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Comparative study of organic solvents and extraction conditions on colour and antioxidant capacity in red cabbage

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Abstract

In industrially natural-dye production, there are many organic solvent with different efficiencies for extracting colour and antioxidants of red cabbage. The objective of this study was to investigate appropriate organic solvent and extract condition on colour and antioxidant activity. Two organic solvents were used for extraction: ethanol for hydrophilic antioxidants and hexane for lipophilic antioxidants. Experimental results showed that red cabbage extracted by ethanol was significantly higher in purple colour, anthocyanin content, total phenolic content, antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl radical assay (DPPH), and ferric reducing/antioxidant power assay (FRAP) than extraction by hexane due to anthocyanins being the main phenolic compound in extract. To find the appropriate concentration and extraction time, the results revealed that the red cabbage extracted by 70% ethanol for 18 h was suitable for extracting bioactive polar compounds, such as hydrophilic antioxidants.

Introduction

Colour is an important sensory property in food products to attract consumer demand. At present, natural dyes from colourful plants for use in food products are increasingly in demand because they are a good choice to avoid potentially mutagenic and carcinogenic effects from synthetic dyes. In the United States, for example, grape extract has been used as a colorant to mix with beverages or soft drinks (Francis, 1989; Bridle and Timberlake, 1997). Yang *et al.* (2008) have reported that the purple maize cob, which was a by-product of the maize industry, could be used as a source of purple colour in food. There are several colour plants, such as red radish, beetroot, carrot, etc. whose extracts could replace synthetic dyes (Sapers, 1994; Chethana *et al.* 2007). Red cabbage is one of many plants that could address the mentioned demand because its leaves are colourful and have a large quantity of phenolic compound, especially anthocyanins.

Anthocyanins are the basis of the red or purple pigments that are a group of natural phenolic compounds. The significant properties of anthocyanins include: antioxidant activity, inhibition of DNA damage in the cancer cell, prevention of coronary heart disease, and preservation of eyesight. Tamura and Yamagami (1994) found that the pericarp of the Muscat Bailey grape is two times more effective than (+)-catechin and α-tocopherol. The anthocyanin can resist the mutation of cells because it inhibits DNA damage in the cancer cells of internal organ of human such as lung, gastric and colon (Chen *et al*., 2006a; Chen *et al.*, 2006b; Wang and Stoner, 2008; Huang *et al*., 2011). Sumner *et al*. (2005) reported that in a patient who had ischaemic coronary heart disease (CHD) daily consumption of pomegranate juice could improve stress-induced myocardial ischaemia within 3 months. The bilberry anthocyanins were used as medicine for the physiological renewal and homeostasis of corneal epithelial cell because of the ability of bilberry anthocyanins to improve nutrition of the retina and restore primary night vision (Song *et al*. 2010; Gottikh and Tashlitskii, 2010). As mentioned above, the advantages of anthocyanins are desirable for cancer patients or consumers who are interested in the maintenance of good health.

Red cabbage is of interest in natural dye functions

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for industrial foods (Markakis, 1982; Malien-Aubert *et al*., 2001). In Japan, anthocyanins from red cabbage were used for industrial extraction because it had a high yield, large quantities and sites compared with berries (Bridle and Timberlake, 1997; Piccaglia *et al*., 2002). In addition, red cabbage anthocyanins were resistant to heat and light and are biologically harmless. In the extraction process, there are many organic solvents with different efficiencies for extracting either hydrophilic antioxidants or lipophilic antioxidants or both (Anwar *et al*., 2013; Chavan and Amarowicz, 2013; Benmeziane *et al*., 2014; Othman *et al.*, 2014; Nur Syukriah *et al.*, 2014). The times and concentrations of solvent during extraction significantly influence the antioxidant activities of the plant extract (Sultana *et al*., 2009; Ahmad *et al*., 2011; Chew *et al*., 2011; Woo *et al.*, 2013; Thoo et al., 2013; Bachir bey et al., 2014; Candrawinata et al., 2014) To obtain the optimum extract, a comparative study of the type of organic solvent and extraction conditions on colour and antioxidant capacity in red cabbage is therefore needed. Until now, information on this subject has not been reported.

The objective of this work was to investigate the effects of polar and nonpolar, extracting duration time and appropriate concentrations of organic solvent on key quality attributes of red cabbage extracted. The quality parameters considered included the colour of the extracts by tristimulus colorimetry as lightness (L^*), redness (a^*), yellowness (b^*) and hue angle (h°) values, anthocyanin content, total phenolic content and antioxidant activities by DPPH and FRAP assays.

Materials and Methods

Plant materials

Fresh red cabbages obtained from the Royal Project Foundation Center, Bangkok, Thailand were used in this study. The plant material, extraction procedures and chemical analyses were conducted at the Postharvest Technology Laboratory, King Mongkut's University of Technology Thonburi, Bangkok. The samples were prepared for extraction with different solvents, extraction times and concentrations.

Sample extraction

The method for extraction was that of Rodriguez-Saona and Wrolstad (2005). The 99.8% hexane (H) and 50, 70, and 95% ethanol (E) solvents were added to fresh red cabbage samples in plastic tubes and homogenised. The extracted sample by hexane was stored for 18 h at 4°C in refrigerator while extracted samples by ethanol were stored for 2, 8, and 18 h. The aqueous red cabbage samples were filtered through Whatman paper No. 1 for separation of the aqueous part from the residue. Then, the aqueous part was collected in plastic vials.

Determination of visual aqueous colours of extracted samples

Tristimulus parameters as lightness (L^*), redness (a^*), yellowness (b^*) and hue angle (h°) values were measured from aqueous samples in 3 replicates using a colorimeter that was calibrated with a standard white plate (Minolta model CR 400, Japan). The results are expressed as L^*, a^*, b^* and h°.

Measurement of anthocyanin content

The pH differential technique of Giusti and Wrolstad (2005) was used for measuring anthocyanin content in this experiment. Potassium chloride buffer (0.025 M KCl, pH 1.0) and sodium acetate (0.4 M $CH_3CO_2Na•3H_2O$, pH 4.5) were used for examining anthocyanin contents. One hundred microliters of extract from red cabbage were added to the test tube. Two tubes of one extract sample were followed by the two buffers. Nine hundred microliters of potassium chloride buffer and sodium acetate buffer were added. The test tubes were mixed and incubated for 15 min at room temperature (25°C). The anthocyanins were measured by spectrum scanning at 320 to 700 nm using a UV-visible spectrophotometer (Shimadzu model 1601, Japan). The absorbance of the diluted extracts was calculated as shown in equation (1)

$$A= (A_{\lambda vis\text{-}max} - A_{\lambda 700})pH\ 1.0 - (A_{\lambda vis\text{-}max} - A_{\lambda 700})pH\ 4.5 \quad (1)$$

Anthocyanin pigment concentration in the red cabbage extract was calculated using equation (2):

$$\text{anthocyanin pigment (mg/L)} = (A \times MW \times DF \times 1000)/(\varepsilon \times l) \quad (2)$$

where A is the absorbance of the mixed solution of the anthocyanin sample, MW is cyanidin-3-glucoside which has molecular weight as 449.2 (g/mol), DF is the dilution factor, ε is the molar absorptive as 26,900 (L/mol*cm), and l is the path length of the cuvette (cm). Then, the values of anthocyanin content in units of mg/L were converted to mg/100 g fresh weight (FW).

Measurement of total phenolic content

Total phenolic content assay was determined by the method of Swain and Hillis (1959). One hundred

and fifty microliters of red cabbage extract and 2400 µL of distilled water were added to the test tubes. Then, 150 µL of 0.25 N of Folin-Ciocalteu reagents was added in the tube. The samples were mixed using a vortex mixer and allowed to react for 3 min. To the mixtures was added 300 µL of 1N Na_2CO_3 solution. The mixtures were vortexed for a few minutes and kept away from light at 25°C for 2 h. Then, the absorbance was analysed at 725 nm, and the data were expressed in gallic acid equivalents (GAE; mg/100 g fresh weight) by a gallic acid concentration of 0.0-0.1 mg/mL.

2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH)

Free radical-scavenging activity of extracts was reacted with the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) which was modified from Brand-Williams *et al.* (1995). The stock solution of DPPH was prepared by twelve milligrams of a purple DPPH powder, which was dissolved in 50 mL of methanol. The working solution was made by mixing 20 mL of stock solution and 90 mL of methanol. Before analysis, the working solution was measured by spectrophotometer at 515 nm to obtain an absorbance of 1.1 ± 0.02 units. Two-thousand eight-hundred and fifty microliters of working solution and 150 µL of extracted anthocyanins were mixed in a plastic tube. The tubes were kept in the dark for 30 min and then recorded at 515 nm. Next, absorbance values of the samples were calculated as shown in formula (3)

$$\% \text{ inhibition} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100 \quad (3)$$

Ferric-reducing antioxidant power assay (FRAP)

The FRAP assay was conducted according to Benzie and Strain (1996) with some modifications. Fresh FRAP reagent consisted of 0.3 M acetate buffer (pH 3.6), 0.01 M TPTZ solution in 40 mM HCl, and 0.02 M $FeCl_3 \cdot 6\ H_2O$ solution in a ratio of 100:10:10 (v/v/v). The reagent was warmed at 37°C before use. The 150 µL of aqueous extract was mixed with 2850 µL of fresh FRAP reagent. Then, the mixture solutions were kept in the dark for 30 min. The samples were measured at 593 nm by spectrophotometer (Shimadzu model 1601, Japan). The absorption result was compared with a standard curve of Trolox at 25 to 800 µM, which was expressed in µM TE / g fresh weight.

Results and Discussion

Colour analysis

Colour parameter analysis of red cabbage extracts at various organic solvents, durations and concentrations shows in the Table 1. In the Tristimulus diagram, the extracted colour could be predicted by the direction of L^*, a^*, b^* and h° value. At an extraction time of 2 h, the colour parameter results reveal that solution extracted using 70% ethanol had the lowest L^* values as 26.4 while 50 and 95% ethanol had values of 28.2 and 28.0, respectively. The L^* values of 50 and 95% ethanol were not significantly different. Increasing the duration of extraction from 2 h to 8 and 18 h caused a significant decrease in L^* values compared to the same concentration of ethanol solvent.

The other colour parameters, a^*, b^* and h°, are quite complicated to describe when the various extraction conditions are compared. At the same durations of 2 and 8 h, the increase in the concentration of ethanol did not significantly affect the a^*, b^* and h° value. The results seem to indicate that the change in those parameters is related to the increase in duration more than the concentration of ethanol. At the same concentration, the value of a^* increased and the values of b^* and h° decreased when the extraction time was 18 h.

With the different organic solvent for extraction, the extracted colours using 95% ethanol and 99.8% hexane for 18 h were obviously dissimilar in appearance. The solution extracted using ethanol contained smaller values for L^*, b^* and h° at 23.2, 0.1 and 0.6, respectively, and larger in a^* values at 14.0 than those of values (L^*, b^*, h° and a^*) of solution extracted using hexane at 75.3, 0.5, 54.4 and 0.1, respectively. The lightness in extracts of hexane was mainly attributed to the greater luminosity. For the colour of red cabbage extracts with 95% ethanol, there was less transmission of light represented by the colour magenta while the other extract scarcely had colour. This result corresponds to previous research by Khandare *et al.* (2011) who studied extraction processing on the colour of black carrot and reported that the extract with small L^* value and large a^* value would show intense coloration in the sample.

Anthocyanin content

Table 2 shows the anthocyanin contents of red cabbage obtained by extraction at different organic solvents, durations and concentrations. The various condition of extract contains anthocyanins in the range 0.0-6.7 mg/100 g fresh weight (FW) with significant differences in each treatment. At the same

Table 1. Colour parameters of red cabbage extracts at various organic solvents, durations and concentrations

Sample	Duration (h)	L*	a*	b*	h°
50% E	2	28.2 ± 1.2^{b}	8.5 ± 1.4^{e}	0.5 ± 0.1^{a}	3.0 ± 0.2^{b}
70% E		26.4 ± 0.6^{c}	9.2 ± 1.0^{de}	0.4 ± 0.1^{ab}	2.4 ± 0.7^{b}
95% E		28.0 ± 1.0^{b}	8.9 ± 0.2^{e}	0.4 ± 0.1^{abc}	2.3 ± 0.7^{b}
50% E	8	26.3 ± 0.6^{c}	10.6 ± 1.4^{cd}	0.3 ± 0.0^{bcd}	1.4 ± 0.2^{c}
70% E		24.8 ± 0.2^{d}	12.1 ± 0.7^{bc}	0.1 ± 0.0^{d}	0.7 ± 0.2^{cd}
95% E		25.2 ± 0.7^{d}	10.8 ± 0.8^{bc}	0.2 ± 0.0^{d}	1.1 ± 0.1^{cd}
50% E	18	24.3 ± 0.1^{d}	12.3 ± 0.6^{b}	0.2 ± 0.1^{cd}	1.1 ± 0.5^{cd}
70% E		22.4 ± 0.3^{e}	15.2 ± 0.4^{a}	-0.1 ± 0.1^{e}	-0.2 ± 0.2^{e}
95% E		23.2 ± 0.1^{e}	14.0 ± 0.3^{a}	0.1 ± 0.1^{d}	0.6 ± 0.2^{d}
99.8% H		75.3 ± 0.4^{a}	0.1 ± 0.0^{f}	0.5 ± 0.0^{a}	54.4 ± 1.3^{a}
F-test		**	**	**	**

Different superscripts in the same column denote that the mean values are significantly different at $p \leq 0.01$

Table 2. Anthocyanin content and total phenolic content of red cabbage extracts at various organic solvents, durations and concentrations

Sample	Duration (h)	ANC*1	TPC*2
50% E	2	2.4 ± 0.1^{i}	168.3 ± 1.1^{f}
70% E		2.8 ± 0.1^{g}	177.8 ± 0.5^{d}
95% E		2.6 ± 0.0^{h}	174.0 ± 1.0^{e}
50% E	8	4.0 ± 0.0^{f}	177.4 ± 1.0^{d}
70% E		4.8 ± 0.1^{d}	185.1 ± 1.0^{b}
95% E		4.3 ± 0.1^{e}	181.6 ± 1.2^{c}
50% E	18	5.1 ± 0.1^{c}	180.8 ± 0.9^{c}
70% E		6.7 ± 0.0^{a}	189.1 ± 1.7^{a}
95% E		6.5 ± 0.1^{b}	184.3 ± 0.9^{b}
99.8% H		0.0 ± 0.0^{j}	2.8 ± 0.2^{g}
F-test		**	**

Different superscripts in the same column denote that the mean values are significantly different at $p \leq 0.01$
Remark *1= Anthocyanin content (mg/100 g FW)
*2= Total phenolic content (mg gallic acid equivalents/100 g FW)

concentration of ethanol, the anthocyanin content increased in extracts with longer extraction times. The duration of 18 h of extraction using 70% ethanol yielded the greatest content of anthocyanin in the extract. Lapornik *et al.* (2005) have reported that the yield of total anthocyanins of black currant extracted using 70% ethanol was enhanced 48% by increasing the extraction time.

When the result of 95% ethanol and 99.8% hexane for 18 h was comparison, the ethanol extract contained an anthocyanin content of 6.5 mg/100 g FW while the hexane extract had no anthocyanin content. The better extraction using ethanol was most likely due to the major polar compounds in red cabbage leaves. Anthocyanins are polar molecules with aromatic rings that are conjugated with multi-hydroxyl, methoxy and glycosyl groups. Harborne and Grayer (1988) reported that the polarity of anthocyanin molecules affected the extraction and separation of anthocyanins from the samples. Commonly, polar organic solvents exhibit a better ability to elute anthocyanins nonpolar solvents. Thus, anthocyanins would be better extracted by a polar solvent, 95% ethanol, than a non-polar organic solvent, 99.8% hexane. This result with regard to the colours of the extracts as shown in the Table 1 is apparently related to the anthocyanin content, which increased with decreases in h° and an increase in the a^* value (Kim *et al*., 2007).

Total phenolic content

Total phenolic contents of red cabbage at various extract conditions are shown in Table 2. The ethanol extract showed the smallest value in 50% ethanol for 2 h, 168.3 mg gallic acid equivalents/100 g FW. At the same concentration i.e., 70% ethanol, total phenolic content of the extract increased to larger values: 177.8, 185.1 and 189.1 mg gallic acid equivalents/100 g FW with durations 2, 8, and 18 h, respectively (Lapornik *et al*., 2005; Turkmen *et al*., 2007). From the result, the extraction obtained using 70% ethanol for 18 h showed the largest total phenolic content, corresponding to the results of anthocyanins. The total phenolic content was greater with greater anthocyanin content as mentioned earlier.

The large amounts of phenolics in the 70% ethanol-extracted solution were due to the optimal combination of water and organic solvent. This combination for extraction contributed to a greater polarity index compared to 95% ethanol. Chandrasekhar *et al.* (2012) reported that the increased proportion of the water in the ethanol enhanced the polarity index of the solvent, subsequently increasing the degrees of extraction. However, the higher proportion of water in the solvent might affect identification and quantification of phenolics and interfere with organic acids, sugars, and soluble proteins (Robards *et al*., 1999; Lapornik *et al*., 2005; Liyana-Pathirana and Shahidi, 2005).

As expected result of total phenolic contents of red cabbage extracts obtained using 95% ethanol and 99.8% hexane, the solution extracted using ethanol contained a large total phenolic content. This result is due to the major polar compounds in red cabbage leaves such as anthocyanins that are a one subgroup of phenolic compounds (Arnnok *et al.* 2012). Thus,

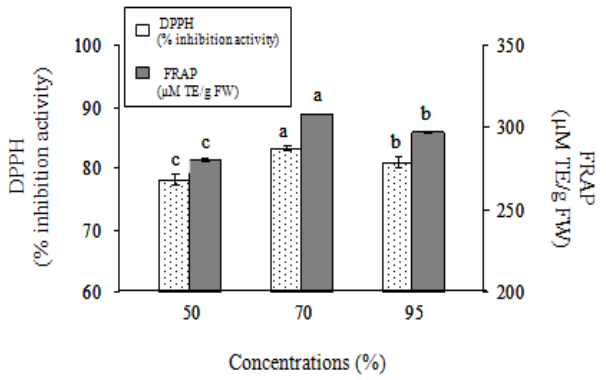

Figure 1. Antioxidant activity of red cabbage extracts obtained using various ethanol concentrations after an extraction time of 2 h

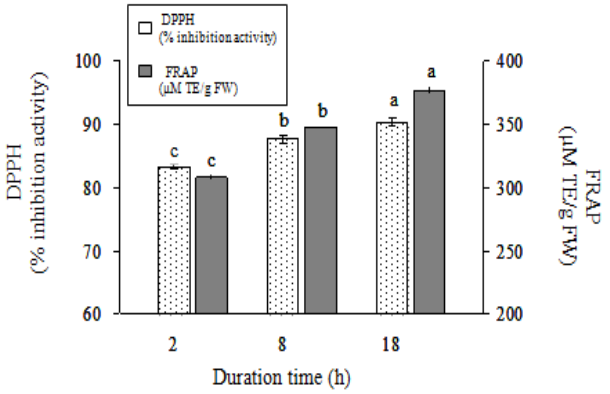

Figure 2. Antioxidant activity of red cabbage extracts obtained using 70% ethanol and various durations

the total phenolic content of flavonoids including anthocyanins would be better extracted by 95% ethanol, a polar organic solvent, than hexane.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Figure 1 shows the antioxidant activity of red cabbage extracts using various ethanol concentrations at a duration of 2 h. That per cent inhibition activity of all extracts illustrated a range of 78.1%-83.2%. The 70% ethanol extract had the largest value of 83.2% while that of 50% ethanol extract had the smallest value. When increasing the duration to 8 and 18 h, the only antioxidant activity obtained from 70% ethanol was compared as shown in Figure 2. The percent inhibition significantly increased with increasing the duration time, and it increased up to 90.4% at a duration of 18 h.

The antioxidant activity at the duration time for 18 h of the sample extracted using 95% ethanol solvent was greater than that obtained using 99.8% hexane (data not shown). The results seem that the percentage of inhibition activity is related to total phenolic content; the inhibition activity was greater with greater total phenolic content. In fact, the phenolic compounds belong to a group of phytochemical substances whose molecules are easy to oxidise such that they can donate hydrogen atoms to reactive compounds and resist free radicals (Castañda-Ovando *et al.* 2009). Fleschhut *et al.* 2006 have been reported that cyanidin, which is a natural organic compound in a particular type of anthocyanins, possesses o-dihydroxy substitution, which would increase the antioxidant activity of the extract.

Ferric-reducing antioxidant power value (FRAP)

From Figure 1, the FRAP values of ethanol of the various concentrations of 50%, 70%, and 95% at 2 h were significantly different. The extracts were in a range of 280.6-308.0 μM TE/g FW. The FRAP value of 70% ethanol had the largest value as 308.0 μM TE/g FW. By increasing the extraction times as shown in Figure 2, extracts had greater antioxidant activity. The results illustrated that a long duration of 18 h with 70% ethanol-extracted solution yielded the greatest the activity as determined in the FRAP assay. An increase in antioxidant capacity in red cabbage extracts was correlated with anthocyanins and total phenolic contents. As a result, solution extracted using 70% ethanol for 18 h was the best condition for extracting the antioxidant properties of red cabbage.

On the other hand, hexane solvent had no antioxidant activity analysed by FRAP assay. Although solution extracted by using hexane had a low total phenolic content, it had no anthocyanins. One could conclude from this result that the anthocyanins play important role in antioxidant activity as tested by FRAP assay.

Conclusions

Ethanol was suitable for extracting natural colorant from red cabbage leaves with significant amounts of antioxidant compounds, such as anthocyanins, and total phenolic contents because of its high degree of polarity compared to the non-polar solvent hexane. The adjustment of polarity properties when adding water showed a moderate portion of 70% ethanol was better than 50 and 95% ethanol. A greater quality of extracts came with an increase in extraction time. The solution extracted using 70% ethanol for 18 h was appropriate for obtaining colorant extracts with large quantities of anthocyanins and hydrophilic antioxidants from red cabbage.

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