

THE COMPARISON OF VITAMIN D CONTENT OF FISH OIL EXTRACTS OF SEVERAL TYPES OF FRESHWATER FISH

Gabriella Septiani Nasution¹, Nita Andriani Lubis², Sri Widia Ningsih³, Adriana
Hamsar⁴

¹²³⁴Politeknik Kesehatan kementerian Kesehatan Medan
Email: gabriellaseptiani01@gmail.com

ABSTRACT

Fish is beneficial for health due to its content of EPA, DHA, linoleic acid, iodine, protein, selenium, omega-3, vitamin A, vitamin D, and vitamin B12. Fish has the potential to be developed into fish oil. According to WHO, the consumption of fish oil can prevent the risk of mortality and improve health functions. However, fish consumption in toddlers is still low. A survey conducted on children in Indonesia showed that 45.1% experienced Vitamin D deficiency. Vitamin D is known to play a role in the immune system as an immunomodulator and enhances innate immunity. This research aims to compare the vitamin D content of fish oil extracts from several types of freshwater fish and determine their fatty acid content. The researched fish will produce different fish oil and vitamin D content, necessitating research to assess the vitamin D content. Furthermore, fish oil with high vitamin D content can be considered a competent candidate for health supplements. Three types of fish, namely snakehead fish, eel, and catfish, were chosen as research samples. The research was conducted at the Integrated Laboratory of Poltekkes Medan and PPKS. This experimental research used a Randomized Block Design (RBD) with three replications. The data were then statistically analyzed using Anova with a significance level of 5%. The research results indicate that the highest vitamin D content is found in eel with alcohol solvent (12.2974 mg/l). The vitamin D content in snakehead fish with alcohol solvent is 7.8832 mg/l, while in catfish with alcohol solvent, it is 3.0069 mg/l. For fish oil with N-Hexane solvent, the vitamin D content in snakehead fish is 1.4096 mg/l, eel is 2.2489 mg/l, and catfish is 4.5706 mg/l.

Keywords: Fish; fish oil; vitamin D; freshwater; fatty acids

INTRODUCTION

Fish is an essential and abundant food source, and Indonesia possesses vast marine potential and abundant freshwater resources for the development of inland fisheries. Freshwater fish such as snakehead fish, eel, and catfish are excellent and prospective sources of protein, fats, vitamins, and minerals. Fish contains unsaturated fatty acids such as EPA, DHA, and linoleic acid, which are beneficial for health. Fish also contains iodine, protein, selenium, omega-3, and various vitamins (A, D, and B12) necessary for maintaining a healthy metabolism. Considering the commodities and unsaturated fatty acid content in these fish, there is great potential for further development into fish oil.

Omega-3 fatty acids in fish play a beneficial role in maintaining arteries, lowering blood pressure, and reducing the risk of heart attacks and strokes. The vitamin D content in fish oil ranges from 50 to 30,000 IU per gram. Vitamin D is involved in bone metabolism, mineral function, muscle function, and enhances innate immunity as an (immunomodulator). ¹

Previous research indicates that fish consumption is beneficial for health, reducing the risk of coronary heart disease, diabetes, child and maternal health, arthritis, cancer, etc. ^{2,3,4} However, national fish consumption in Indonesia is lower compared to other Southeast Asian countries. The Ministry of Marine Affairs and Fisheries reported in 2011 that fish consumption in Indonesia was 31.5 kg per year, significantly lower than Malaysia's 55.4 kg per year. Vitamin D deficiency is a current nutritional concern and a global public health issue. Hypovitaminosis can lead to various diseases, including cardiovascular diseases, diabetes, chronic kidney failure, and asthma. ⁵

Studies abroad show a high prevalence of vitamin D deficiency, exceeding 70% in South Asia and ranging from 6-70% in Southeast Asia across all age groups, including toddlers, schoolchildren, pregnant women, and adult men. ⁶ Studies in Indonesia reveal a 50% prevalence of vitamin D deficiency in women aged 45-55. Surveys conducted on children in Indonesia show 49.3% experiencing insufficiency, while 45.1% have vitamin D deficiency. Although fish is a source of vitamin D, toddlers tend to dislike consuming fish. Advanced fish processing technology allows freshwater fish like snakehead fish, eel, and catfish to be further developed into fish oil, which is also a source of vitamin D. ⁷ Research regarding the nutritional content of these three types of freshwater fish has been carried out, but the vitamin D content in fish oil has never been carried out

This study aims to compare the vitamin D content of extracts from snakehead fish, eel, and catfish oil, as well as their fatty acid compositions. It is suspected that these three freshwater fish may produce different qualities of fish oil and vitamin D, necessitating examination for a comparative analysis of vitamin D content. High vitamin D fish oil also competes as a candidate supplement for immunomodulation and anti-diabetes. The benefits of this research include providing information for the public and government regarding the choice of fish-rich menus for household consumption, promoting family health, and considering the development of fish oil as an innovative and affordable solution for the community.

RESEARCH METHODS

Data Sources and Study Design

This study is an experimental research conducted from June to August 2022. The subjects and objects of the study are Vitamin D in the extract of freshwater fish oil. Experimental research is a method used to investigate the influence of specific treatments on each other in controlled conditions.⁸ The research design employed is a Randomized Block Design (RBD) with three replications. Achieving the objectives requires data collection that supports the analysis process. The collected data consist of primary data obtained directly through laboratory research. Secondary data collection is derived from literature studies and data from relevant institutions. The research is planned to be conducted at the Integrated Laboratory of Poltekkes Medan for fish oil extract preparation and PPKS for the examination of Vitamin D content. The research is expected to last for 2 months. The research samples include three types of freshwater fish: snakehead fish, catfish, and eel.

The research begins with identifying the problem to be studied, setting goals and objectives to be achieved, followed by a literature review involving reading articles, books, journals, and related research on the studied issue. After the proposal submission, the research proceeds with obtaining ethical clearance.

Research Instruments

The process involves sampling freshwater fish (snakehead fish, catfish, and eel), preparing fish samples through washing and steaming, followed by oil extraction using a Soxhlet apparatus. Subsequently, the collected fish oil undergoes two analyses: analysis of Vitamin D content (AOAC, 2002.05) using HPLC and analysis of fatty acid content using Gas Chromatography. The generated data is then statistically analyzed using ANOVA with a significance level of

5%, followed by drawing conclusions. This allows the comparison of Vitamin D content in the extract of fish oil from various types of freshwater fish and the examination of fatty acid content in these three types of freshwater fish. Vitamin D content is obtained after extracting freshwater fish, resulting in crude fish oil, followed by research on the Vitamin D and fatty acid content of freshwater fish (snakehead fish, catfish, and eel).

Data Analysis

The statistical analysis employed in this research is ANOVA to determine whether different types of fish significantly influence Vitamin D content. The data analysis is conducted using the SPSS Program.

Ethical Approval

The methods in this research have obtained approval from the ethical research commission of the Health Polytechnic Ministry of Health Medan with letter No.01.0368/KEPK/POLTEKKES KEMENKES MEDAN 2022.

RESULTS

1. Sample Preparation

Table 1. Wet Weight and Dry Weight of Fresh Fish Samples

No.	Types of Fish	Wet Weight	Dry Weight
1	Snakehead Fish	5 Kg	1.24 Kg
2	Eel	5 Kg	1.53 Kg
3	Catfish	5 Kg	1.34 Kg

The initial phase of the research involved sample preparation to obtain wet and dry weights. Subsequently, extraction was carried out using a solvent (Soxhlet method) to obtain fish oil. Once the fish oil results were obtained, the fatty acid composition was measured using Gas Chromatography, and the Vitamin D content was analyzed using High-Performance Liquid Chromatography (HPLC). The obtained results were then analyzed and summarized.

During the preparation, Snakehead fish, eels, and catfish, obtained in a deceased state, underwent fillet processing. Fillet processing stages included weighing, washing, filleting, skinning, fillet trimming, skin removal, washing, and re-weighing. The by-products from this

process included the head, skin, tailbone, belly flap meat, remaining trimming/flattening meat, and stomach contents. Observations were made on the yield obtained in this fillet process by calculating the fillet meat yield and the yield of each part of the fish waste.

From Table 1, the initial weight or wet weight of the fish samples was standardized to 5 kg. The dry rendering process involved drying the fish in an oven at 60°C for 24 hours, resulting in a constant dry weight for each fish type: Snakehead fish (1.24 kg), Eel (1.53 kg), and Catfish (1.34 kg). Table 1 shows the differing constant dry weights for each fish, with the highest being eel at 1.53 kg. This weight difference is attributed to the heating process stage, which may lead to oil weight loss.

2. Fish Oil Extraction (Soxhlet Method)

The solvent used for the oil extraction process through Soxhlet method was n-hexane. It is important to note that the evaporated or lost solvent should not exceed 5%. If it does, the entire solvent extraction system needs further examination. In this research, different solvents, namely alcohol and n-hexane, were used in the extraction process."



Figure 1. Snakehead fish oil, eel and catfish
Source : Primary Data, 2022

3. Fatty Acid Composition in Fish Oil Formulation Using Alcohol Solvent

Table 2. Fatty Acid Composition in Fish Oil Formulation Using Alcohol Solvent"

Parameter	Test results
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Fatty acid Composition	Unit	Alcoholic Snakehead	Alcoholic Eel	Alcoholic Catfish	Test Method
- Lauric Acid (C12:0)	%	0.3	0.1	0.3	MPOB p3 5 2004 (Gas Chromatography)
- Myristic Acid (C14:0)	%	2,2	3.1	2.0	
- Palmitic Acid (C16:0)	%	30.4	32.0	36.2	
- Palmitoleic Acid (C16:1)	%	7.9	2.0	2.1	
- Stearic Acid (C18:0)	%	9.0	9.9	16.8	
- Oleic Acid (C18:1)	%	40.7	43.3	31.2	
- Linoleic Acid (C18:2)	%	7.6	9.1	10.4	
- Linolenic Acid (C18:3) (Omega - 3)	%	1.5	0.4	0.9	
- Arachidic Acid (C20:0)	%	0.4	0.2	0.2	
- Eicosenoic Acid (C20:1)	%	1.3	0.7	0.9	
- DHA (C22:6)	%	1.6	0.3	2.1	
- EPA (C22:6)	%	1.1	-	1.5	

Table. 3. Fatty Acid Composition in Fish Oil Preparations with Alcohol Solvents

Parameter		Test results			Test Method
Fatty acid Composition	Unit	Cork N- Hexane	Eel N - Hexane	Patin N - Hexane	
- Lauric Acid (C12:0)	%	31.4	0.1	0.3	MPOB p3 5 2004 (Gas Chromatography)
- Myristic Acid (C14:0)	%	11.8	3.1	2.0	
- Palmitic Acid (C16:0)	%	19.2	32.7	36.2	
- Palmitoleic Acid (C16:1)	%	3.7	1.9	3.1	
- Stearic Acid (C18:0)	%	5.8	10.2	15.9	
- Oleic Acid (C18:1)	%	22.9	42.5	31.0	
- Linoleic Acid (C18:2)	%	4.2	9.0	10.3	
- Linolenic Acid (C18:3) (Omega - 3)	%	0.7	0.3	0.9	
- Arachidic Acid (C20:0)	%	0.2	0.2	0.2	
- Eicosenoic Acid (C20:1)	%	0.2	0.7	0.9	
- DHA (C22:6)	%	0.7	0.3	2.0	
- EPA (C22:6)	%	0.5	-	1.5	

In Table 2 (Fish oil with alcohol solvent), it is observed that the Omega-3 content, consisting of DHA (Snakehead fish 1.5%, Eel 0.4%, and Catfish 0.9%), and Omega-6, specifically linoleic acid (Snakehead fish 7.6%, Eel 9.1%, and Catfish 10.4%). This aligns with the findings of Coletta et al., indicating that Omega-3 and Omega-6 can be derived from both fish and vegetable oils.⁹ The recommended ratio for Omega-3 to Omega-6 fatty acids is (1:1) or (2:1), representing an optimal ratio.^{10,11} The fatty acid composition in the table is dominated by Oleic acid in all three types of fish: Snakehead fish (40.7%), Eel (43.3%), and Catfish (31.2%). The high content of oleic and palmitoleic acids is a characteristic feature of freshwater fish oil.¹² EPA was not detected in Eel.

In Table 3, using N-Hexane as the solvent, the Omega-3 content is as follows: DHA (Snakehead fish 0.7%, Eel 0.3%, and Catfish 2.0%), and Omega-6, specifically linoleic acid (Snakehead fish 4.2%, Eel 9.0%, and Catfish 10.3%).

4. Vitamin D Content in Fish Oil

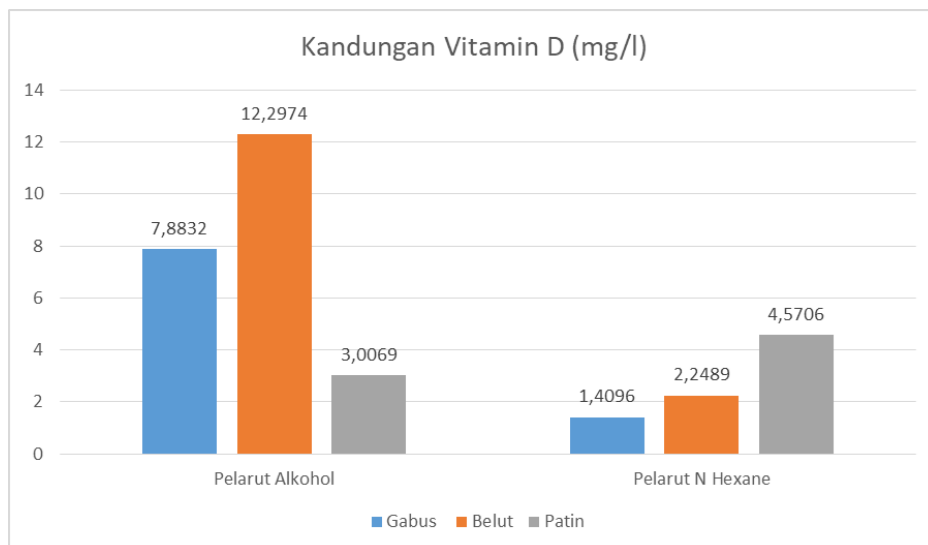


Figure 2. Vitamin D Content with Alcohol and N-Hexane Solvents

In Figure 2, it is observed that the highest Vitamin D content is in Eel with alcohol solvent, measuring 12.2974 ppm, followed by Snakehead fish with alcohol solvent at 7.8832 ppm. Catfish with N-Hexane solvent has a Vitamin D content of 4.5706 ppm, and the lowest Vitamin D content is in Snakehead fish with N-Hexane solvent at 1.4096 ppm.

DISCUSSION

Sample Preparation and Fish Oil Extraction (Soxhlet Method)

During the preparation phase, Snakehead fish, eels, and catfish, obtained in a deceased state, underwent fillet processing. The fillet processing stages included weighing, washing, filleting, skinning, fillet trimming, skin removal, washing, and re-weighing. The by-products from this process included the head, skin, tailbone, belly flap meat, remaining trimming/flattening meat, and stomach contents. Observations were made on the yield obtained from the fillet process by calculating the fillet meat yield and the yield of each part of the fish waste. From Table 1, it is noted that the constant dry weight of each fish differs, with the

highest being eel at 1.53 kg. This weight difference is attributed to the heating process stage, allowing for the loss of oil weight.

Soxhlet extraction is an efficient and effective method for determining the oil or fat content of a substance because the solvent used can be recovered, and the extraction time is relatively short. Soxhlet extraction provides higher extract yields due to the heating process, which is believed to improve extract solubility. Compared to the maceration method, Soxhlet extraction yields higher extract results.

The extraction process utilized the entire fish meat. The extraction principle involves dissolving oil and fat in the oil and fat solvent. According to Vogel (1984), solvent extraction is a process of separating soluble components based on their distribution properties in two immiscible solvents. By exploiting the differences in solubility, the desired compound can be selectively separated. Several factors affecting the extraction process include method differences, solvents, temperature, and extraction time, which influence both the yield amount and the quality of the extract obtained. Using suitable methods, solvents, and times will result in maximum extract yield and quality.

The oil solvent that can be used in the extraction process with an evaporative solvent is n-hexane. It is essential to note that the amount of evaporated or lost solvent should not exceed 5%. If it does, the entire solvent extraction system needs further examination. In this study, different solvents were used, namely alcohol and n-hexane.

Soxhlet extraction is an efficient and effective method for determining the oil or fat content of a substance because the solvent used can be recovered, and the extraction time is relatively short. Soxhlet extraction provides higher extract yields due to the heating process, which is believed to improve extract solubility. Compared to the maceration method, Soxhlet extraction yields higher extract results.

Fatty Acid Composition in Fish Oil Formulation Using Alcohol Solvent

Fish oil contains essential unsaturated fatty acids such as omega-3 (EPA and DHA), omega-6 (linoleic acid and arachidonic acid (ARA)). Additionally, fish also contain vitamin D.^{14,15} After analysis, chromatograms were obtained. Based on the chromatograms obtained, the composition of fish oil fatty acids is presented as a percentage (%). The fatty acid composition obtained from each sample varies, depending on the height and area of the chromatogram for each sample. The composition of fatty acids in the fish oil formulation obtained from the analysis using gas chromatography can be seen in Table 2 for the Fatty Acid Composition in Snakehead Fish, Eel, and Catfish Fish Oil Formulation with Alcohol Solvent, and Table 3 for the Fatty Acid Composition in Snakehead Fish, Eel, and Catfish Fish Oil Formulation with N-Hexane Solvent.

According to Table 2 (fish oil with alcohol solvent) and Table 3 (fish oil with N-Hexane solvent), the analysis results of fatty acids in fish oil formulations using gas chromatography obtained 12 types of fatty acids with different compositions, including saturated fatty acids (SFA) such as Lauric acid (C:12-0), Myristic acid (C:14-0), Palmitic acid (C:16-0), Stearic acid (C:18-0), and Arachidic acid (C:20-0), Behenic acid (C:22:0). Monounsaturated fatty acids (MUFA) such as Palmitoleic acid (C:16-1), Oleic acid (C:18-1), and 9-Eicosenoic acid (C:20-1). Polyunsaturated fatty acids (PUFA) such as Linoleic acid (C:18-2), Oleic acid (C18:1), and DHA (C:22-6) are present in varying compositions in each fish oil formulation. Two types of essential fatty acids are present in Tables 2 and 3. This is consistent with Asfar et al., 2014, stating that snakehead fish consists of saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, and essential fatty acids.¹⁶ The fatty acid composition in the fish oil above is dominated by Oleic acid in all three types of fish: Snakehead fish (40.7%), Eel (43.3%), and Catfish (31.2%). The high content of oleic and palmitoleic acids is a characteristic feature of freshwater fish oil.¹² Other studies by Jacob et al.¹⁷ also state that eel contains high levels of saturated fatty acids, both mono (MUFA) such as oleic acid and poly (PUFA) such as linoleic acid. EPA was not detected in eel. The amount of fat content in fish can also be determined by the color of the fish meat. Red-fleshed fish

contain higher fat than white-fleshed fish.¹⁸ The fat content in freshwater fish is different from that in saltwater fish. This is due to differences in habitat, environment, and food factors.¹⁹ According to the research of Husain et al. (2017); Gunawan, et al. (2014), regarding the difference in fatty acid content between saltwater and freshwater fish, the omega-3 fatty acid content in saltwater fish is higher than that in freshwater fish.^{20,21}

Vitamin D Content

There are two groups of vitamins in fish: water-soluble and fat-soluble. Those soluble in oil include vitamins A and D, found in fish oil. The three types of fish oil studied, namely snakehead fish, eel, and catfish, contain Vitamin D, whether using alcohol or N-Hexane as the solvent. The Vitamin D content in fish oil using alcohol as the solvent is higher compared to using N-Hexane, as alcohol has relatively high solubility and is inert, not reacting with other components. Alcohol has a low boiling point, making it easy to separate the oil from its solvent in the distillation process. The Vitamin D content in snakehead fish oil with alcohol as the solvent is stated to be higher than in other fish, namely 12.2974 ppm.

Statistical Analysis Results

The data obtained are primary data and are ratio-scaled, so quantitative analysis was performed using parametric statistical tests (One-Way ANOVA) using the SPSS 16.0 For Windows program with a significance level (α) of 5%.

Table 4. Statistical Test Results with ANOVA for Vitamin D Content with Alcohol and N-Hexane Solvents.

Variable		Mean	SD	Sig.
	Alcohol	7.7291	4.6471	0.155

content (mg/dl)	n - Hexane	2.7430	1.6374	
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Table 5. Statistical Test Results with ANOVA for Vitamin D Content in Comparison Types of Freshwater Fish.

Variable		Mean	SD	Sig.
content (mg/dl)	Cork	4.6464	4.5775	0.779
	Eel	7.2731	7.1053	
	Patin	3.7888	1.1057	

In Table 4, the Significance value (p-value) in the One-Way ANOVA test is 0.155, which means $p > 0.05$. Therefore, it can be stated that there is no significant difference in the examination results of Vitamin D content using different solvents (Alcohol and N-Hexane).

In Table 5, the Significance value (p-value) in the One-Way ANOVA test is 0.779, which means $p > 0.05$. Thus, it can be stated that there is no significant difference in the examination results of Vitamin D content among various types of freshwater fish.

The results of the ANOVA analysis indicate that the different treatments do not have a statistically significant effect at the 5% significance level on the Vitamin D content values in fish.

CONCLUSION

Vitamin D is present in the examined fish oils, specifically in the oils of snakehead fish, eel, and catfish. There are differences in the Vitamin D content among the three types of fish, with details as follows: snakehead fish oil with alcohol solvent 7.8832 ppm, eel oil 12.2974 ppm, catfish oil 3.0069 ppm, snakehead fish oil with N-Hexane solvent 1.4096 ppm, eel oil with N-Hexane solvent 2.2489 ppm, and catfish oil with N-hexane solvent 4.5706 ppm.

In the samples of snakehead fish, eel, and catfish oil, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) were found.

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