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Extraction of Antioxidant Compounds from Rambutan (*Nephelium lappaceum* L.) Peel as Agricultural Waste in Taiwan

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Abstract

Besides being an agricultural waste, rambutan (*Nephelium lappaceum* L.) peel has been widely known as good antioxidant source. However, no information about the antioxidant or their way of extraction for rambutan grown in Taiwan can be found. Since its major bioactive compound is geraniin, one of polyphenol compounds, so that total phenolic was used as an index to investigate the optimum condition of extraction from rambutan peel (red and yellow) in Taiwan. Extraction was conducted based on solvent ratio (1:10 to 1:30 g/mL), ethanol concentration (0 to 95 %), ultrasonic extraction temperature (30 to 70°C), and extraction method (conventional from 6 to 36 h at room temperature and ultrasonic from 0.5 to 10 min at 50°C). Results showed that the highest total phenolic content found in red rambutan variety was at 1:15 (g/mL) ratio, but no significant difference for yellow rambutan. Forty percent of ethanolic extraction and 50°C for ultrasonic extraction for both varieties. Conventional method (12 h) and ultrasonic method (2 min) resulted in the highest total phenolic recovery. As to their antioxidant activity in this study, FRAP ranged from 3800.25 ± 86.49 to 4116.5 ± 88.41 (μmol Fe^{2+} per g DW), flavonoid from 6.41± 0.48 to 8.57 ± 0.35 (mg Quercetin per g DW), and total phenolic recovery from 297.78 ± 4.06 to 358.42 ± 4.63 (mg GAE per g DW). This is the first paper regarding the study of rambutan peel in Taiwan.

Keywords: Rambutan, geraniin, antioxidant, agricultural waste

Introduction

Demand of antioxidant compounds has been increasing in recent years along with high numbers of researches in this field. Studies about natural antioxidant are expected to replace synthetic antioxidant (e.g. BHT) due to food safety issue (Thitilertdecha et al., 2010). Rambutan peel is one of well-known natural antioxidant sources which also considered as an agricultural waste. In terms of health concern, rambutan peel was proven to act as free radical scavenging activity, anti-hyperglycemic activity by effectively inhibiting alpha-glucosidase, maintaining body weight, antibacterial activities against some pathogenic bacteria (Lestari et al., 2014; Okonogi et al., 2007; Palanisamy et al., 2011; Palanisamy et al., 2008; Thitilertdecha et al., 2008). Rambutan peel was found to have high phenolic content (762 ± 10 mg GAE per g) especially when the fruits are at harvest stage (Palanisamy et al., 2008; Thitilertdecha and Rakariyatham, 2011). Comparing to other fruit peels, such as mangosteen, coconut, banana, longan, passion fruit, and dragon fruit, peel of rambutan fruits exhibited higher antioxidant activity (Palanisamy et al., 2007; Thitilertdecha and Rakariyatham, 2010). Geraniin content reached to 97.80% after purification with 21% yield from crude ethanolic extract, demonstrated much greater antioxidant activity in lipid peroxidation and DPPH assay than synthetic antioxidant (BHT), and possessed hypoglycemic activity *in vitro* (Palanisamy et al., 2007; Thitilertdecha and Rakariyatham, 2010). Isolation of geraniin was suggested to be utilized in medicine and food industry (Thitilertdecha et al., 2010; Perera et al., 2012; Palanisamy et al., 2011). In this paper the optimum condition for total polyphenol extraction and antioxidant capacity from rambutan peel grown in Taiwan were studied for their application in the future.

Materials and methods

Plant materials

Rambutan fruits were obtained from a plantation in Pingtung, Taiwan. The fruit size ranged from 6 to 6.5 cm long and from 5 to 5.5 cm wide for red variety, and from 4.5 to 5 cm long and from 4 to 4.5 cm wide for yellow variety.

Preparation of sample

The fruits were processed immediately following harvest. Fruits were thoroughly washed about twice using tap water, cut, and peels were separated from pulps and seeds. The peels were cut into smaller pieces and dried using vacuum freeze dryer for 2-3 days. The dried peels were powdered using dry blender and stored at -40°C freezer until analysis.

Sample extraction

The extraction was conducted based on optimum condition for both varieties, evaluated by total phenolic content. Optimum condition was carried out based on solvent ratio (1:10, 1:15, 1:20, 1:25, and 1:30 g per mL), ethanol concentration (0, 40, 60, 80, and 95 %), temperature of ultrasonic method (30, 40, 50, 60, and 70°C). The optimum solvent ratio, ethanol concentration, and temperature of ultrasonic method were selected to determine the optimum duration using two methods, conventional (6, 12, 18, 24, and 36 h) and ultrasonic (0.5, 1, 2, 5, and 10 min). The optimum condition of each extraction method was analyzed for its antioxidant activity and flavonoid content.

Conventional extraction

Powdered sample was weighed using analytical balance scale and put into test tube with different conditions of extraction. The extraction was carried out by extracting sample for 6, 12, 18, 24, and 36 h at room temperature in a dark room (Palanisamy et al., 2008). Then, sample was centrifuged at 4°C with 10000 rpm for 10 min using Centrifuge Hitachi CFRX15II and filtered using filter paper (Whatman No. 2).

Ultrasonic extraction

After being weighed, samples in test tube was extracted using ultrasonic Branson 2510 at 42 kHz 100 W for 0.5, 1, 2, 5, and 10 min at 50°C (Prakash Maran et al., 2013). The samples were then centrifuged and filtered under the same condition as conventional method.

Analysis of total phenolic compound (Folin-Ciocalteu)

Total phenolic compound was determined by Folin-Ciocalteu method with minor modification (Thitilertdecha and Rakariyatham, 2011). A sample of 0.1 mL in solvent was mixed with 2 mL of 2% sodium carbonate solution, then 0.1 mL of Folin-Ciocalteu reagent 1N was added to each sample. The mixture was allowed to stand at room temperature for 30 min and then measured at 750 nm using UV-Vis Spectrophotometer Hitachi U-2001. The total phenolic compound was expressed as gallic acid equivalents.

Flavonoid content

Aluminum chloride colorimetric method was used for determining total flavonoid content (Lin and Tang, 2007). Potassium acetate (1M) and aluminum chloride hexahydrate (10%) were prepared; 125 µL of extracted sample was added to 375 µL of 95% ethanol, 700 µL distilled water, and potassium acetate 25 µL, and added with 25 µL of aluminum chloride hexahydrate. Measurement was performed at room temperature for 30 min at 415 nm. The data was expressed as milligram quercetin equivalents (QE) per gram lyophilized powder.

Antioxidant activity using FRAP method

Ferric ion reducing antioxidant power was carried out based on FRAP method with minor modification (Szydłowska-Czerniak et al., 2008). To summarize, FRAP reagent was prepared by mixing 2.5 mL TPTZ (0.3123 g diluted in 0.5M HCl), 25 mL acetate buffer (0.775 g diluted in 4 mL acetate in 250 mL distilled water), and 2.5 mL iron chloride (0.136 g in 25 mL distilled water). The solution was then incubated at 37°C for 10 min. Forty µL of sample was added by 120 µL of distilled water, then 1.2 mL of FRAP reagent was added to each sample. The reaction was kept at room temperature for 6 min and was measured using spectrophotometer at 593 nm with ferrous sulfate as the standard.

Statistical analysis

All analyses were carried out in triplicate and analyzed using SPSS. One-way analysis of variance (ANOVA) and Duncan test (Post Hoc) were used for determining significant difference among the data of ratio, ethanol concentration, temperature of ultrasonic method, as well as duration. Independent T-Test was performed for final assay to compare extraction duration of both methods.

Results and Discussion

Effect of ratio between solid and liquid on total phenolic compound recovery

Effect of different levels of solid and liquid using 40% ethanol at room temperature for 18 h on total phenolic content was shown in Figure 1. The ratio of

1:15 (g per mL) had the highest total phenolic extracted from red variety. These results were similar to previous studies by Perera et al. (2012), and Prakash Maran et al. (2013), i.e. 1:18.6 (g per mL). However, ratio of 1:10 (g per mL) was used in other studies to extract rambutan peel (Palanisamy et al., 2011; Palanisamy et al., 2008).

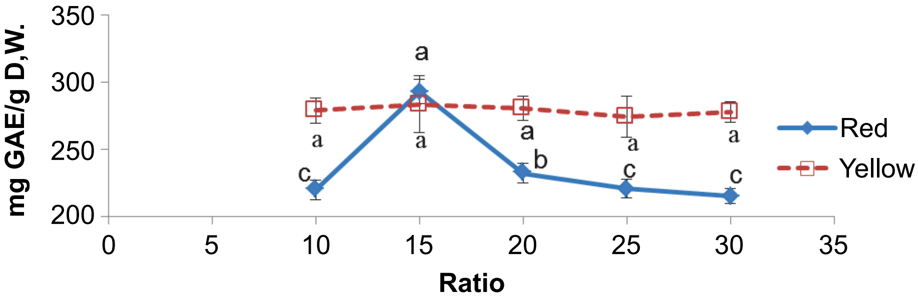

Figure 1. Effect of solid-liquid ratio on the total phenolic recovery (mg GAE per g DW) of the peels of red and yellow rambutan varieties grown in Taiwan. Different letters showed significant differences at $P < 0.05$.

In a study with date seeds, high amount of solvent generated higher gradient concentrations that resulted in increase of diffusion rates up to a peak (Herodež et al., 2003), then the rates decreased gradually (Liu et al., 2013; Feng et al., 2015). On the other hand, yellow variety demonstrated no significant difference among five levels of ratio as reported by Feng et al. (2015), which indicated that phenolic compound had reached to a maximum value and had no significant improvement above a certain ratio.

Effect of ethanol concentration on total phenolic compound recovery

Rambutan peel extraction has been carried out using different types of solvent, such as ether, methanol, aqueous, and ethanol (Okonogi et al., 2007; Palanisamy et al., 2008; Thitilertdecha et al., 2008). Water and ethanol were used as the solvent in this research, since these solvents are safer and less toxic compare to other polar solvents, like ether and methanol.

Rambutan peel was extracted with 1:15 (g per mL) ratio and left for 18 h at room temperature to determine the best ethanol concentration. As shown in Figure 2, ethanol concentration significantly affected the content of total phenolic extracted from both varieties of rambutan peel. Ethanol 40% markedly demonstrated the highest extraction efficiency for both varieties. Since different types of compound have their own solubility against solvent, in terms of polarity, solvent combination determined polarity and solubility, which strongly influenced extraction of antioxidant. It suggested that, there might be some kinds of different phenolic compounds extracted from the rambutan peel grown in Taiwan when compared with other fruit peels by showing different hydrogen bonds and polar sites against solvents (Liu et al., 2013; Feng et al., 2015; Boeing et al., 2014). Furthermore, phenolic compounds may interact with other components from the peel itself, including carbohydrates and proteins during the extraction. This interaction may lead to a complex formation that further lead to insolubility (Naczk and Shahidi, 2004) and perhaps was removed during analysis. In this study, the best ethanol concentration for extraction were the same for both red and yellow rambutan peels. Obviously, phenolic compounds in the peels of these two varieties exhibited similar polarity.

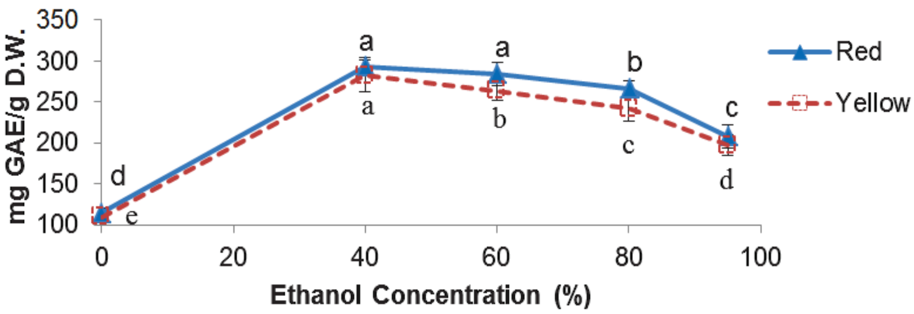

Figure 2. Effect of ethanol concentration on the total phenolic recovery (mg GAE per g DW) of the peels of red and yellow rambutan varieties grown in Taiwan. Different letters showed significant differences at $P < 0.05$.

Effect of temperature using ultrasonic method on total phenolic compound recovery

Temperature significantly altered the recovered phenolic compound from rambutan peel (Figure 3). Higher temperature increased yield of phenolic compound which most likely due to solubility of components and reduction of solvent viscosity which leads to higher mass transfer at certain temperature (equilibrium state) (Fang et al., 2014). However, too high temperature resulted in reduction of phenolic content which might be caused by degradation of thermosensitive phenolic compound (Majd et al., 2014). These results indicated the similar optimum temperature for recovery of rambutan peel polyphenol and antioxidant of spinach extracts as reported by Prakash Maran et al. (2013) and Altermimi et al. (2015) respectively.

Effect of duration using conventional method on total phenolic compound recovery

As shown in Figure 4, the results expressed that at 12 h and 18 h extraction, red variety reached the highest total phenolic content, and it was significantly and gradually decreased after 18 h. Likewise, yellow variety showed the same result, however, still remained unchanged after 24 h and was markedly reduced after 36 h extraction. Antioxidant from rambutan peel reached the peak (optimal time) and tended to decrease when extraction duration was prolonged.

This might be due to oxidation of phenolic compound, or because endogenous enzymes in the plant tissues had damaged phenolic compounds (Naczk and Shahidi, 2004; Kuljarachanan et al., 2009). Despite having similar polarity, both varieties did not seem to have similar types of phenolic compound, since phenolic compounds in the yellow variety degraded slower than red variety at ambient temperature.

Effect of duration using ultrasonic method on total phenolic compound recovery

The total phenolic compound recovery using ultrasonic method was shown in Figure 5.

Total phenolic content gradually increased until reached the highest at 2 min and then markedly decreased when duration was extended for both rambutan varieties. The tendency was similar to Prakash Maran et al. (2013) using ultrasonic method to treat rambutan peel with polyphenols as the parameter. The reduction might have been caused by the decomposition of polyphenols (Lie et al., 2013; Feng et al., 2015).

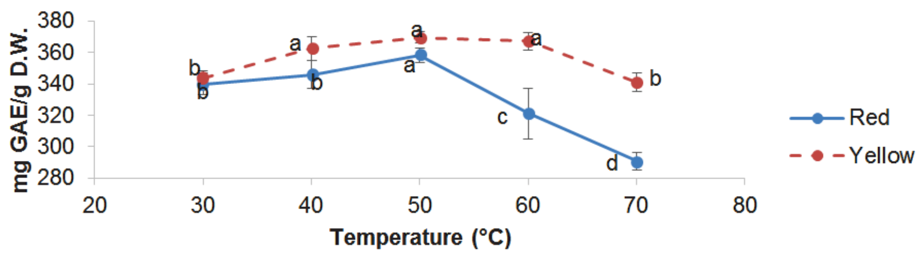

Figure 3. Effect of extraction temperature using ultrasonic method on total phenolic recovery (mg GAE per g DW) of the peels of red and yellow rambutan varieties grown in Taiwan. Different letters showed significant difference at P < 0.05.

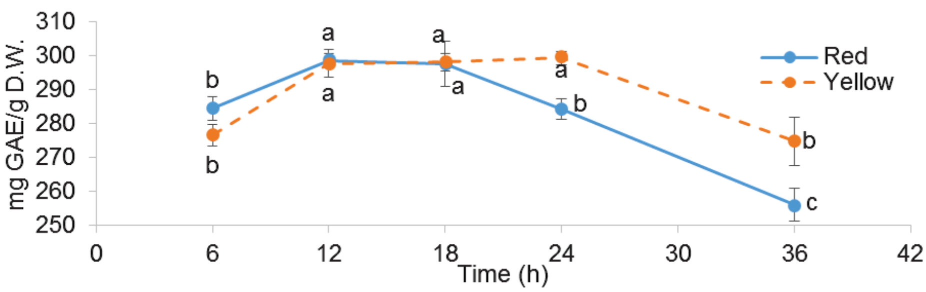

Figure. 4. Effect of extraction duration using conventional method on total phenolic recovery (mg GAE per g DW) of the peels of red and yellow rambutan varieties grown in Taiwan. Different letters showed significant differences at P < 0.05.

Table 1. Antioxidant activity, flavonoid, and total phenolic content of red and yellow rambutan peels using the optimum time duration of conventional and ultrasonic method.

Variety	Method	FRAP (μmol Fe^{2+} per g DW)	Flavonoid (mg Quercetin per g DW)	Total phenolic content (mg GAE per g DW)
Red	Conventional (12 h)	3805.25 ± 33.76[b]	7.74 ± 0.53[a]	298.47 ± 2.10[b]
	Ultrasonic (2 min)	4116.5 ± 88.41[a]	6.41 ± 0.48[b]	358.42 ± 4.63[a]
Yellow	Conventional (12 h)	3800.25 ± 86.49*	8.57 ± 0.35**	297.78 ± 4.06*
	Ultrasonic (2 min)	3977.75 ± 66.52**	7.67 ± 0.22*	354.31 ± 2.36**

Note:
Different letters in the same column showed significant differences at P < 0.05.
[a, b] represent significant difference for red variety, whereas *, ** represent significant difference for yellow variety.

Effects of extraction duration of conventional and ultrasonic methods on antioxidant activity, flavonoid, and total phenolic content

Comparisons between conventional method (12 h) and ultrasonic method (2 min) were determined by measuring their antioxidant activity of red and yellow variety (Table 1). Both varieties showed similar results; ultrasonic for 2 min resulted in a significantly higher FRAP and total phenolic recovery, but lower flavonoid recovery than those of conventional method for 12 h. Higher temperature might increase cavitation of ultrasonic extraction by assisting the breaking of cell wall to release higher yield of phenolic compound, but it could decompose flavonoid. Ferric reducing antioxidant power (FRAP) had strong correlation with phenolic content (Teh and Birch, 2014). Ultrasonic with frequencies higher than 20 kHz was able to facilitate extraction of both organic and inorganic compound from solid matrices by using liquid solvents (Khoddami et al., 2013). Different types of phenolic compounds as well as flavonoids might affect variation on both methods as mentioned earlier. Hence, these results demonstrated that ultrasonic method could be considered as better method than conventional in terms of time efficiency similar to those reported by Teh and Birch (2014) in seed cakes in that ultrasonic extraction method resulted in higher antioxidant activities compared with conventional method.

Conclusion

Ratio, solvent concentration, duration, and method of extraction significantly influenced the recovery of phenolic compounds extracts. The optimum sample to solvent ratio for the best recovery of phenolic was 1:15 using 40% ethanol for 12 h for conventional extraction, and 2 min for ultrasonic extraction method. Ultrasonic extraction for 2 min resulted in a higher FRAP and total phenolic recovery compared with conventional extraction for 12 h. These results suggested that ultrasonic method could be more effective than conventional method to extract antioxidant from rambutan peel.

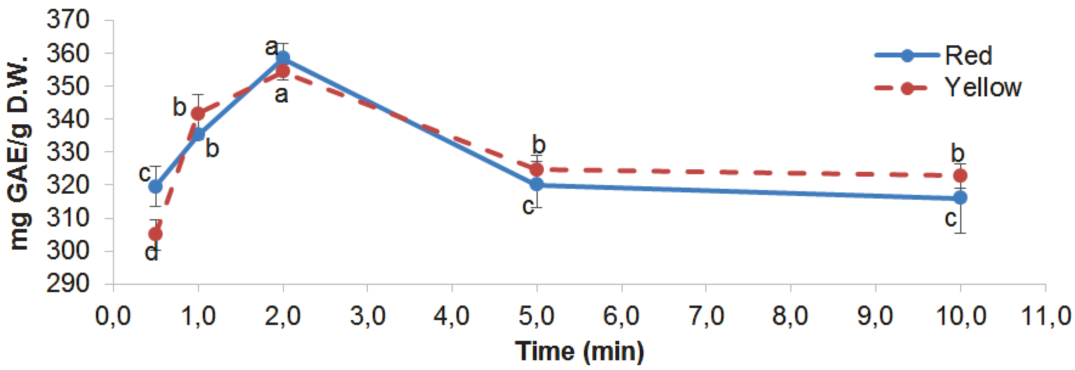

Figure 5. Effect of extraction duration using ultrasonic method on total phenolic recovery (mg GAE per g DW) of the peels of red and yellow rambutan varieties grown in Taiwan. Different letters showed significant difference at P < 0.05.

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