Acceleration of pH Variation in Cloudy Apple Juice Using Electrodialysis with Bipolar Membranes

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The purpose of this study was to accelerate pH variation in cloudy apple juice using electrodialysis (ED). The testing was conducted using two ED configurations. The bipolar and cationic membrane configuration showed that reducing the spacing from 8 to 0.75 mm had little effect on treatment time, whereas stacking eight bipolar membranes reduced acidification time by 30%, although the treatment still took too long (21 min). Furthermore, it was not possible to acidify apple juice to a pH of 2.0 to completely inhibit enzymatic browning. The bipolar and anionic membrane configuration helped to accelerate the acidification step by a factor of 3, increasing the yield from 3.3 to 10 L of juice/m^2 membrane/min. Moreover, treatment time was inversely proportional to the size of the membrane stack. The speed at which the pH of acidified juice returned to its initial value was, however, 4 times slower than the speed of acidification, giving a yield of 2.5 L of juice/m^2 membrane/min. By accelerating the acidification step, ED treatment with bipolar and anionic membranes results in more effective polyphenol oxidase activity and more rapid control of juice browning at pH 2.0. Also, the treatment has very little effect on the chemical composition and organoleptic quality of apple juice.

Keywords: *Cloudy apple juice; browning; electrodialysis; bipolar membrane; acidification*

INTRODUCTION

Demand for cloudy or unclarified apple juice is on the rise (Hervé, 1997). It contains significant quantities of suspended pulp and is perceived as a natural food product that supplies dietary fiber and important nutrients. However, it is very difficult to produce superior quality juice (Lea, 1990). Cloudy apple juice is very sensitive to enzymatic browning (EB) because it contains considerable quantities of polyphenols and polyphenol oxidases (PPO). Enzymatic browning reactions are catalyzed by PPO and result from the oxidation of phenolic compounds to *o*-quinones, which then polymerize to form complex dark pigments, thereby changing the color and aroma of the juice (Macheix et al., 1990).

Temporarily lowering the pH of apple juice to 2.0 and then adjusting it back to its initial value will irreversibly inhibit PPO activity and stabilize juice color. Zemel et al. (1990) added hydrochloric acid concentrate and caustic soda to the juice to adjust its pH; however, the treatment resulted in the formation of salts, which affect the flavor of the juice. In recent studies (Tronc et al., 1997, 1998), we demonstrated the feasibility of acidifying cloudy apple juice and returning the pH to its initial value without altering flavor by using electrodialysis (ED) with bipolar and cationic membranes. However, the process was too lengthy, ~90 min, and required the addition of exogenous KCl to the juice to reach pH 2.0. Furthermore, the voltage applied greatly exceeded the 2 V/membrane average and was not compatible with industrial constraints. Energy consumption was also very high.

The goal of the present study was to accelerate the acidification of cloudy apple juice by ED using bipolar membranes, because this step is critical to the rapid control of enzymatic browning following the extraction stage. The acidified juice is then returned to its initial pH using the same treatment.

MATERIALS AND METHODS

Apple Juice. Juice was extracted from MacIntosh apples that had been stored commercially under controlled atmosphere. The apples were crushed and pressed in a crusher-press model EG 260-X6 (Goodnature Products Inc., Buffalo, NY) under maximum pressure of 1500 psi. About 2 L of juice was extracted from 4 kg of apples for each experiment. The extracted juice underwent ED treatment immediately.

Electrodialysis. In the first experiment, we studied the impact of varying compartment spacing and the membrane stack on the speed of juice acidification.

To study the spacing effect, we employed 2 ED units: the Electrocell AB unit (Electrocell), with 8 mm spacing, as used in previous experiments (Tronc et al., 1997, 1998); and the ED-1-BP unit (Electrosynthesis Co.), with spacings of 1.5 and 0.75 mm. The use of the two different pieces of equipment was unavoidable because, to our knowledge, no laboratory-scale ED units exist for such an extensive range of spacing. The two ED units have the same electrode surface of 100 cm^2. Each ED unit contains eight compartments: four for the juice and four for the electrolyte separated by alternating bipolar and cationic membranes.

To evaluate the effect of the bipolar and cationic membrane stacks on acidification speed, we used four and eight bipolar membranes. In this experiment, the number of compartments varied according to the number of membranes employed. The experiments were done in the ED-1-BP unit with 0.75 mm spacing. In both studies (spacing and stacking), we used the bipolar and cationic membrane configuration (Figure 1A) and the corresponding solutions.

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10.1021/jf991233g CCC: $19.00
Published on Web 05/13/2000

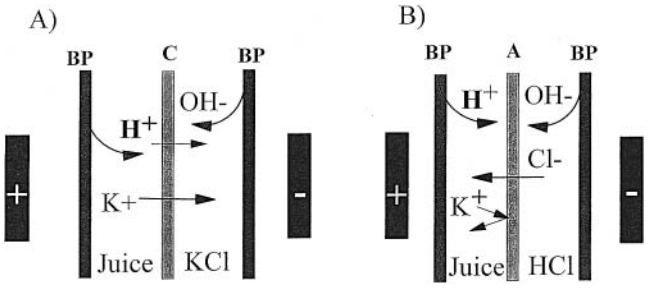

Figure 1. Configuration of ED unit used to modify pH of cloudy apple juice: (A) configuration with bipolar and cationic membranes; (B) configuration with bipolar and anionic membranes. BP, bipolar membrane; C, cationic membrane; A, anionic membrane; 0.25 M KCl; 0.1 M HCl.

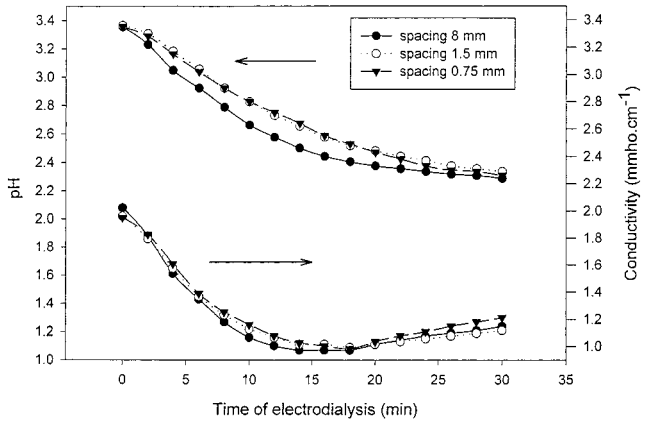

Figure 2. Change in pH and conductivity of apple juice during acidification with different spacings. Conditions: configuration with bipolar and cationic membranes, four bipolar membranes, four cationic membranes, 20 mA/cm^2.

In the second experiment, we used a new configuration with bipolar and anionic membranes and the corresponding solutions (Figure 1B). The testing was done with the ED-1-BP unit, equipped with 0.75 mm spacing and 4, 6, 8, and 10 bipolar membrane stacks. In this configuration, we replaced the KCl solution with a 0.1 N HCl solution.

The bipolar membranes (Neosepta BP-1 model, Tokuyama Soda, Japan) and cationic membranes (Neosepta CMX model, Tokuyama Soda) or anionic membranes (Neosepta AMX model, Tokuyama Soda) each had an effective surface area of 100 cm^2. During treatment, juice temperature was maintained at 25 °C using a Haake G refrigerated bath (Haake, Berlin, Germany). The acidification of the juice by ED was conducted at a constant current density of 20 mA/cm^2 or 40 mA/cm^2, respectively, using a 6024 A DC generator (Hewlett-Packard, Vancouver, BC, Canada). The current density was selected in terms of the applied voltage, which must not exceed 2 V/compartment, according to the manufacturer's instructions in the membrane user guide (Tokuyama Soda Co., Ltd., 1993).

The juice acidified with the bipolar and anionic membrane configuration with 10 bipolar membranes was subsequently stored at room temperature for 1 h to deactivate PPO activity before the pH was returned to its initial value. The ED configuration for raising the pH value was the same as that shown in Figure 2B with a stack of 10 bipolar membranes. However, the juice and HCl compartments were reversed.

During the acidification and pH adjustments, conductivity and juice pH were measured at 1−2 min intervals until the end of the treatments, along with applied voltage. After the treatments, the control juice (prepared prior to the treatment and not treated by ED), the acidified juice (pH 2.00), and the adjusted juice (pH 2.0 with adjustment to pH 3.35) were stored in 300 mL bottles at ambient temperature for 2 h to monitor enzymatic browning and PPO activity. The bottles were left uncovered and exposed to oxygen to encourage EB reactions. The juice samples were frozen to −30 °C immediately following treatment for composition analyses.

Physicochemical Parameters during ED. The juice pH was measured with a Corning 240 pH-meter (Corning Co., Halstead Essex, U.K.). Juice conductivity was determined with a model 35YSI conductivity meter (Yellow Springs Instrument Co., Yellow Springs, OH). Applied voltage was measured with a Micronta 22-185A multimeter (Radio-Shack, Barry, Canada). Specific energy consumption per ED unit was calculated with the equation

$$E = I\int_{t_0}^{t_1}\bar{U}\,\mathrm{d}t/3.6 \times 10^6$$

where E is energy (kW·h); U is average applied voltage (V); I is current intensity (A); and t is treatment time (s).

PPO Activity. PPO activity was assayed following the method developed by Traverso-Rueda and Singleton (1973). Juice (0.1 mL) was added to a mixture containing 1.9 mL of phosphate buffer at pH 6.5 and 1 mL of 0.05 M catechin. Absorbance was measured at 420 nm every 30 s for 2.5 min with a Beckman DU-640 spectrophotometer (Beckman Instruments Inc., Fullerton, CA). PPO activity was expressed in terms of the difference between the initial and maximum absorbance values. The values for PPO activity were reported as a percentage of PPO activity in the control juice immediately after extraction.

Enzymatic Browning. Juice browning was determined with a Labscan Tristimulus colorimeter using Hunter *L*, *a*, and *b* values (Hunter Associate Laboratory Inc., Reston, VA). The results were reported as *L* (luminescence or lightness) and *a* (intensity of brown color varying from green to red) values. Sapers and Douglas (1987) reported that these parameters provide a good indication of browning reactions, showing an increase in *a* and a decrease in *L* during juice browning.

Composition Analyses. Analyses of organic acids and sugars were conducted using HPLC, in accordance with the method established by Doyon et al. (1991). Juice samples (10 mL) were diluted 10-fold, processed in a Sep-Pak C18 cartridge, and filtered through 0.45 μm Millipore filters prior to injection. The organic acid and sugar concentrations were determined on a Waters 510 HPLC (Waters Canada, Mississauga, ON, Canada) equipped with a Waters ion-exchange column (300 × 7.8 mm) and two detectors: a Waters M-590 ultraviolet detector (210 nm) for organic acids and a Waters R-410 refraction index detector for sugars. The injection of 15 μL samples was done with a WISP-710 automatic injector. Optimal separation of juice compounds was achieved with a mobile phase of 0.0049 N sulfuric acid at a flow rate of 0.4 mL/min. Samples were compared to sucrose, glucose, fructose, malic acid, citric acid, and lactic acid standards.

The ash content of the apple juice was determined using a 20 mL juice sample heated to 600 °C for 8 h (AOAC, 1985). Cations were measured in the previously obtained ash samples diluted in 20 mL of 2 N HCl. Assays were carried out by atomic absorption using a SpectrAA-100 flame spectrometer (Varian Australia Pty Ltd., Australia) at 766.5 nm for potassium, 285.2 nm for magnesium, and 317.9 nm for calcium (Varian Australia Pty Ltd., 1989).

Chloride content was determined indirectly by measuring excess Ag concentration after precipitation of silver chloride in a mixture of 10 mL of juice and 10 mL of a 0.1 N silver nitrate solution. After centrifugation, the Ag^+ assays were conducted at 328.1 nm with the same flame spectrometer referred to above.

Phenolic compounds were analyzed by HPLC according to the method devised by Suarez-Valles et al. (1994). The separation of phenolic compounds was achieved using a Waters 510 unit equipped with a Nova-Pak C18 column (300 × 3.9, 4 μm) and a Waters M-590 ultraviolet detector at 280 nm, at ambient temperature. Twenty microliters of juice was filtered through a 0.2 μm Millipore filter prior to injection. The elution consisted of a mixture of water acidified to 2.80 pH with phosphoric acid (solvent A) and methanol (solvent B). The

solvent gradient was as follows:

time (min)	flow (mL/min)	B (%)
0	0.6	2
50	0.6	42
60	0.4	50
75	0.4	50
77	0.6	2

Samples were compared to chlorogenic acid, (−) epicatechin, and phloridzin standards.

The concentration of anthocyanins was determined according to the Wrolstad (1976) method. One milliliter of juice was added to 4 mL of buffer solution at pH 1.0 and 4.5. Turbidity (haze) can be corrected for by measuring the absorbance at 700 nm and subtracting this from the absorbance at the wavelength of maximum absorption (e.g., 510 nm). The optical density of the mixtures at 510 and 700 nm was measured using a Beckman DU-640 spectrophotometer (Beckman Instruments Inc.). The concentration of anthocyanins was determined using the following equation

$$\text{anthocyanin (mg/L)} = A \times 445.2 \times 10d/30.2$$

where $A = (A_1 - A_2) - (A_3 - A_4)$ is the difference in absorbance between pH 1.0 and 4.5 (A_1 is the absorbance at 510 nm and pH 1.0, A_2 is the absorbance at 700 nm and pH 1.0, A_3 is the absorbance at 510 nm and pH 4.5, and A_4 is the absorbance at 700 nm and pH 4.5); 445.2 is the molecular weight of cyanidin 3-galactoside, the main anthocyanin in apples; 30.2 is the molar absorbance of cyanidin 3-galactoside; and d is the dilution coefficient

Organoleptic Tests. The flavor of the ED-treated juice was evaluated by a panel of five industrial tasters from Lassonde Inc. (Rougement, Quebec, Canada). A comparative test was conducted on the control juice and the adjusted juice.

Statistical Analyses. A completely random experimental design was applied. The repeated-measure analysis was performed by ANOVA using SAS software. Orthogonal contrasts were used to separate the effects of the different treatments. The experiments were repeated three times.

RESULTS AND DISCUSSION

Bipolar and Cationic Membrane Configuration. In past research (Tronc et al., 1997, 1998), apple juice was treated in an ED unit with 8 mm spacing and a single bipolar membrane. Acidifying the juice took 30 min, and a pH of 2.0 could be achieved only by adding exogenous KCl to maintain the K^+ concentration in the juice at its initial level. To acidify the juice to pH 2.0 without the addition of KCl and in <30 min, we first reduced the spacing between membranes from 1.5 to 0.75 mm to accelerate the movement of ions through the membranes. A reduction in the spacing alone would not be sufficient to optimize ED treatment (Audinos and Vigneswaran, 1988; Brun, 1989), so we also studied the effect of adding a number of bipolar membranes to increase the source of H_3O^+ ions introduced into the juice.

Figure 2 illustrates the effect of spacing on changes in pH and juice conductivity during acidification. Spacing was observed to have little impact on acidification time. After 30 min of treatment, the juice pH stabilized at 2.25 regardless of the spacing. Treatment time and pH value remained higher than the target levels. At the start of the treatment, the drop in pH was slower with a decrease in spacing. These observations indicate that the reduced spacing does not promote the retention of H_3O^+ ions in the juice. Bear in mind that the principle of this configuration is based on the replacement of K^+ by H_3O^+ in the juice. Because H_3O^+ ions are more mobile than K^+ ions (their mobility is equal to 36.25×10^{-4} cm^2 s^{-1} V^{-1} compared with 7.62×10^{-4} cm^2 s^{-1} V^{-1} for K^+) (Atkins, 1978), they tend to migrate through the juice and pass through the cationic membrane more rapidly, owing to the narrower spacing.

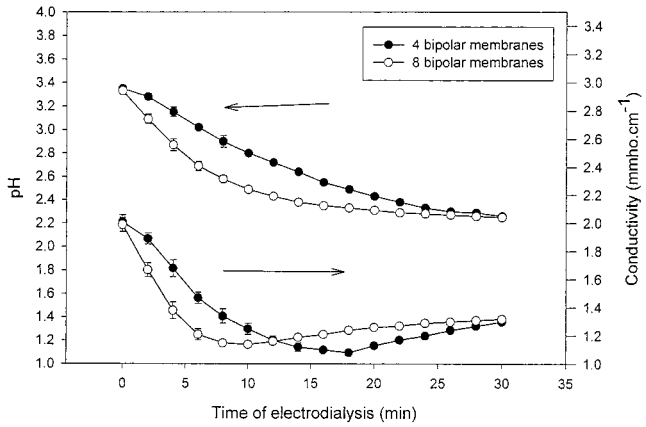

Figure 3. Change in pH and conductivity of apple juice during acidification with a varying number of bipolar membranes. Conditions: configuration with bipolar and cationic membranes, 0.75 mm spacing, 20 mA/cm^2. Vertical bars represent the standard deviations of the means ($N = 3$).

A drop in conductivity in the apple juice was also observed during acidification regardless of the spacing used. It should, however, be noted that apple juice has a much lower conductivity than the electrolytes; consequently, the variations observed in conductivity are also low. The decrease in conductivity results from the depletion of potassium in the juice and the low retention of H_3O^+ ions (Tronc et al., 1997). After 10 min of acidification, the K^+ concentration drops rapidly from 760 to 83 mg/L and then stabilizes until the end of the treatment. Although H_3O^+ ions are better conductors than K^+ ions, most will combine with malic ions in the juice to form malic acid, which breaks down slowly ($K_{a1} = 3.98 \times 10^{-4}$ and $K_{a2} = 7.76 \times 10^{-6}$), compared with salts of malate of potassium, which break down almost completely. This slow breakdown of malic acid results in the observed decrease in conductivity. After this decrease, a slight increase in conductivity was observed, corresponding to a slow drop in juice pH. An analysis of ionic species in the juice showed that the Cl^- concentration increased from 0.7 to 30 mg/L during the ED acidification process. The Cl^- in the juice was introduced from the electrolyte compartment (KCl) and is apparently caused by an imperfection in the selectivity of cationic membranes, which are never completely impermeable to co-ions such as Cl^- (Brun, 1989). Part of the electrical current is generated by the movement of a certain percentage of Cl^- through the cationic membranes (Audinos and Vigneswaran, 1988; Brun, 1989). This small quantity of accumulated Cl^- increases the retention of H_3O^+ in the juice, by forming hydrochloric acid, which increases conductivity and slightly decreases juice pH after 15 min of treatment (Figure 3).

The specific energy consumed by the ED unit diminished considerably with the reduction in membrane spacing (Table 1). At pH 2.25, energy consumption was 20 kWh/m^3 of juice for 0.75 spacing, compared with 97 kWh/m^3 for 8 mm spacing. Despite this decrease, energy consumption remained high from an industrial perspective (Mafart and Béliard, 1992). Moreover, at high voltage, the ionic membranes would have a shorter

Table 1. Average Specific Energy Consumption per ED Unit, in Relation to Membrane Spacing and Stacking at a Juice pH of 2.25

treatment			
spacing (mm)	no. of bipolar membranes	av voltage[a] (V/membrane)	energy consumption[b] (kWh/m^3)
8	4	12	97 ± 4
1.5	4	2.5	22 ± 2
0.75	4	1.8	17 ± 2
0.75	8	1.3	20 ± 2

[a] For a current density of 20 mA/cm^2. [b] Data are averages ± standard deviation ($N = 3$).

useful life. A voltage of ≤2 V per compartment would be acceptable for a current density of 20 mA/cm^2 (Brun, 1989). Only the 0.75 mm spacing met this requirement.

Unlike the spacing variable, membrane stacking considerably accelerated the pH variation and the conductivity of apple juice (Figure 3). Doubling the number of membranes from four to eight accelerated acidification by 9 min to a pH of 2.3, and the drop in juice conductivity was also more rapid. The loss of K^+ during the first 10 min of the treatment was twice as fast, yet a pH of 2.0 was not achieved. At pH 2.25, the apple juice would be in equilibrium, such that ion exchanges would be limited to the introduction of H_3O^+ from the bipolar membrane side and their exit through the cationic membrane, thereby maintaining the electrical neutrality of the apple juice. The 2.25 pH limit would therefore be dependent on the type and concentration of the main organic acid in apple juice, that is, malic acid. If we were to assume, under ideal ED conditions, that all of the K^+ ions were replaced with H_3O^+, that no Cl^- ions were introduced into the juice, that the other juice components (sugars, pectin, etc.) had no impact on pH, and that K_{a2} values of malic acid were negligible, the minimum juice pH could be determined using the equation (Masterton and Slowinski, 1973; Usseglio-Tomasset, 1989)

$$K_{a1} = [H^+]^2/(C - [H^+]) \quad \text{and} \quad pH = -\log [H^+]$$

where C is the malic acid concentration (M) and K_{a1} is a constant for breakdown of malic acid. With $C = 0.058$ M and $K_{a1} = 3.98 \times 10^{-4}$, pH_{min} is 2.44. This value is close to the experimental value of 2.25 we achieved in our studies.

In light of these results, it can be concluded that the acidification of apple juice to a pH of 2.0 is not possible under these conditions. Although we were able to shorten treatment time, it was still too long and the final pH was too high to completely inhibit browning.

Bipolar and Anionic Membrane Configuration. Cationic membranes are replaced with anionic membranes, and the KCl solution is replaced with 0.1 M HCl. In the new configuration, acidification is still triggered by the introduction of protons generated by the bipolar membranes, but the retention of these protons is more effective owing to the continuous introduction of Cl^- counterions from the HCl compartment. The Cl^- ions accumulated in the juice will subsequently serve as counterions for the introduction of OH^-, generated by the anionic side of the bipolar membranes, during the return of the juice pH to its initial value. The accumulated Cl^- ions will thus be eliminated from the juice during the acidification period. Experiments were conducted by varying only the number of membranes,

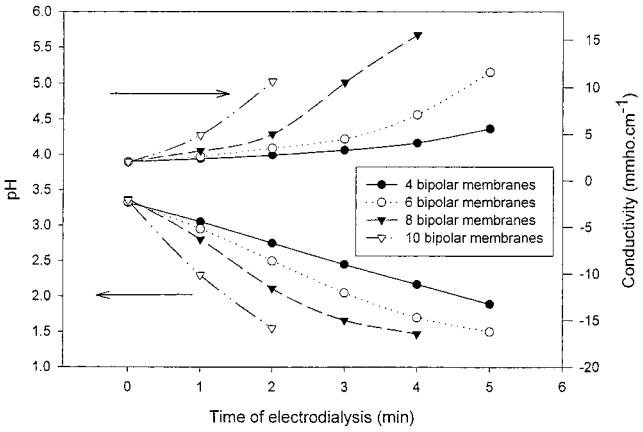

Figure 4. Change in pH and conductivity of apple juice during acidification with a varying number of bipolar membranes. Conditions: configuration with bipolar and anionic membranes, 0.75 mm spacing, 40 mA/cm^2.

Table 2. Specific Energy Consumption and Yield for Different ED Treatments[a]

treatment	energy consumption (kWh/m^3 of juice)	av yield (L of juice/m^2 of membrane/min)	
		acidification	adjusted pH
8 mm separation, addition of KCl[b]	197 ± 5	3.3	2.5
0.75 mm separation, HCl introduced	12 ± 1	10	2.5

[a] Current density of 40 mA/cm^2. [b] Tronc et al. (1998).

at a constant spacing of 0.75 mm, because specific energy is minimal at this spacing (Table 1).

Figure 4 shows the impact of the new configuration on acidification speed. With a four-membrane stack, the juice pH decreased from 3.35 to 2.0 in 4.7 min. When the number of membranes was increased to 10, the required time dropped to ∼1.5 min. Treatment time is therefore inversely proportional to the number of membranes used, which boosts the introduction of Cl^- ions to promote the retention of H_3O^+. The HCl solution also serves to neutralize OH^- produced by the anionic side of the bipolar membranes by hindering their introduction into the juice. Hence, the juice acidification process is more effective and faster than before. This is confirmed by the conductivity results: conductivity is considerably higher because of the accumulation of ions in the juice. At a pH of 2.0, the Cl^- concentration reached 580 mg/L of juice (Table 3). The increase in conductivity improved ED treatment by reducing the applied voltage and energy consumption. The latter was 4−5 kWh/m^3 of juice, which represents very low energy consumption from an industrial standpoint. The yield obtained with configuration B (Figure 1), independent of the number of membranes used, was 3 times higher than that of configuration A (Figure 1) with the addition of KCl (Tronc et al., 1998) (Table 2). Furthermore, when the current density was doubled to 40 mA/cm^2, the average applied voltage did not exceed 2 V/compartment and ranged from 1.5 to 1.8 V.

The time required to return juice pH to its initial value with configuration B, where the juice and HCl compartment were reversd, was longer than the time required to reach pH 2 (Figure 5). The difference is due to the fact that this phase is achieved by replacing the Cl^- accumulated in the juice with OH^-. The low conductivity of Cl^- compared to OH^- (73.5 versus 199 cm^2 Ω^{-1} equiv^{-1}) and the depletion of Cl^- during

Table 3. Impact of ED Treatment[a] on the Chemical Composition of Cloudy Apple Juice[b]

compound	control juice	acidified juice	adjusted juice[c]
ash (mAg/L)	1730 ± 60a	2010 ± 100b	1750 ± 120a
K^+ (mg/L)	764 ± 45a	774 ± 53a	775 ± 41a
Ca^{2+} (mg/L)	27 ± 3a	22 ± 2b	21 ± 3b
Mg^{2+} (mg/L)	35 ± 5a	35 ± 4a	32 ± 4a
Cl^- (mg/L)	0.7 ± 0.3a	580 ± 30c	29 ± 11b
malic acid (g/L)	7.8 ± 0.4a	7.6 ± 0.5a	6.2 ± 0.5b
glucose (g/L)	18.8 ± 3.4a	18.0 ± 2.5a	17.3 ± 3.5a
fructose (g/L)	84.3 ± 4.1a	79.4 ± 3.5a	75.1 ± 3.8a
saccharose (g/L)	13.1 ± 2.1a	11.8 ± 1.5a	11.7 ± 1.8a
anthocyanin (mg/L)	55 ± 3a	55 ± 2a	53 ± 3a

[a] Configuration with bipolar and anionic membranes, 0.75 mm separation, 10 bipolar membranes, 10 anionic membranes, 40 mA/cm^2. [b] Data are averages ± standard deviation ($N = 3$). Data with different letters (a−c) are significantly different, according to orthogonal contrast test ($P < 0.001$). [c] Juice acidified to pH 2.0 readjusted to pH 3.35.

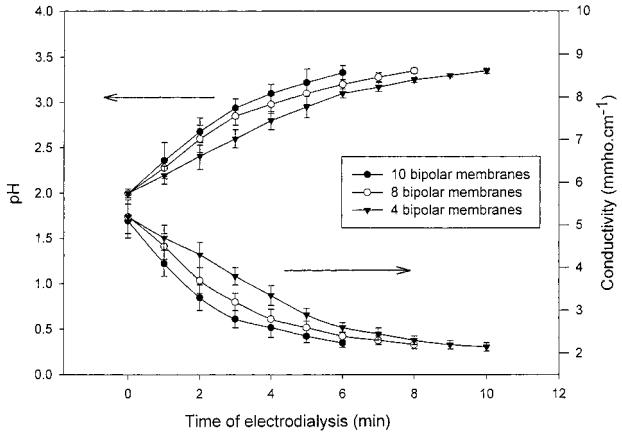

Figure 5. Change in pH and conductivity of apple juice acidified to pH 2.0, during the return of pH to the initial value, with a varying number of bipolar membranes. Conditions: configuration with bipolar and anionic membranes, 0.75 mm spacing, 40 mA/cm^2. Vertical bars represent the standard deviations of the means ($N = 3$).

treatment caused the low retention of OH^- in the juice and made it difficult to eliminate Cl^-. The membrane stacks reduced the time needed to achieve the initial pH of the juice, but the yield remained the same as in the previous study (Tronc et al., 1998), that is, 2.5 L of juice/m^2 of membrane/min (Table 2).

PPO Activity. The acidification of the juice to pH 2.0 completely inhibits PPO activity, but the inhibiting effect was not immediately irreversible (Figure 6). After 1 h of storage at ambient temperature, PPO activity practically stopped in the acidified juice with configuration 2, which is similar to the results obtained by Tronc et al. (1998). However, the rise in pH 1 h after acidification led to a slight temporary reactivation of PPO activity of 0.8%, which is lower than the 25% level reported by Tronc et al. (1998). This result corresponds to the instantaneous treatment using HCl and NaOH, as reported by Zemel et al. (1990), and suggests that the very rapid acidification observed with configuration B (Figure 1) plays an important role in the irreversible PPO inactivation mechanism.

Browning of Juice. The acceleration of acidification by ED provides better control over enzymatic browning compared with previous studies (Tronc et al., 1998). According to the changes in the *L* and *a* values of the control juice, enzymatic browning is very active after

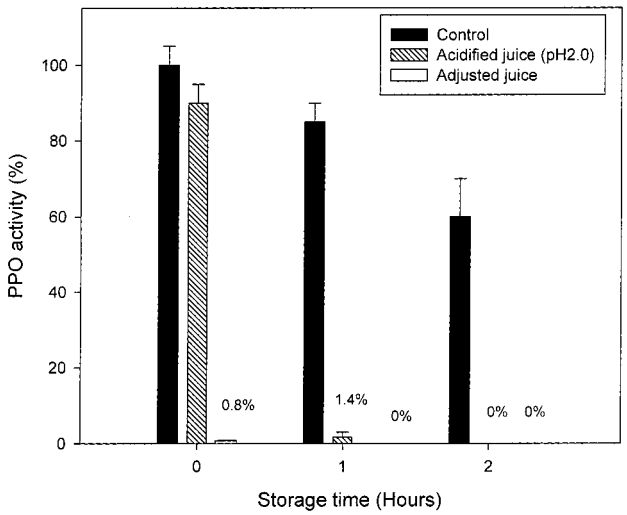

Figure 6. Impact of ED treatment on PPO activity in apple juice during storage at ambient temperature. Vertical bars represent the standard deviations of the means ($N = 3$).

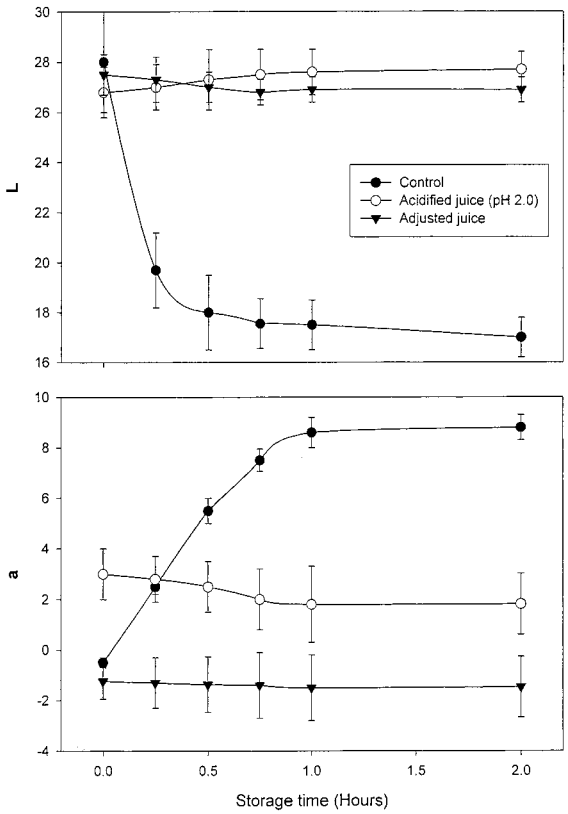

Figure 7. Impact of ED treatment on *L* and *a* values (*L*, *a*, *b*) of apple juice during storage at ambient temperature. Vertical bars represent the standard deviations of the means ($N = 3$).

extraction, and it must be inhibited as quickly as possible in the first few minutes following extraction to keep the color of the treated juice essentially like that of freshly pressed juice (Figure 7). The discrepancies observed between the *L* and *a* values of the control juice and the adjusted juices in our experiments were greater than those obtained by Tronc et al. (1998) for the same storage time following extraction. After the acidification and pH adjustments, the *L* and *a* values stabilized at about the levels for freshly pressed juice (*L* and *a* values for control at time 0) (Figure 7), which demonstrates

Table 4. Concentration (Milligrams per Liter) of Main Phenolic Compounds[a] of Cloudy Apple Juice before and after ED Treatment[b]

compound	control juice	adjusted juice
chlorogenic acid	163.3 ± 4.3	158.8 ± 6.3
(−)-epicatechin	208.5 ± 3.4	198.6 ± 2.8
phloridzin	37.5 ± 1.6	36.6 ± 1.4

[a] Data are averages ± standard deviations ($N = 3$). [b] Configuration with bipolar and anionic membranes, 0.75 mm separation, 10 bipolar membranes, 10 anionic membranes, 40 mA/cm^2.

that browning is completely inhibited. However, value *a* for acidified juice, which varies from green (negative) to red (positive), remained positive and higher than the *a* value for adjusted juice. This is due to the presence of anthocyanins, primarily cyanidin 3-galactoside, the red color of which deepens when the pH is acidic (Mazza and Miniati, 1993), giving acidified juice a redder color. The intensity of this coloration, however, lessens when the juice is at a normal pH, as in the case of adjusted juice.

Chemical Composition of Juices. Chemical analyses demonstrated that ED treatments have variable effects on the composition of cloudy apple juice (Table 3). The percentage of adjusted juice ash remained unchanged by the treatment. A slight increase in ash in acidified juice is due to the fact that the salts formed after the juice was heated to 600 °C are chlorides, not carbonates, owing to the accumulation of Cl^- ions in the juice during acidification. The main cation in the juice, K^+, is preserved owing to the layout of the anionic membranes, which prevent the cations from leaving the juice, representing an advantage from a nutritional standpoint. However, a slight loss in divalent cations (Ca^{2+} and Mg^{2+}) was observed, owing to their low solubility. They could be partially precipitated or adsorbed on the surface of the anionic membranes where the pH at the membrane-solution interface is higher (Brun, 1989). There was no significant change in the concentration of sugars in the juice because the uncharged sugars do not migrate during ED. Only a major osmotic pressure gradient between two compartments could force them to migrate through membranes (Audinos et al., 1985). The malic acid concentration of the adjusted juice decreased by ∼20%. The loss occurred through the anionic membranes because malic acid is negatively charged. Cl^- accumulated in the juice is not totally eliminated after the rise in pH. A residual quantity of 30 mg/L was found in the juice. The use of anionic membranes with greater monovalent anion selectivity would help limit the loss of malic acid and eliminate Cl^- more effectively. Of the four main phenolic compounds [chlorogenic acid, (−)-epicatechin, phloridzin, and procyanidin B2], which represent >90% of the phenolic compounds in apple juice (Varnam and Sutherland, 1994), we quantified the first three (Table 4). The results show that ED treatment preserves phenolic compounds in their original state owing to the instantaneous inhibition of enzymatic browning. Cloudy apple juice that undergoes this treatment remains rich in natural phenolic compounds. These compounds carry little or no charge (Macheix, 1990), and their molecular weight is fairly high (from 290), which makes migration during ED treatment difficult.

Sensory Analysis. Sensory evaluation tests revealed that adjusted juice retained the characteristic flavor and color of freshly pressed juice. The presence of a residual quantity of Cl^- did not seem to affect the flavor of the ED-treated juice. Moreover, the treated juice was slightly fresher, fruitier, and sweeter, with a real apple taste.

Conclusion. The trials with the bipolar and cationic membrane configuration, using bipolar and cationic membranes, demonstrated that a reduction in the spacing between membranes had little impact on acidification time. Although the stack of eight bipolar membranes reduced treatment time by 30%, the treatment is still too lengthy, and the acidification of juice to pH 2.0, the critical value for completely controlling enzymatic browning, was not feasible.

The configuration with bipolar and anionic membranes provides several advantages over the first configuration. This treatment does not require the addition of exogenous KCl; furthermore, there is no chemical waste because all of the electrolytes are reused. It accelerates the treatment and rapidly inhibits enzymatic browning while preserving the nutrients and freshly pressed taste of cloudy apple juice.

ACKNOWLEDGMENT

We thank D. Bélanger and D. Ippersiel for their technical assistance.

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Received for review November 15, 1999. Revised manuscript received March 10, 2000. Accepted March 10, 2000. This study was made possible thanks to financial assistance from the Conseil de recherche en Pêche et Agroalimentaire du Québec (Canada) and A. Lassonde Inc. (Rougement, Canada).

JF991233G