CHAPTER 3

RESULTS AND DISCUSSION

1. Composition and some property of Mungoong

1.1 Chemical compositions and physical property

Proximate compositions of Mungoong, an extract paste from the cephalothorax of white shrimp (*Litopenaeus vannamei*), are shown in Table 2. 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. During shrimp cephalothorax was boiled, some proteins were extracted. After concentrating, water was mostly removed and 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 flavor 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 color as evidenced by low L*- value (9.13). Brown color of Mungoong might be developed via the reaction between sugar and amino acid known as Maillard reaction, which takes place in thermally processed food (Carabasa-Giribet and Ibarz-Ribas, 2000). Mungoong had the 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 food in which A_w is about 0.7 (Fennema, 1996). Mungoong had the neutral pH (7.08).

Table 2 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	28.52 ± 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

† expressed as % (wet basis)

Means ± SD from triplicate determinations.

1.2 Fatty acid profile

The fatty acid profile of Mungoong is shown in Table 3. Mungoong contained 42.3% polyunsaturated and 29.59% saturated fatty acids. Among all fatty acids present, oleic acid (C18: 1 n-9) and linoleic acid (C18: 2 n-6) were predominant. Eicosapentaenoic acid (C20: 5 n-3 (EPA)) and docosahexaenoic acid (C22: 6 n-3 (DHA)) were dominant PUFAs (polyunsaturated fatty acids) in Mungoong lipids, constituting 4.31 and 7.07 g/100g lipids, respectively. For saturated fatty acids, palmitic acid (C16: 0) was most abundant in the lipids extracted from Mungoong produced from the cephalothorax of white shrimp. This is in accordance with the results of Sriket *et al*., (2006) who reported that palmitic acid and stearic acid, EPA and DHA

were the most abundant fatty acids in the lipid extracted from white shrimp meat. Yanar and Celik (2005) reported that palmitic acid, stearic acid, DHA and EPA were found in shrimps (*Nephrops morvegicus, Parapenaeus longirostris, Aristeus antennatus and Penaeus semisulcatus, Metapenaeus monoceros*) in large quantities. From the result, lipids in Mungoong contained a high proportion of PUFAs, which are prone to oxidation. Therefore, Mungoong might be susceptible to rancidity during storage.

1.3 Mineral content of Mungoong

Types and contents of different minerals in Mungoong are shown in Table 4. Among all minerals determined, Na and Ca were the major minerals in Mungoong. P, K, and Mg were also found in high quantities. The prooxidant Fe and Cu were found in very low amounts. Nevertheless, Ni, Cd and Pb, the toxic minerals for consumption (Ichihashi *et al*., 2001), were not detectable. The high content of Na (15.3 g/kg) reflected the addition of salt to improve the taste and flavor of Mungoong. Ca is a major component of shrimp carapace, and it might be leached out during boiling process for Mungoong preparation. The shrimp solid waste contains about 30-35% tissue protein with calcium carbonate and chitin (Gildberg and Stenberg, 2001). Sathivel *et al.* (2003) reported that K, Mg, P, Na, S and Ca were abundant in herring and herring by-product hydrolysates. Cu^{2+} is found in hemocyanin, a pigment in the blood of crustaceans (Decker and Tuczek, 2000). Transition metal ions, particularly Cu and Fe, have been known as the major catalysts for oxidation (Thanonkeaw *et al*., 2006)**.** Due to high content of PUFAs in Mungoong (Table 3), the oxidation might take place via the induction of prooxidants, even at very low concentrations (Table 4).

Table 3 Fatty acid composition (g/100g) of Mungoong from the cephalothorax of white shrimp.

Fatty acids	contents(g/100g)
Myristic acid C14:0	0.88
Myristoleic acid C14:1	0.14
Pentadecanoic acid C15:0	0.53
Palmitic acid C16:0	21.03
Palmitoleic acid C16:1 n-7	2.45
Heptadecanoic acid C17:0	0.73
Cis-10-Heptadecanoic acid C17:1	0.35
Stearic acid C18:0	5.04
Oleic acid C18:1 n-9	21.89
Linoleic acid C18:2 n-6	22.32
a-Linoleic acid C18:3 n-3	1.46
g-Linoleic acid C18:3 n-6	0.21
Moroctic acid C18:4 n-3	0.09
Arachidic acid C20:0	0.21
Cis-11-Ecosenoic acid C20:1 n-9	1.90
Cis-11,14-Eicosadienoic acid C20:2 n-6	1.85
Cis-8,11,14-Eicosatrienoic acid C20:3 n-6	0.09
Cis-11,14,17-Eicosatrienoic acid C20:3 n-3	0.27
Arachidonic acid C20:4 n-6	1.89
Eicosatetraenoic acid C20:4 n-3	0.18
Cis-5,8,11,14,17-Eicosapentaenoic acid C20:5 n-3(EPA)	4.31
Behenic acid C22:0	0.20
Erucic acid C22:1 n-9	0.15
Cetoleic acid C22:1 n-11,n-13	0.51
Docosatetraenoic acid C22:4 n-6	0.20
Docosapentaenoic acid C22:5 n-3	0.58
Docosapentaenoic acid C22:5 n-6	0.34
Cis-4,7,10,13,16,19-Docosahexaenoic acid C22:6 n-3 (DHA)	7.07
Lignoceric acid C24:0	0.12
Nervonic acid C24:1	0.15
Unidentified peak	2.42
Polyunsaturated fatty acid (PUFA)	40.86
n3 PUFA	13.96
n6 PUFA	26.90
Saturated fatty acid (SFA)	28.74

Table 4 Mineral contents of Mungoong from the cephalothorax of white shrimp.

Minerals	Content (g/kg)
P	2.66 ± .005
K	2.09 ± 0.01
Ca	8.07 ± 0.007
Mg	1.00 ± 0.007
Fe	0.07 ± 0.06
Mn	0.002 ± 0.002
Zn	0.02 ± 0.04
Cu	0.01 ± 0.01
Na	15.30 ± 0.02
Ni	ND
Cd	ND
Pb	ND

Average ± SD from triplicate determinations.

ND is not detectable or below detection limit.

1.4 Amino acid compositions

The amino acid compositions of Mungoong are presented in Table 5. Glutamine was found as the most abundant amino acid in Mungoong (2,706 mg/100g). Mungoong also contained high contents of asparagine, alanine and lysine, accounting for 1,406, 1,189 and 1,299 mg/ 100 g sample, respectively. Glutamine and asparagine contribute to the sweet taste (Sikorski, 1990). Hydroxyproline was found in Mungoong, suggesting the extraction of collagen/gelatin during Mungoong preparation from shrimp cephalothorax. Hydroxyproline is the unique amino acid in collagen and its derivatives (Johnston-Banks, 1990). Generally, the shrimp waste hydrolysate has a high content of essential amino acids indicating a high nutritional value (Gildberg and Stenberg, 2001).

Table 5 Amino acid compositions of Mungoong from the cephalothorax of white shrimp.

Amino acids	Content (mg/ 100g sample)
Essential	
Threonine	468.7
Valine	719.8
Methionine	273.8
Isoleucine	603.6
Leucine	849.4
Phenylalanine	618.6
Histidine	349.9
Lysine	1299.8
Tryptophan	ND
Non-essential	
Asparagine	1406.6
Serine	511.5
Glutamine	2706
Glycine	784.6
Alanine	1189.3
Cysteine	26.7
Tyrosine	454.4
Arginine	869.4
Hydroxyproline	29.5
Proline	510.7
Total	13672.3

ND: not detectable.

Mungoong comprised essential amino acids and non essential amino acids at levels of 37.91% and 62.09%, respectively. Certain amino acids are known to exhibit antioxidant activity, which is greater when they are incorporated into dipeptides (Yamaguchi *et al*., 1975). Additionally, peptides derived from many protein sources with increased hydrophobicity have been reported to relate with antioxidative properties (Chen *et al*., 1995). Therefore, hydrophobic amino acids such as leucine, valine and alanine presented in Mungoong might contribute to antioxidative activities. Generally, aromatic amino acids are considered as effective radical scavenger, because they can donate protons easily to electron deficient radicals. At the same time, their antioxidative activity can remain via resonance structures (Rajapakse *et al*., 2005).

2. Effect of extracting media on characteristics and antioxidative activity of soluble fraction from Mungoong

2.1 UV-absorbance and browning intensity

UV-absorbance of different soluble fractions of Mungoong obtained using various extracting media is shown in Table 6. A_{280} and A_{295} have been used to monitor the formation of non-fluorescent intermediate products of Maillard reaction (Farombi *et al*., 2000; Matmaroh *et al*., 2006). Among all fractions tested, the fraction obtained using distilled water as extracting medium showed higher UV-absorbance than others ($p<0.05$). 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 polar and water-soluble.

Browning intensity of different soluble fractions extracted from Mungoong produced from the cephalothorax of white shrimp as monitored by A_{420} is shown in Table 6. The greatest browning intensity was observed in the fraction extracted with distilled water, followed by 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 water soluble fraction suggested that a large proportion of the non-fluorescent intermediate product was converted to 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.

Table 6 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	$2.62 \pm 0.01^{a\dagger\ddagger}$	1.53 ± 0.01^{a}	0.31 ± 0.01^{a}	546.15 ± 2.09^{a}
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}
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}
EtOH	1.24 ± 0.00^{d}	0.40 ± 0.00^{e}	0.16 ± 0.00^{b}	111.98 ± 0.38^{e}

† Means ± SD from triplicate determinations.

‡ Different superscripts in the same column indicate the significant difference ($p < 0.05$)

* The sample was 3-fold diluted prior to measurement.

2.2 Fluorescence intensity

Fluorescence intensity of various soluble fractions from Mungoong extracted using different extracting media is present in Table 6. Among all fractions, water soluble fraction had the highest fluorescence intensity ($p<0.05$), compared with others fractions. The results of fluorescence intensity were in accordance with those of A_{280} and $A_{295.}$ Those fluorescent intermediates were preferably extracted by water. The increase in ethanol proportion in extracting media resulted in the lowered extractability of fluorescent intermediate from Maillard reaction. Both non-fluorescent and fluorescent intermediates are formed and turn to be brown pigments in Maillard reaction (Morales *et al*., 1996).

2.3 DPPH radical scavenging activity

DPPH radical scavenging activity of different soluble fractions of Mungoong using different extracting media is shown in Table 7. Water fraction exhibited the greatest activity, while ethanol fraction had the lowest activity ($p<0.05$). 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 and Becker, 2007). From the result, antioxidant might be water soluble peptides or other antioxidative compounds including MRPs. Peptide have been reported to had the antioxidative activity (Rajapakse *et al*., 2005; Je *et al*., 2005; Wu, *et al*., 2003). Additionally, MRPs also exhibited their antioxidative activity via radical scavenging activity as well as reducing power (Benjakul *et al*., 2005). Those peptides or MRPs were mostly hydrophilic in nature. As a result, they were extracted into water effectively. Apart from peptides and MRPs, phenolic compounds from shrimp shell were found to exhibit the antioxidative activity (Seymour *et al*., 1996).

2.4 ABTS radical scavenging activity

Various fractions obtained by using different extracting media showed different ABTS radical scavenging capacity (Table 7). Water fraction also exhibited the highest activity, whereas the lowest activity was found in ethanol extract. Fractions obtained from media with different water/ethanol ratios differed in their ABTS radical scavenging activities (Tables 7), indicating that extracting media significantly influenced the antioxidant activity of 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, $ABTS^{\bullet}$ and DPPH, differently. From the result, it was postulated that antioxidative compounds were most likely hydrophilic and could be extractable by water. $ABTS^{\bullet}$ 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 and Shui, 2002). Therefore, water soluble fraction from Mungoong was able to scavenge free radicals, thereby preventing lipid oxidation via chain breaking reaction.

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 and Becker, 2007). The ferric reducing antioxidant power generally measures the antioxidant effect of

any substance in the reaction medium as reducing ability. Among all fractions, water fraction from Mungoong had the highest ferric reducing antioxidant power (FRAP) and ethanol fraction showed the lowest FRAP ($p<0.05$) (Tables 7). 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 varying mode of functions. From the result, water fraction showed the pronounced effect in donating electron, in which propagation of lipid oxidation could be retarded. From the result, antioxidant activities of all fractions were in accordance with UV-absorbance, fluorescence intensity as well as browning intensity (Table 6). Therefore, it was most likely that MRPs intermediates and final products might partially contribute to antioxidative activity. Since water soluble fraction had the highest antioxidant activity, it was used for further study.

Table 7 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 ± 0.12[a†‡]	13.51 ± 0.26[a]	8.65 ± 0.03[a]
H_2O : EtOH (2:1)	65.33 ± 0.62[c]	11.30 ± 0.15[b]	7.42 ± 0.03[b]
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]
EtOH	37.61 ± 0.06[e]	7.13 ± 0.12[e]	3.78 ± 0.02[d]

† Means ± SD from triplicate determinations.

‡ Different superscripts in the same column indicate the significant difference ($p<0.05$)

TE: Trolox equivalent

3. Effect of concentrations of water soluble fraction from Mungoong on antioxidative activity and the correlation between antioxidant activities tested by different assays

DPPH, ABTS radical scavenging activities and FRAP of water soluble fraction from Mungoong produced from the cephalothorax of white shrimp increased as the concentrations increased up to 5 mg/ml (Figure 5). Therefore, antioxidative activity of 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. The result was in accordance with Jao and Ko (2002) who reported that 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 (Pokorney and Korczak, 2001). From the result, water soluble fraction from Mungoong showed the antioxidative effect in the concentration ranges used in the study.

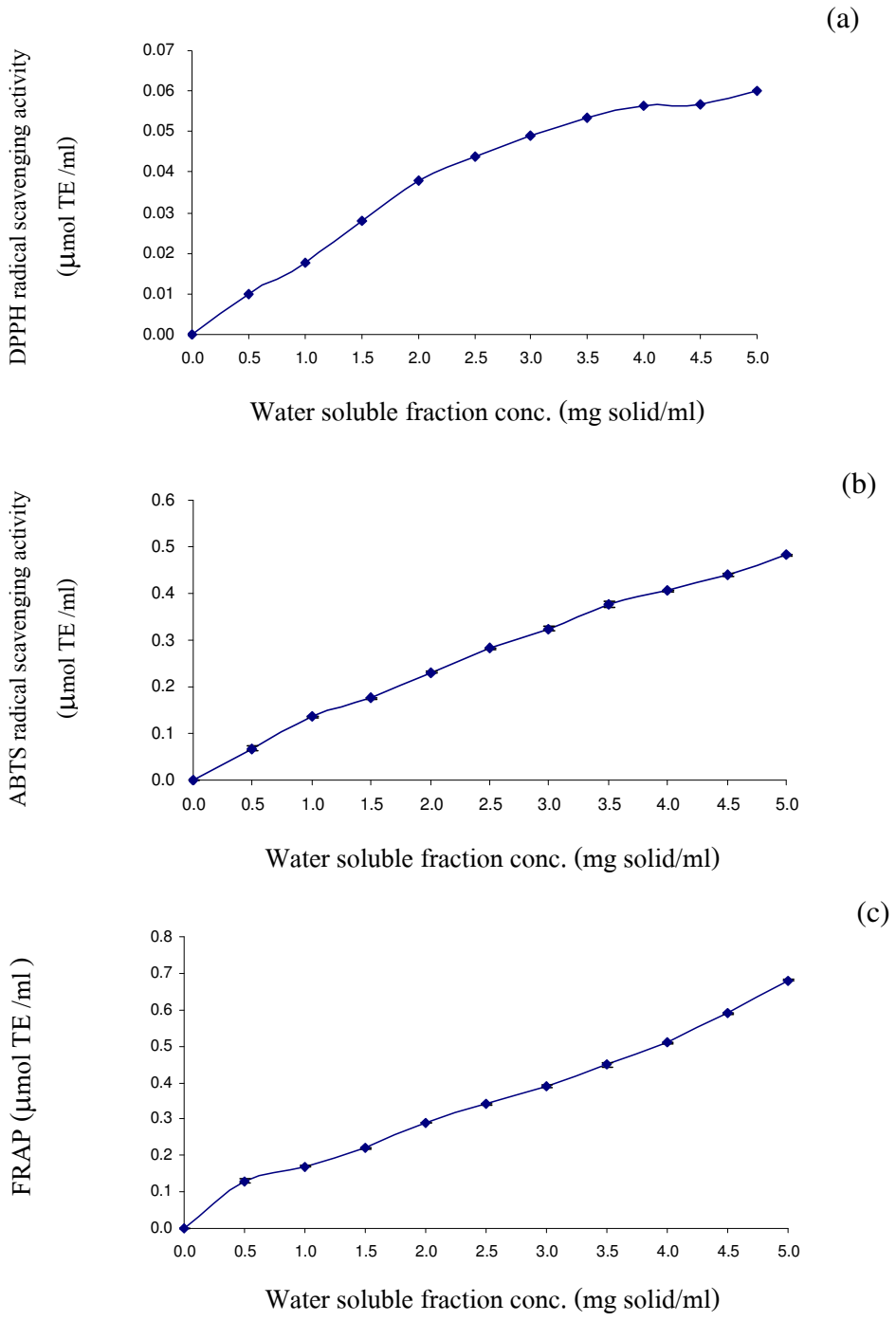

Figure 5 Antioxidative activities of water soluble fraction from Mungoong produced from the cephalothorax of white shrimp at different concentration as determined by the DPPH radical scavenging activity (a) ABTS radical scavenging activity (b) and FRAP (c). Bars represent the standard deviation from triplicate determinations.

The good correlations between antioxidative activities determined by different assays and reported as Trolox equivalent antioxidant capacity (TEAC) were observed (Figure 6). ABTS and DPPH radical scavenging activities correlated very well (R^2 = 0.9630). Both radical scavenging activities showed the good correlation with FRAP. Their linear correlation could be described as: $TEAC_{ABTS}$ = 6.9593$TEAC_{DPPH}$ (R^2 = 0.9630), $TEAC_{ABTS}$ = 0.7697 $TEAC_{FRAP}$ (R^2 = 0.9703) and $TEAC_{FRAP}$ = 8.7364 $TEAC_{DPPH}$ (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: $AEAC_{DPPH}$ = 0.9203 $AEAC_{ABTS}$ (R^2 = 0.9045). Similarly, high correlation was reported between FRAP and DPPH assays in guava fruit extracts (Jimenez-Escrig *et al*., 2001).

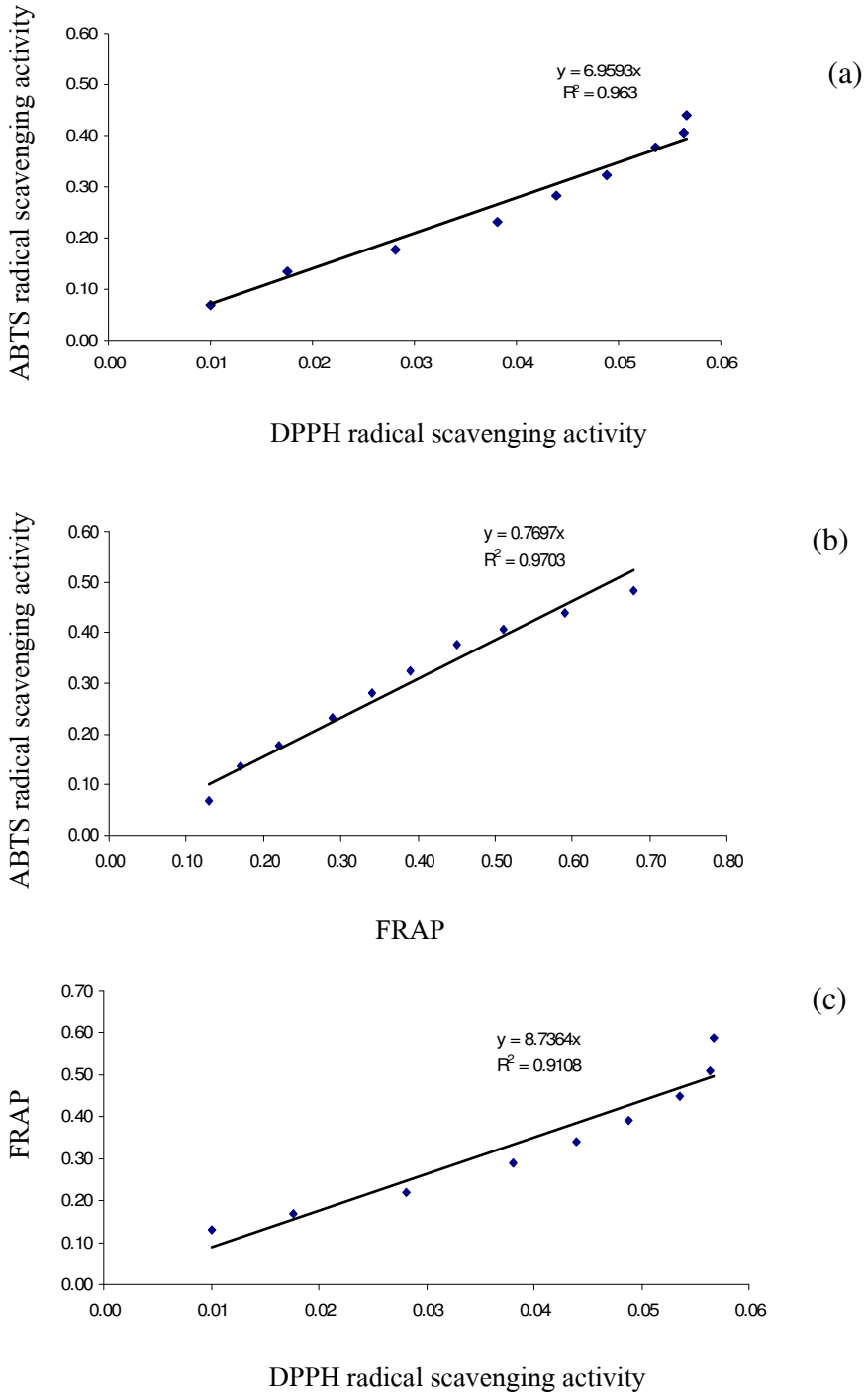

Figure 6 Correlation between ABTS and DPPH radical scavenging activity (a), correlation between FRAP and ABTS radical scavenging activity (b), and correlation between FRAP and DPPH radical scavenging activity (c) of water soluble fraction from Mungoong produced from the cephalothorax of white shrimp. Bars represent the standard deviation from triplicate determinations.

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

The influences of pHs on the antioxidant stability of water soluble fraction from Mungoong produced from the cephalothorax of white shrimp are depicted in Figure 7. DPPH and ABTS radical scavenging activities and FRAP of water soluble fraction remained constant when subjected to pH ranges 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. Additionally, active antioxidative peptides might become negatively charged at alkaline pH. As a result, ABTS radical was possibly quenched with those peptides via ionic interaction. Thus, alkaline pHs mostly affected antioxidative activities of water soluble fraction.

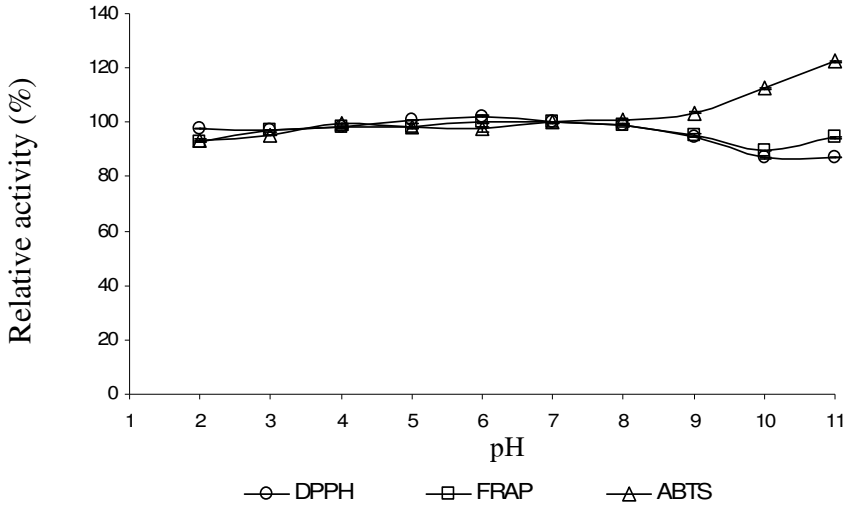

Figure 7 pH stability of water soluble fraction from Mungoong produced from the cephalothorax of white shrimp. Bars represent the standard deviation from triplicate determinations.

Thermal stability of antioxidant activity of water soluble fraction from Mungoong as monitored by DPPH and ABTS radical scavenging activities and FRAP is shown in Figure 8a. From the result, the antioxidant activities of water soluble fraction were stable when heated up to 100°C, where activities of more than 80% were remained. Some losses in ABTS radical scavenging activity was found after 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. Figure 8b. shows thermal stability of water soluble fraction from Mungoong heated at 100 °C for various times. From the result, the antioxidant activity slightly decreased after heated for 30 min and the activity was remained after heating for up to 120 min, in which 80% activity still retained. After 120 min of heating, DPPH radical scavenging activity was decreased to some extent. The denaturation of antioxidative compounds might be enhanced when heated for a longer time. Accumulated energy or enthalpy might be sufficient for antioxidative compounds to undergo denaturation and loss their activities (Pokorny and Schmidt, 2001).

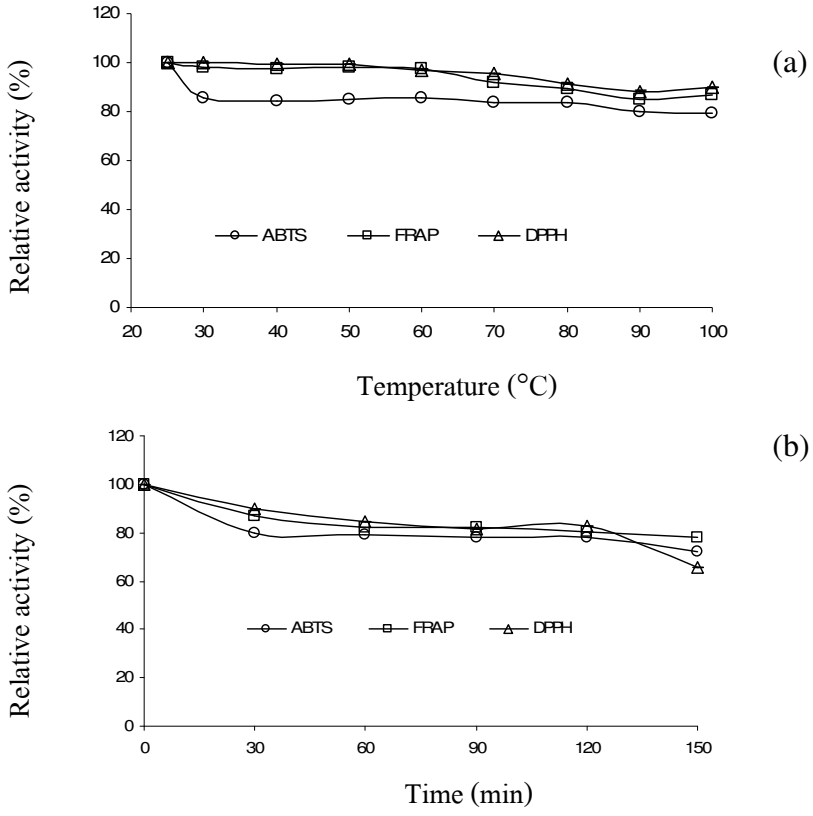

Figure 8 Thermal stability of water soluble fraction from Mungoong produced from white shrimp cephalothorax subjected to heating at various temperatures (a) or subjected to heating at 100°C for various times (b). Bars represent the standard deviation from triplicate determinations.

5. Antioxidative activity and oxidative stability of Mungoong during storage

5.1 Antioxidative activities

Antioxidative activity of Mungoong determined by different assays during the storage at room temperature (28-30°C) and 4°C for 8 weeks is depicted in Figure 9. DPPH and ABTS radical scavenging activities and ferric reducing antioxidant power (FRAP) assays have been widely used for determination of antioxidative activity (Thaipong *et al*., 2006; Siddhuraju and Becker, 2007). The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability (Siddhuraju and Becker, 2007). ABTS 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 and Shui, 2002). FRAP generally measures the antioxidant effect of any substance in the reaction medium as reducing ability. Mungoong water extract showed antioxidative activity determined by ABTS, DPPH radical scavenging activities and FRAP of 100.96, 4.5 and 16.41 μmol Trolox equivalent/g sample, respectively. Antioxidative activity might be attributed to the peptides (Mendis *et al*., 2005; He *et al*., 2006) or Maillard reaction products (Benjakul *et al*., 2005). During the weeks storage of 8 weeks, antioxidative activities determined by all assays remained constant within the first week under both storage conditions ($p>0.05$). Subsequently, slight decreases were noticeable ($p<0.05$). For ABTS assay, no marked changes were observed after 2 weeks of storage ($p>0.05$). The decrease in antioxidative activities of Mungoong water extract might be due to the destruction of antioxidative compounds as the storage time increased, leading to some loss in antioxidative activity. From the result, Mungoong was stable when stored at room temperature as well as at 4°C.

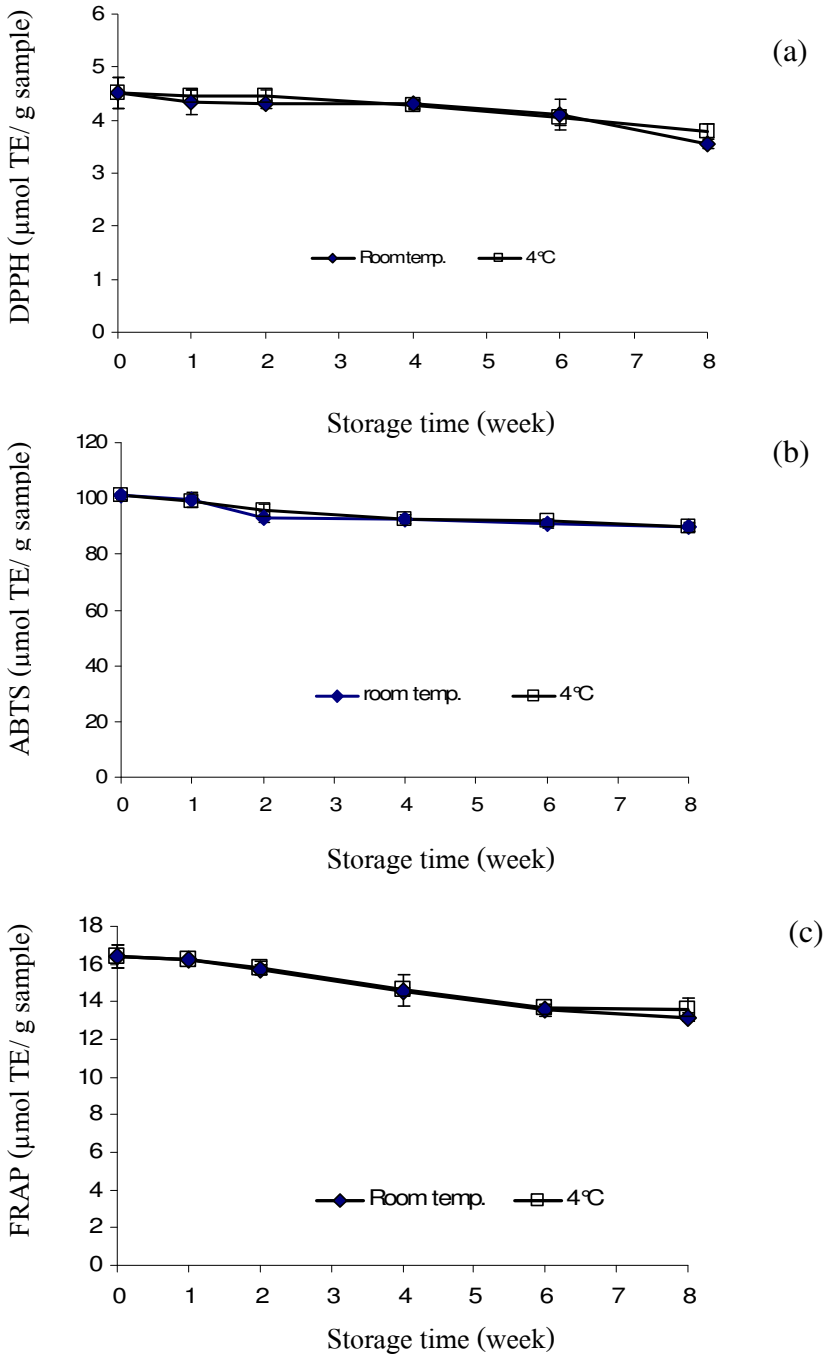

Figure 9 Changes in DPPH radical-scavenging activity (a) ABTS radical-scavenging activity (b) and FRAP (c) of water extract obtained from Mungoong stored at room temperature (28 -30°C) and 4°C for 8 weeks. Bars represent the standard deviation from triplicate determinations. Water soluble fraction at a concentration of 6 mg/ml was used for all assays.

5.2 TBA value

TBA value was used to assess the level of lipid oxidation in Mungoong. TBA value of Mungoong during storage at room temperature (28-30°C) and 4°C for 8 weeks is shown in Figure 10. From the result, TBA values increased up to 2 weeks of storage ($p<0.05$). Thereafter, a slight decrease in TBA value was noted. This might be due to the loss of volatile secondary oxidation products (Stanhnke, 1995). Abnormally low TBA values are noticeable if some TBARS react with proteins in an oxidizing system (Decker, 1998). No marked changes in TBA values were observed between both storage temperatures. Generally, the fat content and fatty acid composition are important in determining the extent of lipid oxidation during storage (Ahn *et al*., 1998). Although trace minerals such as Fe and Cu, the pro-oxidants, were present and Mungoong consisted of polyunsaturated fatty acids, TBA values of Mungoong were very low during the storage of 8 weeks. Amino acid, dipeptides or Maillard reaction products (MRPs) might act as antioxidants (Shahidi and Amarowicz, 1996; Benjakul *et al*., 2005). Thus, the propagation step could be retarded, leading to lower oxidation in Mungoong. From the result, antioxidative peptides or Maillard reaction products (MRPs) in Mungoong might contribute to the retardation of lipid oxidation of Mungoong during storage.

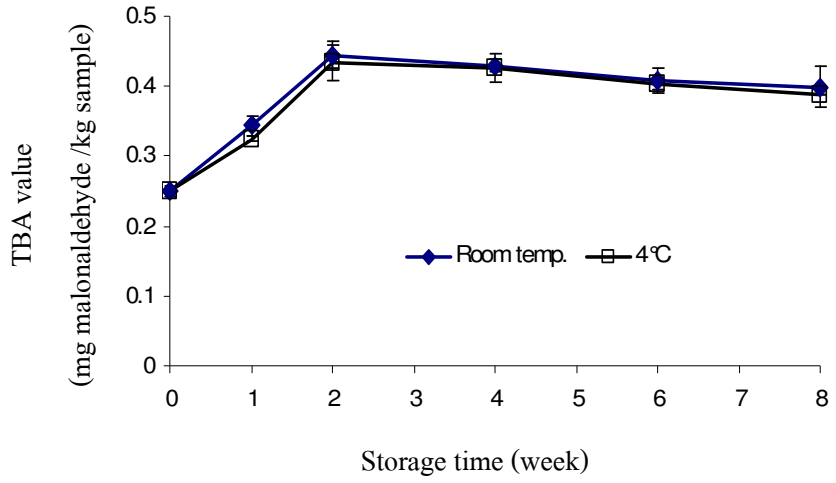

Figure 10 Changes in TBA value of Mungoong during storage at room temperature (28 -30°C) and 4°C. Bars represent the standard deviation from triplicate determinations.

6. Antioxidative activity of water soluble fraction from Mungoong in different systems

6.1 Antioxidative activity in lecithin liposome system

The antioxidant activity of Mungoong water extract in soybean liposome system was tested by monitoring TBARS (Figure 11(a)) and conjugated diene (Figure 11(b)) during incubation at 37°C. During incubation at 37°C, particularly after 42 h, Mungoong water extract showed antioxidant activity, as evidenced by slightly lower TBARS than the control ($p<0.05$). Conjugated diene formation was also lower in the system containing Mungoong water extract, especially when a higher amount of Mungoong water extract was used (Figure 11(b)). During 12-30 h incubation, conjugated diene was higher in the system added with water extract, compared with the control. The result suggest that conjugated diene in the control might undergo changes to produce intermediate or final oxidation products during the incubation period. The formation of dienes occurs during the early stages of lipid oxidation (Frankel, 1997) and conjugated diene hydroperoxides are expected to decompose to secondary products. The decrease or reaching a plateau of conjugated diene was accompanied by an increase in TBARS (Pena-Ramos and Xiong, 2003). Generally, the increase in liposome oxidation was observed when the incubation time increased. During 0-24 h, the sample added with 50 ppm BHT had no changes in both TBARS and conjugated dienes ($p>0.05$). Nevertheless, sharp increases were found thereafter ($p<0.05$). After 42 h incubation, continuous decreases in TBARS were observed in all samples, suggesting the loss of oxidation products as the incubation proceeded. The secondary products with low molecular weight were possibly lost, leading to lower amount of such products (Stanhnke, 1995). For conjugated dienes, the rate of decrease was lower than that of TBARS and the decrease was more pronounced after 30 h of incubation for all samples, except for that containing 200 ppm BHT. This might be caused by the transformation of conjugated dienes during advancement of oxidation. No changes in conjugated dienes and TBARS values in liposome system added with 200 ppm BHT were observed throughout the incubation period of 78 h ($p>0.05$). Therefore, Mungoong water extract showed lower antioxidative activity in lecithin liposome system, when compared with BHT.

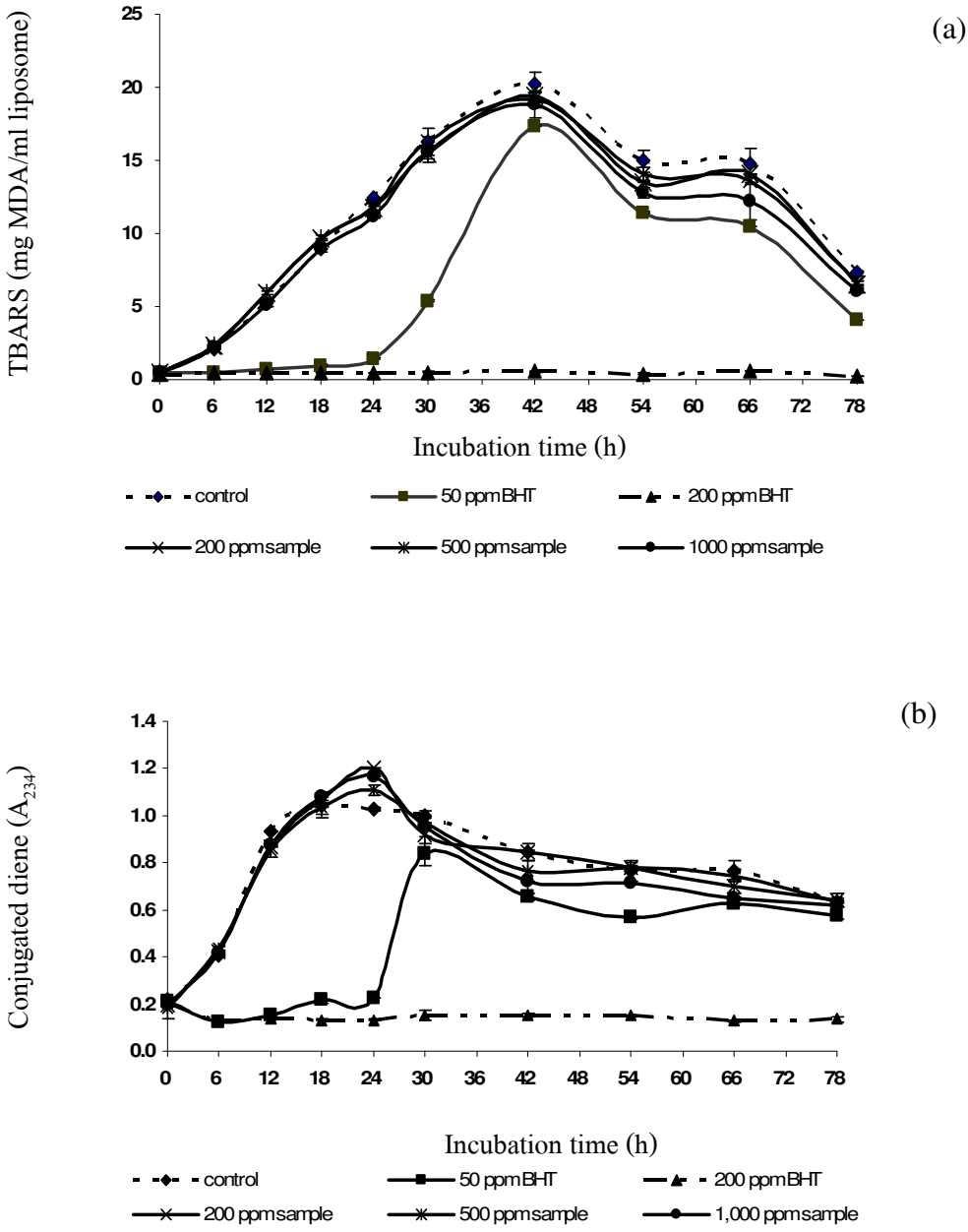

Figure 11 Change in TBARS value (a) and conjugated diene (b) in lecithin liposome system in the absence or presence of water soluble fraction from Mungoong at different levels. Bars represent the standard deviation from triplicate determinations.

6.2 Antioxidative activity in β-carotene linoleic system

The decreases in A_{470} of β-carotene in the absence and the presence of Mungoong water extract and BHT at different levels are shown in Figure 12. The decrease in A_{470} indicates the oxidation of β-carotene in the system caused by the oxidation process. A decrease in A_{470} was effectively retarded when the synthetic antioxidants, BHT (200 ppm), was used. Mungoong water extract at a level of 1,000 ppm showed a higher antioxidant activity than 200 and 500 ppm after 40 min of incubation. No difference in A_{470} was noticeable between the control, the system containing BHT (50 ppm), and the system with Mungoong water extract at levels of 200 and 500 ppm ($p>0.05$). In general, a model system made of β-carotene and linoleic acid in the absence of an antioxidant undergoes rapid discoloration (Matthaus, 2002). This is because of the coupled oxidation of β-carotene and linoleic acid, which generates free radicals. The free radicals of linoleic acid formed upon the abstraction of a hydrogen atom from its diallylic methylene group are able to attack the highly unsaturated β-carotene molecules (Jayaprakasha *et al*., 2001). As a result, β-carotene is oxidized and breaks down. Subsequently the system loses its chromophore and characteristic orange color, which is monitored spectrophotometrically (Wettashinghe and Shahidi, 1999). From the result, Mungoong water extract at 1000 ppm showed antioxidative activity, as indicated by the retardation of β-carotene bleaching in β-carotene/linoleic system, at high concentration (1,000 ppm). From the result, the efficacy in retarding the oxidation between lecithin liposome system and β-carotene linoleic system was different. BHT (50 ppm) showed a higher efficiency in the former system, but lower in the latter, compared with Mungoong water extract at 1,000 ppm. This was possibly governed by the difference in hydrophobicity of compounds and their localization at lipid/ water interface.

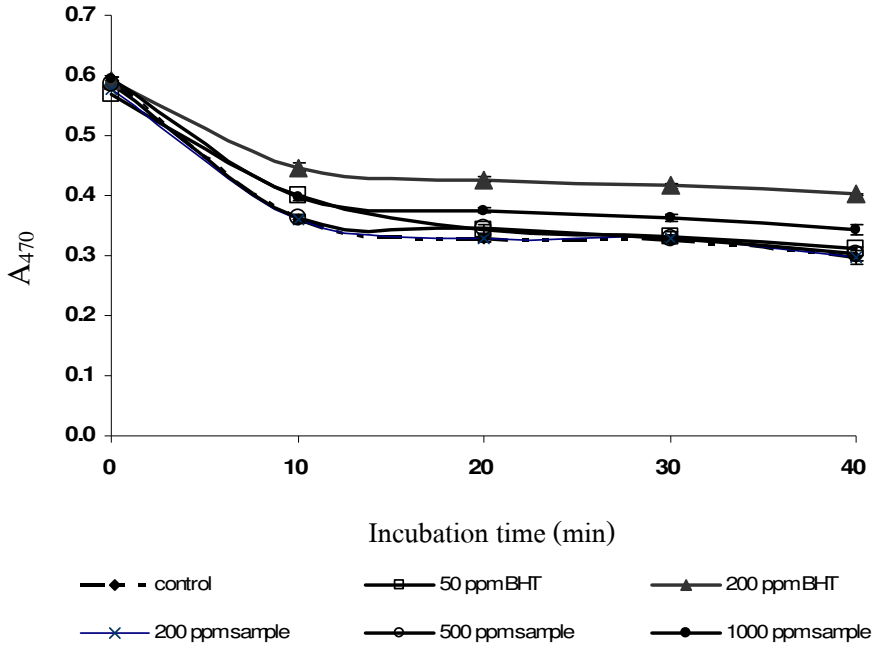

Figure 12 Change in β-carotene bleaching in the absence or presence of water soluble fraction from Mungoong at different levels. Bars represent the standard deviation from triplicate determinations.

6.3 Antioxidative activity in Comminuted fish model system

Lipid oxidation of round scad mince containing different levels (200, 500 and 1000 ppm) of Mungoong water extract was monitored during the storage at 4°C for 8 days as shown in Figure 13. TBARS values of round scad mince samples containing Mungoong water extract at different concentrations (200, 500 and 1,000 ppm) or 200 ppm BHT increased over the entire storage period, but their rate of increases was lower than that of the control ($p<0.05$). Among all samples, the one containing 200 ppm BHT had lower TBARS values throughout the storage period ($p<0.05$). From the result, the higher concentration of Mungoong water extract used, the lower TBARS values in round scad mince were observed. After 6 days of storage, a decrease in TBARS value was found. This might be due to the loss of volatile secondary oxidation products in the samples (Stanhnke, 1995). Round scad is dark fleshed fish containing high amount of fat (5.23 %) (Arthan *et al*., 2007). Also, the meat of this species contained high

amount of myoglobin, a potential source of iron, which might function as a prooxidant. Lipid oxidation in comminuted fish meat can be initiated and promoted by a number of mechanisms including autoxidation, photosensitized oxidation, lipoxygenase, peroxide and microsomal enzymes (Decker and Hultin, 1990; German and Kinsella, 1985). In lipid peroxidation, free radicals abstract a hydrogen from fatty acid double bond to produce fatty acid free radicals, which further react with oxygen to produce fatty acid hydroperoxides. Hydroperoxide are unstable and decomposes readily to shorter chain hydrocarbons such as aldehydes, etc. Those final products can be determined as TBARS (Benjakul *et al*., 2005). Therefore, soluble fractions from Mungoong could retard lipid oxidation in fish mince in a dose-dependent manner.

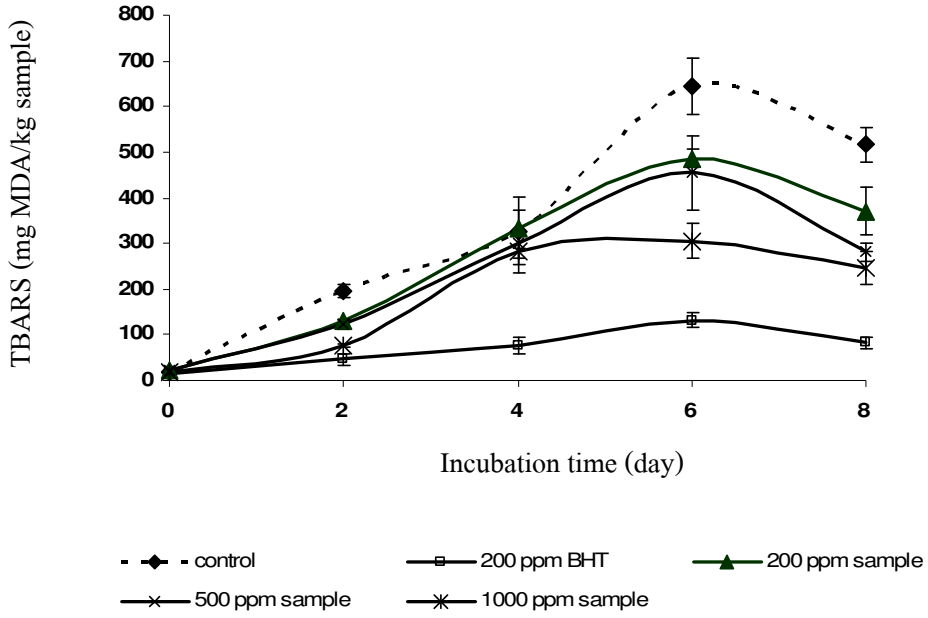

Figure 13 Change in TBARS values of comminuted round scad mince model system in the absence or presence of water soluble fraction from Mungoong at different levels during storage at 4°C. Bars represent the standard deviation from triplicate determinations.

7. Effect of Flavourzyme on the yield, composition and antioxidative activity of Mungoong

7.1 Total soluble solid content in the extract and yield of Mungoong from the cephalothorax of white shrimp prepared by different processes

Total soluble solid content in the extract without and with the aid of Flavourzyme prior to the addition of salt and sugar is shown in Table 8. Soluble solid content in the extract obtained from both raw and cooked cephalothorax using Flavourzyme was higher than that from typical process ($p<0.05$). In general, the extract from raw cephalothorax showed the greater soluble solid content, compared with that from cooked counterpart at both levels of Flavourzyme used ($p<0.05$). Enzyme concentration had no impact on soluble solid content ($p>0.05$). The yields of Mungoong from the cephalothorax of white shrimp, raw and cooked, with the addition of Flavourzyme at 0.15 and 0.30% in comparison with that of Mungoong prepared by typical method are shown in Table 8. In general, the use of Flavourzyme for Mungoong preparation resulted in the higher yield, compared with the typical process ($p<0.05$). At the same level of Flavourzyme used, Mungoong prepared from raw cephalothorax had the higher yield than that produced from cooked cephalothorax ($p<0.05$). Mungoong produced from the typical control process (Mungoong T) rendered the lowest yield ($p<0.05$). For the same state of cephalothorax used, the different levels of Flavourzyme showed no impact on the yield of resulting Mungoong ($p>0.05$). From the result, it was suggested that the state of substrate played an essential role in protein hydrolysis caused by Flavourzyme. The state of the substrate before hydrolysis is of great importance (Šlizyte *et al*., 2005b). During the cooking process, the denaturation of protein together with the removal of water from protein molecules most likely caused the aggregation of proteins. The larger aggregate formed was less susceptible to hydrolysis by Flavourzyme (Thiansilakul *et al*., 2007a). As a consequence, the lowered amount of proteins hydrolyzed by Flavourzyme was attained, when cooked cephalothorax was used as a substrate. In addition, the abilities of enzyme to disperse in the aggregated protein could be lowered, leading to the decreased yield (Šlizyte *et al*., 2005a). Therefore, Flavourzyme at 0.15% was found to be optimal for protein hydrolysis of cephalothorax, especially for raw cephalothorax.

Table 8 Total soluble solid content in the extract and yield of Mungoong prepared by different processes.

Mungoong sampls	Soluble solid content (% dry basis)	Yield (% dry basis)
Mungoong T	$61.71 \pm 0.33^{c\dagger\ddagger}$	86.11 ± 1.33^{c}
Mungoong RF15	74.99 ± 0.24^{a}	106.92 ± 1.57^{a}
Mungoong CF15	63.27 ± 1.28^{b}	89.31 ± 1.73^{b}
Mungoong RF30	74.85 ± 0.27^{a}	106.81 ± 1.56^{a}
Mungoong CF30	63.61 ± 0.77^{b}	90.37 ± 1.48^{b}

†Means ± SD from triplicate determinations.

‡Different superscripts in the same column indicate the significant differences ($p<0.05$).

7.2 Chemical compositions and physical properties of Mungoong from the cephalothorax of white shrimp prepared by different processes

Different Mungoongs obtained from different processes had the varying compositions and physical properties as shown in Table 9. Moisture content of Mungoong samples varied from 29.03% to 36.67 %. Based on dry matter, protein constituted as the major component, indicating that Mungoong could be the good source of proteins. The high protein content was a result of solubilization of protein during hydrolysis, the removal of insoluble undigested non-protein substances and partial removal of lipid after hydrolysis (Benjakul and Morrissey, 1997). Mungoong prepared from raw cephalothorax with the aid of Flavourzyme (Mungoong RF15 and Mungoong RF30) consisted of higher protein content than those prepared from cooked cephalothorax (Mungoong CF15 and Mungoong CF30) and Mungoong from typical process (Mungoong T). However, the lowest fat content was found in Mungoong T ($p<0.05$). Carbohydrate contents in different Mungoong varied with processes used. Carbohydrate most likely were from the sugar added to improve the flavor and taste of Mungoong.

Table 9 Chemical compositions and physical properties of Mungoong from the cephalothorax of white shrimp, prepared by different processes.

Compositions / Properties	Mungoong T	Mungoong RF15	Mungoong CF15	Mungoong RF30	Mungoong CF30
Moisture (%)*	$36.67 \pm 0.02^{a\dagger\ddagger}$	31.44 ± 0.11^{c}	33.86 ± 0.05^{b}	29.03 ± 0.02^{d}	31.18 ± 0.83^{c}
Protein (%)**	41.04 ± 0.54^{b}	46.27 ± 1.83^{a}	39.94 ± 0.74^{b}	46.04 ± 1.12^{a}	39.27 ± 0.56^{b}
Lipid (%)**	16.10 ± 0.24^{bc}	15.69 ± 0.84^{c}	16.95 ± 0.42^{ab}	17.09 ± 0.37^{ab}	17.50 ± 0.71^{a}
Ash (%)**	17.24 ± 0.19^{c}	17.64 ± 0.03^{b}	17.73 ± 0.05^{b}	17.63 ± 0.06^{b}	18.82 ± 0.10^{a}
Carbohydrate (%)**	25.62 ± 0.85^{a}	20.40 ± 1.92^{b}	25.38 ± 0.40^{a}	19.24 ± 1.04^{b}	24.41 ± 0.06^{a}
Salt (%)**	11.76 ± 0.20^{a}	11.31 ± 1.08^{a}	11.21 ± 0.28^{a}	11.14 ± 0.12^{a}	11.73 ± 0.59^{a}
Aw	0.76 ± 0.00^{a}	0.68 ± 0.00^{d}	0.74 ± 0.00^{b}	0.64 ± 0.00^{e}	0.69 ± 0.00^{c}
pH	7.12 ± 0.00^{c}	7.15 ± 0.03^{c}	7.33 ± 0.02^{b}	7.13 ± 0.02^{c}	7.38 ± 0.02^{a}
Color					
L*	18.81 ± 0.04^{a}	15.26 ± 0.01^{d}	17.25 ± 0.14^{b}	14.86 ± 0.04^{e}	16.78 ± 0.06^{c}
a*	13.30 ± 0.13^{b}	13.29 ± 0.23^{b}	15.24 ± 0.14^{a}	12.62 ± 0.16^{c}	10.90 ± 0.10^{d}
b*	19.43 ± 0.15^{b}	16.97 ± 0.25^{d}	20.63 ± 0.42^{a}	17.86 ± 0.10^{c}	16.48 ± 0.13^{e}

*Wet basis.

** Dry basis.

† Means ± SD from triplicate determinations.

‡ Different superscripts in the same row indicate the significant differences ($p<0.05$).

No differences in salt content (11.14-11.76%) were noticeable among all Mungoong samples ($p>0.05$). This contributed to high ash content of all samples (17.24-18.82%). All Mungoong samples were dark brown in color. Browning reaction, particularly Maillard reaction, might take place during evaporating process to obtain the final heavy viscous product. Amino groups in soluble protein or hydrolyzed peptides caused by Flavourzyme could react with carbonyl groups of reducing sugar or lipid oxidation products formed during processing (Ogasawara *et al*., 2006). The varying color of fish protein hydrolysate depended on the composition of the raw material and the hydrolysis condition (Thiansilakul *et al*., 2007b). From the result, the differences in color (L*, a* and b*) of different Mungoong most likely resulted from the differences in number of amine group in peptides generated from hydrolysis process. Water activity (Aw) of Mungoong

was in the range of 0.64-0.76. Aw was in agreement with moisture content. Mungoong was therefore classified as intermediate moisture foods, in which Aw is about 0.7 (Fennema, 1996). Lowered Aw of Mungoong might be associated with the prolonged shelf-life of this product. For the pH, all samples had the neutral pH (7.12-7.38).

7.3 Nitrogen soluble index of Mungoong from the cephalothorax of white shrimp prepared by different processes

Nitrogen soluble index (NSI) of Mungoong produced from raw and cooked cephalothorax without and with the aid of Flavourzyme at different levels is shown in Figure 14. NSI has been used to determine protein solubility, mainly caused by the dispersion of protein in the solvent (Cheftel *et al*., 1985). Solubility is one of the most important physicochemical and functional properties of protein hydrolysates (Kinsella, 1976; Mahmoud *et al.,* 1992). Higher NSI was found in Mungoong RF15 and Mungoong RF30, compared with other samples ($p<0.05$). No differences in NSI were observed between Mungoong T and Mungoong CF15 and Mungoong CF30 ($p>0.05$). It was suggested that Flavourzyme prefered to hydrolyze raw cephalothorax rather than cooked counterpart. During heating process, proteins in the cephalothorax might undergo denaturation and aggregation. Large aggregate formed might be less hydrolyzed by Flavourzyme. Additionally, endogenous proteinase in cephalothorax could be inactivated at high temperature. Cephalothorax is an important source for proteinase (Venugopal, 1995). The result was in accordance with Guérard *et al*. (2001) who reported that the decrease in the concentration of peptide bonds in tuna stomach available for hydrolysis was due to the heating treatment. The denatured proteins are apparently highly resistant to enzymatic breakdown (Mohr, 1980). However, Mungoong produced from raw cephalothorax showed the greater NSI, indicating the greater hydrolysis toward native proteinaceaus substances in cephalothorax. The hydrolytic reaction depends on the availability of susceptible bonds, in which the primary enzymic attack is concentrated, and on the physical structure of the protein molecule (Guérard *et al*., 2001). The higher solubility of Mungoong RF15 and RF30 was possibly due to the size reduction and the formation of smaller, more hydrophilic and more solvated polypeptides generated from enzyme hydrolysis. However, it was noted that NSI of Mungoong was lower than that reported in protein hydrolysate from yellow stripe trevally (*Selaroides leptolepis*) (85%) (Klompong *et al*., 2007) and from round scad (*Decapterus maruadsi*) Muscle (99%) (Tainsilakul *et al*., 2007b). The

polymerization of hydrolyzed proteins during heating to evaporate the water from the products most likely caused the loss in solubility.

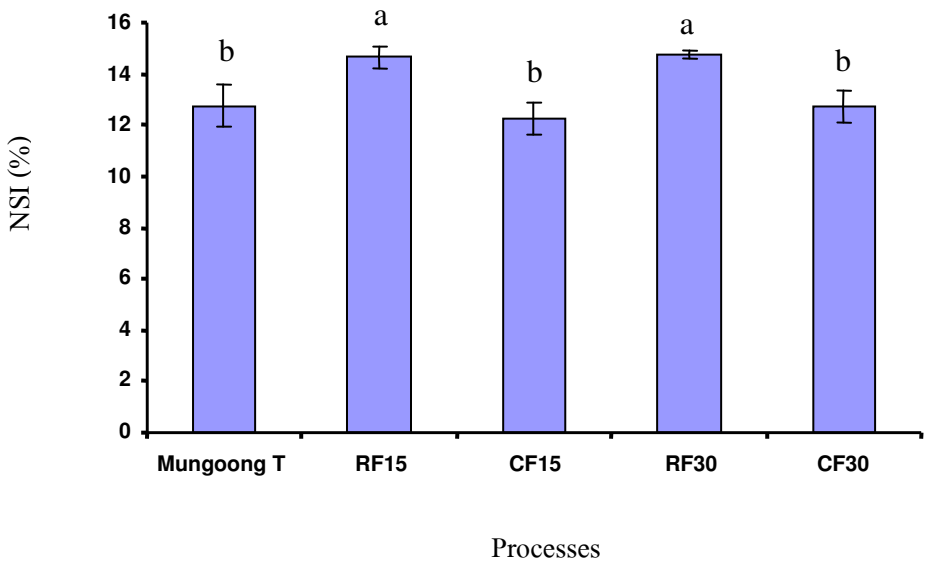

Figure 14 Nitrogen solubility index of Mungoong from the cephalothorax of white shrimp prepared by different processes. Bars represent the standard deviation from triplicate determinations. Different letters indicate significant differences ($p<0.05$). Fz: Flavourzyme; R: Raw cephalothorax; C: Cooked cephalothorax.

7.4 Formal nitrogen content, ammonia nitrogen content and amino nitrogen content of Mungoong from the cephalothorax of white shrimp prepared by different processes

Formal nitrogen contents of Mungoong produced from raw and cooked cephalothorax without and with the aid of Flavourzyme at 0.15 and 0.30% are shown in Table 10. Formal nitrogen content of Mungoong RF15 and RF30 was greater than that of other samples ($p<0.05$). Similar formal nitrogen content was found between Mungoong T and Mungoong produced using cooked cephalothorax with the aid of Flavourzyme (Mungoong CF15 and CF30) ($p>0.05$). With the same level of Flavourzyme, higher formal nitrogen content was noticeable for Mungoong from raw cephalothorax ($p<0.05$). Formal nitrogen contents of all samples were

coincidental with NSI of all samples (Figure 14). Formal nitrogen content is used as a convenient index of the degree of protein hydrolysis (Chaveesuk *et al*., 1993). Formalin can react with alpha amino group and ammonia, releasing the proton which can be titrated with alkaline solution. Thus, formal nitrogen content indicate the cleavage of peptides (Angeles *et al.,* 2002).

Ammonia nitrogen content of all samples are depicted in Table 10. The ammonia nitrogen contents of all samples were from 1.11 to 1.36 mg-N/g sample. The ammonia nitrogen content indicates the breakdown of protein and peptides into free amino acid and volatile nitrogen (Lopetcharat and Park, 2002; Chaveesuk *et al*., 1993). Similar result was observed, when compared with that of formal nitrogen content. In general, a greater ammonia nitrogen content was found in Mungoong produced from raw cephalothorax with addition of Flavourzyme ($p<0.05$).

Amino nitrogen contents of Mungoong produced from different processes are shown in Table 10. Amino nitrogen content was calculated based on the differences between formal and ammonia nitrogen content. The amino nitrogen content represents the amount of primary amino groups in Mungoong. An increase in amino nitrogen concentration is related to the degradation of polypeptide (Lopetcharat and Park, 2002). From the result, Mungoong produced from raw cephalothorax contained the greater amino nitrogen content, compared with other samples. Additionally, no differences in amino nitrogen content were observed between samples with the same process using the different levels of Flavourzyme ($p>0.05$). This result was in agreement with the higher formal nitrogen content (Table 10) and protein content (Table 9) in samples produced from raw cephalothorax with the aid of Flavourzyme.

Table 10 Formal nitrogen, ammonia nitrogen and amino nitrogen contents of Mungoong from the cephalothorax of white shrimp prepared by different processes.

Sample	Formal nitrogen content (mg-N /g sample)	Ammonia nitrogen content (mg-N /g sample)	Amino nitrogen content (mg-N /g sample)
Mungoong T	18.70 ± 0.17[b†‡]	1.78 ± 0.07[b]	16.92 ± 0.24[b]
Mungoong RF15	30.98 ± 0.31[a]	2.06 ± 0.04[a]	28.91 ± 0.35[a]
Mungoong CF15	18.49 ± 0.16[b]	1.70 ± 0.04[b]	16.91 ± 0.18[b]
Mungoong RF30	30.80 ± 0.66[a]	1.99± 0.02[a]	28.81 ± 0.65[a]
Mungoong CF30	18.46 ± 0.66[b]	1.69 ± 0.06[b]	16.77 ± 0.14[b]

† Means ± SD from three experiments.

‡ Different superscripts in the same column indicate the significant differences ($p<0.05$).

*F: Flavourzyme; R: Raw cephalothorax; C: Cooked cephalothorax

7.5 Antioxidative activity of water extract of Mungoong from the cephalothorax of white shrimp prepared by different processes.

7.5.1 DPPH radical scavenging activity

DPPH radical scavenging activity of water extract from Mungoong, produced from different processes, is shown in Figure 15(a). The highest DPPH radical scavenging activity was found in Mungoong RF15 and Mungoong RF30 ($p<0.05$). No differences in DPPH radical scavenging activity were noticeable between Mungoong T and Mungoong CF15 and CF30 ($p>0.05$). The different scavenging activities between Mungoong derived from raw and cooked cephalothorax might be caused by the differences in amino acids or peptides generated. From the result, it was suggested that raw cephalothorax was the suitable substrate for Flavourzyme to hydrolyze protein or peptides as evidenced by a higher NSI (Figure 14), formal and amino nitrogen content (Table 10). Moreover, those peptides or amino acids produced could act as a hydrogen donor to radicals as indicated by the ability to scavenge DPPH radical. Antioxidative activity of protein hydrolysates depends on the proteases (Jun *et al*., 2004) and hydrolysis conditions employed (Jun *et al*., 2004; Pena-Ramos and Xiong, 2003; Jao and Ko, 2002). During hydrolysis, a wide variety of smaller peptides and free amino acids is generated, depending on

enzyme specificity. Changes in size, level and composition of free amino acids and small peptides affect the antioxidative activity (Wu *et al.,* 2003). Even the same number of peptides was cleaved, the resulting peptides might be different in term of amino acid type and sequences. Thus this might contribute to the varying radical scavenging activity. Not only the presence of some favorable amino acids, but also their correct positioning in the peptide sequence is trivial to its activity (Chen *et al*., 1996). The proper positioning of Glu, Leu and His was reported to improve radical scavenging activities of antioxidative peptides (Suetsuna *et al*., 2000).

7.5.2 ABTS radical scavenging activity

Water extract from Mungoong produced from different processes showed different ABTS radical scavenging activity (Figure 15(b)). Mungoong R15 and R30 exhibited the highest activity, whereas the lowest activity was found in Mungoong T. Samples obtained from the same cephalothorax with different Flavourzyme concentration did not differ in ABTS radical scavenging activities ($p>0.05$) (Figure 15(b)), From the result, peptides produced by Flavourzyme in cooked cephalothorax were suggested to contribute to greater ABTS radical scavenging activity, compared with those present in Mungoong T. Therefore, Flavourzyme at 0.15% could hydrolyze the proteins in white shrimp cephalothorax, yielding Mungoong with the highest ABTS radical scavenging activity. This result was in agreement with DPPH radical scavenging activity.

7.5.3 Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP) of water extract from Mungoong samples produced from different processes is depicted in Figure 15(c). Among all samples, Mungoong RF15 had the highest FRAP ($p<0.05$), followed by Mungoong RF30. Conversely, Mungoong CF30 showed the greater FRAP than Mungoong CF15 ($p<0.05$). However, no differences in FRAP were found between Mungoong CF15 and Mungoong T ($p>0.05$). FRAP generally measures the antioxidant effect of any substance in the reaction medium as reducing ability (Siddhuraju and Becker, 2007).

Slight differences found among three different assays indicated that the antioxidative peptides or antioxidative compounds in Mungoong with different processes were slightly different in term of function. For example, Mungoong RF30 might scavenge the radicals, both DPPH and ABTS effectively, but it showed the lower reducing ability than Mungoong

RF15. From the result, it was suggested that enzymatic hydrolysis can be used to improve antioxidant activity of resulting product.

7.6 Fatty acid composition of Mungoong T and Mungoong RF15

The fatty acid profiles of Mungoong T and Mungoong RF15 are shown in Table 11. Similar type and amount of fatty acids were found in both samples. However, polyunsaturated fatty acid (PUFA) content was slightly higher in Mungoong RF15. Mungoong T comprised 38.95% PUFA, 26.54% MUFA and 31.29% SFA, while Mungoong RF15 contained 41.65% PUFA, 25.92% MUFA and 29.79% SFA. Among all fatty acid found, oleic acid (C18: 1 (n-9)) and linoleic acid (C18: 2 (n-6)) were predominant. Docosahexaenoic acid C22:6 (n-3) (DHA) was found in Mungoong T and Mungoong RF15 at levels of 6.72 and 7.44 mg/100g fat, while eicosapentaenoic acid C20:5 (n-3) (EPA) constituted 3.46 and 3.87 g/100g fat, respectively. Kolakowska *et al.* (2002) reported that DHA is usually more abundant than EPA (up to 2-3 times) in fish lipids. From the result, Mungoong prepared with the aid of Flavourzyme had slightly higher PUFA than Mungoong prepared using typical process. Flavourzyme possibly hydrolyzed protein associated with lipid, especially membrane lipid in the cephalothorax. As a result, those lipids containing PUFA could be removed into the soluble fraction, used for Mungoong preparation.

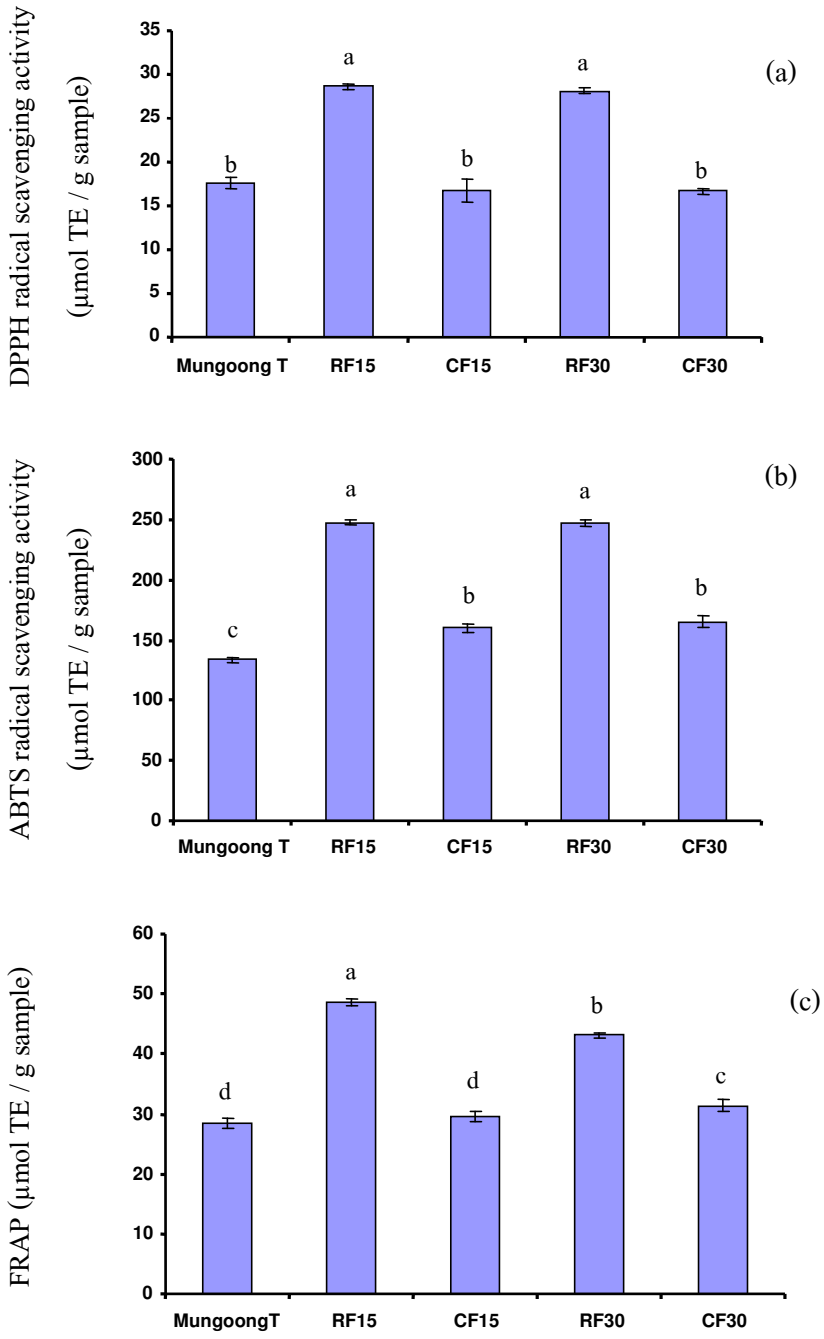

Figure 15 Antioxidative activities of Mungoong from the cephalothorax of white shrimp prepared by different processes. a: DPPH radical-scavenging activity, b: ABTS radical-scavenging activity and c: FRAP. Bars represent the standard deviation from triplicate determinations. Different letters indicate significant differences ($p<0.05$). Fz: Flavourzyme; R: Raw cephalothorax; C: Cooked cephalothorax; TE: Trolox equivalent.

Table 11 Fatty acid composition (g/100g) of Mungoong T and Mungoong RF15 from the cephalothorax of white shrimp.

Fatty acids	Mungoong T	Mungoong RF15
Myristic acid C14:0	0.98	0.97
Pentadecanoic acid C15:0	0.57	0.52
Palmitic acid C16:0	22.52	21.31
Palmitoleic acid C16:1 n-7	2.15	2.33
Heptadecanoic acid C17:0	0.85	0.81
Stearic acid C18:0	5.35	5.16
Oleic acid C18:1 n-9	19.48	18.9
Cis-Vaccenic acid C18:1 n-7	2.36	2.47
Linoleic acid C18:2 n-6	22.73	23.73
α-Linoleic acid C18:3 n-3	1.45	1.39
γ--Linoleic acid C18:3 n-6	0.08	-
Moroctic acid C18:4 n-3	0.1	0.1
Arachidic acid C20:0	0.32	0.3
Cis-11-Eicosenoic acid C20:1 n-9	1.74	1.5
Cis-11,14-Eicosadienoic acid C20:2 n-6	1.18	1.52
Cis-8,11,14-Eicosatrienoic acid C20:3 n-6	0.14	0.18
Cis-11,14,17-Eicosatrienoic acid C20:3 n-3	0.21	0.23
Arachidonic acid C20:4 n-6	1.54	1.54
Eicosatetraenoic acid C20:4 n-3	0.22	0.23
Cis-5,8,11,14,17-Eicosapentaenoic acid C20:5 n-3(EPA)	3.46	3.87
Heneicosanoic acid C21:0	0.11	0.11
Behenic acid C22:0	0.3	0.26
Erucic acid C22:1 n-9	0.18	0.16
Cetoleic acid C22:1 n-11,n-13	0.51	0.44
Docosatetraenoic acid C22:4 n-6	0.26	0.29
Docosapentaenoic acid C22:5 n-3	0.51	0.73
Docosapentaenoic acid C22:5 n-6	0.35	0.4
Cis-4,7,10,13,16,19-Docosahexaenoic acid C22:6 n-3 (DHA)	6.72	7.44
Tricosanoic acid C23:0	0.13	0.19
Lignoceric acid C24:0	0.16	0.16
Nervonic acid C24:1	0.12	0.12
Polyunsaturated fatty acid (PUFA)	38.95	41.65
Monounsaturated fatty acid (MUFA)	26.54	25.92
Saturated fatty acid (SFA)	31.29	29.79

7.7 Mineral content of Mungoong T and Mungoong RF15

The contents of different minerals in Mungoong T and Mungoong RF15 are shown in Table 12. Among all minerals determined, Na, Ca and K were the major minerals in both Mungoong. Fe and Cu, known as pro-oxidants (Thanonkeaw *et al*., 2006), were found at very low content. Nevertheless, Ni, Cd and Pb, the toxic minerals for consumption (Ichihashi *et al*., 2001), were not detectable. However, mineral content was slightly higher in Mungoong T. The high content of Na (15.97 g/kg in Mungoong T and 15.80 g/kg in Mungoong RF15) was associated with the addition of salt. K, Mg, P, Na, S and Ca were abundant in herring and herring by-product hydrolysates (Sathivel *et al*. 2003). Cu was found at very low content and was most likely from hemocyanin in the blood of shrimp (Decker and Tuczek, 2000). Major sources of minerals for marine organisms are sea water and feed (Ichihashi *et al*., 2001). Due to high content of PUFAs in Mungoong (Table 11), the oxidation might take place in the presence of prooxidant (Table 12). Castell *et al*. (1965) found that the relative prooxidant activity of ions in fish muscle decreased in the order: Cu^{2+}>Fe^{2+} >Co^{2+}>Cd^{2+}>Li>Ni^{2+}>Mg^{2+}>Zn^{2+}>Ca^{2+}>Ba^{2+}.

Table 12 Minerals contents (g/kg) of Mungoong T and Mungoong RF15 from the cephalothorax of white shrimp.

Mineral contents (g/kg)	Mungoong T	Mungoong RF15
P	1.60 ± 0.001	0.83 ± 0.003
K	5.87 ± 0.001	4.79 ± 0.001
Ca	6.27 ± 0.00	6.02 ± 0.00
Mg	0.75 ± 0.00	0.53 ± 0.00
Fe	0.05 ± 0.00	0.06 ± 0.009
Mn	0.002 ± 0.00	0.002 ± 0.00
Zn	0.03 ± 0.004	0.03 ± 0.002
Cu	0.05 ± 0.001	0.06 ± 0.01
Na	15.97 ± 0.02	15.80 ± 0.02
Ni	ND	ND
Cd	ND	ND
Pb	ND	ND

Average ± SD from triplicate determinations.

ND: not detectable or below detection limit.