Innovative

# Clarification of apple juice by electroflotation ${ }^{2 / 3}$ 

M. Araya-Farias ${ }^{\text {a }}$, M. Mondor ${ }^{\text {b,* }}$, F. Lamarche ${ }^{\text {b }}$, S. Tajchakavit ${ }^{\text {c }}$, J. Makhlouf ${ }^{\text {a }}$<br>${ }^{\text {a }}$ Department of Food Sciences and Nutrition, Laval University, Sainte-Foy, Quebec, Canada G1K 7P4<br>${ }^{\mathrm{b}}$ Food Research and Development Center, Agriculture and Agri-Food Canada, Saint-Hyacinthe, Quebec, Canada J2S 8E3<br>${ }^{\text {c }}$ A. Lassonde, Inc., 170, 5th ave., Rougemont, Quebec, Canada J0L 1M0

Received 29 March 2007; accepted 21 August 2007


#### Abstract

Apple Juice industry is in search of a simplified technology which enables a quick clarification and stabilisation of apple juice. This study aimed to evaluate the potential of electroflotation as an alternative for the clarification of apple juice. Clarification of apple juice by electroflotation was first done at various current densities $\left(10,20\right.$ and $\left.40 \mathrm{~mA} / \mathrm{cm}^{2}\right)$ with and without addition of gelatin ( $200 \mathrm{mg} / \mathrm{l}$ ). Afterwards, the electroflotation treatments were done at a current density of $20 \mathrm{~mA} / \mathrm{cm}^{2}$ with various concentrations of added gelatin $(0,50,100 \mathrm{and} 200 \mathrm{mg} / \mathrm{l})$. It was shown that electroflotation treatments alone was efficient to reduce the tannin and protein contents of apple juice. However, the decrease in the protein content was in large part due to the use of pectinases prior to the electroflotation treatments. The use of gelatin in combination with the electroflotation aided in the clarification process. The highest gelatin concentration used in this study ( $200 \mathrm{mg} / \mathrm{l}$ ) resulted in a better reduction of tannin and protein levels, while a current density of $20 \mathrm{~mA} / \mathrm{cm}^{2}$ was found to be optimal. Turbidity observed in the juices clarified with electroflotation treatments was in average lower than 10 NTU but higher than 2 NTU which is generally required to produce a stable clarified juice. Brix degree and pH of the apple juice was not affected by the electroflotation treatments while the color was improved.


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Keywords: Apple juice; Clarification; Electroflotation; Stability; Turbidity
Industrial relevance: The production of clarified and stable apple juice is a subject of interest for the beverages industries. The clarification step which remained long and discontinuous implied the addition of a large quantity of pectolytic enzyme and of clarifying agents (such as gelatin) to the freshly pressed juice to induce the precipitation of proteins and other suspended matter in 15-20 h . Fining treatments were followed by a separation step usually consisting of decantation and classical filtration on filter-press, or flotation by dispersed gas. The development of membrane separation processes to replace the traditional approach has enabled the automation of the whole production resulting in lower labor requirement and a considerably shorter process time than the traditional process.

However, the performance of membrane separation processes is influenced by the declining permeate flux with time, which is due to membrane fouling. In some instances, permeate flux decline makes membrane separation processes unattractive for the clarification of apple juice. To our knowledge, we are the first research group to use electroflotation (EF) for clarification of apple juice. It was shown that EF treatments alone were efficient to reduce the tannin and protein contents of apple juice. In addition, the use of gelatin in combination with the EF aided in the clarification process. Turbidity observed in the juices clarified with EF treatments for 30 min was in average lower than 10 NTU. Brix degree and pH of the apple juice were not affected by the EF treatments while the color was improved.

When compared to the values reported in the literature for flotation by dispersed gas, it seems that EF shows better efficiency than flotation in decreasing the juice turbidity ( $99 \%$ decrease for EF as compared to $90 \%$ decrease for flotation). In addition, for experiments carried out by conventional flotation larger amount of fining agent are used ( $70-150 \mathrm{mg}$ of gelatin/l, 400-800 mg/l of silica sol and 200-500 mg/l of bentonite). For these reasons, the new process we propose is advantageous when compared to the traditional flotation approach and it should have a measurable impact on the advancements in the production of clarified apple juice. If used as a pre-treatment to ultrafiltration clarification, it is expected that it would reduce membrane fouling resulting in higher productivity.

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## 1. Introduction

The production of clarified and stable apple juice is a subject of interest for the beverages industries. The clarification step which remained long and discontinuous implied the addition of a large quantity of pectolytic enzyme and of clarifying agents (such as gelatin) to the freshly pressed juice to induce the precipitation of proteins and other suspended matter in 15-20 h. Fining treatments were followed by a separation step usually consisting of decantation and classical filtration on filter-press (Lea, 1995). The development of membrane separation processes to replace the traditional approach has enabled the automation of the whole production resulting in lower labor requirement and a considerably shorter process time than the traditional process (Ben Amar, Gupta, \& Jaffrin, 1990; Rao, Acree, Cooley, \& Ennis, 1987). However, the performance of membrane separation processes is influenced by the declining permeate flux with time, which is due to membrane fouling. In some instances, permeate flux decline makes membrane separation processes unattractive for the clarification of apple juice.

During the past years, clarification processes based on flotation of particles have gained in popularity in the food industries. In Canada, the system « Clarifruit» was developed for the production of clarified apple juice (Lassonde Technology Inc., 1984). The system is based on the flotation of suspended particles using a sursaturated flow of nitrogen (Lassonde Technology Inc., 1984; Lea, 1995). Ferrarini, Celotti, and Zironi (1997) proposed a non-conventional industrial apple juice clarification process combining a flotation system based on the dissolution of gas in the juice to be treated, with crossflow filtration technique. The main drawback of these systems is that usually large amount of flocculant or fining agent is required prior to the flotation step to obtain an efficient clarification of the apple juice.

Electroflotation (EF) could represent a viable alternative for the clarification of apple juice. EF is a solid/liquid separation process based on the suspension of particles by gas bubbles (hydrogen and oxygen) generated at the surface of electrodes, which are immersed in the apple juice, by the application of a current (Burns, Yiacoumi, \& Tsouris, 1997). When compared to other flotation techniques, the EF has the advantage to generate smaller gas bubbles of homogeneous size, which would result in a more efficient clarification process. Furthermore, it is also possible to control the bubbles concentration in the system by varying the current density (Hosny, 1992). The EF was used with success in the mine industry for the separation of small particles from solution (Ahmed \& Jameson, 1985; Ketkar, Mallikaarjunan, \& Venkatachalam, 1991). Good results were also obtained for the separation of oil from water/oil emulsions (Hosny, 1992, 1996), to reduce the concentration of surfactants in aqueous solutions (Moulai-Mosefa \& Tir, 2002), and for the treatment of activated sludge (Choi et al., 2005). Very few works were done in the food industry, however Environment Canada used that technique for the wastewater treatment of meat packaging industry (Environnment Canada, 1982). Okun and Matov (1984) also applied successfully that technique for
the extraction of proteins from alfalfa juice, and Tsai, Hernlem and Huxsoll (2002) applied it to remove the solids from poultry chiller water. For most of these works, a clarifying agent is added to improve the clarification step.

This study aimed to evaluate the use of EF alone or in combination with gelatin for the clarification of apple juice. The effect of these treatments on the solid content of the floc will be discussed, as well as the effect on the physico-chemical parameters of the juice (tannins content, proteins content and turbidity) and on its qualitative parameters (color, pH and Brix degree).

## 2. Materials and methods

### 2.1. Apple juice

Juice was extracted from McIntosh apples that had been stored commercially under controlled atmosphere. The apples were crushed and pressed in a crusher-press model no. EG 260X6 (Goodnature Products Inc., New York, USA) under maximum pressure of 1500 psi . About 20 L of juice was obtained from 30 kg of apples. For each experiment, 4 L of juice was used. The remaining juice was stored at temperature close to $0{ }^{\circ} \mathrm{C}$ prior to use.

### 2.2. Electroflotation cell

A batch electroflotation cell was made for apple juice clarification. The flotation cell was rectangular $(16 \times 17 \mathrm{~cm}$ by 25 cm tall) and made of plexiglass (Fig. 1). A sampling valve was fixed 9 cm above the cell bottom. The cathode was a stainless steel screen (wire diameter of 1.5 mm ), positioned horizontally on top of graphite rods forming the anode. The distance between the two electrodes was 9 mm . The anode was fixed at 1.0 cm above the cell bottom. The anode's area was $153 \mathrm{~cm}^{2}$ and the current density was calculated using this area. Electrical wires were attached to the electrodes with conductive resin and then connected to an external power supply.


Fig. 1. Electroflotation cell.


Fig. 2. EF process for the clarification of apple juice.

### 2.3. Protocols

### 2.3.1. Clarification of apple juice

Before being clarified by EF, the apple juice was treated with pectinases (Pectinex 100, $45 \mathrm{mg} / \mathrm{l}$, and Pectinex Ultra, $10 \mathrm{mg} / \mathrm{l}$ ) and amylases (AMG $300 \mathrm{~L}, 18 \mathrm{mg} / \mathrm{l}$ ) from Novo Nordisk Biochem (Denmark), using dose of enzymes similar to the one used in the industry (Tajchakavit, Boye, \& Couture, 2001). The depectinisation was carried out at $50^{\circ} \mathrm{C} \pm 1^{\circ} \mathrm{C}$ for 50 min . The juice was then clarified by electroflotation (Fig. 2).

EF was done first at various current densities (10, 20 and $40 \mathrm{~mA} / \mathrm{cm}^{2}$ ) with and without addition of gelatin $(200 \mathrm{mg} / \mathrm{l})$. Afterwards, the EF was carried out at a current density of $20 \mathrm{~mA} / \mathrm{cm}^{2}$ with various concentrations of added gelatin $(0,50$,

100 and $200 \mathrm{mg} / \mathrm{l}$ ). A current density of $20 \mathrm{~mA} / \mathrm{cm}^{2}$ was found to be optimal in terms of generation rate of gas bubbles and anode stability (anode starts to degrade at a current density of $40 \mathrm{~mA} / \mathrm{cm}^{2}$ ). Before the EF treatment, a $5 \%(\mathrm{w} / \mathrm{v})$ solution of type A gelatin at $175^{\circ}$ Bloom (Cangel Inc., Ontario, Canada) was hydrated in water at $50^{\circ} \mathrm{C}$ during 1 h . The gelatin solution was added to the apple juice at the required concentration $(0,50$, 100 or $200 \mathrm{mg} / \mathrm{l}$ of apple juice). After mixing for 5 min , the juice was transferred to the electroflotation cell. The treatments were carried out at a temperature varying between 48 and $50^{\circ} \mathrm{C}$, for 30 min . After each treatment and prior to pasteurization, the resulting floc was removed using a dip net and the clarified juice was collected from the sampling valve. Each treatment was compared to a fresh unclarified juice and to a depectinized and centrifuged juice ( 10 min at 3000 rpm ).

### 2.3.2. Juice pasteurisation

The clarified apple juices were conditioned in glass bottles $(200 \mathrm{ml})$ and pasteurised at $95^{\circ} \mathrm{C}$ for 7 min by immersion in a hot water bath. The juices were then cooled under tap water and stored at room temperature. Samples were removed for analysis in duplicate after approximately 24 h of storage.

### 2.4. Floc analysis

The flocs recuperated after the treatment were vacuum dried at $70{ }^{\circ} \mathrm{C}$ using an Isotemp vacuum oven (model 285A, Fisher Scientific Ltd., Ontario, Canada) until a constant weight is obtained (approximately 24 h ) and their solid content was then determined. Due to some juices and sugars remaining in the flocs after the drying step, the real solid content of the flocs


Fig. 3. Floc formation during EF of apple juice without addition of gelatin.

Table 1
Solid content in the floc as a function of the applied current density during the EF treatment

| Treatments | Humid floc | Dry floc |  | Floc without <br> sugars |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |  |
|  |  |  |  |  | $\%^{\mathrm{a}}$ |  |

The values represent the means $\pm$ standard deviations ( $n=2$ ).
${ }^{\text {a }}$ The percentages are estimated based on the weight of the humid floc.
${ }^{\mathrm{b}}$ EF treatments during 30 min .
${ }^{\text {c }}$ Gelatin concentration at $200 \mathrm{mg} / \mathrm{l}$.
${ }^{\mathrm{d}}$ Measurements were carried out in the EF cell.
(without the sugars) was calculated using the following equation:
$w_{\mathrm{f}}=\frac{w_{\mathrm{df}}-w_{\mathrm{hf}} z}{1-z}$
where $w_{\mathrm{f}}$ is the real weight of the floc without the apple juice sugars, $w_{\mathrm{df}}$ is the weight of the dried floc, $z$ is the fraction of sugars in the juice ( 0.11 ) and $w_{\mathrm{hf}}$ is the weight of the humid floc.

### 2.5. Apple juice analysis

Physico-chemical and qualitative analyses were done on the juices including tannin and protein contents, juice turbidity, color, pH and total soluble solids ( ${ }^{\circ} \mathrm{Brix}$ ).

Tannin content was assayed by the absorbance measurement of a mixture containing 0.5 ml of clarified apple juice, 1.5 ml of concentrated HCl and 3 ml of a $4 \%(\mathrm{w} / \mathrm{v})$ solution of vanillin in methanol based on the method developed by Broadhurst and Jones (1978). Catechin solution ( $0-200 \mathrm{mg} / \mathrm{l}$ ) was used as standard. Protein content was determined using the micro-assay method of Bradford (1976). The absorbance of a mixture of $750 \mu \mathrm{l}$ of Cromassie Red and 3 ml of clarified apple juice was measured at 595 nm . Gamma globulin solution ( $0-20 \mathrm{mg} / \mathrm{l}$ ) was used as standard. Turbidity was measured using a Hach turbidimeter (model 2100AN, Hach Company, Colorado, USA) while the color was measured in terms of the $\%$ transmittance at 440 nm . For the unclarified juice, the samples were first centrifuged at 3000 rpm for 10 min before the transmittance measurements. The pH of the juice was measured using a portable pH -meter (model pHi 11 , Beckman, California, USA) while the total soluble solid content was determined using a portable refractometer (Fisher Scientific Ltd., Ontario, Canada).

### 2.6. Statistical analyses

A complete randomized experimental design with two repetitions was applied. An analysis of variance (ANOVA) was performed using SAS software to establish if the different treatments have an effect on the parameters. Furthermore, all pairs of each experimental parameter were compared using a $t$ test analysis to determine whether the different treatments give equivalent results. The $t$-test procedure was applied, as described by Montgomery (1991).

## 3. Results

### 3.1. Effect of the current density

### 3.1.1. Solid content of the floc separated by EF

The EF treatments resulted in the quick formation of a floc even without the addition of gelatin (Fig. 3). The floc brought at


Fig. 4. Effect of the current density on the tannins content of the apple juice treated by EF. Flotation time: 30 min. The vertical bars represent the standard deviation of the means ( $n=2$ ).


Fig. 5. Effect of the current density on the proteins content of the apple juice treated by EF. Flotation time: 30 min . The vertical bars represent the standard deviation of the means ( $n=2$ ).
the surface was compacted by its own weight and also by the gas bubbles. Since water was also displaced by the bubbles, the resulting floc was humid and with a high percentage of solids. As shown in Table 1, the percentage of total solids of the dried flocs vary between 14 and $17 \%$ for the treatments with or without added gelatin. However, if we consider the flocs solids content without the juice sugars, they represent only $3-6 \%$ of the humid flocs. The quantity of floc obtained for the treatments that were carried out with added gelatin was in the order of 3$45 \%, 15-43 \%$ and $29-48 \%$ higher than for the treatments carried out without added gelatin, when expressed in terms of humid floc, dry floc and floc without sugars, respectively (EF treatment without gelatin was used as the reference). However, no significant effect of the current density was observed on the flocs solids content without the juice sugars.

### 3.1.2. Physico-chemical and qualitative parameters of apple juice

The tannin content of the unclarified apple juice was in the order of $275 \pm 26 \mathrm{mg} / \mathrm{l}$ while it was in the order of $225 \pm 7 \mathrm{mg} / \mathrm{l}$ for the depectinized juice. The tannin content of the apple juices submitted to various treatments is illustrated in Fig. 4. An increase of the current density for the EF treatments, with or without added gelatin, resulted in a significant decrease ( $p<0.001$ ) of the tannin content. For the EF treatments without addition of gelatin, a decrease of $10-35 \%$ of the tannin content based on the depectinized juice, and $26-47 \%$ based on the unclarified juice, was observed. The maximum decrease was observed at $40 \mathrm{~mA} / \mathrm{cm}^{2}$. Furthermore, the $t$-test indicates that the EF treatments with added gelatin were significantly more efficient to decrease the tannin content than the EF treatments without added gelatin ( $p<0.05$ ).

The protein content of the unclarified juice was in the order of $208 \pm 26 \mathrm{mg} / \mathrm{l}$ and it was significantly decreased ( $p<0.001$ ) at $119 \pm 12 \mathrm{mg} / \mathrm{l}$ after depectinisation. In contrast with the results obtained for the tannin content, the protein content (Fig. 5) was not significantly reduced when EF treatments alone were applied. Protein contents varying between 113 and $120 \mathrm{mg} / \mathrm{l}$
were obtained, which is not significantly different than for the depectinized juice. However, when gelatin was added to the juice prior to the EF treatments, the final protein contents were significantly lower ( $31-56 \mathrm{mg} / \mathrm{l}$ ). For the same treatments, a slight increase of the protein content was observed with an increase of the current density.

The turbidity values for the different apple juices are given in Table 2. The turbidity was significantly lower ( $p<0.005$ ) for the apple juices clarified by EF without added gelatin than for the unclarified apple juice. However, no significant difference was observed between the EF treatment without added gelatin and the depectinized juice. For the EF treatments carried out with the addition of gelatin, the turbidity values are slightly lower

Table 2
Turbidity and qualitative parameters of apple juices submitted to different clarification treatments

| Treatments | Turbidity (NTU) | Color (\% transmittance at 440 nm ) | pH | Soluble solids ( ${ }^{\circ}$ Brix) |
| :---: | :---: | :---: | :---: | :---: |
| Unclarified juice | $436.0 \pm 2.8$ | $6.8 \pm 3.2$ | $3.3 \pm 0.1$ | $11.0 \pm 0.4$ |
| Depectinized juice | $8.9 \pm 1.4$ | $52.7 \pm 3.4$ | $3.2 \pm 0.1$ | $11.0 \pm 0.2$ |
| EF without gelatin ${ }^{\text {a }}$ |  |  |  |  |
| $10 \mathrm{~mA} / \mathrm{cm}^{2}$ | $9.1 \pm 0.3$ | $51.8 \pm 0.9$ | $3.1 \pm 0.1$ | $10.8 \pm 0.3$ |
| $20 \mathrm{~mA} / \mathrm{cm}^{2}$ | $10.2 \pm 1.4$ | $51.2 \pm 1.3$ | $3.2 \pm 0.2$ | $11.2 \pm 0.2$ |
| $40 \mathrm{~mA} / \mathrm{cm}^{2}$ | $20.0 \pm 0.9$ | $44.5 \pm 3.3$ | $3.0 \pm 0.3$ | $11.0 \pm 0.3$ |
| $\begin{aligned} & \text { Gelatin } \mathrm{b}, \mathrm{c} \\ & 0 \mathrm{~mA} / \mathrm{cm}^{2} \end{aligned}$ | $5.4 \pm 0.2$ | $66.7 \pm 1.8$ | $3.2 \pm 0.1$ | $10.9 \pm 0.2$ |
| EF with gelatin ${ }^{\mathrm{a}, \mathrm{b}}$ |  |  |  |  |
| $10 \mathrm{~mA} / \mathrm{cm}^{2}$ | $4.6 \pm 0.4$ | $68.6 \pm 0.2$ | $3.3 \pm 0.1$ | $11.1 \pm 0.3$ |
| $20 \mathrm{~mA} / \mathrm{cm}^{2}$ | $3.4 \pm 0.5$ | $69.5 \pm 0.9$ | $3.2 \pm 0.2$ | $11.2 \pm 0.1$ |
| $40 \mathrm{~mA} / \mathrm{cm}^{2}$ | $10.9 \pm 2.5$ | $54.9 \pm 2.5$ | $3.1 \pm 0.1$ | $10.9 \pm 0.4$ |

[^1]Table 3
Solid content in the floc as a function of the added concentration of gelatin during the EF treatment at $20 \mathrm{~mA} / \mathrm{cm}^{2}$ during 30 min

| Treatments | Humid floc | Dry floc |  | Floc without sugars |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | (g) | (g) | \% ${ }^{\text {a }}$ | (g) | \% ${ }^{\text {a }}$ |
| Without gelatin | $45.6 \pm 2.9$ | $6.8 \pm 0.2$ | 14.9 | $2.0 \pm 0.1$ | 4.4 |
| With gelatin |  |  |  |  |  |
| $50 \mathrm{mg} / \mathrm{l}$ | $44.9 \pm 0.1$ | $6.6 \pm 0.1$ | 14.7 | $1.8 \pm 0.1$ | 4.0 |
| $100 \mathrm{mg} / \mathrm{l}$ | $56.1 \pm 2.9$ | $8.1 \pm 0.1$ | 14.4 | $2.2 \pm 0.2$ | 3.7 |
| $200 \mathrm{mg} / \mathrm{l}$ | $71.2 \pm 3.1$ | $10.8 \pm 0.1$ | 15.2 | $3.3 \pm 0.2$ | 4.6 |

The values represent the means $\pm$ standard deviations ( $n=2$ ).
${ }^{\text {a }}$ The percentages are estimated based on the weight of the humid floc.
than for the EF treatments without added gelatin. It is worth to mention that the turbidity values measured at a current density of $40 \mathrm{~mA} / \mathrm{cm}^{2}$ were biased due to a problem with the graphite electrode. At this current density, the amount of gas bubbles generated becomes too high and some graphite particles are released in the juice.

A significant increase $(p<0.001)$ in the percentage of transmittance was observed when the apple juice was clarified by EF with added gelatin, when compared to the depectinized juice (Table 2). This is an indication of the improvement of the juice color. As expected, the EF treatments did not affect the pH and the Brix values (Table 2). The values reported for the different clarified apple juices were similar to those of the unclarified apple juice.

### 3.2. Effect of the gelatin concentration

### 3.2.1. Solid content of the floc separated by EF

Table 3 showed that the floc solid content (without the juice sugars) was slightly increased by the increase of gelatin concentration. However, this increase was not proportional to the quantity of gelatin added $(p<0.001)$. An increase in the

Table 4
Turbidity and qualitative parameters of apple juices submitted to different clarification treatments

| Treatments | Turbidity <br> $(\mathrm{NTU})$ | Color (\% transmittane <br> $440 \mathrm{~nm})$ | pH | Soluble solids <br> $\left({ }^{\circ}\right.$ Brix) |
| :--- | :--- | :--- | :--- | :--- |
| Unclarified <br> juice | $359.0 \pm 1.1$ | $6.3 \pm 0.9$ | $3.1 \pm 0.1$ | $11.2 \pm 0.3$ |
| Depectinized <br> juice | $9.5 \pm 1.2$ | $43.8 \pm 2.2$ | $3.2 \pm 0.2$ | $11.2 \pm 0.2$ |
| EF without <br> gelatin ${ }^{\text {a }}$ | $7.9 \pm 0.3$ | $43.0 \pm 0.9$ | $3.0 \pm 0.0$ | $11.2 \pm 0.1$ |
| EF with gelatin |  |  |  |  |
| a |  |  |  |  |
| $50 \mathrm{mg} / 1$ | $4.3 \pm 0.0$ | $54.1 \pm 0.8$ | $3.1 \pm 0.0$ | $11.3 \pm 0.2$ |
| $100 \mathrm{mg} / \mathrm{l}$ | $4.9 \pm 0.5$ | $59.6 \pm 1.3$ | $3.1 \pm 0.1$ | $11.4 \pm 0.0$ |
| $200 \mathrm{mg} / \mathrm{l}$ | $3.4 \pm 0.1$ | $63.5 \pm 0.5$ | $3.0 \pm 0.3$ | $11.0 \pm 0.3$ |

The values represent the means $\pm$ standard deviations ( $n=2$ ).
${ }^{\text {a }}$ EF treatments at $20 \mathrm{~mA} / \mathrm{cm}^{2}$ during of 30 min .
order of $22 \%$ was observed when the gelatin concentration varied from 50 to $100 \mathrm{mg} / \mathrm{l}$, while it was in the order of $50 \%$ when the gelatin concentration was increased from 100 to $200 \mathrm{mg} / \mathrm{l}$. The $t$-test indicates that the EF treatment with added gelatin at $200 \mathrm{mg} / 1$ was significantly different $(p<0.05)$ than the treatments with gelatin at 50 and $100 \mathrm{mg} / \mathrm{l}$ and without gelatin. However, the EF treatments with added gelatin at 50 and $100 \mathrm{mg} / \mathrm{l}$ were not significantly different than the treatment without addition of gelatin.

### 3.2.2. Physico-chemical and qualitative parameters of apple juice

The effects of gelatin concentration on tannin and protein contents of apple juices are illustrated in Fig. 6. An increase in the quantity of gelatin added during the EF treatment at $20 \mathrm{~mA} / \mathrm{cm}^{2}$ resulted in a significant decrease $(p<0.001)$ of the tannin and protein contents.

The turbidity values of apple juices as a function of gelatin concentration are given in Table 4. The turbidity was significantly lower ( $p<0.005$ ) for the apple juices clarified with gelatin, when


Fig. 6. Effect of the gelatin concentration on the tannins and proteins contents of the apple juice treated by EF at $20 \mathrm{~mA} / \mathrm{cm}^{2}$. Flotation time: 30 min. The vertical bars represent the standard deviation of the means $(n=2)$.
compared to the unclarified juice and the juice clarified without gelatin. However, no significant difference was observed between the EF treatment with 50 or $100 \mathrm{mg} / \mathrm{l}$ of added gelatin. The lowest turbidity value was obtained for the EF treatment with $200 \mathrm{mg} / \mathrm{l}$ of added gelatin $(3.4 \pm 0.1 \mathrm{NTU})$. However, this value is slightly higher than the minimal turbidity of 2 NTU required to produce a stable clarified juice (Van Buren, 1989).

An increase in the percentage of transmittance was observed with an increase of the quantity of added gelatin during the EF treatment (Table 4). This increase was significant ( $p<0.001$ ) and indicated an improvement of the juice color. As expected, the EF treatments did not affect the pH and Brix values (Table 4).

## 4. Discussion

### 4.1. Tannin and protein contents

### 4.1.1. Effect of the current density

Our study has demonstrated that the removal of tannins by EF is improved with an increase of the current density. This could be due to the generation of larger amount of gas bubbles, as well as to the increase of the occurrence of oxidation reactions. In fact, an increase in the generation of gas bubbles in the electroflotation cell will bring more particles at the surface of the juice (Hosny, 1992, 1996) while the oxygen generated will catalyze the enzymatic oxidation of the tannins by the polyphenols oxydases (PPO) (Mayer \& Harel, 1979) producing oligomers which are less soluble and easier to be brought at the surface by the gas bubbles.

In contrast with the removal of tannins, it was observed that the removal of proteins by EF was not improved with an increase of the current density. In fact, for the EF treatments with added gelatin, the protein content of the juice was slightly increasing with the current density. It is not clear why this was so; this finding clearly merits further investigation. However, one possible hypothesis is that since the oxidation of tannins is increasing with an increase in current density, and that oxidised tannins polymerize to form larger compounds which are more easily eliminated by gelatin (Metche \& Girardin, 1980), an increasing quantity of excess gelatin would remain in the solution, and as a result a slightly increasing protein content would be measured.

The lowest level of proteins observed in the depectinized juice and the juices treated by EF, when compared to the unclarified juice, resulted probably from the action of pectinase. In fact, the complexes formed of proteins and carbohydrates are mainly responsible for the opalescence of the juice and their protein content is in the order of $36 \%$ (Yamasaki, Yasui, \& Arima, 1964). At the pH of the juice, the surface of these proteins is negatively charged as it is also the case for the pectin and other carbohydrates. However, under the negatively charged layer there are some proteins with positive charges. By using pectinases, a partial hydrolysis of the pectin was achieved which has probably resulted in the exposition of these proteins with positive charges (Grassin, 1992; Kilara \& Van Buren, 1989) and the formation of proteinprotein complexes that could precipitate or be eliminated by EF.

### 4.1.2. Effect of the gelatin concentration

Our study has also demonstrated that the increase of the amount of gelatin added to the apple juice improved the removal of tannins and proteins by EF. It is well known that at the pH of the apple juice ( $\mathrm{pH} \sim 3.3$ ), tannins and native proteins present in the juice are negatively charged as it is the case for most phenolic compounds. This favors the formation of interactions with gelatin which is positively charged and thus the formation of protein-tannin insoluble complexes (Görtges \& Haubrich, 1992). These complexes are eliminated from the juice (into the floc) by the small gas bubbles produced at the electrodes and this would explain the decrease of the tannins and proteins contents with an increase of the added quantity of gelatin.

### 4.2. Turbidity

The clarification treatments show a direct relation between the turbidity values and the tannin and protein concentrations of the apple juices, the lower the turbidity of the juices, the lower their tannin and protein contents. Siebert, Troukhanova, and Lynn (1996) observed that the turbidity (NTU) of model solution is a function of their protein and polyphenol concentrations, as well as the ratio between these concentrations. When the concentration of the proteins (causing sedimentation) is approximately equivalent to the one of the polyphenols, large colloidal particles will form which will result in maximum turbidity. However, when the concentration of proteins or the concentration of polyphenols is in excess, this results in smaller particles and less turbidity. In this work, for the juice treated by EF at $20 \mathrm{~mA} / \mathrm{cm}^{2}$ with $200 \mathrm{mg} / \mathrm{l}$ of added gelatin (lowest turbidity), the protein concentration is approximately $50 \%$ as compared to the concentration of the tannins, while for the juice treated by EF without addition of gelatin (higher turbidity), the protein and tannin concentrations are approximately the same (Fig. 6). However, it is expected that the difference observed between the treatments is also due to the quantity of residual proteins which is higher for EF treatment without addition of gelatin. It is well known that the proteins alone or by association with the phenolic compounds are responsible for juice instability (Heatherbell, 1984).

If we consider that a turbidity of 2 NTU or less is required to ensure a good stability of the juice during storage (Van Buren, 1989), all treatments resulted in an unstable juices. The EF treatment at $20 \mathrm{~mA} / \mathrm{cm}^{2}$ with $200 \mathrm{mg} / \mathrm{l}$ of added gelatin enabled to obtain a turbidity of 3.4 NTU which is still too high. However, if we compare these values with values reported in the literature for flotation by dispersed gas (Ferrarini et al., 1997), it seems that EF is more efficient than flotation in decreasing the juice turbidity. Ferrarini et al. (1997) reported turbidity values after flotation equal to approximately $10 \%$ of the turbidity of the unclarified juice, while our results for EF at $20 \mathrm{~mA} / \mathrm{cm}^{2}$ with $200 \mathrm{mg} / \mathrm{l}$ of added gelatin indicated a $99 \%$ decrease in juice turbidity. Furthermore, for the experiments carried out by Ferrarini et al. (1997) larger amount of fining agent were used ( $70-150 \mathrm{mg}$ of gelatin/l, 400$800 \mathrm{mg} / \mathrm{l}$ of silica sol and $200-500 \mathrm{mg} / \mathrm{l}$ of bentonite). The results reported by the authors also suggest that improvement of the juice
clarification could be achieved by combining the EF treatment with a tangential flow ultrafiltration step.

## 5. Conclusion

Our study has demonstrated that the EF with the addition of small amount of clarifying agents enables a quick flotation of the suspended matter present in the juice, when compared to traditional flotation.

The EF was efficient to partially reduce the tannins content of the juice, while a significant decrease of the protein content was observed between the unclarified juice and the clarified juices. However, this decrease in protein content would be directly attributable to the use of pectinases, which results in the formation of protein-protein complexes and precipitation. Most of the turbidity values reported for the clarified apple juices are lower than 10 NTU but they are higher than the value of 2 NTU required to obtain a stable juice. The pH and Brix degree of the apple juice was not affected by the EF treatments, while the color was improved when EF was carried out with addition of small amount of gelatin. By combining EF with an ultrafiltration step it would be possible to reduce the turbidity value of clarified apple juice to value of less than 2 NTU. The ultrafiltration permeate flux would possibly also be improved by the EF pre-treatment.

## Acknowledgements

The financial support of A. Lassonde, Inc. and Agriculture and Agri-Food Canada is acknowledged. We thank M. Anh Lam Quoc for his assistance with the design of the electroflotation cell.

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    * Corresponding author. Tel.: +1 450768 3297; fax: +1 4507738461.

    E-mail address: mondorm@agr.gc.ca (M. Mondor).

[^1]:    The values represent the means $\pm$ standard deviations $(n=2)$.
    ${ }^{\mathrm{a}} \mathrm{EF}$ treatments during 30 min .
    ${ }^{\mathrm{b}}$ Gelatin concentration at $200 \mathrm{mg} / \mathrm{l}$.
    ${ }^{\text {c }}$ Measurements were carried out in the EF cell.

