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Total phenolic content and antioxidant activity of apple pomace aqueous extract: effect of time, temperature and water to pomace ratio

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Abstract

This study aimed to evaluate the effects of extraction time, extraction temperature and water to pomace ratio on the total phenolic content and antioxidant activity of apple pomace aqueous extracts. Pomace was extracted using water (20-90°C) for 5-60 min. The extracts were evaluated for their total phenolic content (Folin Ciocalteu assay) and antioxidant activity (DPPH, FRAP and ABTS assays). A methanol extract of the pomace was used as control. It was found that water to pomace ratio ($p < 0.001$), extraction temperature ($p < 0.001$) and time ($p < 0.001$) were significant factors in extracting the polyphenolics from apple pomace, with the optimum extraction conditions utilising water to pomace ratio of 20:1 at 90°C for 15 min yielding the most polyphenolic compounds (1148 μg g^{-1} fresh pomace Gallic Acid Equivalents). These results indicated that water was a good solvent for extracting polyphenolics from apple pomace, however, as compared to the methanol extract (control), the aqueous extracts had lower total phenolic content (63%) and antioxidant activity (73-80%).

Introduction

Polyphenolic compounds are regarded as a group of phytochemicals that may promote human health (Boyer and Liu, 2004; Jedrychowski and Maugeri, 2009; Jan *et al*., 2010). This is mainly through their ability to act as antioxidant (Tomás-Barberán and Clifford, 2000; Tomás-Barberan *et al*., 2000; Tsao and Yang, 2003; Clifford, 2004) and also may have anti-inflammatory (Barth *et al*., 2005) activities. Further studies have shown that polyphenolics have the ability to reduce cellular damage and therefore may be beneficial in promoting human health and protecting against numerous diseases linked to oxidative events, such as cardiovascular and respiratory disorders, cancers and diabetes. Many studies have shown a strong relationship between polyphenolic compounds, which have antioxidant activity, and reduced risk of various diseases (Kuhnau, 1976; Hollman and Katan, 1999; Hollman, 2001; Lee *et al.*, 2003; Liu, 2003; Boyer and Liu, 2004; Barth *et al.*, 2005; Martínez-Navarrete *et al*., 2008; Jan *et al*., 2010; Fu *et al*., 2011; Hyson, 2011).

One of the richest sources of dietary phenolics in the Western diet is apple (Boyer and Liu, 2004). In apples, polyphenolic compounds are found throughout the fruit (peel, flesh and seeds) (Schieber *et al*., 2003), where their concentrations are much lower in the flesh compared to the peel, except for chlorogenic acid which tends to be higher in the flesh (Oszmiański *et al.*, 2009). However, quercetin conjugates are exclusive to the peel (Escarpa and González, 1998).

When apples are processed into juice, the polyphenolic content in the juice is significantly decreased in comparison to the fresh apples, this in turn reduces the antioxidant activity of the final product (Van der Sluis *et al*., 2002). In conventional apple juice production, the polyphenolic compound content decreases by at least 58% (Guyot *et al*., 2003) and the antioxidant activity decreases by up to 90% in comparison with the fresh apple (Van der Sluis *et al*., 2002). The clarification and filtration processes affect the polyphenolic content in apple juice as these steps remove the pulp where most of the polyphenolic compounds remain (Van der Sluis *et al*., 2002). Some juices are not clarified and are sold as 'cloudy' apple juice; and have a higher concentration of the polyphenolic compounds and higher antioxidant activity compared to clarified juices (Candrawinata *et al*., 2012). However the concentration of the phenolics in the cloudy juice is still significantly lower compared to fresh apples (Markowski *et al*., 2007), because ultimately, most of the polyphenolic compounds are retained in the pomace, which is the solid remain which is filtered out during juicing (Candrawinata *et al.*, 2013).

Pomace represents approximately 20-35% of the original fruits and is generally composed of remaining carbohydrates, dietary fibres and small

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amount of proteins (Carson *et al.*, 1994; Suárez *et al.*, 2010). Studies have shown that fruit pomace is a rich source of polyphenolic compounds, therefore making it a good source of natural antioxidants (McCann *et al*., 2007; Bhushan *et al*., 2008). Several studies have been conducted extracting polyphenolic compounds from pomace, the most effective being the use of organic solvents such as ethanol, methanol and acetone (Mayya *et al.*, 2003; Van Der Sluis *et al*., 2004; Hayat *et al*., 2010; Suárez *et al.*, 2010; Ajila *et al.*, 2011; Reis *et al*., 2012). These existing solvent methods have not been widely used by apple juice manufacturers due to safety concerns, processing costs and consumer reluctance towards food products exposed to chemicals, including organic solvents.

In the present study, a more environmental friendly approach, using water to extract the phenolics from apple pomace was examined. While extraction of the polyphenolics from apple pomace using water have been evaluated and it was reported that at room temperature (Reis *et al*., 2012), as well as at 100°C (Çam and Aaby, 2010), water is a good solvent for the extraction of phenolics the pomace; this current study utilised an industrial produced apple pomace that was untreated, unlike the freeze dried pomace used in the mentioned studies. Treatment such as freeze drying may affect the phenolic content and antioxidant activity of the pomace. Additionally, the freeze drying treatment adds significant cost to for industry.

The evaluation of extraction time, temperature and water to pomace ratio provides a better understanding of the effect of each extraction parameters. This understanding is important, not only to find out the optimise conditions for the extraction, but also to provide alternative to the industry, in terms of efficiency and cost management. Therefore, the aim of this study is to optimise the extraction of phenolics from industrial untreated apple pomace by evaluating the effect of water to pomace ratio and extraction time and temperature on the efficiency of water as a solvent.

Materials and Methods

Apple pomace

Industrial apple pomace was sourced from a local commercial juice manufacturer (Appledale Processors Co-op. Ltd., Orange, NSW, Australia). The pomace was homogenised and stored at -15°C until use.

Chemicals

Chemical reagents (methanol, Folic Ciocalteu reagent, sodium carbonate, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2, 4, 6-tripyridyl-s-triazine (TPTZ), acetic acid, iron (III) chloride hexahydrate, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), potassium persulfate and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) were all of analytical grade and were purchased from Sigma Aldrich Laboratory Chemicals (Castle Hill, NSW, Australia). Deionised water was prepared on the day of use with a Millipore Milli-Q water purification system (Millipore Australia, North Ryde, NSW, Australia).

Extraction of phenolic compounds from apple pomace

Pomace extracts for the analyses were obtained by adding 5 g apple pomace into 100 mL deionised water at different extraction temperatures. These mixtures were placed into a shaking water bath for different extraction times. Following the extraction, the mixtures were placed into an ice bath for 10 min (Hirun and Roach, 2011). The mixtures were then vacuum-filtered using a double-layer cheesecloth, followed by centrifugation at 12,100 x g (Beckman J2-AC centrifuge, Beckman Instruments Inc., California, USA) (Candrawinata *et al*., 2012). The filtrate obtained would thereafter be referred to as the aqueous extract of the pomace.

As a control, apple pomace was extracted using analytical grade methanol, adapted from a procedure developed by Golding *et al.* (2001). The pomace (5 g) was added into 100 mL methanol and sonicated for 20 min with an UltraSONIK 57X NEY sonificator (Extech Equipment Pty. Ltd., Melbourne, VIC, Australia) before it was vacuum-filtered through a double-layer cheesecloth followed by centrifugation at 12,100 x g (Beckman J2-AC centrifuge, Beckman Instruments Inc., California, USA). The filtrate obtained would thereafter be referred to as the methanol extract of the pomace.

Extraction time and temperature

The extraction was performed by adding 5 g of pomace into 100 mL of deionised water. Extraction at each assigned temperature (20, 50, 60, 70, 80 and 90°C) was performed for 5, 15, 30, 45 and 60 min.

Water to pomace ratio

A series of extractions with different water to pomace ratios were conducted at 90°C for 15 min to evaluate the effect of water to pomace ratio (mLg^{-1}) on the total phenolic content and the antioxidant activity. While keeping the pomace constant at 5 g, the water volume varied from 10-120 mL.

Total phenolic content assay

Total phenolic content was measured by the assay based on a method established by Folin and Ciocalteu (1927). This assay was adapted from Swain and Hillis (1959) and Thaipong *et al*. (2006) with minor modifications. Briefly, 150 µL of extract was mixed with 150 µL of 0.25 N Folin Ciocalteu reagent. The mixture was allowed to react for 2 min before adding 2400 µL of 5% (w v^{-1}) sodium carbonate solution. The incubation time was set for 1 h. The results were expressed in gallic acid equivalents (GAE, µg g^{-1} fresh pomace).

Antioxidant activity assays

Antioxidant activity of the extracts was measured using three different assays, namely DPPH (2,2-diphenyl-1-picrylhydrazyl) , FRAP (Ferric Reducing Ability of Plasma) and ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) assays.

For the DPPH assay, the method was adapted from Brand-Williams *et al*. (1995) and Thaipong *et al*. (2006) with some modifications. The incubation time was set for 30 min. The FRAP assay was performed according to Benzie and Strain (1996) and Thaipong *et al.* (2006). The ABTS assay was conducted based on the method described in Arnao *et al.* (2001) and Thaipong *et al.* (2006). The results from all the antioxidant activity assays are expressed in Trolox equivalents (TE, µg g^{-1} fresh pomace).

Statistical analysis

The data generated from the experiments were subjected to analysis of variance (ANOVA) using Statistical Package for the Social Science (SPSS). The significance of the variables was determined by two-way ANOVA. The significance of the difference between means was determined by one-way ANOVA with Tukey post-hoc tests ($p < 0.05$). Correlation analysis was performed by employing Pearson Correlation ($p < 0.01$). All extractions and measurements were performed in triplicate on different batches of apple pomace.

Results

Extraction time and temperature

The total phenolic content and antioxidant activity of the aqueous extracts, as measured using three different assays, for each extraction temperature and time are presented in Table 1. Regression analysis of the total phenolic content and total antioxidant activity (as measured by DPPH, FRAP and ABTS assays) is shown in Figure 1.

Table 1. Total phenolic content and total antioxidant activity (using DPPH, FRAP and ABTS assay) for different extraction temperature and time (Mean ± Standard Deviation)

		Total Phenolics Concentration (µg g^{-1} GAE)	DPPH Activity (µg g^{-1} TE)	FRAP Activity (µg g^{-1} TE)	ABTS Activity (µg g^{-1} TE)
Control		1847.65 ± 9.36 ^{a}	2038.53 ± 11.9 ^{a}	1883.48 ± 14.8 ^{a}	3472.04 ± 11.39 ^{a}
Temp	**Time**				
20 °C	5	332.68 ± 3.7 ^{b}	233.30 ± 7.8 ^{b}	287.50 ± 9.7 ^{b}	600.70 ± 16.7 ^{b}
	15	455.02 ± 11.0 ^{cd}	331.63 ± 3.3 ^{cd}	377.43 ± 7.6 ^{c}	787.49 ± 23.0 ^{cd}
	30	465.17 ± 25.1 ^{de}	342.20 ± 6.4 ^{cd}	393.40 ± 4.9 ^{c}	800.86 ± 7.6 ^{d}
	45	417.03 ± 23.6 ^{cd}	300.42 ± 4.0 ^{bc}	345.52 ± 0.6 ^{bc}	737.75 ± 4.3 ^{cd}
	60	497.20 ± 12.8 ^{e}	424.49 ± 5.7 ^{e}	485.31 ± 0.1 ^{ef}	935.67 ± 41.8 ^{fg}
50 °C	5	365.05 ± 7.6 ^{b}	315.08 ± 37.6 ^{c}	405.25 ± 11.7 ^{cd}	744.92 ± 6.1 ^{c}
	15	454.47 ± 13.2 ^{cd}	344.92 ± 5.4 ^{cd}	587.49 ± 5.1 ^{gh}	818.22 ± 11.3 ^{d}
	30	571.57 ± 15.9 ^{f}	500.21 ± 4.7 ^{f}	937.23 ± 26.2 ^{kl}	1003.23 ± 12.3 ^{h}
	45	762.97 ± 14.5 ^{hi}	742.58 ± 6.6 ^{i}	982.94 ± 12.4 ^{lm}	1249.70 ± 13.9 ^{k}
	60	724.17 ± 0.3 ^{g}	627.54 ± 20.3 ^{gh}	716.85 ± 14.1 ^{i}	1095.62 ± 12.5 ^{ij}
60°C	5	366.09 ± 4.0 ^{b}	313.70 ± 2.5 ^{c}	468.17 ± 14.8 ^{de}	829.93 ± 5.9 ^{de}
	15	742.47 ± 10.6 ^{gh}	514.60 ± 4.7 ^{f}	637.55 ± 6.8 ^{h}	981.79 ± 0.3 ^{gh}
	30	814.76 ± 11.8 ^{j}	692.15 ± 15.5 ^{hi}	1031.05 ± 3.4 ^{mn}	1080.34 ± 20.8 ^{i}
	45	910.60 ± 14.1 ^{l}	869.76 ± 4.2 ^{j}	1098.33 ± 10.4 ^{n}	1326.67 ± 4.4 ^{l}
	60	798.03 ± 9.9 ^{ij}	676.30 ± 25.8 ^{hi}	813.90 ± 18.4 ^{j}	1139.77 ± 8.7 ^{j}
70°C	5	433.37 ± 5.4 ^{cd}	396.88 ± 28.2 ^{de}	554.87 ± 16.0 ^{fg}	873.05 ± 10.1 ^{e}
	15	864.38 ± 12.5 ^{k}	926.32 ± 3.8 ^{jk}	901.95 ± 10.2 ^{k}	1107.66 ± 23.7 ^{ij}
	30	857.86 ± 9.2 ^{k}	912.24 ± 22.5 ^{jk}	1088.08 ± 7.0 ^{n}	1333.99 ± 12.2 ^{l}
	45	979.22 ± 5.7 ^{mn}	983.01 ± 21.5 ^{kl}	1179.39 ± 24.0 ^{o}	1602.67 ± 2.9 ^{o}
	60	805.96 ± 13.2 ^{j}	746.90 ± 47.1 ^{i}	782.24 ± 18.1 ^{ij}	1354.45 ± 0.8 ^{l}
80°C	5	568.54 ± 9.2 ^{f}	598.00 ± 25.1 ^{g}	536.82 ± 32.8 ^{efg}	929.38 ± 29.8 ^{f}
	15	1008.13 ± 13.3 ^{no}	1394.88 ± 28.9 ^{p}	1184.57 ± 27.5 ^{o}	1351.98 ± 2.9 ^{l}
	30	995.61 ± 6.1 ^{mno}	1427.80 ± 3.3 ^{p}	1169.88 ± 18.1 ^{o}	1428.12 ± 10.4 ^{m}
	45	1096.55 ± 18.2 ^{p}	1093.04 ± 27.1 ^{mn}	1298.62 ± 52.5 ^{p}	1587.14 ± 14.1 ^{o}
	60	1027.93 ± 6.1 ^{o}	1042.83 ± 22.6 ^{lm}	995.71 ± 7.3 ^{lm}	1526.07 ± 17.0 ^{n}
90°C	5	1003.00 ± 7.9 ^{no}	1220.72 ± 68.2 ^{o}	1184.12 ± 41.9 ^{o}	2149.72 ± 29.4 ^{q}
	15	1148.24 ± 6.3 ^{q}	1504.40 ± 8.1 ^{q}	1485.36 ± 8.0 ^{r}	2600.81 ± 12.6 ^{r}
	30	1156.45 ± 6.5 ^{q}	1516.55 ± 22.6 ^{q}	1498.60 ± 17.3 ^{r}	2760.48 ± 3.0 ^{s}
	45	962.48 ± 11.9 ^{m}	1233.42 ± 33.0 ^{o}	1410.88 ± 15.8 ^{q}	2599.66 ± 19.3 ^{r}
	60	829.09 ± 11.8 ^{ik}	1144.64 ± 18.7 ^{n}	1080.09 ± 57.8 ^{n}	1689.38 ± 16.2 ^{p}

For each assay, values that share the same superscript(s) are not significantly different.

Water to pomace ratio

In the evaluation of water to pomace ratio, the extraction time and temperature were chosen based on the data from time and temperature screening experiment, which showed that statistically, 15 min extraction at 90°C was the optimum condition.

Comparison between methanol and aqueous extracts

Figure 3 shows the comparison between the best aqueous extracts and methanol extract (control, 100% extraction), although each assay produced different absolute activities, they all measured the highest content and activity from the extracts generated from 90°C for 15-30 min. In terms of extraction efficiency, the results from Folic-Ciocalteu, DPPH and FRAP assays showed no difference between 15 and 30 min extraction time, although there was a difference between 15 and 30 min for the ABTS assay. In comparison with the control, the best extraction method generated 62.6 ± 1.7% of the phenolics obtained by a methanol extraction.

Discussion

The concentrations of phenolics as measured

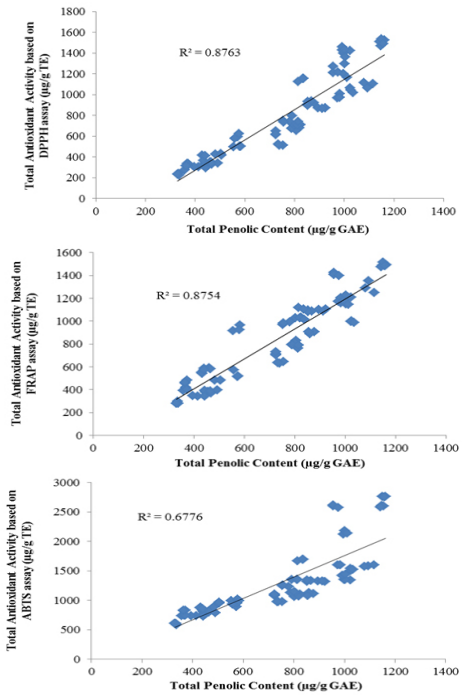

Figure 1. Correlation between total phenolic content and total antioxidant activity of apple pomace aqueous extracts. The total antioxidant activity was measured by DPPH, FRAP and ABTS assays.

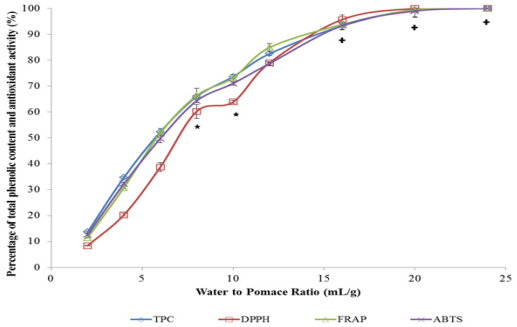

Figure 2. Effect of pomace to water ratio on total phenolic content and antioxidant activity (points that share the same symbols are not significantly different).

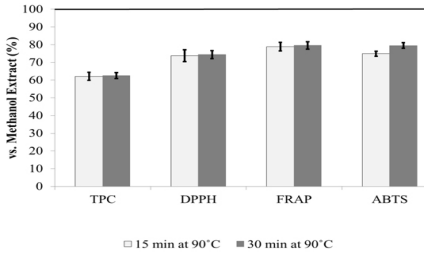

Figure 3. Comparison of the best aqueous extracts (obtained from extraction time 15 and 30 min at 90°C) and methanol extracts. (Mean ± SD)

by the Folin-Ciocalteu assay in the aqueous extract from apple pomace were significantly affected by extraction temperature ($p < 0.001$), time ($p < 0.001$) and both extraction temperature an time ($p < 0.001$). The results show that extraction performed at 90°C for 15 and 30 min resulted in extracts with the highest total phenolic content. In comparison with the methanol extract, these values were 62.2 ± 2.3 and 62.6 ± 1.7 % of the total phenolic content achieved by methanol extraction, respectively. At the lowest extraction temperature (20°C), the most efficient extraction time was 60 min. However at 50, 60, 70 and 80°C, the highest amount of phenolics extracted with the 45 min extraction time (Table 1).

Similar to the total phenolic content, the levels of antioxidant activity (Table 1) were strongly affected by extraction temperature ($p < 0.001$) and time ($p < 0.001$). Aside from the slight differences for the DPPH assay, in general the pattern for the three antioxidant assays followed the same pattern of the total phenolic content. Therefore, as with the total phenolic content, the aqueous extracts obtained using extraction temperature 90°C for 15 and 30 mins consistently exhibited the highest antioxidant activity (Table 1).

The regression analysis shows extremely significant correlations between the total phenolic content assay and the antioxidant activity assays ($R^2 > 0.7$) (Chirinos *et al.*, 2008). Pearson correlation analysis showed that the measured antioxidant activities could be attributed to the total phenolics measure in the extracts, since the correlations between the total phenolic content and each of the antioxidant assay were highly significant ($p < 0.01$). These results did not exclude the possible contribution of other compounds such as vitamin C, however, in this case the phenolic content of the extracts was the main contributor (Malheiro *et al.*, 2011).

The results from the series of water to pomace ratio indicated that water to pomace ratio was a significant factor, which affected the amount of the extracted phenolic from the apple pomace ($p < 0.001$) (Figure 2). As expected, the increase in the amount of water used with a constant amount of pomace led to the increase of extracted phenolics. This in turn produced extracts with higher antioxidant activity (as measured by DPPH, FRAP and ABTS assays). The curves for all assays increased steadily and finally reached a plateau after the water to pomace ratio of 20:1 (Figure 2).

The extraction of phenolics from apple pomace using water is based on a solid-liquid extraction principle. This type of extraction allows the soluble component of the solid material to be removed by a solvent or a mixture of solvents. One of the important aspects of solid-liquid extraction is the ratio of the material to the solvent. The solvent must provide a large exchange surface to facilitate the removal of the soluble components. In general, the more solvent used, the higher the solubility of the solid materials. However, since the solvent will be removed after the

extraction is complete, an optimum solid to solvent ratio must be established (AOAC International, 1999).

Extraction of phenolics from pomace using solid-liquid principle is a process of mass transfer, a process that involves transport of the solvent into the matrix (internal transport), liberating of the solutes and release of solutes from a solid matrix to the global solvent phase (external transport). Water acts as a substance with molecules possessing enough kinetic force to stretch the intermolecular forces of attraction. The increase in temperature will increase the velocity of the solutes in the liquid, hence producing greater separation energy of the solutes from the solid matrices (Rodríguez-Rojo *et al.*, 2012). Indeed, the extractions that were conducted at higher temperatures showed higher phenolic content and antioxidant activity levels, because the efficiency of the water in liberating, transferring and releasing the phenolic compounds from the solid apple pomace matrices was greater compared to extractions at lower temperature.

The length of time for the extraction was also an important factor. The results showed that the longer the extraction time, the more phenolics were extracted from the pomace. This was not unexpected, however upon reaching extraction time at 45 min (for 50-80°C) and 30 min (for 90°C), the phenolic contents and antioxidant activity levels began to decline. This observation suggests that after a critical time, the rate of disintegration and oxidation of the phenolics (due to being exposed to high temperature temperature) is greater than the rate of extraction, causing a decrease in the final phenolic content and antioxidant activity level. Thus, at the highest extraction temperature, only relatively short extraction time of 15 min is required to give the highest total phenolic content and antioxidant activity for the aqueous pomace extracts. Based on the comparison shown in Figure 3, a lower phenolic content was found in the aqueous extract. This observation was expected as water is more polar than methanol and is an inferior solvent to extract phenolic compounds. Many organic compounds, including phenolics have higher solubility in methanol than in water (Alo *et al.*, 2012).

In this study 1.16 GAE kg^{-1} fresh apple pomace was obtained, however Reis *et al*. (2012) obtained 1.72 GAE kg^{-1} dried apple pomace, using only water at room temperature. It should be noted that Reis *et al.* (2012) used freeze-dried apple pomace powder as opposed to the fresh apple pomace. The drying pre-treatment of the pomace was likely to increase the concentration of the phenolics in the samples simply because most of the water would have been removed. The moisture content in the apple pomace used for this study was 65%, which is within the range of the figured reported by other studies (Linskens and Jackson, 1999; Gullón *et al*., 2007), by mathematically eliminating the water component, the amount of phenolics obtained in this present study would be equivalent to 3.29 GAE kg^{-1} dried apple pomace GAE. The difference between these two studies may be due to the drying process involved in the preparation of the samples used in the study of Reis *et al.* (2012), which may negatively affect the phenolic content. In addition, different apple cultivars from which the pomace was produced may also be factor that contributed to the differences, since apple juice manufacturers use a combination of several apple varieties in their juices (based on seasonal availability, yield and production costs).

The other aspect to be considered is the possible effect of the activity of polyphenoloxidase (PPO) in the pomace. This enzyme is suggested to be optimally activated at 50°C (Nantitanon *et al*., 2010), which could potentially lower the total phenolic content and subsequently the antioxidant activity of the extracts. Weemaes *et al*. (1998) established that the breakpoint of PPO in apples was noted at 72.5°C. Another study also found that the activity of PPO is more prominent at around 50°C in comparison with 30°C and this enzyme will be denatured at approximately 70°C (Liu *et al*., 2007).

In the present study, the extractions at 20 and 50°C may have been affected by PPO activity, however, the analysis of the extracts obtained from these two temperatures shows a consistent trend with the rest of the results, indicating that the effect of PPO activity, if there was any, was not significant. Furthermore, the pH of the pomace used in this study was recorded at 4.19, while studies found that PPO in various fruits, including apples, is stable at pH ranging from 6 to 8 and is likely to become unstable below pH 4.5 (Weemaes *et al*., 1998; Liu *et al*., 2007; Mizobutsi *et al.*, 2010). Therefore, it can be concluded that the lower concentration of phenolic content observed from extractions at lower temperature is due to the effect of the extraction temperature, rather than PPO activity.

For a further utilisation of apple pomace extract by the food industry, the extraction technique must be safe. When using organic solvents for the extraction of the compounds, although safety could still be achieved by the removal of the solvents from the final product, consumers prefer products which are not processed using any of the solvents. The use of a water extract gives an opportunity for the manufacturers to match the market demand. In addition to delivering

products that meet consumer's expectations, there are additional considerations by the industry, such as the start-up cost, employee training, safety measures and operating expenses. The water extraction technique requires minimal change to the existing production line in juicing industry thus presenting low start-up cost and operating expenses. Most importantly, compared to using organic solvents, water is cheap and easily accessible.

However, the use of high temperature extraction significantly adds cost for industry, therefore by evaluating the effect of temperature and time on the water extraction of the pomace collectively, as opposed to evaluating the individual effect of time and temperature separately, the efficiency of each temperature and length of extraction can be presented to the industry. Given that there are many points of commercial consideration, although the highest phenolic content and antioxidant activity were obtained through extraction at 90°C, the extraction maybe more cost effective at a lower temperature. For example, by lowering the extraction temperature to 80°C for the same amount of time (15 min), the extraction produced approximately 87% of the phenolics obtained through the extraction at the optimum conditions. Similarly, the water to pomace ratio also plays a crucial role since adequate amount of water is required to extract the maximum phenolics. A lower water to pomace ratio may also be preferable by the manufacturer since less water is required and less energy is needed to remove the water following the extraction. Based on the results, a ratio of 60 mL water for 5 g pomace produced approximately 83% of the phenolics with almost 40% less water compared to the optimum ratio (100 ml per 5 g).

Conclusion

Apple pomace contains phenolic compounds that are readily extracted using water. Length of extraction time, temperature and water to pomace were shown to be significant factors to the amount of phenolic extracted as well as the antioxidant activity of the extracts. In general, the increase in extraction temperature, time and water to pomace ratio increased the levels of the total phenolic content and antioxidant activity of the aqueous extracts. Although the phenolic content and antioxidant activity of water extract were lower when compared to the methanol extract, it was shown that at the best extraction conditions tested, water extracted 62.6 ± 1.7% the total phenolics extracted by methanol. The use of water to extract phenolic compounds from apple pomace provides the food industry with a safe and cheap technique to utilise the waste and in turn add value of juice making process. Future studies should aim at assessing the effects of pre-treatments of the pomace to optimise the use of water to extract phenolics from the pomace.

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