Effect of cooking methods on nutritional composition and antioxidant properties of lotus (*Nelumbo nucifera*) rhizome

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Abstract

Lotus (*Nelumbo nucifera*) rhizome possessed abundant nutrients such as vitamin C, minerals and antioxidants. The consumption of lotus rhizome is limited in Malaysia due to limited information on its nutritional values. Cooking or heat treatment may cause nutritional changes in the food, however, there is a lack of study on the effect of cooking methods on nutritional contents of lotus rhizome. In this study, the effects of cooking methods (steaming, boiling and deep-frying) on nutritional composition (proximate, vitamin C and minerals) and antioxidant properties (total phenolic content, total flavonoid content, DPPH and ABTS scavenging activity) of lotus rhizome were investigated. Results showed that boiling significantly increased moisture and crude fibre but decreased ash, crude fat and crude protein contents of lotus rhizome. Meanwhile, deep frying significantly reduced moisture and increased ash, crude fat, crude protein, crude fibre and carbohydrate contents of lotus rhizome. Steaming induced no significant changes in moisture, crude protein, crude fat, crude fibre, carbohydrate, vitamin C and mineral (potassium, zinc and copper) contents when compared to the raw lotus rhizome. Boiling significantly reduced the amount of vitamin C while deep frying caused a significant reduction in total phenolic content, total flavonoid content and antioxidant activity for both ABTS and DPPH assays of lotus rhizome. In conclusion, steaming would be the best cooking methods for lotus rhizome while deep frying is not recommended to retain the nutritional composition and antioxidant properties of lotus rhizome.

1. Introduction

Lotus (*Nelumbo nucifera*) in the Nelumbonaceae family is an aquatic plant widely grown in Asian countries such as China, Japan and India. It is cultivated for food, ornamental and medicinal purpose (Zhao *et al.*, 2016). According to Wang (2014), lotus rhizome is rich in starch, sugars, lipids, proteins, minerals, vitamins, alkaloids, flavonoids and other biochemical substances. It contains alkaloids that is used in treating arrhythmia, sunstroke, fever, diarrhoea, dysentery, dizziness and stomach problems (Shad *et al.*, 2011). Furthermore, it also has pharmacological activities such as anti-diarrhoeal, anti-inflammatory, antioxidant, antipyretic, diuretic, hypoglycaemic, immunomodulatory and psychopharmacological activities (Bhardwaj and Modi, 2016; Yi *et al.*, 2016). The extract of lotus rhizome especially from its nodes contains a high amount of antioxidant such as phenolic, tannin and flavonoid (Moro, 2012).

Lotus rhizome has potential to be cultivated widely as another important crop in Malaysia, just like sweet potato or cocoyam, as it can be harvested year-round and can be grown in an environment which is unfavourable for other traditional agricultural crops. However, the production of lotus in Malaysia is limited due to the low market demand compared to other vegetables. The consumption of lotus rhizome in Malaysia is limited to Chinese and Indian population. The Malay population in Malaysia rarely consumes the lotus rhizome since not much information on the nutritional value of it is available. The popularity of lotus rhizome is mainly due to its attractiveness white colour and the crispness texture (Li *et al.*, 2017). The fresh lotus rhizome is popular to be sliced and deep-fried as chips for snacks. This is because deep-frying creates unique texture, flavour and taste for the food (Oke *et al.*, 2017).

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According to Metha (2015), vegetables are cooked before consumption to improve their palatability, aroma, taste, appearance, and texture to achieve high acceptance among consumers. Cooking or processing method caused nutrients loss in food product and the amount of loss was depended on the method used. The heat applied during cooking caused the nutrients, which were sensitive to heat, light, oxygen and pH, to be unstable and lead to their thermal degradation (Omotosho *et al.*, 2016).

Most consumers eat lotus rhizome because of its mild sweet flavour or as a part of their traditional food habit without knowing the nutritional value of lotus rhizome. There is a lack of research done purposely to investigate the effect of different cooking methods on the nutritional composition and antioxidant properties of lotus rhizome. Li *et al.* (2017) focused on the effect of steaming and boiling on the physicochemical properties of lotus (*Nelumbo nucifera*) root, such as moisture content, texture and volatile compounds (methylcyclohexane and ethylcyclohexane). Another study conducted by Zhao *et al.* (2016) investigated the effect of heat blanching and calcium immersion on physicochemical properties related to the texture of rhizome. Meanwhile, Vora and Srinivasan (2015) studied on the nutritional benefits of raw and fried lotus stems. Frying caused the reduction of iron, calcium, phosphorus, sodium and vitamin C content and increased the amount of carbohydrate, protein and dietary fibre in lotus stem (Vora and Srinivasan, 2015).

The objectives of this study are to determine the effect of cooking methods on the proximate composition, vitamin C, minerals content, and antioxidant properties of lotus (*Nelumbo nucifera*) rhizome. Information obtained from this study would be beneficial to consumers in choosing the good cooking method for lotus rhizome and is expected to encourage its consumption among various communities in Malaysia.

2. Materials and methods

2.1 Sample preparation and cooking treatments

Five (5) kg of lotus (*Nelumbo nucifera*) rhizomes with an average length of 45.0-57.0 cm and width of 4.0-5.0 cm were purchased from the farmer at Ipoh, Perak. The samples were washed, peeled and sliced into 0.3 cm of thickness. The lotus rhizome slices were divided into four portions, where one portion was left as raw for control while the other three portions were cooked by steaming, boiling and deep-frying. The cooking conditions were as follows:

Steaming: Lotus rhizome slices (0.9 kg) were steamed for 30 mins in 3 L of $100^{\circ}C$ boiling water in a steamer until they were tender.

Boiling: Lotus rhizome slices (0.9 kg) were boiled for 30 mins in 2 L of $100^{\circ}C$ boiling water in a stock pot until they were tender.

Deep frying: Lotus rhizome slices (0.9 kg) were deep-fried for 1.5 mins in a saucepan with 350 mL palm oil (Bagus, Sime Darby Plantation, Malaysia) at $170\pm2.0^{\circ}C$ until they were crispy.

All samples were cooked without adding any salt or additional ingredients. The cooked samples were taken for quick freezing in a blast freezer (Technomac, Malaysia) at -20°C for two hrs and stored in a freezer (Panasonic, Malaysia) at -20°C before further analysis.

2.2 Proximate analysis

Proximate analysis of lotus rhizome was carried out according to AOAC (2005) methods. The samples were dried in an oven (Chemopharm, Malaysia) at $105^{\circ}C$ for overnight for moisture determination. Analysis of ash was carried out by heating the samples in a muffle furnace (Carbolite, Malaysia) at $550^{\circ}C$ for overnight. Crude fat content was analysed using Labtec ST310 (Foss, Malaysia) with petroleum ether (EMSURE, Germany) as extraction solvent. Crude protein content was measured using a Kjeldahl method with Turbotherm digestion unit (Gerhardt, Malaysia) and Vapodest 30S distillation unit (Gerhardt, Malaysia). The conversion factor for nitrogen-protein percentage used was 6.25. The samples were boiled with 0.13 M H_2SO_4 (R&M Chemical, Malaysia) and 0.31 M NaOH (R&M Chemical, Malaysia) in Gerhardt Fibre Bag system for determination of crude fibre content. The carbohydrate content of the sample was calculated by subtracting 100% with the sum of percentage of moisture, ash, crude fat, crude protein and crude fibre contents.

2.2.1 Oil uptake and moisture loss

The oil uptake and moisture loss of deep-fried lotus rhizome were determined according to Kim *et al.* (2015) method and calculated as follows:

$$Oil\ uptake\ (\%) = \frac{W_D \times F_D - W_d \times F_d}{W_D} \times 100$$

$$Moisture\ loss\ (\%) = \frac{W_d \times M_d - W_D \times M_D}{W_D} \times 100$$

Where W_D = mass of cooked sample (g); W_d = mass of raw sample (g); F_D = fat amount of cooked sample (g/1g wet basic); F_d = fat amount of raw sample (g/1g wet basic); M_D = moisture content of cooked sample (g/1g

wet basic); and M_d = moisture content of raw sample (g/1g wet basic).

2.3 Vitamin C analysis

Lotus rhizome juice was prepared by grounding 10 g of chopped lotus rhizome slices with 50 mL distilled water (Hussain *et al.*, 2016). The mixture was filtered and added with 3% metaphosphoric acid (EMSURE, Germany) until the volume became 100 mL. Determination of vitamin C was carried out according to Pegg *et al.* (2010) method. Lotus rhizome juice (10 mL) was titrated with 2,6-Dichlorophenolindophenol (DCPIP) dye solution until pink colour exists for 15 s. The ascorbic acid content was calculated using the formula below:

$$\text{Ascorbic acid content (mg/100 g)} = (X - B) \times \left(\frac{0.0543}{10}\right) \times \left(\frac{100}{10}\right)$$

Where X = volume for test solution titration (mL) and B = volume for test blank titration (mL).

2.4 Mineral analysis

Mineral extraction of lotus rhizome samples was carried out according to AOAC (2005) method. Lotus rhizome (1.5 g) was incinerated in a muffle furnace to form ash to be evaporated to dryness on a hot plate (Favorit, Malaysia) with 2 mL of concentrated HCl (EMSURE, Malaysia). Subsequently, 10 mL of 20% concentrated HNO_3 (Merck, Germany) was added. After incubation in water bath at 60°C for 1 hour, the mixture was diluted into 100 mL with distilled water and filtered using Whatman No.42 filter paper prior to analysis. Determination of mineral content of lotus rhizome was carried out using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP–OES) (Optima 8300, PerkinElmer, USA) according to Akpinar-Bayizit *et al.* (2010) method.

2.5 Antioxidant analysis

2.5.1 Antioxidant extraction

Antioxidant extraction of lotus rhizome slices was carried out according to Leong *et al.* (2012) method. Chopped lotus rhizome samples were dried in an oven (Southstar, Malaysia) at 50°C for 48 hrs until almost 5-7% moisture content left. They were grounded to pass a 1 mm sieve. Lotus rhizome powder (5.0 g) was mixed with 50 mL of methanol (EMSURE, Germany) for overnight incubation in darkness at room temperature (28°C). The mixture was centrifuged (Hettich Zentrifugen, Malaysia) at 3000 r/min for 15 mins. The supernatant was separated through filtration with Whatman No.1 filter paper and was evaporated at 40°C using a rotary evaporator (Buchi, Malaysia). The yield of antioxidant extraction of lotus rhizome was calculated using the following equation:

$$\text{Yield (\%)} = \frac{\text{Weight of dry extract (g)}}{\text{Weight of lotus rhizome powder (g)}} \times 100\%$$

2.5.2 Total phenolic content (TPC)

Total phenolic content (TPC) in lotus rhizome was determined using the Folin Ciocalteu method according to Zhao *et al.* (2014). Lotus rhizome extract solution of 0.1 mL of 5 mg/mL was mixed with 2 mL distilled water and 1 mL of Folin Ciocalteu phenol reagent (R&M Chemical, Malaysia) for incubation in darkness at room temperature (28°C) for 5 mins. A total of 5 mL of 20% aqueous Na_2CO_3 solution (Bendosen, Malaysia) was added followed by incubation for 60 mins. The absorbance was measured at 735 nm using UV-Vis spectrophotometer (Interscience, Malaysia) with distilled water as blank. Gallic acid (R&M Chemical, Malaysia) was used as standard. TPC (GAE/g DE) in lotus rhizome was calculated through a linear regression equation obtained from gallic acid standard graph.

2.5.3 Total flavonoid content (TFC)

Aluminium chloride coloration method was adopted for the estimation of total flavonoid content (TFC) in lotus rhizome according to Zhao *et al.* (2014). Lotus rhizome extract solution of 0.6 mL of 10 mg/mL was mixed with 3.75 mL distilled water and 0.225 mL of 5% aqueous $NaNO_3$ solution for incubation in darkness at room temperature (28°C) for 6 mins. A total of 0.45 mL 10% $AlCl_3$ solution (Sigma-Aldrich, USA), 1.5 mL of 1 mol/mL NaOH solution (R&M Chemical, Malaysia) and 0.975 mL of distilled water were added to the mixture before incubation for 15 mins. The absorbance was measured at 500 nm using UV-Vis spectrophotometer (Interscience, Malaysia) with distilled water as blank and catechin (TGI, Tokyo) as standard. TFC (CE/g DE) in lotus rhizome was calculated through a linear regression equation obtained from catechin standard graph.

2.5.4 DPPH radical scavenging activity

DPPH radical scavenging activity of lotus rhizome extract was measured as in accordance to Zhao *et al.* (2014) with slight modification. A series of lotus rhizome extract solution (0.5 to 3.0 mg/mL) was mixed with 5 mL of 0.1 μmol/L 2, 2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich, Germany) solution in methanol for incubation in darkness at room temperature for 30 mins. The absorbance was measured at 517 nm using UV-Vis spectrophotometer (Interscience, Malaysia) with methanol as blank. The following equation was used for the calculation of DPPH free radical scavenging activity:

$$\text{Scavenging ability (\%)} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100\%$$

Where $A_{control}$ = absorbance of the control and A_{sample} = absorbance of the sample

A graph of scavenging ability against the concentration of the sample extract was plotted to determine the amount of lotus rhizome extract with 50% scavenging activity (IC_{50}) values.

2.5.5 ABTS radical scavenging activity

Analysis of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity of lotus rhizome extract was conducted using Wu *et al.* (2011) method. $ABTS^+$ radical solution was prepared by incubating the mixture of 3 mL of 7 mM ABTS stock solution (Sigma-Aldrich, USA) and 3 mL of 2.45 mM potassium persulfate (EMSURE, Germany) at room temperature for 16 hrs in darkness. The mixture was diluted with 80% ethanol (HmbG Chemical, Malaysia) to obtain absorbance of 0.70±0.005 at 734 nm using UV-Vis spectrophotometer (Interscience, Malaysia). A total of 0.3 mL of a series of lotus rhizome extract solution (0.5 to 3.0 mg/mL) was mixed with 2.7 mL of $ABTS^+$ radical solution and incubated for 30 mins. The absorbance was measured at 734 nm using UV-Vis spectrophotometer with methanol as blank. The scavenging ability (%) and IC_{50} values were determined as previously described for DPPH.

2.6 Statistical analysis

All analyses were carried out in the triplicate and data was expressed in mean ± standard deviation form. Statistical analysis was carried out using one-way ANOVA and Tukey's-b using SPSS version 16 (SPSS Inc., USA).

3. Results and discussion

3.1 Proximate composition

Table 1 shows the raw lotus rhizome contained 86.86% moisture, 1.05% ash, 0.14% crude fat, 2.34% crude protein, 0.80% crude fibre and 8.81% carbohydrate. This finding is in accordance with Wang (2014) findings on lotus rhizome samples. Meanwhile,

Read (1982) reported that lotus rhizome contained 1.70% protein, 0.10% fat, 9.7% carbohydrate and 1.10% ash. The variation in the proximate composition of lotus rhizomes in the present and previous studies may be due to differences in the maturity level and planting location of lotus rhizome samples. This finding is supported by Agunbiade *et al.* (2017) and Chinomso *et al.* (2018) on orange-fleshed sweet potato and on *Moringa oleifera* leaves in their studies, respectively.

The moisture content of steamed (89.20%) and boiled lotus rhizome (91.51%) was significantly different with raw (86.86%) lotus rhizome (p < 0.05). A similar result was reported by Bembem and Sadana (2013) and Okibe *et al.* (2016) where the moisture content of their potato and pumpkin leaves were higher after boiled, respectively. According to Lola (2009), fibre and other chemical compounds presented in the vegetables absorbed water during boiling.

In this study, the moisture content of the deep-fried lotus rhizome was lowest (15.37%) compared to raw and other cooked lotus rhizomes. This finding is in agreement with Shunmugapriya and Kalaiselvan (2017) who reported that fried garlic possessed the lowest percentage of moisture content compared to steamed and boiled samples. The high heat applied during deep-frying caused the moisture escaped from food through evaporation (Omotosho *et al.*, 2016; Oke *et al.*, 2017). On the other hand, the ash content of deep-fried sample was the highest (3.71%), followed by raw (1.05%), steamed (0.69%) and boiled sample (0.17%). Boiling might reduce more ash content than steaming due to the leaching out of mineral into boiling water (Lola, 2009; Wang *et al.*, 2012). The ash increment occurred in deep-fried samples was related to the moisture lost. Dehydration concentrated the nutrient left in the sample and thus increased the ash content in the fried product (Yodkraisri and Bhat, 2012).

There was no significant difference (p > 0.05) found between crude fat content of raw and steamed (0.11%) samples and between crude fat content of raw and boiled (0.06%) lotus rhizome (Table 1). A similar result was reported by Hwang *et al.* (2012) on red pepper. A large increase of crude fat content was found on the deep-fried lotus rhizome in the present study and this is in

Table 1. Proximate composition on a wet weight basis for raw and cooked lotus rhizomes

Lotus rhizome	Proximate composition					
	Moisture (%)	Ash (%)	Crude fat (%)	Crude protein (%)	Crude fibre (%)	Carbohydrate (%)
Raw	86.86±0.35[c]	1.05±0.05[b]	0.14±0.01[b]	2.34±0.17[b]	0.80±0.02[c]	8.81±0.51[b]
Steamed	89.20±0.25[b]	0.69±0.08[c]	0.11±0.01[b]	2.22±0.12[b]	1.01±0.06[c]	6.76±0.46[b]
Boiled	91.51±0.53[a]	0.17±0.01[d]	0.06±0.01[b]	0.77±0.05[c]	2.06±0.07[b]	5.43±0.46[b]
Deep fried	15.37±1.90[d]	3.71±0.25[a]	31.41±1.07[a]	8.33±0.38[a]	12.28±0.20[a]	28.91±2.67[a]

Mean ± SD, n = 3. Values with different superscript letters in the same column are significantly different at p<0.05

accordance with Omotosho *et al.* (2016) on unripe plantain. Deep-fried food increased its fat content through extra fat absorption from the frying oil and decreased its moisture content through moisture escaping from food due to high heat (Bordin *et al.*, 2013).

The moisture loss and oil uptake of deep-fried lotus rhizome were 73.88% and 31.27%, respectively. This finding is in agreement with Manjunatha *et al.* (2012) on Gethi (*Dioscorea kammonensis kunths*) strips. According to Kita (2014), moisture loss would cause dehydration which contributed to changes in cell structure and formation of small channel matrix occupied subsequently by the frying oil. Vigorous water escaped from vegetable had limited the oil absorption during the frying process, causing a higher rate of moisture loss than oil uptake (Bouchon *et al.*, 2003).

The highest crude protein content was found on deep -fried (8.33%) lotus rhizome followed by raw (2.34%), steamed (2.22%) and boiled (0.77%) lotus rhizome. The large increment in crude protein content of deep-fried food was contributed by the moisture loss which concentrated the organic materials left (Vora and Srinivasan, 2015) and by the hydrolysis of insoluble protein compound which increased the protein availability (Reid *et al.*, 2016). Meanwhile, boiling decreased crude protein content of lotus rhizome, which is in accordance with the previous studies on potato (Bembem and Sadana, 2013) and red pepper (Hwang *et al.*, 2012). Soluble nitrogenous substance leached out into boiled water when cooked, causing more loss rather than denaturation (Reid *et al.*, 2016).

Deep frying increased the crude fibre content of lotus rhizome from 0.80% to 12.28%. Similar results were observed on four varieties of potato (Murniece *et al.*, 2011), lotus stem (Vora and Srinivasan, 2015) and purple flesh potatoes (Tian *et al.*, 2016). This is probably due to structural damage to the cells, which induced a marked loss of other liposoluble compounds and increased fibre content (Sun *et al.*, 2014). Furthermore, the increase in fibre content in cooked samples were due to dehydration because of moisture loss (Vora and

Srinivasan, 2015). The highest carbohydrate content was also found in the deep-fried lotus rhizome, followed by steamed, raw and boiled lotus rhizome.

3.2 Vitamin C content

Table 2 shows the amount of vitamin C and minerals in the raw and cooked lotus rhizomes in the present study. The raw lotus rhizome contained 15.02 mg/100 g vitamin C. This amount was higher than the vitamin C in the lotus *(Nelumbo nucifera)* rhizomes reported by Shad *et al.* (2011; 0.26-0.35 mg/g) but lower than USDA (2016; 44 mg/100 g). The differences probably due to cultural practice, maturity, preharvest climatic conditions, harvesting method influenced the vitamin C level in vegetables as stated by Lee and Kader (2000) and Duya (2017).

Boiling and deep-frying significantly decreased the vitamin C content in lotus rhizome. The amount of vitamin C in the boiled and deep-fried lotus rhizomes in the present study were 3.80 mg/100 g and 1.45 mg/100 g, respectively. This finding is in contrast with Ikanone and Oyekan (2014) who reported that boiled Irish potato and sweet potato lost more vitamin C than the fried samples. Meanwhile, previous studies showed that the steaming cooking method retained more vitamin C in potato tubers (Bembem and Sadana, 2013), and broccoli, spinach and lettuce (Zeng, 2013) compared to the boiling method. This probably due to vitamin C is a heat-sensitive water-soluble nutrient as reported by Igwemmar *et al.* (2013).

Apart from thermal degradation, vitamin C would be lost through leaching out during boiling (Yuan *et al.*, 2009). Thus, boiling contributed to the highest reduction of vitamin C content for lotus rhizome compared to deep frying as it caused both thermal degradation and leaching out of vitamin C. Moreover, oxidation of vitamin C occurred easily in aqueous solution and could be enhanced by higher temperature, physical damage and relative humidity (Lee and Kader, 2000). In the deep-fried sample, the loss of vitamin C was due to the higher temperature (170°C) applied during frying. The rate of vitamin C retention in cooked food was influenced by

Table 2. Vitamin C and mineral composition of raw and cooked lotus (*Nelumbo nucifera*) rhizome.

Lotus rhizome	Vitamin C (mg/100 g)	Amount of mineral (mg/100 g)								
		K	Ca	Mg	Fe	Zn	Cu	Mn	Na	P
Raw	15.02 ±1.36[a]	2437.53 ±37.95[a]	151.75 ±1.76[c]	134.61 ±1.34[b]	13.27 ±0.32[b]	5.08 ±0.07[a]	2.64 ±0.05[a]	1.49 ±0.02[b]	74.14 ±0.89[b]	804.63 ±5.63[b]
Steamed	13.40 ±1.14[a]	2512.60 ±37.06[a]	201.20 ±1.67[b]	148.89 ±1.31[a]	24.34 ±1.37[a]	5.55 ±0.45[a]	2.96 ±0.45[a]	2.57 ±0.03[a]	76.97 ±1.27[a]	914.58 ±3.29[a]
Boiled	1.45 ±0.31[b]	617.30 ±8.18[c]	263.27 ±1.49[a]	113.95 ±0.61[c]	12.02 ±0.55[b]	3.52 ±0.03[b]	1.61 ±0.01[b]	2.61 ±0.03[a]	43.70 ±0.71[d]	376.50 ±2.04[d]
Deep fried	3.80 ±0.54[b]	1481.53 ±29.92[b]	92.06 ±1.92[d]	69.19 ±1.07[d]	7.27 ±0.73[c]	2.53 ±0.27[c]	1.29 ±0.01[b]	1.09 ±0.01[c]	52.12 ±0.57[c]	442.28 ±4.52[c]

Mean ± SD, n = 3. Values with different superscript letters in the same column are significantly different at p<0.05

the size of cut, use of cooking media, time and temperature of cooking (Severi *et al*., 1997).

3.3 Mineral composition

Raw lotus rhizome was composed of potassium (2437.53 mg/100 g), calcium (151.75 mg/100 g), iron (13.27 mg/100 g), zinc (5.08 mg/100 g), copper (2.64 mg/100 g), manganese (1.49 mg/100 g), sodium (74.14 mg/100 g), and phosphorus (804.63 mg/100 g) as shown in Table 2. These findings were contradicted with USDA (2016) which reported that there were about 556 mg potassium (K), 45 mg calcium (Ca), 1.16 mg iron (Fe), 0.39 mg zinc (Zn), 0.26 mg copper (Cu), 0.26 mg manganese (Mn), 40 mg sodium (Na) and 100 mg phosphorus (P) presented in lotus rhizome. Jongrungruangchok *et al*. (2010) stated that the minerals of *Moringa oleifera* leaves obtained from different regions in Thailand varied in mineral content because of several reasons such as cultivated regions, growing conditions, nature of soil, seasonal changes, genetically different cultivars and storage conditions. Pongrac *et al*. (2016) also found that the amounts of mineral compounds for grains and sprouts were different from each other when they were cultivated in tap water or moderately mineral-rich water.

Three types of trend were observed for the mineral content in lotus rhizomes in the present study. The trends were steamed > raw > deep-fried > boiled for K, Na and P; steamed > raw > boiled > deep-fried for Mg, Fe, Zn and Cu; and boiled > steamed > raw > deep-fried for Ca and Mn. According to Ikanone and Oyekan (2014), boiling caused a higher loss of Zn, Mg, Na and Ca and lower loss of Cu and Fe for Irish and sweet potato compared to frying. Contrastingly, boiling caused more loss for Na and Ca but not for Zn and Mg compared to frying in the lotus rhizome in the present study. Vora and Srinivasan (2015) reported that frying reduced Na and Fe in lotus stem whereas Okibe *et al*. (2016) reported that steaming retained K, Na, Ca, Mg, Fe and P better than boiling as minerals were soluble in water and could leach out into boiling medium during cooking (Okibe *et al*., 2016). The findings in Vora and Srinivasan (2015) and Okibe *et al*. (2016) were in accordance with the present study.

Generally, different type of mineral would undergo different rate of losses even though they were treated with the same cooking method. This condition was contributed by different proportion of soluble salt, which leach out easily to the water when heating, and insoluble salt, which retained in the plant when heating (Faboya and Aku, 1996). However, the increment of mineral content might occur after the breaking down of plant tissue which increased the mineral extractability. The heat applied degraded the antinutrient hindered the availability of minerals, causing minerals to be found in the cooked medium (Oulai *et al*. 2013).

3.4 Antioxidant content

The highest antioxidant extraction yield was obtained from raw lotus rhizome (4.03%), followed by steamed (3.29%), boiled (2.23%) and deep-fried (1.19%) lotus rhizome (Table 3). A similar trend was reported by Sultana *et al*. (2008) on peas, carrot, spinach and white turnip. The antioxidant extraction yield of raw lotus rhizome was in agreement with Yang *et al*. (2007) who reported that the extract yield was ranged from 4 to 5% when using methanol as solvent. Contrastingly, the antioxidant extraction yield of lotus rhizome in Wu *et al*. (2011) and Zhao *et al*. (2014) studies were 10.70% and 5.30 to 6.84%, respectively. Soil nature and agro-climatic condition caused different bioavailability of extractable components in plant tissue and hence contributed to the distinct values of extract yield of antioxidant (Hsu *et al*., 2006).

Total phenolic content (TPC) of raw lotus rhizome (50.66 mg GAE/g) was higher compared to the cooked sample of lotus rhizomes (11.96 – 43.80 mg GAE/g). This finding is in agreement with Zhao *et al*. (2014) who found the TPC of lotus rhizome in their studies ranged from 31.63 to 70.01 mg GAE/g. Contrastingly, Wu *et al*. (2011) reported a lower result with 16.14 mg GAE/g of TPC in the raw lotus rhizome. The variation in the TPC was contributed by the distinct environmental and geological conditions of planting region as reported by Sultana *et al*. (2008). This is supported by Podsedek (2005) who stated that the polyphenols content for vegetable was varied due to factors such as varieties, climatic conditions, cultural practices, maturity at harvest

Table 3. Yield of antioxidant extraction, total phenolic content, total flavonoid content, IC_{50} values of DPPH and ABTS radical scavenging activity for raw and cooked lotus (*Nelumbo nucifera*) rhizome.

Lotus rhizome	Antioxidant extraction yield (%)	Total phenolic content (mg GAE/g DE)	Total flavonoid content (mg CE/g CE)	IC_{50} values of DPPH radical scavenging activity (µg)	IC_{50} values of ABTS radical scavenging activity (µg)
Raw	4.03±0.11[a]	50.66±4.49[a]	11.98±0.38[a]	1027	495
Steamed	3.29±0.13[b]	43.80±2.94[a]	10.65±0.65[b]	1186	569
Boiled	2.23±0.14[a]	20.78±1.70[b]	5.13±0.39[c]	1468	694
Deep fried	1.19±0.03[a]	11.96±0.85[c]	2.54±0.13[d]	1705	799

Mean ± SD, n = 3. Values with different superscript letters in the same column are significantly different at p<0.05

and storage conditions. Furthermore, boiling and deep-frying decreased the amount of TPC of lotus rhizome which was 20.78 mg GAE/g and 11.96 mg GAE/g, respectively. Boiling contributed to the loss of antioxidant content in vegetable due to leaching out of phenolic compounds into boiling water (Sultana *et al.*, 2008; Hwang *et al.*, 2012; Sengul *et al.*, 2014). Meanwhile, high heat applied during frying would lose most phenolic content compared to other cooking methods, i.e. which caused thermal decomposition of the phenolic compound during frying (Turkmen *et al.*, 2005; Kalkan and Yucecan, 2013).

Total flavonoid content (TFC) of lotus rhizome showed a similar trend with TPC, at which raw lotus rhizome (11.98 mg CE/g DE) contained the highest value of TFC, followed by steamed (10.65 mg/g DE), boiled (5.13 mg CE/g DE) and deep-fried (2.54 mg CE/g DE) lotus rhizome. Flavonoid compound which is soluble in water could leach out during boiling (Bembem and Sadana, 2012). Thus, boiled vegetable had a lower TFC than steamed samples. The TFC of raw lotus rhizome in the present study was in agreement with Zhao *et al.* (2014) where the TFC in the lotus rhizome ranged from 7.33 to 13.21 mg CE/g DE.

3.5 Antioxidant activity

Inhibitory concentration (IC_{50}) values of DPPH (1027 µg) and ABTS (495 µg) assay for raw lotus rhizome were the lowest among all samples (Table 3). The lowest IC_{50} value indicated the highest antiradical activity (Leong *et al.*, 2012). Thus, the raw lotus rhizome contained the highest antioxidant activity compared to the cooked samples. Meanwhile, Wu *et al.* (2011) reported that the IC_{50} value of DPPH and ABTS assay for the lotus rhizome extract in their studies were 458.58 µg and 4917.51 µg, respectively. Yang *et al.* (2007) suggested that the extracting solvent used affected the antioxidant activity of plant extract. The solvent methanol used in the present study was found to have higher antioxidant extraction yield and hence higher antioxidant activity compared to the petroleum ether used by Wu *et al.* (2011). In addition, the growing region also influenced the antioxidant content and activity for lotus rhizome extract (Zhao *et al.*, 2014).

The IC_{50} value for ABTS assay of lotus rhizome in the present study was lower than of DPPH due to the different reaction of ABTS assay and DPPH assay. This is in accordance with Al-Laith *et al.* (2015) findings on three wild medical plants (*Aizoon canariense, Asphodelus tenuifolius* and *Emex spinosus*). For instances, ABTS cation radical could react with hydrophilic and lipophilic antioxidant while DPPH radical can only react with hydrophilic antioxidant (Boligon *et al.*, 2014).

Zhang and Hamauzu (2004) reported that cooking reduced the antioxidant components and activity of broccoli. In the present study, the IC_{50} value of DPPH assay for steamed, boiled and deep-fried lotus rhizome were 1186 µg, 1468 µg and 1705 µg, respectively; while of ABTS assay were 569 µg, 694 µg and 799 µg, respectively (Table 3). The steaming method caused the least loss of antioxidant activity, followed by boiling in both DPPH and ABTS assay. This is due to polyphenols compound could dissolve in boiled water, thus boiling caused a higher reduction in antioxidant activity in vegetables compared to steaming (Hwang *et al.*, 2012; Sengul *et al.*, 2014). Meanwhile, Kalkan and Yucecan (2013) reported that frying caused the highest loss of antioxidant activity in vegetables. Zhang *et al.* (2011) suggested that long term exposure to the high temperature degraded the antioxidant compound in bamboo shoot.

4. Conclusion

Methods of cooking affect the nutritional composition and antioxidant properties of lotus (*Nelumbo nucifera*) rhizome. Boiling and deep-frying brought prominent change on the moisture, ash, crude fat, crude protein, crude fibre and carbohydrate content of lotus rhizome whereas steaming brought only a small change of proximate composition when compared to raw lotus rhizome. In addition, steamed lotus rhizome caused the lowest loss of vitamin C and minerals (K, Na, P, Mg, Fe, Zn, Cu, Ca and Mn) while boiling gave the highest loss of vitamin C and deep-frying gave the highest loss of most of the minerals in the lotus rhizome. Deep frying caused the significant degradation of antioxidant properties, followed by boiling and steaming. Thus, deep-frying is not recommended for healthy preparation as it increased the fat content significantly in the final product and contributed to a high loss of moisture, vitamin C, minerals and antioxidant properties of lotus rhizome. Overall, steaming is considered as the best cooking methods for lotus rhizome as it retained proximate composition, vitamin C, minerals, TPC, TFC, and antioxidant activity better than boiling and deep-frying.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

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