PHYSICOCHEMICAL CHARACTERISTICS OF TERUBOK, *Tenualosa toli* FISH OIL

Nurasyikin Abdul Rahman & Jumat Salimon

Oleochemistry Programme, School of Chemical Science and Food Technology
University Kebangsaan Malaysia, Bangi, 43600 Selangor Darul Ehsan.

Abstract

The physical and chemical characteristics of terubok, *Tenuolosa toli* fish oil were analyzed. These parameters include iodine value (IV), peroxide value (PV), acid value (AV), saponification value (SV), percentage of free fatty acid (%FFA), refractive index (RI) and colour. The extracted lipid obtained from dried muscles was about 41.65%. The percentages of unsaturated fatty acids were higher than saturated fatty acids accounting for 52.79±0.23% and 47.22±0.23% respectively. Monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) contained approximately 27.69±1.53% and 25.10±1.76%. PUFA consisting of omega-3 and omega-6 fatty acids in terubok oil which are linoleic acid (C18:2n-6, LA), α-linolenic acids (18:3n-3, ALA), stearidonic acid (C18:4n-3, SA), arachidonic acid (20:4n-6, AA), eicosapentaenoic acids (20:5n-3, EPA) and docosapentaenoic acids (22:6n-3, DHA).

Abstrak

Analisis ciri-ciri fizikal dan kimia minyak ikan terubok, *Tenualosa toil* yang telah dijalankan. Parameter diukur adalah seperti nilai iodin (IV), nilai peroksida (PV), nilai keasidan (AV), nilai penyabunan (SV), nilai bahan tak tersabun, peratus asid lemak bebas (%FFA), indeks kebiasan (RI) dan penentuan warna minyak. Peratus lipid yang diekstrak daripada isi ikan yang telah dikeringkan adalah sebanyak 41.65%. Peratusan asid lemak tak tepu dalam minyak ikan terubok didapati lebih tinggi berbanding peratusan asid lemak tepu iaitu 52.79±0.23% dan 47.22±0.23% masing-masingnya. Kandungan asid lemak monotak tepu (MUFA) dan asid lemak politak tepu (PUFA) pula adalah sebanyak 27.69±1.53% dan 25.10±1.76%. PUFA yang didapati dalam minyak ikan terubuk terdiri dari asid lemak omega-3 dan asid lemak omega-6 iaitu asid linoleik (C18:2n-6, LA), asid α-linolenik (18:3n-3, ALA), asid stearidonik (C18:4n-3, SA), asid arakidonik (20:4n-6, AA), asid eikosapentaenoik (20:5n-3, EPA) dan asid dokosapentaenoik (22:6n-3, DHA).

Keywords: Terubok, *Tenualosa toli* , Polyunsaturated Fatty acids (PUFA), Physicochemical charactistics.

Introduction

The Clupeid fish, *Tenualosa toli* or known as Terubok is one of the marine commercial and important consumption tropical shad in Malaysia [1]. Table 1 shows the five *Tenualosa sp* which are found widespread in Asian region. This fish is rich in lipid and provide valuable fatty acids which play a significant role in human health. Polyunsaturated omega-3 fatty acids (ω-3 PUFAs) EPA and DHA especially obtained from fish oil are reported to be potential in reducing coronary heart diseases, stroke, hypertension, cardiac arrhythmias, diabetes, rheumatoid arthritis, brain development, photoreception system, cancer and depression [2-4].

PUFA omega-6 (ω-6) groups are important for a healthy skin, premenstrual syndrome, migraine and multiple sclerosis [5]. Since human and vertebra can produce only saturated and ω-9 fatty acids [6], therefore ω-3 fatty acids must be consumed from foods. Only marine seaweed and some fungi are able to synthesis ω-3 fatty acids [7-8]. Marine fish can neither biosynthesis ω-3 fatty acids de novo nor from shorter chain precursors such as ALA. Therefore ω-3 fatty acids are important in fish. Fish itself needs

PUFA for cell membrane biochemistry and for maintaining physiological function such as osmoregulation, and eicosanoid metabolism [9-10].

Table 1: The variety of *Tenualosa sp* in tropical Asian region

Common name	Scientific name	Origin
Terubok	*Tenualosa toli*	Malaysia
Terubuk	*Tenualosa macrura*	Indonesia
Hilsa	*Tenualosa ilisha*	India
Pha Mak Pang	*Tenualosa thibaudeaui*	Mekong
nd	*Tenualosa reevesii*	Southern China

Source: Blaber et al. 1999.

The interest in PUFA in human diet has led to exploit some of local marine fish lipids which were recognized as having similar PUFA as other commercial source of ω-3 and ω-6 PUFA such as menhaden oil and cod liver oil. The PUFA obtained from local fish oil may be found easily in a large scale with lower cost. PUFA from local fish are highly potential to be used in food industry and therefore promoting the expending fish industry in Malaysia. The aims of this study are to characterize the physicochemical characteristics of crude terubok fish oil and also to determine the compositions of PUFA in terubok oil.

Material and methods

2.1 Sample preparation

Fishes were gutted and cleaned. The skinless fish muscles were homogenized before dried in freeze-dryer at -50 ^{0}C for 3 days.

2.2 Crude oil extraction

Dried fish muscles were weight and crushed using an electric grinder. Samples were extracted with sohxlet apparatus using a mixture of methanol/chloroform (1:2, v/v) as solvents. The extraction was carried out for 6 hours in mild temperature (55-60 ^{0}C).

2.3 Physical and chemical characteristics

Refractive index (RI) was determined using a refractometer (Atago, Japan) and crude fish oil color was estimated by a chromameter (Cielab Coordinate Minolta Chromameter CR100). Iodine value (IV, Wijs), acid value (AV), peroxide value (PV), free fatty acids content (%FFA, oleic acid), saponification number (SV) and unsaponifiable matter were measured according to Porim Test Method [11].

2.4 Fatty acid methyl esters (FAMEs) analysis

The extracted lipid was first transesterified with sodium methoxide to produce fatty acid metal ester [11]. Fatty acids composition was determined by using a gas chromatograph (Shimadzu), equipped with a column BPX 70 (30m x 0.25mm x 0.25μm) and FID detector. The oven, injector and detector temperature were held at 240 ^{0}C, 260 ^{0}C and 280 ^{0}C respectively. Nitrogen as carrier gas was used and flowed at 0.3 mL/min. Identification of the fatty acids composition was based on Menhaden oil and standard individual fatty acids methyl esters C12:0, C14:0, C14: 1, C16:0, C16:1, C18:0, C18:1n-9, C18:2n-6, C18:3n-3, C20:4n6, C20:5n-3 and C22:6n-3 which were purchased from Sigma.

Results and Discussion

3.1 Physical and chemical characteristics

Terubok fish contains high lipid percentage (dry wt/wt) compared to other tropical fish which is about 58.6%. Physicochemical characteristics of Terubok and Menhaden oils are showed in Table 2. The refractive index of Menhaden oil (1.48±0.01) was higher than Terubok (1.45±0.01). This indicated that

Menhaden oil is having more unsaturated fatty acids and less colored compared to Terubok fish oil. Terubok oil contains more saturated fatty acids and reddish in color whereas Menhaden oil was yellowish.

Table 2 : Physicochemical characteristics of menhaden and terubok oil

Characteristics	Menhaden	Terubok
% lipid	-	58.6±1.2
Iodine value	188.35±2.84	170.43±0.18
Peroxcide value	1.47±0.11	8.4±1.98
Acid value	1.02±0.08	7.0±0.23
% FFA	0.49±0.03	3.52±0.11
Saponification number	119.92±6.94	166.90±1.98
Unsaponifiable matter	3.28±0.04	13.90±1.70
Refractive index	1.48±0.01	1.45±0.01
Color	L=33.43± 0.14	L=29.67± 0.01
	a=-0.92± 0.01	a=-0.32 ±0.09
	b=+7.19± 0.11	b=+1.63± 0.03

M±S.D= mean of duplicate samples ± standard deviation
*L= (Black) 0-100 (White)
*a= (Greenness) -100--+100 (Redness)
*b= (Blueness) -100--+100 (Yellowness)

In general the quality of oil was determined by its chemical characteristics. The iodine value, IV for Terubok oil (170.43±0.18) is slightly lower than of Menhaden oil (188.35±2.84). This indicates that Terubok oil is comparable with Manhaden oil that contains high percentage in polyunsaturated fatty acids. The extracted Terubok oil obtained in this study was not refined, unbleached and deodorized (RBD). It is in a form of crude oil and show higher value of peroxide value, PV (8.4±1.98), acid value (7.0±0.23) and %FFA (3.52±0.11) compared to commercial RBD Menhaden oil that exhibits lower values with 1.47±0.11, 1.02±0.08, 0.49±0.03 respectively. However according the Typical Crude Fish Oil Guidelines Specification [12], crude fish oil should content %FFA in the range of 2-5%, PV (3-20 Meq/kg) and IV in the range of 95-200 (Wijs method). This indicates that the Terubok crude oil obtained is comparable to the guideline. The saponification value for Terubok oil was 166.90±1.98 whereas for Menhaden oil was 119.92±6.94. This indicates that Terubuk oil contain lower average of molecule weight fatty acid chain length compared to Manhaden oil. Shorter chain fatty acid glycerides show higher in saponification value than those with longer chain fatty acid glicerides [13]. Fish oils usually show a relatively low unsaponifiable matters value such as in Menhaden oil which is about 3.28±0.04. However crude Terubok oil yields an average high unsaponifiable matter of 13.90±1.70 that due to phospholipids and other minor lipid groups.

3.2 Fatty acids composition

The gas chromatograpy results show that there are more than 20 fatty acids types observed in Terubok fish oil. However, in this report only 12 dominant fatty acids are identified as shown in Table 3. The major saturated fatty acids (SFA) were myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$) and stearic acid ($C_{18:0}$). Unsaturated fatty acids (USFA) dominated by monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Oleic acid ($C_{18:1}$), palmitoleic acid ($C_{16:1}$) and myristoleic acid ($C_{14:1}$) were contributing in high amount of MUFA. Terubuk's PUFA consist of linoleic acid ($C_{18:2n-6}$, LA), α-linoleate acid ($C_{18:3n-3}$, ALA), stearidonic acid ($C_{18:4n-3}$, SA), arachidonic acid ($C_{20:4n-6}$, AA), eicosapentaenoic acid ($C_{20:5n-3}$, EPA) and docosapentaenoic acid ($C_{22:6n-3}$, DHA). The fatty acids percentage in Terubok oil increased in the following order, USFA>SFA>MUFA>PUFA. This is different from Menhaden oil which increased in following order, USFA>PUFA>MUFA>SFA. The different in fatty acids compositions in fish is due to the geographic factor, species, periods of fasting, diet, level of maturity and spawning cycles [14].

Table 3: Fatty acids composition of menhaden and terubok

Fatty acids	Menhaden		Terubok	
	Average	*S. D	Average	* S. D
C14:0	10.44	0.52	9.22	0.51
C16:0	23.17	0.38	31.46	0.06
C18:0	3.92	0.29	6.54	0.33
C14:1	0.18	0.21	0.22	0
C16:1	13.36	0.21	10.28	0.25
C18:1	9.42	0.14	17.3	1.13
C18:2n-6	1.6	0.01	1.56	0.15
C18:3n-3	1.59	0.01	1.42	0.27
C18:4n-3	3.24	0.01	3	0.16
C20:4n-6	1.77	0.04	1.3	0
C20:5n-3	16.35	0.25	8.62	0.86
C22:6n-3	14.98	0.56	9.2	1.16
Saturated	37.53	0.62	47.22	0.23
Unsaturated	62.48	0.62	52.79	0.23
Monounsaturated	22.96	0.28	27.69	1.53
Polyunsaturated	39.52	0.89	25.1	1.76
(ω-3)	36.15	0.83	22.24	1.91
(ω-6)	3.37	0.06	2.86	0.15
(ω3/ω6)	10.73	0.06	7.82	1.07

Average= mean of duplicate samples
*S. D= standard deviation

Lipids from marine fish are generally recognised by low level of linoleic acids (18:2n-6) and linolenic acids (18:3n-3) but high level of EPA and DHA [15]. This is in agreement for both examined fish oils. The percentages of EPA and DHA in Menhaden oil (16.35± 0.25, 14.98 ±0.56) and in Terubok oil (8.62±0.86, 9.2±1.16) were significantly in range as reported in previous research [16]. The report has identified fatty acids composition of others selected Malaysian marine fish and claimed that EPA and DHA were in the range of 27.3-9.36% and 6.76-0.82% respectively, which contributed by yellow striped scad and striped sea catfish. On the other hand, stearidonic acid, SA was found in small amount in most Malaysian fish (0.98-0.19%), however for Terubok oil contains about 3.0±0.16%. This is higher percentage compared to extracted cod liver oil that gave only 2.29% [17]. However, SA in Terubok oil was lower compared to Menhaden oil approximately 3.24±0.01%. These differences may due to their diet and environmental factors. Plankton is the primary source of ω-3 fatty acids and transferred via the nutrition chain [18].

In addition, aracidonic acid, AA was used as a precursor of eicosanoid acid which is important to produce prostaglandin [19]. Nonetheless AA is a minor compound in marine fish compared to freshwater fish that are capable to elongate and desaturase from LA [9]. Lenoliec acid, LA and AA found in Terubok oil were 1.56±0.15 and 1.30±0.00% compared to Menhaden oil that was 1.60 ±0.01 and 1.77± 0.04% respectively. The ω-3/ω-6 fatty acids ratio is a superior index in comparing relative nutritional value of fish oil from different species [20]. In general, the ω-3 fatty acids especially SA, EPA and DHA contain are higher than ω-6 fatty acids (LA and AA) in many marine fish. The ω-3/ω-6 ratio found in Terubok oil was 7.82±1.07% while Menhaden give bigger percentage of 10.73±0.06%. The ratio was relatively comparable to Menhaden oil. This indicates that Terubok oil can be as a good source of PUFA especially EPA, DHA and SA obtained from local shad fish.

Conclusions

Terubok oil shows high composition in PUFA comparable to commercial Manhaden fish oil. It is suggested that the Terubok oil can be further exploit for it commercial value. Its high content in PUFA can be used as food supplement, pharmaceutical products and also babies feed active ingredients.

Acknowledgement

We would like to thank UKM and the Ministry of Science, Technology and Innovations (MOSTI) for project funding under research grant # 09-02-02-0115 EA 277

References

1. Blaber, S. J. M., Brewer, D.T., Milton, D. A., Merta, G. S., Efizon, D., Fry, G. & van der Velde, T. (1997) "The Life History of The Protandrous Tropical Shad *Tenualosa macrura* (Alosinae: Clupeidae): Fishery Implication", *Estuarine, Coastal and Shelf Science 49*. 689-701.
2. Furst, P., & Kuhn, K. S. (2000) "Fish Oil Emulsions: What Benefits Can They Bring?", *Clinical Nutrition 19*(1). 7-14.
3. Sidhu, K. S. (2003) "Health Benefits and Potential Risks Related to Consumption of Fish or Fish Oil", *Regulatory Toxicolog And Pharmacy 38*. 336-344.
4. Holiloglu, H. I. Bayir, A. Sirkecioglu, A. N. Aras, A. M., & Atamanalp, M. (2004) "Comparison Of Fatty Acids Composition Some Tissues of Rainbow Trout (*Oncorhynchus mykiss*) Living In Seawater and Freshwater", *Food Chemistry 86*. 55-59.
5. Senanayake, N. & Shahidi, F. (2002) "Lipase-Catalyzed Incorporation of DHA into Borage Oil Optimization Using Response Surface Methodology", *Food Chemistry 77*. 115-123.
6. Pigott, G. M., & Tucker, B. W. (1990) "Seafood: Effects of Technology on Nutrition", Mercel Dekker, Inc. New York.
7. Uauy, R. Valenzuela, A., (2000) "Marine Oils: The Health Benefits of n-3 Fatty Acids", *Nutrition 16*. 680-684.
8. Shahidi, F., & Wanasundara, U. N. (1998) "Omega-3 Fatty Acids Concentrates: Nutritional Aspects and Production Technologies", *Food Science and Technology 9*. 230-240.
9. Sargent, J. Bell, G. McEvoy, L. Tocher, D., & Estevez, A. (1999) "Recent Development in The Essential Fatty Acid Nutrition of Fish", *Aquaculture 177*. 191-19
10. Smith, D. M. Hunter, B. J. Allan, G.L. Roberts, D. C. K. Booth, M. A., & Glencross, B.D.(2004) "Essential Fatty Acids in The Diet of Silver Perch (*Bidyanus bidyanus*): Effects of Linolenic Acid on Growth and Survival", *Aquaculture 236*. 377-390.
11. Siew, W. L.,Tang, T. S. & Tan, Y. A. (1995) "Methods of Test For Palm Oil and Palm Oil Products", PORIM.
12. Stansby, M. E. (1990) "Fish Oils in Nutrition", New York : Van Nostrand Reinhold.
13. O'Brien, R. D. (1998) "Fats and Oils: Formulating and Processing for Application", Lancaster: Technomic Publishing Co. Inc.
14. Hunter, B. J., & Robert, D. C. K. (2000) Potential Impact of the Fat Composition of Farmed Fish on Human Health", *Nutrition Research 20* (7). 1047-1058.
15. Steffens, W. (1997) "Effects of Variation in Essential Fatty Acids in Fish Feeds on Nutritive Value of Freshwater Fish for Humans", *.Aquaculture 151*. 97-119.
16. Osman, H., Suriah, A. R. & Law, E.C. (2001) "Fatty Acid Composition and Cholesterol Content of Selected Marine Fish in Malaysian Waters", *Food Chemistry 73*. 55-60.
17. Guil-Guerrero, J. L. & Belarbi, E. H. (2001) "Purification Process for Cod Liver Oil Polyunsaturated Fatty acids", *JAOCS 78*. 477-481.
18. Grun, I. U. Shi, H. Fernando, L. N., & Clarke, A. D. (1999) "Differentiation and Identification Of Cultured and Wild Crippie (*Pomoxis Spp*.) Based on Fatty Acids Composition", *Technol 32*. 305-311.

19. Bell, J.G. Ghioni, C., & Sargent, J. R. (1994) "Fatty Acid Compositions of Ten Freshwater Interverbrates Which are Natural Food Organisms of Atlantic Salmon Parr (*Salmo salar*): A Comparision with Commercial Diets", *Aquaculture 128*. 301-313.
20. Suriah, A.R, Huah T. S, Osman, H., & Nik Mat, D. (1995) "Fatty Acids Composition of Some Malaysian Freshwater Fish", *Food Chemistry 54*. 45-49. ↵