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Evaluation of solvent extraction of *Amaranth betacyanins* using multivariate analysis

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Abstract

The present study was aimed at assessing the effect of solvents on the yield and the color properties of amaranth extract. Two species of amaranth namely *Amaranthus gangeticus* and *Amaranthus blitum* were extracted with water, methanol and ethanol. Seven parameters like betacyanin content, total soluble solids, lightness (L^*), redness (a^*), yellowness (b^*), hue angle (h^*) and chroma (c^*) were analyzed to assess extraction efficiency. Correlation analysis was carried out to assess the linear association among the analytical variables. Principal component analysis was used to establish the relationships between the different analytical variables and to detect the most important factors of variability. Among the two varieties, *Amaranthus gangeticus* extract contained about two and half time more betacyanin with half of total soluble solids compared to *Amaranthus blitum.* Water is the best as solvent for extracting betacyanin from *Amaranthus gangeticus* and ethanol in case of *Amaranthus blitum.* Among the analytical parameters, a^* and c^* were perfectly correlated. Three principal components were found among the seven analytical variables accounting 88% of total variability. The first principal components mostly reflected the redness (a^*), whereas the second principal components reflected the betacyanin content, total soluble solids and lightness (L^* value).

Introduction

Colour is one of the crucial factors for the consumer's acceptability of any processed foods. One of the attributes contribute to the appearance of food is colour. For this reason, food industries focus on physical quality of food. However, food processing will affect the physical appearance of food by losing the colour compound. So food colourant becomes important either as additive to food which the colour lost during processing or intensify the appearance of food. There is a growing tendency in the food industry to replace synthetic dyes with natural pigments. Natural food colourants have been criticised for being more expensive, less consistent and for having less potential. However, as the list of approved artificial colours has diminished under increasing regulation by the FDA, manufacturers have begun to devote more time and resources into developing natural colour additives (Burrows, 2009). Chlorophylls, carotenoids, anthocyanins and betalains are the common and widely studied natural pigments. Betacyanin is a water soluble nature colourant responsible for red or violet colour (Strack *et al.*, 2003). The potential sources of betacyanins so far reported by other researchers are red beet (Stintzing and Carle, 2004), cactus pear (Saénz *et al*., 2009), pitaya (Ng *et al*., 2012) and Amaranth (Cai *et al*., 2005). To date, the most common sources of betalains is the red beet, but it is restricted by its earthy smell as well as considerable nitrate level (Herbach *et al*., 2006). Betalains from red amaranth becomes an advantage over this.

Betacyanin content of *Amaranthus* species, extraction and production of powder by spray drying were reported by Cai and Corke (2000) and Cai *et al*. (1998a), respectively. Antioxidant potential, cooking stability and application of amaranthus betacyanins in modeling food system has also been studied. Previous studies have shown that betacyanins exhibit antioxidant activity. Cai *et al.* (1998b) identified 19 different types of betalains from family *Amaranthaceae* which possessed high antioxidant activity. In Malaysia, five species of Amaranthus have been identified: *Amaranthus gangeticus*, *Amaranthus blitum*, *Amaranthus paniculatus*, *Amaranthus viridis*

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and *Amaranthus spinosus* (Amin *et al*., 2006). Among them, the first two only are red in color and are assumed to contain betacyanin.

For exploratory data analysis, principal component analysis is a very useful technique for the research in many applied fields. It is widely used in researches of food application and product developments and sensitive compounds like food colourants. This statistical tools aids in simplifying data interpretations, establishing relationships among the variables and detecting the most important factors accounting the highest variability. In this study the betacyanin content of these two species and the effect of different solvents on the yield of betacyanins and colour parameters of the extract have been compared and the principal components among the analytical variables are extracted.

Materials and Methods

Raw materials

Amaranthus gangeticus and *Amaranthus blitum* were the two red amaranth species and collected from Pasar Borong, Seri Kembangan, Selangor Darul Ehsan, Malaysia. It was cleaned by removing the root, soil as well as rinsing by tap water. After that it was stored in the freezer at -20°C.

Methods

Water, methanol and ethanol extractions were carried out in order to investigate which type of extraction could exhibit the highest amount of betacyanin pigments. Water extraction was performed by following the method described by Cai *et al*. (1998b) with simple modifications. Both species were taken out from the freezer and cut into small pieces which expose greater surface area to facilitate extraction. Exactly 100 g of fresh sample was weighed and 200 ml of distilled water was added in a beaker. The sample was heated up to 80°C and held for 5 min. After that it was immediately placed in an ice bath to cool down to room temperature. The vegetables were removed and the extracted solution was obtained. The same conditions applied to methanol and ethanol extractions, except that constant stirring every 10 min for 1 hour instead of the blanching process.

The extracts was firstly purified to remove soil and small particles by centrifuging using centrifuge (Kubota 2010, Japan) at 4000 rpm for 15 min. Further purification was done by vacuum filtration system. The purified solution was then concentrated to achieve total soluble solid content of 6 by using rotary evaporator (Heidolph Laborota 4001 efficient, Germany). Total soluble solid content was determined by using a pocket refractometer (Atago, Japan). The pH of concentrated extracts was adjusted to 5.6 by adding ascorbic acid. The extracts were then stored in a freezer at -20°C and ready for analysis.

Betacyanin determination

Betacyanin content was determined by using a spectrophotometer (Ultrospec 3100 pro, England) at a wavelength of 538 nm before and after spray drying. The betacyanin content before spray drying was 34.08 mg/100 g of fresh weight. Mcilvaine buffer was prepared and it was used to replace with distilled water that normally dissolved powder for betacyanin determination (Cai and Corke, 2005). The quantification of betacyanin was described by Lim *et al.* (2011). The betacyanin content (mg/100 g of fresh weight) was calculated using Eq.2:

$$\frac{A_{538}(MW)\times V\times(DF)}{\varepsilon LW}\times 100$$

where A_{538} = absorbance at 538 nm (λ_{max}), L (path length) = 1.0 cm, DF = dilution factor, V = volume extract (mL), W = fresh weight of extracting material (g). For betanin, ε (mean molar absorptivity) = 6.5 x 10^4 L/mol cm in H_2O and MW (molecular weight) = 550 g/mol.

Colour measurement

The colour parameters L^*, a^*, b^* of powder were measured using a colour spectrophotometer (UltraScan Pro Hunter Lab). L^*, a^*, b^* are lightness, redness and yellowness, respectively. Hue angle H^o and Chroma C^* are calculated using the following equations:

$$H^o = [\tan^{-1}(b^*/a^*)]$$

$$C^* = (a^{*2} + b^{*2})^{1/2}$$

Total soluble solid content determination

Total soluble solid content of betacyanin extracts were determined using a pocket refractometer (ATAGO, Japan).

Statistical analysis

For the evaluation of the yield and color parameters, the multivariate statistical methods like correlation analysis, principle component analysis and cluster analysis were applied. The analysis was carried out by using Statgraphics Centurian VI, 2011 software.

Results and Discussion

Extract properties

The results of the betacyanin yield and colour

Table 1. Comparison between *Amaranthus blitum* and *Amaranthus gangeticus*

Extract properties	*Amaranthus blitum*			*Amaranthus gangeticus*		
	WE	ME	EE	WE	ME	EF
BE (mg/100 g)	62.07	65.46	68.57	152.5	141.7	111.7
TSS	0.3	1.1	2.9	0.4	0.6	1.4
*L**-value	25.63	25.59	25.47	26	25.68	25.95
*a**-value	6.24	7.01	6.65	6.23	7.29	7.38
*b**-value	0.65	-0.45	-0.22	-0.19	0.86	-1.06
h-value	0.1	359.9	359.9	358.3	353.3	351.8
*c**-value	6.27	7.02	6.65	6.23	7.34	7.46

BE= Betacyanin content, WE = Water Extraction, ME = Methanol Extraction, EE = Ethanol Extraction

parameters of the extract extracted using three extraction solvents (water, methanol and ethanol) from two species of amaranth (*Amaranthus blitum* and *Amaranthus gangeticus*) are presented in Table 1. According to Table 1, higher extracted betacyanin content was found in *A. gangeticus*, which was about two times more than that of extracted from *A. blitum.* This was because of the higher betacyanin pigment content in the whole plant of *Amaranthus gangeticus* species which appeared as red in colour. However, for *Amaranthus blitum* species, the betacyanin pigment does not distributed to whole plant, which only presents in some areas located at leaves as well as stem. The colour of whole plant is a combination of red from betacyanins and green from chlorophylls. As shown in Table 1, there were insignificant differences of betacyanin content extracted from *A. blitum* for different types of extraction. Surprisingly, highest betacyanin content (152.48 mg/100 g) was found in *A. gangeticus* by water extraction. This might be due to the blanching process at 80^{o}C for 5 min to facilitate the betacyanins to be driven out from cell walls. Besides, betacyanin is a water soluble compound and it can be easily extracted and dissolved in water so that high betacyanin pigments are obtained. Betacyanin pigments from 21 genotypes and 7 species were analyzed for colour properties and stability by Cai *et al.* (a, b). The dried extract of *Amaranthus tricolor* contained the highest betacyanins as reported by the researchers.

Concerning the colour parameters, the L*-value between the two species and three different extraction methods was from 25.47 to 26.00, a* value from 6.23 to 7.38, and C* from 6.23 to 7.46. The value of these parameters would be increased after spray drying process. As a conclusion, the species and method of extraction do not affect the colour parameters from betacyanins. It could be noticed that there were no much differences for the total soluble solid content between two species. Nevertheless, the lowest value of total soluble solid content was found in water extraction from both species. Methanol and ethanol increased small amount of total soluble solid content during methanol and ethanol extractions. So water extraction was preferred, which resulted in fewer impurities to the extracts.

Table 2. Correlation coefficients for the analytical parameters of the solvent extract

	Betacyanin content	TSS	L* - value	a*- value	b*- value	h value	c* value
Betacyanin content	1	-0.4077	0.7385*	0.1554	0.0786	0.4501	0.1625
TSS	-0.4077	1	-0.4761	0.2060	-0.4403	0.4194	0.1863
L* value	0.7385	-0.4761	1	0.0278	-0.3691	0.1983	0.0490
a*-value	0.1554	0.2060	0.0278	1	-0.2827	0.5277	0.9991**
b*-value	0.0786	-0.4403	-0.3691	-0.2827	1	-0.4937	-0.2787
h value	0.4501	0.4194	0.1983	0.5277	-0.4937	1	0.5022
c* value	0.1625	0.1863	0.0490	0.9991	-0.2787	0.5022	1

Table 3. Component weights of the analytical variables

Analytical variables	*Component 1*	*Component 2*	*Component 3*
betacyanin content	-0.21	0.58	0.03
TSS	-0.21	-0.54	-0.33
L* value	-0.17	0.59	-0.29
a*-value	-0.51	-0.09	0.43
b*-value	0.36	0.09	0.62
h value	-0.49	0.02	-0.21
c* value	-0.50	-0.08	0.44

Statistical evaluation of colour properties of the extracts

To measure the linear association among the variables studied, the correlation analysis was carried out. The correlation coefficients between the analytical variables are presented in Table 2. This table shows Pearson product moment correlations between each pair of variables. These correlation coefficients range between -1 and +1 and measure the strength of the linear relationship between the variables. Except a* value and c* value, there is no perfect correlation among the analytical variables studied. Only these two variables showed a high indirect dependence with each other. Betacyanin content and colour parameters of pitaya peel and flesh were showed highly dependent as reported by Phebe *et al.* (2009). In case of taste evaluation of lactic acid fermented vegetable juices, many of the taste variables used for the evaluation were not found correlated. Among eight variables, only three variables were found statistically correlated (Karovicova and Kohajdova, 2002).

Principal component analysis (PCA) was performed to obtain a small number of linear combinations of the 7 variables which account for most of the variability in the data. Furthermore, it will detect the most important factors of variability. Variables were previously standardized and orthogonal rotation was used to apply the PCA. The PCA of seven variables yielded three principal components with eigenvalues greater than 1, which is a common statistical cut-off point (Dixon, 1992). Together they account for 87.8% of the variability in the original data, with 41% of the total variance explained by the first principal components (PC1), 30% by the second (PC2) and 17% by the third principal components (PC3). PC1 denotes the linear combination of the analytical variables for

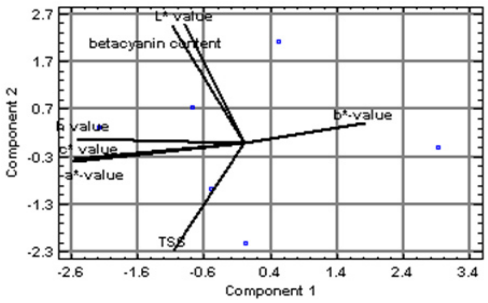

Figure 1. Representation of analytical attributes as a function of PC1 and PC2

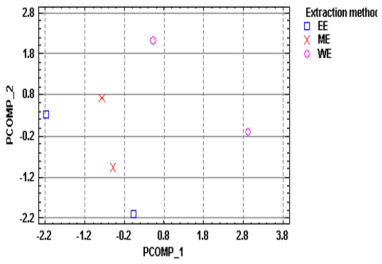

Figure 2. Representation of the extraction methods (water, methanol and ethanol extraction) versus PC1 and PC2

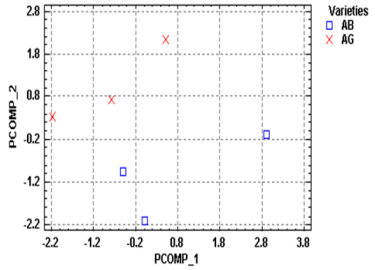

Figure 3. Representation of the two varieties of amaranth (*Amaranthus blitum* and *Amaranthus gangeticus*) versus PC1 and PC2

the extraction and it summarises the variations in the original data matrix in a single number, whereas the second and third components summarizes the remaining information better. With loses of only 12% variation, the dimensionality of the original data is therefore reduced from seven variables to three uncorrelated components.

The analytical parameters are presented as a function of principal components 1, 2 and 3 in Table 3. Principal component 1 (PC1) and principal component 2 (PC2) represents most of the variance (71%). Among these two, maximum weightage was found in case PC1 (41%). Therefore, two dimensional component plots have been generated for PC1 and PC2 and have been shown in Figure 1. As shown in Figure 1, the first principal component (PC1) reflected mostly the a*-value variable (component weight -0.51). Therefore, the first principal component mainly evaluated the extraction solvents based on the a*-value. The negative part of the PC2 reflected mostly TSS and the L*-value and the positive part reflected mostly the betacyanin content. It revealed that PC2 evaluated the solvent extraction based on betacyanin content and the lightness (L* value). It is also important that with increasing betacyanin content lightness of the sample decreased. The third principal components resulted in a linear combination of b* value, a* value and c* with the rest variability. There are six principal components were reported in case of sensory attribute data of fermented food products, which accounted for more than 90% of the variance (Frau *et al.*, 2009). The two varieties of amaranth extracts extracted by three different extraction methods are plotted as a function of PC1 and PC2 and shown in Figure 2 and Figure 3. In Figure 2, samples are presented as extraction methods. Water extraction was mainly represented by the PC1 and methanol whereas ethanol extraction were represented by the PC2.

Figure 3 represents the samples extracted from two varieties of amaranth. It is revealed from figure that extracted samples are hardly distinguished based on the varieties *A. blitum* and *A. gangeticus* used to extract color compounds. However, they are represented by the principal component 2. In case of classifying Mohon, cheese traditionally and industrially manufactured cheeses could not be distinguished based on physical parameters analyzed by principal component analysis. However, the existence of significant differences among the four types of Mahon cheese (fresh, half-ripened, ripened and old-ripened) with regard to physical parameters has been demonstrated (Ghosh and Chattopadhayay, 2012).

In conclusion, *A. gangeticus* extract contained the highest betacyanin and the lowest total soluble solids compared to *A. blitum.* Extraction methods are highly influenced by the amaranth varieties. Water extraction yielded the highest betacyanin from *A. gangeticus* whereas ethanol was found the best in case of *A. blitum*. The a* and the c* were found perfectly correlated among the analytical parameters, Three principal components were found among the seven analytical variables accounting 88% of total variability with losing only 12%. The first principal components mostly reflected the redness (a*), whereas the second principal components reflected the betacyanin content, total soluble solids and lightness (L* value). As a conclusion, *A. gangeticus* could be used as potential source of betacyanin content, a major water soluble natural colorant and multivariable analysis could successfully be used to reduce the analytical variables to assess the extraction factors.

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