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Antioxidative activity of Mungoong, an extract paste, from the cephalothorax of white shrimp (*Litopenaeus vannamei*)

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Abstract

The antioxidative activity of Mungoong, an extract paste, from the cephalothorax of white shrimp (*Litopenaeus vannamei*) was studied. Extraction media were shown to affect the antioxidative activity and properties of resulting extracts from Mungoong. Distilled water exhibited the highest efficacy in extracting the antioxidants from Mungoong, as evidenced by the highest ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (2,2-diphenyl-1-picryl hydrazyl) radical scavenging activity as well as ferric reducing activity power (FRAP), compared with distilled water/ethanol mixture (1:1, 1:2 and 2:1) and ethanol. UV-absorbances at both 280 and 295 nm (A_{280}, A_{295}), browning intensity (A_{420}) and fluorescence intensity were also highest in the extract using distilled water. ABTS and DPPH radical scavenging activity and FRAP of water extract, increased linearly with increasing concentration. Good correlation between ABTS and DPPH radical scavenging activity; DPPH radical scavenging activity and FRAP; ABTS radical scavenging activity and FRAP were observed, suggesting that antioxidants in the extract, possessed the capability of scavenging the radicals together with reducing power. Antioxidants in the water extract from Mungoong showed high stability over the wide pH ranges (2–11) and temperature up to 100 °C, in which the activity of more than 80% remained. MALDI-TOF analysis revealed that water extract contained the peptides having the mass ranges of *m/z* 400–1000 and 4000–7000.

Keywords: Antioxidant; Extraction; ABTS; DPPH; FRAP; Stability; White shrimp; Extract

1. Introduction

Oxidation directly affects food quality, and is commonly associated with changes of flavour and texture. Therefore, prevention of lipid oxidation has been of concern in the food industry. The use of synthetic antioxidants is an old practice and their safety could be questioned by the consumer. The alternative natural compounds with efficient antioxidative activity, have been paid increasing attention.

Shrimp and shrimp products have attracted considerable attention due to their delicacy. Additionally, they are enriched in amino acids, peptides, proteins and other useful nutrients. Penaeid shrimps have become the economically important species for Thailand and are widely cultured in ponds. Black tiger shrimp (*Penaeus monodon*) and white shrimp (*Litopenaeus vannamei*) are commonly cultured and exported with a catch volume over 1000 tons per year. Thailand exported 249,570 tons of shrimp and products with the value of 2.19 billions US dollars in year 2001 (Suphamongkhon, 2002). By-products from the processing of shrimp may account for up to 80% of the

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original weight of raw material (Shahidi, Synowiecki, & Naczk, 1992). Those by-products consist of 71.4% head and 28.6% shell (Meyers, 1986) and may pose a disposal problem due to the ease of spoilage. Generally, shrimp wastes are processed as animal feed and as protein feedstuff in aquaculture diets (Fagbenro, 1996; Nwanna, Balogun, Ajenifuja, & Enujigha, 2004; Sudaryono, Hoxey, Kailis, & Evans, 1995; Sudaryono, Tsvetnenko, & Evans, 1996). To increase the market value of the discards, many approaches have been used. Protein and amino acids (Mandeville, Yaylayan, & Simpson, 1992), colourant (Chen & Meyers, 1982), flavourant (Pan, 1990) and chitin and chitosan (Coward-kelly, Agbogbo, & Holtzapple, 2006) can be recovered from shrimp head and shell. Enzymatic protein hydrolysates from shrimp waste have also been produced (Ruttanapornvareesakul et al., 2005). Protein hydrolysate or peptides from fish and shellfish, as well as their by-products, have been shown to exhibit antioxidative activity (He, Chen, Sun, Zhang, & Gao, 2006; Mendis, Rajapakse, Byun, & Kim, 2005). Natural antioxidant, mainly phenolic compound, from shrimp shell waste was characterised by Seymour and Li (1996).

Mungoong is a traditional Thai fishery product produced from the cephalothorax of shrimp. The product is manufactured by boiling the raw materials to extract the soluble substances, followed by evaporating to a paste form with approximate 70% dry matter. Some ingredients, including sugar, are added to improve the taste (TCPS 324, 2004). Thus, Mungoong can be a potential source of nutrients, flavourants and natural antioxidants. However, no information regarding antioxidants of a protein concentrate from shrimp waste has been reported. Therefore, the objective of this investigation was to characterise and determine antioxidative activities of Mungoong from the cephalothorax of white shrimp.

2. Materials and methods

2.1. Mungoong sample

Freshly prepared Mungoong samples were purchased from local market in Songkhla, Thailand. The sample was stored at 4 °C until use and the storage time was not greater than 1 month.

2.2. Chemicals

Ethanol and methanol were obtained from Merck (Darmstadt, Germany). 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2-diphenyl-1-picryl hydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2,4,6-Tripyridyl-*s*-triazine (TPTZ), ferric chloride hexahydrate and potassium persulfate were procured from Fluka Chemical Co. (Buchs, Swizerland).

2.3. Proximate analysis and determination of physical properties of Mungoong

Moisture, protein, fat, ash and salt contents of Mungoong were determined according to the method of AOAC (1999). Chitin was determined according to the method of Spinelli, Lehman, and Wieg (1974). Colour was measured by Hunter lab and reported in CIE system. L^*, a^* and b^* parameters indicate lightness, redness/greenness and yellowness/blueness, respectively. Water activity was measured using water activity analyser (Thermoconstanter, Novasina, Swizerland). pH was determined by pH meter CG 842 (Schott, Germany) as described by Benjakul, Seymour, Morrissey, and An (1997).

2.4. Preparation of soluble fractions from Mungoong

Different extracting media including distilled water, distilled water/ethanol mixture (1:1, 1:2 and 2:1) and ethanol were used to extract the soluble substances from Mungoong. Mungoong (1 g) was mixed with extracting medium (100 ml) and the mixture was stirred at room temperature for 30 min. The mixture was then centrifuged at 3000*g* for 10 min at room temperature using a Sovall Model RC-5B Plus refrigerated centrifuge (Newtown, CT, USA) to remove undissolved debris. The supernatant was used for further analyses.

2.5. Analyses

2.5.1. Measurement of UV-absorbance and browning intensity

UV-absorbance and browning intensity of soluble fractions from Mungoong were determined according to the method of Ajandouz, Tchiakpe, Ore, Benajibas, and Puigserver (2001). The formation of Maillard reaction intermediate products was monitored by measuring the absorbance at 280 and 295 nm using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan). The browning intensity was measured as the absorbance at 420 nm. Prior to absorbance measurement, samples were 3-fold diluted with the same solvents used for extraction.

2.5.2. Measurement of fluorescence intensity

Fluorescent intermediate products from the Maillard reaction of soluble fractions from Mungoong, were determined as described by Morales and Jimennez-Perez (2001). The fluorescence intensity was measured at an excitation wavelength of 347 nm and emission wavelength of 415 nm using a RF-1501 Fluorescence spectrophotometer (Shimadzu, Kyoto, Japan).

2.5.3. Antioxidant activity determination

2.5.3.1. DPPH radical scavenging activity. DPPH radical scavenging activity was determined by DPPH assay as described by Wu, Chen, and Shiau (2003) with a slight

modification. Sample (1.5 ml) was added with 1.5 ml of 0.15 mM 2,2-diphenyl-1-picryl hydrazyl (DPPH) in 95% ethanol. The mixture was mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance of the resulting solution was measured at 517 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The blank was prepared in the same manner, except that distilled water was used instead of the sample. A standard curve was prepared using Trolox in the range of 10–60 μM. The activity was expressed as μmol Trolox equivalents (TE)/g Mungoong.

2.5.3.2. ABTS radical scavenging activity. ABTS radical scavenging activity was determined by ABTS assay as per the method of Arnao, Cano, and Acosta (2001) with a slight modification. The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulphate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in dark. The solution was then diluted by mixing 1 ml ABTS solution with 50 ml methanol in order to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). Fresh ABTS solution was prepared for each assay. Sample (150 μl) was mixed with 2850 μl of ABTS solution and the mixture was left at room temperature for 2 h in dark. The absorbance was then measured at 734 nm using the spectrophotometer. A standard curve of Trolox ranging from 50 to 600 μM was prepared. The activity was expressed as μmol Trolox equivalents (TE)/g Mungoong.

2.5.3.3. FRAP (Ferric reducing antioxidant power). FRAP was assayed according to Benzie and Strain (1996). Stock solutions included 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-*s*-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. A working solution was prepared freshly by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ solution and 2.5 ml of $FeCl_3 \cdot 6H_2O$ solution. The mixed solution was incubated at 37 °C for 30 min and was referred to as FRAP solution. A sample (150 μl) was mixed with 2850 μl of FRAP solution and kept for 30 min in dark. The ferrous tripyridyltriazine complex (coloured product) was measured by reading the absorbance at 593 nm. The standard curve was prepared using Trolox ranging from 50 to 600 μM. The activity was expressed as μmol Trolox equivalents (TE)/g Mungoong.

2.6. The effect of the concentration of Mungoong soluble fraction on antioxidative activities and the correlation between antioxidative activities tested by different assays

Selected soluble fraction from Mungoong, rendering the highest antioxidative activity at various concentrations (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mg/ml) was determined for DPPH, ABTS and FRAP assays. The correlations between ABTS and DPPH; DPPH and FRAP; ABTS and FRAP were examined.

2.7. pH and thermal stability

For the pH stability study, the selected soluble fraction (3 mg/ml) was adjusted to different pHs (2, 3, 4, 5, 6, 7, 8, 9, 10 and 11) using HCl and NaOH and incubated at room temperature for 30 min. Thereafter, the pHs of the sample were adjusted to pH 7.0. The residual antioxidative activities were determined by DPPH, ABTS and FRAP assays.

For the thermal stability study, the selected soluble fraction (pH 7.0) was incubated at 30, 40, 50, 60, 70, 80, 90 and 100 °C in the temperature controlled-water bath (Memmert, Schwabach, Germany) for 30 min. The treated samples were suddenly cooled in iced water. The sample without incubation (25 °C) was used as the control. The residual antioxidative activities were determined by DPPH, ABTS, and FRAP assays. The effect of heating times at 100 °C on antioxidative activity of selected soluble fraction from Mungoong was also investigated. Soluble fraction (3 mg/ml) was heated at 100 °C for 30, 60, 90, 120 and 150 min. The treated samples were immediately cooled in iced water after the designated time. The sample without incubation (25 °C) was used as the control. The residual antioxidative activities were measured by DPPH, ABTS, and FRAP assays.

2.8. MALDI-TOF analysis

Mungoong (1 g) was extracted with 100 ml of water and the mixture was stirred at room temperature for 30 min. The mixture was centrifuged at 3000*g* for 10 min at room temperature using a Sorvall Model RC-5B Plus refrigerated centrifuge (Newtown, CT, USA) to remove undissolved debris. The supernatant was filtered through a Minisart RC4 filter (0.45 μm) and analysed by a matrix assisted laser desorption ionisation (MALDI) mass spectrometer equipped with a delayed extraction source and a 337 nm pulsed nitrogen laser. An instrument Bruker Reflex IV MALDI was run in the linear mode using 20 kV acceleration. The 100 times diluted sample was mixed with 1 volume of matrix solutions (either saturated 2,5-dihydroxybenzoic acid (DHBA) matrix solution or 20 mg/ml of sinapinic acid (SA) in acetonitrile/water, 50:50, v/v). Finally, 0.5 μl of the mixture was deposited onto the MALDI target plate. All spectra were the results of signal averaging of 200 shots. The MALDI-TOF MS was run in the positive refractor mode.

2.9. Statistical analysis

All data were subjected to Analysis of Variance (ANOVA) and the differences between means were evaluated by Duncan's Multiple Range Test (Steel & Torrie, 1980). SPSS statistic program (Version 10.0) (SPSS, 1.2, 1998) was used for data analysis.

Table 1
Chemical compositions and some physical properties of Mungoong from the cephalothorax of white shrimp

Compositions/properties	Contents/values
Moisture	31.08 ± 0.07
Protein	24.24 ± 0.49
Lipid	6.83 ± 0.856
Ash	9.18 ± 0.02
Carbohydrate	28.67 ± 0.64
Salt	4.96 ± 0.10
Chitin	0.15 ± 0.01
Aw	0.76 ± 0.00
pH	7.08 ± 0.05
Color	
L^*	9.13 ± 0.22
a^*	7.21 ± 0.25
b^*	11.13 ± 0.34

Means ± SD from triplicate determinations.

3. Results and discussion

3.1. Chemical composition and physical property of Mungoong

Proximate compositions of Mungoong paste are shown in Table 1. Mungoong consisted of 31.08% moisture, 24.24% protein, 9.18% ash and 6.83% fat. It was found that protein and carbohydrate were the major components in Mungoong. Mungoong is generally produced by boiling the cephalothorax in clean water. After the extract was concentrated, water was mostly removed and the protein became concentrated, as indicated by the high protein content in the finished product. The high content of carbohydrate (28.67%) most likely resulted from the addition of sugar to improve the flavour and taste of Mungoong. Mungoong contained 4.96% salt. This might contribute to high ash content (9.18%) found in the sample. Chitin constituted in the sample at very low content (0.15%). Since chitin was not soluble in water, it could be removed during the filtration process of the extract before evaporation.

Mungoong was dark brown in colour as evidenced by low L^*-value (9.13). The brown colour of Mungoong might be developed via the reaction between sugar and amino acid known as the Maillard reaction, which takes place in thermally processed food (Carabasa-Giribet & Ibarz-Ribas, 2000). Mungoong had a^*-value of 7.21 and b^*-value of 11.13. Water activity (A_w) of Mungoong was 0.76. Normally, the browning rate is usually maximum in intermediate moisture foods in which A_w is about 0.7 (Fennema, 1996). Mungoong had neutral pH (7.08).

3.2. Effect of extracting media on characteristics and antioxidative activity of Mungoong

3.2.1. UV-absorbance and browning intensity

UV-absorbance of different soluble fractions of Mungoong obtained using various extracting media, is shown in Table 2. A_{280} and A_{295} have been used to monitor the formation of non-fluorescent intermediate products of the Maillard reaction (Farombi, Britton, & Emerole, 2000; Matmaroh, Benjakul, & Tanaka, 2006). Among all the fractions tested, the fraction obtained using distilled water as extracting medium, showed higher UV-absorbance than others ($p < 0.05$). The ethanol fraction had the lowest A_{280} and A_{295} ($p < 0.05$). The results suggested that different intermediate products with different absorbance maxima were extracted by different extracting media. Water showed the greater ability in extracting the non-fluorescent intermediate from Mungoong, possibly due to the similar polarity. The increase in ethanol portion of extracting media led to the lower A_{280} and A_{295}. Therefore, non-fluorescent intermediates were more likely to be polar and water-soluble.

The browning intensity of different soluble fractions extracted from Mungoong as monitored by A_{420} is shown in Table 2. The greatest browning intensity was observed in the fraction extracted with distilled water, followed by the ethanol fraction. The lower A_{420} was noticeable in fractions obtained using water/ethanol mixture. From the result, the relationship between UV-absorbance and browning intensity (A_{420}) of the water soluble fraction suggested that a large proportion of the non-fluorescent intermediate product was converted into a brown polymer (Ajandouz et al., 2001). However, it was noted that the hydrophobicity and hydrophilicity of brown products were possibly different from those of non-fluorescent intermediates to some extent.

3.2.2. Fluorescence intensity

Fluorescence intensity of various soluble fractions from Mungoong extracted using different extracting media is present in Table 2. Among all fractions, the water soluble fraction had the highest fluorescence intensity ($p < 0.05$), compared with others fractions. The results of fluorescence

Table 2
A_{280}, A_{295}, browning intensity (A_{420}) and fluorescence intensity of different soluble fractions of Mungoong produced from the cephalothorax of white shrimp

Extracting media	A_{280}*	A_{295}*	Browning intensity (A_{420})	Fluorescence intensity
H_2O	2.62 ± 0.01[a†,‡]	1.53 ± 0.01[a]	0.31 ± 0.01[a]	546.15 ± 2.09[a]
H_2O:EtOH (1:1)	1.90 ± 0.00[b]	0.96 ± 0.00[c]	0.08 ± 0.00[d]	316.13 ± 0.51[c]
H_2O:EtOH (1:2)	1.58 ± 0.00[c]	0.71 ± 0.00[d]	0.05 ± 0.00[e]	274.84 ± 1.15[d]
H_2O:EtOH (2:1)	1.89 ± 0.01[b]	1.07 ± 0.00[b]	0.09 ± 0.00[c]	384.92 ± 2.58[b]
EtOH	1.24 ± 0.00[d]	0.40 ± 0.00[e]	0.16 ± 0.00[b]	111.98 ± 0.38[e]

* The sample was 3-fold diluted prior to measurement.
† Means ± SD from triplicate determinations.
‡ Different superscripts in the same column indicate the significant difference ($p < 0.05$).

intensity were in accordance with those of A_{280} and A_{295}. Those fluorescent intermediates were preferably extracted by water. The increase in the ethanol proportion in the extracting media resulted in lowered extractability of fluorescent intermediate from the Maillard reaction. Both non-fluorescent and fluorescent intermediates are formed and turn into brown pigments in the Maillard reaction (Morales, Romeo, & Jimenez-Perez, 1996).

3.2.3. DPPH radical scavenging activity

DPPH radical scavenging activity of different soluble fractions of Mungoong using different extracting media is shown in Table 3. The water fraction exhibited the greatest activity, while the ethanol fraction had the lowest activity. The decrease in polarity of extracting media caused the decrease in DPPH radical scavenging activity of resulting fractions. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability (Siddhuraju & Becker, 2007). From the results, antioxidants may be water soluble peptides or other antioxidative compounds including MRPs. Peptides have been reported to have antioxidative activity (Je, Park, & Kim, 2005; Rajapakse, Mendis, Jung, Je, & Kim, 2005; Wu et al., 2003). Additionally, MRPs also exhibited their antioxidative activity via radical scavenging activity, as well as reducing power (Benjakul, Lertittikul, & Bauer, 2005). Those peptides or MRPs were mostly hydrophilic in nature. As a result, they were extracted into water effectively. Apart from peptides and MRPs, the phenolic compounds from shrimp shell were found to exhibit the antioxidative activity (Seymour & Li, 1996).

3.2.4. ABTS radical scavenging activity

Various fractions obtained by using different extracting media showed different ABTS radical scavenging capacity (Table 3). The water fraction exhibited the highest activity, whereas the lowest activity was found in the ethanol extract. Fractions obtained from media with different water/ethanol ratios differed in their ABTS radical scavenging activities (Table 3), indicating that the extracting media significantly influenced the antioxidant activity of the resulting fractions. Similar results of ABTS radical scavenging activity of fractions were generally observed, compared with those of DPPH radical scavenging activity. Nevertheless, slight differences in activities determined by both assays were observed among fractions obtained using media with different water/ethanol ratios. The result suggested that those fractions might scavenge two different radicals, ABTS$^{\cdot}$ and DPPH, differently. From the results, it was postulated that antioxidative compounds were most likely hydrophilic and could be extractable by water. ABTS$^{\cdot}$ assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants (scavengers of aqueous phase radicals) and of chain breaking antioxidants (scavenger of lipid peroxyl radicals) (Leong & Shui, 2002). Therefore, the water soluble fraction from Mungoong was able to scavenge free radicals, thereby preventing lipid oxidation via a chain breaking reaction.

3.2.5. Ferric reducing antioxidant power (FRAP)

Antioxidant potential of different fractions from Mungoong was estimated from their ability to reduce TPTZ–Fe (III) complex to TPTZ–Fe (II) complex (Siddhuraju & Becker, 2007). The ferric reducing antioxidant power, generally measures the antioxidant effect of any substance in the reaction medium as its reducing ability. Among all the fractions, the water fraction from Mungoong had the highest ferric reducing antioxidant power and the ethanol fraction showed the lowest FRAP ($p < 0.05$) (Table 3). This result was in agreement with DPPH and ABTS radical scavenging activities. However, FRAP of fraction obtained using different water/ethanol ratios was found to differ slightly from those found from DPPH and ABTS assays. Thus, media with different polarity might contribute to the extraction of different antioxidative compounds with a varying mode of functions. From this result, the water fraction showed the pronounced effect in donating electrons, in which propagation of lipid oxidation could be retarded. From the results, antioxidant activities of all fractions were in accordance with UV-absorbance, fluorescence intensity as well as browning intensity (Table 2). Therefore, it was most likely that MRP intermediates and final products might partially contribute to the antioxidative activity. Since the water soluble fraction had the highest antioxidant activity, it was used for further study.

3.3. Effect of concentrations of the water soluble fraction from Mungoong on antioxidative activity and the correlation among different assays tested

DPPH, ABTS radical scavenging activities and FRAP of the water soluble fraction from Mungoong, increased

Table 3
Antioxidative activities of soluble fractions of Mungoong produced from the cephalothorax of white shrimp determined by different assays

Extracting media	ABTS (μmol TE/g sample)	FRAP (μmol TE/g sample)	DPPH (μmol TE/g sample)
H_2O	$75.33 \pm 0.12^{a\dagger,\ddagger}$	13.51 ± 0.26^{a}	8.65 ± 0.03^{a}
H_2O:EtOH (1:1)	68.07 ± 0.77^{b}	8.90 ± 0.06^{d}	7.14 ± 0.55^{bc}
H_2O:EtOH (1:2)	59.80 ± 1.33^{d}	10.31 ± 0.35^{c}	6.86 ± 0.04^{c}
H_2O:EtOH (2:1)	65.33 ± 0.62^{c}	11.30 ± 0.15^{b}	7.42 ± 0.03^{b}
EtOH	37.61 ± 0.06^{e}	7.13 ± 0.12^{e}	3.78 ± 0.02^{d}

TE: Trolox equivalent.
† Means ± SD from triplicate determinations.
‡ Different superscripts in the same column indicate the significant difference ($p < 0.05$).

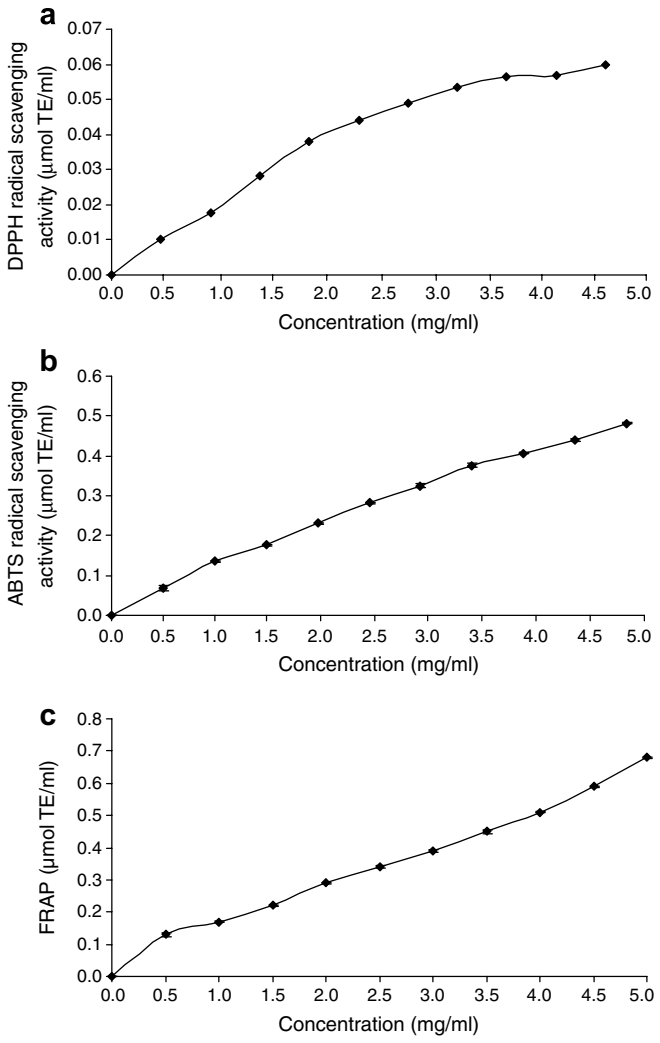

Fig. 1. Antioxidative activities of water soluble fraction from Mungoong produced from the cephalothorax of white shrimp at different concentrations as determined by the ABTS radical scavenging activity (a) DPPH radical scavenging activity (b) and FRAP (c). Bars represent the standard deviation from triplicate determinations.

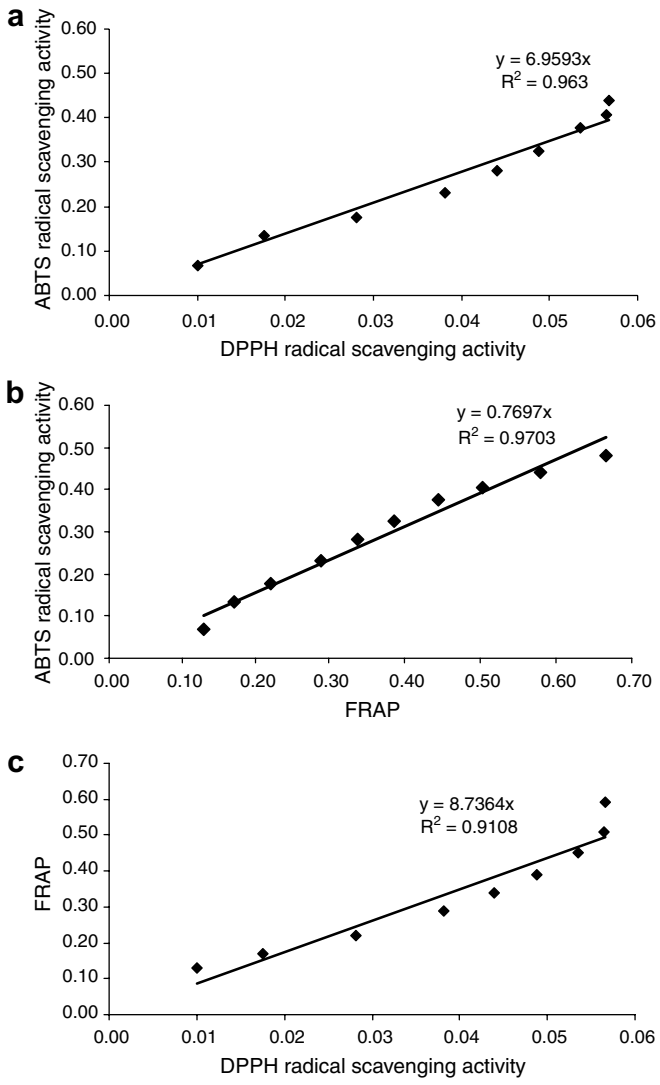

Fig. 2. Correlation between ABTS and DPPH radical scavenging activity (a), correlation between FRAP and ABTS radical scavenging activity (b), and correlation between FRAP and DPPH radical scavenging activity (c) of the water soluble fraction from Mungoong produced from the cephalothorax of white shrimp. Bars represent the standard deviation from triplicate determinations.

as the concentrations increased up to 5 mg/ml (Fig. 1). Therefore, antioxidative activity of the water soluble fraction was in the concentration-dependent manner. The results suggested that antioxidative compounds in the fraction tested were capable of radical scavenging with reducing power to a greater extent when higher concentrations were used. This result was in accordance with Jao and Ko (2002) who reported that the DPPH radical scavenging activity of protein hydrolysates from tuna cooking juice, increased when the concentration increased from 17% to 75%. Protein hydrolysates from aquatic species contain both antioxidative and prooxidative components, and their final effect depends on their concentration (Pokorny & Korczak, 2001). From the results, the water soluble fraction from Mungoong showed the antioxidative effect in the concentration ranges used in the study.

The good correlations between antioxidative activities determined by different assays and reported as Trolox equivalent antioxidant capacity (TEAC) were observed (Fig. 2). ABTS and DPPH radical scavenging activities correlated very well ($R^2 = 0.9630$). Both radical scavenging activities showed good correlation with FRAP. Their linear correlation could be described as: $TEAC_{ABTS} = 6.9593TEAC_{DPPH}$ ($R^2 = 0.9630$), $TEAC_{ABTS} = 0.7697TEAC_{FRAP}$ ($R^2 = 0.9703$) and $TEAC_{FRAP} = 8.7364TEAC_{DPPH}$ ($R^2 = 0.9108$). Leong and Shui (2002) studied the antioxidant capacity of a group of fruits by DPPH and ABTS radical scavenging assays and expressed as L-ascorbic acid equivalent antioxidant capacity (AEAC). A good correlation existed between two assays: $AEAC_{DPPH} = 0.9203AEAC_{ABTS}$ ($R^2 = 0.9045$). Similarly, high correlation was reported between FRAP and DPPH

assays in guava fruit extracts (Jimenez-Escrig, Rincon, Pulido, & Saura-Calixto, 2001).

3.4. pH and thermal stability of water soluble fraction from Mungoong

The influences of pH on the antioxidant stability of the water soluble fraction from Mungoong are depicted in Fig. 3. DPPH and ABTS radical scavenging activities and FRAP of the water soluble fraction remained constant when subjected to the pH range of 2–8. At pH above 8, DPPH radical scavenging activity and FRAP slightly decreased. Conversely, the increase in ABTS radical scavenging activity was noticeable. At alkaline pH, antioxidative compounds exhibiting ABTS radical scavenging activity might be activated, while those with DPPH radical scavenging activity and FRAP lost their activity to some extent. Thus, alkaline pHs mostly affected antioxidative activities of the water soluble fraction.

The thermal stability of antioxidant activity of the water soluble fraction from Mungoong as monitored by DPPH and ABTS radical scavenging activities and FRAP is shown in Fig. 4a. From the results, the antioxidant activities of the water soluble fraction were stable when heated up to 100 °C, where activities of more than 80% remained. Some losses in ABTS radical activity was found after the water soluble fraction was incubated at 30 °C for 30 min. Thereafter, no further changes in activity were observed with increasing temperature up to 100 °C. This result reconfirmed that the fraction contained different antioxidative compounds with different thermal stability. Fig. 4b shows thermal stability of the water soluble fraction from Mungoong heated at 100 °C for various times. From the result, the antioxidant activity slightly decreased after being heated for 30 min and the activity remained after heating for up to 120 min, in which 80% activity was still retained. After 120 min of heating, the DPPH radical scavenging activity decreased to some extent. The denaturation of antioxidative compounds might be enhanced when heated for a longer time. Accumulated energy or enthalpy

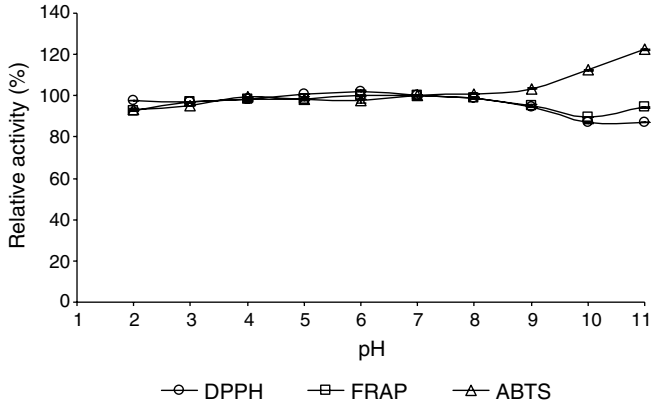

Fig. 3. pH stability of the water soluble fraction from Mungoong produced from the cephalothorax of white shrimp. Bars represent the standard deviation from triplicate determinations.

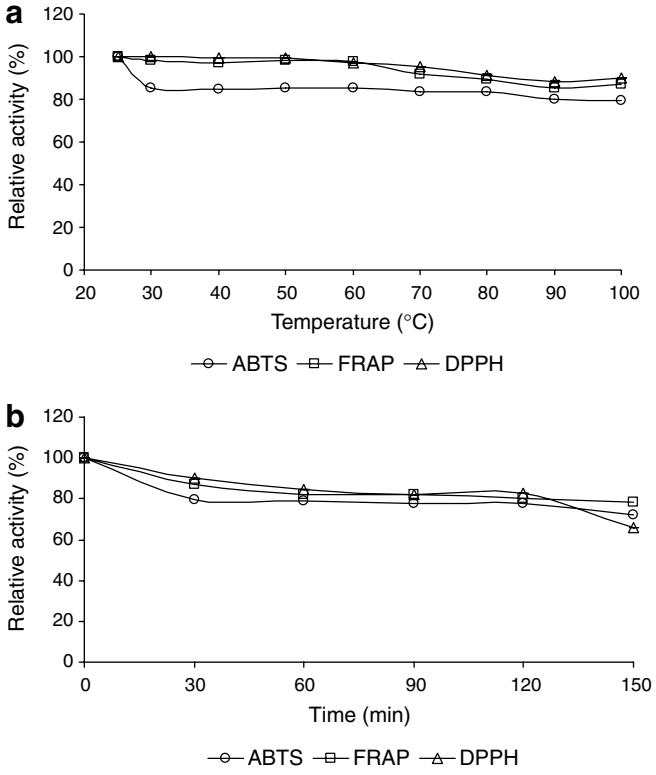

Fig. 4. Thermal stability of the water soluble fraction from Mungoong produced from the cephalothorax of white shrimp subjected to heating at various temperatures (a) or subjected to heating at 100 °C for various times (b). Bars represent the standard deviation from triplicate determinations.

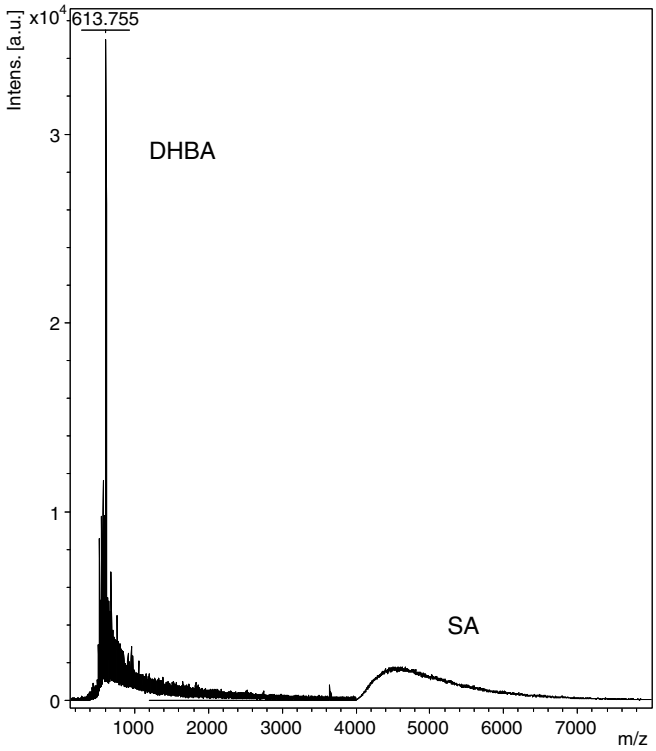

Fig. 5. Representative mass spectra of the water soluble fraction of Mungoong measured by MALDI-TOF MS.

might be sufficient for antioxidative compounds to undergo denaturation and loss of their activities (Pokorny & Schmidt, 2001).

3.5. MALDI-TOF analysis

The typical spectra obtained for the water soluble extract of Mungoong with two different matrices are presented in Fig. 5. DHBA was a relatively efficient matrix for the ionization and desorption of lower molecular mass peptides. Many intense signals were found within the mass range of *m*/*z* 400–1000. Higher molecular mass peptides were only detected when SA was used as the matrix. The molecular masses of these peptides were in the mass range of *m*/*z* 4000–7000. From the results, it is evident that the water soluble extract of Mungoong contained mainly peptides and water soluble proteins.

4. Conclusion

Mungoong, shrimp extract paste, was an important source of antioxidants. The water soluble fraction from Mungoong exhibited antioxidant activity as evidenced by its high DPPH and ABTS radical scavenging activities and FRAP. Antioxidative activities of the water soluble fraction were dependent on concentrations. Generally, antioxidant activity of the water soluble fraction containing the peptides with the mass range of *m*/*z* 400–1000 and 4000–7000 was stable over a wide pH and temperature range. It could be a promising novel natural antioxidant, especially for neutraceutical purposes.

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