

## COMPARISON OF FAT COMPOSITION AND CHEMICAL PROPERTIES OF FAT EXTRACTS BETWEEN FISH FILLETS OF SELECTED WARM-WATER AND COLD-WATER FISH

### *COMPARAÇÃO DA COMPOSIÇÃO DA GORDURA E PROPRIEDADES QUÍMICAS DOS EXTRATOS DE GORDURA ENTRE FILÉ DE PEIXE DE ÁGUA QUENTE E DE ÁGUA FRIA*

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**ABSTRACT:** The purpose of this study was to determine and compare fat composition and chemical properties of fish fillets of selected warm-water fish obtained from Straits of Malacca. A cold water fish, namely salmon was used for comparison. Moisture content, crude fat, fatty acids composition and chemical characteristics of fish fillets of Yellowstripe scad, Japanese threadfin bream and salmon were determined. Japanese threadfin bream fillet had highest moisture and crude fat contents, followed by fillets of Yellowstripe scad and salmon. A significantly strong and negative correlation was found between moisture and crude fat contents of these fish fillets. Fillets of Japanese threadfin bream and Yellowstripe scad also had higher total saturated fatty acids than total unsaturated fatty acids. Although salmon fillet had lowest percentage of saturated fatty acids, it had highest monounsaturated fatty acids and polyunsaturated fatty acids (PUFA) compared with the two warm-water fish. Palmitic acid and oleic acid were the major fatty acids in the fish fillets. Chemical properties of the oils extracted from the warm-water fish fillets were varied compared to salmon. The selected warm-water fish fillets offer favorable fatty acids composition and chemical properties, which can potentially be used as good sources of PUFA.

**KEYWORDS:** Chemical properties. Japanese threadfin bream. Salmon. Saturated fatty acid. Unsaturated fatty acid. Yellowstripe scad.

### INTRODUCTION

Fish is a common source of protein that is regularly consumed by everyone all over the world, especially in Southeast Asian region (AGUSA et al., 2007). According to the online commentary of Maritime Institute of Malaysia on maritime issues, Malaysia is identified as one of the highest fish-consuming countries in Asia with the consumption of more than 40 kg/capita/year. Over the years, demand for fish and fish consumption has increased from 20 kg in 1970 to 54 kg in 2010 (TEH, 2012).

Nutritional composition of fish varies among different types of fish (OSMAN et al., 2001; NUR AIRINA; JAMALUDIN, 2012). Fish does not only provide a good protein and a variety of vitamin and mineral in our diet, it is also an excellent source of fat especially polyunsaturated fatty acids (DEEPIKA et al., 2014). Fish contains a high level of polyunsaturated fatty acid (PUFA). PUFA is known as essential fatty acid. It needs to be obtained

from the diet because it cannot be produced by human body (RUBIO-RODRÍGUEZ et al., 2010). Fish is also known to be a rich source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (HARRIS, 2004; MAQSOOD et al., 2012), where these fatty acids have several health benefits (SWANSON et al., 2012).

Yellowstripe scad and Japanese threadfin bream are warm-water fish having the highest total PUFA especially DHA (NURNADIA et al., 2013). Therefore, these two types of fish were selected for determination of fat composition and chemical properties. There are also limited studies on chemical properties of the oils extracted from these fish fillets. Hence, understanding of fat acids composition and chemical properties of oils extracted from the fish fillets helps to estimate oxidation value of the fish oil (DAVE et al., 2014). Studies related to fatty acids composition and chemical properties of different types of warm-water fish are needed because the fish have high

DHA and EPA that suitable for pharmaceutical and food industrial use. Also, cold water fish could have different chemical properties compared to the warm water fish. Therefore, this study aimed to compare fat composition and chemical properties of fillets of selected warm-water fish and salmon.

## MATERIAL AND METHODS

### Chemicals and reagents

All chemicals and reagents used were of analytical and gas chromatography grades. Petroleum ether, methanol, chloroform, 95% ethanol and n-hexane were purchased from R & M Marketing (Essex, UK). Butylated hydroxytoluene (BHT), sodium methoxide, cyclohexane, glacial acetic acid and Wijs solution were purchased from Merck Malaysia (Selangor, Malaysia). Potassium iodide, sodium iodide, sodium thiosulphate ( $\text{Na}_2\text{S}_2\text{O}_3$ ), potassium hydroxide (KOH), phenolphthalein indicator, starch indicator and sodium hydroxide were obtained from Sigma-Aldrich Malaysia (Selangor, Malaysia). 37-component fatty acid methyl esters (FAME) mix standard 47885-U (Supelco, Germany) was used as the external standard for determination of fatty acids composition.

### Preparation of samples

Fillets of Yellowstripe scad, Japanese threadfin bream and salmon were purchased from a local supermarket in Selangor, Malaysia. Prior to purchasing, all fish samples were checked for sample freshness by observing the texture of the fish fillets. Our expert panel evaluated the freshness of all fish samples. The assessment was measured through "finger method", by pressing on the fillet of fish by finger. This assessment is an indication of the suitability for further processing and is mainly dependent on the firmness (CHENG et al. 2014). Additionally, all fish samples in the market were well handled and kept at cold storage at 4°C. Yellowstripe scad (n = 5) weighed 50-100g g and length range from 16 to 20 cm, Japanese threadfin bream (n = 5) weighed 100-230 g and length range from 18 to 25 cm and salmon (n = 2) weighed 1200-2000 g and length range from 60 to 84 cm were purchased to obtain fish fillets. To obtain fish fillet, the fish were beheaded, gutted, skinned, washed and filleted. All samples were sealed in different plastic bags before kept at -80°C prior to analysis. Triplicate analytical samples were obtained for each experiment for the validity and accuracy of the results.

### Determination of moisture content

Moisture content of the fish fillets was determined according to the method described by AOAC Method 930.15 (AOAC, 2000). Briefly, 5.0 g of fresh fish fillet was weighed and added into an aluminum disc, and were dried in a laboratory oven at 105°C for 3 h. The covered disc and its dried sample were weighed and moisture content was calculated using the equation as follows:

Moisture content (%) =

$$\frac{\text{Loss of weight of sample (g)}}{\text{Weight of sample taken (g)}} \times 100$$

### Extraction of crude fat

Standard Soxhlet method was used for extraction of crude fat according to the method described by AOAC 948.15 (AOAC, 2000). Briefly, 1 g of fresh fish fillet was weighed and extracted with 250 mL of petroleum ether for 8 h (heating rate of 150 drop/min) on a Soxhlet instrument. The flask and its dried sample were weighed and crude fat value was calculated using the equation as follows:

$$\text{Crude Fat (\%)} = \frac{\text{Final weight of sample (g)}}{\text{Initial weight of sample (g)}} \times 100$$

### Lipid extraction for analysis of fatty acids and chemical properties

Extraction of lipid was done based on the method described by BLIGH and DYER (1959) with some modifications. Briefly, 30 g fish fillet was homogenized with a mixture of 60 mL of methanol and 30 mL of chloroform for 2 min. One part of chloroform (30 mL) were added to the mixture and stirred for 30 sec before addition of 30 mL of distilled water. To minimize oxidation, the extracted fats were kept in the solvent containing 0.05% BHT in amber glass bottles, flushed with nitrogen, and wrapped with aluminum foils to avoid light exposure.

### Preparation of fatty acid methyl esters

Fatty acid methyl esters (FAMES) were prepared by pipeting the extracted fat (0.1 mL) into clean 10-mL screw-top glass bottles and diluted using 1 mL of hexane before addition of 0.5 mL of sodium methoxide. The mixture was homogenized by vortex for 15 sec. Clear upper phase layer was removed, filtered using a 0.45 µm syringe filter and transferred to a gas chromatography (GC) vial before gas chromatography analysis.

### Gas chromatography determination of fatty acids

Gas chromatography (GC) was used to determine methyl esters of FAMES and fish fillets, which was according to the method described by NURNADIA et al. (2013). A capillary gas chromatograph model Agilent 6890 (Agilent Technology, USA) equipped with a split injector and flame ionisation detection system were used to separate and quantify each FAME component. FAMES were separated using a highly polar HP88 column, 100 m × 0.25 mm, 0.20 µm ID (Agilent, USA). Split injection with a split ratio (volume of gas passing to waste : volume of gas passing down capillary column) of 10 : 1 and 99.9 mL/min split flow were applied. Helium was used as the carrier gas at a linear velocity of 30.0 mL/min. The operating temperatures were 250°C for injection port, 250°C for flame ionisation detector and 200°C for column temperature. FAME components were identified by comparing retention time of sample with each of the 37-component of FAME mix 47885-U (Supelco, Germany).

Area normalization method was used to identify every single fatty acid in the samples. Based on the literature (VISENTAINER et al., 2014), content of fatty acids (%) was calculated using the peak area of fatty acid in relation to the total peak area of all eluted fatty acids in the sample.

$$\% \text{ of total fatty acids} = (A/B) \times 100$$

Where A is area of specific fatty acid and B is total area of fatty acids present.

Calibration curves were obtained from the plotted graphs of 37-component FAME mix, with the dilution factors of 10×, 20×, 30×, 40×, and 50×. Fatty acids were quantified based on equation obtained from the calibration curves. Fatty acids content of the fish fillets was presented as mg per 100 g fresh weight (FW).

### Iodine value

Iodine value (IV) was determined according to AOCS Official Method Cd 1d-92 (AOCS, 2004) with slight modification. Briefly, 0.1 g of the filtered fish oil was weighed and added with 10 mL of chloroform before addition of 20 mL of sodium iodide solution. The mixture was titrated with 0.1 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution. IV of the fish oil was calculated according to the equation as follows:

$$IV \text{ (g/100 g FW)} = \frac{(B-S) \times N \times 12.69}{W}$$

Where B is titration of blank (mL), S is titration of test solution (mL), N is normality of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> and W is weight of fish oil (100 g).

### Peroxide value

Peroxide value (PV) was measured using AOCS Official Method Cd 8-53 (AOCS, 1990a). Briefly, 2 g of the filtered fish oil was weighed into a 250 mL Erlenmeyer flask. Approximately 30 mL of acetic acid-chloroform solution (3:2, v/v) were added and swirled to mix. The mixture was then added with 0.5 mL of saturated potassium iodide solution and allowed to stand for 1 min. Then, 30 mL of distilled water were immediately added to the oil samples and swirled to mix. The samples were titrated against 0.01 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> until the yellow of iodine almost disappears. Starch indicator (2.0 mL) were added and the titration were continued until the blue disappeared. Two blank samples were simultaneously prepared without addition of the fish oil. The volume of titrate used was recorded and PV was calculated using the equation as follows:

$$PV \text{ (mEq/100 g FW)} = \frac{(S-B) \times N \times 100}{W}$$

Where S is volume of titrated sample (mL), B is volume of titrated blank (mL), N is normality of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution and W is weight of fish oil (100 g)

### Saponification value

Saponification value (SV) was determined following AOCS Official Method Cd 3-25 (AOCS, 2004a). Approximately 1 g of the fish oil was weighed into 250 mL round bottom flask and added with 50 mL of alcoholic KOH. Duplicate blank samples were prepared with 50 mL of the KOH solution. The mixture was boiled gently on a hot plate for 1 h until it became clear. The mixture was allowed to cool at room temperature. For titration, 1 mL of phenolphthalein indicator was added and titrated against 0.5 N HCl until the pink completely disappeared. SV of the fish oil was calculated using the formula as follows:

$$SV \text{ (mg KOH/g FW)} = \frac{56.1 \times N (A-B)}{W}$$

Where N is normality of HCl, A is titration of blank (mL), B is titration of sample (mL), and W is weight of fish oil (g).

### Acid value

Acid value (AV) was determined according to AOCS Official Method Ca 5a-40 (AOCS, 1990). Briefly, 5.0 g of the filtered oil was weighed into a 250 mL Erlenmeyer flask and added with 100 mL of hot neutralized 95% ethanol, followed by 2 mL of 1% phenolphthalein. Then mixture was titrated against 0.1 N NaOH and shaken vigorously until pink persisted for at least 30 sec during titration. The volume of titrate used were recorded. Percentage of AV was calculated applying the formula as follows:

$$AV (\%) = \frac{V \times N \times 28.2}{W}$$

Where V is volume of sample, N is normality of sodium hydroxide solution, and W is weight of fish oil (g).

### Statistical analysis

All data obtained were analyzed using Statistical Package for Social Science (SPSS) Version 21.0. All analyses were performed in triplicate. Descriptive statistics were presented as mean  $\pm$  standard deviation (SD). Statistical significance among the fish samples was determined using one-way analysis of variance (ANOVA) coupled with post-hoc Tukey's test at  $p < 0.05$ .

**Table 1.** Moisture content and crude fat of the fish fillets.

Common name	Scientific name	Local name	Moisture content	Crude fat
Japanese threadfin bream	<i>Nemipterus japonicus</i>	Kerisi	81.98 $\pm$ 0.22 <sup>a</sup>	1.28 $\pm$ 0.14 <sup>a</sup>
Yellowstripe scad	<i>Selaroides leptolepis</i>	Selar kuning	77.50 $\pm$ 0.24 <sup>b</sup>	1.84 $\pm$ 0.08 <sup>b</sup>
Salmon	<i>Salmo solar</i>	Salmon	71.62 $\pm$ 0.53 <sup>c</sup>	9.45 $\pm$ 0.05 <sup>c</sup>

All values are presented as mean  $\pm$  standard deviation of three replicates (%). Different superscript lowercase letters (<sup>a-c</sup>) in same column show significant differences at  $p < 0.05$  (post-hoc Tukey's test).

Moisture content of these fish fillets ranged between 71% and 82%. Result showed that Japanese threadfin bream had highest moisture content (81.98%) followed by Yellowstripe scad (77.50%) and salmon (71.62%). Moisture content in the fish fillets was significantly different at  $p < 0.05$ . Post-hoc test showed that moisture content of salmon fillet (71.62%) was significantly lower than the fillets of Yellowstripe scad (77.50%) and Japanese threadfin bream (81.98%). Literature reported that fish fillet contained 70-80% of moisture content (NURNADIA et al., 2011). According to Savitri (2011), white fish fillet has higher moisture content (80%) than fatty fish (70%). Therefore, moisture content of marine fish should have not vary much between fish species. However, prolonged exposure to high pressure and temperature during cooking will cause a loss of water in fish fillet (STOECKER; STOECKER, 1998).

The result showed that crude fat content (%) was highest in salmon fillet (9.45%), followed by the fillets of Japanese threadfin bream (1.28%) and Yellowstripe scad (1.84%). The fillets of two warm-water fish had significantly lower crude fat content than salmon fillet. Based on our previous finding, crude fat content of warm-water fish varies from 0.7% to 23.2% (NURNADIA et al., 2011). However, the crude fat for all fish fillets studied was less than 10%.

Pearson correlation test revealed that there was a significantly strong and negative correlation

between moisture and crude fat content of the fish fillets ( $r = -0.937$ ,  $p < 0.01$ ). The result is supported by previous studies that fish samples with lower moisture content tend to have a greater percentage of crude fat (OSMAN et al., 2001; JAKHAR et al., 2012; ŠIMAT; BOGDANOVIĆ; 2012)

## RESULTS AND DISCUSSION

### Moisture content and crude fat analysis

Moisture and crude fat content of the selected fish fillets and correlation between moisture and crude fat content of the fish fillets were determined in this study. Table 1 shows the moisture and crude fat content of the fillets of Yellowstripe scad, Japanese threadfin bream and salmon.

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### Fatty acids content

In this study, fatty acids content of the fish fillets was presented as quantitative and qualitative determination. Qualitative analysis of fatty acids was determined based on the area normalization method, whereas quantitative analysis was performed based on the standard calibration method. Salmon fillet was tested for comparison with the two warm-water fish fillets because it is one of cold-water fish that has high omega-3 content (DEEPIKA et al., 2014). According to Exler and Pehrsson (2007), salmon is an important contributor of many nutrients among the seafood that are consumed in United States.

Table 2 depicts percentages of fatty acids of fillets of Yellowstripe scad and Japanese threadfin bream and salmon. Result showed that fillet of Japanese threadfin bream had significantly higher percentages of palmitic, stearic and oleic acids than Yellowstripe scad at  $p < 0.05$ . Conversely, fillet of Yellowstripe scad had significantly higher percentages of DHA and EPA than fillets of Japanese threadfin bream and salmon.

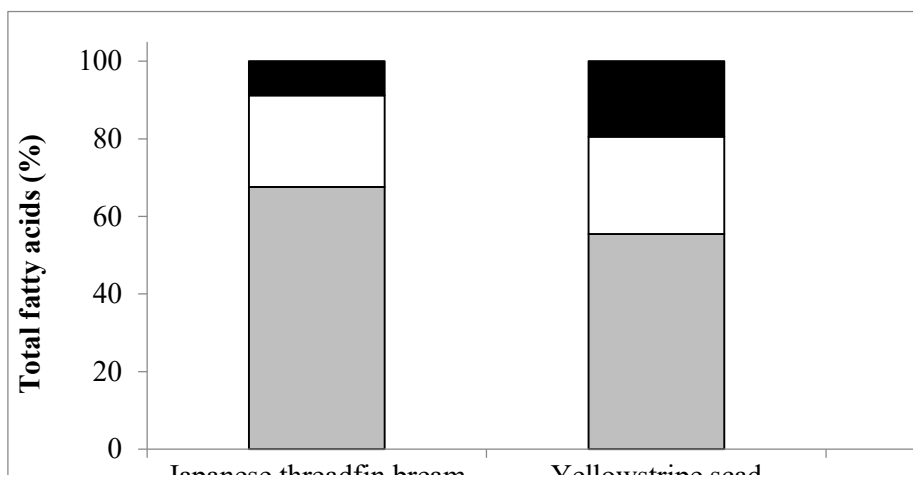
**Table 2.** Estimation of fatty acids level in the fish fillets.

Fatty acid	Japanese threadfin bream	Yellowstripe scad	Salmon
<i>SFA</i>			
Lauric acid (C12:0)	0.54 ± 0.01 <sup>a</sup>	0.62 ± 0.06 <sup>a</sup>	0.25 ± 0.01 <sup>bc</sup>
Myristic acid (C14:0)	2.52 ± 0.05 <sup>a</sup>	3.35 ± 0.01 <sup>b</sup>	2.34 ± 0.06 <sup>a</sup>
Pentadecanoic acid (C15:0)	1.40 ± 0.28 <sup>a</sup>	1.04 ± 0.08 <sup>a</sup>	0.22 ± 0.01 <sup>bc</sup>
Palmitic acid (C16:0)	40.48 ± 0.76 <sup>a</sup>	34.86 ± 0.01 <sup>b</sup>	19.87 ± 0.03 <sup>c</sup>
Heptadecanoic acid (C17:0)	2.68 ± 0.73 <sup>a</sup>	1.29 ± 0.07 <sup>a</sup>	1.10 ± 0.04 <sup>a</sup>
Stearic acid (C18:0)	17.17 ± 0.40 <sup>a</sup>	10.93 ± 0.01 <sup>b</sup>	5.16 ± 0.12 <sup>c</sup>
Lignoceric acid (C24:0)	2.81 ± 0.74 <sup>a</sup>	3.35 ± 0.01 <sup>a</sup>	1.56 ± 0.05 <sup>ab</sup>
<i>MUFA</i>			
Myristoleic acid (C14:1)	0.15 ± 0.01 <sup>a</sup>	0.21 ± 0.02 <sup>a</sup>	0.08 ± 0.01 <sup>ab</sup>
cis-10-Pentadecenoic acid (C15:1)	0.31 ± 0.01 <sup>a</sup>	0.98 ± 0.13 <sup>b</sup>	0.15 ± 0.01 <sup>ac</sup>
Palmitoleic acid (C16:1)	3.57 ± 0.12 <sup>a</sup>	8.76 ± 0.01 <sup>b</sup>	3.70 ± 0.03 <sup>ac</sup>
cis-10-Heptadecenoic acid (C17:1)	1.07 ± 0.04 <sup>a</sup>	1.09 ± 0.44 <sup>a</sup>	0.47 ± 0.01 <sup>a</sup>
Oleic acid (C18:1n9c)	18.51 ± 0.23 <sup>a</sup>	14.17 ± 0.01 <sup>b</sup>	34.10 ± 0.13 <sup>c</sup>
<i>PUFA</i>			
Linoleic acid (C18:2)	3.55 ± 0.08 <sup>a</sup>	3.31 ± 0.07 <sup>a</sup>	18.89 ± 0.02 <sup>b</sup>
γ-Linolenic acid (C18:3)	0.93 ± 0.02 <sup>a</sup>	1.20 ± 0.01 <sup>a</sup>	7.06 ± 0.28 <sup>b</sup>
cis-11,14,17-Eicosatrienoic acid (C20:3n3)	2.49 ± 0.06 <sup>a</sup>	6.81 ± 0.06 <sup>b</sup>	2.54 ± 0.12 <sup>ac</sup>
cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3)	0.95 ± 0.05 <sup>a</sup>	4.80 ± 0.56 <sup>b</sup>	2.07 ± 0.01 <sup>ac</sup>
cis-4,7,10,13,16,19-Docosahexaenoic acid (C22:6n3)	0.91 ± 0.01 <sup>a</sup>	3.28 ± 0.04 <sup>b</sup>	0.49 ± 0.01 <sup>c</sup>

All values are presented as mean ± standard deviation of three replicates as % total fatty acids. Percentages of fatty acids were estimated based on area normalization method. Different superscript lowercase letters (a-c) in same column show significant differences at p<0.05 (post-hoc Tukey's test)

As shown in Fig. 1, fillets of Yellowstripe scad and Japanese threadfin bream had the highest saturated fatty acid (SFA) content compared to unsaturated fat acids content which were monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) of 67.58% and 55.41%, respectively. Percentage of SFA of salmon

fillet was 30.49%. However, percentages of MUFA and PUFA of salmon fillet were higher than the other fish fillets. Based on the results obtained, the warm-water fish fillets had higher SFA than unsaturated fatty acids. However, salmon fillet had higher unsaturated fatty acids than the warm-water fish fillets.



**Figure 1.** Qualitative determination of total fatty acids content of the selected fish fillets.

Based on the result presented in Table 3, total SFA in fillet of Japanese threadfin bream (561.42 mg per 100 g FW  $\pm$  24.50) was significantly higher than the other two fillets. Fillets of salmon and Yellowstripe scad had similar total SFA content of 496.98 and 494.41 mg/100 g FW respectively. The most dominant SFA in fish species was palmitic acid (C16:0) (NURNADIA et al., 2013). However,

the result obtained from this study showed that palmitic acid content was significantly differed between the fish fillets at  $p < 0.05$ , where Japanese threadfin bream fillet (313.32 mg/100 g FW) had the highest palmitic acid content, followed by the fillets of salmon (301.29 mg/100 g FW) and Yellowstripe scad (285.73 mg/100 g FW).

**Table 3.** Quantitative determination of fatty acids level in the fish fillets.

Fatty acid	Japanese threadfin bream	Yellowstripe scad	Salmon
Lauric acid (C12:0)	1.38 $\pm$ 0.02 <sup>ab</sup>	1.83 $\pm$ 0.24 <sup>a</sup>	1.18 $\pm$ 0.09 <sup>b</sup>
Myristic acid (C14:0)	18.24 $\pm$ 0.04 <sup>a</sup>	26.27 $\pm$ 0.05 <sup>b</sup>	34.36 $\pm$ 0.95 <sup>c</sup>
Pentadecanoic acid (C15:0)	9.87 $\pm$ 2.31 <sup>a</sup>	7.58 $\pm$ 0.63 <sup>ab</sup>	2.48 $\pm$ 0.08 <sup>b</sup>
Palmitic acid (C16:0)	313.32 $\pm$ 0.01 <sup>a</sup>	285.73 $\pm$ 0.36 <sup>b</sup>	301.29 $\pm$ 0.28 <sup>c</sup>
Heptadecanoic acid (C17:0)	22.31 $\pm$ 6.74 <sup>a</sup>	10.98 $\pm$ 0.69 <sup>a</sup>	17.69 $\pm$ 0.52 <sup>a</sup>
Stearic acid (C18:0)	145.57 $\pm$ 0.65 <sup>a</sup>	97.67 $\pm$ 0.06 <sup>b</sup>	85.00 $\pm$ 1.96 <sup>c</sup>
Lignoceric acid (C24:0)	50.73 $\pm$ 14.73	64.35 $\pm$ 0.37	54.98 $\pm$ 1.75
$\Sigma$ SFA	561.42 $\pm$ 24.5 <sup>a</sup>	494.41 $\pm$ 2.4 <sup>b</sup>	496.98 $\pm$ 5.63 <sup>bc</sup>
Myristoleic acid (C14:1)	0.45 $\pm$ 0.09 <sup>a</sup>	1.01 $\pm$ 0.17 <sup>a</sup>	0.49 $\pm$ 0.28 <sup>a</sup>
cis-10-Pentadecenoic acid (C15:1)	1.62 $\pm$ 0.02 <sup>a</sup>	7.31 $\pm$ 1.09 <sup>bc</sup>	1.47 $\pm$ 0.02 <sup>a</sup>
Palmitoleic acid (C16:1)	27.14 $\pm$ 0.38 <sup>a</sup>	71.71 $\pm$ 0.07 <sup>b</sup>	55.79 $\pm$ 0.48 <sup>c</sup>
cis-10-Heptadecenoic acid (C17:1)	7.86 $\pm$ 0.10 <sup>a</sup>	8.58 $\pm$ 3.71 <sup>a</sup>	6.70 $\pm$ 0.17 <sup>a</sup>
Oleic acid (C18:1n9c)	153.48 $\pm$ 0.95 <sup>a</sup>	124.13 $\pm$ 0.16 <sup>b</sup>	559.77 $\pm$ 2.42 <sup>c</sup>
$\Sigma$ MUFA	190.55 $\pm$ 1.54 <sup>a</sup>	212.74 $\pm$ 5.2 <sup>b</sup>	624.22 $\pm$ 3.37 <sup>c</sup>
Linoleic acid (C18:2)	29.26 $\pm$ 0.15 <sup>a</sup>	28.86 $\pm$ 0.62 <sup>a</sup>	319.38 $\pm$ 0.53 <sup>b</sup>
$\gamma$ -Linolenic acid (C18:3)	3.35 $\pm$ 0.07 <sup>a</sup>	7.45 $\pm$ 0.10 <sup>a</sup>	156.28 $\pm$ 6.34 <sup>b</sup>
cis-11,14,17-Eicosatrienoic acid (C20:3n3)	39.02 $\pm$ 1.89 <sup>a</sup>	118.14 $\pm$ 1.25 <sup>b</sup>	80.44 $\pm$ 3.90 <sup>c</sup>
cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3)	12.19 $\pm$ 0.47 <sup>a</sup>	68.28 $\pm$ 7.95 <sup>b</sup>	54.35 $\pm$ 0.04 <sup>bc</sup>
cis-4,7,10,13,16,19-Docosahexaenoic acid (C22:6n3)	12.99 $\pm$ 0.03 <sup>a</sup>	50.75 $\pm$ 0.70 <sup>b</sup>	13.48 $\pm$ 0.13 <sup>a</sup>
$\Sigma$ PUFA	96.81 $\pm$ 2.61 <sup>a</sup>	273.48 $\pm$ 10.62 <sup>b</sup>	623.93 $\pm$ 10.94 <sup>c</sup>

All values are presented as mean  $\pm$  standard deviation of three replicates as mg/100 g FW based on calibration curve method. Different superscript lowercase letters (<sup>a-c</sup>) in same column show significant differences at  $p < 0.05$  (post-hoc Tukey's test).

For the two selected warm-water fish fillets (Table 3), Yellowstripe scad had a significantly higher total MUFA content (212.74 mg/100 g FW) than Japanese threadfin bream (190.55 mg/100 g FW) at  $p < 0.05$ . However, salmon fillet had the significantly highest total MUFA (624.22 mg/100 g FW,  $p < 0.05$ ). Major MUFAs in the fish fillet were oleic acid and palmitoleic acid. Salmon fillet had a significantly higher amount of oleic acid which was 559.77 mg/100 g FW, followed by palmitoleic acid of 55.79 mg/100 g FW. For the selected warm-water fish fillets, both Yellowstripe scad and Japanese

threadfin bream had significantly highest amounts of oleic acid which were 124.13 and 153.48 mg/100 g FW respectively, followed by palmitoleic acid of 71.71 and 27.14 mg/100 g FW respectively.

Previous studies indicated that MUFA in fish and shellfish was represented by oleic acid (NURNADIA et al., ÖZOGUL; ÖZOGUL, 2007). As depicts in Table 3, salmon fillet (623.93 mg/100 g FW) had the significantly highest total PUFA content at  $p < 0.05$ , followed by fillets of Yellowstripe scad (273.48 mg/100 g FW) and Japanese threadfin bream (96.81 mg/100 g FW).



Besides these findings, the fillet of Yellowstripe scad had the highest DHA and EPA content compared to the other two fish fillets. DHA and EPA are essential fatty acids in prevention of several chronic diseases. The high DHA and EPA content in the fish oil shows that the fat extracted Yellowstripe scad is a rich source of omega-3 fatty which is comparable to cold-water fish oil.

As shown in Table 4, salmon fillet had the significantly highest total omega-6 content (319.39 mg/100 g FW,  $p < 0.05$ ). It also contained the highest

total omega-3 content (C18:3, C20:3n3, C20:5n3, and C22:6n3) of 304.55 mg/100 g FW, followed by fillets of Yellowstripe scad (244.62 mg/100 g FW) and Japanese threadfin bream (67.55 mg/100 g FW). Omega-6/omega-3 ratio was significantly lower for fillets of Japanese threadfin bream (0.43) and Yellowstripe scad (0.18) compared to salmon fillet (1.05) at  $p < 0.05$ . Therefore, the result obtained from this study shows that the selected warm-water fish fillets had higher proportion of omega-3 than omega-6.

**Table 4.** Omega-3 and omega-6 content and ratio of the fish fillets.

Sample	n3	n6	n6/n3
Japanese threadfin bream	67.55	29.26	0.43
Yellowstripe scad	244.60	28.86	0.18
Salmon	304.55	319.38	1.05

Values of omega-3 (n3) and omega-6 (n6) are presented as mean  $\pm$  standard deviation of three replicates (mg/100 g FW).

### Chemical analysis

Table 5 shows the selected chemical values of fish oil extracted from the fish fillets. In this study, IVs for all fish fillet oils were within the standard value. According to BAKO et al. (2014), standard IVs for fish oil were between 160 and 190

g/100 g sample; ADENIYI and BAWA (2006) stated that the standard IVs were between 135 and 190 g/100 g sample. A high IV indicates that the sample has greater number of double bonds, and IV is used to measure the degree of unsaturation of lipid (BAIÃO; LARA, 2005).

**Table 5.** Selected chemical properties of the fish fillets.

Parameters	Japanese threadfin bream	Yellowstripe scad	Salmon
Iodine value (g/100 g FW)	138.81 $\pm$ 20.37 <sup>a</sup>	140.03 $\pm$ 24.21 <sup>ab</sup>	203.02 $\pm$ 10.61 <sup>c</sup>
Peroxide value (mEq/kg FW)	4.14 $\pm$ 0.74 <sup>a</sup>	3.15 $\pm$ 0.76 <sup>ab</sup>	1.64 $\pm$ 0.73 <sup>bc</sup>
Saponification value (mg KOH/g FW)	329.75 $\pm$ 10.36 <sup>a</sup>	460.39 $\pm$ 28.65 <sup>b</sup>	378.99 $\pm$ 2.10 <sup>c</sup>
Acid value (%)	0.40 $\pm$ 0.03 <sup>a</sup>	0.46 $\pm$ 0.03 <sup>ab</sup>	1.77 $\pm$ 0.44 <sup>c</sup>

All values are presented as mean  $\pm$  standard deviation of three replicates. Different superscript lowercase letters (<sup>a-c</sup>) in same column show significant differences at  $p < 0.05$  (post-hoc Tukey's test).

Based on this study, the oil extracted salmon fillet (203.02 g/100 g) had a significantly higher IV than the fish fillet oils of Yellowstripe scad (140.03 g/100 g) and Japanese threadfin bream (138.81 g/100 g). Therefore, we can conclude that the oil extracted from salmon fillet had a higher degree of unsaturation compared to the selected warm-water fish fillets. Diets that have a high unsaturated fat are able to reduce CVD risk and have better health benefits for human. It is because saturated fatty acid raised the levels of total cholesterol and low-density lipoprotein (LDL)-cholesterol (DANIELS; GREER, 2008), whereas monounsaturated and polyunsaturated fatty acids reduced LDL-cholesterol level (MENSINK; KATAN, 1989).

The result showed that there was a statistically significant difference between the oils extracted from the fish fillets determined by one-way ANOVA at  $p < 0.05$ . Post-hoc test revealed that IV of salmon fillet oil (71.62 g/100 g) was significantly lower than the oils extracted from Japanese threadfin bream (138.81 g/100 g) and Yellowstripe scad (140.03 g/100 g) at  $p < 0.05$ . However, there was no significant difference between the oils extracted from the selected warm-water fish fillets.

Peroxide value (PV) of the oils extracted from the selected fish fillets were within the range of standard value (Table 5) which was between 3 and 20 mEq/kg fish oil (BIMBO, 1998), except for salmon fillet oil. As shown in Table 5, the fillet oils

of Japanese threadfin bream (4.14 mEq/kg) and Yellowstripe scad (3.15 mEq/kg) had higher PV than salmon fillet oil (1.64 mEq/kg). These values indicated level of rancidity of fish oils was higher in the selected warm-water fish fillets than salmon fillet. However, the lower PV might be due to the fact that the tested fish was not fresh. According to HUSS (1995), a low PV might be due to breakdown of hydroperoxides over time. Low PV also acts as an indicator for early phase of auto-oxidation and late stage of the severely oxidized product where most hydroperoxides are degraded.

As shown in Table 5, saponification value (SV) for the fillet oils of Japanese threadfin bream, salmon and Yellowstripe scad were 329.75, 378.99, and 460.39 mg KOH/g, respectively. These results showed that the fillet oils had higher SV than the standard range of SV. According to Bimbo (1998), average range of SV for fish oil is 165-195 mg KOH/g.

SV is used as a measure of average molecular weight of fatty acids present in a lipid sample (XIE et al., 2006). Based on the result obtained, the high SV is most likely due to the present of impurities in the fish oils. As reported in the literature, crude fish oil contained some non-triglyceride substances (DO NASCIMENTO et al., 2015). Method described by Bligh and Dyer (1959) was used to extract fish oil from the selected fish samples instead of using Soxhlet extraction method. The high SV might also be contributed by the unsaponifiable matter present in crude marine fish oil such as sterols, glyceryl ethers, hydrocarbons, fatty alcohols and some minor quantities of pigments and vitamins (DO NASCIMENTO et al., 2015).

AV in fillet oils extracted from both warm-water and cold water fish is presented in Table 5.

Result showed that the fillet oil of Japanese threadfin bream (0.40%) had lowest AV, followed by the fillet oils of Yellowstripe scad (0.46%) and salmon (1.77%). The fish fillet oils had lower AV than the standard AV range for fish oil, which is between 2% and 5% (BAKO et al., 2014). The result obtained shows that fillet oil of cold-water fish had a significantly higher AV than the fillet oils of warm-water fish. In contrast, a previous study reported that lipid extracted from a freshwater catfish (*Clarias batrachus*) had AV of 1.93% (ISLAM et al., 2013) Therefore, AV in tropical fish is varied among tropical fish species.

## CONCLUSIONS

Selected warm-water fish fillets have a good composition of fatty acids and are comparable to salmon. Fillet of Yellowstripe scad had the highest EPA and DHA which is comparable to salmon.

The oils extracted from the selected warm-water fish fillets had lower ratio of omega-6 to omega-3, and an ideal chemical properties especially lower iodine and acid values than salmon fillet. Therefore, these fish fillets have a high quality of oils. Future studies are suggested to explore different storage conditions on lipid rancidity of fillets of Yellowstripe scad and Japanese threadfin bream in order to obtain better quality fish oils extracted from the fish fillets intended to be kept in cold storage.

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**RESUMO:** O objetivo deste estudo foi determinar e comparar a composição gordurosa e as propriedades químicas de filés de peixes selecionados de água quente obtidos no Estreito de Malaca. Um peixe de água fria, o salmão, foi usado para comparação. Foram determinados o teor de umidade, a gordura bruta, a composição de ácidos graxos e as características químicas dos filés de yellowstripe scad, sargo japonês e salmão. Os filés de sargo japonês apresentaram maior teor de umidade e de gordura bruta, seguidos por filés de yellowstripe scad e de salmão. Foi encontrada uma correlação significativamente forte e negativa entre a umidade e o teor de gordura bruta desses filés de peixe. Filés de sargo japonês e de yellowstripe scad também apresentaram ácidos graxos saturados totais mais altos do que os ácidos graxos insaturados totais. Embora o filé de salmão tenha menor porcentagem de ácidos graxos saturados, ele apresentou os maiores ácidos graxos monoinsaturados e ácidos graxos poliinsaturados (PUFA em comparação com os dois peixes de água quente. O ácido palmítico e o ácido oleico foram os principais ácidos graxos dos filés de peixe. As propriedades químicas dos óleos extraídos dos filés de peixe de água quente foram variadas em comparação ao salmão. Os filés de peixe de água quente selecionados oferecem composição favorável de ácidos graxos e propriedades químicas, que podem ser potencialmente usadas como boas fontes de PUFA.



**PALAVRAS-CHAVE:** Propriedades químicas. Sargo japonês. Salmão. Ácido graxo saturado. Ácido graxo insaturado. Yellowstripe scad.

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