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Optimization of Wet Rendering and Alkali Neutralization for Omega-3-Rich Oil Production from Milkfish (Chanos chanos)

Ilham Ahmad[1*], Rahmawati Saleh[2], Nurlaeli Fattah[2], Imran Muhtar[1], Luthfiah[2]

[1] Agroindustry Study Program, Department of Agricultural Technology, State Agricultural Polytechnic of Pangkajene Islands, Indonesia.
[2] Fishery Product Processing and Storage Study Program, Department of Agricultural Technology, State Agricultural Polytechnic of Pangkajene Islands, Indonesia.

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Corresponding Author:
Ilham Ahmad
ilham.ahmad@polipangkep.ac.id

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Abstract: This study addresses the instability, off-flavors, and variable quality that limit the use of fish oil as an omega-3 source, particularly from milkfish (Chanos chanos). The research aims to produce omega-3-rich milkfish oil that meets international quality standards and is suitable for microencapsulation. Fresh milkfish was processed using wet rendering at four temperatures (70, 80, 90, and 100°C) and three heating times (30, 40, and 60 min). The crude oil was then refined by alkali neutralization with different NaOH concentrations. Oil yield and oxidative quality parameters—peroxide value, p-anisidine value, total oxidation (TOTOX), and acid value—were measured and analyzed using factorial ANOVA. The highest yield (0.29%) was obtained at 100°C for 60 min. Peroxide and anisidine values (3.44 and 11.75 meq/kg) and TOTOX (<20 meq/kg) complied with International Fish Oil Standards, whereas acid values (2.43–2.68 mg KOH/g) exceeded the limit at prolonged high-temperature extraction. These results indicate that higher temperatures increase oil recovery but promote hydrolytic degradation. Careful optimization of time and temperature is therefore required to obtain stable, high-quality omega-3 milkfish oil for further microencapsulation and application in health-oriented food products.

Keywords: Alkali neutralization; Microencapsulation; Milkfish oil; Omega-3 fatty acids; Wet rendering

Introduction

Omega-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have gained global recognition for their critical roles in promoting human health. These bioactive lipids are associated with cardiovascular protection, modulation of blood lipids, neuroprotection, and anti-inflammatory effects. During pregnancy and early infancy, DHA is particularly essential for optimal development of the brain and retina, and supplementation has been linked with improved neurodevelopmental outcomes (Comitini et al., 2020; Khan et al., 2021). Marine fish oils remain the most efficient and accessible natural sources of EPA and DHA and therefore continue to underpin the development of functional foods, nutraceuticals, and therapeutic formulations (Yi et al., 2023).

Indonesia, with its vast marine biodiversity and strong aquaculture sector, holds considerable potential for developing high-value omega-3-based products. One promising resource is Chanos chanos (milkfish), an aquaculture commodity widely cultivated in coastal regions such as Pangkep Regency. Recent studies have shown that milkfish flesh and head oils possess high levels of unsaturated fatty acids and physicochemical properties that can meet international criteria for edible fish oil when appropriately processed (Hidayah et al., 2022; Sasongko et al., 2025). Nevertheless, nutritionally valuable parts such as viscera and belly fat are often

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underutilized or avoided due to perceived risks associated with fat and cholesterol. In fact, omega-3-rich oils derived from these fractions may help reduce blood triglycerides and improve cardiovascular risk markers when consumed in appropriate amounts (Khan et al., 2021; Palabiyik et al., 2025; Santos et al., 2020; Visioli et al., 2020).

Industrial utilization of fish oil, however, is challenged by its intrinsic instability. Long-chain omega-3 PUFAs are highly susceptible to oxidation, leading to the formation of primary and secondary oxidation products that cause rancid flavour, off-odours, and nutrient degradation. In addition, the composition and quality of fish oil are influenced by species, habitat, raw-material freshness, and extraction conditions, which complicates standardization for food and nutraceutical applications (Yi et al., 2023). These limitations reduce consumer acceptance and restrict the incorporation of fish oil into a wider range of products, making technological strategies to improve stability and sensory quality indispensable. One of the most widely explored strategies is microencapsulation.

Microencapsulation transforms unstable liquid oils into fine, dry microcapsules that provide controlled release, enhanced oxidative stability, and improved sensory properties. Encapsulated fish oil can be more easily incorporated into functional foods, beverages, and dietary supplements without imparting a strong fishy taste or odour, while also improving shelf life and handling (Perez-palacios et al., 2022). Wall materials such as maltodextrin, gum arabic, and protein hydrolysates are commonly used because of their good emulsifying and film-forming properties, allowing the formation of protective matrices around oil droplets (Annamalai et al., 2020; Perez-palacios et al., 2022). The stability of the resulting microcapsules is strongly influenced by emulsion droplet size distribution and homogenization conditions, which determine microcapsule morphology, encapsulation efficiency, and reconstitution behavior in food systems (Perez-palacios et al., 2022).

Besides microencapsulation, the initial extraction and purification steps play a decisive role in determining the final quality of omega-3-rich fish oil. Among various extraction methods, wet rendering is frequently used for fish by-products due to its simplicity, low cost, and suitability for small- to medium-scale operations. However, the combined effects of heat and water can promote hydrolysis and oxidation, resulting in elevated acid and peroxide values if the process is not carefully controlled (Hidayah et al., 2022; Sasongko et al., 2025). Crude fish oils obtained via wet rendering typically contain free fatty acids, pigments, oxidation products, and other impurities that must be removed to comply with standards such as those of the Food and Agriculture Organization (FAO) and the International Fish Oil Standards (IFOS). In this context, chemical refining through alkali neutralization using sodium hydroxide (NaOH) is widely applied to reduce free fatty acids and improve oxidative stability and sensory characteristics of fish oils (Purnamayati et al., 2023).

A growing number of studies has investigated the extraction, refining, and quality evaluation of fish oils derived from various species and by-products, demonstrating that appropriate combinations of wet rendering and alkali neutralization can bring parameters such as acid value, peroxide value, p-anisidine value, and total oxidation (TOTOX) within FAO and IFOS limits while maintaining acceptable sensory properties (Hidayah et al., 2022; Purnamayati et al., 2023; Sasongko et al., 2025). However, systematic integration of extraction, refining, and microencapsulation steps specifically for milkfish oil is still limited, particularly for omega-3-rich oils recovered from viscera and belly fat. There remains insufficient information on how wet rendering temperature and time, followed by NaOH-based neutralization and subsequent encapsulation, affect key quality indicators relative to international benchmarks and the technological requirements of functional food applications.

Therefore, the objective of this study is to extract, refine, and microencapsulate omega-3-rich fish oil from milkfish using the wet rendering method followed by NaOH-based neutralization. The novelty of this research lies in its integrated approach that spans from crude oil extraction to microencapsulation while systematically assessing quality indicators in accordance with international standards. By establishing a process framework tailored to milkfish by-products, this work aims to support the development of affordable, locally sourced omega-3 ingredients and to strengthen the utilization of Indonesian marine resources for functional marine-based products with global market potential.

Method

Time and Location of the Research

This study was conducted between April and July 2024 at several institutions: the Laboratory of Fishery Product Processing and the Biochemistry Laboratory at Politeknik Pertanian Negeri Pangkajene Kepulauan, the Chemical Engineering Laboratory at Politeknik Negeri Makassar, the Integrated Laboratory of the Department of Aquatic Product Technology at IPB University, and the Health Laboratory in Makassar.

Type of Research

This research is an experimental laboratory study using a Completely Randomized Design (CRD) with a factorial treatment structure. The experiment was designed to evaluate the effects of extraction temperature and time on the yield and quality of omega-3-rich oil from milkfish (Chanos chanos) obtained by wet rendering, followed by alkaline neutralization.

Research Methods

The primary material used was fresh milkfish sourced from fish farmers in Pangkep Regency, with average weights ranging from 200 to 300 g per fish. Chemical reagents included sodium hydroxide (NaOH), potassium hydroxide (KOH), acetic acid (CH_3COOH), chloroform, sodium sulfite (Na_2SO_3), trimethylpentane, and p-anisidine solution, all of analytical grade and purchased from Merck and Sigma Aldrich. The equipment employed comprised UV–Vis spectrophotometers (Shimadzu and Agilent 8453), gas chromatography systems (Shimadzu GC 2010 Plus), digital balances, water baths, and centrifuges (Hettich EBA-20 and Hitachi).

Research Stages

Sample preparation

Fresh milkfish were eviscerated, and the edible parts were washed, filleted, and minced. The minced fish tissue was used as the raw material for oil extraction.

Oil extraction (wet rendering)

Fish oil extraction was carried out using a modified wet rendering method. Minced milkfish was mixed with water at a 1:2 ratio (w/v) and subjected to steam cooking at different temperatures (70, 80, 90, and 100 °C) for three extraction times (30, 40, and 60 min). After heating under controlled conditions, the mixture was filtered and centrifuged to separate the oil from the aqueous phase. Oil yield was calculated based on the mass or volume of extracted oil relative to the total mass of the fish material, and the optimum treatment was selected for further analysis. This procedure follows recent applications of wet rendering for fish-oil production (Purnamayati et al., 2023:Ayeloja et al., 2024).

Oil purification (alkali neutralization)

Crude oil obtained from each treatment was purified by alkali neutralization. The oil was mixed with aqueous NaOH solution at Baumé values of 16–26 °Bé (approximately 11–20% w/w NaOH), which are within the ranges commonly used for neutralization of fish oils from sardine, red snapper and tilapia viscera (Bija et al., 2017; Purnamayati et al., 2023). The mixture was heated at 60 °C for 30 min and then allowed to settle for 15 h. The soapstock formed during neutralization was removed, and the clarified oil phase was collected for further evaluation, following approaches reported for refined fish oils in recent studies (Ningsih et al., 2024).

Quality analysis of purified oil

Purified oils were characterized using standard quality parameters commonly applied in recent fish-oil quality studies. Peroxide value (PV) was determined by iodometric titration to quantify primary oxidation products, using procedures described in recent fish-oil research (Purnamayati et al., 2023; Ayeloja et al., 2024; Arias et al., 2022). The p-anisidine value (AV), representing secondary oxidation, was measured spectrophotometrically according to the p-anisidine test conditions used for edible fish oils (Purnamayati et al., 2023; Bannenberg et al., 2017; Ningsih et al., 2024). Free fatty acid (FFA) content and acid value were determined by titration with standardized NaOH, following AOAC-based procedures for edible oils as implemented in recent studies (Purnamayati et al., 2023; Ningsih et al., 2024). Acid value, indicating hydrolytic degradation, was calculated by multiplying FFA values (expressed as oleic acid) by a factor of 1.99, which represents the milligrams of KOH required to neutralize 1 g of fat.

Data Analysis

The experimental data on oil yield, peroxide value, p-anisidine value, acid value, and TOTOX were analyzed using a Completely Randomized Design (CRD) with a factorial arrangement of treatments (temperature × time). Analysis of Variance (ANOVA) was performed using SPSS version 17.0 at a 5% significance level ($\alpha = 0.05$). When significant differences among treatments were detected, Duncan's Multiple Range Test was applied to compare mean values and identify the optimal extraction conditions.

Result and Discussion

Result

The research aimed to investigate the extraction and purification of omega-3-rich fish oil from milkfish using the wet rendering method followed by alkali neutralization. The parameters evaluated include oil yield, peroxide value, anisidine value, total oxidation value, and acid value—each representing essential indicators of oil quality and oxidative stability.

Oil Yield

The yield of milkfish oil varied significantly with extraction temperature and time. The highest yield, recorded at 0.29%, was achieved at 100°C with an extraction time of 60 minutes. This trend confirmed that both temperature and duration positively influenced oil extraction efficiency, as heat facilitates the breakdown of lipid–cell complexes and improves lipid release into the aqueous phase. The results demonstrate a statistically significant increase in oil recovery with elevated thermal input, in line with recent observations in fish-oil extraction studies, which also reported higher yields at increased temperatures and longer extraction times (Purnamayati et al., 2023; Ayeloja et al., 2024).

Despite the increase, the yield remained considerably lower than that obtained from other fish species processed under different extraction conditions, such as tilapia or catfish, where higher oil recoveries have been documented (Purnamayati et al., 2023; Ayeloja et al., 2024). This discrepancy may be attributed to the inherent lipid composition of milkfish, extraction limitations inherent in the wet rendering process, and biological factors such as fish maturity, diet, or seasonal lipid variation. Moreover, the use of water as a solvent, while safe and cost-effective, likely diluted the lipid concentration, impeding optimal oil liberation from fish tissue (Getahun et al., 2025; Hussain et al., 2025; Samarajeewa, 2024; Swetha et al., 2024).

Peroxide Value

Peroxide value (PV) is a primary indicator of lipid oxidation. In this study, the peroxide values increased with higher temperatures and longer extraction times, indicating a direct correlation between thermal exposure and oxidative degradation. The highest PV, recorded at 3.44 meq/kg, was obtained at 100°C with 30 minutes of extraction. Although elevated, this value remained within the International Fish Oil Standards (IFOS) limit of ≤3.75 meq/kg, suggesting that the oxidation of primary products, such as hydroperoxides, was still under control during the thermal process, consistent with recent work on edible fish oils (Purnamayati et al., 2023; Bannenberg et al., 2017; Ningsih et al., 2024).

These findings corroborate the oxidative sensitivity of polyunsaturated fatty acids under heat exposure, as demonstrated in recent fish-oil quality studies (Bannenberg et al., 2017; Ayeloja et al., 2024). The increase in PV can be attributed to the cleavage of double bonds in unsaturated fatty acids, which react with oxygen to form hydroperoxides. However, since PV alone cannot fully reflect total oxidative degradation, further indicators such as anisidine value and TOTOX were also assessed.

Anisidine Value

Anisidine value (AV) reflects the level of secondary oxidation products, particularly aldehydes such as 2-alkenals and 2,4-dienals, formed through hydroperoxide decomposition. The study revealed that AV also increased significantly with temperature and time, consistent with the trends observed in PV. The highest anisidine value was 11.75 meq/kg at 100°C for 30 minutes—still well within the IFOS acceptable limit of <15 meq/kg.

The increase in AV supports findings that prolonged heat application accelerates hydroperoxide decomposition into reactive aldehydes ((Purnamayati et al., 2023; Ayeloja et al., 2024; Ningsih et al., 2024)). The combined increases in both PV and AV indicate that oxidative degradation occurred at both primary and secondary stages, although controlled within acceptable boundaries under the optimal extraction conditions.

Total Oxidation Value (TOTOX)

The TOTOX index, which integrates both primary and secondary oxidation values using the formula TOTOX = 2PV + AV, offers a comprehensive measure of oil quality. The results indicated that TOTOX values also increased with rising temperatures and durations. However, across all treatments, TOTOX values remained under the IFOS upper threshold of 20.

These results suggest that while some degree of oxidative damage occurred, the oil quality was still acceptable for consumption and further processing. TOTOX serves as a robust parameter in assessing the overall oxidative load of fish oil, as emphasized in the Codex Standard for Fish Oils and recent evaluations of commercial fish oils (Codex Alimentarius Commission, 2024; Bannenberg et al., 2017; Ningsih et al., 2024). Maintaining TOTOX within safe limits ensures the functionality of omega-3 oils, particularly in encapsulated forms, where stability during storage and application is critical (Perez-palacios et al., 2022).

Acid Value

Among the evaluated parameters, acid value (AV) showed the most critical deviation from standards, particularly at elevated temperatures and longer extraction times. The highest acid values, 2.43% and 2.68%, were recorded at 100°C for 40 and 60 minutes, respectively. These values exceeded the IFOS maximum of 2.25 mg KOH/g, indicating significant hydrolysis and the presence of free fatty acids.

The rise in acid value reflects hydrolytic degradation resulting from water-based processing and excessive heating, which can cleave triglycerides into glycerol and free fatty acids. Similar increases in free fatty acids and acid value under harsh extraction or storage conditions have been reported in more recent work on viscera and refined fish oils (Purnamayati et al., 2023; Ayeloja et al., 2024; Ningsih et al., 2024). The degradation not only lowers oil quality but may also affect its taste and stability in applications.

While the acid value in several treatments remained within acceptable levels, the deviation under high-temperature conditions highlights the importance of optimizing extraction parameters. These findings underscore the trade-off between maximizing yield and preserving oil quality.

Discussion

Influence of Extraction Conditions on Oil Yield

The yield of milkfish oil increased markedly with higher extraction temperatures and longer processing times, peaking at 0.29% at 100°C for 60 minutes. This observation aligns with the thermally enhanced diffusion of lipids from tissue matrices, a phenomenon noted in recent thermal extraction processes for tilapia and other species, where higher temperatures and longer times improved oil recovery within controlled ranges (Purnamayati et al., 2023; Ayeloja et al., 2024). Higher temperatures disrupt cell membranes and facilitate the release of intracellular fats, explaining the consistent trend observed in Figure 1.

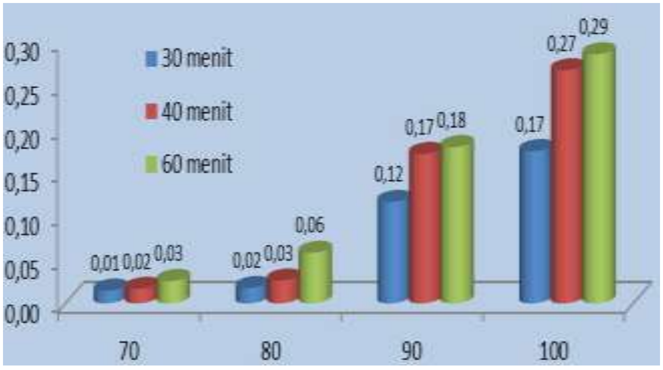

Figure 1. Graph of milkfish oil yield

Nevertheless, the relatively low yield compared to other fish species suggests several limitations. First, the intrinsic lipid content in *Chanos chanos* may be lower than in oil-rich species such as tilapia or catfish. Second, the wet rendering method inherently involves the presence of water, which can dilute lipids and impair full extraction efficiency. Furthermore, factors such as fish age, reproductive stage, and diet are known to influence lipid profiles and yield in fish-oil production (Ayeloja et al., 2024). These findings highlight a critical trade-off: while wet rendering is safe and cost-effective, it may not maximize yield potential compared to solvent-based or assisted (e.g., ultrasound) methods (Purnamayati et al., 2023).

Oxidative Stability: Peroxide and Anisidine Values

Peroxide value (PV) and anisidine value (AV) are primary indicators of lipid oxidative quality. Both values increased with elevated temperature and extended exposure time, indicating a degradation process initiated by heat-induced peroxidation. The PV trend shown in Figure 2 demonstrates that hydroperoxide formation intensifies at higher thermal loads, consistent with the oxidation of polyunsaturated fatty acids (PUFAs) like EPA and DHA (Purnamayati et al., 2023; Bannenberg et al., 2017; Ayeloja et al., 2024). Nonetheless, PV values remained below 3.75 meq/kg, suggesting primary oxidation was controlled.

Figure 2. Graph of peroxide numbers in milkfish oil

The subsequent rise in AV, as seen in Figure 3, reinforces the hypothesis that hydroperoxides decompose into aldehydes with continued heat application. Similar relationships between temperature, PV, and AV have been reported for refined and purified viscera oils (Purnamayati et al., 2023; Ayeloja et al., 2024; Ningsih et al., 2024). The degradation pathway from primary to secondary oxidation products is well documented in recent fish-oil quality literature (Bannenberg et al., 2017; Ayeloja et al., 2024), and the combined PV and AV trends here reflect a progressive but manageable level of oxidation.

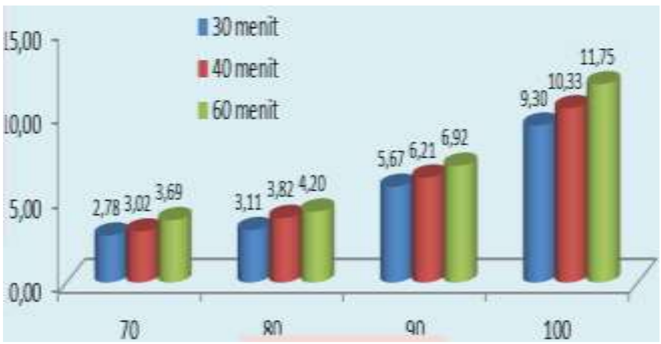

Figure 3. Graph of anisidine values in milkfish oil

Maintaining PV and AV within the IFOS limits is essential for ensuring that the oil remains suitable for human consumption and encapsulation. These oxidative markers not only affect oil safety but also influence sensory quality, as aldehydes contribute to rancidity and off-flavors (Bannenberg et al., 2017; Ningsih et al., 2024).

Total Oxidation Value (TOTOX) and Quality Implications

The integration of PV and AV into a composite TOTOX index provides a more complete assessment of oil degradation. As shown in Figure 4, TOTOX increased steadily with processing severity, yet all values remained within the 20 meq/kg IFOS threshold. This demonstrates that, under the tested extraction conditions, milkfish oil maintained a level of oxidative quality that aligns with international standards for fish-oil safety (Codex Alimentarius Commission, 2024; Ningsih et al., 2024).

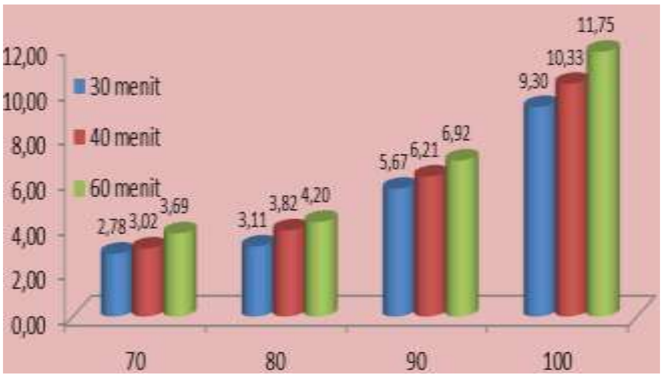

Figure 4. Graph of total oxidation of milkfish oil

From a formulation perspective, these findings are encouraging. The stability profile supports the potential use of this oil in microencapsulated formats, where oxidative integrity during storage and application is critical. Recent reviews on fish-oil microencapsulation emphasize that low initial PV, AV, and TOTOX values are pivotal to preserving bioactivity and preventing rancidification in functional foods (Annamalai et al., 2020; Perez-palacios et al., 2022).

Acid Value and Hydrolytic Degradation

Acid value (AV), representing the extent of hydrolysis into free fatty acids (FFAs), exceeded IFOS limits in several high-temperature treatments. As shown in Figure 5, values reached 2.43% and 2.68% at 100°C for 40 and 60 minutes, respectively, surpassing the 2.25 mg KOH/g threshold. This result suggests that wet rendering, particularly when extended or excessively heated, promotes lipid hydrolysis—an effect also observed in recent studies on viscera and refined fish oils where high temperature and moisture increase FFA content (Purnamayati et al., 2023; Ningsih et al., 2024).

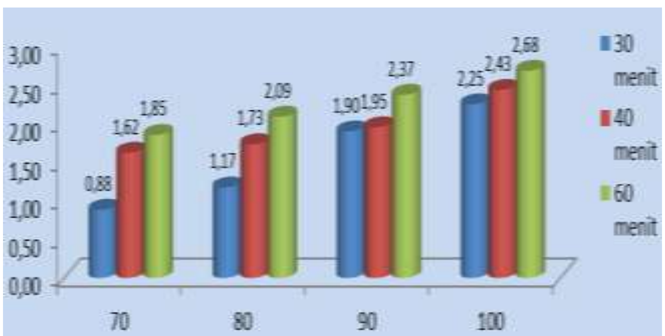

Figure 5. Acid number graph milkfish oil

Hydrolysis may be exacerbated by the aqueous environment of wet rendering, which facilitates enzymatic or non-enzymatic cleavage of triglycerides. The resulting FFAs can accelerate secondary oxidation, degrade taste and odor, and reduce oil stability. These factors are critical in determining the shelf life and functional performance of encapsulated oils (Bannenberg et al., 2017; Perez-palacios et al., 2022).

While shorter heating durations preserved acid values within acceptable limits, the data highlight the importance of thermal control. A processing temperature of 90–100°C for ≤ 30 minutes appears optimal for balancing yield and oil quality without compromising AV, consistent with controlled extraction and refining conditions recommended in recent work (Purnamayati et al., 2023; Ayeloja et al., 2024).

Balancing Yield and Quality for Encapsulation

The key implication of these findings is the need to balance oil recovery with quality preservation for encapsulation purposes. Although longer and hotter extractions yield more oil, they simultaneously trigger oxidative and hydrolytic degradation. For microencapsulation, this presents a challenge: encapsulating degraded oil would compromise stability, flavor, and nutritional efficacy.

Thus, the ideal extraction protocol must maximize yield while maintaining PV, AV, TOTOX, and acid value within strict thresholds. Recent studies show that the quality of the core oil directly impacts microcapsule stability, particle size, and uniformity: oils with low oxidation levels and appropriate physicochemical properties produce finer emulsions and better encapsulation efficiency (Annamalai et al., 2020; Perez-palacios et al., 2022). Homogeneous, low-oxidation oils are therefore preferred for spray drying or other microencapsulation techniques.

Additionally, the purification step using NaOH neutralization was essential in achieving oil that met oxidative standards. Recent work on viscera-oil refining demonstrates that alkaline neutralization can significantly reduce FFAs and oxidation products, thereby improving both chemical and sensory quality (Purnamayati et al., 2023; Ningsih et al., 2024). Such refined oil is more suitable for conversion into powder form, allowing efficient masking of undesirable sensory properties and facilitating incorporation into health-oriented food products (Perez-palacios et al., 2022).

Conclusion

This study demonstrated that wet rendering followed by alkali neutralization is a viable method for extracting omega-3-rich oil from milkfish (Chanos chanos), with most oxidation parameters falling within international standards. Extraction temperature and duration had a significant influence on oil yield and quality. The highest yield (0.29%) was achieved at 100°C for 60 minutes, but this condition also led to increased peroxide, anisidine, total oxidation, and acid values—indicating a decline in oil stability. While peroxide and anisidine values remained within IFOS guidelines, acid values exceeded acceptable limits under extended heating, suggesting hydrolytic degradation due to water-based extraction. These findings emphasize the need to optimize extraction parameters to balance yield and quality. The results contribute to a better understanding of processing conditions required for high-quality fish oil production suitable for microencapsulation. The oil's oxidative profile supports its potential for application in functional food and nutraceutical formulations, especially when stabilized through encapsulation technology. Further studies are recommended to refine encapsulation processes and explore bioavailability and shelf-life improvements.

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