the intermediate polarity fraction of extract rich in apolar phenolic compounds of fruit presented the highest antiradical activity while the most hydrophobic fractions of the anthocyanin-rich extract from fruit and pomace appeared to be the most efficient at inhibiting the lipid peroxidation. The antioxidant or pro-oxidant activity of fractions increased with the concentration. The phenol polarity and the technological process to manufacture cranberry juice can influence the antioxidant and antiradical activities of fractions.

Keywords: HPLC Fractions; Cranberry Juices; Phenolic Extracts; Free Radical Scavenging Capacity; Antioxidant Activity

1. Introduction

Stresses, physical damage, viral infection, cytotoxic or carcinogenic compounds, as a consequence of chemical or biological aggression, may cause peroxidation of cell membrane lipids and liberation of toxic substances, such as free radicals [1]. Studies concerning the relationship between the morbidity due to cancer and heart diseases and the consumption of fruits and vegetables indicated that polyphenols present in large amount in fruits and vegetables have a significant impact on the morbidity decrease from these diseases [2]. Fruits, including berries, are one of the most important sources of phenolic compounds in our diets. Especially hydroxybenzoic and hydroxycinnamic acid derivatives, anthocyanins, flavonols, catechins, and tannins, hydrolyzable or condensed, are frequently present [3]. Many of these compounds exhibit a wide range of biological effects, including antioxidant, antimicrobial, anti-inflammatory, and vasodilatory actions [4]. Phenolic extracts of berries (blackberries, red raspberries, sweet cherries, blueberries, and strawberries) inhibited human low-density lipoprotein (LDL) and liposome oxidation [5]. Berries have also shown a remarkably high scavenging activity toward chemically generated active oxygen species [6]. Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, and also by acting as oxygen scavengers [7].

The antioxidant properties of cranberries are documented in the literature and cranberries are ranked one of the highest antioxidant activities among many other fruits [8]. Cranberry phenolics have been shown to have free radical-scavenging properties against superoxide radical (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl radicals (•OH), and singlet oxygen (¹O₂), and they can also inhibit lipid peroxidation, as well as protein and lipid oxidation in liposomes [6]. The anthocyanin pigments responsible for the fruit brilliant red color and are among the principal antioxidant constituents [5]. Other phytochemicals found in cranberries (flavonols, flavanols and benzoic and cinnamnic acid derivatives) have attracted a great deal of attention because of their antioxidant activity [8,9]. Porter et al. [10] demonstrated that proanthocyanidin found in cranberry inhibits the oxidation of human LDL catalyzed by copper ions in vitro. Although, the antioxidants of cranberry products in lipid systems and the free radical-scavenging capacity have been studied, investigations...
on the fractions of phenolic compounds isolated from cranberry products are very scarce [10,11]. Also, there has been an increasing interest in exploring new antioxidants of natural origins because of the potential toxicity of synthetic antioxidants and consumers’ preference [11]. In addition, some fractions of cranberry polyphenols could present significant potential benefits for human health [12,13].

Thus, the aim of the present study was to evaluate the antioxidant and antiradical activities of fractions of different polarity obtained from two cranberry juices (clarified juice and juice concentrate) and three extracts from cranberry fruits and pomace containing water-soluble and apolar phenolic compounds, and anthocyanins. In this work, an HPLC method for the separation of fractions was established, and a rapid colorimetric method for measurement of free radical-scavenging capacity was applied and a non-enzymatic method of liposome peroxidation was used for evaluating the ability of a sample to inhibit oxidation and to prevent damage to cellular membranes.

2. Materials and Methods

2.1. Raw Material and Cranberry Processing

Frozen cranberries (Vaccinium macrocarpon) and three main cranberry processing products (pomace, clarified juice and juice concentrate (final product)) were provided by Atoka Cranberries Inc. (Manseau, QC, Canada) and were stored at −80°C until used. The initial processing step to make juice involves reducing frozen cranberries to a mash using a fruit mill. Then, the raw juice recovery from mash was done using a fruit press at 1.90 bar. During the juice pressing step, high amounts of press cake were obtained: cranberry pomace is the main byproduct of the cranberry processing industry. It is composed primarily of skin, seeds, and stems left over after pressing the fruit for juice. During the filtering process, a cross-flow membrane filtration through a 0.45 µm filter disk. The flow rate was 3 ml·min⁻¹ and the detection was achieved by photodiode array (250 nm - 550 nm). Between three and four fractions per extract or juice were recovered (Table 1). The fractions were defined to obtain well delimited peaks...
Table 1. Times of elution and solvent percentages used for HPLC-DAD fractionation* of cranberry juices and three extracts from cranberry fruits and pomace.

| Sample | Fractions | Time (min) | Solvent A/Solvent B (%) |
|--------|-----------|------------|-------------------------|
| 1      | 0 - 13    | 85/15 - 68/32 |
| 2      | 13 - 27   | 68/32 - 50/50  |
| 3      | 27 - 45   | 50/50 - 26/74  |
| 4      | 45 - 65   | 26/74 - 0/100  |
| 1      | 0 - 10    | 85/15 - 72/28  |
| Fruit E1 | 10 - 38   | 72/28 - 35/65  |
| 3      | 38 - 65   | 35/65 - 0/100  |
| 4      | 45 - 65   | 26/74 - 0/100  |
| 1      | 0 - 5     | 85/15 - 78/22  |
| 2      | 5 - 25    | 78/22 - 52/48  |
| 3      | 25 - 37   | 52/48 - 37/63  |
| 4      | 37 - 65   | 37/63 - 0/100  |
| 1      | 0 - 13    | 85/15 - 68/32  |
| 2      | 13 - 27   | 68/32 - 50/50  |
| 3      | 27 - 45   | 50/50 - 26/74  |
| 4      | 45 - 65   | 26/74 - 0/100  |
| Pomace E1 | 0 - 5     | 85/15 - 65/35  |
| 2      | 15 - 31   | 65/35 - 44/56  |
| 3      | 31 - 46   | 44/56 - 25/75  |
| 4      | 46 - 65   | 25/75 - 0/100  |
| 1      | 0 - 15    | 85/15 - 65/35  |
| Pomace E2 | 15 - 33   | 65/35 - 42/58  |
| 3      | 33 - 65   | 42/58 - 0/100  |
| 1      | 0 - 13    | 85/15 - 68/32  |
| 2      | 13 - 27   | 68/32 - 50/50  |
| Clarified juice | 27 - 45  | 50/50 - 26/74  |
| 4      | 45 - 65   | 26/74 - 0/100  |
| Juice concentrate | 13 - 27  | 68/32 - 50/50  |
| 3      | 27 - 45   | 50/50 - 26/74  |
| 1      | 0 - 10    | 85/15 - 68/32  |
| 2      | 13 - 27   | 68/32 - 50/50  |
| 3      | 27 - 45   | 50/50 - 26/74  |
| 4      | 45 - 65   | 26/74 - 0/100  |

*The fractions were defined to obtain well delimited peaks: one major peak or several peaks with similar polarity (Figure 1). E1, E2 and E3 are three extracts from cranberry fruits and cranberry pomace: 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/v); E3: anthocyanins extracted with methanol/water/acetic acid (85/14.5/0.5, v/v/v). A linear gradient was carried out between solvent A (water/acetic acid, 97/3, v/v) and the solvent B (methanol/acetic acid, 97/3, v/v) for 65 min. After lyophilization, fractions were re-dissolved in their corresponding solvent of fractionation (average percentage) to evaluate their functional properties.

2.4. Total Phenol Concentration

Total phenolic compound content in each fraction from cranberry extracts or juices was determined by spectrophotometry (absorbance at 760 nm) according to the Folin-Ciocalteu procedure [17]. Total phenolic compound content of samples was estimated from a calibration curve ($r^2 = 0.9986$) by plotting known solutions of gallic acid (10, 20, 40, 60, 80, 100 and 500 µg/mL).

2.5. Determination of Free Radical-Scavenging Capacity

Free radical scavenging capacity of each fraction from cranberry extracts or juices was evaluated following a modified procedure of the DPD (N,N-diethyl-p-phenylenediamine) (Sigma-Aldrich Ltd, Oakville, ON, Canada) colorimetric method, as reported by Caillet et al. [18]. Two hundred (200) µL of sample (312.5 µg/ml in final concentration) were added in a cell containing 3 mL of 0.15 M NaCl and submitted to electrolysis for 1 min (10 mA DC, 400 V) using a power supply (Bio-Rad, model 1000/500, Mississauga, ON, Canada). After electrolysis, a volume of 200 µL of solution was sampled and added to 2 mL of DPD solution (25 mg/mL). The generated oxidative species (superoxide anion (O$_2^-$), singlet oxygen (1O$_2^-$) and OH radicals) and their by-products (hydrogen peroxide (H$_2$O$_2$) and hypochlorite ion (OCl$^-$)) react instantly with DPD, producing a red coloration that can be measured at 515 nm using a DMS 100S spectrophotometer (Varian Canada Inc., Mississauga, ON, Canada). The antiradical activity describes the capacity of polyphenols to inhibit the accumulation of oxidative species (able to oxidize DPD) and consequently the red coloration at 515 nm. The reaction advancement was quantified 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 antioxidants). The scavenging percentage was calculated according to the following equation:

$$\text{Scavenging} (%) = 100 - \left[ \frac{OD_{sample}}{OD_{control}} \right] \times 100$$
Figure 1. Chromatogram obtained from cranberry juices and three extracts of cranberry fruits and pomace on a Zorbax SB-C18 column (250 × 9.4 mm D.I.). Fruit E1 (a), fruit E2 (b), fruit E3 (c), pomace E1 (d), pomace E2 (e), pomace E3 (f), clarified juice (g) and juice concentrate (h). E1, E2 and E3 are three extracts from cranberry fruits and cranberry pomace. 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/v); E3: anthocyanins extracted with methanol/water/acetic acid (85/14.5/0.5, v/v/v). A linear gradient was carried out between solvent A (water/acetic acid, 97/3, v/v) and the solvent B (methanol/acetic acid, 97/3, v/v) for 65 min. The DAD was set at 220 - 550 nm. Between three and four fractions (#1 - 4) of different polarities were separated from each extract or juice.

where OD\text{control} represents the OD of electrolyzed solution in the absence of sample. In fact, OD is directly related to the degree of oxidation of DPD reagent by the oxidative species. Thus extracts or juice able to reduce completely the level of reactive oxidative species will have a 100% scavenging capacity.

The antiradical activity of extracts and juice was estimated from calibration curve ($r^2 = 0.9973$) constructed by plotting known solutions of Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Sigma-Aldrich Ltd); 0.8, 1.6, 2.4, 3.2, and 4.0 mM) against % scavenging capacity. Then, data were reported to the quantity of dry matter of each sample and the quantity of phenolic compounds and results were expressed as mM Trolox® equivalent/mg of dry matter and mM Trolox® equivalent (TE)/mg of phenol.
2.6. Determination of the Lipid Peroxidation Inhibition Capacity

The determination of the antioxidant activity of each fraction from cranberry extracts or juices was done using a microtechnique based on the non-enzymatic peroxidation of rat liver microsomes method modified [19] 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 (thiobarbituric reactive substances) 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 lipid peroxidation inhibition capacity.

Liposomes preparation: Liposomes were formed by an injection method, as described by Batzri and Korn [20]. 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 an hypodermic syringe fitted with a fine needle (G26).

Microplate preparation: Twenty five µl of samples and controls were added to a microplate (96 wells). Three final concentrations for each fraction (19.5, 78.2, 313 µg/ml) were tested [19]. 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 µL of reaction mixture was added to a microplate using a multichannel pipette. Finally, 10 µL of FeCl (Sigma-Aldrich) solution (4.3 mg/mL) was added to the wells. The microplate was then incubated at 37°C for 15 minutes. One hundred fifty µL of a fresh solution of 10% (v/v) SDS (Sigma-Aldrich) and 0.67% (v/v) thiobarbituric acid (Sigma-Aldrich) in a 1:2 ratio was added in the microplate. The colorimetric reaction was produced at 80°C for 30 min. The TBARS of the controls and samples were evaluated at 540 nm with a Microplate Autoreader (model EL 309, Biotek Instruments, Winooski, VT). The positive control was represented by the reaction mixture in presence of Trolox without the sample, and the optical density of the chroomogen formed denoted complete peroxidation. The negative control contained only the phosphate buffer without liposomes. The relative antioxidant activity was calculated using the following equation:

\[ \text{AA} (\%) = \left( \frac{\text{OD}_{\text{negative control}} - \text{OD}_{\text{sample}}}{\text{OD}_{\text{negative control}}} \right) \times 100 \]

Thus extracts or juice able to inhibit completely the lipid peroxidation will have a 100% antioxidant capacity. Then, data were reported to the quantity of dry matter of each sample and the quantity of phenolic compounds and results were expressed as Trolox® equivalent (TE)/mg of dry matter or Trolox® equivalent/mg of phenol relatively to the positive control.

2.7. Statistical Analysis of Data

Analysis of variance and Duncan’s multiple-range was done using Stat-Packets Statistical Analysis software (Walonick Associates Inc., MN, USA) for the determination of antiradical and antioxidant activities. Differences between means were considered significant when \( p \leq 0.05 \). These experiments were repeated three times.

3. Results and Discussion

3.1. Antiradical Activity

HPLC analysis (Figure 1) allowed according to their polarity the separation of the phenolic compounds obtained from two cranberry juices and three extracts isolated from frozen cranberries and pomace containing anthocyanins, water-soluble and apolar phenolic compounds. The most polar phenolic compounds presented the shortest elution times and low molecular weight (MW). Between three and four fractions from each juice or extract were collected according to conditions defined in Table 1, in order to collect compounds with close polarities. The phenolic content of fractions might be evaluated through standard phenolic compounds and their retention time: phenolic acids (gallic acid: 6.27 min, chlorogenic acid: 7.14 min, caffeic acid: 10.41 min, p-coumaric acid: 17.25 min), anthocyanins (cyanidin 3-galactoside: 19.04 min, cyanidin 3-arabinoside: 22.96 min, peonidin 3-galactoside: 24.22 min, peonidin 3-arabinoside: 26.12 min), flavonoids (myricetin 3-galactoside: 29.12 min, quercetin 3-galactoside: 31.79 min, myricetin 3-arabinoside: 32.14 min, quercetin 3-arabinoside: 34.48 min) and proanthocyanidins (with DP of 5: 48.19 min, with DP of 6: 56.33 min). Thus in fractionation into 4 fractions, Fraction 1 was enriched in phenolic acids, Fraction 2 was enriched in anthocyanins, Fraction 3 was enriched in flavonoids and Fraction 4 was enriched in proanthocyanidins. In fractionation into 3 fractions, Fraction 1 was enriched in phenolic acids, Fraction 2 was enriched in anthocyanins and flavonoids, Fraction 3 was enriched in flavonols and fraction 4 was enriched in proanthocyanidins.

The free radical-scavenging capacity of these fractions is presented in Table 2. When data were reported to the quantity of dry matter of each sample, the results showed that the fraction 2 (enriched in anthocyanins and flavonoids) of extract rich in apolar phenolic compounds (E2) of fruit presented the highest free radical-scavenging activities (39.58 mM TE/mg dry matter) followed by the fraction 2 (enriched in anthocyanins) of clarified juice (7 mM TE/mg dry matter). Conversely, fractions 1 (enriched in phenolic acids) and 4 (enriched in proanthocyanidins) of extract rich in water-soluble phenolic compounds (E1) obtained from fruit, fraction 1 (enriched in phenolic acids)
Table 2. Radical-scavenging capacity of each fraction from cranberry juices and three extracts of cranberry fruits and pomace.

| Samplesa | Fractions | Free radical scavenging capacityb,c,d (mM TE/mg) |
|----------|-----------|-----------------------------------------------|
|          |           | Free radical scavenging capacity (mM TE/mg) |
|          | fraction  | phenol                                       |
| Fruit E1 | 1         | 0.43 ± 0.05h 43.42 ± 4.70t                    |
|          | 2         | 3.94 ± 0.13m 30.82 ± 2.41qr                   |
|          | 3         | 4.02 ± 0.12m 14.21 ± 0.92m                    |
|          | 4         | 0.38 ± 0.03gh 19.80 ± 1.66g                   |
|          | 1         | 0.74 ± 0.05i 7.40 ± 0.51j                     |
| Fruit E2 | 2         | 39.58 ± 3.16o 40.39 ± 4.07st                  |
|          | 3         | 2.02 ± 0.34j 111.61 ± 12.81v                   |
|          | 4         | −1.52 ± 0.10a −38.48 ± 2.99a                   |
|          | 1         | 0.38 ± 0.03gh 111.61 ± 12.81v                  |
|          | 2         | 3.85 ± 0.12m 49.29 ± 5.21t                     |
|          | 3         | 4.04 ± 0.44m 33.45 ± 3.01rs                    |
|          | 4         | 3.91 ± 0.15m 15.37 ± 1.67mn                    |
| Fruit E3 | 1         | 0.01 ± 0.001e 0.12 ± 0.01f                     |
|          | 2         | 0.33 ± 0.03fg 1.14 ± 0.12g                     |
|          | 3         | 0.38 ± 0.03gh 2.79 ± 0.34h                     |
|          | 4         | 0.30 ± 0.03f 25.00 ± 1.66p                     |
|          | 1         | 2.95 ± 0.19k 25.21 ± 2.08p                     |
|          | 2         | 2.79 ± 0.21jkl 25.36 ± 2.11lm                  |
|          | 3         | 2.99 ± 0.30k 11.50 ± 1.02l                     |
|          | 4         | 3.08 ± 0.31k 18.01 ± 2.04ano                   |
| Pomace E1| 1         | 2.87 ± 0.25k 26.09 ± 2.49p                     |
|          | 2         | 3.28 ± 0.31kl 10.93 ± 1.05kl                   |
|          | 3         | 3.25 ± 0.30kl 9.02 ± 0.89k                     |
|          | 4         | 0.49 ± 0.31bl −3.06 ± 2.15b                    |
|          | 1         | 7.36 ± 0.40n 56.61 ± 4.08a                     |
|          | 2         | 3.84 ± 0.34lm 3.84 ± 0.38i                     |
|          | 3         | 2.82 ± 0.27k 25.63 ± 2.19p                     |
|          | 4         | 0.0026 ± 0.0005d 0.075 ± 0.007c                 |
| Pomace E3| 1         | 0.0103 ± 0.0014e 0.026 ± 0.004d                 |
|          | 2         | 0.0017 ± 0.0007cd 0.017 ± 0.002c                |
|          | 3         | 0.0011 ± 0.0003c 0.068 ± 0.005e                 |
| Clarified juice | 4         |                                                     |
| Juice concentrate | 4         |                                                     |

aE1, 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/v); E3: anthocyanins extracted with methanol/water/acetic acid (85/14/5, v/v/v). bTE: Trolox® equivalent. cValues are means ± standard deviations. Within each column, means bearing the same lowercase letter are not significantly different (P > 0.05). dA negative result indicates that the fraction is pro-oxidant.

Of fruit E2, all fractions of pomace E1 showed a low free radical-scavenging capacity. Moreover, all fractions of concentrate showed a very low free radical-scavenging capacity (between 0.001 and 0.01 mM TE/mg dry matter). It appears that conditions of the evaporation to obtain a juice concentrate exerted a significant effect (P ≤ 0.05) on bioactive molecule content and their antiradical properties. Also, two fractions (i.e. fractions 1 enriched in phenolic acids from clarified juice and anthocyanin-rich cranberry extract (E3) of fruit) were shown slightly pro-oxidant. The other fractions showed an average free radical-scavenging capacity (between 2 and 4 mM TE/mg dry matter).

Among the samples, phenolic compounds of fraction 3 (enriched in flavonols and proanthocyanidins) of fruit E2 showed the highest free radical-scavenging activity (111 mM TE/mg phenol) when results were expressed in mM Trolox® equivalent/mg phenol. The extraction conditions of the extract rich in water-soluble phenolic compounds (E1) (water/methanol (85:15, v/v)) were similar to those employed for juice (water). All fractions of fruit E1 showed an important free radical-scavenging activity (between 14 and 43 mM TE/mg phenol) while antiradical activity of phenol compounds in all fractions of juice concentrate was extremely low (below 0.75 mM TE/mg phenol).

Thus, the data obtained reveal that the technological process to manufacture cranberry juice has influenced the phenolic compound content. Also, the free radical-scavenging capacity of phenolic compounds was reduced in fractions 1 (enriched in phenolic acids) and 3 (enriched in flavonols) from clarified juice and fractions 1 to 3 from presscake E1 compared to that obtained with the corresponding fractions of fruit E1. It tends to prove that extraction has led to the recovery of most bioactive molecules, except for molecules of the less polar fraction (enriched in proanthocyanidins) of pomace E1. Moreover, it is also important to note that most fractions of E2 and E3 contained phenolic compounds with good or very good free radical-scavenging activities, except for fraction 1 (enriched in phenolic acids) of fruit E3 which phenolic compounds were pro-oxidant.

3.2. Antioxidant Activity

The capacity of lipid peroxidation inhibition of each fraction at three concentrations (19.5, 78.1 and 313 µg/mL) is presented in Table 3. The results showed that the antioxidant or pro-oxidant activity of fractions increased with the concentration. Of all the samples tested, the most hydrophobic fractions (enriched in proanthocyanidins) of the anthocyanin-rich cranberry extract (E3) from fruit and pomace appeared to be the most efficient at inhibiting the lipid peroxidation (above 2 TE/mg dry matter) when results were expressed in Trolox® equivalent/mg.

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Table 3. Lipid peroxidation inhibition capacity of each fraction from cranberry juices and three extracts of cranberry fruits and pomace.

| Samples | Fractions | TE/mg fraction | TE/mg phenol |
|---------|-----------|----------------|--------------|
|         |           | 19.5 µg/ml | 78.2 µg/ml | 313 µg/ml |
|         |           | 19.5 µg/ml | 78.2 µg/ml | 313 µg/ml |
| Fruit E1 |           |           |           |           |
| 1       | -0.03 ± 0.01Bc | -0.02 ± 0.01Bd | -0.19 ± 0.01Ac | -2.39 ± 0.24Ba |
| 2       | 0.18 ± 0.03Ag | 0.35 ± 0.01Bi | 0.52 ± 0.06Cij | 1.40 ± 0.13Ai |
| 3       | 0.56 ± 0.07Ak | 1.35 ± 0.29Bo | 1.54 ± 0.15Bn | 1.99 ± 0.23Aj |
| 4       | 0.37 ± 0.03Aij | 0.46 ± 0.04Bjk | 0.76 ± 0.06Ckl | 19.79 ± 1.40Ao |
| Fruit E2 |           |           |           |           |
| 1       | 0.02 ± 0.01Cd | -0.008 ± 0.001Bc | -0.11 ± 0.01Ac | 0.25 ± 0.02Be |
| 2       | 0.18 ± 0.01Ag | 0.43 ± 0.03Bj | 0.92 ± 0.05Cm | 0.19 ± 0.01Ad |
| 3       | 0.93 ± 0.09Bm | -0.50 ± 0.09Ab | -0.51 ± 0.09Ab | 51.77 ± 3.83Bq |
| Fruit E3 |           |           |           |           |
| 1       | 0.03 ± 0.01Ad | 0.26 ± 0.09Bhi | 0.33 ± 0.02Bh | 3.12 ± 0.36Akl |
| 2       | 1.13 ± 0.11Amn | 1.56 ± 0.15Bb | 1.48 ± 0.23Bn | 9.38 ± 0.56An |
| 3       | 0.04 ± 0.01Ae | 0.11 ± 0.02Bg | 0.81 ± 0.06Clm | 0.34 ± 0.03Af |
| 4       | 1.49 ± 0.31An | 2.10 ± 0.21Bp | 2.30 ± 0.29Bo | 19.07 ± 1.93Ao |
| Pomace E1 |           |           |           |           |
| 1       | -0.09 ± 0.01Ab | -0.09 ± 0.01Ac | -0.15 ± 0.01Ad | -1.10 ± 0.11Bb |
| 2       | 0.37 ± 0.03Aij | 0.53 ± 0.05Bkl | 0.64 ± 0.06Bjk | 1.28 ± 0.11Ahi |
| 3       | 0.20 ± 0.03Ag | 0.84 ± 0.08Bn | 1.72 ± 0.16Cn | 1.47 ± 0.13Ai |
| 4       | 0.31 ± 0.03Ahi | 0.57 ± 0.04Blm | 0.76 ± 0.06Ckl | 26.41 ± 2.32Ap |
| Pomace E2 |           |           |           |           |
| 1       | 0.07 ± 0.03Cef | 0.004 ± 0.001Bf | -0.16 ± 0.01Ad | 0.32 ± 0.02Cf |
| 2       | 0.29 ± 0.02Ah | 0.60 ± 0.05Blm | 0.86 ± 0.07Clm | 1.15 ± 0.10Ah |
| 3       | 0.02 ± 0.01Ad | 0.20 ± 0.02Bb | 0.31 ± 0.03Ch | 0.10 ± 0.01Ac |
| 4       | 0.70 ± 0.06Bl | -1.27 ± 0.12Aa | -1.30 ± 0.14Aa | 2.75 ± 0.26Bk |
| Pomace E3 |           |           |           |           |
| 1       | 0.15 ± 0.02Ag | 0.44 ± 0.03Bj | 0.61 ± 0.06Cj | 1.42 ± 0.13Ai |
| 2       | 1.10 ± 0.09Amn | 1.43 ± 0.13Bo | 1.79 ± 0.15Cn | 3.68 ± 0.34Al |
| 3       | 1.26 ± 0.11An | 2.17 ± 0.18Bp | 2.52 ± 0.24Bo | 3.50 ± 0.33Al |
| Clarified juice |           |           |           |           |
| 1       | -0.47 ± 0.03Ab | -0.48 ± 0.03Ab | -0.49 ± 0.03Ab | -2.94 ± 0.31Aa |
| 2       | 0.19 ± 0.02Ag | 0.26 ± 0.04Bhi | 0.36 ± 0.03Ch | 1.45 ± 0.13Ai |
| 3       | 0.44 ± 0.04Aj | 0.65 ± 0.04Bm | 0.93 ± 0.06Cm | 0.45 ± 0.04Ag |
| 4       | 0.42 ± 0.04Aj | 0.58 ± 0.05Blm | 0.88 ± 0.07Clm | 3.82 ± 0.34Al |
| Juice concentrate |           |           |           |           |
| 1       | 0.16 ± 0.02Ag | 0.20 ± 0.02Ah | 0.25 ± 0.02Bg | 1.83 ± 0.14Aj |
| 2       | 1.10 ± 0.11Amn | 1.45 ± 0.12Bo | 1.70 ± 0.15Bn | 1.13 ± 0.13Ah |
| 3       | 0.50 ± 0.01Ak | 0.48 ± 0.02Ajk | 0.51 ± 0.01Ai | 0.12 ± 0.02Ac |
| 4       | 0.09 ± 0.01Af | 0.09 ± 0.01Ag | 0.10 ± 0.01Af | 6.01 ± 0.53Am |

*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/v); E3: anthocyanins extracted with methanol/water/acetic acid (85/14.5/0.5. v/v/v). TE: Trolox® equivalent. Values are means ± standard deviations. Means in the same row bearing the same uppercase letter are not significantly different (P > 0.05). Means in each column bearing the same lowercase letter are not significantly different (P > 0.05). A negative result indicates that the fraction is pro-oxidant.
dry matter. However, most of the fractions showed a weak antioxidant activity (below 1 TE/dry matter). Also, fractions 1 (enriched in phenolic acids) of E1, E2 and clarified juice and fraction 4 (enriched in proanthocyanidins) of E2 were slightly pro-oxidant.

Among the samples, the phenolic compounds present in the more hydrophobic fractions (enriched in proanthocyanidins) from fruit and pomace E1 showed the highest antioxidant activity (63 and 39 TE/mg phenol, respectively) when results were expressed in Trolox® equivalent/mg phenol. Antioxidant activities of phenolic fractions of E1 and juices increased as their polarities decreased. Also, it is interesting to note that phenols present in the most hydrophobic fraction (enriched in flavonols and proanthocyanidins) of fruit E2 at 19.5 µg/ml showed a very good capacity of lipid peroxidation inhibition (51 TE/mg phenol) while they were strongly pro-oxidant at 313 µg/ml (∼28 TE/mg phenol). Moreover, the phenols of E3 fractions showed a good antioxidant activity while the phenolic compounds present in fraction 1 (enriched in phenolic acids) of E1 and in many fractions of E2 and two juices showed a low capacity of lipid peroxidation inhibition or pro-oxidant properties.

The data obtained reveal that many fractions of cranberry extracts and juices are free radical-scavengers and primary antioxidants, which react with free radicals and inhibit the lipid peroxidation. However, the phenolic compounds of most fractions of cranberry extracts and juices contributed substantially to the radical scavenging activity, while the lipid peroxidation inhibition activity was attributable to the most hydrophobic fractions composed mainly of large polyphenols (i.e. proanthocyanidins and flavonoid oligomers) and this in spite of the low total amounts of these compounds present. Also, the more polar fraction of cranberry extracts and juices, containing mainly phenolics acids, organic acids and sugars, promoted generally lipid oxidation. Numerous classes of phenolic compounds are present in cranberry juice and by-products of cranberry-juice processing (e.g., press cake) [21,22]. The wide range of phenolics in cranberry known to contribute to the characteristic antioxidant activity profile include the catechins, quercetin, p-coumaric acid, chlorogenic acid, myricetin, trans-resveratrol and cyanidin/peonidin 3-galactoside/arabinoside [23-25]. The concentration of these compounds in cranberry will vary depending on the maturity and variety of the cranberry fruit [26]. Moreover, the extent to which particular phenolic compounds contribute to the total antioxidant capacity of cranberry may also depend on both the relative concentration of individual antioxidant compounds, as well as possible synergistic interactions between different fruit constituents [25].

The antioxidant and antiradical activities of phenol fractions from aqueous extract (E1) of cranberry differ from those of phenol fractions from solvent extracts (E2 or E3). Antioxidant activities of phenol fractions of E1 increased as their polarities decreased, but this is not the case with phenol fractions from solvent extracts. Also, antiradical activities of phenol fractions from fruit E1 decreased as their polarities decreased, whereas those of phenol fractions from fruit E2 increased as their polarities decreased. The reason is certainly the variation of solubility of compounds extracted in water or solvents, which is connected to their hydrophilic or hydrophobic character. In the present study, it appears that the polarity of phenolic compounds is a determinant of antioxidant and antiradical activities. Thus, antioxidant activity will differ depending on the phenolic molecular structure [9], and antiradical activity is dependent on the structure of the free radical-scavenging compounds and the substituents present on the ring of the flavonoids [27]. The polarity of the flavonoids depends primarily on the nature of the radicals on rings, and in particular on the number of OH groups [2]. Thus, the spatial arrangement of substituents is a greater determinant of antiradical and antioxidant activities than the flavan backbone alone [28]. The differences in antioxidant activity between polyhydroxylated and polymethoxylated flavonoids are most likely due to differences in both hydrophobicity and molecular planarity [2]. Consistent with most polyphenolic antioxidants, both the configuration and total number of hydroxyl groups substantially influence several mechanisms of antiradical activity [28]. Our results indicate that the phenols of polar fractions (1 and 2) from fruit E1 have free radical-scavenging capacities which are more significant than those of phenols of less polar fractions (3 and 4). Free radical-scavenging capacity is primarily attributed to the high reactivities of hydroxyl substituents. Hydroxyl groups on the B-ring donate hydrogen and an electron to hydroxyl, peroxy, and peroxynitrite radicals, stabilizing them and giving rise to a relatively stable flavonoid radical. Among structurally homologous flavones and flavanones, peroxy- and hydroxyl-scavenging increases linearly and curvilinearly, respectively, according to the total number of OH groups [29]. The glycosylated derivatives also have an influence on the polarity of the molecule. In general, the glycosylated compounds have a weaker antiradical activity than their aglycone equivalent [30]. The key role of flavonoids, as scavengers of free radicals, is emphasized in several reports [31]. Flavonoids with adjacent dihydroxy substituents on the B ring have been shown to be effective in radical scavenging [24]. This was the case, in particular, for catechol unit found in quercetin of flavonols, cyanidin of anthocyanidins, catechins of flavan-3-ols, and procyanidins of proanthocyanidins. In cranberry, anthocyanins are among the principal antioxidant constituents, although hydroxycinnamates such as chlorogenic acid, hydrolyzable tannins and con-
densed tannins are also effective antioxidants. The con-
tribution of other flavonoids, such as flavonols, to the an-
tioxidant effect of cranberry is generally much less sig-
nificant compared to the activity of anthocyanins and

tannins [8]. 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 [8].

Our results indicate that the cranberry phenols of frac-
tions are good free radical-scavengers, but several cran-
berry phenol fractions were less efficient at inhibiting the
lipid peroxidation. The method used to evaluate the lipid
peroxidation inhibition activity of phenols is based on the
Fenton reaction and detects non-enzymatic autoxidation
[19]. The oligomeric proanthocyanidins have been noted
for their ability to inhibit low molecular weight iron-me-
diated lipid oxidation compared to their monomeric counter-
parts [10,32] and the number of catechol units in the re-
action mixture was found to positively correlate with the
ability of catechins and procyanidins to protect against
lipid oxidation [33]. Among cranberry phenol fractions
of different polarity, Lee et al. [11] showed that the most
apoar fraction was the most effective fraction in inhibiting
thiobarbituric acid reactive substances (TBARS) for-
mation. This could explain the high capacity of lipid per-
oxidation inhibition observed in fraction 4 from fruit and
pomace E1. The smaller flavonol aglycones such as quer-
cetin on the other hand have been shown to orient readily
into membrane bilayers [34]. Interestingly, quercetin, which
is a major phenolic phytochemical present in cran-
berries, can completely suppress Fe-promoted Fenton chem-
istry at micromolar levels even in the presence of the
major cellular iron chelators ATP or citrate [35]. How-
ever, the radical scavenging activity of quercetin pro-
vides only partial protection against Fenton chemistry-
mediated damage while Fe chelation by quercetin can
completely inhibit Fenton chemistry, indicating that the
chelation may be key to its antioxidant activity [36]. Also,
several reports indicate that some phenolic acids exert
apparently conflicting effects on the Fenton reaction de-
pending on the oxidation conditions and that they are
potential prooxidants [37]. Thus, benzoic acid, caffeic acid
and chlorogenic acid can enhanced lipid oxidation and
exhibited prooxidant and antioxidant activities depending
on the lipid oxidation phases (incubation time) and their
concentration [38]. This could explain the prooxidant
activities observed in fractions 1 from fruit E1 and E2,
pomace E1 and juices.

The technological process to manufacture cranberry
juice can also influence the antioxidant and antiradical
activities, since fractions from clarified juice, juice con-
centrate and pomace showed activities much lower than
those observed with corresponding fractions from cran-
berry fruit extracts, in particularly with fractions from E1
which the extraction conditions were similar to those
used to obtain the juice. Apart from the genetic charac-
ters of raw materials, also the conditions of the techno-
logical process exert a significant effect on the concen-
trations of antioxidants and the free radical-scavenging
compounds in juices and on their final properties [39].

The release of antioxidants and free radical-scavenging
compounds into the juice is considerably affected by the
parameters of unit operations during processing, such as
fruit crushing and mash heating, as well as by the type of
enzymatic preparation used for mash maceration, and
juice pressing conditions [40].

4. Conclusion

Our results showed that the phenolic compounds present
in cranberry fractions have antioxidant and antiradical
activities, and suggest that even a partial purification of
these compounds has an important impact on their anti-
oxidant and free radical scavenging activities. Antiradical
and antioxidant activities of fractions reflect the struc-
ture-activity relationship, it appears that the polarity of
phenolic compounds is a determinant of these activities.
The present work indicated that the cranberry phenols are
good free radical-scavengers, but they were less efficient
at inhibiting the lipid peroxidation. The antioxidant effect
was dose dependent at the concentration levels used, in
general, fractions were good antioxidant only at the
higher concentration of 313 µg/mL. Also, the technolo-
gical process to manufacture cranberry juice has nega-
tively affected the antioxidant and antiradical activities
of all fractions regardless of polarity. However, phenols pre-

sent in most fractions of fruit extracts have shown very
interesting antiradical properties while phenols in the
most hydrobolic fractions of extract rich in water-soluble
phenolic compounds and the anthocyanin-rich cranberry
extract had a remarkable action against lipid oxidation.
These fractions represent antioxidant sources that may
have potential for inducing beneficial effects on human
health, and in general, our results showed that the cran-
berry fractions contain significant amounts of antioxidant
active compounds, which may be regarded as a promis-
ing natural additive for health beneficial functional foods
and nutraceuticals.

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