Characterization of Refined Oils from Atlantic Salmon Belly as Affected by Degumming

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ABSTRACT

This study was carried out to produce crude salmon oil from salmon bellies and purified oil by three purification stages: degumming with three different agents (hot water, 85% phosphoric acid, and 0.3% citric acid), neutralization with alkali, and bleaching with activated carbon. The crude and purified oils were analyzed for yield, peroxide value, *p*-anisidine value, free fatty acid, heavy metal and fatty acid composition. The results showed that yield of refined salmon oil was 33.7% of raw material used. Free fatty acid content, and peroxide and *p*-anisidine values were within acceptable ranges which indicated that the refined oil produced was of an acceptable quality. The degummed oil using citric acid and phosphoric acid had reduced iron and copper contents when compared to those formed in oil degummed with hot water, and the values were within the standard for edible fish oil. Besides fatty acid composition, refined oil showed a higher percentage of monounsaturated fatty acids and polyunsaturated fatty acids. The ratio of omega-3 to omega-6 fatty acids was about 1.58-2.17.

Keywords: salmon oil, refining process, degumming, neutralization, bleaching

INTRODUCTION

Commercial processing of frozen salmon generally requires the removal of viscera, heads, tails, frame, and belly, which accounts for approximately 40% of the wet weight of salmon (Cooper *et al*., 2014). The belly itself constitutes about 5-9% and could be used to provide higher valued product such as fish oil. Normally, the main production of fish oil in Thailand comes from by-product of tuna processing. Therefore, salmon belly can serve as an interesting alternate material for fish oil production. Recently, fish oil is used in nutraceutical or dietary supplement in the form of capsule for supporting health and preventing some diseases. Fish oil has high levels of eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3). These fatty acids are long chain *n*-3 polyunsaturated fatty acids (*n*-3 PUFA) and

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cannot be synthesized in the human body. They must be obtained through diet or supplementation (Zatsick and Mayket, 2007). These fatty acids have beneficial and/or preventative effects toward neurological disorders in adults or elderly (Freemantle *et al*., 2006), and in the etiology of metabolic pathologies such as cardiovascular, hypertension, hyperlipidemia (Zhang *et al*., 2010), immunologic, inflammatory diseases (Eslick *et al*., 2009), diabetes and obesity (Horrocks and Young, 1999).

For fish oil production, the refining process is considerably important to obtain high quality of the oil. The refined fish oils must have natural-flavor and oxidative stability, or be free from rancid odor and taste. Generally, the refining process is composed of degumming, neutralization, and bleaching, which are operations to remove undesirable components of crude oils. Degumming is a process to remove phosphatides, consisting of two types: hydratable phosphatides (i.e. phosphatidylcholine and phosphatidylinositol) and non-hydratable phosphatides (i.e. calcium and magnesium salts of phosphatidylethanolamine, and phosphatidic acid) (Johnson, 2002). Degumming usually uses water and the amount of water should be balanced with the hydratable phosphatide content of the oil to cause much soluble hydratable phosphatides in sludge or gums. However, water could not remove nonhydratable phosphatide, which would still be dissolved in the oil. Therefore, this research aimed to increase efficiency of the degumming process by using phosphoric acid and citric acid compared to hot water.

There have been reports on using degumming agents for the degumming step in oils, but few have been done on salmon oil. Sathivel *et al*. (2003) reported that using 0.3% citric acid solution in the degumming process of catfish oil from viscera and menhaden oil could separate the oil and gums off. The gums included both hydratable and nonhydratable phosphatides and proteinaceous compound. The other study of Crexi *et al*. (2010) indicated that using phosphoric acid (85 % v/v) in the degumming step of croaker oil resulted in high soluble nonhydratable phosphatide. Degumming can be conducted either as a separate operation or simultaneously with neutralization, a step to remove most of the free fatty acids by reacting with sodium hydroxide (NaOH) to become sodium soaps. The amount and strength of NaOH used depend on the amount of free fatty acids present in the oil due to excessive NaOH can hydrolyze triglycerides and reduce the yield of neutralized oil. The proper amount of NaOH used is determined by free fatty acid content or acid values to evaluate operation efficiency that affects further refining step. For bleaching, it is mainly used to improve the color quality of the oil by eliminating the pigment present in the initial material. It also contributes to the breakdown of peroxide and removal of residual trace of soap.

The aims of this study were to produce fish oil from salmon belly and to examine the effect of degumming agents (water, phosphoric acid, and citric acid) on the chemical properties of the refined oil.

MATERIALS AND METHODS

Raw materials

Atlantic salmon (*Salmo salar*) bellies were obtained from Food Project (Siam) Company Limited, Samut Sakorn Province. They were transported in frozen condition to the laboratory of the Department of Fishery Products, Faculty of Fisheries, Kasetsart University. Upon arrival, they were kept in a freezer at -18°C for further study.

Production of crude salmon oil

Frozen salmon bellies were thawed at room temperature (30-32°C), trimmed to remove bones and fins, cut into small pieces, heated with steam cooker at 95-100°C for 15 min, and crushed with a silent cutter for 5-10 min. The solids were separated from the liquids by primary filtering with a cheesecloth. The remaining solids were pressed by hydraulic press to separate the pressed cake from the pressed liquid. The pressed liquid was composed of salmon oil, soluble protein, and water. The upper phase (salmon oil) was heated at 80°C for 15 min to separate the remaining soluble protein by centrifugation at 6,000 rpm (5,640 x g), 4°C for 20 min. The obtained crude oil was kept in amber bottles, flushed with nitrogen gas, and stored at -18°C in a freezer until the crude oil was further analyzed for chemical properties.

Determination of chemical properties of crude salmon oil

Yield

Yield of crude salmon oil was expressed as percentage of raw material used.

Peroxide value

Peroxide value (PV) was determined following AOCS Official method Cd 8-53 (1998). PV was expressed as milliequivalent (meq) peroxide to 1 kg sample.

ρ-Anisidine value

ρ-Anisidine value (ρ-AV) was determined in terms of millimoles of 2-alquenal to kilogram of sample oil by AOCS Official method Cd 18-20 (1998).

Free fatty acids

Free fatty acids (FFA) analysis was determined and expressed as the percentage of oleic acid according to AOCS Official method Ca 5a-40 (1998).

Heavy metals

Heavy metals (HM) including copper (Cu) and iron (Fe) ions of crude salmon oil and degummed oils were determined according to AOAC Official method TE-CH-134 (2005). The oil sample was digested with nitric acid and hydroperoxide under pressure in closed Teflon vessels and heated by microwave oven (Ethos Sel, Milestone, Italy). The temperature was set to digest the samples in three steps, starting from room temperature (25°C) to 80°C for 4 min, from 80°C to 130°C for 7 min, and from 130°C to 170°C for 5 min, and held at 170°C for 10 min. Then the sample solution was diluted with Mili-Q water and analyzed by an inductively coupled plasma optical-emission spectrometer (ICP-OES, Optima 4,300 DV, Perkin-Elmer, USA), which was expressed as mg. kg^{-1} by comparison with

calibration standards of Cu and Fe. The limits of detection were 0.10 mg. kg^{-1} of Cu and 0.65 mg. kg^{-1} of Fe.

Fatty acid composition

Fatty acid composition (FAC) was determined using fatty acid methyl esters (FAME), prepared as described by AOCS Official method Ce 1b-89 (1998). The oil sample was saponified with methanolic NaOH, and FFAs were methylated with boron trifluoride in methanol. The FAME was extracted with iso-octane, dried over anhydrous sodium sulphate and identified by gas chromatography (GC, 17A, Shimadzu, Japan) equipped with flame ionization detector. The capillary column (30 m x 0.25 mm, 0.25 µm film thickness) was used for analysis. The FAME analysis was carried out in triplicate by injecting 1.0 µl; split ratio 1:80. The injection and detector temperatures were held at 250°C and 260°C, respectively. The flow rate of nitrogen carrier gas was at 1.0 ml/min, linear speed was 30 cm/s, the oven temperature held at 170°C for 5 min and increased to 240°C at 5°C/min, and then held at 240°C for 30 min. FAME sample (1 µl) was injected into GC in splitless mode. The peak of FAME sample was identified and compared to the retention times of each peak of fatty acid standard PUFA No. 3 from Menhaden oil (Sigma-Aldrich, USA), which was expressed as the percentage area of each FAME mixture. Saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), omega-3 fatty acid (*n*-3), omega-6 fatty acid (*n*-6), and ratio of omega-3 to omega-6 fatty acids (*n*-3/*n*-6) were also calculated.

Production and characterization of refined salmon

Degumming

The crude salmon oil sample was heated in a water bath at 80-85°C for 15 min and agitated by stirrer with two-bladed propellers at 250 rpm for 15 min. The hot crude oil was equally divided into 3 portions. Three different agents were used for the degumming process, namely, (1) hot water at 100°C (1% w/w of oil), (2) 85% phosphoric acid (1% w/w of oil), and, (3) 0.3% of citric acid (1% w/w of oil). Each degumming agent was added into hot crude oil with agitation at 250 rpm for 15 min. After cooling down at room temperature, the mixture was centrifuged at a speed of 6,000 rpm for 20 min at 4°C to separate the hydrated gum of phosphatides from degummed oil. The degummed oils were weighed, kept in amber bottles, flushed with nitrogen gas, and stored at -40°C in a freezer for further analysis. The degummed oils were then subjected to neutralization.

Neutralization

Degummed oils were heated at 85°C and agitated at 250 rpm for 10 min. Then 20° Baumé (Bé) of NaOH (14.07-14.65% of actual NaOH by weight) was added following AOCS Official Method Ca 9b-52 (1998). The oil was heated again at 85°C for 60 min and centrifugedat 6,000 rpm, 20 min at 4°C to separate neutralized oil from soapstock. The neutralized oils were washed with hot water (10% of oil), followed by agitation at 250 rpm for 10 min. This step was repeated three times until the oils were

clear. The subsequent neutralized oils were maintained at 50°C and centrifuged at 6,000 rpm to separate the residue. The neutralized oils were weighed, kept in amber bottles, flushed with nitrogen gas, and stored in a freezer at -40°C for further analysis. The neutralized oils were further processed for bleaching.

Bleaching

The neutralized oils were added with 2% (w/w) of activated carbon. The mixtures were agitated at 500 rpm for 15 min (room temperature). Filtration was done with Buchner funnel and filter paper No. 5 containing diatomaceous earth, and then the water was removed by using a rotary evaporator (decanter). The bleached oils were weighed, kept in amber bottles, flushed with nitrogen gas, and stored at -40°C in a freezer until further analysis.

Statistical analysis

The experiment was run in triplicate with a completely randomized design (CRD). Means and deviations of the data collected were reported. Analysis of variance (ANOVA) was performed (SPSS statistics 22.1, IBM Corporation, Armonk, NC., USA.) to detect differences in data among samples, followed by Duncan's Multiple Range Test with $P<0.05$ level of significance.

RESULTS AND DISCUSSION

Yield of salmon oil

Percentage of severed salmon products derived from crude salmon oil processing is shown in Table 1. The products included crude salmon oil, pressed cake, soluble protein,and water, resulting in 33.7±6.1, 36.4 ±1.2,10.3±0.6, and17.6±4.9%, respectively. The crude salmon oil had one third of the initial salmon belly flesh, which was higher than the finding of Strobel *et al*. (2010), who reported that the total fat content of salmon fillets (wild Atlantic salmon, farmed Atlantic salmon, rainbow trout, and herring) had 2.1±1.1, 12.3±4.7, 7.9±6.3, and 12.7±3.1%, respectively. The products derived from crude salmon oil production, such as pressed cake and soluble protein could be further used for protein hydrolysate production (Zhong *et al*., 2002). The results indicated that salmon belly is suitable to be used as raw material for salmon oil production due to the high amount of lipid accumulated within bellies.

Table 1. The percentage of salmon products derived from salmon oil production

Products	Percentage (%)
Fish oil	33.7 ± 6.1
Pressed cake	36.4 ± 1.2
Soluble protein	10.3 ± 0.6
Water	17.6 ± 4.9

Values are given as mean ±standard deviation from triplicate determination.

The crude salmon oil was transferred to undergo purification process including degumming, neutralization, and bleaching. The degumming process was aimed to remove hydrated gum or phosphatides, which consisted of complex lipids, resin, sugar, and proteinaceous compound (Sikorski, 2002). The percentage of weight loss compared to initial crude oil is shown in Table 2. The results showed that the effect of using hot water, phosphoric acid, and citric acid on percentage of weight loss in degumming process was not significantly different ($p \geq 0.05$), but there was a significant difference ($p < 0.05$) compared to the oil after neutralization and bleaching steps. The neutralization step resulted in a higher weight loss than the degumming process because neutralization can eliminate free fatty acid, carotenoid pigment, phospholipids, sulfur compound, insoluble substances in oil, and soluble matter in water to become soapstock (Sikorski, 2002). During the bleaching process, activated carbon can trap undesirable odors from the oxidized product and absorb pigment, residual trace of soap, and trace metals (Rossi *et al*., 2003). This resulted in a higher weight loss. After purification process, weight loss of purified salmon oil was almost 67-70 % or the obtained purified oil was approximately 30% of initial crude salmon oil. The results indicated that a high amount of undesirable compounds could be eliminated by the purification process.

Table 2. The percentage of weight loss in each purification step of salmon oil

Steps	Degumming agent	Weight loss (%)
Degumming	Hot water	6.70 ± 0.15^{a}
	Phosphoric acid	4.51 ± 0.17^{a}
	Citric acid	5.95 ± 0.08^{a}
Neutralization	Hot water	38.99 ± 0.33^{b}
	Phosphoric acid	38.59 ± 0.67^{b}
	Citric acid	37.58 ± 0.26^{b}
Bleaching	Hot water	67.02 ± 1.25^{c}
	Phosphoric acid	69.80 ± 2.32^{c}
	Citric acid	68.32 ± 2.23^{c}

Values are given as mean ±standard deviation from triplicate determination. Values in each column with different superscript (a-c) indicate significant differences ($p<0.05$).

Free fatty acid

The presence of free fatty acid (FFA) in the oil is an indication of insufficient process, lipase activity, or hydrolyzed contents. High quality oils should have a low FFA content. The FFA content of crude oil and purified oil are shown in Table 3. The crude oil gave an initial FFA content of 1.39%, as oleic acid. The content was not significantly different ($p \geq 0.05$) after degumming with hot water and citric acid (1.42±0.06 and 1.45±0.06 % as oleic acid, respectively), but was slightly higher ($p<0.05$)

Table 3. Chemical properties of crude salmon oil and refined oil in each purification step using different degumming agents

Steps	Degumming agent	Free fatty acid (% as oleic acid)	Peroxide value (meq. kg^{-1})	Anisidine value	Heavy metals (mg. kg^{-1})	
					Copper	Iron
Crude oil		1.39±0.00^{d}	6.16±0.02^{bc}	1.85±0.04^{c}	<1.00	2.30
Degumming	Hot water	1.42±0.06^{d}	10.95±0.20^{d}	1.55±0.06^{b}	<1.00	6.70
	Phosphoric acid	1.85±0.06^{e}	7.53±0.08^{c}	0.93±0.02^{a}	ND	1.40
	Citric acid	1.45±0.06^{d}	10.96±0.05^{d}	1.47±0.04^{b}	ND	1.38
Neutralization	Hot water	0.27±0.06^{b}	7.51±0.07^{c}	2.01±0.03^{c}	-	-
	Phosphoric acid	0.20±0.00^{a}	4.11±0.01^{a}	2.96±0.01^{e}	-	-
	Citric acid	0.20±0.00^{a}	4.10±0.01^{a}	2.87±0.05^{e}	-	-
Bleaching	Hot water	0.40±0.02^{c}	9.59±0.01^{d}	1.39±0.02^{b}	-	-
	Phosphoric acid	0.39±0.02^{c}	4.10±0.01^{a}	2.44±0.01^{d}	-	-
	Citric acid	0.37±0.01^{c}	4.47±0.02^{a}	2.12±0.02^{c}	-	-

Values are given as mean ±standard deviation from triplicate determination. Values in each column with different superscript (a-e) indicate significant differences ($p<0.05$). ND = Not detected.

after degumming with phosphoric acid (1.85±0.06% as oleic acid). This could be explained by the fact that phosphoric acid is decomposed to give hydrogen ions three times (as 7.25×10^{-3}, 6.31×10^{-8}, and 4.89×10^{-13} at 25°C, respectively), which was similar to the dissociation of citric acid (as 7.10×10^{-4}, 1.70×10^{-5}, and 6.4×10^{-6} at 25 °C, respectively) (Zumdahl and Zumdahl, 2007). Phosphoric acid gives hydrogen ions in a wide period more than citric acid and can hydrolyze ester bond of triglycerides more than citric acid and water.

The FFA content of crude salmon in this study was lower than those derived from viscera, head, and skin (3.48±0.04 %) reported by Huang and Sathivel (2010), but was higher than crude sardine oil (0.21± 0.009 %) as reported by Noriega-Rodriiguez *et al*. (2010). The results obtained on the FFA content of crude salmon oil from this study were in accordance with the guidelines and requirements of quality for producing oil, which should range from 1 to 7 (Young, 1986). However, the FFA content in crude oil significantly decreased after neutralization and bleaching. Most of the FFA in degummed oils was eliminated by reacting with NaOH and forming soapstock to precipitate off and the residue FFA in neutralized oils was absorbed with activated carbon. The results of FFA in neutralized oils after degumming with phosphoric acid and citric acid were similar to neutralized oil derived from viscera, head, and skin reported by Huang and Sathivel (2010). The FFA of bleached oils slightly increased. This is similar to the bleached sardine oil from the study of Noriega-Rodriiguez *et al*.

(2010). However, the FFA content of salmon oil after neutralization and bleaching did not exceed 0.5 % as oleic acid. Refined fish oil is recommended to have FFA between the range of 1.8 to 3.5 % as oleic acid (Young, 1986).

Peroxide value

Peroxide value (PV) is an indication of primary changes in oil, which is oxidized to generate hydroperoxide. Incident peroxide compound is not an odoriferous substance so it could not be identified by sensory evaluation. However, peroxide compounds could be decomposed and changed to small volatile compounds, such as carbonyl, aldehyde, ketone, alcohol, and hydrocarbons, which are the products of the secondary changes to affect the rancid off-flavors of oxidized oil (Boran *et al*., 2006). Table 3 shows PV of crude oil compared to purified oil. PV of degummed oils with 3 degumming agents significantly increased when compared to crude oil. Particularly, using hot water during degumming gave the highest PV in the oil due to the broken molecule of water and repeated heat in this step were catalysts to the formation of hydroperoxide from the reaction between double bonds of released free fatty acid and oxygen by autoxidation. PV decreased during neutralization because NaOH reacted with most of the FFA and oxidation products, and slightly increased during bleaching due to the oxygen restarted to attach double bond of long chain unsaturated fatty acid again. PV of crude salmon oil was 6.16 ± 0.02 meq.kg^{-1}, which was in agreement with the guidelines for required quality for producing oil (3 to 20 meq. kg^{-1} for crude oil) (Young, 1986). PV of degummed oils was slightly higher than crude oil, but it decreased after neutralization and bleaching by reacting with NaOH to form soapstock and absorption with activated carbon. The results indicated that PV of the refined salmon oil after purification process did not to exceed 10 meq. kg^{-1} according to the standards of edible oils (Codex, 2009).

ρ-*Anisidine value*

ρ-Anisidine value (ρ-AV) is a measure of the secondary products of fat and oil oxidation especially aldehydes (principally 2-alkenals and 2, 4-dienals) and could be evaluated through sensory means, given the strong rancid smell of fish oil (Wrolstad *et al*., 2005). The ρ-AVs of crude salmon oil and purified oil are shown in Table 3, which are significantly different ($p<0.05$). The ρ-AV of crude oil was 1.85±0.04, which was in agreement with the guideline of required quality for producing oil with a desirable and acceptable shelf life (4 to 60 for crude oil) (Young, 1986). The ρ-AV of all fish oil samples in every purification process did not exceed 3 which is in accordance with the standard of fish oils which should be lower than 20 (Codex, 2013). The results of ρ-AV of refined salmon oil after purification process was acceptable, which indicated that the oil was oxidized to a low extent.

Heavy metals

The remaining residue of heavy metals (HM), such as copper (Cu) and iron (Fe) in fish could come from environmental conditions resulting in HM accumulation in fish body. Consumption of fish oil contaminated with high HM residues might

cause harm to the health of consumers from accumulated toxin in the body (Rossell, 2009). The HM of crude oil and refined oil are shown in Table 3. Cu and Fe ions in crude salmon oil derived from the belly were less than 1.00 and 2.30 mg. kg^{-1}, respectively, which was in agreement with the suggestion for required crude oil after extraction that it should be less than 2.0 mg. kg^{-1} of Cu (Rubio-Rodríguez *et al*., 2012) and 0.50 to 7.00 mg. kg^{-1} of Fe (Young, 1986). After degumming, the residual of Cu in degummed oil with hot water was less than 1.00 mg.kg^{-1}, but they were not detected in degummed oils with phosphoric and citric acids. The residual of Fe in degummed oil with hot water was up to 6.70 mg. kg^{-1} due to hydration caused by phosphatides insoluble in the oil, especially phosphatidylethanol-amine and phosphatidic acid which can combine with Fe ions resulting in increased nonhydratable phosphatides (Johnson, 2002). However, it was lower for degummed oils with phosphoric acid and citric acid (1.40 and 1.38 mg. kg^{-1}, respectively). These results agreed with the guidelines for food quality control in refined oil, which should be 0.1 mg. kg^{-1} of Cu and 2.5 mg. kg^{-1} of Fe (Codex, 2009). Cu and Fe ions in degummed oils with phosphoric acid and citric acid were significantly lower than in degummed oil with hot water. Citric and phosphoric acids could chelate metals by reducing their redox potentials and stabilizing the oxidized form of the metal. Moreover, carboxyl groups of citric acids and phosphate groups of phosphoric acids bind with metals and form complexes to precipitate off during process (Akon and Min, 2002). The results in this study demonstrated that degummed oils with phosphoric acid and citric acid had lower Cu and Fe contamination in the oils.

Fatty acid composition

The fatty acids compositions (FAC) of crude salmon oil and purified oil are shown in Table 4. The FAC in purified oil was not significantly different ($p>0.05$) from that in crude oil. There are 3 groups of fatty acids found in salmon oil,namely: 1) saturated fatty acids (SFA) consisting of myristic acid (MRA, C14:0), plamitic acid (PMA, C16:0), and stearic acid (STA, C18: 0); 2) monounsaturated fatty acids (MUFA) including plamitoleic acid (PTA, C16:1 *n*-7), veccenic acid (VCA, C18:1 *n*-7), oleic acid (OA, C18:1 *n*-9), gadoleic acid (GDA, C20 :1 *n*-9), and eucic acid (EUA, C22:1 *n*-9); and, 3) polyunsaturated fatty acids (PUFA) consisting of linoleic acid (LA, C18:2 *n*-6), alpha–linolenic (ALA, C18:3 *n*-3), stearidonic acid (SDA, C18:4 *n*-3), eicosatetraenoic acid (ETA, C20:4 *n*-3), arachidonic acid (AA, C20:4 *n*-6), eicosapentaenoic acid (EPA, C20:5 *n*-3), docosapentaenoic acid (DPA, C22:5 *n*-3), and docosahexaenoic acid (DHA, C22:6 *n*-3). There were 16 fatty acids found in salmon oil for this study which were different from the findings of Ackman (2000) who only found 14 fatty acids excluding VCA and ETA in salmon oil obtained from fillet. Strobel *et al*. (2010) also showed that salmon oil derived from fillets of wild Atlantic salmon, farmed Atlantic salmon, rainbow, and herring had only 13 fatty acids excluding MRA, VCA, and ETA. The major fatty acids found in salmon oil derived from the belly included OA, PMA, LA, DHA, EUA, EPA, and GDA accounting for 74.70 to 77.08% of the total fatty acids. Table 5

Table 4. Fatty acid composition (%) of crude salmon oil and refined oil in each purification step using different degumming agents

Fatty acids	Crude oil	Degumming			Neutralization			Bleaching		
		Hot water	Phosphoric acid	citric acid	Degummed with hot water	Degummed with phosphoric acid	Degummed with citric acid	Degummed with hot water	Degummed with phosphoric acid	Degummed with citric acid
C14:0	3.81±0.51^{a}	4.26±0.28^{a}	4.09±0.21^{a}	3.81±0.72^{a}	3.74±0.19^{a}	4.12±0.31^{a}	4.02±0.10^{a}	3.97±0.23^{a}	3.99±0.08^{a}	3.95±0.15^{a}
C16:0	10.70±0.23^{a}	13.30±1.15^{a}	12.71±1.00^{a}	12.09±1.10^{a}	11.72±1.75^{a}	11.96±0.86^{a}	12.02±0.72^{a}	12.28±0.81^{a}	11.91±0.82^{a}	11.99±1.12^{a}
C18:0	2.29±0.05^{a}	2.85±0.29^{a}	2.68±0.27^{a}	2.53±0.27^{a}	2.59±0.46^{a}	2.29±0.49^{a}	2.23±0.79^{a}	2.66±0.37^{a}	2.54±0.22^{a}	2.50±0.21^{a}
C16:1 *n*-7	4.44±0.15^{a}	4.62±0.04^{a}	4.57±0.14^{a}	4.70±0.16^{a}	4.58±0.13^{a}	4.66±0.07^{a}	4.53±0.43^{a}	4.65±0.16^{a}	4.40±0.18^{a}	4.51±0.09^{a}
C18:1 *n*-7	3.58±0.28^{a}	3.74±0.63^{a}	4.00±0.18^{a}	3.59±0.47^{a}	4.11±0.74^{a}	3.81±0.96^{a}	3.30±0.42^{a}	3.84±0.06^{a}	4.03±0.13^{a}	3.66±0.17^{a}
C18:1 *n*-9	29.87±0.93^{a}	29.51±1.19^{a}	29.56±0.76^{a}	29.09±1.17^{a}	28.50±0.84^{a}	27.79±1.18^{a}	29.51±1.08^{a}	30.04±0.80^{a}	29.29±1.08^{a}	30.69±1.21^{a}
C20:1 *n*-9	5.34±0.20^{bc}	5.52±0.12^{bc}	5.49±0.08^{bc}	5.59±0.42^{bc}	5.14±0.34^{bc}	5.62±0.27^{ab}	5.83±0.08^{b}	4.91±0.31^{bc}	5.29±0.25^{bc}	4.47±0.09^{c}
C22:1 *n*-9	6.22±0.69^{a}	5.47±0.14^{a}	5.56±0.20^{a}	5.46±0.40^{a}	5.34±0.61^{a}	5.89±0.29^{a}	5.71±0.25^{a}	5.17±0.14^{a}	5.31±0.19^{a}	5.53±0.29^{a}
C18:2 *n*-6	9.78±0.75^{a}	8.84±0.53^{a}	10.05±1.03^{a}	10.61±1.22^{a}	11.70±1.27^{a}	10.53±0.37^{a}	10.42±0.90^{a}	10.07±0.56^{a}	9.92±0.48^{a}	10.43±0.33^{a}
C18:3 *n*-3	3.60±0.37^{a}	3.07±0.13^{a}	3.50±0.67^{a}	3.68±0.55^{a}	3.61±0.80^{a}	3.94±0.34^{a}	3.66±0.31^{a}	3.77±0.24^{a}	3.84±0.35^{a}	3.88±0.35^{a}
C18:4 *n*-3	1.32±0.42^{a}	0.80±0.08^{a}	0.95±0.23^{a}	0.85±0.23^{a}	1.01±0.30^{a}	1.04±0.08^{a}	0.97±0.10^{a}	1.09±0.07^{a}	1.09±0.02^{a}	1.10±0.03^{a}
C20:4 *n*-3	1.41±0.13^{a}	1.14±0.09^{a}	1.22±0.08^{a}	1.28±0.04^{a}	1.16±0.17^{a}	1.34±0.04^{a}	1.30±0.15^{a}	1.29±0.05^{a}	1.29±0.11^{a}	1.27±0.06^{a}
C20:4 *n*-6	0.94±0.18^{a}	1.41±0.21^{a}	1.26±0.47^{a}	1.56±0.67^{a}	1.55±0.54^{a}	1.72±0.79^{a}	2.03±0.22^{a}	1.06±0.24^{a}	1.22±0.19^{a}	1.10±0.32^{a}
C20:5 *n*-3	5.49±0.44^{a}	4.30±0.29^{bc}	4.79±0.45^{abc}	4.44±0.67^{abc}	4.22±0.61^{bc}	4.58±0.56^{abc}	4.09±0.05^{c}	5.26±0.13^{bc}	5.13±0.12^{abc}	5.11±0.18^{abc}
C22:5 *n*-3	2.84±0.59^{a}	2.55±0.16^{a}	2.49±0.06^{a}	2.23±0.23^{a}	2.27±0.13^{a}	2.49±0.11^{a}	2.41±0.06^{a}	2.66±0.47^{a}	2.77±0.14^{a}	2.61±0.12^{a}
C22:6 *n*-3	8.55±1.00^{a}	8.30±0.26^{a}	8.92±0.46^{a}	8.58±0.33^{a}	8.75±0.99^{c}	8.33±1.16^{a}	7.97±1.01^{a}	7.25±0.34^{a}	7.85±0.77^{a}	7.03±0.47^{a}

Values are given as mean ±standard deviation from triplicate determination. Values in each row with different superscript (a-c) indicate significant differences ($p<0.05$).

Table 5. Lipid classes of crude salmon oil and refined oil in each purification step using different degumming agents

Fatty acids	Crude oil	Degumming			Neutralization			Bleaching		
		Hot water	Phosphoric acid	citric acid	Degummed with hot water	Degummed with phosphoric acid	Degummed with citric acid	Degummed with hot water	Degummed with phosphoric acid	Degummed with citric acid
Σ SFA	16.80±0.76^{a}	20.41±1.67^{a}	19.48±1.47^{a}	18.43±1.95^{a}	18.05±1.29^{a}	18.37±1.06^{a}	18.27±1.36^{a}	18.91±1.06^{a}	18.44±1.11^{a}	18.44±1.42^{a}
Σ MUFA	49.45±0.93^{a}	48.86±1.98^{a}	49.18±0.56^{a}	48.43±1.21^{a}	47.67±1.72^{a}	47.77±1.18^{a}	48.88±0.55^{a}	48.61±0.66^{a}	48.32±0.93^{a}	48.86±0.78^{a}
Σ PUFA	33.93±1.18^{a}	30.41±1.24^{a}	33.18±1.05^{a}	33.23±1.02^{a}	34.27±1.29^{a}	33.97±1.53^{a}	32.85±1.04^{a}	32.45±0.70^{a}	33.11±1.47^{a}	32.53±0.79^{a}
Σ*n*-3	23.21±1.20^{a}	20.16±1.40^{a}	21.87±1.29^{a}	21.06±0.87^{a}	21.02±0.78^{a}	21.72±1.30^{a}	20.40±1.38^{a}	21.32±0.81^{a}	21.97±1.42^{a}	21.00±0.84^{a}
Σ*n*-6	10.72±0.57^{bc}	10.25±0.08^{c}	11.31±0.66^{abc}	12.17±0.57^{abc}	13.25±0.74^{a}	12.25±0.57^{abc}	12.45±0.84^{ab}	11.13±0.41^{bc}	11.14±0.3^{bc}	11.53±0.13^{abc}
n-3/*n*-6	2.17±0.00^{a}	1.97±0.34^{a}	1.93±0.12^{a}	1.73±0.28^{a}	1.58±0.34^{a}	1.77±0.12^{a}	1.64±0.21^{a}	1.92±0.12^{a}	1.97±0.09^{a}	1.82±0.08^{a}

Σ SFA = sum of saturated fatty acid,
Σ MUFA = sum of monounsaturated fatty acid,
Σ PUFA = sum of polyunsaturated fatty acid,
Σ*n*-3 = sum of omega-3 fatty acid,
Σ*n*-6 = sum of omega-6 fatty acid,
n-3/*n*-6 = ratio of omega-3 fatty acid to omega-6 fatty acid.
Mean value from triplicate data±standard deviation.
Values in each row with different superscript (a-c) indicate significant differences ($p<0.05$).

shows the percentages of SFA, MUFA, and PUFA of crude salmon oil and purified oil in the range of 16.80 – 20.41, 47.67 – 49.45, and 30.41 – 34.27%, respectively. The results were similar to the report of Blanchet *et al.* (2005), who suggested that FAC of wild and farmed Atlantic salmons were 19.0±1.0 and 25.6±2.9% of SFA, 53.7±3.9 and 33.4±7.9% of MUFA, and 27.3±3.0 and 41.0±5.8% of PUFA, respectively.

Dietary ratios of *n*-3/*n*-6 PUFA have been considered to implicate controlling markers of metabolic syndrome. The long chain *n*-3 PUFA has been shown to decrease insulin resistance, triglyceride levels, heart rate, and blood pressure, and increase high density lipoprotein cholesterol (Carpentien *et al*., 2006). Conversely, *n*-6 PUFA can increase inflammatory signals and have been associated with cardiovascular heart disease (Hibbeln *et al*., 2006). The ratio of *n*-3/*n*-6 of salmon oil in this study was in the range of 1.58 – 2.17 that was in agreement with the study of Strobel *et al*. (2010) who denoted that ratio of *n*-3/*n*-6 of salmon oil from fillet was 2.89±1.96. In addition, AA, EPA, and DHA are considered to be the important fatty acids as precursors for eicosanoids in the human body. Normally, AA, DHA and EPA can be synthesized from LA and ALA, respectively, by enzymes (δ-5 and δ-6 desaturases). However, LA and ALA are essential fatty acids andcould not be synthesized in the human body, so they must be obtained from plants and animals. Although humans have the capacity to convert ALA to EPA and DHA, the efficiency of conversion is low, in particular to DHA. Generally, ALA intake increases EPA and DPA, but there is very little increase in DHA in plasma fractions (platelets, white cells and red blood cells) or breast milk (Francois *et al*., 2003; Burdge and Calder, 2005). The results showed that AA in salmon oils of this study were 1.06 – 1.22 %, which were higher than that reported by Ackman (2000) in which AA of salmon oil derived from fillet was 0.5 %. Strobel *et al*. (2010) also indicated that salmon oils from both wild and farmed Atlantic salmon were found to have AA of 0.40±0.11 and 0.40±0.13%, respectively. EPA of refined salmon oils was found to be in the range of 5.11 - 5.26%, which were also higher than the findings of Ackman (2000) who found that EPA was 4.6 %. DHA of refined salmon oils was found to be in range of 7.03 - 7.85%, which was lower than that of a study by Ackman (2000) who reported that DHA was up to 11.9 %. The results were slightly different from the report of Strobel *et al*. (2010) who showed that salmon oils from both wild and farmed Atlantic salmons had DHA at 16.90±6.18 and 8.04±2.67 %, respectively. Therefore, the difference between fatty acid composition and fatty acid content in refined salmon oil depends on the types of initial raw material, extraction process, and refining process, which keep the essential fatty acid and the specific chemical structures.

CONCLUSION

Salmon belly is a good raw material source for fish oil production with a high percentage of yield (33.7±6.1 %). Most of the chemical properties of oils by using three different degumming agents (water, phosphoric acid, and citric acid) were not significantly different ($p \geq 0.05$), except the

degummed oils using citric acid and phosphoric acid which had higher capacity in lowering Cu and Fe in the oils than water. Free fatty acid content, peroxide value, and ρ-anisidine value for all oil samples were within acceptable ranges, indicating that acceptable quality of refined oil can be obtained from salmon belly. Fatty acid composition showed that the percentages of monounsaturated fatty acids and polyunsaturated fatty acids were higher than saturated fatty acids. The ratio of omega-3 to omega -6 was found to be 1.58-2.17.

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