Pulsed electric field assisted juice extraction from alfalfa

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Gachovska, T.K., Ngadi, M.O. and Raghvan, G.S.V. 2006. **Pulsed electric field assisted juice extraction from alfalfa.** Canadian Biosystems Engineering/Le génie des biosystèmes au Canada **48**: 3.33 - 3.37. The objective of this study was to evaluate the effect of pulsed electric field (PEF) treatment on alfalfa juice extraction. Alfalfa mash was produced by blending alfalfa leaves. Approximately 40 g of the mash were subjected to PEF treatment of 200 pulses at 1 Hz in two successions. Each PEF treatment was followed by pressure application at 2 and 4 MPa, respectively. White protein was separated from green protein and the mineral content of the various components of the samples was determined. PEF treatment increased extraction of juice from alfalfa mash by 38% compared to non-PEF-treated samples. Dry matter, protein content, and mineral content of PEF treated samples were significantly ($P<0.05$) higher than non-PEF samples. Extraction of white protein was enhanced by the PEF treatment. **Keywords:** pulsed electric field, extraction, alfalfa juice, protein, minerals.

L'objectif de cette étude fut d'évaluer l'effet d'un traitement par champ électrique pulsé (CEP) sur l'extraction de jus de luzerne. Des feuilles de luzerne ont été hachées au boyeur-mélangeur. Des échantillons de 40 g de luzerne hachée ont été traités par CEP en utilisant 200 impulsions à une fréquence de 1 hertz, et ce en deux successions. Chaque traitement de CEP a été suivi d'une application de pression de 2 ou de 4 MPa. La protéine blanche a été séparée de la protéine verte et la teneur en minéraux des diverses composantes des échantillons a été déterminée. Le traitement par CEP a augmenté de 38% l'extraction du jus des feuilles de luzerne en comparaison des échantillons traités par pression seulement. La matière sèche, la teneur en protéines et la teneur en minéraux des échantillons traités par CEP furent significativement ($P<0.05$) plus élevées que pour les échantillons de luzerne traités par pression seulement. Le traitement de la luzerne par CEP a augmenté l'extraction de la protéine blanche. **Mots-clés:** champ électrique pulsé, extraction, jus de luzerne, protéine, minéraux.

INTRODUCTION

Alfalfa juice has been noted for its high nutritional qualities especially in terms of its crude protein (15 to 20%), vitamins (A, D, E, K, C, B1, B2, B6, B12, Niacin, Panthothanic acid, and others) and different kinds of minerals like phosphorus, calcium, sulfur, magnesium, and others (Ensminger and Olentine 1978). Alfalfa sprouts are directly consumed by humans whereas alfalfa juice is widely used as a supplement in some health food products. In particular, concentrates that are high in protein can be obtained from alfalfa juice. Whole leaf protein concentrate (LPC) from alfalfa has been shown to cure symptoms of kwashiorkor, the protein deficiency disease (Savoie and Beauregard 1991). This places alfalfa juice products in the category of foods that can be referred to as nutraceuticals or functional foods.

Raw extracted alfalfa juice has two types of protein namely “green” and edible “white” (Koegel and Straub 1996). The white protein has good nutritional properties while the green has not been accepted as a food because of its texture, color, and grassy flavor (Knuckles and Kohler 1982). The separation of green protein from the white type can be achieved by heating, centrifugation, and filtration (Betschart 1977; Knuckles and Kohler 1982).

Much research work has been done on alfalfa juice extraction (Duran and Nunez-Arenas 1988; Mills 1984; Sinha et al. 2000) using different mechanical methods of pressing, but the full quantity of juice has not been extracted. Sinha et al. (2000) extracted approximately 52% of total water content of alfalfa using pressures greater than 4 MPa. Juice extraction during pressing of plant material such as apple was reported to increase when pressure was held constant for more than 5 min (Bazhal et al. 2001).

High pulsed electric field has been used to enhance juice yield from apple, carrot, beet, and other fruits and vegetables (Bazhal and Vorobiev 2000; Eshtiaghi and Knorr 2002). When a plant is treated with high pulsed electric field, the cell membranes are ruptured leading to an increase in permeability of the cell walls and subsequent increase in juice yield (Eshtiaghi and Knorr 2002). A combination of pressing method and pulsed electric field may enhance juice extraction.

The objective of this work was therefore to compare juice quantity, protein, and mineral content of alfalfa juice extracted using two methods, namely: mechanical pressing and combination of high pulsed electric field pre-treatment and mechanical pressing.

MATERIALS and METHODS

Approximately 200-mm long fresh alfalfa stems with leaves were obtained at pre-blossoming stage from Macdonald Campus farm, McGill University in the fall of 2004. They were sorted, cleaned, and comminuted with a domestic food processor (Oster, Sunbeam Products Inc.) for 5 min to obtain a homogenous mash. The mash was kept in a closed two-liter vessel to prevent evaporation prior to use. The mash was properly mixed before every experiment to obtain a homogenous mix. The mash had initial moisture content of 68% wet basis.

Alfalfa pressing

Approximately 40 g of the alfalfa mash was introduced into a cylindrical treatment chamber which was used for both high

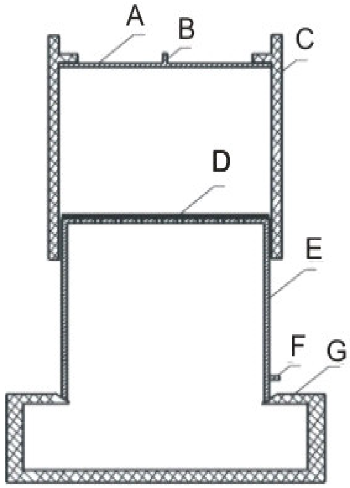

Fig. 1. Schematic of pulsed electric field treatment and pressing chamber. Labels: A - stainless steel disc base (upper electrode), B - electrical connection outlet on disc electrode, C - plastic cylinder, D - stainless steel sieve, E - stainless steel perforated cylindrical plunger (lower electrode), F - electrical connection outlet on cylindrical electrode, G - plastic container for collection of expressed juice. During pressing, the plunger sits on top of the juice container (G). The plastic cylinder (C) containing samples is pushed through the plunger to extract juice.

voltage treatment and juice extraction. Figure 1 is a schematic diagram of the chamber and a juice collector. The treatment chamber consisted of an insulated short cylinder made of plastic (polyoxymethylene, derlin), cylindrical plunger, and a disc base that formed the electrodes during PEF treatment as well as rigid structure for juice pressing. The perforated cylindrical plunger and the base were made of stainless steel. Extracted juice was filtered through a stainless steel sieve placed on top of the perforated plunger. Juice extracted during pressing was collected in a plastic collector placed under the treatment chamber. The volume of the treatment chamber was 78.5 mL Pressure was applied in two stages using the Instron Universal Testing Machine (Model 4502, Instron, Norwood, MA) with the Series IX software. In the first stage of pressing, the filled treatment chamber was pressed until a pressure of 2 MPa was recorded. It was then held at this pressure for 5 min. In the subsequent second stage, the pressure was increased to 4 MPa and held for 5 min for further tissue breakdown and juice yield (Bazhal and Vorobiev 2000).

The weights of alfalfa juice and mash residue were measured after each stage of pressing and the percentages were calculated. Alfalfa juice was collected in flacon tubes and the mash residue was kept in plastic zipped bags for chemical analyses. All procedures were carried out in triplicates and the mean values and standard deviations were calculated.

High pulsed electric field treatment

Both pressing and pulsed electric field treatment steps were accomplished using the same treatment chamber. This type of setup should be more amenable to industrial application. Pulsed electrical field treatment of the alfalfa mash was achieved by using a pulsed electric field generator. The circuitry of the generator is shown in Fig. 2. A variable autotransformer (Powerstat Type 3PN116C 0-140 V, Superior Electric Co., Bristol, CT) was used to supply the voltage to the circuit. The input voltage was regulated by the autotransformer to obtain a pulse frequency of 1 Hz (Eshtiaghi and Knorr 2002). The voltage was then transformed by a high voltage transformer (Model L 9862159A –115/10,000V, Canadian General Electrical Company Ltd., Toronto, ON) and rectified by a high voltage diode. The resistor was used to limit the current that passed through the bank of capacitors used to store the energy. The initial treatment voltage applied to the treatment chamber depended on the distance between the 15-mm diameter stainless steel sphere discharger. The break voltage for this diameter can be calculated using Eq. 1.

$$V_0 = 4.85\ell_{sph}^{0.75} \quad (1)$$

where:

ℓ_{sph} = distance between the spheres (mm), and
V_0 = break voltage, that is voltage applied to the treatment chamber.

For PEF treatment, the first stage of mechanical pressing was preceded by application of 200 pulses electric field with charged 1μF capacitor supplying 6 kV. The distance between the treatment electrodes was 40 mm. This gap between the electrodes was reduced to approximately 10 mm after the first pressing stage. Additional PEF treatment was applied before the second pressing stage. All treatments (pressing and PEF) were completed within 25 min.

Protein separation process

The separation of green protein from white protein was achieved by heating the alfalfa juice in a water bath to 62°C and holding for 2 min (Knuckles and Kohler 1982). The temperature was monitored using a thermocouple (Type K, Omega Engineering Inc., Stamford, CT). The juice was centrifuged at 900 rpm for 10 min and then filtered with a Nalgene 0.45 μm syringe filter. The filtrate (white protein) and the residue (green protein) were collected for chemical analysis.

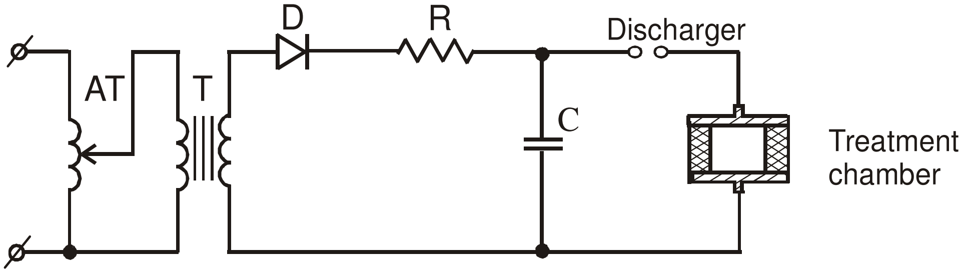

Fig. 2. Electrical circuit diagram for the pulsed electric field generator used to treat alfalfa mash.

Chemical analysis

The moisture contents of four different groups of samples were

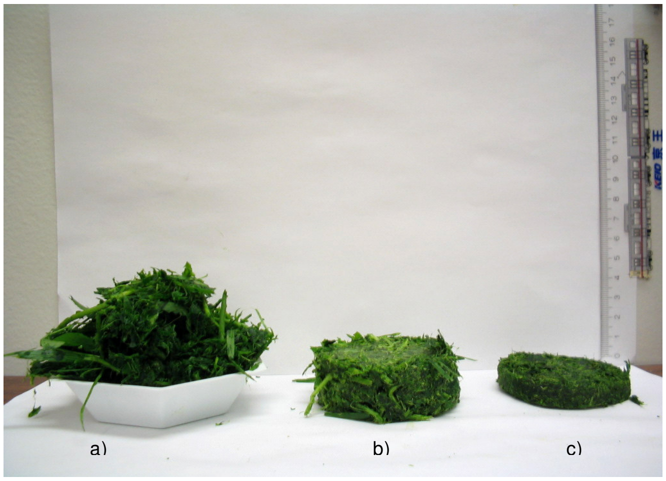

Fig. 3. Typical pictures of (a) alfalfa mash before pressing, (b) untreated mash residue after pressing, and (c) treated mash residue after pressing.

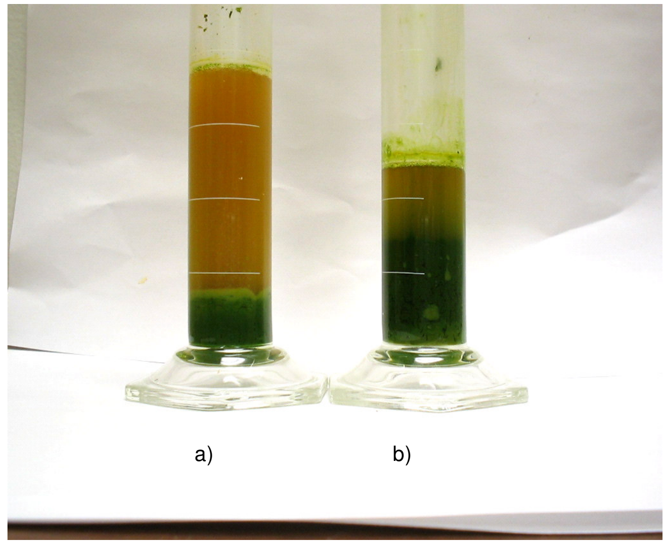

Fig. 4. Illustration of the relative quantities of juice extracted from alfalfa mash: (a) juice from PEF-treated alfalfa mash and (b) juice from untreated alfalfa mash.

measured. The first group included untreated alfalfa mash, residue from both the untreated and treated alfalfa mash after pressing. The second group was juices extracted from both untreated and treated alfalfa. The third group consisted of residue from filtration of untreated and treated juice, and the fourth group was made up of filtrates from the untreated and treated juice. Freeze-drying was used to determine moisture content of the samples. Samples were frozen to -50ºC in a freezer (-86°C ULT Freezer ThermoForma, Thermo Electron Corporation, Waltham, MA). They were then transferred into a freeze-dryer (Thermo Savant ModulyoD 115, Thermo Electron Corporation, Waltham, MA) to dry for 48 h. Moisture content was determined based on the wet weight.

Protein and mineral contents of three groups of samples were measured. The first group was untreated alfalfa mash, residue from both the untreated and treated alfalfa mash after pressing. The second group was juices extracted from both untreated and treated alfalfa. The third group was filtrates from both the untreated and treated juice. The micro - Kjeldahl method was used for crude protein determination (Chang 1998). About 0.5 g of ground dry product was weighed accurately into a digestion tube. Two 3.5-g K_2SO_4 tablets were added with selenium, which acted as a catalyst. Twelve milliliters of concentrated sulfuric acid and 5 mL of hydrogen peroxide were also added. The digestion process continued for 20 min at 420°C. The solution was then cooled and distilled and the steam was collected in a flask containing 25 mL of 4% boric acid solution. Titration was made with 0.2N HCl. The total nitrogen was calculated based on corrected acid volume used and the mass of the sample (Chang 1998).

Mineral content for the four groups was determined by ashing approximately 0.5 g of each dry sample. All measurements were made in duplicate; mean and standard deviation were calculated.

RESULTS and DISCUSSION

Visual observation of the pressed alfalfa cake showed that the PEF-treated samples were smaller than the non-PEF-treated (Fig. 3). The samples were compressed more than the non treated samples. This is indicative of higher weakening and disruption of the plant cellular structure as a result of electroplasmolysis. It was also observed that the PEF-treated mash cake dripped water after storing for a few minutes (within 20 min) in a zipped bag whereas the non-PEF-treated mash cake did not show any dripped water until after 24 h. Again, this observation supports the notion that the PEF treatment reduced the turgor pressure and elastic modulus of the plant membranes (Bazhal et al. 2004). Other authors have suggested that elastic collapse of cellular materials induced by electric field can result in significant reduction in membrane thickness and influence their osmo-regulation mechanism (Coster et al. 1976). The reduction in turgor pressure and decrease in membrane thickness apparently decreased the moisture holding capacity of the PEF-treated samples and allowed them to easily give up moisture without further application of pressure. Thus, PEF may enhance drying of the pressed mash cake. Similar effect of PEF treatment on water drip and subsequent acceleration of drying was observed for tobacco leaves (Armyanov et al. 2001) and potato (Arevalo et al. 2004).

More juice was extracted from the PEF-treated mash as shown in Fig. 4. Sedimentation of solids occurred in the juice extracted from the PEF-treated samples after about 12 h while no separation was noticed in the juice extracted from non-PEF-treated mash until after about 36 h. Also, there was a quick separation of the white and green fractions in the alfalfa juice. PEF treatment enhanced separation of white and green protein in the alfalfa juice.

Results of mean yield of pressed alfalfa mash are presented in Table 1. Statistical analysis showed that there were significant differences ($P<0.05$) between average values of extracted juice, dry material, and mineral content for PEF-treated and non-treated alfalfa mash. The data showed that pulsed electric field treatment increased quantity of extracted alfalfa juice by 37.6% compared with untreated. After the first stage of pressing at 2 MPa, the increase was 46.1%. However, the increased juice

Table 1. Average yield of pressed alfalfa mash at two pressures. Moisture content of fresh alfalfa was 68% wb.

	Juice extraction (% by mass)		
	2 MPa	4 MPa	Total
Untreated alfalfa	18.8	8.5	27.3
Treated alfalfa	27.5	10.1	37.8

yield was reduced to 19.8% after the second pressure (4 MPa) application. Approximately 33.1 and 44.9% of total water in the alfalfa mash were removed from the non-PEF-treated and the PEF-treated samples, respectively. Sinha et al. (2000) reported a higher juice extract of about 51.2% from a process of double maceration of alfalfa leaves and pressing at 4 MPa. The moisture content of the alfalfa samples used in the study by Sinha et al. (2000) was 75.7% (wet basis) whereas the moisture content of samples used in this study was 68.1% (wet basis). The difference between juice extraction from alfalfa in the different studies may be attributed to differences in initial moisture content of the samples (Mills 1984; Savoie and Beauregard 1991). The overall result showed a good prospect for PEF application in extraction of juice from alfalfa leaves.

Approximately 40 g of alfalfa mash was used for each extraction and results of quantity of the samples are shown in Table 2. Treated alfalfa residue had a lower moisture content of 58.8% compared to untreated alfalfa residue and alfalfa mash. This is again due to increased juice yield as a result of PEF treatment (Bazhal and Vorobiev 2000; Eshtiaghi and Knorr 2002). The moisture contents of the untreated and treated juice were similar at 82.7 and 81.3%, respectively. Also, similar moisture contents were obtained for filtrates from the treated and untreated juice (82.2 and 82.0%, respectively). This implies that the percentages of dry matter (DM) in treated and untreated juice are the same. PEF treatment only enhanced the quantity of extractable juice and not the dry matter. The moisture content ratio remained the same for both treated and untreated samples. Chemical composition of the different alfalfa samples are presented in Table 3.

Protein content of the alfalfa mash was 25.5%. There was a slight but significant ($P<0.05$) reduction in the protein content of the treated mash residue. The resulting juice from the treated mash had higher protein content. Thus, PEF treatment increased the quantity of protein extractable from alfalfa leaves by 57%.

Table 2. Mass balance of the different alfalfa samples obtained from 40 g of alfalfa mash with initial moisture content 68% wb.

	Mass (g)	Moisture content (% wb)
Mash	40.1 (0.08)*	68.1 (0.3)
Untreated mash residue	29.1 (0.23)	62.3 (0.86)
Treated mash residue	25.0 (0.25)	58.8 (0.88)
Untreated juice	10.9 (0.15)	82.7 (1.34)
Treated juice	15.8 (0.19)	81.3 (1.25)
Untreated juice residue	2.2 (0.21)	74.3 (0.43)
Treated juice residue	2.7 (0.16)	67.8 (0.55)
Untreated filtered juice	7.3 (0.13)	82.2 (1.01)
Treated filtered juice	11.4 (0.18)	82.0 (0.98)

*Values in parentheses are standard deviations.

The mineral content of treated mash residue was significantly lower than the mineral content of the untreated mash residue. Consequently, the mineral content of the juice extracted from the treated mash was higher than the juice from the non-treated mash. PEF apparently enhanced mineral extraction from alfalfa by 73%.

Table 3. Chemical composition of the different alfalfa samples obtained from 40 g of alfalfa mash with initial moisture content 68% wb.

	Protein (% mass DM)	Protein (g)	Minerals (% mass DM)	Minerals (g)
Mash	25.5 (0.25)*	3.3	7.0 (0.12)	0.9
Untreated mash residue	26.0 (0.48)	2.9	6.0 (0.07)	0.7
Treated mash residue	24.3 (0.71)	2.5	5.7 (0.05)	0.6
Untreated juice	29.4 (0.15)	0.6	10.3 (0.12)	0.2
Treated juice	27.2 (0.05)	0.8	11.4 (0.11)	0.3
Untreated filtered juice	21.9 (0.42)	0.3	11.4 (0.12)	0.2
Treated filtered juice	21.4 (0.32)	0.4	12.8 (0.10)	0.3

CONCLUSION

From the conducted experiments and analysis we can conclude that PEF treatment resulted in the breakdown of the cell membrane of alfalfa leaves which in turn enhanced juice extraction by 37.8% more than the untreated sample. PEF treatment increased the quantity of dry matter obtainable from alfalfa juice, alfalfa juice residue, and alfalfa juice filtrate. Protein and mineral contents in the treated samples were significantly ($P<0.05$) higher than the untreated samples.

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