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Effects of Solvent and Temperature on the Extraction of Colorant from Onion (*Allium cepa*) Skin using Pressurized Liquid Extraction

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Abstract: This study focused on the influence of some operative parameters of Pressurized Liquid Extraction (PLE) employed for the extraction of colorants from onion (*Allium cepa*) skin. Extractions were conducted at temperatures ranging from 50 to 150°C with pressure of 1000 psi and two extraction cycles of 15 min. The effect of two extraction solvents (distilled water and 0.1% HCl in methanol (v/v)) was examined. The separation and determination of anthocyanin was carried out by high performance liquid chromatography with diode array detector. Major anthocyanin compounds identified include cyanidin 3-glucoside, cyanidin 3-rutinoside and cyanidin chloride. Video Spectral Comparator (VSC) was used to measure the color quality of the extracts based on CIE system (International Commission on Illumination, Vienna). It was found that the type of extraction solvent and extraction temperature influenced the yield, color and the composition of anthocyanins extracted. Using pressurized solvent extractor, specific color can be obtained by manipulating the type of solvent and temperature of extraction.

Key words: Onion skin, anthocyanins, pressurized liquid extraction

INTRODUCTION

There has been an increasing trend towards replacement of synthetic colorants by natural colorants in the last 20 years because of safety and health benefits. Although, natural colorants are generally less stable and costly than synthetic colorants, their developments and utilization is attracting more and more attention. The continuous increasing markets for natural colorants make it worthwhile to search for and develop new or alternative sources of natural colorants (Zhong *et al*., 2005).

Earlier study reported that the color of red onions is due primarily to anthocyanins present in the epidermal cells of the scale leaves of the bulb and four major anthocyanins, cyanidin 3-glucoside, cyanidin 3-laminaribioside, cyanidin 3-malonylglucoside and cyanidin 3-malonyllaminaribioside were identified (Donner *et al*., 1997). As anthocyanins are soluble in polar solvents, extraction of anthocyanins from plant materials is commonly done using methanol with an addition of small amounts of hydrochloric or formic acid. The acid lowers the pH of solution and prevents the degradation of the non-acylated anthocyanin pigments (Da Costa *et al*., 2000). Conventional extractions of anthocyanins were typically conducted at temperature ranging from 20 to 50°C as temperatures more than 70°C caused rapid degradation of anthocyanins (Ju and Howard, 2003).

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Pressurized Liquid Extraction (PLE) has been used to extract anthocyanins and polyphenols from plant materials (Ju and Howard, 2003; Salces *et al*., 2001). A major advantage of PLE over conventional solvent extraction methods conducted at atmospheric pressure is that pressurized solvents remain in a liquid state well above their boiling points, allowing for high-temperature extraction. The PLE is attracting interest as it features short extraction time, low solvent use, high extraction yields and provides a high level of automation (Breithaupt, 2004). This study aimed at assessing the effect of solvents and temperature on PLE of colorant from onion skin. The yield, color and composition of colorants obtained using PLE were compared to that of conventional soaking method.

MATERIALS AND METHODS

Samples

Onion (*Allium cepa*) skins collected from local stores in September 2006. The samples were dried at room temperature for several days and cut into small pieces (approximately 0.5× 0.5 cm) prior to analysis.

Chemicals

A mixture of anthocyanins standards was prepared containing cyanidin 3-glucoside, cyanidin 3-rutinoside and cyanidin chloride, purchased from BioChemica USA. The solvents used were methanol (Merck, Germany), methanol LiChrosolv (Merck, Germany), methanol Baker Analyzed HPLC solvent (J.T. Baker, USA), hydrochloric acid fuming 37% (Merck, Germany), formic acid 98-100% GR for analysis (Merck, Germany). The dispersing agent used was diatomaceous earth, non-washed, SiO_2 approximately 90% purchased from Sigma-Aldrich, Germany.

Extractions

Pressurized Liquid Extraction (PLE)

Extractions were performed using a pressurized liquid extraction system (Model ASE200, Dionex Corporation) using 22 mL extraction cells with a cellulose filter paper placed at the bottom of each cell. Dried onion skin sample (approximately 2.5 g) was mixed with Diatomaceous Earth (DE) (ratio of 1 g sample to 0.2 g DE), to prevent aggregation of sample particles during extraction, was added into each cell. Each sample was extracted twice with static extraction time of 15 min for each extraction at 1000 psi using the selected solvent and temperature. Extracts were filtered and dried using rotary evaporator at 35-40°C and redissolved in methanol for the extraction using acidified methanol, while extracts obtained from water extraction were redissolved in distilled water for UV-Vis spectroscopic analysis, color measurement and liquid chromatographic analysis.

Soaking Method using Water

Dried samples were soaked in distilled water at 90°C for 3 h (Bhuyan and Saikia, 2005). A liquor ratio of 1:20, corresponding to a ratio of 1 g plant material to 20 mL of water was applied. The extract obtained was filtered using Whatman filter paper No. 1 and dried using a rotary evaporator at 35-40°C. The extract was redissolved in distilled water for UV-Vis spectroscopic analysis, color measurement and liquid chromatographic analysis (Bhuyan and Saikia, 2005).

Soaking Method Using Acidified Methanol

Colorants were extracted from dried samples by soaking in 0.1% HCl (v/v) in methanol for 48 h at room temperature in dark condition (Longo and Vasapollo, 2006). A liquor ratio of 1:20, corresponding to a ratio of 1 g plant material to 20 mL of solvent was applied. The extract was filtered and dried and redissolved in methanol for UV-Vis spectroscopic analysis, color measurement and liquid chromatographic analysis.

Chemical Analysis of Extracts

UV-Vis Absorption

The absorbance of the extracted samples was measured using Shimadzu UV-160 1PC UV-Visible spectrophotometer at wavelength range from 200 to 700 nm.

Color Measurement

Color was measured with Video Spectral Comparator (VSC 5000, Foster and Freeman, UK) and expressed as the CIEL*a*b* coordinates, representing lightness and darkness (L*), redness (+a*), greenness (-a*), yellowness (+b*) and blueness (-b*).

HPLC Analysis

Analysis were performed using Agilent Technologies 1200 series High Performance Liquid Chromatography (HPLC) system equipped with quaternary pumps and a diode array detector. Chromatographic separation was carried out using a ZORBAX SB-C18 column (5 μm, 4.6×250 mm) at 26°C. All samples were filtered through Whatman 0.45 μm prior to liquid chromatography. Compounds were separated by gradient elution using HPLC grade methanol (solvent A) and formic acid:water (5:95) (solvent B). The gradient condition started with 17% A, linearly increased to 30% A at 15 min, then to 23% A at 20 min, followed with 40% A at 30 min and 17% A at 40 min. A volume of 20 μL of sample was manually injected into the system with the flow rate of 1.0 mL min^{-1}. Absorbance spectra was recorded between 250-700 nm. The wavelength used for quantification was 525 nm (4 nm bandwidth; reference wavelength at 700 with 100 nm bandwidth).

RESULTS AND DISCUSSION

Based on preliminary study, the effect of extraction pressure studied at three different pressure settings (1000, 1500 and 2000 psi) was not significant and extraction pressure of 1000 psi was chosen in this study. Similar observations were reported by Luthria (2008) and Zaibunnisa *et al.* (2009).

Effect of Solvent

Extraction of colorant compounds from plant materials was commonly performed by boiling in water without the addition of chemicals or solvents (Bechtold *et al.*, 2003). In this study, the effect of solvent on the yield and quality of colorant was studied using two solvents, 0.1% HCl in methanol (v/v) and water. At various extraction temperatures, the yields of colorant (g colorant/100 g sample) obtained using acidified methanol were slightly higher than those using water (Fig. 1).

Acidified methanol extract showed two absorption maxima (λ_{max}) at 434 and 534 nm, with highest absorption observed for extract extracted at 80°C (Fig. 2). For extraction using water, lower absorption at both wavelengths was observed. The visible maximum absorbance wavelength (λ_{max}) at 534 nm supported the identity of the anthocyanins as no other plant phenolics show absorption between 520 and 546 nm (Da Costa *et al.*, 2000).

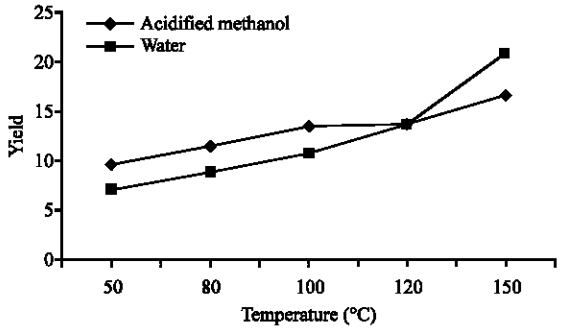

Fig. 1: Yields of extracted colorants (colorant g/100 g sample) using acidified methanol and water at different temperatures with pressure of 1000 psi

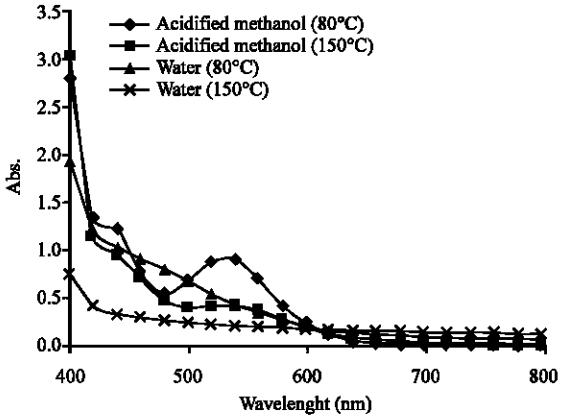

Fig. 2: UV/Vis absorption spectra of onion skins extracts extracted with acidified methanol and water using PLE at 80 and 150°C

Based on earlier studies, the main anthocyanin in the red onion was identified as cyanidin 3-glucoside (Fossen *et al*., 2003). Besides cyanidin 3-glucoside, other major anthocyanins reported were cyanidin 3-laminaribioside, cyanidin 3-malonylglucoside and cyanidin 3-malonyllaminaribioside identified in 4 red onion cultivars (Donner *et al*., 1997). Table 1 compares the composition of anthocyanins extracted using acidified methanol with those extracted using water at various extraction temperatures. Three anthocyanins, cyanidin 3-glucoside, cyanidin 3-rutinoside and cyanidin chloride, were successfully separated from extracts using acidified methanol. However, only two anthocyanins, cyanidin 3-glucoside and cyanidin 3-rutinoside were present in all extracts using water as extraction solvent.

The color parameters in Table 2 indicated that extractions using acidified methanol at various extraction temperatures produced an interesting red shade as the a* values (redness) were higher than the b* values. For extractions using water, the a* values were slightly lower compared to those of acidified methanol extracts obtained at similar extraction temperatures (Table 2). As reported, at low pH, anthocyanins predominantly present in the form flavylium cation giving a reddish color and stable at highly acidic medium and this has probably led to a worldwide use of solvents containing acids for the extraction of anthocyanins from plant organs (Revilla *et al*., 1998).

Table 1: Correlation between composition of anthocyanins and calorimetric data of extracted colorants

	Composition of anthocyanins [ppm (μg mL^{-1})]			
Extraction methods	Cyanidin 3-glucoside	Cyanidin 3-rutinoside	Cyanidin chloride	Calorimetric data
PLE with acidified methanol (80°C, 1000 psi)	43.68±0.24	3.86±0.04	1.55±0.03	L*: 53.10±0.44 a*: 33.33±0.25 b*: 13.37±0.15
PLE with acidified methanol (150°C, 1000 psi)	12.02±0.12	1.31±0.02	0.78±0.02	L*: 55.53±0.32 a*: 25.17±0.11 b*: 12.23±0.21
PLE with water (80°C, 1000 psi)	22.86±0.12	3.43±0.01	nd	L*: 41.93±0.64 a*: 26.60±0.56 b*: 1.87±0.67
PLE with water (150°C, 1000 psi)	0.60±0.003	0.14±0.01	nd	L*: 67.4±0.56 a*: 14.00±0.10 b*: 17.20±0.44

Values are given as Mean±SD (n = 3), nd: Not detected

Table 2: Calorimetric data of extracted colorants obtained from PLE using acidified methanol and water at different temperatures with pressure of 1000 psi

	Calorimetric data					
	Acidified methanol extraction			Water extraction		
Extraction temperature (°C)	L*	a*	b*	L*	a*	b*
50	49.53±0.21	30.70±0.17	9.50±0.20	52.97±0.12	29.07±0.15	12.07±0.06
80	53.10±0.44	33.33±0.25	13.37±0.15	41.93±0.64	26.60±0.56	1.87±0.67
100	47.87±0.25	33.90±0.00	8.30±0.17	48.27±0.45	27.20±0.46	6.53±0.50
120	50.73±0.15	32.70±0.46	8.97±0.29	56.40±0.44	27.37±0.35	12.43±0.31
150	55.53±0.32	25.17±0.11	12.23±0.21	67.40±0.56	14.00±0.10	17.20±0.44

Values are given as Mean±SD (n = 3)

Effect of Extraction Temperature

The effect of temperature on extraction efficiency was investigated, since, it impacts the equilibrium (solubility) and mass transfer rate (diffusion coefficient) (Spigno *et al*, 2007). In PLE extraction, temperature can be set above the normal boiling point of the solvent. In this study, 5 different temperatures (50, 80, 100, 120 and 150°C), were selected to evaluate the influence of temperature on the extraction efficiency and quality of colorants from onion skin. Using both acidified methanol and water extraction, the yield of extracted colorant increased with an increase in extraction temperature (Fig. 1). In acidified methanol extraction, the yields (g colorant/100 g sample) varied from 9.56±0.46 to 16.71±1.60 while using water; the yield varied from 7.06±1.30 to 20.99±0.75. Highest amount of colorant was extracted at 150°C for both acidified methanol and water extraction.

Using both solvents, acidified methanol and water, the amount of identified anthocyanins decreases as the extraction temperature increases from 80 to 150°C (Table 1). These observations may be due to degradation of anthocyanins at high temperature as reported by Laleh *et al*. (2006) and Türker and Erdoğdu (2006). Thermal degradation of anthocyanins could occur via two mechanisms: (1) hydrolysis of the 3-glycoside linkage to form the more labile aglycon and (2) hydrolyzation of the pyrilium ring resulted in production of chalcone which are responsible for brown color developed in food containing anthocyanins (Ju and Howard, 2003; Laleh *et al*., 2006). As expected, using both solvents, the a* values (redness) decrease at high temperature (150°C) (Table 2). In addition, the UV-Vis absorption spectra for acidified methanol extract showed highest absorption at 434 and 534 nm, at extraction temperature of 80°C and decreases at high temperature (Fig. 2).

Table 3: Yields and anthocyanins composition of extracted colorants obtained from soaking and PLE methods

Extraction methods	Yields (colorant g/100 g sample)	Composition of anthocyanins [ppm (μg mL^{-1})]			
		Cyanidin 3-glucoside	Cyanidin 3-rutinoside	Cyanidin chloride	Calorimetric data
PLE with acidified methanol (80°C, 1000 psi)	11.57±0.76	43.68±0.24	3.86±0.04	1.55±0.03	L*: 53.10±0.44 a* : 33.33±0.25 b* : 13.37±0.15
PLE with acidified methanol (150°C, 1000 psi)	16.71±1.60	12.02±0.12	1.31±0.02	0.78±0.02	L*: 55.53±0.32 a* : 25.17±0.11 b* : 12.23±0.21
PLE with water (80°C, 1000 psi)	8.93±0.68	22.86±0.12	3.43±0.01	nd	L*: 41.93±0.64 a* : 26.60±0.56 b* : 1.87±0.67
PLE with water (150°C, 1000 psi)	20.99±0.75	0.60±0.003	0.14±0.01	nd	L*: 67.40±0.56 a* : 14.00±0.10 b* : 17.20±0.44
Soaking with acidified methanol (room temperature)	10.41±1.46	20.04±0.57	2.47±0.05	0.81±0.03	L*: 53.70±0.10 a* : 40.30±0.53 b* : 7.13±0.29
Soaking with water (90°C)	8.97±0.17	5.62±0.03	0.92±0.01	nd	L*: 67.10±0.26 a* : 24.43±0.40 b* : 19.10±0.10

Values are given as Mean±SD (n = 3), nd: Not detected

Comparison of Yield and Color of Extracted Colorants Obtained from PLE with those of Conventional Water and Solvent Extraction

Using conventional soaking technique, the yields obtained were 10.41±1.46 for acidified methanol extraction and 8.97±0.17 for water extraction (Table 3), comparable with those obtained using PLE. It can be concluded that the composition of anthocyanins extracted depended on the type of solvent used. As reported earlier, cyanidin chloride could not be extracted using water in both PLE and conventional soaking techniques. The color measurement indicated that the a* value for acidified methanol extractions using soaking technique was high which corresponds to a stronger reddish shade. The a* values for water extraction using PLE at 80°C and conventional soaking were comparable.

CONCLUSION

The data obtained demonstrated that extraction solvent has a great impact on the quality and yield of colorants from onion skin. An increase in extraction temperature increases the yield but at high temperature some anthocyanins may degrade, thus changing the color of extracts. The PLE has shown to be a good alternative for the extraction of natural colorants as the type of solvent and temperature can be adjusted to optimize the yield of specific color from onion skin.

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