

Optimization of Extraction of Anthocyanins from Roselle (*Hibiscus sabdariffa* var. *sabdariffa*) in Aqueous Medium

GHANENDRA GARTAULA* and DHAN BAHADUR KARKI

Central Department of Food Technology, Tribhuvan University, Dharan, Nepal

The dried roselle (*Hibiscus sabdariffa* var. *sabdariffa*) calyces were studied for the extraction of anthocyanin in aqueous solution. The total anthocyanin content in dried roselle powder was found to be 310.48mg/100g of dry matter. The extraction temperature at 70°C gave the highest yield (66.69-70.61%), while at 90°C the degradation of anthocyanins occurred. The solid-liquid ratio revealed no significant effect ($p>0.05$), whereas the particle size showed a significant effect ($p<0.05$) on the extraction of anthocyanin. The yields of anthocyanin extraction for 300 and 600 μm particle powders were significantly higher ($p<0.5$) than that of 1180 μm powder. Reduction in the particle size inversely increased the yield of anthocyanins. In conclusion, the extraction of anthocyanin from dried roselle calyces with an average particle size of 600 μm using a solid-liquid ratio of 1:50 in aqueous medium with pH 7.0 at 70°C for 20min showed the maximum yield (69.84%).

Keywords: Anthocyanin, Roselle, Calyx, Extraction condition, Solid-liquid ratio

Introduction

Color is an important factor in influencing consumers' acceptability of any food products. It is highly appreciated for its aesthetic role as well as a basis for the quality. In this latter respect, color gives visual cues to flavor identification and taste thresholds, influencing food preferences, food acceptability and ultimately food choice. Most food nowadays is processed in some way before reaching the consumer and manufacturers have a need to replace colour lost during processing or to colour products which would otherwise be colorless and unappealing. With increasing public concern on the safety of synthetic colorants, natural pigments are assuming greater prominence (Bridle and Timberlake, 1997). The pigments and colour precursors found in fruits and vegetables occur for the most part in cellular plastid inclusions (e.g. chloroplasts and other chromoplasts) and to a lesser extent dissolved in fat droplets or water within the cell protoplast and vacuole. These pigments can be classified into four major groups; chlorophylls, carotenoids, anthocyanins and anthoxanthins. Pigments belonging to the latter two groups are also referred to as flavonoids (Potter and Hotchkiss, 1996). Anthocyanins are the water-soluble pigments responsible for the brilliant orange red through deep purple colors in flowers and fruits (Wong, 1996). In food plants, anthocyanins are widespread occurring in at least 27 families, 73 genera and a multitude of species. It is reasonable to assume; therefore, that human beings are well conditioned to ingesting anthocyanins; further, it may be argued that the present day diet with its increasing dependence on processed foods has become deficient in anthocyanins (Bridle and Timberlake, 1997). At present, the role of anthocyanins as food colorant is becoming increasingly important. Not only do they contribute to the most important attributes of food—both for aesthetic value and for quality judgment, but also they tend to yield potential health effects, as they have been

observed to possess potent antioxidant properties (Tsai *et al.*, 2002). The biological effect of anthocyanins in human low density lipoprotein and lecithin-liposome systems have been reported (Meyer *et al.*, 1997; Satue-Gracia *et al.*, 1997). The anthocyanins are not only non-toxic and non-mutagenic but also have positive therapeutic properties for instance, in ophthalmology for the treatment of various circulatory disorders and inflammatory diseases (Bridle and Timberlake, 1997).

Materials and Methods

Raw materials: Fresh mature roselle (*Hibiscus sabdariffa* var. *sabdariffa*) calyces were brought from Gaurigunj-8 of Jhapa District, Nepal. The calyces were separated manually, cleaned in clean water and drained. The calyces were then dried intermittently in the cabinet drier (REICO®) at 55±5°C. The dried calyces were packed in double Polythene (PE) pouches, kept in black PE bag and stored at -28°C (deep fridge) until use. Prior to analysis, the calyces were milled in a mixer blender (PICASSO®). The powdered calyces were then sieved through three different standard sizes of 300, 600 and 1180 μm (Sethi Standard Test Sieves).

Methodology: Moisture content (Hot air oven method), crude fat (Ether extraction method), protein (Micro-Kjeldahl), crude fiber, ash content and total acidity were determined as per Ranganna, (2007). The aqueous extraction of the powdered calyces was carried out at three different pH, viz. pH 4, 7 and 9 at different times and temperatures. The pH was maintained using citric acid monohydrate (Qualigenes Fine Chemicals, India), sodium bicarbonate (Merck Limited, India) and sodium hydroxide (Qualigenes Fine Chemicals, India) respectively. A portable pH tester (HANNA®, Hanna Instruments HI96107) was used to monitor the pH. The extraction of the roselle powder was carried out in water bath (SHAKER BATH SBS 30®, UK (for 90±2°C) and Clifton® Nickel Electro Limited, England (for 70±2°C and 50±2°C). The incubation temperature

*Correspondent author, E-mail: ghanendra@gmail.com

and heating time used were $90\pm 2^{\circ}\text{C}$ (10, 20 and 30 min), $70\pm 2^{\circ}\text{C}$ (20, 40 and 60min), $50\pm 2^{\circ}\text{C}$ (20, 40 and 60 min) and $32\pm 2^{\circ}\text{C}$ (30, 60 and 90 min). Once the thermal treatment was completed the conical flasks were taken out and brought to room temperature ($32\pm 2^{\circ}\text{C}$) immediately then filtered. A particle size of $1180\mu\text{m}$ and solid-liquid ratio of 1:100 was used in the first set of experiment. In the second set of experiment, the extraction was carried out for 20min using solvent of pH 7, at $70\pm 2^{\circ}\text{C}$. The particle sizes were varied, viz., 300, 600 and $1180\mu\text{m}$. Similarly, the solid-liquid ratio was also varied, as 1:50, 1:100 and 1:150. The aqueous extraction was carried out as per the method described by Tsai *et al.*, (2002). For the extraction of total anthocyanins roselle powder was soaked in distilled water and ground in a mortar and pestle. The process was continued until the residue was colourless. The volume was made up 50 ml in a conical flask. The extract was then filtered for further analyses purposes.

The pH of the extract was measured using portable pH tester (HANNA®). The total acidity of the extract was measured as per Ranganna, (2007). From the total acidity, the percentage (%) extraction of acid was calculated. The total anthocyanin of the extract was measured as per Srivastava and Kumar, (2002) with some modification. Three ml of the extract was taken and diluted to 15 ml with 0.1 N HCl. It was then allowed to equilibrate in dark for 1hr. The absorbance was measured at $32\pm 2^{\circ}\text{C}$ and 510 nm using a spectrophotometer (Digital Spectrophotometer, Model 305).

Calculation: Total OD/ 50 ml = (OD* volume made up* 50/ ml juice taken),

Total anthocyanins (mg/50ml) = (Total OD/50ml)/87.3

The total anthocyanin was expressed on percentage dry basis.

Statistical analysis: The analysis were carried out in triplicates and all the data were calculated using Microsoft Office Excel (2003), and reported as means \pm SD (standard deviation). The statistics significance was evaluated by Analysis of Variance (ANOVA) (two-way, no blocking, all interactions) using Genstat Discovery Edition 3.0. The means of the data were compared using Least Significant Difference (LSD) method at 5% level of significance ($p < 0.05$).

Results and Discussion

Proximate composition: The crude protein, ether extract, crude fiber, ash and acidity content (dry basis) of roselle was found to be $13.57\pm 0.85\%$, $2.38\pm 0.22\%$, $8.80\pm 0.45\%$, $6.23\pm 0.31\%$ and $33.05\pm 1.58\%$ respectively. The ethereal extract and the crude fiber was found quite lower than that given in The Wealth of India (1997). However, the results were at par with that given by Babalola *et al.* (2001).

Total anthocyanins: The total anthocyanins of the roselle was found to be 310.48 mg/100g (dry basis). This constitutes only about 20.7% of that reported by Du and Francis (1973).

This might be due to the longer storage time which caused the degradation of anthocyanins to such an extent (Marti *et al.*, 2001; Alighourchi and Barzegar, 2009). Though degradation might have occurred, this is considered a good yield compared with some natural pigments reported in the literatures, such as in grapes (Dieci, 1967), red cabbage (Idaka, 1987-88), elderberry (Bronnum-Hansen *et al.*, 1985), blackberry (Fan-Chiang and Wrolstad, 2005), strawberry (da Silva *et al.*, 2007), wood sorrel (Pazmino-Duran *et al.*, 2001) and black sorghum bran (Awika *et al.*, 2004), to name a few.

Extraction of the powder: In the preliminary experiment, pH of the solvent, extraction time and extraction temperature were varied. Extraction was carried out at solvent pHs of 9, 7 and 4, extraction temperatures of 32 ± 2 , 50 ± 2 , 70 ± 2 and $90\pm 2^{\circ}\text{C}$, and extraction times varying from 10 to 90min. The particle size was $1180\mu\text{m}$ and solid-liquid ratio (S/L) was 1:100.

Effect of particle size and solid-liquid ratio on pH, acidity and anthocyanins of the extract: From the preliminary experiment, it was found that the temperature is the only factor that affected the extraction of anthocyanins from the roselle powder. The pH of the solvent and the extraction time had no significant effect ($p > 0.05$) on the extraction of anthocyanins. Extraction temperature of 70°C gave the highest yield. Hence, the second study was conducted using the solvent of pH 7 (neutral pH), extraction temperature of 70°C and the lowest extraction time at 70°C , i.e. 20 min. The result was in close support to the results reported by Fan *et al.*, (2008) and Ruenroengklin *et al.*, (2008).

The particle size and solid-liquid ratio (S/L) were varied. The particle sizes used were 300, 600 and $1180\mu\text{m}$. The solid-liquid ratio was 1:50, 1:100 and 1:150. Here, the particle size refers to the size of the roselle powder, and S/L refers to the ratio of powder to the solvent. The extraction processes were the same as that conducted in the first set of the experiment. The effect of particle size and solid-liquid ratio on the pH of the extract, the extraction of acid and the extraction of anthocyanins from the roselle powder were studied.

Effect on pH of the extract: The effect of particle size and solid-liquid ratio on the pH of the extract is shown graphically in Figure 1. The pH of the extract increased as the particle size was increased from 300 to $600\mu\text{m}$ but remained constant at $1180\mu\text{m}$, for solid-liquid ratio 1:50. At solid-liquid ratio of 1:100, pH increased first and then again decreased to the initial level with change of particle size from 300 to $1180\mu\text{m}$ through $600\mu\text{m}$, as shown in Figure 1. At S/L of 1:150, the pH of the extract remained fairly constant for 300 and $600\mu\text{m}$ while it decreased slightly for $1180\mu\text{m}$ particle size.

Statistical analysis revealed that the particle size and solid-liquid ratio had no significant effects ($p > 0.05$) on the pH of the extract. The interaction effect of particle size and solid-liquid ratio was also insignificant ($p > 0.05$).

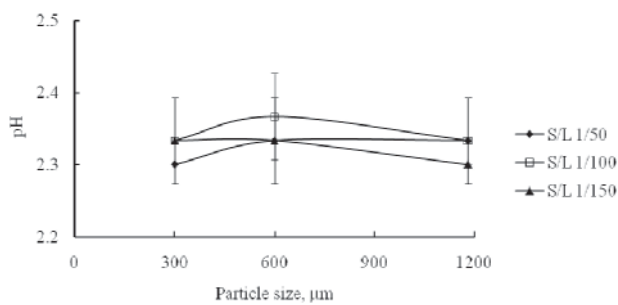


Figure 1. Effect of particle size and solid-liquid ratio on pH of the extract

Effect on extraction of acid: Figure 2, shows the effect of particle size and solid-liquid ratio on the extraction of acid. With the solid-liquid ratio of 1:50, a decreasing trend of the extraction of acid was observed for all particle sizes. The extraction of acid remained constant with particle sizes 300 and 600µm, and increased slightly when the particle size was increased to 1180µm, when solid-liquid ratio of 1:100 was used. When solid-liquid ratio was varied to 1:150, the extraction decreased initially with a rise later on as the particle sizes were varied as 300, 600 and 1180µm respectively. Statistical analysis revealed that particle size and the interaction of particle size and solid-liquid ratio had no significant effect at 5% level of significance on the extraction of acid. But, solid-liquid ratio had a significant effect ($p < 0.05$).

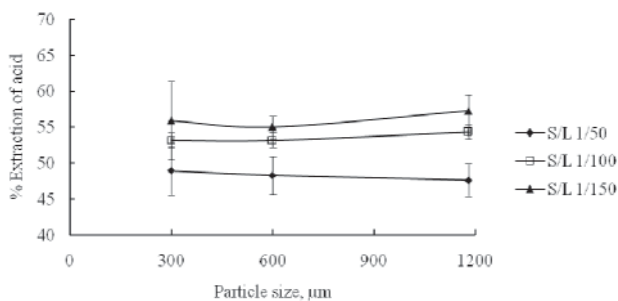


Figure 2. Effect of particle size and solid-liquid ratio on the extraction of acid

The extraction of acid from the roselle powder with 1:50 solid-liquid ratio was significantly lower than those of 1:100 and 1:150 for all particle sizes. Extractions done at solid-liquid ratio of 1:100 and 1:150 were not different statistically in all the three cases. This could not be explained due to the unavailability or supporting literatures. The highest yield achieved with the ratio 1:100 was 54.34% and that with 1:150 was 57.24%.

Effect on anthocyanins extraction: The effect of particle size and solid-liquid ratio on the extraction of anthocyanins is graphically presented in Figure 3. As seen from the graph, with solid-liquid ratio of 1:50, the extraction decreased with increase in particle size from 300 to 1180 µm. With solid-liquid ratio of 1:100, the extraction of anthocyanins increased as the

particle size increased from 300 to 600µm, but again decreased when the particle size was 1180µm. A similar pattern was seen with the solid-liquid ratio of 1:150.

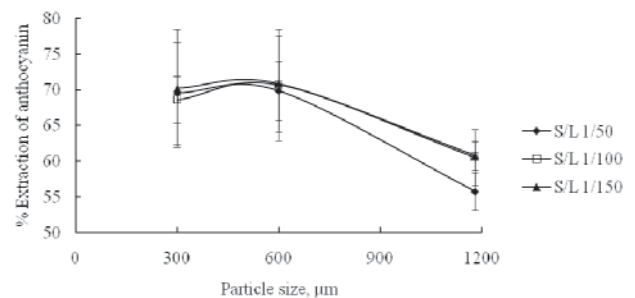


Figure 3. Effect of particle size and solid-liquid ratio on the extraction of anthocyanins

Statistical analysis revealed that the particle size of the roselle powder had a significant effect ($p < 0.05$) on the extraction of anthocyanins. However, the solid-liquid ratio and the interaction of particle-size and solid-liquid ratio had no significant effect at 5% level of significance ($p > 0.05$).

The extraction of anthocyanins in the extract with 300 and 600µm powders differed significantly with that of 1180µm powder, irrespective of the solid-liquid ratio; the former two being statistically the same and higher than the latter one. The extraction yield of 69.84% was obtained with the S/L of 1:50 and 600µm powder.

It was seen from the result that the smaller the particle size, the more was the extraction of anthocyanins. The result agrees with the facts given by Gao and Mazza (1996) and Landbo and Meyer, (2001) reported that the reduction in particle size increases the yield of anthocyanins. No significant effect ($p > 0.05$) of solid-liquid ratio was seen. Gao and Mazza (1996) reported that the yield of anthocyanins was unaffected by the solid-liquid ratio. In contrary, Cacace and Mazza (2003) reported that the major factor affecting the extraction of anthocyanins was solvent-to-solid ratio.

Since the extraction of anthocyanins from 300 and 600µm powder were statistically the same, it could be better to choose the latter, as it would be more energy efficient process. Furthermore, the solid-liquid ratio of 1:50 would be appropriate to choose due to the use of less amount of solvent.

Conclusion

The total anthocyanins content in the calyces was found to be 310.48 mg/100g (db). From the experimental result and consideration on the extraction of anthocyanins, the best extraction time, temperature and pH of the solvent were found to be 20min, 70°C and 7.0 respectively. The particle size of powder significantly affected ($p < 0.05$) on the extraction of anthocyanins. The maximum anthocyanins extraction was obtained by using a solid-liquid ratio of 1:50 with solvent pH of 7.0 by using particle size of 600µm at 70°C for 20min.

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