

# Comparison of Soybean Oils, Gum, and Defatted Soy Flour Extract in Stabilizing Menhaden Oil during Heating

X. YUE, Z. XU, W. PRINYAWIWATKUL, J.N. LOSSO, J.M. KING, AND J.S. GODBER

**ABSTRACT:** Capabilities of crude soy oil, degummed oil, gum, and defatted soy flour extract in preventing the oxidation of menhaden oil and its omega-3 fatty acids, DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid), during heating were evaluated. The menhaden oil mixed with defatted soy flour extract demonstrated the greatest stability by producing the lowest TBA reactive oxidation products and retaining the highest concentrations of DHA and EPA after heating at 150 °C for 30 min. A range of 62.8% to 71.5% of DHA and 67.7% to 75.9% of EPA remained in the fish oil with defatted soy flour extract, while only 29.9% of DHA and 37.2% of EPA were retained in the fish oil with no addition. Stabilizing capability from highest to lowest was defatted flour extract > gum > degummed oil = crude oil. The defatted flour extract had the highest level of total phenolic content (11.3 μg catechin equivalent/g), while crude oil, degummed oil, and gum contained 7.1, 6.1, and 6.0 μg catechin equivalent/g, respectively. The level of isoflavones in the defatted soy flour extract was 55 mg/g, which was over 100 times higher than in the crude oil or gum. Although isoflavones were not detected in the degummed oil, it contained the highest level of tocopherols (414 μg/g), whereas the lowest level (215 μg/g) was found in the defatted flour extract. The order of free radical scavenging capability measured from high to low was the defatted soy flour extract, crude oil, degummed oil, and gum.

**Keywords:** antioxidant, fish oil, gum, isoflavone, phenolic, soy, tocopherol

## Introduction

Many epidemiological and clinical studies confirmed that daily intake of omega-3 long chain polyunsaturated fatty acid (PUFA) from fish oil is beneficial for preventing various cardiovascular diseases (Dyerberg and others 1978; Crombie and others 1987; Wang and others 2006). Food products enriched with fish oil or omega-3 PUFA as a health promoting component offer potential in the burgeoning area of functional food product development. However, the omega-3 PUFAs in fish oil are readily oxidized to produce off- or rancid-flavor volatiles when exposed to light, oxygen, prooxidants, and high temperatures (McClements and Decker 2000). Thus, the quality of fish oil or foods fortified with fish oil usually deteriorates rapidly if not stabilized. While the instability of omega-3 PUFA has been solved for some food applications, there are serious hurdles for using fish oil in other foods, such as many functional foods, that still need to be overcome. Synthetic antioxidants such as TBHQ (tertiary butyl hydroquinone), BHA (butylated hydroanisole), and BHT (butylated hydroxytoluene), alpha-tocopherol acetate, and EDTA (ethylenediaminetetraacetic acid) are used for retarding the fish oil oxidation. However, the addition of those artificial chemicals is restricted by the FDA because of food safety concerns, not to mention emerging trends for consumer preferences toward more “green” food processing applications. Kotsonis and others (2001) have suggested that even small amounts of artificial antioxidants could have potentially harmful health effects from long-term consumption.

Lately, the safety and health benefits of antioxidants from natural sources, such as grains and cereals, have been reported in numerous studies and recognized by the FDA and many consumers (Truswell 2002; Martinez-Tome and others 2004; Nystrom and others 2005). The antioxidants from natural sources are generally considered as safe food ingredients. However, information on using the natural antioxidants to replace synthetic antioxidants in preventing fish oil oxidation is limited. Several antioxidants, including isoflavones, have been identified in soybeans (Meng and others 1999). Soy isoflavones were well recognized to play an important role in reducing the formation and progression of certain types of cancers and some chronic diseases such as cardiovascular disease, Alzheimer's disease, and osteoporosis (Messina 1999). They are effective antioxidants because of their phenol structure and redox potential (Meng and others 1999). Thus, the antioxidants in soybean could be used as a natural “green” antioxidant ingredient to effectively stabilize fish oil and other food systems containing omega-3 PUFA.

In this study, the capabilities of intermediate products and byproducts of soybean oil refining, including crude soy oil, degummed oil, gum, and defatted soy flour, for preventing long chain fatty acid (DHA and EPA) oxidation and stabilizing menhaden oil during heating were investigated. The DPPH (2,2'-diphenyl-1-picrylhydrazyl) free radical quenching activity and total phenolic, isoflavone, and tocopherol contents in the oils, gum, and defatted soy flour extract were determined. This information would be helpful in the development and utilization of soy products as a food antioxidant or an antioxidant nutritional supplement. Furthermore, this application will also increase the potential health benefits of fish oil with the additional health promoting functions of the soy antioxidants.

MS 20070580 Submitted 7/24/2007, Accepted 9/10/2007. Authors are with Dept. of Food Science, Louisiana State Univ. Agricultural Center, Baton Rouge, LA 70803, U.S.A. Direct inquiries to author Xu (E-mail: zxu@agctr.lsu.edu).

## Materials and Methods

### Materials

Hexane, methanol, and butanol were HPLC grade and purchased from Fisher Scientific Inc. (Fair Lawn, N.J., U.S.A.).  $\text{BCl}_3$ -methanol and 2,2-dimethoxypropane were purchased from Supelco (Bellefonte, Pa., U.S.A.). Menhaden fish oil (without any stabilizers), DPPH (2,2'-diphenyl-1-picrylhydrazyl), Folin-Ciocalteu reagent, catechin, and 2-thiobarbituric acid were purchased from Sigma-Aldrich (St. Louis, Mo., U.S.A.). Heptadecanoic acid (C17:0), docosahexaenoic acid C22:6 (DHA), eicosapentaenoic acid C20:5 (EPA), soy isoflavones (daidzin, glycitin, and genistin), and alpha and gamma tocopherol standards were obtained from Sigma-Aldrich. Soybeans were purchased from a local farmers market.

### Crude soy oil, degummed oil, gum, and defatted soy flour extract preparation

**Crude soy oil.** Soybeans were ground for 3 min at a medium speed in a kitchen blender. The particles of the ground whole soy flour were able to pass through a 1-mm sieve. The soy flour (200 g) was weighed into a 1000-mL glass beaker and extracted using 400 mL hexane. The mixture was incubated at 60 °C in a water bath and stirred gently for 20 min. After the incubation, the solvent layer was separated from solid residue by centrifuging at 2000 × g for 10 min using a Hermel Z383 K table top centrifuge (Nat'l. Labnet Co., Woodbridge, N.J., U.S.A.). The clear supernatant was transferred to a clean round bottom flask. Then the solid residue was extracted with another 400 mL of hexane. The separated hexane extracts were combined and dried using a vacuum evaporator at 50 °C. The dried extract was crude soy oil, which was weighed to obtain the crude oil yield. The solid residue was dried under a ventilation hood overnight to obtain defatted soy flour.

**Defatted soy flour extract.** Methanol (400 mL) was mixed with the defatted soy flour and incubated at 60 °C in a water bath and stirred gently for 20 min. After the incubation, the supernatant was separated from the residue by centrifuging at 2000 × g for 10 min. The defatted soy flour extract was obtained after the supernatant was dried using the vacuum evaporator at 50 °C. The extract was weighed to obtain the defatted soy flour extract yield.

**Degummed oil and gum.** Twenty grams of crude oil were weighed and transferred into a clean centrifuge test tube and degummed using 3% (w/w) distilled water in the oil. The mixture of oil and water was vortexed for 5 min and incubated at 60 °C in a water bath for 30 min with shaking. The incubated crude oil and water mixture was centrifuged at 3000 × g for 10 min. The separated upper oil phase was degummed oil, which was transferred to a clean test tube and weighed. The lower phase was a mixture of white gum and water, and subsequently was dried using the vacuum evaporator at 80 °C to remove the moisture and was then weighed.

### Determination of total phenolic content

The total phenolic contents of the oils, gum, and defatted soy flour extract were determined using the Folin-Ciocalteu reagent (Velioglu and others 1998). The Folin-Ciocalteu reagent was diluted 10 times with deionized water. The oils, gum, or defatted soy flour extract (50 mg each) was redissolved in 10 mL methanol, and 0.1 mL of this solution was mixed with 0.75 mL diluted Folin-Ciocalteu reagent. The reaction solution was left at 25 °C for 5 min. Then 0.75 mL of sodium bicarbonate solution (60 g/L) was added. The mixture was incubated at 25 °C for 90 min and filtered through a 0.45- $\mu\text{m}$  syringe filter (Pall Corp., Ann Arbor, Mich., U.S.A.). The

absorbance of the solution was determined at 750 nm using a UV-Visible SpectraMax Plus<sup>384</sup> spectrophotometer (Molecular Devices, Sunnyvale, Calif., U.S.A.). Catechin was used to prepare a standard curve. The total phenolic content was calculated and expressed as  $\mu\text{g}$  catechin equivalent/g of the oil, gum, or defatted soy flour extract.

### Determination of total tocopherol content using HPLC

The total tocopherol concentration was determined using the method of Xu (2002). The HPLC system consisted of Waters (Milford, Mass., U.S.A.) 510 pumps, a 715 Ultra WISP injector, and 470 fluorescence detector. Chromatograms were recorded and processed using Waters Millennium chromatography software. The samples were injected into a 25 cm × 4.6 mm i.d. 5- $\mu\text{m}$  Supelcosil LC-Si (Supelco) column. The column was preceded by a 5 cm × 4.6 mm i.d. guard column packed with 40- $\mu\text{m}$  pellicular silica. The mobile phase consisted of 0.5% ethyl acetate and 0.5% acetic acid in hexane at a flow rate of 1.5 mL/min. The fluorescence detector was set at 290 nm excitation and 330 emission. The oils, gum, and defatted soy flour extract (50 mg each) were dissolved in 10 mL of hexane and vortexed. One hundred microliters of the solution were injected into the HPLC system. Each tocopherol concentration was calculated based on their standard curves. The total tocopherol content was calculated by summing each tocopherol concentration.

### Determination of total isoflavone content using HPLC

The oils, gum, or defatted soy extract solution (50 mg in 10 mL methanol) was transferred to HPLC vials. The HPLC system consisted of a Supelco Discovery C18 column (i.d. 3 mm × 25 cm), a Waters 2690 separation module, a 996 photodiode array detector, and a Millennium32 chromatography manager. The mobile phase was a mixture of water and ethanol, with percentage of water in ethanol ramped from 90% to 50% in 40 min with a constant flow rate of 0.3 mL/min (Xu and others 2002). The chromatograms obtained at a wavelength of 254 nm were used to quantify the isoflavones. The concentration of the 3 major isoflavones, daidzin, glycitin, and genistin, was calculated based on their standard curves. The total isoflavone content was calculated by summing the 3 isoflavone concentrations.

### Determination of free radical scavenging capability using the DPPH method

The solution for the DPPH test was prepared by redissolving 50 mg each of crude oil, degummed oil, gum, and defatted soy flour extract in 20.0, 10.0, 5.0, and 2.5 mL methanol. The concentration of DPPH solution was 0.025 g in 1000 mL of methanol. Two milliliters of the DPPH solution were mixed with 100  $\mu\text{L}$  of each methanol solution and transferred to a cuvette. The reaction solution was monitored at 515 nm for 30 min at 25 °C using a UV-Visible SpectraMax Plus<sup>384</sup> spectrophotometer (Molecular Devices, Sunnyvale). The inhibition percentage of the absorbance of DPPH solution was calculated using the following equation (Moure and others 2000):

$$\text{Inhibition\%} = (\text{Abs}_{t=0\text{min}} - \text{Abs}_{t=30\text{min}}) / \text{Abs}_{t=0\text{min}} \times 100$$

where  $\text{Abs}_{t=0\text{min}}$  was the absorbance of DPPH at zero time and  $\text{Abs}_{t=30\text{min}}$  was the absorbance of DPPH after 30 min of incubation.

### Preparation of fish oil samples oxidized by heating

Menhaden fish oil solution was prepared by dissolving 1000 mg oil in 100 mL of hexane. Each 250 mg of crude oil, degummed oil, gum, and defatted soy flour extract were dissolved in 50 mL of methanol. Two milliliters of the menhaden fish oil solution (10.0 mg/mL of hexane) were added to each test tube (13 × 100 mm). Two-tenths (for 5% [w/w] adding level) or 1.0 mL (for 25% [w/w] adding level) of the crude oil, degummed oil, gum, or defatted soy flour extract solution (5.0 mg/mL of methanol) was mixed with the fish oil solution by vortexing for 1 min. Then, all solutions in the test tubes were evaporated at 30 °C by a centrifuge vacuum evaporator (CentriVap Mobile System; Labconco, Kansas City, Mo., U.S.A.). Thus, the dried samples are the fish oil mixed with 5% or 25% added level of the soy oils, gum, and defatted soy flour extract. The dried samples from only 2.0 mL of menhaden oil without any additive served as the control.

Twelve fish oil samples from each added level of the crude and degummed oils, gum, and defatted soy flour extract and control were prepared. Three of them were used for obtaining an average initial TBA value and another 3 for measuring an average initial DHA and EPA concentrations before heating. The remaining 6 samples were heated at 150 °C in a sand bath for 30 min. Then, 3 of them were used for determining their final TBA value and another 3 for measuring their final DHA and EPA concentrations after heating.

### Determination of fish oil oxidation using TBA method

The TBA values were determined using the method of Pegg (2002). Fish oil sample in the test tube was dissolved in 4 mL butanol and mixed thoroughly. Four milliliters of TBA (0.2% in butanol) solution were added to the tubes. The tubes were capped and vortexed for 1 min. The TBA reaction was carried out at 95 °C in a water bath for 1 h. After the reaction, the tubes were cooled down in ice water. The absorbance of each solution was measured at 523 nm using the UV-Visible SpectraMax Plus<sup>384</sup> spectrophotometer. Each fish oil oxidation after heating was expressed by the increase of TBA absorbance value, which was the difference of the final TBA absorbance value from each heated sample and its corresponding average initial TBA absorbance value before heating.

### Determination of DHA and EPA changes using GC method

The DHA and EPA were determined using the method of Li and Watkins (2002). Fish oil sample was mixed with heptadecanoic acid (C17:0) (0.1 mg/mL in hexane), as an internal standard, for the DHA and EPA analyses. After adding 2 mL BCl<sub>3</sub>-methanol and 1 mL 2,2'-dimethoxypropane, all test tubes were capped and incubated at 60 °C in a water bath for 10 min to perform the derivatization of fatty acid methyl esters. Then, 1 mL hexane and 1 mL water were added to the tubes and vortexed for 30 s. The upper hexane layer was transferred to another tube, dried with anhydrous sodium sulfate, and transferred to a GC vial.

A gas chromatograph (Hewlett Packard 5890, Agilent Technologies, Palo Alto, Calif., U.S.A.) with an FID detector was used to deter-

mine the DHA and EPA concentration. Helium was used as a carrier gas with a column flow rate of 1.2 mL/min. The injection volume was 5 μL and the split ratio was 1:100. The injector and detector temperatures were 250 and 270 °C, respectively. The oven temperature program was set to hold at 50 °C for 3 min and then increased at 4.0 °C/min to 250 °C. The column was a Supelco SP2380 (30 m × 0.25 mm). The concentrations of DHA and EPA were calculated using the C17:0 internal standard as a reference. The percentage of retained DHA or EPA in the fish oil after heating was obtained by comparing its final concentration in each heated sample to its corresponding average initial concentration before heating.

### Statistical analysis

The determination of the total phenolic, tocopherol, and isoflavone contents and the DPPH free radical scavenging test were performed in triplicate. The TBA, DHA, and EPA analyses were performed 3 times as well. The means and standard deviations were calculated and the data were analyzed by 1-way ANOVA with multiple comparisons at  $\alpha = 0.05$  by using SAS (SAS Inst. Inc., Cary, N.C., U.S.A.).

## Results and Discussion

### Total phenolic, isoflavone, and tocopherol contents of the crude and degummed oils, gum, and defatted soy flour extracts

The yield of soybean crude oil, degummed oil, and gum was  $16.2 \pm 0.7$ ,  $12.5 \pm 1.8$ , and  $1.9 \pm 0.4\%$  (based on whole soy flour weight), respectively. These values are similar to the yields produced by industrial scale soybean oil extraction and refining. Soybean usually contains 17% to 22% lipid and 1.5% to 2.5% of gum (Liu 1997). Soybean also consists of about 35% protein and 30% carbohydrate, which are hexane insoluble components and the major composition of the defatted soy flour (Liu 1997). In this study, the yield of defatted soy flour extract using methanol was  $7.5 \pm 0.8\%$  (based on whole soy flour weight).

The defatted soy flour extract had the highest concentration of phenolic compounds and isoflavones among the soy products (Table 1). The total phenolic content in the defatted soy flour extract was 11.3 μg catechin equivalent/g and approximately 1.5 times higher than that of the crude oil and twice as high as degummed oil or gum (Table 1). The concentration of isoflavones in defatted soy flour extract was 55 mg/g, with 0.3 and 0.2 mg/g in the gum and crude oil, respectively, and was under the detection limit in degummed oil (Table 1). The polarities of the phenolics and isoflavones are higher than triglyceride and other lipids. They are readily extracted by more polar solvents such as methanol rather than hexane. A similar observation was reported in the study by Sun and others (2006), in which the total phenolic content in oat extract obtained by methanol was over 3 times higher than the extract produced by hexane, although the yield of hexane extract was higher than the methanol extract. Opposite to the total phenolic and isoflavone contents, the total tocopherol content (215 μg/g) in the defatted flour extract was significantly lower than the oils or

**Table 1 – Total phenolic, tocopherol, and isoflavone contents in crude and degummed oils, gum, and defatted soy flour extract.**

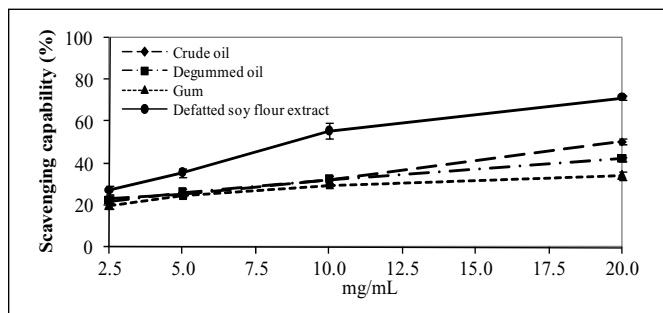
Samples	Crude oil	Degummed oil	Gum	Defatted soy flour extract
Phenolics (μg/g)	$7.1 \pm 0.4^a$	$6.1 \pm 0.6^a$	$6.0 \pm 0.6^a$	$11.3 \pm 1.5^b$
Tocopherols (μg/g)	$387 \pm 16^a$	$414 \pm 23^a$	$238 \pm 15^b$	$215 \pm 10^b$
Isoflavones (mg/g)	$0.2 \pm 0.1^a$	ND	$0.3 \pm 0.1^a$	$55.0 \pm 4.0^b$

ND = not detected. Data with different letters in a row are significantly different ( $P < 0.05$ ).

gum (Table 1). The degummed oil had the highest tocopherol concentration (414  $\mu\text{g/g}$ ), followed by crude oil (388  $\mu\text{g/g}$ ) and gum (238  $\mu\text{g/g}$ ). The tocopherol content in soybeans varies significantly by different varieties and usually ranges from 190 to 300  $\mu\text{g/g}$  (dry basis) (Liu 1997). Compared to phenolics and isoflavones, tocopherols are less polar and largely extracted by a nonpolar solvent such as hexane. Higher tocopherol content in the extract by using hexane than acetone or methanol was reported by several studies (Moreau and others 2003; Sun and others 2006). Thus, in this study, more hydrophilic antioxidants may be highly concentrated in defatted soy flour extract while more lipophilic antioxidants may be concentrated in the oils and gum.

### Free radical scavenging capability of the crude and degummed soy oils, gum, and defatted soy flour extract

The results of free radical quenching capabilities (TEAC) of the oils, gum, and defatted soy flour extract measured by the DPPH method are shown in Figure 1. The free radical scavenging capability of defatted soy flour extract was significantly higher than the oils or gum, and increased to 71.5% at 20 mg/mL of concentration. At that concentration, the scavenging capabilities were 50.7%, 42.3%, and 34.2% in the crude oil, degummed oil, and gum, respectively. The order of the free radical scavenging capability from high to low was defatted soy flour extract, crude oil, degummed oil, and gum. The DPPH free radical scavenging test has been widely used for evaluating antioxidant activity of grain and cereal extracts (Bryngelsson and others 2002; Sun and others 2006). These studies also indicated that antioxidant activities of methanol extracts from grains and cereals determined using the DPPH test were positively correlated with the concentration of phenolic acids in the extracts. In this study, the order of the DPPH radical quenching capabilities is in agreement with their concentration of phenolic compounds (Table 1). The higher DPPH radical scavenging capability of defat-



**Figure 1**—Free radical quenching capabilities (TEAC) of crude or degummed oil, gum, and defatted soy flour at different concentrations measured by the DPPH method.

**Table 2**—Retained DHA and EPA in menhaden oils mixed with crude or degummed oil, gum, and defatted soy flour extract after heating at 150 °C for 30 min.

Sample	Control	Fish oil mixed with 5% (w/w)			
		Crude oil	Degummed oil	Gum	Defatted soy flour extract
DHA (%)	29.9 ± 4.1 <sup>a</sup>	31.5 ± 1.6 <sup>a</sup>	32.8 ± 2.4 <sup>a</sup>	46.7 ± 1.0 <sup>b</sup>	62.8 ± 9.2 <sup>c</sup>
EPA (%)	37.2 ± 2.1 <sup>a</sup>	38.0 ± 0.6 <sup>a</sup>	38.9 ± 1.3 <sup>a</sup>	52.9 ± 0.7 <sup>b</sup>	67.7 ± 3.7 <sup>c</sup>
Sample	Control	Fish oil mixed with 25% (w/w)			
		Crude oil	Degummed oil	Gum	Defatted soy flour extract
DHA (%)	29.9 ± 4.1 <sup>a</sup>	36.4 ± 2.2 <sup>b</sup>	39.0 ± 2.1 <sup>b</sup>	49.6 ± 0.3 <sup>c</sup>	71.5 ± 2.3 <sup>d</sup>
EPA (%)	37.2 ± 2.1 <sup>a</sup>	45.6 ± 0.2 <sup>b</sup>	46.0 ± 3.5 <sup>b</sup>	55.6 ± 1.3 <sup>c</sup>	75.9 ± 4.1 <sup>d</sup>

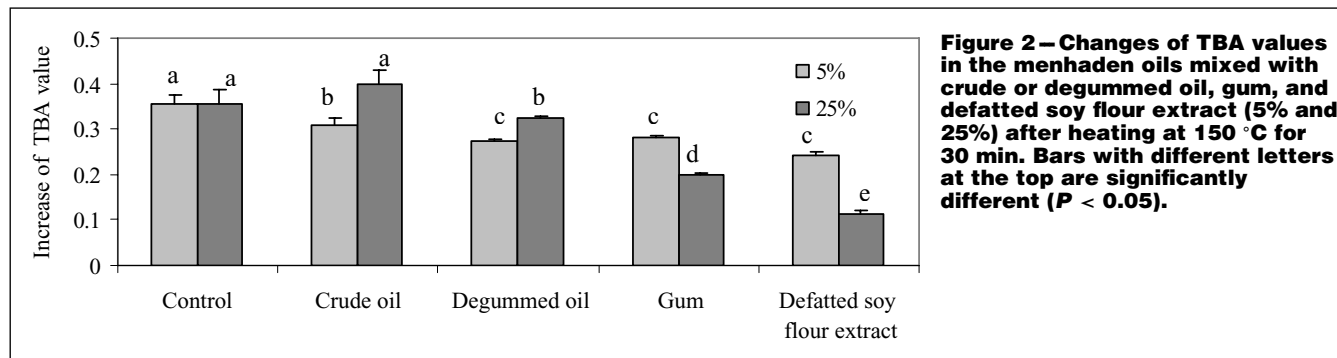
Initial levels of DHA and EPA are 2.3 and 2.7 mg before heating, respectively. Data with different letters in each row are significantly different ( $P < 0.05$ ).

ted soy flour extract suggests that most antioxidants in soybean are hydrophilic compounds and not likely extracted by hexane. Even though the hexane extract (the crude oil) would have contained a higher level of the lipophilic tocopherols, their antioxidant activity was not as important as the hydrophilic antioxidants, which remained in the defatted soy flour. Another possible reason could be a dilution effect brought about by greater extraction of neutral lipid material such as triglyceride and fatty acids. Adom and Liu (2005) found that the hydrophilic antioxidant activity in grains and cereals contributed over 98% of the total antioxidant activity.

### Capabilities of the crude and degummed oils, gum, and defatted soy flour extract in preventing DHA and EPA oxidation and stabilizing menhaden oil during heating

The capabilities of the oils, gum, and defatted soy flour extract in preventing DHA and EPA oxidation during heating are shown in Table 2. The original EPA and DHA in used menhaden oil are 13.5% and 11.5%, respectively. Significantly higher retained DHA and EPA were found in the fish oils mixed with the gum and defatted soy flour extract. In the control group, only 29.9% of DHA and 37.2% of EPA in the fish oil were retained after being heated at 150 °C for 30 min, while 49.6% to 71.5% DHA and 55.6% to 75.9% EPA were retained in the fish oils mixed with 25% gum or 25% defatted soy flour extract. For the crude and degummed oils, the fish oil stabilizing capabilities were achieved only when they were added to the fish oil at a level above 25%. It has been reported that crude soybean oil showed a stronger capacity to inhibit lipid oxidation than refined oil because it contains higher phenolic pigments, which are removed during oil refining (Xu and others 2005). However, because of a high concentration of susceptible unsaturated fatty acids (C18:1 and C18:2) in the soy oils, the increase of TBA reactive oxidation products in the fish oil mixed with 25% crude or degummed oil was significantly higher than the fish oil with the 5% addition level after heating (Figure 2). The significantly higher antioxidant capability of defatted soy flour extract in stabilizing fish oil was also reflected by the lower increase of TBA reactive oxidation products than the oils or gum (Figure 2). Some studies indicated that the TBA reactive substances in fish oil were increased 3 to 4 times at 4 °C and 2 to 3 times at -18 °C during 30 d of storage (Boran and others 2006). The level of *n*-3 fatty acids dropped from 120 mg to 40 mg/g oil after 7-d storage at 25 °C (Borquez and others 1997). In this study, the change of TBA value in the fish oil mixed with 25% of defatted soy flour extract was 3 times lower than the control group, although the fish oil oxidation was accelerated at 150 °C.

The higher level of phenolics in defatted soy flour extract may have contributed to its higher antioxidant capability in stabilizing the menhaden oil during heating. It was reported that the phenolic antioxidants are most likely responsible for preventing bulk oil



**Figure 2 – Changes of TBA values in the menhaden oils mixed with crude or degummed oil, gum, and defatted soy flour extract (5% and 25%) after heating at 150 °C for 30 min. Bars with different letters at the top are significantly different ( $P < 0.05$ ).**

oxidation (Frankel 1996). The significantly higher soy isoflavones in defatted soy flour extract are the most important antioxidants because of their phenol structure and redox potential that provide excellent antioxidant activity (Meng and others 1999). The capabilities of Chardonnay grape and black raspberry seed flour extracts rich in phenolic antioxidants in preventing *n*-3 fatty acids in menhaden oil oxidation were reported by Luther and others (2007). In their study, the oxidation of EPA and total *n*-3 fatty acids in menhaden oil mixed with 1.5% of Chardonnay grape and black raspberry seed flour was retarded after 80 °C incubation for 4.5 h. They also found that the DHA and EPA in fish oil with tocopherols at 130 ppm were oxidized as fast as the control group without any stabilization. This result is in agreement with our study in that a lower capability of the crude and degummed oil in stabilizing the fish oil was observed, although the oils had higher level of tocopherols. Sun and others (2006) also found that a methanol extract of oat demonstrated greater capability in inhibiting the DHA oxidation during heating than the hexane extract with higher tocopherol content. Some studies found that an oat extract with higher level of phenolics significantly improved the stability of vegetable oils at a frying temperature of 180 °C and reduced peroxide values during 26 d of storage at 60 °C (Duve and White 1991; Tian and White 1994).

## Conclusions

The defatted soy flour extract showed the greatest capability for stabilizing menhaden oil and preventing DHA and EPA oxidation during heating. The extract could significantly reduce the degradation of health beneficial omega-3 long-chain polyunsaturated fatty acids in foods during cooking. This would also prevent the production of undesirable and toxic lipid oxidation products and maintain the health function and quality of foods fortified with fish oil. Therefore, defatted soy flour could be utilized to extract soy antioxidants in order to stabilize fish oil and also enhance health promoting potential with the inclusion of soy isoflavones.

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