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Extraction, Identification and Thermal Stability of Anthocyanins from Eggplant Peel as a Natural Colorant

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ABSTRACT

The aim of this research is to evaluate the anthocyanin content of local eggplant peels and its potential as a food colorant. In the present study, various mixtures of aqueous ethanol and acetone solutions were utilized to extract anthocyanins from eggplant peel by a discontinuous process to obtain a natural red-purple colorant. First, the most appropriate combination for extraction solvent was selected as 70 (v/v) % ethanol in water acidified with 3 (v/v) % acetic acid, which was determined by analysis of extraction yields and total anthocyanins content of extracts utilizing UV-Vis spectrophotometer. Then, the extracted anthocyanins were identified and quantified by high performance liquid chromatography with diode array detector and characterized by high performance liquid chromatography equipped with three-dimensional mass spectrometry measurements. According to data analyses the major anthocyanin in eggplant skin was delphinidin-3-rutinoside (91.43%). Eventually, the thermal stability of extracted anthocyanins was evaluated during 15 days at different temperatures. Results demonstrated that the amount of anthocyanins decreased further with increasing of storage period and temperature. Prog. Color Colorants Coat. 8(2015), 59-67 © Institute for Color Science and Technology.

1. Introduction

Fruits, vegetables and flowers contain anthocyanin colorants, which are responsible for purple, red and blue colors. These colorants are water soluble and classified as phenolic compounds named flavonoids.

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They have various structural forms, and their colors and stabilities depend on physico-chemical phenomena such as temperature and pH (Figure 1) [1-6]. Recently, anthocyanin colorants have attracted more attention owing to their natural antioxidant properties. Due to electron lack of anthocyanins, they are very reactive toward generated free radicals in the body to neutralize them [7-9]. Liver safe-keeping, coronary heart diseases reduction and anticancer properties are instances of their health benefits in foods [10-16]. Textile and food industries are interested in anthocyanins due to their deep red-purple color as a source of natural colorant [17]. Eggplant is a vegetable with uncertain origin, which is mostly believed to be originated in China or Iran and then introduced to the Middle East by Arab traders or armies in the 8th century [18-20]. The eggplant skin contains various types of anthocyanins.

The researches of studying six and eight cultivars of eggplant skin showed that Nasunin was the main anthocyanin of eggplant skin [21]. Further in a recent research, delphinidin-3-rutioside has been identified as major anthocyanin in eggplant as well [20]. Beside, low stability of anthocyanins against variations of practical parameters including temperature and pH has restricted their applications as colorants in food and textile industries [22].

In the present study, various combinations of solvents were utilized for extraction of anthocyanins from eggplant peel (Iran). First, the optimized extraction process was determined according to the extraction yields and total anthocyanins content in extracts. Then, high performance liquid chromatography (HPLC) combined with mass spectrometry (MS) were carried out for identification and characterization of anthocyanins in extract. Finally, thermal stability of anthocyanins obtained from eggplant skin was evaluated in a 15-days period by varying the temperature.

2. Experimental

2.1. Materials

Fresh eggplants were obtained from local market in Tehran, Iran. The eggplants peel was manually removed. The slices were finally reduced to 20 mm^2 size and stored at -20℃. Other reagents and solvents were purchased from Merck with analytical grade. The experiments were repeated three times and the average values were reported.

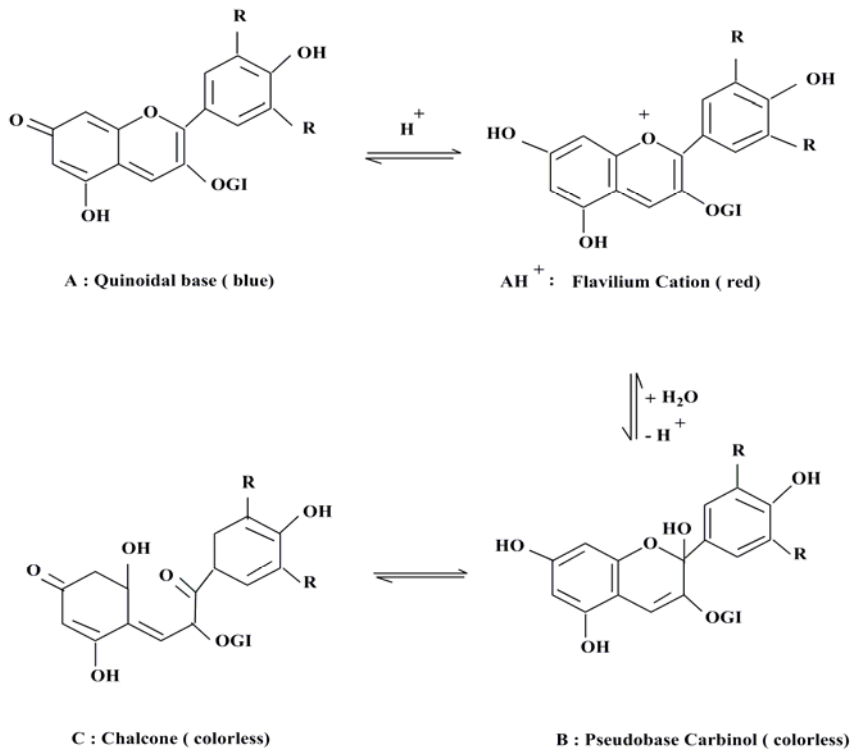

Figure 1: Structural transformation of anthocyanins according to pH [5].

2.2. Extraction of anthocyanins

The extraction procedure was performed in batch mode. Ethanol (70% (v/v)) or acetone (70% (v/v)) in water was used as solvents, which were acidified with hydrochloric acid (1% (v/v)) or acetic acid (3% (v/v)) individually to gain four kinds of solvents. Acetic acid was used due to easy availability as a weak acid. In each experiment, certain amount of eggplant peels (50 g) was placed in the beaker and 100 mL of prepared solvent was added to it. Extraction of samples was carried out at room temperature for eight hours. Vacuum filter was applied for filtration of extracts and then rotary evaporator at 50 ºC was employed for evaporation of solvent and finally drying the samples at room temperature while protected against light. The obtained extracts were weighed and compared with initial weight of eggplant peel for calculation of extraction yield from Eq. (1) [23]:

$$\text{Extraction yield} = \frac{\text{weight of anthocyanins extract(g)}}{\text{weight of fresh eggplant peels}} \times 100 \quad (1)$$

The UV-Vis spectra of extracted anthocyanins are shown in Figure 2.

2.3. Qualitative evaluation of anthocyanins

Thin layer chromatography (TLC) was used for qualitative analysis of anthocyanins. Analysis was performed using silica gel F 254 and the mixture of ethyl acetate, glacial acetic acid, formic acid and water with volume ratio of 100:11:11:26 as mobile phase to evaluate the presence of anthocyanins in extracted solutions.

2.4. Determination of total anthocyanins content

The total anthocyanins content of an extracted solution was determined according to pH differential method and expressed as cyanidin-3-glucoside equivalents [24, 25]. This method can be used for determination of total monomeric anthocyanins content by measuring the change in absorbance at two different pH values (1 and 4.5). At a pH of 1.0, anthocyanins are in the colored flavylium cation form; however, they are in the colorless hemiketal forms at pH 4.5. Thus, the difference in UV-Vis absorbance at the λ_{max} for cyanidin-3-glucoside (510 nm) is proportional to the concentration of total anthocyanins. In this method, First dilution factor for each extracted solution was defined by diluting it by KCl buffer (pH 1.0) until its absorbance at 510 nm reach less than 1.2 against the same buffer solution (pH 1.0) as blank. Then, each extracted solution was diluted based upon the defined dilution factor with aqueous buffers (potassium chloride-hydrochloric acid solution, pH 1.0 and sodium acetate trihydrate, pH 4.5), individually.

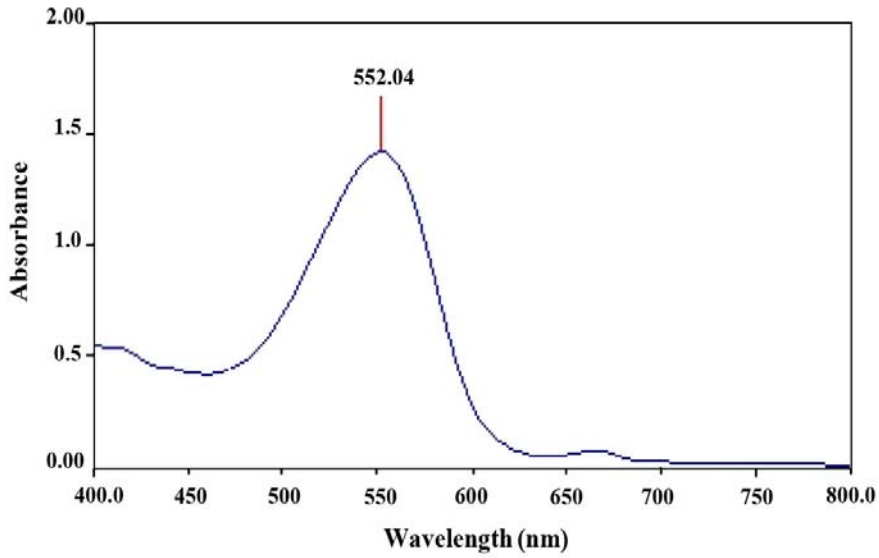

Figure 2: UV-Vis spectra of extracted anthocyanins.

The absorbance for each sample was then found against the buffer solution with pH 1.0 and buffer solution with pH 4.5 as the blank at λ = 510 nm (λ_{-max} for the cyaniding-3-glucoside) and λ= 700 nm (for correction factor). The final absorbance (A) was calculated from Eq. (2):

$$A = (A510\text{-}A700)_{pH\ 1.0} - (A510 - A700)_{pH\ 4.5} \quad (2)$$

Then total anthocyanins content was calculated using Eq. (3):

$$TAC = (A/\varepsilon \times L) \times M_W \times DF \times 1000 \quad (3)$$

Where TAC is total anthocyanins content (mg/L) which was then converted to g/100g of fresh peel (%w/w); M_W is molecular weight of cyaniding-3-glucoside (449.2 g/mol) Also, ε is molar absorption coefficient of cyanidin 3-glucoside (226, 900 L/mol.cm); L is width of cell (1cm); DF is dilution factor.

2.5. Characterization of extracted anthocyanins by HPLC-DAD and HPLC-MS3

Using a HPLC System, Knauer WellChrom equipped with a pump K-1001, a diode array detector K-2600 (HPLC-DAD) and a Knauer column oven, anthocyanins were separated on an analytical Perfectsil Target ODS-3 C18 column (250×4.6 mm, 5 µm) with a Phenomenex C18 pre-column (4×3.0 mm) at a constant temperature of 25 °C and a flow rate of 1 mL/min. Eluent A and eluent B were aqueous in formic acid 5 (v/v)% and acetonitrile 100 (v/v)%, respectively. Starting with 100 (v/v)% A for 5 min, linear gradients were followed to 10 (v/v)% B at 20 min, 13 (v/v)% B at 40 min, 20 (v/v)% B at 44 min, 25 (v/v)% B at 50 min, and finally 100 (v/v)% B at 55 min. Monitoring was performed at 520 nm. By utilizing the same method, three-dimentional mass spectrometry (HPLC-MS3) detection was carried out on an Agilent 6410 Triple Quadruple HPLC-MS coupled to an Agilent 1200 series liquid chromatography equipped with an auto sampler (1200 series) and diode array detector (1200 series). Mass spectrometric conditions were as follow: nitrogen as drying gas, flow rate of 6 mL/min; nebulizing gas adjusted at 10 psi, desolvation gas temperature at 300℃ and Capillary voltage adjusted at -4000 V. Electro spray Ionization (ESI+) was used as the method of ionization and Mass spectrometer was operating on positive mode, over the mass range of 100-2000 m/z. Mass Hunter software was applied for data processing.

2.6. Thermal stability test of anthocyanins

The thermal stability of the extracted anthocyanins from eggplant peels was estimated by changing the temperature. The extract with maximum yield of 0.49% (w/w) (extracted by ethanol 70% (v/v) and acidified by acetic acid (3% (v/v)) were covered with aluminum foil and incubated at 20, 30 and 40℃ in the presence of air. Samples were kept at 1, 3, 6, 9 and 15 days, and the total anthocyanin contents were measured by spectrophotometery with the same method as mentioned in section 2.4. Then losses of anthocyanin contents toward initial value (0.49% (w/w)) were calculated. There was not significant color change in samples with temperature variations. The effect of the ambient humidity was ignored due to its very low content.

3. Results and discussion

3.1. Extraction of anthocyanins

Ethanol and acetone in water were used due to proper solubility of anthocyanins in polar solvents. The acids were added to enhance stability of anthocyanins owing to their phenolic structures [26]. These extracts contain monomeric and polymerized anthocyanins and other unknown substances. The highest yield of anthocyanins extraction from eggplant peels was obtained by ethanol 70% (v/v) ethanol acidified with 3% (v/v) acetic acid solvent (Table 1). Experiments suggested that the ethanol provides strong hydrogen bonding between alcoholic solvent and the anthocyanins. However, acetone showed the lower extraction response in comparison with ethanol [27, 28].

Table 1. The yield of anthocyanin extraction from eggplant peel with different combinations of solvents.

Solvent	Extract [g]	Yield [%w/w]
Ethanol 70% (v/v) + acetic acid 3% (v/v)	0.56	1.12
Acetone 70% (v/v) + acetic acid 3% (v/v)	0.44	0.88
Ethanol 70% (v/v) + HCl 1% (v/v)	0.21	0.42
Acetone 70% (v/v) + HCl 1% (v/v)	0.18	0.36

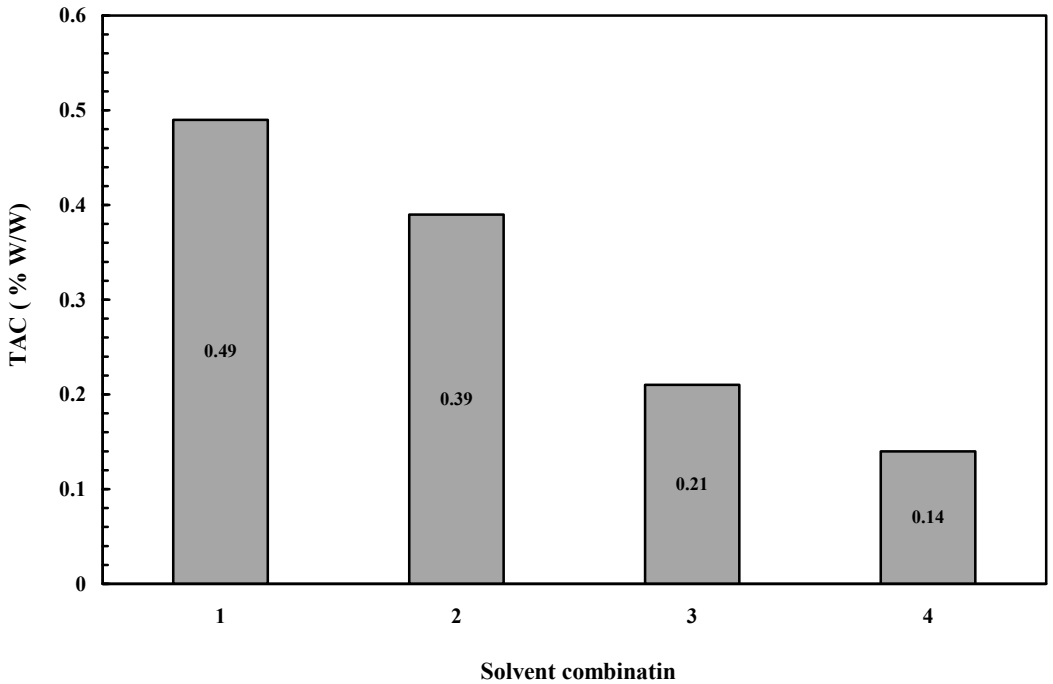

Figure 3: Total anthocyanins Content (TAC) and color intensity of anthocyanin extracts: (1) ethanol 70% (v/v) and acetic acid (3% (v/v)) extract (2) acetone 70% (v/v) and citric acid (3 (v/v)%) extract (3) ethanol 70% (v/v) and HCl (1% (v/v)) extract, and (4) acetone 70% (v/v) and HCl (1% (v/v)) extract.

Extraction with solvent contained HCl resulted in lower yield value than that using acetic acid mixture. This can be related to anthocyanins degradation via hydrolysis and obscuring a realistic profile of anthocyanins [23]. Use of weaker organic acids such as acetic acid declined degradation of anthocyanins.

3.2. Qualitative determination of anthocyanins

Thin-layer chromatography (TLC) was carried out to confirm the presence of anthocyanins in extracts. TLC results exhibited that eggplant peel extracts contained anthocyanins. This was verified by the presence of red spots on the TLC plate under visible light. This result was in consistent with the report of Wagner [29], stating that anthocyanin shows red to blue-violet colors under visible light.

3.3. Determination of total anthocyanins content of extracts

Visible spectrophotometry was used to determine the

total monomeric anthocyanins content in eggplant peels extract. In fact, total anthocyanin content is only related to concentration of monomeric anthocyanins, because polymerized anthocyanins do not exhibit reversible behavior with pH, and thus are excluded from the absorbance calculation. Figure 3 shows that the total anthocyanins content varies from 0.14 (%w/w) (hydrochloric acid 1(v/v)%) to 0.39 (%w/w) (acetic acid 3 (v/v)%) in the case of acetone 70 (v/v)% in water as extracting solvent, and from 0.21 (%w/w) (hydrochloric acid 1 (v/v)%) to 0.49 (%w/w) (acetic acid 3 (v/v)%) in the case of ethanol 70 (v/v)% in water. Ethanol 70 (v/v)% acidified with citric acid (3 (v/v)%) provided the greater total anthocyanins content than other solvents according to the reasons mentioned in section 3.1.

3.4. HPLC-DAD and HPLC-MS3 detection of anthocyanin

Figure 3 shows the typical HPLC chromatogram of eggplant peel extract monitored at 520 nm. Also to assign the chemical structures of the individual anthocyanin peaks, HPLC-MS^3 measurements were performed and the obtained results were compared with literature data (Ando et al. [30], Ichiyanagi et al. [31], Wu and Prior [21]). Four glycosylated structures were detected in eggplant. According to the results obtained by the above mentioned techniques, the major anthocyanin was delphinidin-3-rutinoside (91.43%, 4) refering to peak area in Figure 4 and Table 2, followed by delphinidin-3-rutinoside-5-glucoside (5.68%, 1), delphinidin-derivative isobaric to 1 (1.63%, 2) and delphinidin-3-glucoside (1.25%, 3), respectively (Figure 4, Table 2).

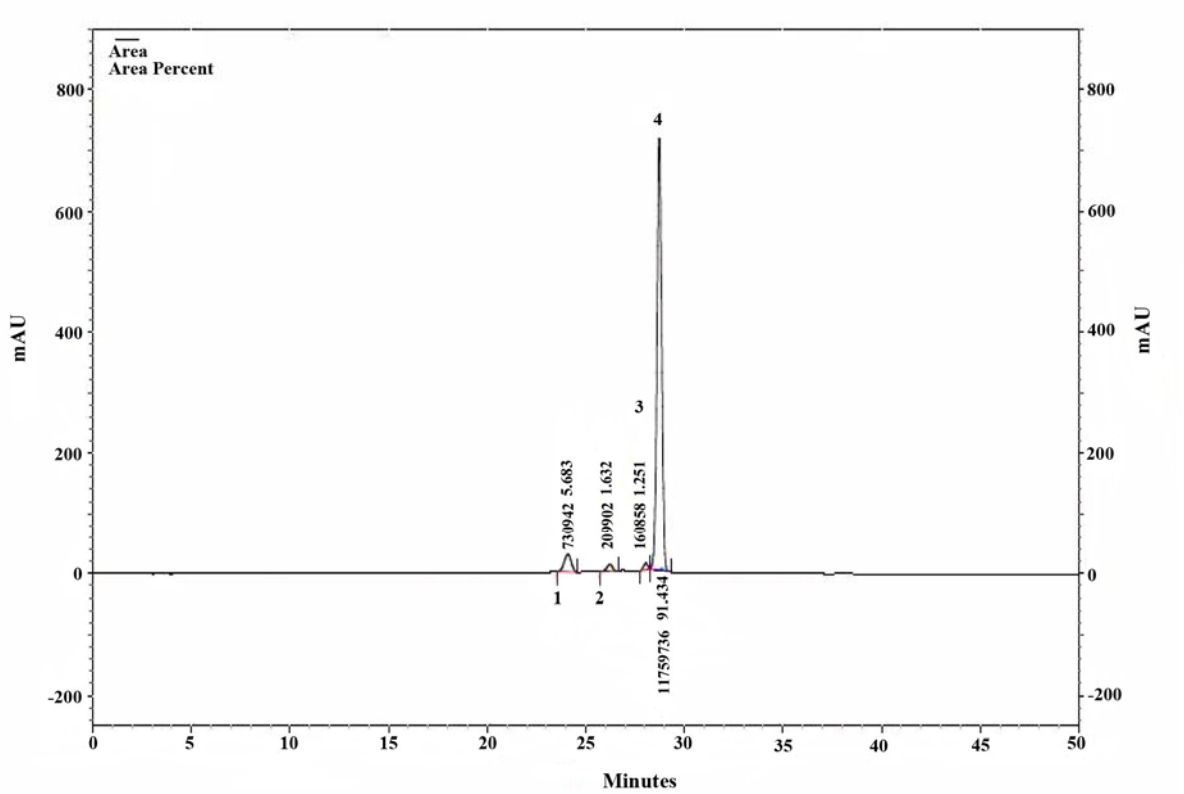

Figure 4: HPLC-DAD chromatogram of anthocyanins extracted from eggplant, monitored at 520 nm (peal assignment is given in Table 2).

Table 2: HPLC-DAD-MS3 data for anthocyanins from eggplant peels.

Anthocyanin	Rt [min]	λ_{max} [nm]	m/z $[M^+]$	MS^2 m/z$[M^+]$	MS^3 m/z $[M^+]$
Delphinidin-3-rutinoside-5-glucoside (1)	23.7	526	773	611 465 303	465 303
Delphinidin-3-rutinoside-glucoside (2)	26.3	522	773	627 465 303	465 303
Delphinidin-3-glucoside (3)	27.9	527	465	303	303
Delphinidin-3-rutinoside (4)	28.8	525	611	465 303	303 -

Table 3: Effect of temperature and storage time in losses (%wt) of anthocyanins extracted from eggplant peel.

Time [days]	Temperature [℃]		
	20℃	30℃	40℃
1	8.23	7.22	8.12
3	12.41	25.31	22.87
6	17.36	35.78	77.92
9	22.44	48.54	90.12
15	35.89	56.98	97.58

3.5. Thermal stability of anthocyanins

Temperature is a factor in destabilizing the anthocyanin molecular structure. By increasing the temperature, a greater degree of destruction in anthocyanins is observed. Losses of anthocyanins of extract in the dark and at different temperatures are represented in Table 3. The initial anthocyanins content were 0.49% (w/w). The data clearly show that the loss of pigments is higher at elevated temperatures and increases with increasing of storage time [32].

4. Conclusions

In this study, extraction of anthocyanins from eggplant peel was carried out using different combinations of solvents. The yield of each extract was calculated and the total anthocyanins content of each extract was also measured by spectrophotometry. HPLC-DAD and HPLC-MS3 analyses were carried out for characterization and identification of extracted anthocyanins. Furthermore, thermal stability of extracted anthocyanins was investigated during 15 days by changing the temperature. According to the total anthocyanins content and extraction yield of products, it can be concluded that among four extraction solvents, ethanol 70% (v/v) acidified with acetic acid (3% (v/v)) was the best solvent for extraction of anthocyanins from eggplant peel due to effective hydrogen bonding between hydroxyl groups of anthocyanins and ethanol. According to the results

obtained from HPLC and HPLC-MS^3 analyses, delphinidin-3-rutinoside was the major anthocyanin in eggplant peel (91.4%) which shows that the percent of this anthocyanin in our eggplant peels is higher than that of other reported amounts in literature (less than 90%). Finally, the data demonstrated that the total anthocyanins content of extracts decreased with increasing the temperature and the time of storage and the best storage temperature for extracted products was 20℃.

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