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Enhanced Anthocyanin Extraction from Red Cabbage Using Pulsed Electric Field Processing

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Abstract

This study was conducted to evaluate the effect of pulsed electric field (PEF) treatment on anthocyanin extraction from red cabbage using water as a solvent. Mashed cabbage was placed in a batch treatment chamber and subjected to PEF (2.5 kV/cm electric field strength; 15 μs pulse width and 50 pulses, specific energy 15.63 J/g). Extracted anthocyanin concentrations (16 to 889 μg/mL) were determined using HPLC. Heat and light stabilities of the control and PEF-treated samples, having approximately the same initial concentrations, were studied. PEF treatments enhanced total anthocyanin extraction in water from red cabbage by 2.15 times with a higher proportion of nonacylated forms than the control ($P < 0.05$). The heat and light stabilities of the PEF-treated samples and control samples were not significantly different ($P > 0.05$).

Keywords: anthocyanin, extraction, pulsed electric field, red cabbage

Practical Application: An innovative pretreatment technology, pulsed electric field processing, enhanced total anthocyanin extraction in water from red cabbage by 2.15 times. Manufacturers of natural colors can use this technology to extract anthocyanins from red cabbage efficiently.

Introduction

Anthocyanins are a group of natural phenolic compounds found in plants. They are water-soluble pigments responsible for the blue, purple, and red color of plant fruits, flowers, and leaves (Strack and Wray 1993). They can be found in grapes, red currants, black currants, raspberries, strawberries, apples, cherries, red cabbage, red-flashed potato, radish, and aubergines (Henry 1996; Rodriguez-Saona et al. 1999).

More than 250 different anthocyanins have been found in nature and 8 to 23 of them have been found in red cabbage (Markakis 1982; Baublis et al. 1994; Wu et al. 2006; Charron et al. 2007). The anthocyanin composition of red cabbage is very complex, but the major anthocyanins are based on a core structure of cyanidin-3-O-diglucoside-5-O-glycoside (Wu and Prior 2005).

There has been an increased interest in red cabbage pigments as a source of natural food colorants since the mid-1970s (Salunkhe and Kadam 1998). Pigments from red cabbage are used widely in Japan and are available from European and Canadian companies. Applications for red cabbage colorants include reconstituted juices, beverages, jams (with pectin), fruits preparations, yogurts, confectionery, candies, dry mixes, chewing gum and a variety of sauces (Chigurupati et al. 2002).

The daily consumption of anthocyanins in human diets has been estimated to be up to 200 mg/d (Wu and Prior 2005). Consumption of anthocyanin-rich fruit and vegetables and drinking of juice or wine have been associated with lower risk of coronary heart disease and cancer (Duthie et al. 2000). It also has been demonstrated that anthocyanins protect against DNA damage induced by oxidative agents (Lazzè et al. 2003). The health benefits of anthocyanins are well known due to their antioxidant properties. The antioxidant and antihyperglycemic properties of red cabbage extract may offer a potential therapeutic source for the treatment of diabetes (Kataya and Hamza 2007). In contrast, some artificial colorants used in food processing have been shown to have adverse health effects (Damasceno 1988). For these reasons, consumer demand for natural colorants has been on the increase. Several technologies have been proposed for enhancing extraction of phytochemicals of plant and vegetable origins. Usaquén-Castro et al. (2006) used ultrasound to assist in extraction of polyphenols from red-grape residues. Microwave pretreatments improved extraction of anthocyanins in red raspberries (Sun et al. 2007). High-pressure extraction of anthocyanins from red grape pomace with carbon dioxide and cosolvents has been shown to increase their yield significantly (Mantell et al. 2003).

Pulsed electric field (PEF) has been studied widely for microbial inactivation (Jin and Zhang 1999; Walkling-Ribeiro et al. 2009), enhanced juice extraction (Jemai and Vorobiev 2006; Lopez et al. 2008), and accelerating drying processes of fruits and vegetables (Ade-Omowaye et al. 2003; Shynkaryk et al. 2008). Applying PEF to biological cells above a threshold level can result in electroporation of the cell membrane or wall. This process leads to an increase in permeability of the cell walls and easier release of the intracellular contents (Knorr and Angersbach 1998). The degree of electroporation depends on many factors including product constituents, electric field intensity, type of pulse waveform, treatment time, and the pulse number (Jeyamkondan et al. 1999). PEF treatments in conjunction with fruit and vegetables processing were first reported in the mid-1900s (Flaumenbaum 1949). Enhanced juice yields from apples, carrots, beets, alfalfa, and

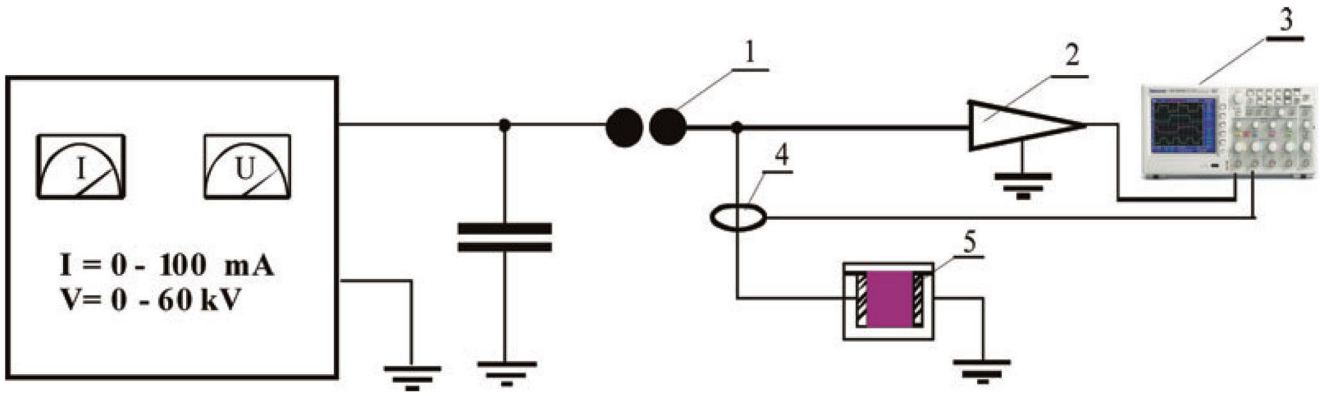

Figure 1. Electrical circuit for pulsed electric field treatment of cabbage: 1 = spark gap switch; 2 = high voltage probe; 3 = oscilloscope; 4 = current probe; 5 = treatment chamber.

other fruits and vegetables with PEF pretreatment have been reported (Gachovska et al. 2006). Chalermchat et al. (2004) used PEF to increase pigment extraction in red beet root. Gachovska et al. (2006) reported increased minerals extraction from alfalfa after PEF treatment.

The use of PEF to enhance anthocyanin extraction in red cabbage has not been reported. In this study, the use of PEF treatment of red cabbage mash to enhance the release of anthocyanins was evaluated. The objective of this study was to determine the effect of PEF treatment on the yield of anthocyanins from red cabbage and to compare the temperature and light stability of the anthocyanin obtained by PEF-treated cabbage mash and control, nontreated mash.

Materials and Methods

Fresh red cabbage was purchased from a local supermarket and stored at 4 °C for less than 2 d. The cabbage was removed from the refrigerator and held at room temperature (22 °C) for 2 h before processing. Preparation of the cabbage prior to PEF extraction included of cabbage chopping and discarding of the stems, which contained little pigment, and subsequent mashing with a domestic food processor (MFP 200, Mini-Pro™, Black & Decker, China) for 1 min to obtain a homogenous mash without any addition of water. There was no measurable temperature increase during mashing. Approximately 42 g of the mash were placed in a PEF treatment chamber.

PEF treatment

PEF treatment of the cabbage was accomplished using an exponential decay PEF generator. The generator contained a DC power supply (CF60/25-12C, Hipotronics, Inc., Brewster, N.Y., U.S.A.), a capacitor (General Atomics Electronic systems, San Diego, Calif., U.S.A.) and a spark gap switch (Figure 1). The energy stored in the capacitor was discharged through a parallel plate treatment chamber (Figure 2). The voltage applied to the chamber was measured with a voltage probe (Model, P6015 A, Tektronix, Inc., Beaverton, OR, U.S.A.). The chamber was made from 2 stainless steel electrodes separated by an insulation material (Deorin, American Plastics Supply & Fabrication, Omaha, Nebr., U.S.A.). The distance between the electrodes was 2 cm and the electrode area was 30 cm^2. PEF treatment parameters of the cabbage samples were chosen based on preliminary experiments. In the literature, researchers have used the voltage in the range of 0.5 to 5 kV/cm for treating plant materials (Eshtiaghi and Knorr 2002; Gachovska et al. 2006) using PEF. For the treatment chamber with

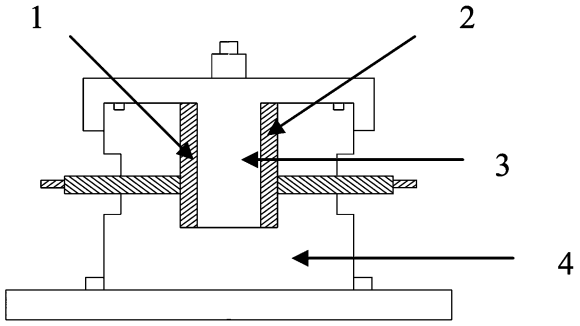

Figure 2. Sketch of the pulsed electric field treatment chamber: 1 = high voltage electrode; 2 = ground electrode; 3 = sample; 4 = insulator/spacer.

samples used in this study, there was no dielectric breakdown until the electrical field strength of 2.5 kV/cm for 1 μF capacitance of the discharge capacitor and therefore 2.5 kV/cm was used in the further study. For selecting the number of pulses, the electrical impedance of the treatment cell with the sample was measured every 5 pulses. Impedance is an indirect measure of damage done to plant cells. After 50 pulses, the impedance did not change. Therefore, the number of pulses was selected as 50 in this study. The pulse width was approximately 15 μs and the frequency of the pulse was 1 Hz. Treatment time is the product of pulse width and the number of pulses and therefore the treatment time was 0.75 ms. There was negligible temperature raise (<1 °C) due to the PEF treatment.

Color extraction

A mass of 40 g of the mashed, PEF-treated, or untreated cabbage were weighed and placed in a 250 mL glass flask. ASTM Type II, distilled deionized organic-free purified water (100 mL) was added and the contents in the flasks were rotated end-over-end a few times and placed in a dark box for 4 h at 22 °C. Prefiltering was done using a coffee filter to remove large particles. The samples were separated by filtration using Whatman Puradisc™ 13 mm (0.2 μm pore size) syringe filters and held at 4 °C until HPLC analyses and for evaluation of the thermal and light stabilities.

HPLC analysis

Chromatographic analyses were performed using HPLC (Waters 2695, Milford, Mass., U.S.A.) equipped with an autosampler/injector and a photodiode array (PDA, Waters 2996) detector as per the method by Wu and Prior (2005). Separa-

tion of the gradient was conducted using a reversed phase HyPURITY C18 column (4.6 mm × 250 mm, 5 μm Thermo Scientific, Waltham, Mass., U.S.A.). Before HPLC analyses, the samples were acidified by adding 0.5 mL of concentrated (12 N) HCl to the extract (20 mL). The acidified extract (2 mL) was filtered through a 0.45 μm poly (tetraflurothylene) membrane (Pall Life Science, Ann Arbor, Mich., U.S.A.) and transferred to amber 12 × 32 mm autosampler vials immediately prior to HPLC analysis. The solvents used were mobile phase A—5% formic acid in water and mobile phase B—5% formic acid in methanol, with a flow rate of 0.8 mL/min. The method was optimized to provide separation and detection of standard compounds (3-O-β-glucopyranoside conjugates of pelargonidin, cyaniding, delphinidin, petunidin, and malvidin (Polyphenols Laboratories, Sandnes, Norway). The gradient settings are listed in Table 1.

Mass spectrometric analyses of selected cabbage extracts were obtained using a Waters 2695 HPLC coupled to a Finnegan LCQ IT/MS (San Jose, Calif., U.S.A.) operated in a positive electrospray mode. A HyPURITY C18 column (2.1 × 250 mm, 5 μm, Thermo Scientific) was used to separate the cabbage pigments at a flow rate of 0.2 mL/min. Binary gradients of 5% formic acid in water (solvent A) and a 5% formic acid in methanol (solvent B) were used, as listed in Table 2. The mass spectrometer settings were: capillary temperature of 150 °C, sheath gas flow rate of 70 (N_2, arbitrary units); auxiliary gas flow rate of 15 (N_2, arbitrary units); spray voltage of 4.5 kV and capillary voltage of 25 V.

Thermal and light stability

The thermal and light stabilities of the extracts from the control (untreated) and PEF-treated samples were studied. The absorbance of the extracts from the PEF-treated and untreated samples were measured using a UV spectrophotometer (Spectronic Genesys 5 UV/Vis, Milton Roy Co., Ivyland, Pa., U.S.A.). Because the PEF-treated samples had approximately twice the concentration of anthocyanins, water was added to the PEF-treated sample extracts to obtain approximately the same absorbance as the control samples. From each solution, 2 mL were transferred to each of 12 vials (6 for thermal and the other 6 for light stability). To minimize contamination with oxygen, each vial was closed with a crimped cap and used for only 1 measurement.

Dyrby et al. (2001) studied thermal degradation of anthocyanins from 4 sources (red cabbage, blackcurrants, grape skins, elderberries) at temperatures of 25, 40, 60, and 80 °C and reported that the anthocyanins from red cabbage were more stable than those from other sources and that the thermal degradation of red cabbage was significant only at 80 °C. Therefore, thermal degradation of PEF-treated and control cabbage extracts, having approximately the same concentration, were studied at 80 °C for 5 h in this study.

The vials were placed in a water-circulating bath (W19, Thermo Haake, Germany) and, every hour, one vial of the control and PEF-treated samples was removed, cooled rapidly in ice water and the quantity of anthocyanin was determined using HPLC. The data for the PEF-treated and control samples were fitted using the 1st-order rate law which was in agreement with the results of Sapers et al. (1981) and Shi et al. (1992) for cabbage. The equation for anthocyanin determination is

$$C(t)/C_0 = e^{-kt} \quad (1)$$

where C_0 and $C(t)$ are the anthocyanin concentrations at time 0 and t, respectively, and k is the 1st-order rate constant.

Light is another parameter that affects anthocyanin stability. To investigate the sensitivity of the PEF-treated and control samples to light, all samples (PEF-treated and control samples) were placed randomly under a fluorescent lamp in a light box (106/36/30 cm) at room temperature (22 °C). The light box was lined with aluminum foil and illuminated with a fluorescent lamp (F20T12/D, 20 W, Philips, Salisbury, N.C., U.S.A.). The distance between the samples and the lamp was 30 cm. The test was conducted for 5 d. Every 24 h, 1 vial of each solution was removed and the concentration of anthocyanin was determined using HPLC.

Statistical analyses

Statistical analysis was carried out using Statistical Analysis System (Release 9.1, SAS Inst. Inc., Cary, N.C., U.S.A.). All experiments were conducted in triplicate and a paired t-test was used to test the difference in anthocyanin concentrations between the PEF-treated and control samples at $P \leq 0.05$. A paired t-test was used to test the difference between the total anthocyanin concentrations for the 2 biggest peaks between PEF-treated and control samples for the same time point.

Results and Discussion

As reference standards are unavailable for most anthocyanins, identification of individual chromatographic peaks in the plant extracts was based on comparison of their retention times and mass spectrometer data with the standards used and data published by Wu and Prior (2005) and McDougall et al. (2007). The aqueous extractions were performed for 4 h at room temperature using water as a solvent. It was observed (visually) that not all of the color was removed from the cabbage. The LC/MS analyses of PEF-treated and control samples showed 12 peaks in the chromatograph (Figure 3A and 3B). Wu and Prior (2005) identified 23 anthocyanain compounds in red cabbage. The data from Wu and Prior (2005) were used to identify the anthocyanin compound for each peak (Table 3).

Table 1. Gradient settings used for high-performance liquid chromatography-ultraviolet analyses at a flow rate of 0.8 mL/min.

Time (min)	5% (v/v) formic acid H_2O	5% (v/v) formic acid methanol
0 to 2	84%	16%
2 to 10	84% to 83%	16% to 17%
10 to 15	83% to 82%	17% to 18%
15 to 22	82% to 80%	18% to 20%
22 to 40	80% to 58%	20% to 42%
40 to 54	58% to 30%	42% to 70%
54 to 54.9	30%	70%
55 to 60	84%	16%

Table 2. Gradient settings for high-performance liquid chromatography-mass spectrometry analyses at flow rate 0.2 mL/min.

Time (min)	5% (v/v) formic acid H_2O	5% (v/v) formic acid methanol
0 to 2	87%	13%
2 to 10	87% to 86%	13% to 14%
10 to 15	86% to 85%	14% to 15%
15 to 22	85% to 83%	15% to 17%
22 to 40	83% to 53%	17% to 47%
40 to 49	53% to 30%	47% to 70%
49 to 49.9	30%	70%
50 to 60	87%	13%

Reverse-phase HPLC chromatograms of red cabbage anthocyanin profiles, detected at 520 nm for the control and PEF-treated samples, are presented in Figures 3A and 3B. Concentrations of each compound were estimated by total absorption at 520 nm. PEF treatment of the mashed red cabbage resulted in 2.15 times ($P < 0.05$) the total anthocyanin extraction in water compared to the untreated samples. Improvement in the extraction efficiency of the PEF-treated samples could be attributed to the better mass transfer for the PEF-treated samples compared to the untreated samples. Chalermchat et al. (2004) reported an increase in the total extractable red pigment (betanin) from PEF-treated red beet root presumably due to increased permeability. Gachovska et al. (2006) also reported a 73% enhancement in minerals extracted from alfalfa after PEF treatment. PEF treatment of plant tissue leads to the breakage of the cell membranes resulting in an enhancement of mass transfer and free movement of the intercellular liquid, where most of the anthocyanins are located.

HPLC and LC/MS analyses of the extracts provided an indication of whether or not PEF increased extraction of individual polyphenols. Although acylated compounds typically comprise a larger proportion of anthocyanins, the nonacylated varieties are more available for absorption (Charron et al. 2007). The chromatograms at 520 nm for the control and PEF-treated cabbage samples indicated the presence of at least 12 pigments similar to the anthocyanin peaks described by Wu and Prior (2005). Based on a comparison of LC/MS and HPLC chromatograms, peak 1 from the HPLC chromatograms (Figure 3) was tentatively identified as cyanidin 3-diglucoside-5-glucoside ($[M]^+(m/z) = 773$). The cyanidin 3-diglucoside-5-glu-

Table 3. Tentative identification of anthocyanin peaks in red cabbage and their concentrations for PEF-treated and control samples.

Peak nr	t_R (min) MS	$[M]^+$ (m/z)	t_R (min) HPLC	Anthocyanin	PEF µg/mL	Control µg/mL
1	4.5	773	5.4	cyanidin 3-diglucoside-5-glucoside	646	296
2	5.52	979	8.4	cyanidin 3-(sinapoyl)diglucoside-5-glucoside	180	86
3	9.74	1081	20.95	cyanidin 3-(caffeoyl)(p-coumaroyl)diglucoside-5-glucoside	105	37
4	11.2	1111	23.7	cyanidin 3-(glycopyranosyl-feruloyl)diglucoside-5-glucoside	89	32
5	12.9	1141	26.5	cyanidin 3-(glycopyranosyl-sinapoyl)diglucoside-5-glucoside	44	16
6	13.49	979	29.9	cyanidin 3-(sinapoyl)diglucoside-5-glucoside	72	19
7	21.24	935	31.47	cyanidin 3-(caffeoyl)diglucoside-5-glucoside	105	40
8	31.36	919	36.14	cyanidin 3-(p-coumaroyl)diglucoside-5-glucoside	334	249
9	33.2	979	36.91	cyanidin 3-(sinapoyl)diglucoside-5-glucoside	889	315
10	36.09	1125	38.58	cyanidin 3-(feruloyl)(feruloyl)diglucoside-5-glucoside	209	113
11	36.9	1155	39.16	cyanidin 3-(sinapoyl)(feruloyl)diglucoside-5-glucoside	233	164
12	37.51	1185	39.54	cyanidin 3-(sinapoyl)(sinapoyl)diglucoside-5-glucoside	556	281
13[a]		949		cyanidin 3-(sinapoyl)diglucoside-5-xyloside		
14[a]		1185		cyanidin 3-(sinapoyl)diglucoside-5-(sinapoyl)glucoside		

a. Peaks 13 and 14 were observed only for light stability experiment after 96 of light exposure or longer.

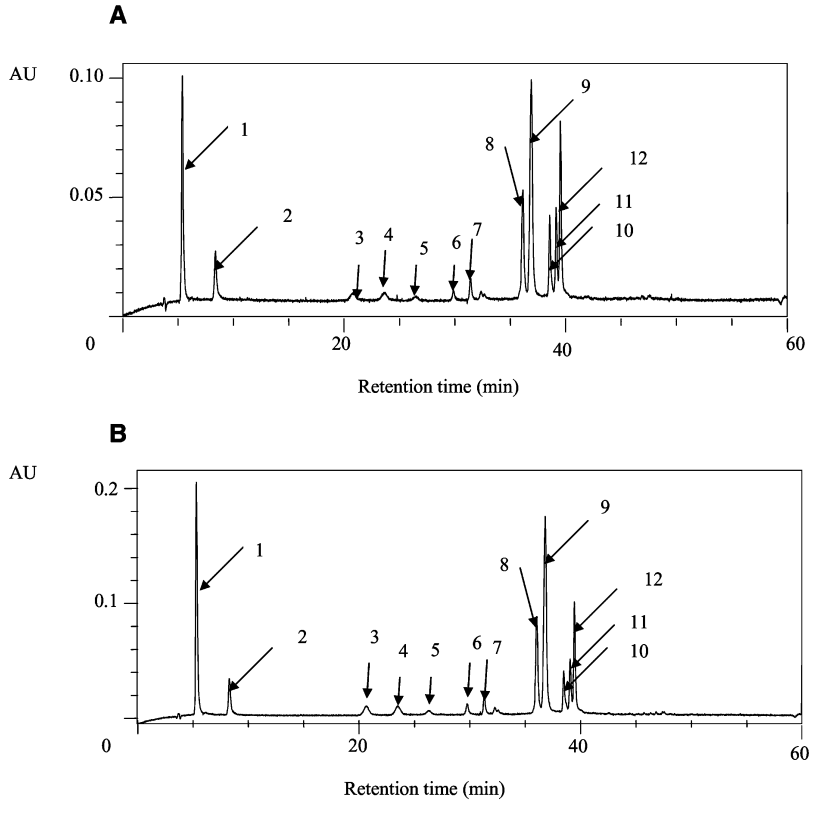

Figure 3. Reverse-phase high pressure liquid chromatography chromatograms of anthocyanin profiles of red cabbage detected at 520 nm for (A) control and (B) pulsed electric field treated samples. The *y*-axis is absorbance unit (AU). Peaks 1 to 12 are defined in Table 3.

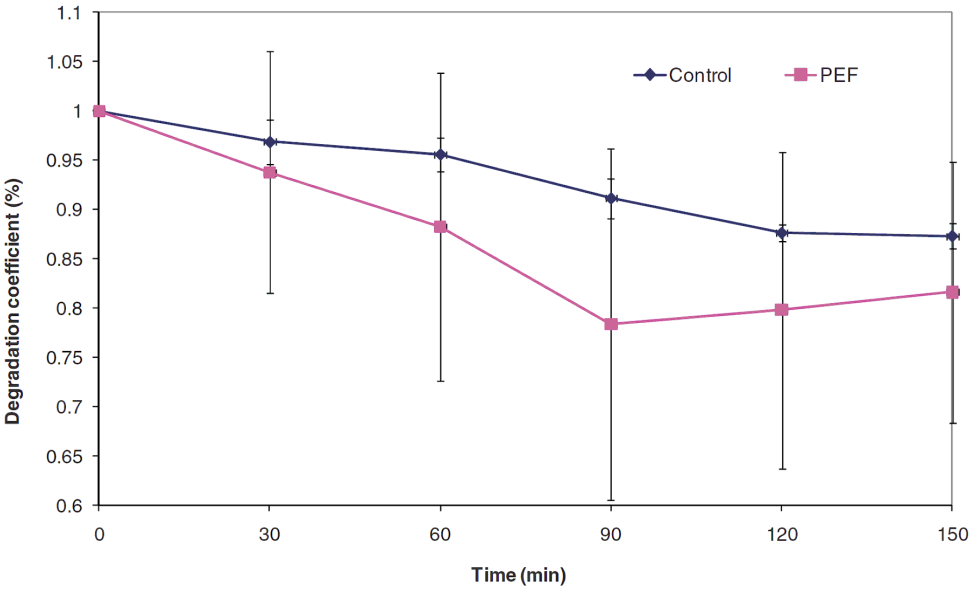

Figure 4. Thermal degradation of the total anthocyanin extracted from control and pulsed electric field (PEF)-treated samples during heating at 80 °C.

coside concentration in PEF-treated samples was 2.18 times higher than that in control samples ($P < 0.05$). McDougall et al. (2007) reported cyanidin 3-diglucoside-5-glucoside as the 1st major peak in red cabbage extracts with photo diode array spectrum maxima at 510 and 280 nm.

The molecular ion of peak 2 ($[M]^+(m/z)$ = 979) was identified as cyanidin 3-(sinapoyl)diglucoside-5-glucoside. There was a significant difference ($P < 0.05$) between the concentrations of the extracted anthocyanin-peak 2 of the PEF-treated samples was 2.11 times higher than that of control. Peak 3 from the chromatograms was identified as cyanidin 3-(caffeoyl)(p-coumaroyl)diglucoside-5-glucoside and had $[M]^+(m/z)$ = 1081, with PEF treatment significantly enhancing this anthocyanin (P = 0.004) by 2.81 times when compared to the control. Peak 4 ($[M]^+(m/z)$ = 1111) was identified as cyanidin 3-(glycopyranosyl-feruloyl)diglucoside-5-glucoside and peak 4 of the PEF treatment was 2.90 times higher than that of control ($P < 0.05$). Peak 5 (cyanidin 3-(glycopyranosyl-sinapoyl)diglucoside-5-glucoside) was found in all 3 replicates of the PEF-treated samples at 44 µg/mL. Only 1 replicate of the control sample contained a small amount of this anthocyanin (16 µg/mL), while the other 2 replicates did not have this anthocyanin. Therefore we could not determine statistical significance due to lack of replicates. Peak 6 ($[M]^+(m/z)$ = 979), identified as cyanidin 3-(sinapoyl)diglucoside-5-glucoside, had 3.77 times higher concentration of this anthocyanin for PEF-treated sample when compared to the control ($P < 0.05$).

Peaks 7 and 8, with retention times of 31.47 and 36.14 min and masses of $[M]+(m/z)$ = 935 and 919, respectively, were identified as cyanidin 3-(caffeoyl)diglucoside-5-glucoside and cyanidin 3-(p-coumaroyl)diglucoside-5-glucoside. The concentration of these 2 cyanidins (peaks 7 and 8) for the PEF-treated samples (105 and 334 µg/mL) were significantly higher than those for control samples (40 and 249) with P value < 0.01. Peak 9, the largest component of both the control and the PEF-treated samples, with a $[M]+(m/z)$ = 979, was identified as cyanidin 3-(sinapoyl)diglucoside-5-glucoside. PEF enhanced this compound significantly by 2.09 times ($P < 0.05$)

Peaks 10 and 11 had $[M]+(m/z)$ = 1125 and 1155 and were identified as cyanidin 3-(feruloyl)(feruloyl)diglucoside-5-glucoside and cyanidin 3-(sinapoyl)(feruloyl)diglucoside-5-glucoside occurring in PEF-treated samples in amounts approximately 1.85 and 1.42 times higher than that in the control samples ($P < 0.05$). Peak 12 in the HPLC chromatograms had $[M]+(m/z)$ = 1185 and was identified as cyanidin 3-(sinapoyl)(sinapoyl)diglucoside-5-glucoside. PEF enhanced the concentration of this compound by 2.05 times ($P < 0.05$). Wu and Prior (2005) detected other pigment peaks which were not identified in either PEF or control extracts.

PEF increased the concentration of all anthocyanins from 142% to 379%, as shown in Figure 3. PEF treatment led rather to an increase of smaller peaks (> 250%) than to bigger peaks. The maximum increase in extraction rate (3.79 times) due to PEF treatment was observed for peak 6, tentatively identified as the nonacylated cyanidin 3-(sinapoyl)diglucoside-5-glucoside. The minimum increase in extraction rate (1.42 times) was observed for peak 8, identified as cyanidin 3-(p-coumaroyl) diglucoside-5-glucoside.

Thermal stability

In most cases, color extracts are used as food ingredients that are thermally processed. Hence, the thermal stability of the color extracts is important. The 1st-order rate constant for thermal degradation of total anthocyanin for PEF-treated samples at 80 °C was 1.88×10^{-3}/h (R^2= 0.99) and for the control samples it was 1.61×10^{-3}/h (R^2= 0.98). The calculated 1st-order rate constants for PEF-treated and control samples were a little smaller but in the same range as calculated by Dyrby et al. (2001) for solutions of cabbage extract in McIlvaine buffer and noncarbonated soft drink ($9 \times 10^{-3}/h^{-1}$).

The degradation coefficients (that is, ratio of the concentration at time t to the initial concentration) for the total anthocyanin and for the 2 largest peaks were calculated. The effects of the degradation coefficients on total anthocyanin in PEF-treated and control samples, as a function of heating time, are shown in Figure 4. The results from the experiment showed that the degradation coefficient decreased, as the heating time increased, as much for the total anthocyanin as for the 2 largest peaks (peak 1: cyanidin 3-diglucoside-5-glucoside and peak 9: cyanidin 3-(sinapoyl) diglucoside-5-glucoside).

The results from the statistical analysis (paired t-test) for the total anthocyanin and 2 of the biggest chromatographic peaks showed that there were no significant differences ($P <$ 0.05) in the degradation coefficients for the same heating time

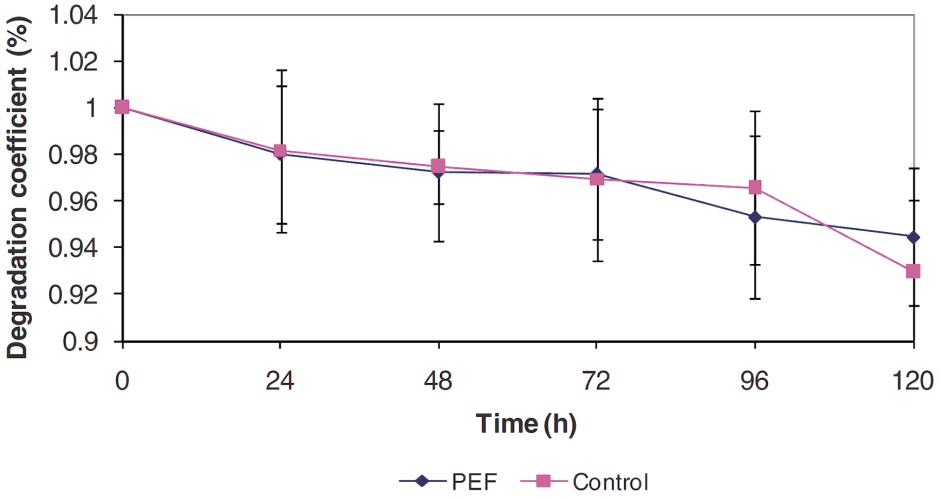

Figure 5. Light degradation of the total anthocyanin extracted from control and pulsed electric field-treated samples during 5 d of exposure to 20-W fluorescent lamp.

between the PEF-treated and control samples. This means that although PEF treatment led to a better extraction efficiency, the degrees of degradation for the PEF and control samples were the same. There was no indication that PEF treatment selectively causes extraction of pigments that are more susceptible to accelerated degradation.

Zhang et al. (2008) studied the stability of PEF-treated cyanin-3-glucoside during various storage temperatures (4, 24, and 37 °C). At 37 °C, they reported a degradation rate constant of 6.24×10^{-4}/h. In this study, we found the degradation rate constant at 80 °C to be 1.88×10^{-3}/h, which is about 3 times higher than the rate reported by Zhang et al. (2008). As the temperature increases, the degradation rate increases and therefore the results of this study are in line with the results reported by Zhang et al. (2008).

Light stability

The resulting degradation coefficients for the total anthocyanin due to light, as a function of time, for PEF-treated and control samples are presented in Figure 5. The statistical analysis (paired *t*-test) showed no significant difference ($P < 0.05$) in the total anthocyanin contents and the 2 largest chromatographic peaks (peaks 1 and 9) for the PEF-treated and control samples for the same illumination time. This suggested that PEF treatment of cabbage did not cause any changes to anthocyanins that would have accelerated light degradation.

Approximately 15% of the total anthocyanin was destroyed after 5 d of illumination of the PEF and controls samples. Sapers et al. (1981) studied storage stability of colorants prepared from red cabbage for 9 mo and concluded that the solutions gradually lost color and the degradation coefficient was approximately 80%. Palamidis and Markakis (1975) studied light stability of anthocyanin from grapes and concluded that more than 50% of total pigments were destroyed when the samples were placed in presence of light for 135 d. The higher degradation rate in our experiment could be explained by the fact that the samples were stored under a fluorescent lamp where the light intensity was 230 ft-c, while the light intensity for Sapers et al. (1981) experiment was 90 to 100 ft-c. The degradation coefficient is proportional to the absorbed intensity (Wayne 1988) and, therefore, a higher degradation was obtained in our study.

The total anthocyanin data for PEF and control samples from the light experiment also were fitted to the 1st-order rate law using equation 2. The 1st-order rate constant for light degradation of total anthocyanin for PEF and for control samples were 1.9×10^{-3}/h (R^2= 0.95) and 1.6×10^{-3}/h (R^2= 0.98), respectively. Ochoa et al. (2001) also fitted the light degradation data from their study to 1st-order rate law and the coefficients of degradation of anthocyanins from the different fruits were $0.036 - 0.055 \times 10^{-3}$/d. The differences in the rate constants between the 2 studies could have been due to the use of approximately 12 times lower light intensity than that used in the current study. Due to the photochemical reaction during the light experiment conducted in the present study, 2 new peaks were observed in the photo diode array spectrum at 520 nm for control and PEF-treated cabbage samples after 96 and 120 h of exposure to light (peaks 13 and 14 in Table 3 and Fig-

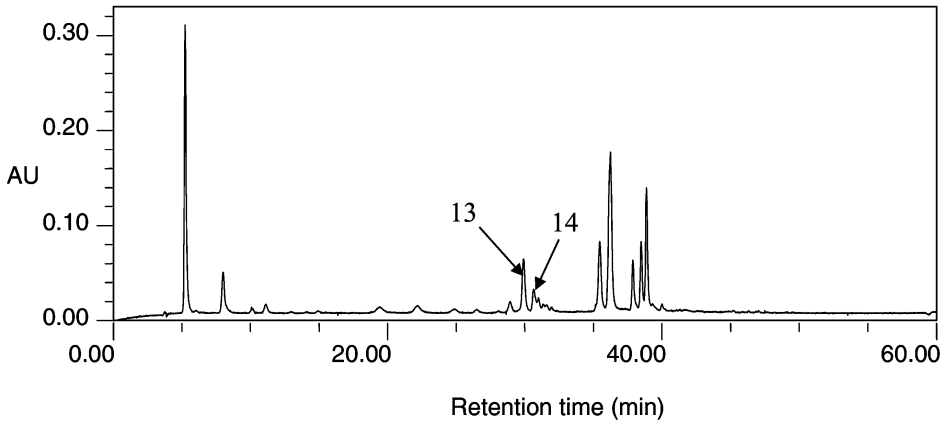

Figure 6. Reverse-phase high pressure liquid chromatogram of anthocyanin profiles of red cabbage detected at 520 nm after 120 h of light exposure. Additional peaks 13 and 14 were observed after light treatment and are defined in Table 3. The *y*-axis is absorption units (AU).

ure 6). The exposure to light not only accelerated the degradation of anthocyanins, but also produced degradation products. Using LC/MS, the peaks were tentatively identified as cyanidin 3-(sinapoyl)diglucoside-5-xyloside and cyanidin 3-(sinapoyl) diglucoside-5-(sinapoyl)glucoside. Their mass-to-charge ratios ([M]+(*m/z*)) were 949 and 1185, respectively. These 2 peaks also were identified by Wu and Prior (2005). The placement of the peaks in the photo diode array spectrum was the same.

Conclusions

Pulsed electrical field (2.5 kV/cm; 15 μs and 50 pulses) enhanced the total anthocyanin extraction from red cabbage by 2.12 times. The enhancement of anthocyanins due to PEF treatment varied for different anthocyanins with a range of 1.34 to 3.77 with higher efficiencies for nonacylated anthocyanins. Thermal and light stabilities of the PEF-treated samples and control samples, having approximately the same initial concentrations, were equivalent. PEF treatment of cabbage did not cause any chemical changes that would accelerate light and thermal degradation.

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