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Effect of ion exchange resin weight and extract flow rate on the properties of starfruit (*Averrhoa carambola* L.) extract

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Abstract

This research was carried out to determine the effect of resin weight and extract flow rate during deacidification on the oxalic acid content, physicochemical and antioxidative properties of starfruit extract. Nine treatments which consisted of different combinations of resin weight (3, 5 and 8 g) and flow rate (6, 9, and 12 ml/min) were carried out. Parameters measured were oxalic acid content, pH, total polyphenol content, vitamin C and free radical scavenging activity (DPPH). Increasing the weight of resin and decreasing the flow rate increased the pH of starfruit juice. The pH of starfruit juice increased from 3.6 for control to 9.9 for sample deacidified with 8 g of resin at flow rate of 6 ml/min. Oxalic acid content of starfruit juice showed a significant ($p<0.05$) reduction with deacidification. Deacidified samples showed significant decrease ($p<0.05$) in total polyphenol content, vitamin C and free radical scavenging activity compared to control. However, different combinations of resin weight and flow rate during deacidification did not have any significant effect ($p>0.05$) on total polyphenol content, vitamin C content and free radical scavenging activity of starfruit juice.

Introduction

Starfruit (*Averrhoa carambola* L.) is a very good source of natural antioxidants. On average, it contains 33 mg of polyphenols per gram of starfruit (Shui and Leong, 2004). It contains several polyphenols which include L-ascorbic acid, gallic acid, oxalic acid, tartaric acid, citric acid, α-ketoglutarate, succinate and fumaric acid (Morton, 1987; Shui and Leong, 2004). Several epidemiological studies showed that natural antioxidant intake is associated with reduction of many chronic diseases like cardiovascular disease and cancer (Thaipong *et al.*, 2006; Tsai *et al.*, 2007). Antioxidant plays an important role in maintaining homeostasis in human body by interacting with free radicals and terminating the chain reaction before vital molecules are damaged.

While consuming starfruit and its extracts are beneficial to most, it has been reported to cause adverse health effects on renal compromised individuals. In May 2008, a 66-year old Malaysian fell into a coma after consuming starfruits (Anon, 2008). In the literature, there have been 20 reported cases of star fruit intoxicating patients with renal failure (Chang *et al*., 2000; Fang *et al*., 2001).

Starfruit, a member of the Oxalidaceae family, is popular in many tropical countries and contain high level of oxalate (Chen *et al*., 2001). Although the chemical nature of the star fruit neurotoxin remains obscure, oxalic acid has been proposed as a putative candidate (Chen *et al*., 2001). In addition, because of the similar neurological manifestation caused by ingestion of many high oxalic acid containing plant extracts, it is prudent to consider oxalic acid as a causal agent of starfruit associated toxicological encephalopathy in dialysis patients (Sanz and Reig, 1992). Fang *et al.* (2008) also found that acute oral administration of starfruit or oxalate solution could directly cause renal tubular cell apoptosis that leads clinically to acute renal shutdown. Kidney seems to be one of the main target organs of oxalate toxicity. Chang *et al.* (2000) reported that starfruit ingestion by patients with renal failure may have a high resultant mortality, even when supportive care and dialysis are promptly administered. Fang *et al.* (2008) concluded that oral ingestion of star fruit can produce acute renal injury, not only through the obstructive effect of crystals of calcium oxalate but also by inducing apoptosis of renal epithelial cells.

Deacidification is a process that has been used to reduce the level of acid in food systems based on several methods such as microbiological and also chemical (Devatine *et al*., 2001). Oxalic acid content in starfruit extract may be removed by the deacidification method. Several studies had been done to deacidify fruit extracts. Couture and Rouseff

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(1992) used a deacidification process via ion exchange resin to deacidify sour orange juice. Deacidification using calcium salt precipitation was used to remove tartaric and malic acid (Devatine *et al.*, 2001). Vera *et al.* (2002) investigated the various methods such as calcium precipitation, ion exchange resin and electrodialysis to deacidify yellow passion fruit juice. Vera *et al*. (2003) concluded that ion exchange is fairly satisfactory for deacidifying passion fruit juice. Deacidification by activated charcoal powder has been used to reduce the levels of caproic, caprylic and capric acids in noni extract (Norma *et al.*, 2004). Thus, this study was carried out to determine the effect of deacidification using ion exchange resin on the removal of oxalic acid in starfruit.

Materials and Methods

Raw material

Starfruits (*Averrhoa carambola* L.) was purchased from a local market at a maturity index of WA 6 (Tarmizi and Pauziah, 2005). Amberlite IRA 67 resin was obtained from Fluka. 1,1-diphenyl-2-picrylhydrazyl (DPPH) was from Sigma-Aldrich (St. Louis, Mo). Folin Ciocalteu reagent (FC), sodium carbonate, gallic acid and methanol were purchased from Merck. All the other chemicals and reagents used were of analytical grade and used without any purifications.

Sample preparation

Starfruits were washed with distilled water and cut into small pieces. The cut fruits (10 gm) was mixed with 10 ml distilled water using a blender. The extract was filtered using Whatman No 4 filter paper before deacidification and physicochemical analyses.

Deacidification with ion exchange resin

Deacidification was carried out by pumping the extract into a column filled with the ion exchange resin and the effluent collected in a bottle. For the effect of resin weight, three different weight (3, 5 and 8 g) was used. While for the effect of flow rate, three different flow rates (6, 9 and 12 ml/m) were applied. Deacidification was conducted for nine combinations of the mass and flow rates.

Determination of oxalic acid content

Oxalic acid content was determined using high performance liquid chromatography with UV detector (Waters). Sulphuric acid (5 mM) was used as mobile phase. Samples was filtered through 0.45 µm nylon filter and 10 µl of sample was injected into the column. Mobile phase was ran at a flow rate of 0.6 ml/min. Oxalic acid was detected using a UV detector at 214 nm and absorption of 0.1 AUFS (Trevaskis and Trenery, 1996).

Determination of total phenolic content

The total phenolic content in starfruit extract was determined using the Folin-Ciocalteu procedure according to Maisuthisakul *et al.* (2007) with some modification. Starfruit extracts (0.1 ml) were mixed with 0.75 mL of the diluted Folin–Ciocalteu reagent and incubated for 10 min at room temperature. After 5 min, 0.75 ml of 7.5% sodium carbonate solution was added. The mixture was allowed to stand in the dark for 60 min before measuring the absorbance at 765 nm using an UV-Visible spectrophotometer (Pharmacia Biotech, Cambridge, UK) against a blank. Total phenolic content values were determined from a calibration curve prepared with a series of gallic acid standard and results were expressed as mg of gallic acid equivalents/g fresh weight (mg GAE/g fresh weight).

Determination of percentage of free radical elimination activity (DPPH)

DPPH free radical elimination activity in extracts was determined according to the method of Tsai *et al*. (2007). Samples (100 µl) was mixed with 400 µl 0.01 mM methanolic DPPH. Subsequently, the mixture is shaken for 1 min and allowed to react at room temperature for 30 minutes. The absorbance was measured by VERSAmax Tunable Microplate Reader (California, USA) at 517 nm. The percentage of free radical elimination activity of the tested samples were compared with controls. Each sample was measured at least thrice. The percentage of free radical elimination activity was calculated based on the following formula:

$$\text{Percentage of free radical elimination activity (FRSA)} = \frac{(Ac - As)\ x\ 100\ \%}{Ac}$$

Where Ac = uptake of control; As = absorption of the samples tested after 60 minutes.

Determination of vitamin C

Vitamin C in the extract of starfruit were determined using the method of AOAC (1990). Two ml of sample was added into a conical flask which contained 5 ml metaphosphoric-acetic acid solution. This solution was titrated with a dye solution and the volume of endophenol was recorded. Ascorbic acid content was calculated using the formula below:

Ascorbic acid content (mg/100 ml juice) =

$$\frac{[F\ x\ (V-Vo)x\ 100]}{SV}$$

Where; F=factor of dye (mg ascorbic acid/ml dye); V=average volume (ml) of dye used for titration; Vo=average volume (ml) of dye used for blank titration; SV=sample volume

Statistical analysis

All analyses was done in triplicate and analyzed using analysis of variance test (ANOVA) through the Statistical Analysis System (SAS) version 6.12. Duncan multiple range test (DMRT) was used to compare differences in mean values between treatments. Significant level was set at ($p < 0.05$).

Results and Discussion

Oxalic acid content

Table 1 shows the effect of resin weight and flow rate on oxalic acid content of starfruit extracts. Oxalic acid content for the control sample was 0.185 N, while after deacidification ranged between 0.059-0.088 N. A significant reduction ($p<0.05$) of oxalic acid content was observed in deacidified samples compared to control. However, changes in resin weight and flow rate did not result in any significant difference in oxalic acid content among deacidified samples. The ion exchange resin adsorbed positive ions thus reduced the level of acidity in the starfruit extract. However, resin weight and flow rate did not show any significant differences may be due to the resin becoming saturated. Thus, the weight of resin should be increased to be more than 8 g.

pH

Table 2 shows the pH values of control sample and samples deacidified at different resin weight and flow rate. All the extracts after deacidification exhibited significantly higher ($p<0.05$) pH value compared to control samples. At each level of resin weight, decreasing the flow rate provides a significant increase ($p<0.05$) in pH value. Chung *et al*. (2007) documented that the flow rate of juice through resin affected the efficiency of resin to adsorb ions present in the juice. When flow rate is high, time of contact between resin and juice is less, thus ions adsorbed by the resin is less resulting in less deacidification of the extract. Increasing the weight of resin used during deacidification lowers the acidity in starfruit extract significantly ($p<0.05$). Higher quantity of resin used enabled more ions to be adsorbed in a particular time period.

Table 1. Oxalic acid content of starfruit extracts deacidified at different extract flow rate and ion exchange resin weight

	Oxalic acid content (N)		
Control	$0.185^{a} \pm 0.290$		
	Resin weight (g)		
Flow rate (ml/min)	3	5	8
6	$0.059^{b} \pm 0.069$	$0.071^{b} \pm 0.085$	$0.064^{b} \pm 0.080$
9	$0.091^{b} \pm 0.119$	$0.078^{b} \pm 0.100$	$0.076^{b} \pm 0.101$
12	$0.081^{b} \pm 0.103$	$0.070^{b} \pm 0.088$	$0.088^{b} \pm 0.109$

[a-b] Means with different alphabet indicates significant differences ($p<0.05$).

Table 2. pH values of starfruit extracts deacidified at different extract flow rate and ion exchange resin weight

	pH value		
Control	$3.65^{j} \pm 0.015$		
	Resin weight (g)		
Flow rate(ml/min)	3	5	8
6	$6.95^{g} \pm 0.015$	$7.95^{d} \pm 0.025$	$9.95^{a} \pm 0.015$
9	$6.55^{h} \pm 0.021$	$7.56^{e} \pm 0.020$	$9.55^{b} \pm 0.026$
12	$6.14^{i} \pm 0.015$	$7.15^{f} \pm 0.015$	$9.14^{c} \pm 0.026$

[a-j] Means with different alphabet indicate significant difference ($p<0.05$).

Total phenolic content (TPC)

Table 3 shows the TPC of control and deacidified samples. TPC for the control was 147.82 ± 5.16 mg GAE/100g. The value of TPC in this study was similar to the values reported by Cieslik *et al.* (2006). TPC for deacidified samples ranged from 98.56 to 103.89 mg GAE/100g. There was no significant differences for TPC between all deacidified samples. However, there was a significant reduction ($p<0.05$) in total polyphenol content in deacidified samples compared to control. The reduction of TPC in deacidified samples may be due to the adsorption of phenolic compounds to the resin (Vera *et al*., 2003).

Vitamin C content

Table 4 shows vitamin C content of control and deacidified samples. Vitamin C for control sample was 5.39±0.02 mg/100g. This value was similar to the previously reported value (5.2 ± 1.9 mg/100g)

Table 3. Total polyphenol content of control and deacidified samples of starfruit extracts deacidified at different extract flow rate and ion exchange resin weight

	Total polyphenol content (mg GAE/100 g)		
Control	147.82^{a} ± 5.16		
	Resin weight (g)		
Flow rate (ml/min)	3	5	8
6	101.01^{b} ± 5.68	103.24^{b} ± 1.38	103.33^{b} ± 5.01
9	103.89^{b} ± 6.40	98.56^{b} ± 4.76	102.09^{b} ± 5.26
12	100.36^{b} ± 5.75	105.45^{b} ± 3.32	102.01^{b} ± 4.38

$^{a-b}$ Means with different alphabet indicate significant difference ($p<0.05$).

Table 4. Vitamin C content of control and deacidified samples of starfruit extracts deacidified at different extract flow rate and ion exchange resin weight

	Vitamin C content (mg/100 g)		
Control	5.39^{a} ± 0.02		
	Resin weight (g)		
Flow rate (ml/min)	3	5	8
6	4.65^{b} ± 0.29	4.65^{b} ± 0.17	4.69^{b} ± 0.06
9	4.74^{b} ± 0.21	4.64^{b} ± 0.15	4.75^{b} ± 0.21
12	4.52^{b} ± 0.30	4.56^{b} ± 0.12	4.57^{b} ± 0.09

$^{a-b}$ Means with different letters indicate significant difference ($p<0.05$).

Table 5. Percentage removal of free radical activity of control and deacidified samples of starfruit extracts deacidified at different extract flow rate and ion exchange resin weight

	Free radical elimination (%)		
Control	87.74^{a} ± 1.00		
	Resin weight (g)		
Flow rate (ml/min)	3	5	8
6	76.87^{b} ± 1.17	78.26^{b} ± 0.99	77.55^{b} ± 1.60
9	77.44^{b} ± 0.21	77.23^{b} ± 0.82	77.71^{b} ± 1.11
12	76.60^{b} ± 0.86	78.37^{b} ± 0.58	77.73^{b} ± 0.90

$^{a-b}$ Means with different letters indicate significant difference ($p<0.05$).

by Cieslik *et al.* (2006). The content of vitamin C in experimental samples ranges from 4.52-4.75 mg/100g. There were no significant differences ($p>0.05$) in vitamin C content among all the deacidified samples. However, the values in deacidified samples were significantly lower ($p<0.05$) than control. These results indicate that the deacidification process significantly ($p<0.05$) reduced vitamin C content. It has been documented that ion exchange resin is very effective in removing amino acids and ascorbic acid in fruit juices (Lee and Kim, 2003). However, different mass of resin and flow rate used for deacidification process did not have any significant impact on the content of vitamin C.

Free radical elimination activity (DPPH)

Table 5 shows the percentage of free radical elimination activity of control and deacidified samples. The percentage of free radical elimination activity for the control sample is 87.74%, while for experimental samples between 76.60%-78.37%. The results showed that there were no significant differences ($p>0.05$) among all deacidified samples. However, these values were significantly ($p<0.05$) lower when compared to control samples. These results demonstrated that deacidification reduced free radical elimination activity of the starfruit extracts. The reduction of free radical elimination activity was in agreement with the results of TPC and vitamin C thus suggesting that the reduction may be due to the adsorption of phenolic and ascorbic acid to the ion exchange resin. However, difference in resin weight and flow rate did not have significant effects on its free radical elimination activity.

Conclusion

Based on the results it may be concluded that deacidification significantly ($p<0.05$) reduced oxalic acid content in starfruit extract. Total soluble solids of star fruit extracts did not show significant changes ($p>0.05$) with deacidification using ion exchange resin. The pH value of deacidified starfruit extract significantly increased ($p<0.05$) compared with control samples. However, the total polyphenol content, vitamin C content and free radical elimination activity (DPPH) of starfruit extract decreased significantly ($p<0.05$) when deacidified using ion exchange resins. Thus, this study showed that deacidification using ion exchange resin can be considered for the reduction of oxalic acid in starfruit extract but with some loss of antioxidative properties.

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