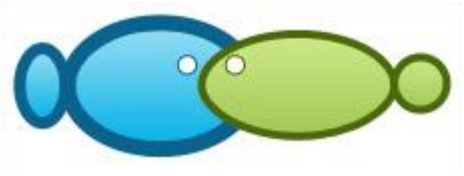

Exploration of seluang fish (*Rasbora argyrotaenia*) oil extraction methods by enzyme extraction and wet pressing with quality analysis

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Abstract. Seluang fish (*Rasbora argyrotaenia*) is a fish found in South Sumatra, Indonesia. Previous studies on seluang fish oil revealed high contents of omega 3 and cholecalciferol (vitamin D3). This study aimed to assess the efficacy of wet pressing (WP) and enzyme extraction (EE) methods in optimizing the quality of extracted seluang fish oil. It was expected to obtain an optimal extraction method in producing fish oil. In the WP method, grinded seluang fish was previously thawed at room temperature for 8 hours, then water was added, and heated at 95°C for 30 minutes. Grinded fish was then pressured and water and oil were co-extracted. In the EE method, grinded fish and papain were mixed then heated at 60°C for 120 minutes. Next, the enzyme was deactivated by heating. Neutral lipids were determined using liquid chromatography via centrifugation in a high performance liquid chromatography (HPLC) system. Determination of the fatty acid profile was carried out by the AOAC method. The fatty acid methyl esters were determined by gas chromatography (GC). The acidity value was determined according to the AOCS Official Methods Ca 5a-40. The yield in the EE methods was higher than that from the WP methods ($p<0.05$). The levels of wax esters, triacylglycerides, and free fatty acids in the WP methods were significantly lower than those in the EE methods. Meanwhile, there was no significant difference for cholesterol levels between the two methods. Analysis of fatty acids content showed no significant difference related to fatty acid content of the two extraction methods. The EE method was more effective than the WP method, both in terms of quantity and quality of fish oil.
Key Words: DHA, EPA, neutral lipids, papain.

Introduction. Seluang fish (*Rasbora argyrotaenia*) is a fish found in South Sumatra, Indonesia. Seluang fish possesses has a body 12-15 cm long, rich in oil. The oil content presents potential for further exploration related to its benefits, efficacy and safety (Partan & Hidayat 2017). Previous studies of seluang fish oil exhibited high contents of omega 3 and cholecalciferol (vitamin D3) (Partan & Hidayat 2017). Omega 3 has the potential to inhibit cellular inflammatory processes, where inflammation is believed to play a role in the initiation of various degenerative health disorders, both cardiovascular related (acute coronary syndrome), or endocrine (diabetes mellitus and dyslipidemia). In addition, omega 3 has the potential to activate neuronal growth in the brain, so that it can improve cognitive abilities and intelligence. Cholecalciferol (vitamin D3) is important in the regulation of the human immune system. Cholecalciferol has the potential to regulate the immune system to increase the synthesis of immune cells and decreases the activity of the immune system when it is too active. Previous studies had shown the potential of fish oil in the field of reducing the inflammatory response that occurred in patients with autoimmune disorders, like Systemic Lupus Erythematosus (SLE) (Partan et al 2018; Partan et al 2019).

Better techniques and methods to extract seluang fish oil are required to obtain the optimum quality of omega 3 and cholecalciferol in seluang fish oil. Wet pressing (WP) extraction technique is a fish oil extraction technique that consists of three main processes: heating at high temperatures (85-95°C), pressing and centrifugation. This technique is very common and is often used for fish oil extraction processes, but sometimes the WP technique is still not optimal in producing fish oil. Meanwhile, the

enzyme extraction (EE) technique is a fish oil extraction technique that utilizes digestive enzymes to optimize fish oil extraction. Papain is a digestive enzyme obtained from papaya plant (*Carica papaya*), where this enzyme has the potential and meets the criteria for a safe and effective digestive enzyme (Suseno et al 2013; Suseno et al 2017; Bako et al 2017).

This study aimed to assess the efficacy of WP and EE methods in optimizing the quality of extracted fish oil. To our knowledge, this was one of the first studies conducted in order to optimize the extraction method from seluang fish oil. It was expected to obtain an optimal extraction method in producing high quality fish oil.

Material and Method

Fish processing. About 30 kg of seluang fish was obtained from the Palembang Fish Auction Center, South Sumatra, Indonesia, in May 2019. The fish was kept in a refrigerator at -25°C to minimize biochemical changes during fish transportation from the Auction Center to the Biotechnology Laboratory, Faculty of Medicine, Sriwijaya University (Palembang, Indonesia). Next, the whole body of the seluang fish was grinded and stored at -25°C until use.

Wet pressing. Grinded seluang fish were previously thawed at room temperature for 8 hours, then 1 L of water was added to every 200 g of flesh of the seluang fish, and heated at 95°C for 30 min. Grinded fish were pressured and water together with oil were co-extracted. The third step was centrifugation at 5000 rpm, temperature 25°C, for 10 minutes (Bako et al 2017; Taati et al 2018).

Enzyme extraction. A total of 1 kg of grinded whole fish and 10 g of papain were mixed then heated at 60°C for 120 minutes. Next, the enzyme was deactivated by heating at 95°C for 30 min, followed by centrifugation (5000 rpm), at 25°C, for 10 min (Taati et al 2018).

Determination of yield. The yield was expressed as a percentage of oil separated by seluang fish. Yield was calculated as follows (Taati et al 2018):

%Yield = Weight of fish oil/Weight of whole fish x 100

Neutral lipids evaluation. Total neutral lipids were determined using liquid chromatography in the HPLC system (Agilent 1200). Lipid separation was carried out at room temperature in a column (Lichrospher Diol 5 mm, 4x250 mm) and detection was carried out at an evaporative light scattering detector (Agilent 1200 series) at 45°C and a pressure of 3.5 bar. The mobile phase consists of a mixture of solvents: (A) hexane/acetic acid (99.5/0.5 by volume) and (B) hexane/1-propanol/acetic acid/water (85/14.4/0.5/0.1 by volume). The solvent gradient used was as follows: first, solvent A was flowing for 2 min, after that, solvent B was added in three steps, up to 10% in 10 min, to 44% in 15 min and to 100% in 9 min. The stationary phase was rinsed with solvent A during 6 min. The total solvent flow rate was kept constant at 1 mL min^{-1} throughout the analysis. Calibration was carried out using standards of palmityl palmitate (99%), tripalmitin (>99%), dipalmitin (99%), monopalmitin (99%) and palmitic acid (99%) in hexane. The calibration curves showed a good correlation according to the exponential relationship described for an evaporative light scattering detector (Taati et al 2018).

Determination of fatty acids. Determination of the fatty acid profile was carried out by the AOAC method. The fatty acid methyl esters were first prepared and then analyzed by gas chromatography (GC) in a Hewlett Packard gas chromatograph (6890N Network GC System) equipped with an auto-sampler (7683B series) and a flame ionization detector (FID). The separation was carried out with helium (1.8 mL min^{-1}) as a carrier gas. A fused silica capillary column (OmegawaxTM-320, 30 m × 0.32 mm i.d.) was used. The

column temperature was programmed starting at a constant temperature of 180°C for 20 min, heated to 200°C at 1°C min^{-1}, held at 200°C during 1 min, heated again to 220°C at 5°C min^{-1} and finally held at 220°C for 20 min. A split injector (50:1) at 250°C was used. The FID was also heated at 250°C. Most of the fatty acid methyl esters were identified by comparison of their retention times with those of chromatographic standards (Sigma Chemical Co). Their quantification was made by relating the peak area to the area of an internal standard (methyl tricosanoate), as indicated by the AOAC method (Latimer 2016). Calibration curves were made for each pair of internal standards and chromatographic standards in order to find the appropriate response factors (Taati et al 2018).

Determination of acid value. The acidity value was determined according to the AOCS Official Methods Ca 5a-40 (Latimer 2016; Taati et al 2018).

Statistical analysis. Statistical analysis was performed with IBM SPSS 25 software. Data was presented as Mean±SD (standard deviation). Then, a bivariate analysis was performed with a T-test, to assess differences in the mean content and content of each extraction method. Significance was set for $p < 0.05$.

Results and Discussion

Yield. The amount of oil obtained by WP and EE methods is presented in Figure 1. The yield in the EE method was higher than in the WP method ($p<0.05$).

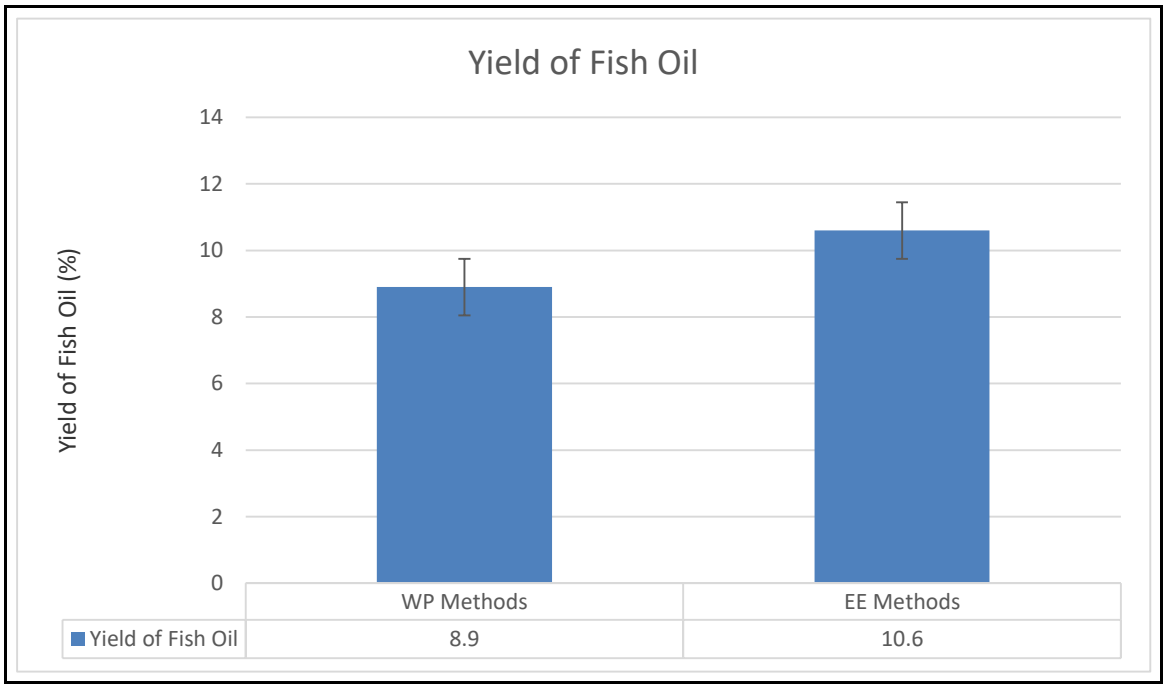

Figure 1. The yield (%) of seluang fish (*Rasbora argyrotaenia*) oil obtained by wet pressing (WP) and enzyme extraction (EE) methods.

Neutral lipids. Table 1 showed the level of neutral lipids with significant differences between WP and EE methods. The levels of wax esters, triacylglycerides, and free fatty acids in the WP method were significantly lower than in the EE method. Meanwhile, there was no significant difference for cholesterol levels between the two methods.

Table 1

The levels of neutral lipids (%) in seluang fish (*Rasbora argyrotaenia*) oil by wet pressing (WP) and enzyme extract (EE) methods

Components	*WP method*	*EE method*
Wax esters (WE)	1.82±0.3	1.32±0.2
Triacylglycerides (TAG)	90.21±3.02	92.32±4.34
Free fatty acids (FFA)	3.56±0.32	4.54±1.12
Cholesterol (CHOL)	2.21±2.01	2.34±2.02

Free fatty acids profile. Analysis of fatty acids content showed no significant difference between the two extraction methods. However, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels showed significant differences between the two extraction methods. EPA and DHA levels are higher in EE than WP method.

Table 3

The fatty acids profile in seluang fish (*Rasbora argyrotaenia*) oil obtained by wet pressing (WP) and enzyme extraction (EE) methods

Fatty acids	*WP method*	*EE method*
C12:0	0.08±0.01	0.06±0.03
C14:0	5.11±1.02	3.02±1.03
C15:0	0.87±0.2	0.89±0.35
C16:0	11.76±0.5	12.65±0.43
C16:1	6.28±2.4	6.45±3.32
C17:0	1.75±1.2	1.86±1.01
C18:0	4.89±2.54	3.89±2.94
C18:1c	16.23±4.56	16.89±5.54
C18:2c	1.29±1.54	1.82±1.54
C18:3 n6	1.09±1.14	1.59±1.21
C18:3 n3	1.86±0.04	2.19±0.02
C20:0	0.89±0.54	0.99±0.67
C20:1	0.59±0.44	0.79±0.43
C20:4 n3	1.85±0.89	1.89±0.87
C20:4 n6	1.95±0.98	1.96±0.77
C20:5 n3	4.19±2.99	4.29±3.54
C22:0	0.89±0.54	0.77±0.76
C22:5 n3	4.76±2.54	4.99±3.34
C22:6 n3	19.89±9.54	20.87±8.98
EPA+DHA	8.89±0.34	10.97±0.94

Acid value. Figure 2 showed a significant difference in acid values between WP and EE methods. Acid values in EE methods were almost close to recommended rates, compared to WP methods.

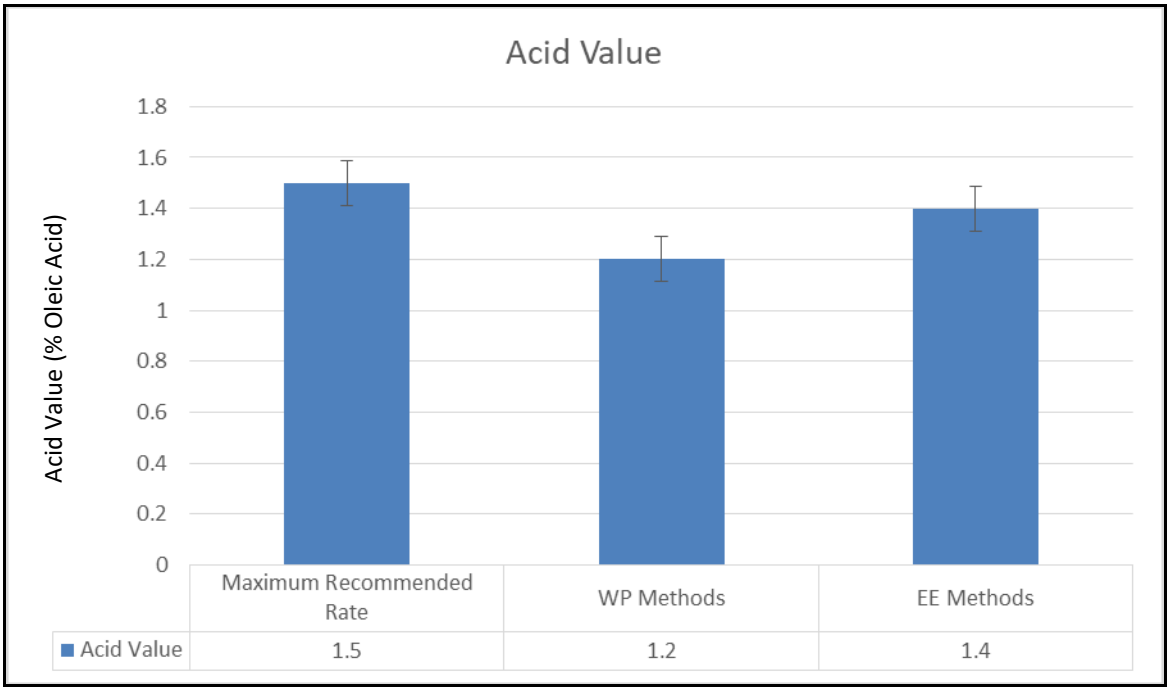

Figure 2. Acid value determination.

Fish oil is rich in essential fatty acids, which play a role in optimizing the metabolism of cells, further improving the quality of health. Essential fatty acids are the main precursors in the production of various hormones and growth factors, especially sexual hormones, such as testosterone, estrogen, and androgens. EPA and DHA essential fatty acids play a role in maintaining the integrity of cell membranes, preventing damage from cells and increasing the survival of cells, especially neuronal cells. Thus, EPA and DHA are very important in increasing the transmission of impulses in nerve cells and have an impact on improving cognitive function. Because of enormous potential and benefits of fish oil, optimal efforts are needed to explore extraction methods, in order to obtain fish oil with optimal both quantity and quality (Ramakrishnan 2013; Deepika et al 2014; Ivanovs & Blumberga 2017).

Whole fish oil from the EE method showed a higher yield than the WP method. In the WP method, fish are first roasted at high temperatures and then extracted with mechanical pressure. The heat contributes to the denaturation of the protein matrix from the fish meat tissue bound by oil. After this process, solid particles and liquids can be extracted mechanically. In addition, heat causes the opening of clots of oil and fat cells, resulting in the release and fluidity of oil. EE methods provide the presence of protease enzymes that play a role in the hydrolysis of proteins dissolved from fish meat in fish oil. The hydrolysis carried out by the protease enzyme plays a more effective and efficient role in the separation of oil clots from the free tissue protein compared with high temperature and mechanical pressure. Therefore, the results are much higher than the results of the WP method. A study presented the results of oil extraction from fish by-products by 4 methods: WP, supercritical fluid extraction (SFE), EE and cold extraction (CE). The studied showed that the SFE method was better than EE method, and EE was better than WP and CE (Rubio-Rodriguez et al 2012). Other studies showed similar results to this study, with significant differences between the WP and EE methods, where the EE method was more effective in extracting fish oil (Azmir et al 2013; Hajeb et al 2015; Bonilla-Méndez & Hoyos-Concha 2018; Wenwei et al 2019).

The results of neutral lipids analysis in this study showed significant differences in the levels of wax esters (WE), triacylglycerides (TAG) and free fatty acids (FFA) in oil extracted from seluang fish by the WP and EE methods. Low levels of WE and high TAG levels can indicate low intracellular fat levels and high levels of extracellular fat that are bound to proteins. Weak bonding of extracellular oil with protein causes an increase in

fish oil extraction, so that it will be followed by an increase in triacylglycerol and reduced WE. A high TAG in fish oil indicates high PUFAs in fish oil. On the other hand, the hydrolysis of TAG causes the formation of FFA. Therefore, higher levels of TAG and PUFA produce more FFA. In a study on the effect of cooking time and temperature on quantitative and qualitative parameters of extracted oil from tuna (*Thunnus albacares*) meat showed that with increasing TAG, the level of FFA also increased (Latip et al 2014; Baehaki et al 2015; Ahmed et al 2017; Rachmawati & Samidjan 2018).

This study showed that the EE method was able to obtain oils with higher EPA and DHA content compared to the WP method. EPA and DHA are unsaturated fatty acids with double chains, where high temperature heating carried out in the WP method was believed to play a role in causing damage or breaking the fatty acid double chains, so that saturated fatty acids were formed (Hajeb et al 2015; Nugraheni et al 2017).

Acidity is an important parameter in oil quality, which is influenced by the amount of FFA and other non-lipid acids, such as acetic acid. In general, oils that contain high amounts of TAG and PUFA have high FFA levels to reduce acidity. In this study, TAG and FFA levels in the EE method were slightly higher than in the WP method. Therefore, higher acidity value in the EE method was caused by FFA (Taati et al 2018).

Conclusions. The enzyme extraction method was a method of extracting fish oil seluang fish (*R. argyrotaenia*) that was more effective than the wet pressing method, both in terms of quantity and quality of fish oil.

Acknowledgements. The authors are grateful to the Department of Internal Medicine and Department of Biology, Faculty of Medicine, Universitas Sriwijaya, in the cooperation for the conduction of this study.

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Received: 06 November 2019. Accepted: 01 March 2020. Published online: 30 August 2020.
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How to cite this article:
Partan R. U., Hidayat R., 2020 Exploration of seluang fish (*Rasbora argyrotaenia*) oil extraction methods by enzyme extraction and wet pressing with quality analysis. AACL Bioflux 13(4):2283-2289.