Effect of pH Variation by Electrodialysis on the Inhibition of Enzymatic Browning in Cloudy Apple Juice

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The pH of cloudy apple juice was reduced temporarily and then returned to its initial value using electrodialysis (ED) technology by water splitting with a bipolar membrane. The juice was treated in a small scale unit at temperatures between 16 and 24 °C and a constant current density of 40 mA/cm^2. The pH of the juice was reduced from 3.5 to 2.0 in 30 min. However, this level of acidification required the addition of 12.3 mM K^+ (KCl) every 5 min during the treatment. Exogenous K^+ compensated for the K^+ loss from the juice and maintained the electric charge at neutrality, which favored the accumulation of H_3O^+ in the juice. The reduction of juice pH to 2.0 resulted in the complete inhibition of polyphenol oxidase (PPO) activity compared with the control. Following acidification, the pH of the juice was returned to its initial value in 60 min by introducing OH^- produced by water splitting. The pH adjustment of the juice slightly reactivated the PPO, but browning inhibition was complete and irreversible. The treatment enhanced the color of cloudy apple juice during storage without modifying the flavor or sugar content. However, the ED treatment slightly reduced the malic acid content and substantially reduced the mineral contents.

Keywords: *Electrodialysis; bipolar membranes; apple juice; enzymatic browning; polyphenol oxidase*

INTRODUCTION

Cloudy or unclarified apple juice has increasing market potential due to its superior sensory and nutritional qualities (Guierre, 1975). However, the production of high-quality juice is difficult because of its sensory instability (Lea, 1990). Cloudy apple juice is very sensitive to enzymatic browning (EB) since it contains considerable quantities of polyphenols and polyphenol oxidases (EC 1.14.18.1, PPO) that are bound to suspended particles. Enzymatic browning reactions are catalyzed by PPO and result in the oxidation of phenolic compounds into *o*-quinones which then polymerize into complex dark-colored pigments (Macheix et al., 1990).

Zemel et al. (1990) showed that PPO activity could be irreversibly inhibited by temporarily lowering the pH of apple juice to 2.0 with HCl. Afterward, the pH was adjusted to its initial value by the addition of NaOH solution. This treatment inhibited EB and stabilized the apple juice. However, both the dilution effect from adding acid and base and the formation of salts greatly affected juice flavor, rendering it unacceptable.

Electrodialysis (ED) is a membrane technique that results in the separation of ions (Lopez-Leiva, 1988). When ED is used with bipolar membranes, H_3O^+ and OH^- ions are generated. This method has been used to regenerate mineral acids and bases (Mani, 1991). The application of ED with bipolar membranes appears to be a promising approach for obtaining changes in the pH of cloudy apple juice. The pH can be modified by gradually incorporating protons or hydroxyl ions that are derived from the dissociation of water, without affecting juice flavor.

In a recent study (Tronc et al., 1997), we have demonstrated the feasibility of acidifying cloudy apple juice to pH 2.7 using ED with a bipolar membrane. The treatment partially inhibited PPO activity and reduced EB. However, further acidification to pH 2.0, required for irreversible denaturation of PPO, was impossible under our experimental conditions. It was hypothesized that further reductions in apple juice pH might be attained by maintaining a relatively stable concentration of K^+ during the ED treatment. This could be achieved by supplementing the juice with exogenous K^+ during acidification by ED.

In the present study, the pH of cloudy apple juice was temporarily reduced to pH 2.0 and then returned to its initial value using ED technology by water splitting. The effects of pH variation on the PPO activity, EB, and composition of cloudy apple juice were determined.

MATERIALS AND METHODS

Juice Samples. Juice was extracted from MacIntosh apples, traditionally used in Quebec. Apples were from commercial storage under controlled atmospheres and were stored at 4 °C, for a maximum of 2 weeks. Prior to extraction, apples were washed with cold water and screened to discard fruit of lower quality. For each experiment, ~3 L of juice was extracted from 6 kg of apples. The apples were crushed in a Butcher Boy meat grinder (Lasar Manufacturing Co. Inc.) equipped with a screen with 9 mm diameter perforations. After crushing, the mixture was pressed in a vertical hydraulic press (PF 6510, Perform, Gram, Denmark) under a maximum pressure of 4 bar. To eliminate large particles, extracted juice was filtered through six layers of cheesecloth. Immediately following the extraction, which lasted ≈15 min, the juice was transferred to the ED unit.

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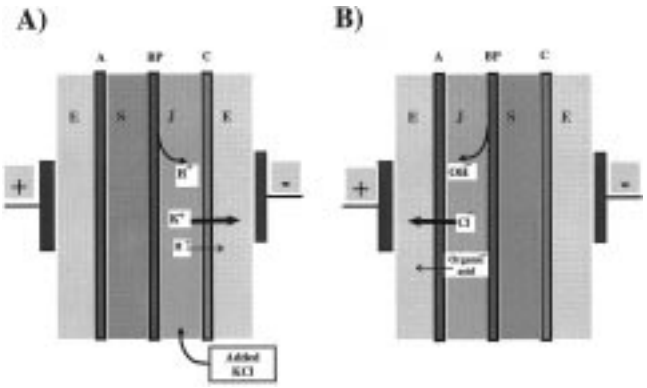

Figure 1. Electrodialysis cell for pH variation of cloudy apple juice: (A) configuration of ED cell for the acidification of cloudy apple juice; (B) configuration of ED cell for the adjustment of pH of cloudy apple juice to its initial value. E, electrolyte; J, juice; S, KCl solution; A, anion-exchange membrane; C, cation-exchange membrane; BP, bipolar membrane.

Electrodialysis Treatments. The ED unit used for modifying the pH of cloudy juice (Figure 1) consisted of a laboratory cell with 0.6 cm between membranes (Electrocell AB, Taby, Sweden) and four compartments: two at each extremity contained electrolyte (0.25 M KCl), one for juice and one for a 0.25 M solution of KCl. A bipolar membrane with an effective area of 100 cm^2 (Neosepta BP-1 model, Tokuyama Soda Co., Tokyo, Japan) separated the KCl solution and juice compartments.

The system was equipped with two conventional ion-exchange membranes each having an effective area of 100 cm^2, i.e., one anion-exchange (AMX Neosepta model) and one cation-exchange membrane (CMX Neosepta model) on each side of the bipolar membrane. The juice, the electrolyte, and the KCl compartments were fed from 600 mL double-jacket glass containers (School, Duran, Germany) using centrifugal pumps (WMD-30RT-220 Iwaki Walchem, Tokyo, Japan).

For each experiment, 2 L of apple juice was used; 1 L was used for the control and the remaining juice (1 L) for ED treatment. The change in juice pH was carried out by incorporating protons or OH^- from the dissociation of water molecules at a constant current density of 40 mA/cm^2 and a voltage of 35−45 V. A 6024A generator (Hewlett-Packard, Vancouver, Canada) supplied the electric current. During the treatment, juice temperature was controlled at 16−24 °C by a Haake G refrigerated reservoir equipped with a Haake D8 programming unit (Haake, Berlin, Germany). Experiments were carried out in batch by recirculating juice in the ED system at a flow rate of 0.8 gpm and a pressure of 24.1 kPa.

The apple juice was acidified by ED to pH 2.0 in 30 min as shown in Figure 1A. The juice circulated on the cationic side of the bipolar membrane where the protons were generated, while the KCl solution circulated on the anionic side. However, acidification to pH 2.0 required the addition of 12.3 mM K^+ (5 mL of KCl solution of 20 g/100 mL) to the juice every 5 min. Indeed, in a previous study (Tronc et al., 1997) it was shown that the decrease in K^+ concentration in apple juice during ED might be responsible for the limitation in pH reduction; pH could not be lowered to <2.7.

Following acidification, the pH of the juice was returned closer to its initial value of pH 3.45 in 60 min by reversing the compartments of the juice and the KCl solution (Figure 1B). The juice circulated on the anionic side of the bipolar membrane where OH^- ions were generated, while the KCl solution circulated on the cationic side.

A control juice, which was not acidified, was prepared prior to the treatment by circulating apple juice in the ED unit for 5 min without an applied potential difference.

During the acidification and pH adjustments, changes in the physicochemical parameters of the juice were monitored. For this, pH, conductivity, dissolved oxygen, and potassium concentrations were measured at intervals varying from 30 s to 5 min until the end of the treatment.

After the treatments, the acidified (pH 2.0) and adjusted (pH 2.0 with adjustment to pH 3.45) juices and controls were stored at ambient temperature for 6 h in glass bottles (200 mL) that were left uncovered and exposed to oxygen in ambient air to encourage EB reactions. Before storage, juice samples were taken to determine sugar, organic acids, and mineral concentration. During storage, juice samples were taken every hour and analyzed for PPO activity and enzymatic browning.

Analyses of Physicochemical Parameters during ED. The pH was measured with a 240 Corning pH-meter (Corning Co., Halstead Essex, England). Juice conductivity was determined with a conductivity meter (model 35YSI, Yellow Springs Instrument Co., Yellow Springs, OH) using a probe constant of 1 cm^{-1}. Dissolved oxygen was measured using an oximeter equipped with a 55/12 oxygen probe (Yellow Springs Instrument Co.).

The potassium concentration was determined on 10 mL juice samples. They were diluted 20-fold in 2 N hydrochloric acid, filtered through a 5 μm Baxter filter to eliminate particles, and again through a 0.45 μm disposable Baxter filter (cellulose acetate). Assays were carried out on a plasma emission spectrometer (ICP spectrometer 3510, ARL, Sunland, CA) at 766.5 nm.

Composition Analyses. The concentration of malic acid and sugars was determined by HPLC, following the method of Doyon et al. (1991). Juice samples (20 mL) were diluted 10-fold in distilled water; 1 mL of the dilution was then eluted on a preconditioned Sep-Pak C_{18} (with 5 mL of methanol, HPLC quality, followed by 6 mL of distilled water) and filtered through 0.45 μm Baxter filters (cellulose acetate). Samples of 15 μL were injected at 5 °C, using an automatic injector (WISP-710B) equipped with a cooling unit, into a Waters HPLC (model 510) assembled with an ion-exchange column (ION-300, 300 mm × 7.8 mm, Mandel Science, Rockwood, ON) and two detectors: a Waters M-590 ultraviolet detector for malic acid and a Waters R-410 refraction index detector for sugars (Waters Canada, Mississauga, ON). The optimal separation of juice components was achieved with a mobile phase of 0.0049 N sulfuric acid, a flow rate of 0.4 mL/min, and a detection wavelength of 210 nm. The samples were compared to standards of malic acid, sucrose, glucose, and fructose.

Calcium and magnesium concentrations were determined on 20 mL juice samples following the method described above for potassium. These ions are less mobile than potassium in ED (Perez et al., 1994), but they contribute to the stability of cloudy apple juice. Assays were carried out on ICP at 317.9 nm for Ca^{2+} and at 279 nm for Mg^{2+}. The concentrations of Ca^{2+} and Mg^{2+} in juice were calculated using standard curves from four concentrations (0, 10, 50, and 100 ppm).

Activity of PPO. Enzymatic activity was assayed following the method of Traverso-Rueda and Singleton (1973). An aliquot (0.05 mL) of juice was added to a mixture containing 1.9 mL of 0.05 M phosphate buffer (pH 6.5), 1 mL of 0.05 M catechin, and 0.05 mL of distilled water. After agitation with a magnetic stirrer, the reaction was monitored by spectrophotometry (Varian DMS-100 spectrophotometer, Varian, Springvale, Australia). Absorbance was recorded at 420 nm every 10 s for 10 min. PPO activity was then determined by the difference between initial and maximum absorbance after 10 min of reaction. The relative activity of PPO was reported as a percentage of enzymatic activity in the juice control at time 0, corresponding to the end of ED (Zemel et al., 1990).

Browning in Juice. Browning in apple juice was estimated on 10 mL of well-homogenized juice, by a Labscan Tristimulus colorimeter using Hunter *L*, *a*, and *b* values (Hunter Associates Laboratory Inc., Reston, VA). 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.

Statistical Analysis. The experiment was a conventional split-plot design. Using the procedures of SAS, repeated measurements were subjected to analysis of variance and least significant difference (LSD) comparisons which enabled the

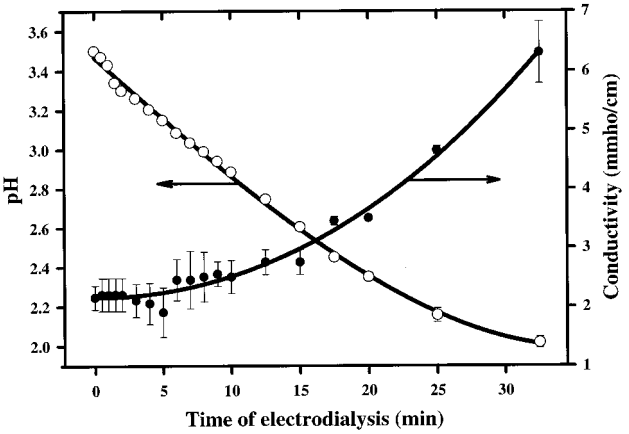

Figure 2. Changes in pH and conductivity as a function of acidification time of cloudy apple juice with the addition of 12.3 mM KCl every 5 min. Vertical bars represent ± standard deviations of the means ($N = 3$).

separation of treatment effects at each sampling time (Steel and Torrie, 1980). Three treatments were compared: the juice control, the acidified juice (pH 2.0), and the adjusted juice (pH 2.0 with adjustment to pH 3.45). The concentrations of malic acid, sugars, and minerals were compared using orthogonal contrasts. The experiments were repeated three times, and the analyses were duplicated.

RESULTS AND DISCUSSION

Acidification of Cloudy Apple Juice. When KCl was added every 5 min to cloudy apple juice during ED acidification, the pH curve as a function of a time became quasi-linear ($R = 0.99$) (Figure 2). This pattern was different from the asymptotic decrease in pH observed in apple juice during ED treatment without addition of KCl (Tronc et al., 1997). After 30 min of electroacidification, the pH of cloudy apple juice, initially at pH 3.5, decreased to pH 2.0. The limitation in pH reduction reported in earlier studies was overcome by maintaining an almost constant flow of K^+ ions across the cationic membrane, which resulted in a greater retention of H_3O^+ ions in juice; potassium ions acted as counterions against protons entering the solution.

During juice acidification with the addition of KCl, a large increase in juice conductivity was observed as juice pH decreased to <2.7 (Figure 2). As referred to earlier (Tronc et al., 1997), at this pH, malic acid, which is responsible for buffering the juice pH, is almost completely in the protonated form (Usseglio-Tomasset, 1989). Therefore, the ions accumulating in juice remain in a free state and largely contribute to increases in conductivity. Indeed, the conductivity of free protons is much greater than that of K^+ leaving the juice (349.6 and 73.5°/Ω^{-1} cm^2 mol^{-1} at 25 °C, respectively). The accumulation of Cl^- from the added KCl might also contribute to increases in juice conductivity, because they are slightly better conductors than K^+ ions (76.3°/Ω^{-1} cm^2 mol^{-1} at 25 °C) (Atkins, 1978). The addition of KCl to apple juice every 5 min maintained the K^+ concentration that was relatively stable and close to its original value (Figure 3).

The concentration of dissolved oxygen in the apple juice decreased during the treatment from 1.5 to 0.8 mg/L and afterward stabilized at pH 2.5 and lower. This level was 40 times higher than that of the apple juice acidified to pH 2.7 without addition of KCl (Tronc et al., 1997). These results indicate that oxidation reactions seem to be greatly inhibited by the low pH reached.

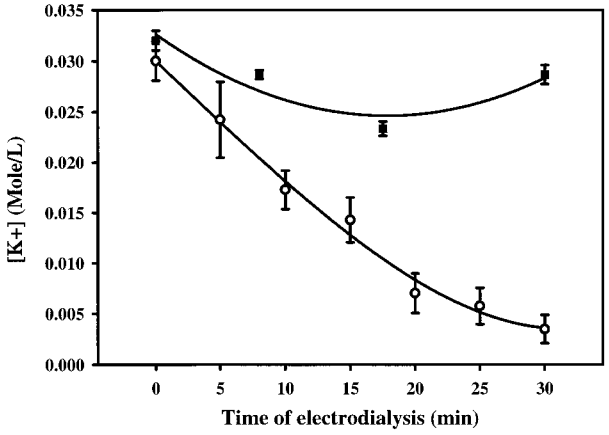

Figure 3. Kinetics of K^+ migration during the acidification of cloudy apple juice with or without addition of KCl: (■) acidified juice (pH 2.0) with addition of KCl; (○) acidified juice (pH 2.7) without addition of KCl. Vertical bars represent ± standard deviations of the means ($N = 3$).

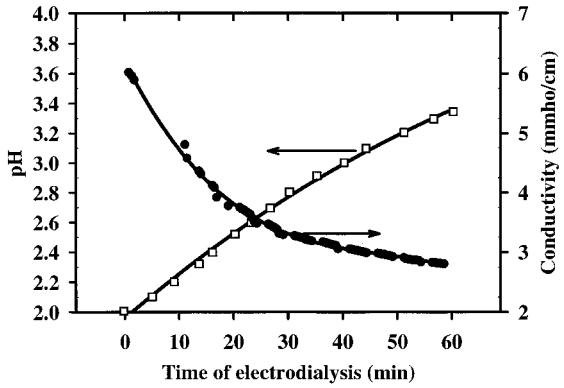

Figure 4. Changes in pH and conductivity as a function of pH adjustment of cloudy apple juice previously acidified at pH 2.0. Variation coefficients for pH and conductivity were below 1.5 and 7%, respectively ($N = 3$).

Adjustment of Juice pH to Its Initial Value. The time required for adjusting the apple juice pH closer to its initial value (3.45 vs 3.5, respectively) using ED was almost twice (60 min) that required for the acidification (Figure 4). This might be explained by the different ionic species, with differences in conductivity and mobility, present in the second phase of the treatment (mainly hydroxyl and chloride ions) compared with the first phase. It is known that protons are much better conductors than hydroxyl ions (349.6 and 199.1°/Ω^{-1} cm^2 mol^{-1} at 25 °C, respectively) and chloride ions, from KCl addition, are just slightly better conductors than K^+ ions (Atkins, 1978).

PPO Activity and Color Stability of Cloudy Apple Juice Treated by ED. By decreasing the pH of cloudy apple juice to 2.0, a drastic decrease in PPO activity was observed (Figure 5). After 1 h of storage at pH 2.0 (corresponding to time 0 in Figure 5), the enzymatic activity remaining in the juice was 4% of the initial value of the control. Thereafter, the PPO activity was completely inhibited and remained so during 6 h of storage.

Similar results were reported by Zemel et al. (1990) with HCl, following the reduction of apple juice pH to 2.0 (Figure 5). However, slightly more time was required to totally inactivate PPO in the present study (60 vs 45 min). This difference might be explained by a greater accumulation of Cl^- derived from acidifying

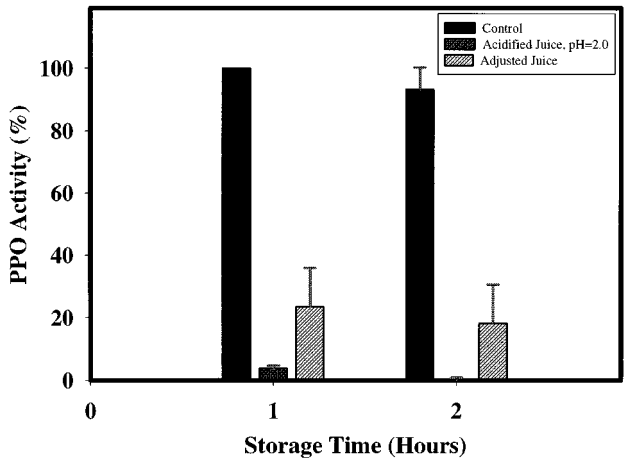

Figure 5. Effect of pH variation of cloudy apple juice using ED on relative activity of PPO during apple juice storage at room temperature. Time 0 represents the end of ED after pH adjustment of the juice. Vertical bars represent ± standard deviation of the means ($N = 3$).

the juice with HCl (Zemel et al., 1990). According to Janovitz-Klapp et al. (1990), halogen-including chlorides have an inhibiting effect on PPO activity. However, this inhibition is very dependent on pH (Rouet-Mayer and Philippon, 1986); the lower the pH, the greater the inhibiting effect of the halogen. These researchers also observed that apple juice PPO was completely inhibited at pH 4.0 and at NaCl concentration of 400 mM. However, the experimental conditions differed from those of the present study; instead of directly using apple juice, purified enzyme extracts from apple and specific buffering conditions were employed.

The addition of KCl at a rate of 1 g/L every 5 min during ED leads to an accumulation of ≈67 mM of chloride ions in the cloudy apple juice at the end of the treatment (calculated values). To verify the effect of adding this concentration of Cl^- on PPO enzymatic activity, the same quantity of KCl was added to apple juice acidified to pH 2.0 with malic acid as in ED-treated juice acidified to pH 2.0. At this KCl concentration, no difference in enzymatic activity was detected between the acidified juices (unpublished results). Although the effect of Cl^- on juice PPO activity should not be entirely excluded, the present results suggest that its influence is secondary compared with the effect of reduced pH.

A significant reduction in browning was observed for the juice acidified to pH 2.0 by ED (Figure 6). This was confirmed by the measurements of L ($p < 0.0001$) and a ($p < 0.0002$) values, which were affected by the pH reduction.

When the pH of the juice was adjusted to its initial value, a slight increase in PPO activity was noted (Figure 5). The adjusted juice presented a higher enzymatic activity (25%) compared with the acidified juice (4%). Contrary to observations by Zemel et al. (1990), the inhibition of this enzyme under the conditions of the present study was not found to be completely irreversible. This difference might be due to a partial extraction of chloride ions, which contribute to enzyme inhibition (Rouet-Mayer and Philippon, 1986), as pH returned to its initial value using ED; that was not the case in the approach used by Zemel et al. (1990).

This revival of PPO activity did not affect the stability of juice color. On the contrary, the adjustment of pH using ED led to an improvement in juice color as shown

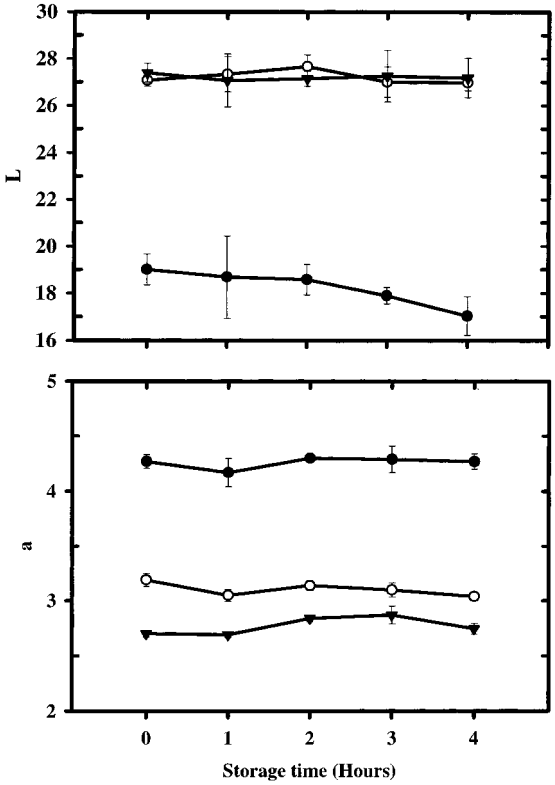

Figure 6. Effect of pH variation of cloudy apple juice using ED on the color indices L and a during apple juice storage at room temperature: (●) control; (○) acidified juice (pH = 2.0); (▼) adjusted juice. Time 0 represents the end of ED after pH adjustment of the juice. Vertical bars represent ± standard deviation of the means ($N = 3$).

by a decrease in the browning index compared with juice acidified to pH 2.0 (Figure 6). This difference was confirmed by a significant ($p < 0.0001$) decrease in the a value of the adjusted juice compared with that of the acidified juice. It is possible that the inhibition of browning in apple juice, while PPO is still partially active, is due to the reduction of some important substrate of the enzyme, such as chlorogenic acid and (+)-catechin (Lea, 1990). The observed difference in a index between juices might also originate from the partial destruction or the adsorption of juice browning pigments on electrodialysis membranes.

Changes in the Composition of Cloudy Apple Juice Treated by ED. The concentration of sugars was slightly modified by ED treatment. However, these changes were not significant ($p < 0.1$). The concentrations of glucose and fructose were slightly reduced, while that of sucrose was slightly increased. These changes occurred only after the pH adjustment. The concentration of malic acid in apple juice decreased during ED treatment (Table 1); the decrease was significant ($p < 0.0001$) only after pH adjustment of the juice. The effect of ED treatment on the concentration of minerals in cloudy apple juice was more noticeable (Table 1). The major loss in minerals was observed only after acidification. Calcium and magnesium concentrations were reduced by 68 and 61%, respectively. As all the positively charged ions such as Ca^{2+} and Mg^{2+} were extracted from the juice, which also occurred for K^+, this decrease was expected. However, since the mobility of Ca^{2+} and Mg^{2+} is inferior to that of K^+, less of these ions were lost during the juice acidification. The low mobility of Ca^{2+} and Mg^{2+} has been explained by their

Table 1. Effect of pH Variation on the Concentration of Minerals and Malic Acid in Cloudy Apple Juice[a]

compound	control	acidified juice	adjusted juice[b]
calcium (mg/L)	60 ± 4a	37 ± 5b	41 ± 7b
magnesium (mg/L)	51 ± 3a	31 ± 5b	26 ± 4b
malic acid (g/L)	6.6 ± 0.4a	6.3 ± 0.3a	5.2 ± 0.3b

[a] Data represent the means ± standard deviations ($N = 3$). Means with different letters are significantly different ($P < 0.0001$ for orthogonal contrast). [b] Juice pH 2.0 with readjustment at pH 3.45.

steric hindrances (Perez et al., 1994). From a nutritional point of view, the decreases in Ca^{2+} and Mg^{2+} content of ED juice are undesirable. However, apple juice is not normally considered to be a significant source of these ions (Southgate et al., 1990). A preliminary sensory analysis (data not shown) indicated that the characteristic flavor of apple juice was preserved, but it was milder and less acidic.

Conclusion. This study demonstrated the feasibility of acidifying cloudy apple juice to pH 2.0 while maintaining the K^+ ions concentration at its initial level during ED. Exogenous addition of K^+ compensated for the loss of K^+ in juice and maintained the electrical neutrality of the medium; this results in a greater accumulation of H_3O^+ ions in juice. This treatment completely inhibited PPO activity and, moreover, led to an improvement in the juice color during storage. The stabilization of juice color is mainly due to the pH effect and possibly to the accumulation of chloride ions in the medium.

After the apple juice was acidified to pH 2.0, the adjustment of juice pH, to a level close to its initial value, did not result in a change in juice color even though a slight increase in PPO activity was observed. The inhibition of browning reactions by ED treatment is consequently irreversible. The ED treatment of apple juice resulted in a large decrease in mineral concentration as well as a slight loss of malic acid. By contrast, no noticeable change in the composition of sugars (sucrose, fructose, and glucose) was observed. In terms of sensory characteristics, ED does not appear to affect cloudy apple juice; its characteristic flavor is preserved, but it is less acidic and somewhat milder.

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