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Optimisation of Radio Frequency Assisted Extraction of Apple Peel Extract: Total Phenolic Contents and Antioxidant Activity

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The objective of this study is to determine the optimum operating conditions of apple peel extraction process using a novel extraction method known as radio frequency assisted extraction (RFAE). Apple peel extract with the highest total phenolic contents and antioxidant activity is targeted in this study. RFAE is a green extraction method which operates based on the dielectric heating concept. Response surface methodology was employed for designing the experiment, analysing the effects and optimising the extraction conditions for RFAE of apple peel phenolic compounds. The effects of ethanol concentration (10 – 70 %), mixing speed (10 – 320 mL N_2/min), solid to liquid ratio (0.002 - 0.02 g/mL) and RF power (200 – 400 W) on total phenolic compounds and DPPH radical scavenging activity efficiency were investigated in order to understand and improve the RFAE performance. All processing parameters exert significant effects on the total phenolic compounds and radical scavenging activity of the apple peel extract. The highest recovery of TPC with a value of 121.87 mg GAE/ g DW can be achieved if the extraction is performed using 42.67 % ethanol concentration, mixing speed of 320 mL N_2/min, solid to liquid ratio of 0.0114 g/mL and power at 400 W. The maximum value of DPPH (94.54 %) can be achieved if the extraction is performed using an ethanol concentration, mixing speed, solid to liquid ratio and power of 37.33 %, 165 mL N_2/min, 0.02 g/mL and 400 W.

1. Introduction

Phenolic compounds are becoming important as more and more research findings are showing their association with potential health improvements. Research has shown that these compounds possess several beneficial properties such as antioxidant, antimicrobial, anti-inflammatory, anti-allergenic, anti-atherogenic, anti-thrombotic, cardioprotective and vasodilatory effect (Balasundram et al., 2006). Phenolic compounds are present in many food sources such as fruits, vegetables, cereals, nuts, honey, maple syrup, etc. Much work has been done on recovering phenolic compounds from fruits and vegetables waste during postharvest handling or food processing since these wastes contain large concentrations of phenolic compounds which can be extracted and converted into beneficial functional ingredients.

Tonnes of apple peels are being discarded each year from the apple processing industry, which, if not processed into useful products, might lead to waste disposal issues. Chlorogenic acid, procyanidins, epicatechin, quercetin, cyanidins, phloretin and phloridzin are among the phenolic compounds detected in apple peel (Tsao et al., 2003). *In vitro*, *in vivo* and human trials have demonstrated the strong antioxidant activity of these apple phenolic compounds and their potential therapeutic effects for neurodegenerative diseases (Hyson, 2011), cancers, cardiovascular diseases (Boyer and Liu, 2004) and etc. Recently many studies have been published on the utilisation and commercialisation of apple phenolics as functional ingredients in yogurt (Sun-Waterhouse et al., 2012), snack bar (Sun-Waterhouse et al., 2010), beverage (Wegrzyn et al., 2008) cosmetics, textile (Alonso et al., 2013) and etc.

Apart from highlighting the importance of phenolic compounds from apple peel, the main focus of this paper is to introduce a newly developed radio frequency assisted extraction system (RFAE) as a new method of extraction for bioactive compounds. Radio frequency assisted extraction (RFAE) is being developed because

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it has several benefits over existing extraction systems. First of all, RFAE can be considered as a green extraction process because it focuses on minimising the usage of solvent and can restrict the selection of the solvent to food grade solvents only. Rapid heating, low solvent utilisation, no product surface burning/ overheating, no direct contact between the sample and the heating element, ambient pressure working environment, and better heating uniformity are some of the advantages offered by RFAE. The way RFAE functions is similar to microwave assisted extraction (MAE), however the major difference is that RFAE functions at a lower frequency (27.12 MHz). With lower frequency, RF has higher penetration depth which might be an advantage for a larger scale industrial application as compared to the sample size restriction encountered with MAE and UAE (ultrasound assisted extraction). The objective of the paper is to obtain the optimum processing conditions for apple peel extract using a novel radio frequency extraction method which this optimum condition produces the crude apple peel extract of highest total phenolic compounds and antioxidant activity.

2. Experimental

2.1 Apple Peel Sample Preparation

McIntosh apples from Quebec which were harvested in October 2011 were used for this experiment. The apples were washed and surface dried before the skin was removed. The peeling of apple skin was performed using an Apple Pro-Peeler (Starfrit Atlantic Promotions Inc., Canada). The fresh peel was subjected to drying using a microwave-hot air dryer (Post-Harvest Laboratory, McGill University) for 40 min at a microwave power of 150 W with maximum temperature of 50 °C. The final moisture content of the peel was 17 %. Subsequently, the dried peel was ground using a domestic blender Magic Bullet (Homeland Housewares LLC, USA) and sieved to obtain apple peel powder with particle sizes ranging between 500 - 750 μm. This size was selected based on previous screening study (Mohd Jusoh, 2015).

2.2 Chemicals

Hydrochloric acid (HCl), Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), aluminium chloride ($AlCl_3$), sodium hydroxide (NaOH), 2,4,6-tris-2-pyridyl-1,3,5-triazine (TPTZ), acetic acid (CH_3COOH), sodium acetate (CH_3COONa), ferric chloride hexahydrate ($FeCl_3.6H_2O$), methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, catechin, ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethanol was obtained from Greenfield Ethanol Inc. (Canada).

2.3 Radio Frequency Assisted Extraction

Figure 1 shows a radio frequency assisted extraction system. In order to perform the extraction, 50 mL of extraction solvent (10, 40 or 70 % ethanol in aqueous hydrochloric acid maintained at 1 mM) was added into the RF extraction tube. Then a 50 g of ground apple peel samples were added to the solvent and the sample-solvent mixture was stirred for 15 s using a stirring rod to enhance mixture uniformity. After that, the extraction tube was placed between the RF electrodes in the applicator. The RF generator was then switched on and tuned accordingly to ensure maximum energy coupling and avoid power reflectance as this significantly impacts the efficiency and lifespan of the generator. The machine has maximum input power of 600 W. The control of RF power was done manually by turning the RF power knob. All extractions were performed for 10 min with final temperature of 50 °C. It took approximately 2 to 3 min for the sample to reach final temperature of 50 °C. The samples were continuously stirred during the extraction process using a nitrogen bubble stirrer which was developed solely for this RFAE application. Nitrogen gas was used in this extraction process since it is an inert gas that does not participate in causing oxidation of compounds during extraction. The extraction was performed at three levels of ethanol contents (10, 40 and 70 %), with three different nitrogen bubble stirring speed (10, 165 and 320 mL N_2/min), solid to liquid ratio (0.1, 0.5 and 1.0 g/mL) and RF power (200, 300 and 400 W) to evaluate the effects of these processing factors on the quality of apple peel extract.

2.4 Total Phenolic Compounds Determination

The total phenolic contents in the apple peel extract were determined using the method described in Waterhouse (2005) with minor modifications. 0.1 mL of extract was mixed with 7.9 mL of distilled water in a 15 mL centrifuge tube. Then, 0.5 mL Folin-Ciocalteu reagent was added into the mixture. The tube containing the mixture was agitated for 1 minute using a shaker and left aside for 7 min. After that, 1.5 mL of $NaCO_3$ was added and mixed again. The mixture was incubated for 2 h at room temperature. All tubes were properly covered with aluminum foil to prevent them from light exposure. A Biochrom US 1000 UV/VIS spectrophotometer (Biochrom, Massachusetts) was used to measure the absorbance of the sample with the wavelength set at 765 nm. The quantification of total phenolic compounds in the samples was performed using gallic acid standard. Initially, three gallic acid curves in 1 mM hydrochloric acid with different amounts of ethanol content (10, 40 and 70 %) were prepared, however all the three curves shared the same equation ($y = 0.0008x$) and the same r^2 value (0.98). The amount of phenolic content obtained was expressed as milligram gallic acid equivalent per gram of dry mass (µg GAE/g DM).

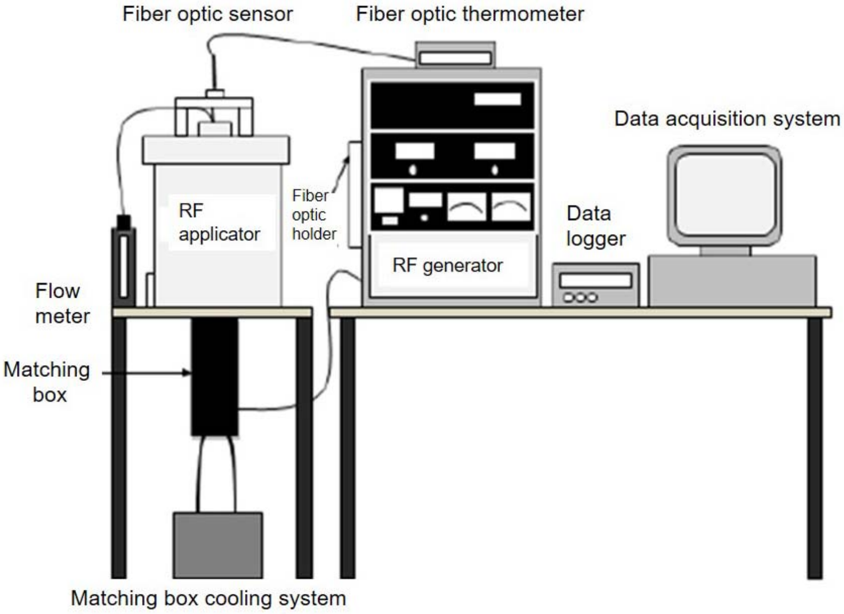

Figure 1: Radio frequency assisted extraction system (RFAE) (Mohd Jusoh, 2015)

2.5 Antioxidant Determination Using DPPH Assay

The method used to evaluate the antioxidant activity of the apple peel extract was through DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Experimental procedures were performed by modifying the methods described in Sharma and Bhat (2009). DPPH solution of 100 µM was prepared in extraction solvent of 1 mM HCl with 10, 40 and 70 % ethanol concentrations. 3 mL of extract was mixed with 1 mL DPPH solution in 15 mL tube covered with aluminum foil. This mixture was shaken vigorously and left aside for 30 min at room temperature. The absorbance of the sample at 517 nm was measured via spectrophotometry. Eq(1) was used to calculate the percentage inhibition of DPPH by the apple peel phenolic compounds:

$$\text{DPPH inhibition } \% = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) \times 100\ \% \tag{1}$$

Abs control is the absorbance of 100 µM DPPH solution without extracts.

2.6 Design of Experiment and Statistical Analysis

A face centred central composite design (CCD) with uniform precision was applied for this experiment. This design offers a lower number of experimental runs to be performed compared to other designs. Through CCD, the number of experimental runs performed was only 31 including 7 central points. The experimental design and analysis was performed using JMP version 8 (SAS Institute Inc., North Carolina). Significance of each effect was determined by p-value < 0.05. Table 1 summarises the experimental factors and levels for this experiment.

Table 1: Face Centered CCD experimental design

Factor	Unit	Low level (-1)	Medium level (0)	High level (+1)
Ethanol content (X_1)	%	10	40	70
Stirring speed (X_2)	mL N_2/min	10	165	320
Solid to liquid ratio (X_3)	g/mL	0.002	0.011	0.02
Power (X_4)	W	200	300	400

3. Results and Discussion

3.1 Effect of Extraction Parameters on Total Phenolic Contents (TPC) of Apple Peel Extract

Figure 2 highlights the effects of ethanol concentration, solid to liquid ratio, mixing speed and RF power on the TPC yield. Figure 2(a) illustrates the increasing values of these two factors up to a certain point (ethanol 10 – 42 %, solid to liquid ratio 0.002 – 0.0114 g/mL) which yielded the maximum TPC value. If these maximum operational values are increased beyond the stated value, a reduction in TPC yield will be observed. The plausible explanation for the quadratic effects of these two factors is explained by the fact that aqueous ethanol at 42 % may have an intermediate polarity between pure ethanol and water which is more suitable for extracting the phenolic compounds from the apple peel. Work carried out by Bai et al. (2010) highlighted that aqueous ethanol is a better solvent rather than pure water or pure ethanol for the extraction of phenolic compounds. Adding some water to ethanol can enhance the rate of extraction by causing the raw material to swell, allowing the solvent to penetrate the solid particles more easily (Gertenbach, 2002). In terms of solid to liquid ratio, lowering the solid to liquid ratio increases the concentration gradient between the inside and the surface of the particles which leads to higher extraction rates (Gertenbach, 2002). With RFAE, an increase of TPC up to a maximum point caused by a higher solid to liquid ratio was observed. A logical explanation is that if the appropriate surface contact between the solid and solvent is maintained, an increase in TPC value, caused by a high solid to liquid ratio is possible and expected. As the solvent surface contact subsides due to the high solid to liquid ratio, the TPC recovery reduces. Even though this finding contradicts the mass transfer principle, nonetheless, an improvement of yield due to the high solid to liquid ratio was also observed and reported by Gupta et al. (2013). Increases in the mixing speed and RF power increased the TPC yield as the mixing action increases uniformity and contact between solid and solvent, meanwhile higher power induces faster volumetric heating that plausibly accelerates diffusivity of target compounds into the solvent. Based on the experimental data and mathematical computation, it is suggested that the highest recovery of TPC with a value of 121.87 mg GAE/g DW can be achieved if the extraction is performed using 42.67 % ethanol concentration, mixing speed of 320 mL N_2/min, solid to liquid ratio of 0.0114 g/mL and power at 400 W.

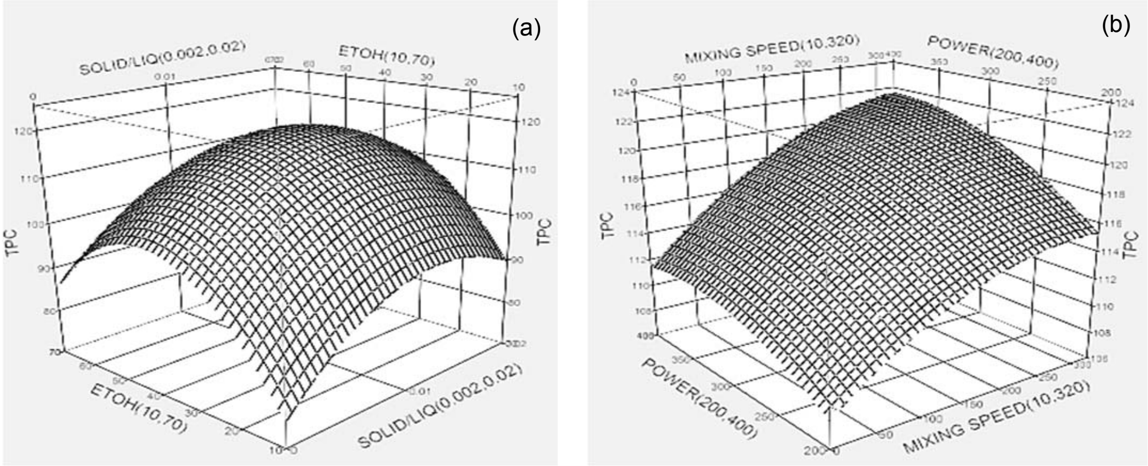

Figure 2: Three-dimensional representation of the effects of ethanol concentration and solid to liquid ratio (a) and mixing speed and RF power (b) on TPC yield

Through a multiple regression analysis, a relationship between the extraction parameters to the TPC yield was obtained and described in Eq(2). The statistical analysis indicates that this TPC model (Eq(2)) is significant ($p < 0.05$), and the good fit between the model and observation data was proven through its lack of fit value which was not significant. This also means that the model is reliable for predicting TPC under similar experimental conditions. An R^2 of 0.99 indicates that all variation in the model are well accounted for by the model.

$$\begin{aligned}\mathrm{TPC} = {} & 117.78 + 3.03X_1 + 4.12X_2 + 2.79X_3 + 2.47X_4 + 1.55X_1X_2 - 1.49X_1X_3 - 1.60X_2X_3 \\ & + 0.68X_2X_4 - 20.43X_1^2 - 1.87X_2^2 - 9.20X_3^2 - 1.62X_4^2\end{aligned} \tag{2}$$

3.2 Effect of Extraction Parameters on DPPH Scavenging Activity of Apple Peel Extract

Figure 3 illustrates the effect of ethanol concentration, mixing speed, solid to liquid ratio and power on DPPH. Increasing the solid to liquid ratio increases the DPPH value plausibly because more phenolic compounds are liberated in the solvent (Figure 3(b)). Increasing the mixing speed from 10 to 189.1 mL N_2/min causes an increase in the DPPH value however if the speed is increased beyond that, the DPPH value will decrease (Figure 3(a)). This is likely due to the insufficient time of contact between the solid particle and solvent with high intensity agitation as experienced as well in the case of FRAP. Highest DPPH can be achieved when ethanol concentration is between 10 - 47 %, beyond that value, the DPPH value starts to decrease. One plausible explanation is that by increasing the ethanol content the polarity changes, which thus reduces the tendency of the solvent to aptly extract compounds that can react with DPPH. Increasing the power had no significant impact on DPPH (Figure 3(b)). From the optimisation analysis for single response, the maximum value of DPPH (94.54 %) can be achieved if the extraction is performed using an ethanol concentration, mixing speed, solid to liquid ratio and power of 37.33 %, 165 mL N_2/min, 0.02 g/mL and 400 W.

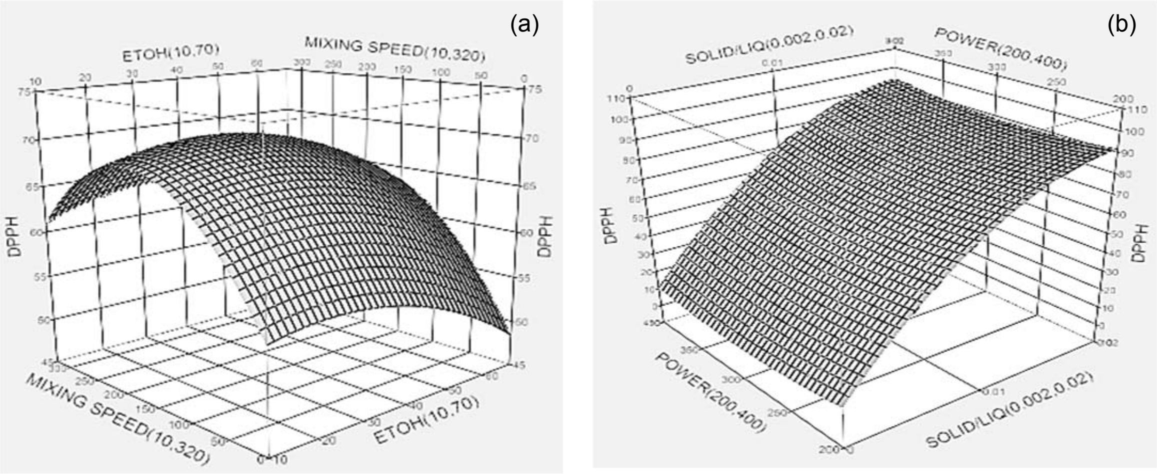

Figure 3: Three-dimensional representation on the effect of ethanol concentration and mixing speed (a) and solid to liquid ratio and power (b) on DPPH

The relationship between all tested factors and their effect on the DPPH value is formulated in Eq(3). Based on the lack of fit value which was not significant and R^2 value of 0.99, it is concluded that the model obtained for DPPH is well fitted and suitable for predicting the DPPH value.

$$\mathrm{DPPH} = 69.81 - 2.67X_1 + 3.47X_2 + 33.65X_3 - 3.72X_1^2 - 9.42X_2^2 - 14.81X_3^2 + 4.33X_4^2 \tag{3}$$

4. Conclusion

There is a great potential for the development of radio frequency heating applications for the extraction of bioactive compounds from biomass. Ethanol concentration, mixing speed, solid to liquid ratio and power, all exert significant effects on the quantity and quality of the extract obtained. The highest recovery of TPC with a value of 121.87 mg GAE/g DW can be achieved if the extraction is performed using 42.67 % ethanol concentration, mixing speed of 320 mL N_2/min, solid to liquid ratio of 0.0114 g/mL and power at 400 W. the maximum value of DPPH (94.54 %) can be achieved if the extraction is performed using an ethanol concentration, mixing speed, solid to liquid ratio and power of 37.33 %, 165 mL N_2/min, 0.02 g/mL and 400 W.

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