JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 10245–10251 10245
DOI:10.1021/jf9021114

Production of a Phenolic Antioxidant Enriched Cranberry Juice by Electrodialysis with Filtration Membrane

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The fruit of the American cranberry is known for its benefits on human health and its nutraceutical potential. The enrichment of cranberry products with functional phytochemicals may improve their functionality for enhancing health benefits, but this enrichment may be difficult due to the commercial rarity and difficulty of purification of anthocyanins and other phytochemicals from natural sources. The aim of this work was to evaluate the feasibility of the production of a cranberry juice enriched with natural phenolic antioxidant compounds using an ultrafiltration (UF) membrane stacked in an electrodialysis (ED) cell. Total concentrations of proanthocyanidins and anthocyanins increased by 34.8% and 52.9%, respectively, in cranberry juice treated with the EDUF system. Moreover, an 18% increase of the antioxidant capacity of the enriched cranberry juice was obtained by the EDUF treatments. The EDUF process might be used for natural enrichment of cranberry juice with antioxidant phenolics.

KEYWORDS: Cranberry juice; ultrafiltration membrane; electrodialysis; antioxidant capacity; proanthocyanidin; anthocyanin

INTRODUCTION

Cranberry fruit is a rich source of phenolic phytochemicals including phenolic acids, flavonoids, and ellagic acids (*1*). These phytochemicals may contribute to the beneficial effects of cranberry on human health because many of these compounds have antioxidant activity that may help protect cells against oxidative damage caused by free radicals (*2*). Some of the specific anthocyanidins in cranberry have antioxidant strength equal to or greater than that of vitamin E (*3*). Moreover, proanthocyanidin molecules in cranberry have been shown to inhibit *Escherichia coli* adhesion to human body cells (*4*). They can also inhibit the formation of bacterial complexes responsibles of dental plaque (*5*, *6*), prevent gastric ulcers caused by *Helicobacter pilori* (*7*), and display potent anticancer activity (*8*).

The enrichment of cranberry products with functional phytochemicals may improve their functionality for enhancing health benefits (*1*), but this enrichment may be difficult due to the commercial rarity and limitation in the processes having the capacity to purify anthocyanins (*9*) and other phytochemicals from natural sources. The cranberry juice could be concentrated in phenolic phytochemicals to decrease the volume of juice to be drunk to have the effective health benefits or to decrease the cost of drying to obtained a natural extract. It would be especially interesting to selectively concentrate the juice in certain beneficial fractions without otherwise modifying the composition in sugar or minerals. For instance, a cranberry juice enriched in proanthocyanidins could be a useful means for preventing urinary tract infections, whereas a juice enriched in anthocyanins might be useful to reduce cardiovascular diseases (*10*).

Membrane filtration techniques are used for the large-scale isolation of bioactive molecules from complex feeds, but, unfortunately, they have a too low selectivity. To overcome this drawback, a new method to separate bioactive molecules has been developed: electrodialysis with ultrafiltration membrane (EDUF). The ultrafiltration membrane is stacked as a molecular barrier in an electrodialysis cell, and the driving force in the process for the migration of molecules is an external electric field (*11*). This technology has been applied with success for the separation of charged molecules such as tobacco polyphenols (*12*) and green tea polyphenols (*13*), for bioactive peptide purification (*14*, *15*), and for the electroseparation of chitosan oligomers (*11*). As proanthocyanidins and anthocyanins are charged molecules under specific pH conditions, this technology might be used to enrich cranberry juice in bioactive components.

The objectives of the present work were to (1) determine the direction of migration of the proanthocyanidin and anthocyanin molecules at the pH of cranberry juice, (2) determine the appropriate configuration of the EDUF system for the separation of the proanthocyanidins and anthocyanins in cranberry juice, and (3) evaluate the feasibility of separating and concentrating natural phenolic antioxidant compounds from cranberry juice with an EDUF process.

MATERIALS AND METHODS

Material. *Cranberry Juice*. Treatments were carried out on commercially available pasteurized 100% pure cranberry juice (Gourmet Atoca, Maison Bergevin, Quebec, Canada).

Electrodialysis Cell. The electrodialysis cell was a Microflow type cell with an effective area of 10 cm^2 (ElectroCell AB, Karlskoga, Sweden)

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Published on Web 10/16/2009

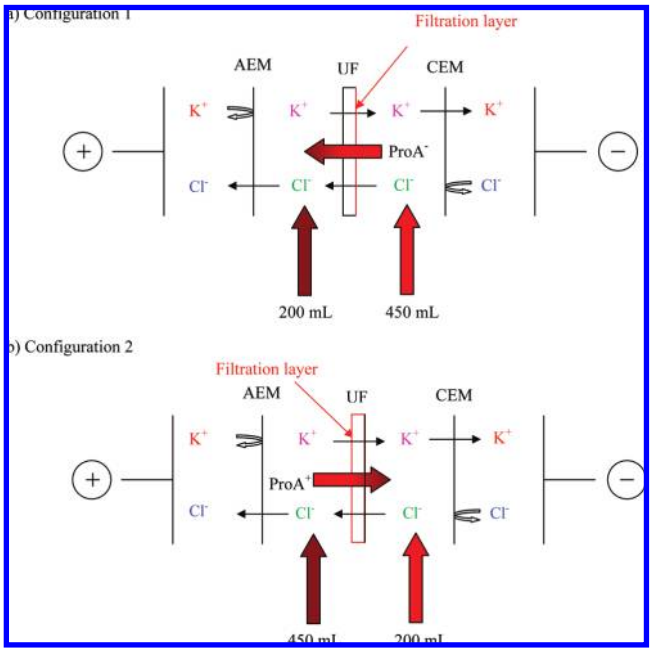

Figure 1. Configurations of the EDUF system used to enrich cranberry juice with the filtrating side of the ultrafiltration membrane in the cathode direction (**a**) or in the anode direction (**b**). AEM: anion-exchange membrane, UF: ultrafiltration membrane, CEM: cation-exchange membrane, ProA: proanthocyanidins and anthocyanins.

with one Neosepta CMX-SB cationic membrane (Tokuyama Soda Ltd., Tokyo, Japan), one Neosepta AMX-SB anionic membrane (Tokuyama Soda Ltd.), and one filtration membrane. The cell had three closed loops, and each was connected to a separate external reservoir to allow recirculation of the solutions during treatments. The solutions were circulated using three centrifugal pumps, and the flow rates were controlled using flowmeters (Aalborg Instruments and Controls, Inc., Orangeburg, NY). The anode was a dimensionnally stable electrode (DSA), and the cathode was a 316 stainless steel electrode. The anode/cathode voltage difference was supplied by an electrical power supply (model HPD 30-10, Xantrex, Burnaby, Canada).

Protocol. *Electrodialysis with Ultrafiltration Membrane (EDUF) Treatments*. On the basis of preliminary studies, a polyether−sulfone ultrafiltration (UF) membrane with a molecular weight cutoff (MWCO) of 500,000 Da (Millipore Corp., Bedford, MA) was chosen for the treatments of cranberry juice. Treatments were performed in batch process using a constant voltage difference of 30 V. The duration of the treatments was 4 h, and three replicates of each treatment were performed. As the proanthocyanidin and anthocyanin migration directions at the pH of the cranberry juice were unknown at the beginning of this study, two different cell configurations (**Figure 1**) were used. In both configurations, raw juices were added in both compartments of the EDUF cell. In configuration 1, the filtration layer of the ultrafiltration membrane was on the cathode side to facilitate the migration of these molecules if they were negatively charged in cranberry juice. However, in the case that the proanthocyanidin and anthocyanidin molecules were positively charged at cranberry juice pH, they would migrate toward the cathode. Consequently, in a second configuration (configuration 2), the filtration layer of the ultrafiltration membrane was placed on the anode side to facilitate the migration of these molecules toward the cathode. In the electrodialysis cell, two of the three compartments contained 450 or 200 mL of cranberry juice (**Figure 1**). There was a 20 g/L KCl solution (350 mL) in the third compartment for electrode rinsing. Cranberry juices flow rates were 100 mL/min, whereas the flow rate of the electrolyte solution was 300 mL/min. Conductivity and pH of cranberry juices were recorded throughout the process as well as the global system resistance. Moreover, measurements of the titrable acidity, color, and antioxidant capacity were made on treated juices and on a control. Total proanthocyanidin and anthocyanin contents of treated cranberry juices were also determined.

Analyses. *Freeze-Drying*. Samples (2.5 mL) of control and treated juices were frozen at −18 °C and then freeze-dried at room temperature for 12 h in a Labconco freeze-dryer (model 7750000, Kansas City, MO). Samples of juices were freeze-dried before the oxygen radical absorbance capacity (ORAC), total anthocyanin, and total proanthocyanidin were analyzed.

Total Proanthocyanidin Content. Freeze-dried samples were diluted with 10 mL of methanol. Diluted samples were vortexed, sonicated for 45 min in a water bath at room temperature, and centrifuged at 5000 rpm for 5 min. Then, 2.5 mL of 20% sulfuric acid solution in methanol and 2.5 mL of a 12 g/L vanillin solution in methanol were added to 1 mL of the resulting supernatants. The mixtures were incubated at 30 °C during 15 min, and the absorbance of the resulting solutions was read at 500 nm in a Hewlett-Packard model 8453 spectrophotometer (Palo Alto, CA) (*16*, *17*). Total proanthocyanidin content of the treated juices was calculated on the basis of calibration curve obtained with a catechin solution in methanol and expressed as catechin equivalents in milligrams per milliliter.

Total Anthocyanin Content. Total anthocyanin content of the cranberry juices was determined by HPLC. The system used was an Agilent 1100 series (Agilent Technologies, Palo Alto, CA) equipped with a diode array detector. Anthocyanins were analyzed with a Luna 5 μm C_{18} column (2 mm × 250 mm, Phenomenex, Torrance, CA). Acetic acid/acetonitrile/phosphoric acid (10%/5%/1% v/v) in water was used as solvent for elution at a 1 mL/min flow rate. The detection wavelength was 520 nm. The total anthocyanin content in samples was expressed as percentage of the initial anthocyanin quantity in cranberry juices before treatments (*18*).

pH. The pH was measured using a VWR Symphony electrode (VWR Scientific, Ville Mont-Royal, Canada) equipped with an automatic temperature compensation device and connected to a VWR Symphony SR601C benchtop pH-meter.

Conductivity. A YSI conductivity meter, model 3100, was used with a YSI immersion probe (model 3252, cell constant $K = 1\ cm^{-1}$; Yellow Springs Instrument, Yellow Springs, OH) (*11*−*13*).

Global System Resistance. The global resistance of the system was calculated using the Ohms law ($U = RI$) for the values of current and voltage read directly from the power supply indicators (*12*).

Titrable Acidity. Titrable acidity of a control and of treated juices was determined on 20 mL of juice with a solution of NaOH (1 mol/L) until pH 7.0 was reached (*19*).

Juice Color. Color parameters of control and treated juices were determined with a chromameter (model Minolta meter CR-300, Konica Minolta Inc., Mississauga, ON, Canada). Results were reported as *L* (luminescence or lightness), *a* (intensity of color varying from red to green), and *b* (intensity of color varying from yellow to blue) (*20*−*22*).

Oxygen Radical Absorbance Capacity. Freeze-dried samples were diluted in 10 mL of acetone/water/acetic acid (AWA; 70%/29.5%/0.5%). Diluted samples were centrifuged two times at 5000 rpm during 5 min. The supernatants were removed and diluted with AWA to obtain a final volume of 25 mL. A dilution (1:80) of the diluted supernatants was also made with 0.075 M phosphate buffer (pH 7.0). The automated ORAC assay was carried-out with a Galaxy fluorometer (BGM LabTech, Durham, NC). Trolox, a water-soluble analogue of vitamin E, was used as a control standard. The experiment was conducted at 37 °C under pH 7.0 condition with a blank sample in parallel. For the analysis, 200 μL of a fluorescein solution (0.036 mg/L) and 20 μL of the diluted samples were added in wells. Then, 75 μL of a 2,2′-azobis(2-aminopropane dihydrochloride) (AAPH) solution at a concentration of 8.6 mg/L was added in wells. AAPH was used as a source for the peroxyl radical, which is generated as a result of the spontaneous decomposition of AAPH at 37 °C (*2*). The analyzer was programmed to record, at the emission wavelength of 520 nm and excitation wavelength of 485 nm, the fluorescence 35 times during the 120 min analysis. ORAC results were calculated on the basis of Trolox standard curve obtained and are reported as micromoles of Trolox equivalents (TE) per gram of freeze-dried samples (*23*).

Statistical Analyses. Data obtained during treatments were subjected to multivariate analysis of variance using JMP IN software (version 5.1, SAS Institute Inc., Cary, NC). A Tukey test was also performed on data using Sigma Stat software (version 2.03, SPSS Inc., Chicago, IL).

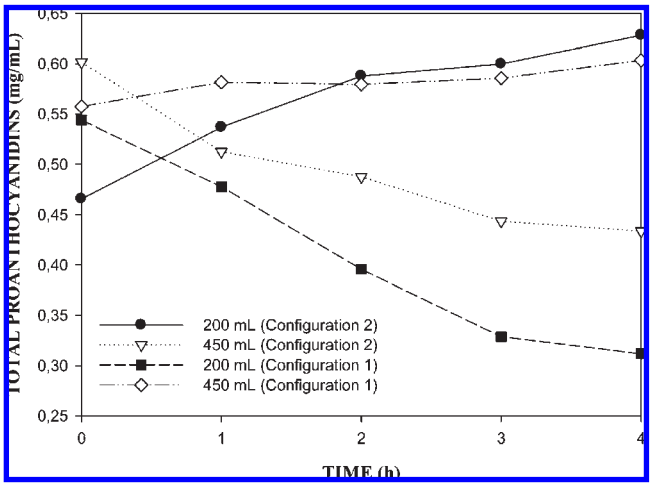

Figure 2. Evolution of total proanthocyanidin content of cranberry juices as a function of time during EDUF treatments with configurations 1 and 2.

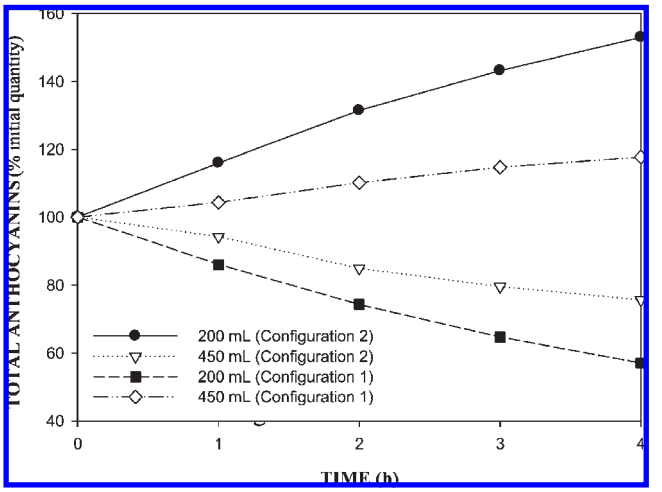

Figure 3. Evolution of total anthocyanin content of cranberry juices as a function of time during EDUF treatments with configurations 1 and 2.

RESULTS AND DISCUSSION

Proanthocyanidin Content. Statistical analysis of proanthocyanidin data revealed that the processing time ($P = 0.0003$) and the juice volume ($P = 0.0416$) as well as the dual interaction time/configuration ($P = 0.0008$) and time/juice volume ($P < 0.0001$) had significant effects on the electromigration rates of proanthocyanidins from cranberry juices.

When configuration 1 was used (**Figure 2**), the total proanthocyanidin content of the 450 mL juice compartment increased from 0.557 to 0.603 mg of catechin equiv/mL, which for an initial juice volume of 450 mL corresponds to a total increase of 20.7 mg of catechin equiv in that compartment. A decrease of the total proanthocyanidin content from 0.544 to 0.321 mg of catechin equiv/mL was measured for the 200 mL juice compartment. This corresponds, for an initial juice volume of 200 mL, to a total decrease of 46.4 mg of catechin equiv in that compartment. However, when the EDUF treatments were carried out with configuration 2, the total proanthocyanidin content of the 450 mL juice compartment decreased from 0.601 to 0.434 mg of catechin equiv/mL, whereas the total proanthocyanidin contents in the 200 mL juice increased from 0.466 to 0.628 mg of catechin equiv/mL. Therefore, a total increase of 32.4 mg of catechin equiv and a total decrease of 75.15 mg of catechin equiv in the proanthocyanidin contents occurred, respectively, in the 200 and 450 mL cranberry juice compartments when configuration 2 was used.

Results obtained showed that the proanthocyanidins always migrated in the cathode direction. These molecules are then positively charged at the pH of cranberry juice. Moreover, the total increase in the proanthocyanidin content was, for the same process duration and the same surface of the membrane in contact with the juice, more important when configuration 2 was used for the EDUF treatments. This could be explained by the cell configuration. In the two system configurations used, the filtrating layer of the UF membrane was always on the side of the 450 mL juice compartment. As the proanthocyanidin migration always migrated in the cathode direction, the migration should have been facilitated in configuration 2.

Only a limited part (about 44%) of the total proanthocyanidins content that had left the feed juice was recovered in the enriched juice after EDUF of cranberry juices with the two configurations. This could be caused by a fouling of the UF membrane or by an accumulation of proanthocyanidin molecules inside the UF membrane. At the end of the 4 h treatments, some proanthocyanidin molecules might not have entirely crossed through the UF membrane and stayed in the membrane because the electric field application was stopped.

Total Anthocyanin Content. The multivariate analysis of variance of the data showed that the configuration ($P < 0.0001$), the juice volume ($P < 0.0001$), the dual interactions configuration/juice volume ($P = 0.0114$), time/configuration ($P < 0.0001$), and time/juice volume ($P < 0.0001$), and the triple interactions time/configuration/juice volume ($P = 0.0020$) had significant effects on the evolution of the total anthocyanin contents in cranberry juices during EDUF treatments. Moreover, results of the multivariate analysis of variance performed on individual anthocyanin compound evolution during the EDUF treatments were similar to those obtained for the data of total anthocyanin contents except that the triple interaction time/configuration/juice volume had not a significant effect ($P = 0.051$) on the evolution of the concentration of the anthocyanin cyanidin-3-glucoside.

As previously observed for the total proanthocyanidin contents of the cranberry juices, the anthocyanins always migrated in the cathode direction. These molecules are then also positively charged at the pH of cranberry juice. Moreover, when configuration 1 was used during EDUF treatments, the total anthocyanin contents increased by 17.7% in the 450 mL juice compartment (**Figure 3**) and decreased by 42.9% in the 200 mL cranberry juice compartment. However, in the EDUF system with configuration 2, an increase of 52.9% of the total anthocyanin content was observed in the 200 mL juice and the concentration dropped by 24.4% in the 450 mL juice. The increase of the total anthocyanidin contents in cranberry juice was more important when configuration 2 was used for the EDUF treatments for the same process duration and the same surface of membrane in contact with the juices. This could be explained in the same way as for the total proanthocyanidin contents in treated juices. In the two system configurations used, the filtrating layer of the UF membrane was always on the side of the 450 mL juice compartment. The anthocyanin migration should then have been facilitated in configuration 2. The higher volume in this configuration could also explain this observation.

The individual anthocyanins detected in the cranberry juice were cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside, peonidin-3-galactoside, peonidin-3-glucoside, and peonidin-3-arabinoside (**Table 1**). These anthocyanins are the same as those identified in cranberry fruits by Vvedenskaya and Vorsa (*24*). The evolution of individual anthocyanin molecules in

Table 1. Total Anthocyanin Content (in Percent of Initial Concentration) of Cranberry Juices Treated by EDUF[a]

configuration	juice	cyd-3-gal	cyd-3-glu	cyd-3-arab	pnd-3-gal	pnd-3-glu	pnd-3-arab
1	450 mL	117.16 ± 2.93	122.16 ± 10.88	116.78 ± 3.15	117.07 ± 2.84	117.02 ± 2.96	116.13 ± 2.42
	200 mL	56.29 ± 3.18	59.57 ± 7.02	55.44 ± 3.22	57.26 ± 3.05	57.70 ± 3.42	56.28 ± 3.09
2	200 mL	150.55 ± 3.44	167.84 ± 24.14	150.51 ± 3.81	149.71 ± 3.21	149.32 ± 3.16	149.47 ± 3.30
	450 mL	75.44 ± 2.22	75.40 ± 5.80	74.62 ± 2.22	76.03 ± 2.31	76.99 ± 1.93	75.43 ± 2.38

[a] cyd-3-gal, cyanidin-3-galactoside; cyd-3-glu, cyanidin-3-glucoside; cyd-3-arab, cyanidin-3-arabinoside; pnd-3-gal, peonidin-3-galactoside; pnd-3-glu, peonidin-3-glucoside; pnd-3-arab, peonidin-3-arabinoside.

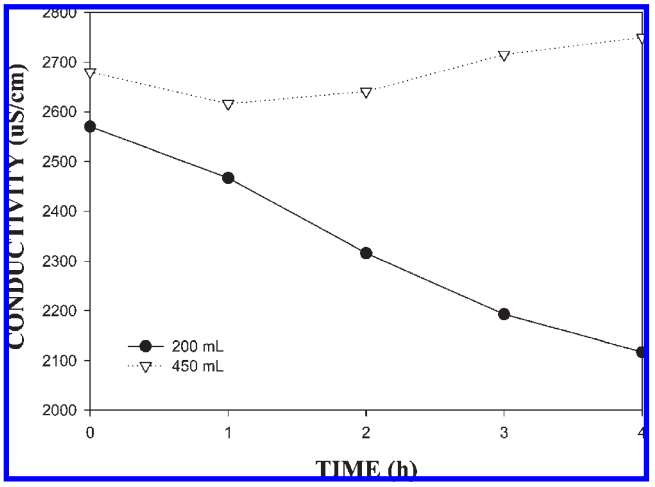

Figure 4. Evolution of juice conductivity as a function of time during EDUF treatments with configuration 2.

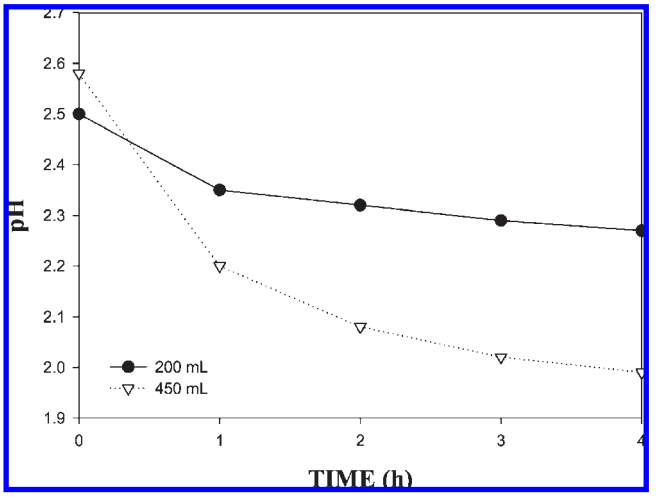

Figure 5. Evolution of juice pH as a function of time during EDUF treatments with configuration 2.

cranberry juices during EDUF treatments was similar to the evolution of the total anthocyanin content in the treated juices (results not shown).

Because proanthocyanidins and anthocyanins were positively charged at cranberry juice pH and the migration of these compounds was more important in EDUF system with configuration 2, only the results concerning electrodialytic parameters and juice characteristics obtained during EDUF treatments with configuration 2, which was more efficient, will be presented hereafter.

Conductivity. The multivariate analysis of variance of the data showed that time ($P < 0.0001$) had a significant effect on conductivity during EDUF.

The averaged initial electrical conductivity of the cranberry juice was 2625 μS/cm. As presented in **Figure 4**, the conductivity of the 450 mL juice increased from 2655 to 2749 μS/cm, whereas the conductivity of the 200 mL juice dropped over time from 2568 to 2117 μS/cm during treatments. This decrease in conductivity corresponded to a 17.6% demineralization of the 200 mL juice. According to Perez et al. (*25*) and Hiroaka et al. (*26*), the conductivity of a solution is a suitable criterion for following a demineralization process.

Variations in conductivity data of treated cranberry juices are consistent with the cell configuration (**Figure 1b**). In the 200 mL juice in contact with the cationic membrane, K^+ ions migrated through this membrane during the EDUF treatments. At the same time, proanthocyanidins, anthocyanins, and K^+ ions from the 450 mL juice compartment migrated to the 200 mL juice compartment and the anions (Cl^-) in the 200 mL juice would migrate through the ultrafiltration membrane toward the 450 mL juice. As the mobility of the proanthocyanidin and anthocyanin molecules is supposed to be much lower than that of the K^+ (67×10^{-5} cm^2/s·V (*19*, *20*)) and that of Cl^- (68×10^{-5} cm^2/s·V (*27*, *28*)) ions, the conductivity of the 200 mL juice decreased during the treatments. This led to a demineralization of the 200 mL cranberry juice. Moreover, at the beginning of treatments, the arrival of each anion in the compartment must be compensated by the loss of another ion with the same charge or by the arrival of cation to maintain electroneutrality during EDUF (*29*). This phenomenon may explain, in part, the relatively constant conductivity in the 450 mL juice compartment in contact with the anionic membrane during the first 2 h of the treatments. Such a phenomenom of cation/anion balance migration was observed previously during EDUF (*11*, *14*, *29*). However, the quantity of ions available for the migration was less important in the 200 mL juice compartment than in the 450 mL juice compartment, and the drop in conductivity in the 200 mL juice probably resulted in water dissociation at the surfaces of the anionic and cationic membranes. In addition, because water dissociation is usually higher for anion-exchange membranes than for cation-exchange membranes (*30*, *31*), this would explain why the 450 mL juice became acidified. More H_3O^+ was probably produced in the 450 mL juice compartment at the anionic membrane interface than OH^- in the 200 mL juice compartment at the cationic membrane interface. The protons generated at the anionic membrane interface, which have the highest electrical mobility (325×10^{-5} cm^2/s·V (*27*, *28*)) may have contributed to the increase in conductivity of the 450 mL juice observed after 2 h of treatment.

pH. The multivariate analysis of variance of the data showed that time ($P < 0.0001$) and the dual interaction time/juice volume ($P = 0.0002$) had significant effects on the evolution of pH of juices during EDUF treatments.

The mean initial pH value of the cranberry juices was 2.54. As can be seen in **Figure 5**, the pH of the 450 and 200 mL cranberry juices dropped 0.59 and 0.23 pH units, respectively, during treatments. Therefore, the pH of the two juices decreased during treatments, but the drop in pH was more important in the 450 mL juice compartment. As previously mentioned, water dissociation

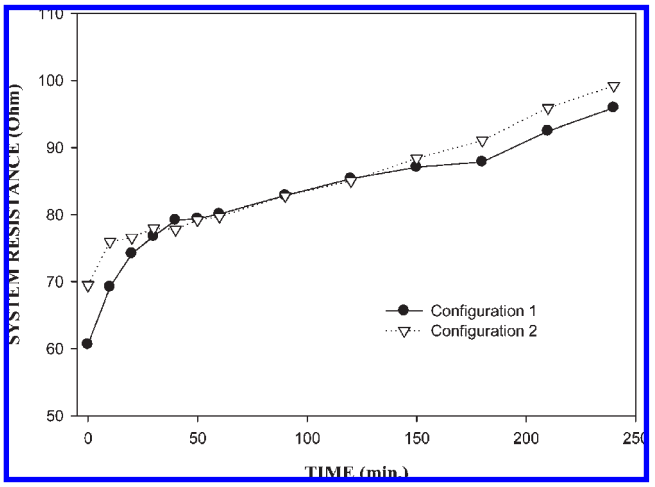

Figure 6. Evolution of system resistance as a function of time during EDUF treatments with configurations 1 and 2 of cranberry juices.

Table 2. Titrable Acidity of Control and Treated Cranberry Juices with an EDUF Process (Configuration 2)

juice	titrable acidity[a] (mL of NaOH)
control	6.98 ± 0.03 a
200 mL	5.40 ± 0.51 b
450 mL	6.87 ± 0.31 a

[a] Means that are followed by the same letter are not significantly different ($P > 0.05$).

should have occurred at the anionic membrane surface. In this situation, H^+ and OH^- would have been generated at the anionic membrane interface and the amount of H^+ in the 450 mL juice compartment should have increased; this would have resulted in the important decrease in pH measured in this compartment. The H^+ electrogenerated at the interface of the anionic membrane was used to acidify the 450 mL juice solution, whereas K^+ ions, present as intrinsic ionic species in cranberry juice, would migrate through the ultrafiltration membrane toward the cathode to the 200 mL juice compartment. K^+ ions participated in the compensation of electroneutrality in response to proanthocyanidin and anthocyanin migration as well as H^+ electrogeneration. When almost all of the K^+ ions from the 450 mL juice have migrated, the migration of the H^+ ions to the 200 mL juice happens, and this results in a decrease of the pH within this compartment. The pH of the 200 mL cranberry juice compartment decreased during the first 90 min of EDUF but stabilized thereafter. The equilibrium between protons entering and leaving this compartment explains the resulting stabilization of the pH as already observed when there was a lack of intrinsic cations during EDUF (*29*) and bipolar membrane electrodialysis (*32*−*34*).

Global System Resistance. The multivariate analysis of variance of the data showed that time ($P < 0.0001$) had a significant effect on the evolution of the global system resistance during EDUF treatment.

The global system resistance increased from 69.5 ± 5.0 to 99.2 ± 7.0 Ω in the ED cell with configuration 2. The increase in the global system resistance was important during the first 10 min of the treatments (**Figure 6**) and was slower thereafter. These results confirm the hypothesis mentioned earlier that water dissociation might have occurred at membrane interfaces. Water dissociation might result in a slower increase in the system global resistance after the first 10 min of the process, due to the high electrical conductivity of H^+ ions.

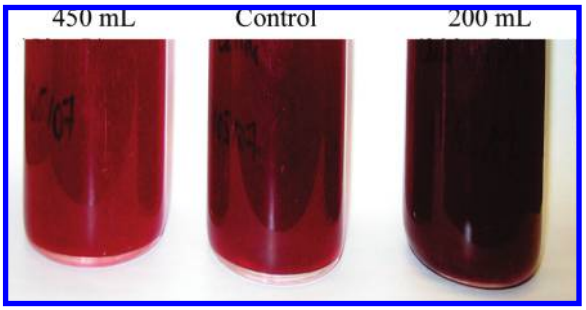

Figure 7. Photographs of cranberry juices before (control) and after EDUF treatments.

Table 3. Color Parameters Measured for Control and Treated Cranberry Juices with EDUF System (Configuration 2)[a]

juice	*L*	*a*	*b*
control	23.21 ± 0.05 a	2.00 ± 0.15 a	0.22 ± 0.14 a
200 mL	23.22 ± 0.05 a	1.74 ± 0.07 a	0.03 ± 0.13 a
450 mL	23.33 ± 0.22 a	2.81 ± 0.28 b	0.28 ± 0.11 a

[a] For each column, means that are followed by the same letter are not significantly different ($P > 0.05$).

Titrable Acidity. The multivariate analysis of variance showed that the titrable acidity values of all samples were significantly different ($P < 0.0001$).

Titrable acidity values of all treated juices decreased during the EDUF treatments (**Table 2**) in comparison with the control, but the drop in titrable acidity of the 200 mL juice was more important than in the 450 mL juice compartment. The decrease in titrable acidity of the two treated juices might be explained by the cell configuration. The dissociated organic acids ($R-COO^-$) in the 200 mL cranberry juice compartment should have migrated in the anode direction through the UF membrane to reach the 450 mL juice compartment. This could explain, in part, the important decrease in the titrable acidity of the 200 mL juice. However, to maintain the electroneutrality of the 450 mL juice, for each negatively charged organic acid received from the 200 mL juice compartment, one anion and/or organic acid would have migrated to the electrolyte. Then the titrable acidity value for the 450 mL juice remained similar that for the control.

Cranberry Juice Color. The multivariate analysis of variance of colorimetric data showed that samples of treated juices were significantly different for the *a* ($P < 0.0001$) parameter values and not significantly differents for the *L* ($P > 0.530$) and *b* parameters ($P > 0.114$).

As can be seen in **Figure 7**, the 200 mL enriched juice seems to be darker than the control and the 450 mL juice. On the contrary, the 450 mL juice, with a lower concentration in proanthocyanidins and anthocyanins, appears lighter than the other juices. This might be explained by the migration, during the EDUF treatments, of the anthocyanins in the cranberry juice from the 450 mL juice compartment to the 200 mL juice compartment. Four major anthocyanin pigments are responsible for the red color of the cranberry, cyanidin galactoside, peonidin galactoside, cyanidin arabinoside, and peonidin arabinoside (*35*). However, luminescence values of the control and treated juices were very similar, and the *a* color parameter value for the 450 mL juice was significantly higher than for the control and the 200 mL juices (**Table 3**). The dark color of the 200 mL juice after treatments might have interfered with the measurement of the *a* color parameter of that juice.

To evaluate in a preliminary way the effect of the EDUF treatments on the organoleptic properties of cranberry juice, the

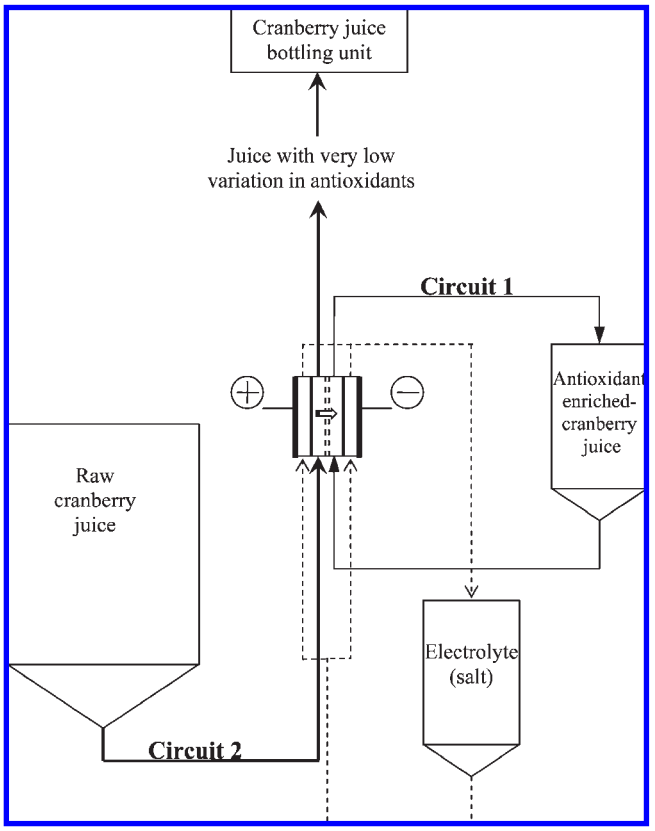

Figure 8. Schematic flow diagram for the production of antioxidant-enriched cranberry juices.

Table 4. Antioxidant Capacity (in Micromolar Trolox Equivalents per Gram of Freeze-Dried Juice Samples) of Control and Treated Juices with EDUF System (Configuration 2)[a]

juice	0 h	4 h
control	180.53 ± 8.95 a	
200 mL	212.09 ± 47.08 a	250.71 ± 37.97 b
450 mL	256.01 ± 21.48 a	238.41 ± 31.80 a

[a] For each row, means that are followed by the same letter are not significantly different from the control ($P > 0.05$).

taste of treated cranberry juices was compared with the taste of the control juice, by five untrained people. The 450 mL juice, characterized by a reduced concentration in proanthocyanidins and anthocyanins, was bitter and had a bad taste, different from the control juice. On the contrary, the taste of the 200 mL juice, characterized by a higher concentration in proanthocyanidins and anthocyanins, tasted better than the control juice. The good taste of the enriched juice was surprising because proanthocyanidin are very astringent (*36*, *37*). However, in addition to polyphenols, the major organic acids in cranberry juice (malic, citric, and quinic acid) are also expected to contribute to astringency. The addition of acid to wines or tannic acid solutions has been shown to increase astringency (*38*−*40*). Because the titratable acidity of the 200 mL juice decreased during the EDUF treatments due to a migration of organic acids from that compartment to the 450 mL one, the astringency of the 200 mL could also be less important after treatments.

Antioxidant Capacity. The multivariate analysis of variance of the data showed that time ($P < 0.003$) had a significant effect on the antioxidant capacity of the juices treated by EDUF.

The antioxidant capacity of the control juice was 180.53 μM Trolox equiv (TE)/g of freeze-dried juice samples (**Table 4**). The antioxidant capacity of the 450 mL juice decreased slightly (7% drop) during the treatments. However, an 18% increase of the antioxidant capacity of the 200 mL juice was obtained by the EDUF treatments of cranberry juices. This result suggests that phenolic antioxidants (proanthocyanidins and anthocyanins) migrated from the 450 mL juice to the 200 mL juice during EDUF, and that would have had an effect on the antioxidant capacity of the 200 mL juice. Earlier studies also found a positive correlation between ORAC antioxidant capacity of berries and their total phenolic content (*41*−*43*).

Conclusion. Results obtained in this study confirm that proanthocyanidin and anthocyanin molecules are positively charged, at cranberry juice pH (pH 2.54), because they always migrated in the cathode direction. When configuration 2 of the EDUF system was used, migration yields of proanthocyanidin and anthocyanin molecules were higher. The total concentrations of proanthocyanidins and anthocyanins increased by 34.8% and 52.9%, respectively, in cranberry juice when the EDUF system was used with that configuration. Moreover, an 18% increase of the antioxidant capacity of the 200 mL enriched cranberry juice was obtained by the EDUF treatments. Then, the EDUF process might be used for natural enrichment of cranberry juice with phenolic antioxidant. Moreover, the taste of the enriched cranberry juice seems to be improved in comparison with the nontreated juice. However, this process should be optimized to increase the migration rates of proanthocyanidins and anthocyanins through the UF membrane, and the electromigration time must be increased to allow the complete migration of these compounds.

In all previous studies on EDUF, the molecules of interest were recovered in a KCl solution to obtain an enriched permeate that could be used to fortify other products. However, in this study, EDUF has been used to concentrate a cranberry juice in its own specific compounds. The production of phenolic antioxidant enriched cranberry juice could be feasible at a large scale with no production of juice with low content in antioxidants and bad taste. Indeed, as presented in **Figure 8**, the EDUF process could be directly integrated to the bottling process of cranberry juice to produce antioxidant-enriched cranberry juices in batch process (circuit 1) and cranberry juice with very low variation in antioxidant content in a continuous process. In fact, the residence time of raw cranberry juice in the anode side of the ultrafiltration compartment of the EDUF cell allows the migration of a restricted quantity of proanthocyanidin and anthocyanin. In addition, due to the volume of raw cranberry juice treated, the increase in these phenolics antioxidants of juice recirculating in circuit 1 would effectively produce an antioxidant-enriched cranberry juice.

This technology is a sustainable and environnementally friendly technology and appears to be a one-step process. Another advantage of this technology is that compounds of interest would be directly transferred from one juice to another without the need for previous solvent extraction, and without sugar concentration or migration.

ACKNOWLEDGMENT

We give special thanks to Monica Araya-Farias for technical support. We also thank Pascal Dubé for technical help with HPLC and ORAC sample analyses.

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Received June 22, 2009. Revised manuscript received September 17, 2009. Accepted September 28, 2009. The financial support of the Natural Sciences and Engineering Research Council of Canada (NSERC) is acknowledged.