Technical paper

Anthocyanin Recovery from Mangosteen (*Garcinia mangostana* L.) Hull using Lime Juice Acidified Aqueous Methanol Solvent Extraction

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The recovery of anthocyanin from mangosteen (*Garcinia mangostana* L.) hull was investigated using an aqueous methanol solvent acidified by Mexican lime (*Citrus aurantifolia* Swingle) juice in comparison with the conventional HCl acidified methanol solvent. The addition of 0.20 mL lime juice/mL to an aqueous methanol extraction solvent gave a maximum total monomeric anthocyanin (TMA) recovery of 4.742 ± 0.590 mg cy-3-glu/g hull powder compared to 2.950 ± 0.265 when using an HCl acidified extraction solvent at 0.20%. This acidified aqueous methanol extraction solvent using lime juice produced an increase of 60.75% anthocyanin which suggests that this natural lime juice can be a good acidifying agent. The quantity of lime juice or HCl added to the extraction solvent was found to be a more important factor than its pH value in influencing TMA yield. A lower particle size of hull powder of 250 μ m to 500 μ m was also found to give the highest recovery of anthocyanin ($p < 0.05$).

Keywords: *Garcinia mangostana* L., extraction, *Citrus aurantifolia* Swingle, particle size, total monomeric anthocyanin, hydrogen bond formation

Introduction

Anthocyanins in plants have important roles in attracting insects for pollination and seed dispersal, for which it is crucial to maintain an eco-biology interaction and balance (Kong *et al.*, 2003). Numerous studies have revealed that anthocyanins extracted from various plants, such as the Nasturtium flower (Garzón and Wrolstad, 2009), *Crataegus monogyna* flower (Froehlicher *et al.*, 2009), tart cherry products (Kirkosyan *et al.*, 2009), black and red mulberry fruits (Özgen *et al.*, 2009), purple corn (Yang and Zhai, 2009), and even *Garcinia indica* Choisy (Nayak and Rastogi, 2011), possess antioxidant properties. In addition, anthocyanins have also been reviewed for their anti-cancer properties (Wang and Stoner, 2008). More commonly, anthocyanins are widely used as a source of natural colourants in the food processing industry and for this reason, and their contributive function towards

health, studies of enhancing the anthocyanin yield from plant materials have been explored.

Various methods have been used to obtain anthocyanins from plant materials. These include extraction methods of fractionated high speed pressure from elderberry pomace (Sae-bra *et al.*, 2010), subcritical solvent from dried grape pomace (Monrad *et al.*, 2010), and ultrasound-assisted from *Garcinia indica* Choisy (Nayak and Rastogi, 2013). Despite the different methods, solvent extraction of anthocyanins from plant materials is the most widely employed method and has been well documented in two standard protocols. They are the acetone extraction and chloroform partition, and the acidified methanol extraction protocol (Giusti and Wrolstad, 2005). The latter protocol is more commonly used by researchers probably due to it being simple, fast and effective. It involves maceration or soaking of plant material in methanol containing a small concentration of mineral acid such as hydrochloric acid (HCl) (Burin *et al.*, 2011; Du and Francis, 1977; González-Paramás *et al.*, 2006; Koh *et al.*, 2011; Palapol *et*

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al., 2009).

The purpose of acidifying the extraction solvent in the process of extracting anthocyanins from plant materials is to denature the membranes of the cell tissue in which most of the anthocyanin pigments are located (Giusti and Wrolstad, 2005). Although the use of a HCl acidified extraction solvent enhances the anthocyanin yield, it may result in pigment degradation, as noted by a few researchers during the evaporation process to concentrate crude extracts for analytical needs (Froehlicher *et al.*, 2009; Garzón and Wrolstad, 2009; González-Paramás *et al.*, 2006). Hence, in reducing pigment degradation, weaker organic acids, namely acetic acid (Kirakosyan *et al.*, 2009; Özgen *et al.*, 2009), formic acid (Sun *et al.*, 2009), and trifluoroacetic acid (Baublis *et al.*, 1994) have been tried. While the juice of the Mexican lime (*C. aurantifolia* Swingle) contains mostly citric and malic acids (Vandercook, 1977), this research has attempted its use as another source of natural and organic acid for extracting anthocyanins from mangosteen (*Garcinia mangostana* L.) hulls. Another reason for the use of Mexican lime juice is because of its additional health benefits. It is well known not only as a good source of vitamin C, but also other vitamins such as thiamin, riboflavin, niacin, vitamin A and mineral elements such as potassium and calcium (Ortiz, 2002). Reports from the literature mention that all the freeze-dried citrus juice of Mexican lime extracted with chloroform, acetone, methanol and 80% aqueous methanol exhibited inhibitory activity against human pancreatic cancer cells. The methanolic extract showed the best effect (Patil *et al.*, 2009b). Further, the Mexican lime oil, either extracted from the peel (Razzaghi-Abyaneh *et al.*, 2009) or the whole fruit (Patil *et al.*, 2009a), has been discovered to have an anti-aflatoxigenic activity and the medicinal property of inhibition of human colon cancer cells. Recent studies by Rammanee and Hongpattarakere (2011) demonstrated that hydro-distilled essential oil extracted using ethyl acetate from Mexican lime peel, exhibited stronger anti-fungal activity compared to shogun mandarin, pomelo and acidless orange peels.

The objective of this paper is to compare the recovery of total monomeric anthocyanin (TMA) yield from mangosteen hull using an aqueous methanol solvent acidified with natural Mexican lime juice and the yield using HCl. The effect of different mangosteen hull powder particle sizes on the TMA yield is also to be investigated.

Materials and Methods

Mangosteen hull powder preparation Fresh and fully ripened mangosteen fruits were harvested for the hull as the red pigments of cyanidin-3-sophoroside and cyanidin-3-glucoside identified from mangosteen hull increase as the colour

of the mangosteen fruits change from green to purple black as they ripen (Du and Francis, 1977; Palapol *et al.*, 2009). The preparation of dried mangosteen hull was as described by Cheok *et al.* (2012) where small pieces of mangosteen hulls were dried under a fan at an ambient temperature of 28°C for at least two days to achieve a moisture loss of about 48.5% from the initial weights measured. Two kilograms of dried hull were ground into powder with a particle size below 500 µm using a dry grinder (RT-02A, Taiwan) for the acidified aqueous methanol extraction study while another one kilogram was ground into powder with five segregated sizes for studying the effect of particle size on extraction efficiency.

The ground powder was sieved using an analytical sieve shaker (AS200, Retsch, Germany) operated for 10 min under continuous mode to obtain particle sizes smaller than 500 µm and also the five size ranges of ≤ 250 µm (X_1), 250 µm – 500 µm (X_2), 500 µm – 1 mm (X_3), 1 mm – 2 mm (X_4), and 2 mm – 4 mm (X_5). The particle size distribution of mangosteen hull powder below 500 µm was determined using a particle size analyzer (MAL1009117, Mastersizer 2000, Malvern, U.K.) and reported as volume percentage ± standard error, $n = 9$; 3.6 – 15.21 (0.844 ± 0.038%), 15.21 – 64.28 (8.216 ± 0.025%), 64.28 – 271.63 (62.706 ± 0.108%) and 271.63 – 515.41 (28.237 ± 0.064%) µm.

Preparation of Mexican lime juice and HCl acidified aqueous methanol extraction solvents Fifty pieces of green fresh Mexican lime fruits (Figure 1a) with an average diameter of 5 cm, were plucked and collected directly from a local farm located at Sungai Gadut, Negeri Sembilan, Malaysia (2°43' North, 101°53' East) in April 2010 for this experiment. The collection of fresh fruits followed the standard harvesting method for commercial distribution, i.e. all fruits were plucked and collected at their green and glossy stage before ripening to a pale-yellow. The fresh fruits were cut into half and the citrus juice was squeezed manually before filtering using a 1-mm kitchen sieve and kept in a refrigerator at 10°C (Figure 1b). Six different levels of lime juice acidified aqueous methanol extraction solvents were prepared using 70 mL methanol absolute with 5, 10, 15, 20, 25 or 30 mL of Mexican lime juice and distilled water to make-up a 100 mL. These six levels of Mexican lime juice acidified aqueous methanol solvents were labelled as 0.05, 0.10, 0.15, 0.20, 0.25, and 0.30 mL lime juice/mL aqueous methanol.

Another set of acidified aqueous methanol extraction solvent was prepared using hydrochloric acid (HCl) 36% (Fisher Scientific, Leicestershire, UK). The six levels of 100 mL-HCl acidified aqueous methanol extraction solvent were 0.01%, 0.05%, 0.10%, 0.15%, 0.20%, and 0.30%. The control extraction solvent was prepared with 70 mL methanol absolute

Fig. 1. (a) Mexican lime fresh fruits, (b) i. Half fruit; ii. Squeezing of the juice; iii. Juice kept in a bottle.

and 30 mL of distilled water without adding any acidic agent. The pH of each acidified aqueous methanol solvent and the control was measured using a digital pH meter (HI 8424, Hanna Instruments Inc, Romania). These extraction solvents were used for anthocyanin extraction immediately after each preparation and the extraction experiment was repeated at each acidified level.

Determination of organic acids in Mexican lime juice

The predominant organic acids in Mexican lime juice were determined using the titration method following the procedure of the Association of Analytical Chemists (AOAC) (2000) (method 940.15: Final Action). Phenolphthalein was used as the indicator where its colourless-to-pink transition appears at pH 8.0 – 9.6 during titration with 0.1M NaOH. The sample was analyzed in duplicate and the results averaged. The results obtained were expressed mg/100mL.

Extraction of anthocyanins The anthocyanins were extracted from the mangosteen hull powder prepared using acidified aqueous methanol solvents either from lime juice or HCl. Five grams of mangosteen hull powder were mixed with 100 mL acidified aqueous methanol solvents in a 250-mL conical flask. The extraction was conducted at room temperature ($25 \pm 1^\circ\text{C}$) on a stirring hot plate using a magnetic stirrer for two hours. The mixture was then centrifuged at 5000 rpm for 10 min, and filtered through a 6- μm filter paper (No.1, Advantec, Toyo Roshi Kaisha Ltd, Japan) with a vacuum pump aspirator (DOA-P604-BN, Cast Manufacturing Inc, USA). The extract was immediately taken for total

monomeric anthocyanin determination. Subsequently, using similar extraction procedures, mangosteen hull powder with different particle sizes ranging from $X_1 - X_5$ was used in the extractions using a 100 mL (70 mL methanol: 20 mL lime juice: 10 mL distilled water) extraction solvent and repeated for every particle size range.

Total monomeric anthocyanin (TMA) determination

The total monomeric anthocyanin (TMA) content of mangosteen hull powder was determined using the pH differential method (Lee *et al.*, 2005) which required two types of buffer solution, i.e., potassium chloride (KCl) and sodium acetate (CH_3COONa) buffers. The preparation of potassium chloride buffer of 0.025M, pH 1.0 and sodium acetate buffer of 0.4M, pH 4.5 followed the method of Giusti and Wrolstad (2005). In brief, the potassium chloride buffer was prepared by mixing 1.86 g of KCl powder (99.5% purity, Merck, Darmstadt, Germany) with 980 mL of distilled water in a beaker. The pH mixture was slowly and carefully adjusted to pH 1.0 by adding concentrated HCl while measuring the pH using a digital pH meter (HI 8424, Hanna Instruments Inc, Romania). For the sodium acetate buffer, this was prepared using 54.43 g of sodium acetate powder (99% purity, Merck, Darmstadt, Germany) mixed with 960 mL of distilled water. The pH of the mixture was also carefully adjusted using concentrated HCl until a pH of 4.5 was achieved. Both the buffer mixtures were transferred into their own respective 1-litre flask respectively, and filled with distilled water to make up to 1 litre.

The filtered crude extract was diluted to a factor of 200 using the two prepared buffer solutions; the potassium chloride buffer at pH 1.0 and the sodium acetate buffer at pH 4.5 in triplicate. The absorbance of these diluted extracts were measured at $\lambda_{\text{vis-max}}$ at 510 nm and 700 nm using a UV-Vis spectrophotometer (DR2800, Hach, U.S.A) against distilled water as a blank after allowance of 15 min equilibration after the dilutions. The total monomeric anthocyanins were calculated as cyanidin-3-glucoside using the following equation:

$$\text{TMA (mg/L)} = \frac{A \times MW \times DF \times 1000}{(\epsilon \times 1)} \quad (1)$$

where A (absorbance) = $(A_{510} - A_{700})_{\text{potassium chloride}} - (A_{510} - A_{700})_{\text{sodium acetate}}$; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside; DF (dilution factor) = 200; 1000 = conversion from g to mg; ϵ (molar absorptivity coefficient in L/mol/cm for cyanidin-3-glucoside) = 26,900; 1 = pathlength in cm. The TMA results in mg/L were then expressed as mg/g of hull powder. The triplicate results were averaged to arrive at a single figure.

Statistical analysis Single factor ANOVA was used to determine the significant difference of total monomeric anthocyanin from repeated trials in triplicate using the Data Analysis program in Microsoft Excel.

Results and Discussion

TMA yield using lime juice and HCl acidified aqueous methanol extraction solvents Figure 2a shows that the TMA yield increased 545% significantly ($p < 0.05$) from the control, from 0.735 ± 0.174 to 4.742 ± 0.590 mg cy-3-glu/g hull powder when 0.20 mL of lime juice acidified aqueous methanol solvent was used. Further addition of lime juice of 0.25 and 0.30 mL, however, did not give a further significant increment in anthocyanin yield. This suggests that the addition of lime juice up to 20 mL into the aqueous methanol extraction solvent is sufficient to give the maximum anthocyanin recovery and this could be due to the extraction system approaching an equilibrium stage. When using HCl, the TMA yield also increased significantly ($p < 0.05$) from the control but at a lower percentage of 301.36% for the addition of 0.20% HCl (Figure 2b).

The influence of acidified extraction solvents on anthocyanin yield using mineral acid, HCl has been reported by Chirinos *et al.* (2007). In their work, four HCl acidified 90% methanol concentrations of 0.005% (pH 5.00), 0.01% (pH 3.08), 0.10% (pH 2.11), and 0.15% (pH 1.45) were investigated and they discovered that the best conditions giving the highest yield of anthocyanin were within the range of 0.10% and 0.15%, where no significant differences were found between them. The anthocyanin yield increased significantly by 60.53% and 35.62%, respectively for the two types of purple

Fig. 2. TMA yield extracted from (a) lime juice, and (b) HCl acidified aqueous methanol solvents.

colour mashua tubers of ARB 5241 and DP 0224 when using 0.10% HCl acidified solvent compared to those using 0.005%. They explained that the addition of acid into the extraction solvent acted on the plant material in three different ways, i.e. firstly, it may have increased the stability of the anthocyanins, secondly, it may have favoured the dissolution of phenolic compounds via a hydrolysis mechanism, which are initially part of polymers or bound to other cell wall constituents, and thirdly, it may have improved the disintegration of the cell walls, which facilitated the solubilization and diffusion of phenolic compounds from the plant material. Their study however, was limited to using HCl in the acidified aqueous methanol extraction solvent.

While the use of acid in the extraction solvent enhances anthocyanin recovery, it may vary with plant materials. Chirinos *et al.* (2007) found that 90% aqueous methanol solution acidified with 0.10% HCl gave the highest anthocyanin recovery from mashua tubers while González-Paramás

et al. (2006) used 5% HCl acidified methanol for red runner bean and grape skins, and 0.1% for strawberries. For anthocyanin recovery from mangosteen hull, 0.20% HCl gave the highest content of 2.950 ± 0.265 mg cy-3-glu. The replacement of HCl with Mexican lime juice at 0.20 mL surprisingly increased the anthocyanin recovery further by 60.75%, yielding a TMA of 4.742 ± 0.590 mg cy-3-glu. This significant improvement in anthocyanin recovery suggests that this natural lime juice is viable as a replacement for HCl as an acidified agent which is a mineral acid in nature. Natural acids are by nature a good food additive and are more widely used in the food processing industry. In this regard, lemon juice has been proven by Yapó (2009) for its enhancement of galacturonic acid content, degree of esterification, viscosity-average molecular weight and the gelling power of pectin from a yellow passion fruit by-product as compared to commercial citric acid extractant. Additionally, Rusak *et al.* (2008) also revealed that the extraction of phenolics from white tea by water could be accelerated by the addition of lemon juice.

The improvement of anthocyanin recovery when using Mexican lime juice compared with mineral acid HCl can be linked to the hydrogen bond formations (Anslyn and Dougherty, 2006) of the carboxyl groups ($-\text{COOH}$) of the organic acids in the lime juice with the anthocyanin molecules of the mangosteen hull. The predominant organic acids present in the lime juice were citric acid (13.60 g/100 mL), followed by lactic (9.50 g/100 mL), tartaric (7.90 g/100 mL), malic (7.10 g/100 mL) and acetic acid (6.35 g/100 mL) as determined and listed in Table 1. All the organic acids are carboxylic acids which contain the carboxyl group ($-\text{COOH}$), a functional group in which a hydroxyl group ($-\text{OH}$) is directly bonded to the carbon atom of a carbonyl ($-\text{C}=\text{O}$) (Ege, 2004). The hydrogen atom of this carboxyl group is covalently bonded to a strongly electronegative atom of

oxygen, therefore, the affinity of this hydrogen atom with other nonbonding electrons is very great (Ege, 2004; Marechal, 2007). As such, the carboxyl groups of the lime juice acidified extraction solvent have a greater chance to form hydrogen bonds with the molecules of anthocyanin compared to HCl in the extraction solvent.

Effect of extraction solvent pH on TMA yield Even though the minor acidic condition of the extraction solvent could enhance anthocyanin yield by denaturing the cell wall membrane of the plant material where most of the pigment is located, the enhancement of the anthocyanin yield, whether due to its pH value or the quantity of acid agent present in the extraction solvent, has not been elucidated thus far. Despite no significant changes in pH values of the different concentrations of lime juice or HCl added, the TMA increased following the amount added. This is particularly obvious for the lime juice acidified extraction solvent (Figure 3a) where the addition of 0.05 mL of lime juice significantly ($p < 0.05$) reduced the pH value of the extraction solvent from 6.91 to 3.60. Further juice addition from 0.05 to 0.30 mL did not give any reduction in pH value. Likewise, the pH value of the HCl acidified extraction solvent significantly ($p < 0.05$) decreased from pH 6.91 to 2.07 at 0.10% (Figure 3b). These TMA yields suggest that the lime juice or HCl quantity in the

Table 1. Organic acids in Mexican lime (*C. aurantifolia* Swingle) juice

	(g/100 mL)
Lactic acid	9.50 ± 0.000^d
Acetic acid	6.35 ± 0.005^a
Citric acid	13.60 ± 0.000^c
Malic acid	7.10 ± 0.000^b
Tartaric acid	7.90 ± 0.000^c

Value expressed as mean $\pm$ standard deviation ($n = 2$).

Different letters indicate significantly different ($p < 0.05$).

Fig. 3. The pH of extraction solvents at different levels of (a) lime juice and (b) HCl addition.

acidified extraction solvent were predominating the pH effects.

The pH value displayed on the pH meter was the product of the measurement of H^+ activity in the extraction solvent with the oxygen atoms of the glass membrane of the gel filled glass electrode of the pH meter (Harris, 2007). The voltage of the electrode changed by 59.16 mV for every pH-unit change of H^+ activity, resulting lower pH values displayed on the pH meter for acidic conditions (Harris, 2007). H^+ activity is expected to be higher if the lime juice or HCl is increased in the acidified extraction solvent. However, when the oxygen atoms of the glass membrane are saturated and bound with H^+ , a further induction of acid agent into the extraction solvent might not stimulate H^+ activity. Consequently, no pH changes were observed above 0.05 mL/mL for the lime juice and 0.10% for HCl.

Effect of particle sizes of mangosteen hull powder on TMA yield For the 0.20 mL juice/mL acidified aqueous methanol extraction solvent, the maximum TMA yield obtained was 5.60 ± 0.48 mg cy-3-glu/g hull powder at a particle size of 250 to 500 μm (Figure 4). No significant difference ($p > 0.05$) of TMA yield was observed above 500 μm to 4 mm and also below 250 μm . This result indicates that a particle size of less than or equal to 500 μm was suitable for maximum anthocyanin yield. Particle size is expected to influence extraction yield as the specific surface area of a sample increases as the particle size decreases, and this subsequently decreases the solvent penetration path length and enhances the mass transfer or reaction between solute and solvent that influences bioactive compound yield recovery. This agrees with Li *et al.* (2009) who highlighted that the extraction of bioactive compounds from plant materials is directly affected by the particle size distribution where the specific surface area is closely tied to particle size distribution.

The selection of particle size in other bioactive compound extraction studies varied with its research objectives where Chirinos *et al.* (2007) extracted phenolic compounds from mashua using lyophilized powder at a particle size less than or equal to 0.1 mm. Gião *et al.* (2009) discovered that a particle size of 0.2 mm was sufficient to obtain the maximum antioxidant power for three types of medicinal plants, i.e., agrimony, sage, and savoury, which are used as traditional medicines in Portugal. Li *et al.* (2009) found that the decrease of particle size from 30 to 70 mesh resulted in a rapid increase in the extraction yield of essential oils of oleoresin from *Cuminum cyminum*, while a decrease of particle size from 60 to 90 mesh did not result in an increase in the oil yield. The authors revealed that excessively small particle sizes resulted in difficulty in filtering the extraction solution

Fig. 4. Effect of particle sizes of mangosteen hull powder on TMA yield.

of high density particles, thus lowering the yield.

Conclusions

The maximum TMA yield of 4.742 ± 0.590 and 2.950 ± 0.265 mg cy-3-glu/mg hull powder was achieved using 100 mL of acidified 70% aqueous methanol solvent with 20 mL of lime juice and 0.20% HCl, respectively. The TMA yield obtained using lime juice acidified aqueous methanol solvent was 60.75% higher compared to the HCl acidified solvent. This result suggests that natural lime juice can act as an organic acidic agent for anthocyanin extraction from mangosteen hull replacing mineral acid HCl. The anthocyanin yield extracted from mangosteen hull powder was influenced by the type and quantity of the acidic agent present in the solvent. The quantity of acidic agent added to the extraction solvent attributed to the maximum anthocyanin recovery was not influenced by its pH value. A particle size of less or equal to 500 μm greatly enhanced the anthocyanin yield.

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References

- AOAC International (Association of Official Analytical Chemists). (2000). In: Horwitz W (eds) *Official methods of analysis of AOAC International*, 17th edn., Vol. II., Maryland: USA.
- Anslyn, E.V. and Dougherty, D.A. (2006). "Modern physical organic chemistry." University Science Books, California, pp.145-202.
- Baublis, A., Spomer, A. and Berber-Jimenez, M.D. (1994). Anthocyanin pigments: Comparison of extract stability. *J. Food Sci.*, **59**, 1219-1221.

- Burin, V.M., Rossa, P.N., Ferreira-Lima, N.E., Hillmann, M.C.R. and Boirdignon-Luiz, M.T. (2011). Anthocyanins: optimisation of extraction from Cabernet Sauvignon grapes, microcapsulation and stability in soft drink. *Int. J. Food Sci. Technol.*, **46**, 186-193.
- Cheok, C.Y., Chin, N.L., Yusof, Y.A. and Law, C.L. (2012). Extraction of total phenolic content of *Garcinia mangostana* Linn. Hull I. Relationship between direct UV-Vis spectrophotometer absorbance and Folin-Ciocalteu measurement method. *Food Bioprocess Technol.*, **5**, 2928-2933.
- Chirinos, R., Rogez, H., Campos, D., Pedreschi, R. and Larondelle, Y. (2007). Optimization of extraction conditions of antioxidant phenolic compounds from mashua (*Tropaeolum tuberosum* Ruiz & Pavón) tubers. *Sep. Purif. Technol.*, **55**, 217-225.
- Du, C.T. and Francis, F.J. (1977). Anthocyanins of mangosteen, *Garcinia mangostana*. *J. Food Sci.*, **42**, 1667-1668.
- Ege, S. (2004). "Organic chemistry - structure and reactivity." Houghton Mifflin Company, Boston, pp. 29, 31-35, 55, 103-108, 594-625.
- Froehlicher, T., Hennebelle, T., Martin-Nizard, F., Cleenewerck, P., Hilbert, J.-L., Trotin, F. and Grec, S. (2009). Phenolic profiles and antioxidative effects of hawthorn cell suspensions, fresh fruits, and medicinal dried parts. *Food Chem.*, **115**, 897-903.
- Garzón, G.A. and Wrolstad, R.E. (2009). Major anthocyanins and antioxidant activity of Nasturtium flowers (*Tropaeolum majus*). *Food Chem.*, **114**, 44-49.
- Gião, M.S., Pereira, C.I., Fonseca, S.C., Pintado, M.E. and Malcata, F.X. (2009). Effect of particle size upon the extent of extraction of antioxidant power from the plants *Agrimonia eupatoria*, *Salvia* sp. and *Satureja montana*. *Food Chem.*, **117**, 412-416.
- Giusti, M. and Wrolstad, R.E. (2005). Characterization and measurement of anthocyanins by UV-Visible spectroscopy. In "Handbook of food analytical chemistry," ed. by R.E. Wrolstad, *et al.*, Wiley, New Jersey, pp. 19-31.
- González-Paramás, A.M., Lopes da Silva, F., Martín-López, P., Macz-Pop, G., González-Manzano, S., Alcalde-Eon, C., Rivas-Gonzalo, J.C. and Santos-Buelga, C. (2006). Flavanol-anthocyanin condensed pigments in plant extracts. *Food Chem.*, **94**, 428-436.
- Harris, D.C. (2007). "Quantitative chemical analysis" 7th edn. WH Freeman and Company, New York, pp. 298-321.
- Kirakosyan, A., Seymour, E.M., Llanes, D.E.U., Kaufman, P.B. and Bolling, S.F. (2009). Chemical profile and antioxidant capacities of tart cherry products. *Food Chem.*, **115**, 20-25.
- Koh, K., Youn, J.-E. and Kim, H.-S. (2011). Identification of anthocyanins in black soybean (*Glycine max* (L.) Merr.) varieties. *J. Food Sci. Technol.*, doi: 10.1007/s13197-011-0493-y.
- Kong, J.-M., Chia, L.-S., Goh, N.-K., Chia, T.-F. and Brouillard, R. (2003). Analysis and biological activities of anthocyanins. *Phytochem.*, **64**, 923-933.
- Lee, J.-M., Durst, R.W. and Wrolstad, R.E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *J. AOAC Int.*, **88**, 1269-1278.
- Li, X.-M., Tian, S.-L., Pang, Z.-C., Shi, J.-Y., Feng, Z.-S. and Zhang, Y.-M. (2009). Extraction of *Cuminum cyminum* essential oil by combination technology of organic solvent with low boiling point and steam distillation. *Food Chem.*, **115**, 1114-1119.
- Marechal, Y. (2007). "The hydrogen bond and the water molecule - the physics and chemistry of water, aqueous and bio media." Elsevier, Amsterdam.
- Monrad, J.K., Howard, L.R., King, J.W., Srinivas, K. and Mauro-moustakos, A. (2010). Subcritical solvent extraction of anthocyanins from dried red grape pomace. *J. Agric. Food Chem.*, **58**, 2862-2868.
- Nayak, C.A. and Rastogi, N.K. (2013). Optimization of solid - liquid extraction of phytochemicals from *Garcinia indica* Choisy by response surface methodology. *Food Res. Int.*, **50**, 550-556. doi: 10.1016/j.foodres.2011.02.033.
- Ortiz, J.M. (2002). Botany: taxonomy, morphology and physiology of fruits, leaves and flowers. In "Citrus - The genus citrus," ed. by G Dugo, and A Di Giacomo, Taylor and Francis, London, pp. 34.
- Özgen, M., Serçe, S. and Kaya, C. (2009). Phytochemical and antioxidant properties of anthocyanin-rich *Morus nigra* and *Morus rubra* fruits. *Sci. Hortic.*, **119**, 275-279.
- Palapol, Y., Ketsa, S., Stevenson, D., Cooney, J.M., Allan, A.C. and Ferguson, I.B. (2009). Colour development and quality of mangosteen (*Garcinia mangostana* L.) fruit during ripening and after harvest. *Postharvest Bio. Technol.*, **51**, 349-353.
- Patil, J.R., Jayaprakasha, G.K., Murthy, K.N.C., Tichy, S.E., Chetti, M.B. and Patil, B.S. (2009a). Apoptosis-mediated proliferation inhibition of human colon cancer cells by volatile principles of *Citrus aurantifolia*. *Food Chem.*, **114**, 1351-1358.
- Patil, J.R., Murthy, K.N.C., Jayaprakasha, G.K., Chetti, M.B. and Patil, B.S. (2009b). Bioactive compounds from Mexican lime (*Citrus aurantifolia*) juice induce apoptosis in human pancreatic cells. *J. Agric. Food Chem.*, **57**, 10933-10942.
- Rammanee, K. and Hongpattarakere, T. (2011). Effects of tropical citrus essential oils on growth, aflatoxin production, and ultra-structure alterations of *Aspergillus flavus* and *Aspergillus parasiticus*. *Food Bioprocess Technol.*, **4**, 1050-1059. doi: 10.1007/s11947-010-0507-1.
- Razzaghi-Abyaneh, M., Shams-Ghahfarokhi, M., Rezaee, M.-B., Jaimand, K., Alinezhad, S., Saberi, R. and Yoshinari, T. (2009). Chemical composition and antiaflatoxic activity of *Carum carvi* L., *Thymus vulgaris* and *Citrus aurantifolia* essential oils. *Food Contrl.*, **20**, 1018-1024.
- Rusak, G., Komes, D., Likić, S., Horžić, D. and Kovač, M. (2008). Phenolic content and antioxidative capacity of green and white tea extracts depending on extraction conditions and the solvent

- used. *Food Chem.*, **110**, 852-858.
- Seabra, I.J., Braga, M.E.M., Batista, M.T.P. and de Sousa, H.C. (2010). Fractioned high pressure extraction of anthocyanins from elderberry (*Sambucus nigra* L.) pomace. *Food Bioprocess Technol.*, **3**, 674-683.
- Sun, J., Yao, J., Huang, S., Long, X., Wang, J. and García-García, E. (2009). Antioxidant activity of polyphenol and anthocyanin extracts from fruits of *Kadsura coccinea* (Lem.) A.C. Smith. *Food Chem.*, **117**, 276-281.
- Vandercook, C.E. (1977). Organic acids. In "Citrus science and technology Vol.1-Nutrition, anatomy, chemical composition and bioregulation," ed. by Nagy *et al.*, The AVI Publishing Company Inc, Connecticut, pp. 208-224.
- Wang, L-S. and Stoner, G.D. (2008). Anthocyanins and their role in cancer prevention. *Cancer Letters*, **269**, 281-290.
- Yang, Z. and Zhai, W. (2009). Identification and antioxidant activity of anthocyanins extracted from the seed and cob of purple corn (*Zea mays* L.). *Innov. Food Sci. Emerg. Technol.*, **11**, 169-176.
- Yapo, B.M. (2009). Lemon juice improves the extractability and quality characteristics of pectin from yellow passion fruit by-product as compared with commercial citric acid extractant. *Biores. Technol.*, **100**, 3147-3151.