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Total lipid and omega-3 content in Pangasius catfish (*Pangasius pangasius*) and milkfish (*Chanos chanos*) from Indonesia

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ABSTRACT

Introduction: Supplementation of the diet with fish oil omega-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has been associated with multiple health benefits. This study aimed to determine total lipid and omega-3 content in two fishes from Indonesia, which were *Pangasius pangasius* (*P. pangasius)* and *Chanos chanos (C. chanos).* **Methods:** Total lipid was extracted from *P. pangasius* and *C. chanos* and the lipid content was then analysed using gas chromatography–mass spectrometry (GC-MS). **Results:** Lipid content of *C. chanos* (4.63±3.84%) was higher than *P. pangasius* (3.94±1.43%) but less than that found in *Salmo salar* (*S. salar)* which was found to contain 6.98±2.56% lipid. Furthermore, polyunsaturated fatty acid omega-3 (EPA and DHA) analysis showed that *C. chanos* oil contained 0.36% EPA and 1.17% DHA. These levels are lower than that found in *S. salar*, often referred to as the "gold standard" for omega-3 fatty acids. **Conclusion:** *C. chanos* contains considerable amounts of EPA and DHA. As it is widely available in Indonesia, it may be used as source of omega-3 fatty acids instead of salmon.

Keywords: *Chanos chanos*, *Pangasius pangasius*, omega-3 fatty acids

INTRODUCTION

Omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been associated with various health benefits. These dietary polyunsaturated fatty acids (PUFAs) play a role in the immunological (Ergas *et al.*, 2002), neuronal (Katakura *et al.*, 2013) and muscular system (Smith, 2016). They also support adult and foetal development (Starling *et al.*, 2015). The supplementation of omega-3 fatty acids through fish oil has been proven to reduce blood pressure in patients with systolic hypertensions significantly (Minihane *et al.*, 2016). Furthermore, population that resides in fishing village or those who consume large amount of fish are found to have lower chance of suffering from cardiovascular diseases (Bjerregaard & Dyerberg, 1988).

The dietary intake demands of omega-3 fatty acids EPA and DHA are met mainly through the consumption of fish, fish oil and krill oil since only a limited percentage of EPA and DHA (<15%) is formed in the body from the shorter 18-carbon-fatty acids, alpha-linoleic acid (ALA). Unlike EPA and DHA, ALA is derived from plant oil such as

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canola oil, flaxseed oil as well as chia seed and soya bean (Harris, 2010). The composition of DHA and EPA present in fish depends on the types of food it consumes (Sprague, Dick & Tocher, 2016). USDA reported that *Salmo salar* (*S. salar*), especially the Atlantic salmon, contain high levels of EPA and DHA. Unfortunately, salmon is known to be expensive due to their susceptibility to sea lice and changes in ocean condition. The act of over harvesting and changes in land use has reduced the number of salmon overtime (Hilborn, 2013). Efforts have been developed to attend to and resolve such problem (Gormaz *et al.*, 2014).

Although Indonesia accounts for 4.6% of global food fish aquaculture production, it depends on imports for salmon (FAO, 2014). Consequently, efforts have been made to analyse local fishes to find a substitute for salmon as a source omega-3 fatty acids. Indonesia has been the second producer of *Chanos chanos* (*C. chanos*) after Philippines and the second producer of *Pangasius* sp. after Vietnam. Due to toxicity concern as a result of heavily polluted Mekong River in Vietnam (Murk, Rietjens & Bush, 2016), Ministry of Marine Affairs and Fisheries Indonesia (2016) reported the increase in production of *Pangasius* sp. during the period 2011-2015. Furthermore, a report by Food and Agriculture Organization (FAO) Regional Office for Asia and The Pacific (Needham & Funge-Smith, 2015) showed that the amount of fish consumption in Indonesia was 187,200 tons for *C. chanos* and 130,000 tons for *P. pangasius*. In view of the high production and consumption of *C. chanos* and *Pangasius pangasius (P. pangasius)* in Indonesia, this study assessed the lipid and omega-3 content of *C. chanos*, *P. pangasius*, and *S. salar*.

MATERIALS AND METHODS

Sample preparation

Raw fish samples, including four *P. pangasius* and three *C. chanos*, were obtained from traditional markets in Tangerang, Indonesia. Meanwhile, three *S. salar* were obtained from supermarket in Tangerang, Indonesia. The samples were then halved equally, but only one piece was used. Since *S. salar* is bigger than *P. pangasius* and *C. chanos*, only the middle part (around one third) of one piece was used (Figure 1). The cut samples were incised and the ventral and dorsal muscles were taken. Later, a sharp knife was used to separate the skin from the meat of the cuttings. The meat was then partitioned into smaller cubes (Figure 2).

Lipid extraction

The lipid content of fish samples was extracted and purified according to Bligh & Dyer (1959). Each 100 g sample of the fresh or frozen fish tissue was homogenised for 1 min with a mixture of 100 ml chloroform and 200 ml methanol, followed by the addition of 100 ml chloroform. After blending for 1 min, 180 ml distilled water was

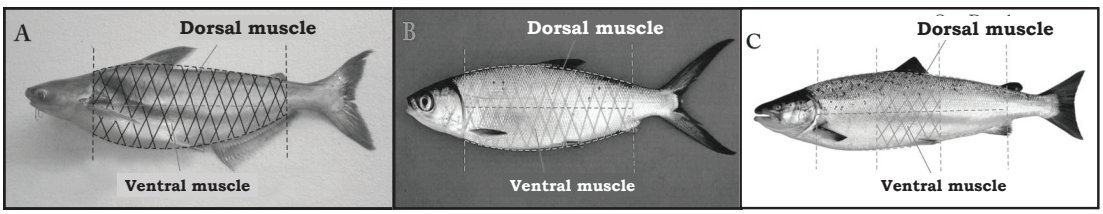

Figure 1. Incision of (A) *P. pangasius*, (B) *C. chanos*, and (C) *S. salar*. ××× refers to cutting area.

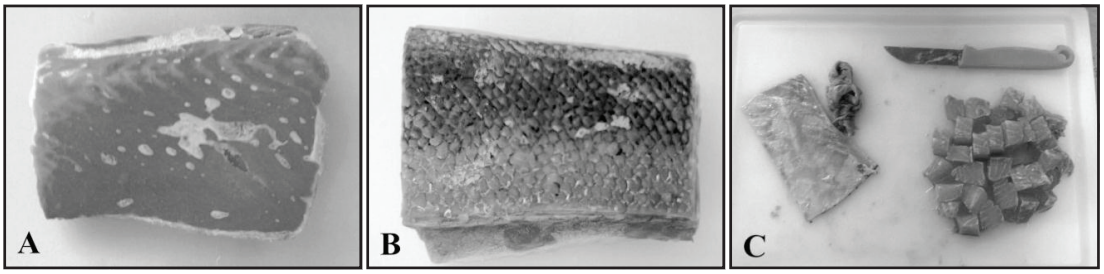

Figure 2. Separation of fish meat and skin sample. (A) Layer of meat on large pieces of *S. salar*; (B) Layer of skin on large pieces of *S. salar*; (C) Skin and small pieces of *S. salar*.

added and blended for another 1 min. The homogenate was then filtered and centrifuged at 1,000 rpm for 10 min. The chloroform layer formed at the bottom of the tube contained the purified lipid was then aspirated and filtered. Upon filtration, the solvent was evaporated using a rotary evaporator at 50 °C with agitation at 120 rpm. To ensure all the solvent was removed, the concentrated lipid was dried at 100 °C for 1 h and then cooled to room temperature. The lipid that was obtained was weighed and placed in a vial.

Gas chromatography–mass spectrometry (GC-MS) preparation

Fatty acid methyl esters (FAME) of total lipid were prepared for GC-MS analysis (Harynuk, Wynne & Marriott, 2006). The ampoule containing the mixture of 15 mg lipid and 1 ml of 14% BF3-MeOH was flame-sealed. It was then placed in boiling water for 7 min. After cooling to room temperature, the ampoule was broken and the lipid was poured into a new ampoule. One ml of hexane was added to the lipid and the ampoule was then filled entirely with distilled water and flame sealed. The mixture was homogenised with vigorous shaking and then incubated till phase layers were formed. After allowing for complete separation, ampoule was broken and the organic layer on top was moved into a new vial. For GC-MS analysis, standard methyl ester EPA and DHA were used.

Gas chromatography–mass spectrometry (GC-MS) procedure

Prior to sample injection, hexane was injected three times to rinse GC-MS machine. Lipid samples (1 µl) and standard methyl ester EPA-DHA (1 µl) were separately injected and the chromatograms that were obtained were analysed. The EPA and DHA concentration in sample were calculated based on relative abundance by comparing the peak of EPA and DHA on sample's chromatogram with standard's chromatogram, which showed the peak of known concentration of EPA and DHA.

RESULTS

The meat was analysed for its total lipid as it was the most commonly consumed part of the fish. Total lipid content in raw fish samples as compared to other fishes is shown in Table 1. Salmon had the highest lipid content (6.98±2.56%) followed by *C. chanos* (4.63±3.84%) and *P. pangasius* (3.94±1.43%). Since *C. chanos* had a higher lipid content, its omega-3 fatty acid component was then analysed using GC-MS. Figure 3 is a typical GC-MS chromatogram of fatty acids in lipid samples of *C. chanos.* Table 1 shows the polyunsaturated fatty acid omega-3, EPA and DHA content in *C. chanos* and *S. salar* analysed in this study, alongside content reported by other sources.

Table 1. Total lipid and omega-3 fatty acid content in various fishes

Fish species	*English name*	*Country*	*Total lipid (%)*	*Omega-3 (g/ 100 g)*		*Reference*
				EPA	*DHA*	
Salmo salar†	Atlantic Salmon	Indonesia	6.98±2.56	1.60	3.89	this study
Salmo salar, wild	Atlantic Salmon	United States	6.34	0.29	1.12	Exler, 2007
Salmo salar, farmed	Atlantic Salmon	United States	10.80	0.62	1.29	Exler, 2007
Salmo salar, wild	Atlantic Salmon	n/a	2.54	0.32	1.12	USDA, 2018 (NDB_No:15076)
Salmo salar, farmed	Atlantic Salmon	n/a	13.42	0.86	1.10	USDA, 2005 (NDB_No:15236)
Salmo salar, farmed	Atlantic Salmon	Scotland	12.40±3.50	0.60±0.30	0.80±0.30	Henriques *et al.*, 2014
Salmo salar, farmed	Atlantic Salmon	Norway	10.00±2.30	0.40±0.10	0.60±0.20	Henriques *et al.*, 2014
Salmo salar, farmed	Atlantic Salmon	Faroe Island	11.70±0.90	0.80±0.10	1.20±0.10	Henriques *et al.*, 2014
Chanos chanos†	Milkfish	Indonesia	4.63±3.84	0.36	1.17	this study
Chanos chanos	Milkfish	n/a	6.73	n/a	n/a	USDA, 2018 (NDB_No:15053)
Pangasius pangasius†	Pangasius Catfish	Indonesia	3.94±1.43	n/a	n/a	this study
Pangasius pangasius	Pangasius Catfish	Indonesia	3.83±0.77	0.21-2.48	0.95-9.96	Panagan, Yohandini & Gultom, 2011
Pangasius hypothalamus	Striped catfish	Malaysia	6.23	1.41±0.03	0.14±0.01	Muhamad & Mohamad, 2012
Ictalurus punctatus, wild	Channel Catfish	n/a	2.82	0.13	0.23	USDA, 2018 (NDB_No:15010)
Nile tilapia	Tilapia	n/a	1.70	0.01	0.09	USDA, 2002 (NDB_No:15261)

†fish samples in this study

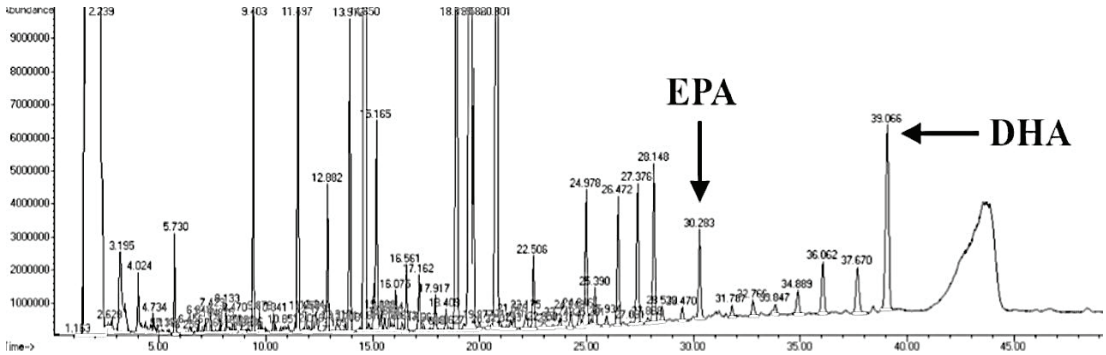

Figure 3. GC-MS chromatogram of fatty acids in lipid samples from *C. chanos*. Peaks of EPA and DHA from lipid sample of *C. chanos* are displayed at RT 30.283 and 39.066 min, respectively

DISCUSSION

The lipids of the fish samples were extracted and purified in a single-step procedure (Bligh & Dyer 1959). The complex mixture of lipid was fractionated according to polarity or solubility in different solvents. Chloroform, which is hydrophobic solvent, was used to extract neutral lipids with low polarity. The more polar solvent, methanol, was used to extract the amphiphilic membrane lipids. Optimum lipid extraction was achieved when fish tissue was homogenised with a mixture of chloroform and methanol. The resulting homogenate was then added to water and this resulted in monophasic solution with methanol, but produced a biphasic layer with chloroform. As a result, the chloroform layer contained lipids and the methanol-water layer contained the non-lipids. When chloroform layer was isolated, a purified lipid extract was obtained.

Salmon, especially the *S. salar* (the Atlantic salmon), was reported to have high lipid content, while other local fresh water fishes, such as *P. pangasius* and *C. chanos*, were reported to have lower lipid content (Table 1). Nevertheless, high amount of total lipid does not mean a high content of omega-3 fatty acids since the fish oil contains both unsaturated fatty acids (UFA) and saturated fatty acids (SFA) (Panagan, Yohandini & Gultom, 2011). Foods with high SFA and low UFA content are not recommended for consumption. Omega-3 fatty acids are one of the UFA commonly found in fish oil.

USDA Food Composition Databases provided data on the nutritional composition, including total lipids and PUFAs, of *C. chanos*. However, data on EPA and DHA content was not mentioned, suggesting the lack of information on EPA and DHA in this fish. On the other hand, the Atlantic Salmon was reported to contain 0.32-0.86% EPA and 1.10-1.11% DHA (Table 1). The EPA and DHA content in the Atlantic Salmon that was used in this study was found to be higher than that reported by the USDA. These differences of the omega-3 fatty acids content are due to the different samples used. A previous study, for example, reported differences in EPA and DHA content between wild and farmed Salmon (Sprague, Dick & Tocher, 2016). Like humans, salmon are inefficient at converting the shorter-chain fatty acids, α-linolenic acid (ALA; 18:3n-3), into EPA and DHA. As such, they need to obtain the omega-3 through their diet. Wild salmon usually consume foods with high levels of marine ingredients, including other pelagic fish. Farmed salmon are usually fed with terrestrial food such as those from plant sources which are mainly of oilseed origin (Bell *et al.*, 2004). The fatty acid profiles of vegetable oils differ from those of fish oil, the former being richer in omega-6 and devoid of omega-3 fatty acids, resulting in changes to the fatty acid composition of farmed fish (Torstensen, 2005).

The Atlantic salmon, *S. salar,* often referred to as the "gold standard" for omega-3 fatty acids were found to have higher EPA and DHA ie 1.60% and 3.89% respectively than *C. chanos* oil. Nevertheless, the sample of *C. chanos* analysed was found to contain a considerable amount of these important omega-3 fatty acids, ie 0.36% EPA and 1.17% DHA.

CONCLUSION

The study was conducted due to the vast availability and production of both *C. chanos* and *P. pangasius* in Indonesia in contrast to the scarce availability of salmon. To date, there is no information on omega-3 fatty acid content of *C. chanos* and *P. pangasius,* both of which are widely available in Indonesia. The analysis showed that *C. chanos* has

considerable content of EPA and DHA which can be used as an alternative source of the beneficial fatty acids.

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Authors' contributions

MS, advised on the data analysis and interpretation, prepared the draft of the manuscript and reviewed the manuscript; PFW, conducted the study, performed data collection, analysis and interpretation; JL, assisted in drafting of the manuscript and reviewed it; TTJ, conceptualised and designed the study, advised on the data analysis and interpretation, assisted in the drafting and reviewing the manuscript.

Conflict of interest

The authors declare no conflict of interest.

References

Bell JG, Henderson RJ, Tocher DR & Sargent JR (2004). Replacement of dietary fish oil with increasing levels of linseed oil: modification of flesh fatty acid compositions in Atlantic salmon (*Salmo salar*) using a fish oil finishing diet. *Lipids* 39:223–232.

Bjerregaard P & Dyerberg J (1988). Mortality from ischaemic heart disease and cerebrovascular disease in Greenland. *Int J Epidemiol* 17:514-519.

Bligh EG & Dyer WJ (1959). A rapid method of total lipid extraction and purification. *Can J Physiol Pharmacol* 37:911-917.

Ergas D, Eilat E, Mendlovic S & Sthoeger ZM (2002). N-3 fatty acids and the immune system in autoimmunity. *Isr Med Assoc J* 4:34-38.

Exler J (2007). *Nutrient content and variability in newly obtained salmon data for USDA Nutrient Database for Standard Reference*. Experimental Biology, Washington, D.C.

FAO (2014). *Global aquaculture production volume and value statistics database updated to 2012*. Fisheries and Aquaculture Department, Food and Agriculture Organization.

Gormaz JG, Fry JP, Erazo M & Love DC (2014). Public Health Perspectives on Aquaculture. *Curr Environ Health Rep* 1:227-238.

Harris WS (2010). Omega-3 fatty acids. In: Coates PM, Betz JM, Blackman MR, Cragg GM, Levine M, Moss J, White JD (eds). *Encyclopedia of Dietary Supplements*, 2nd ed. Informa Healthcare, New York.

Harynuk J, Wynne PM & Marriott PJ (2006). Evaluation of new stationary phase for the separation of fatty acid methyl esters. *Chromatographia* 63:S61-S66.

Henriques J, Dick JR, Toucher DR & Bell JG (2014). Nutritional quality of salmon products available from major retailers in the UK: content and composition of n-3 long-chain PUFA. *Br J Nutr* 112:964-975.

Hilborn R (2013). Ocean and dam influences on salmon survival. *Proc Natl Acad Sci USA* 110:6618-6619.

Katakura M, Hashimoto M, Okui T, Shahdat HM, Matsuzaki K & Shido O (2013). Omega-3 Polyunsaturated Fatty Acids Enhance Neuronal Differentiation in Cultured Rat Neural Stem Cells. *Stem Cells Int* 2013:490476.

Minihane AM, Armah CK, Miles EA, Madden JM, Clark AB, Caslake MJ, Packard CJ, Kofler BM, Lietz G, Curtis PJ, Mathers JC, Williams CM & Calder PC (2016). Consumption of Fish Oil Providing Amounts of Eicosapentaenoic Acid and Docosahexaenoic Acid That Can Be Obtained from the Diet Reduces Blood Pressure in Adults with Systolic Hypertension: A Retrospective Analysis. *J Nutr* 146:516-523.

Ministry of Marine Affairs and Fisheries Indonesia (2016). Peta Sentra Produksi Perikanan Budidaya. From https://www.ojk.go.id/sijaring/id/sektor-kelautan-dan-perikanan/usaha-perikanan budidaya/Dokumen%20Usaha/peta%20sentra%20budidaya%202016.pdf [Retrieved December 28 2018].

Muhamad NA & Mohamad J (2012) Fatty Acids Composition of Selected Malaysian Fishes. *Sains Malaysiana* 41:81-94.

Murk AJ, Rietjens IMCM & Bush SR (2016). Perceived versus real toxicological safety of pangasius catfish: a review modifying market perspectives. *Rev Aquacult* 10:123-134.

Needham S & Funge-Smith (2015). *The consumption of fish and fish products in the Asia-Pacific region based on household surveys*. FAO-UN Regional Office for Asia and the Pacific, Bangkok.

Panagan AT, Yohandini H & Gultom JU (2011). Analisis kualitatif dan kuantitatif asam lemak tak jenuh omega-3 dari minyak ikan patin (*Pangasius pangasius*) dengan metoda kromatografi gas. *Jurnal Penelitian dan Sains* 14:38-42.

Smith GI (2016). The Effects of Dietary omega-3s on muscle composition and quality in older adults. *Curr Nutr Rep* 5:99-105.

Sprague M, Dick JR & Tocher DR (2016). Impact of sustainable feeds on omega-3 long-chain fatty acid levels in farmed Atlantic salmon, 2006–2015. *Sci Rep* 21892:1-9.

Starling P, Charlton K, McMahon AT & Lucas C (2015). Fish intake during pregnancy and foetal neurodevelopment–a systematic review of the evidence. *Nutr* 7:2001-2014.

Torstensen BE, Bell JG, Rosenlund G, Henderson RJ, Graff IE, Tocher DR, Lie & Sargent JR (2005). Tailoring of Atlantic salmon (*Salmo salar* L.) flesh lipid composition and sensory quality by replacing fish oil with a vegetable oil blend. J. *Agric Food Chem* 53:10166–10178.

USDA (2002). *Nutrient Data Laboratory, ARS, USDA National Food and Nutrient Analysis Program Wave 6l, Beltsville MD.* From http://ndb.nal.usda.gov/ndb/foods. [Retrieved May 30 2019].

USDA (2005). *Nutrient Data Laboratory, ARS, USDA National Food and Nutrient Analysis Program, Wave 9k, Beltsville MD.* From http://ndb.nal.usda.gov/ndb/foods. [Retrieved May 30 2019].

USDA (2018). *National Nutrient Database for Standard Reference.* From http://ndb.nal.usda.gov/ndb/foods. [Retrieved May 30 2019].