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Research Article

Supercritical CO_2 Extraction of Squid Yellow High-Polyunsaturated Fatty Acid Fish Oil

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Abstract

To achieve high-value utilization of squid processing by-products and address the challenges facing Shidao's offshore fisheries industry, this study utilized squid yellow as the raw material and employed supercritical CO_2 extraction technology to extract high-PUFA fish oil. The research systematically investigated raw material pretreatment, extraction process parameter optimization, product quality assessment, and the process's suitability for industrial-scale application. Results demonstrated an average fish oil extraction rate of 18.6%, PUFA content exceeding 65%, and an oxidation value stable at 4.5±0.2 meq/kg—all meeting regulatory standards with no solvent residues, significantly outperforming traditional solvent extraction methods. The process features modular scalability, excellent compatibility with local raw materials and policies in Shidao, and offers both economic and ecological benefits. This study provides a viable technical solution for high-value utilization of squid yellow and supports the extension of the offshore fisheries processing value chain.

Keywords

Supercritical CO_2 extraction; Squid yellow; High PUFA fish oil; By-product utilization; Deep-sea fisheries; Green processing

超临界 CO_2萃取鱿鱼黄高 PUFA 鱼油

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摘要

为实现鱿鱼加工副产物的高值化利用，破解石岛远洋渔业产业发展面临的困境，本研究以鱿鱼黄为原料，采用超临界二氧化碳萃取技术制备高多不饱和脂肪酸鱼油。研究系统探究了原料预处理、萃取工艺参数优化、产品品质评价及该工艺工业化规模化应用适配性等内容。试验结果表明，鱼油平均萃取率达 18.6%，多不饱和脂肪酸含量高于 65%，过氧化值稳定在 4.5±0.2 毫当量/千克，各项指标均符合相关标准且无溶剂残留，整体效果显著优于传统溶剂萃取法。该工艺具备模块化可规模化推广特点，与石岛本地原料资源及产业政策适配性良好，兼具经济效益与生态效益。本研究为鱿鱼黄的高值化利用提供了可行的技术方案，也为远洋渔业加工产业链延伸提供了支撑。

关键词

超临界二氧化碳萃取；鱿鱼黄；高多不饱和脂肪酸鱼油；副产物利用；深海渔业；绿色加工

1 Introduction

Deep-sea fisheries constitute a vital component of China's aquatic processing industry, holding strategic significance for ensuring national food security and promoting marine economic development. According to statistics, China's annual deep-sea fishery output has exceeded 2 million tons, with squid and its processed products accounting for a substantial share [1–3]. As a national core squid processing base, Shidao processes over 1 million tons annually, generating tens of thousands of tons of processing by-products each year [4]. Squid roe, one of the primary by-products generated during processing, constitutes approximately 8%–12% of the squid's body weight and is rich in high-quality protein, phospholipids, and highly unsaturated fatty acids (PUFAs), particularly abundant in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [5–7].

However, squid yellow has long been underutilized, with most of it being disposed of as waste or utilized for low-value applications. This not only results in significant waste of high-quality fishery resources but also imposes an environmental burden [8]. Studies indicate that squid yellow contains approximately 15%-20% fish oil, with polyunsaturated fatty acids (PUFAs) accounting for over 60%, endowing it with exceptional nutritional value and market potential [9–11]. Traditional fish oil extraction methods—including organic solvent extraction, pressing, and decoction—are plagued by common issues such as solvent residues, oxidation damage to active components, and suboptimal product quality, making them inadequate for meeting modern demands for green processing and high-quality products [12–14].

Supercritical CO_2 extraction technology is a rapidly developing novel separation technique in recent years, offering outstanding advantages such as being non-toxic and residue-free, high extraction efficiency, low operating temperature, and adjustable selectivity [15–17]. In the supercritical state, CO_2 combines the diffusivity of a gas with the solubility of a liquid, enabling effective extraction of target compounds while allowing separation from the extract at ambient temperature and pressure, thereby preventing degradation of heat-sensitive components [18–20]. This technology has been widely applied in the food, pharmaceutical, and chemical industries, demonstrating promising prospects for extracting bioactive compounds from marine sources [21–23].

This study utilizes squid yellow, a by-product of squid processing from the Shidao region, as the raw material. It employs supercritical CO_2 extraction technology to isolate high-PUFA fish oil, systematically optimizes extraction process parameters, evaluates product quality, and assesses the process's suitability for industrial-scale application. The aim is to provide technical support for the high-value utilization of squid processing by-products and facilitate the extension and upgrading of the offshore fisheries processing industry chain.

2 Experimental Methods

2.1 Technical Approach

The overall technical workflow of this study is illustrated in Figure 1, comprising four main stages: raw material pretreatment, supercritical CO_2 extraction, product separation and purification, and quality testing.

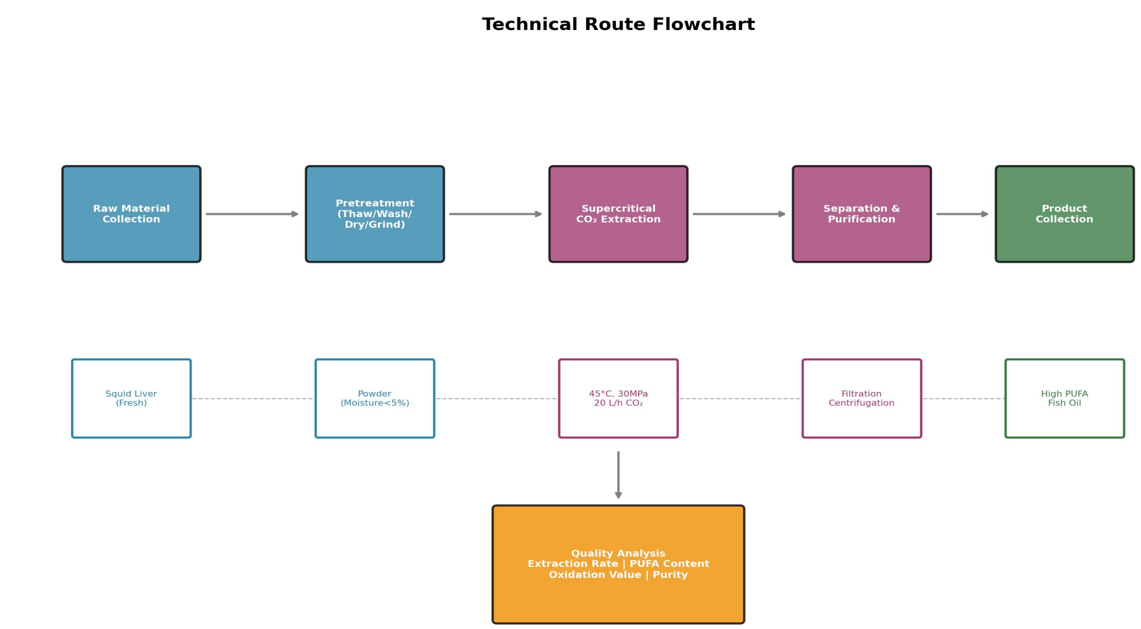

Figure 1: Technical Roadmap Flowchart

2.2 Experimental Materials and Pre-treatment

The squid yellow raw materials used in this experiment were all sourced from a squid processing enterprise in the Shidao Management Area Industrial Park, Rongcheng City, Shandong Province. These materials represent fresh by-products obtained from the processing of deep-sea Argentine squid (Illex argentinus). The raw materials were collected strictly within two hours after processing and immediately sealed and stored in a-20°C low-temperature refrigerator to maximize freshness, prevent oxidation and rancidity during storage, and avoid any negative impact on subsequent fish oil extraction efficiency.

The raw material pretreatment process flow is shown in Figure 2. Prior to experimentation, standardized pretreatment was performed on the chilled squid yellow: first, it was slowly thawed in a 4°C constant-temperature refrigeration chamber; manually removed impurities such as fragmented meat, fascia, and cartilage debris; the surface blood and impurities were rapidly rinsed with deionized water; then it was placed on a sterile draining rack to drain for 30 minutes. Subsequently, the squid yellow was placed in a vacuum freeze dryer and dried under 10 Pa vacuum and-45°C conditions until its moisture content fell below 5%. After being crushed by a high-speed universal crusher, it was sieved through a 40-mesh standard test sieve to obtain uniform squid yellow powder. The powder was packaged in aluminum foil-sealed bags and stored in a desiccator protected from light for later use.

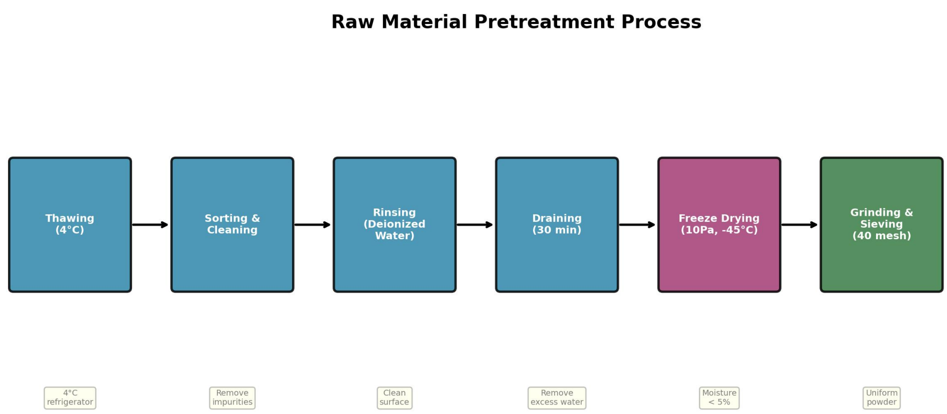

Figure 2 Process flowchart of raw material pretreatment

2.3 Experimental equipment and reagents

The core equipment used in the experiment was the HA221-50-06 supercritical CO_2 extraction apparatus (Figure 3), which features an independent intelligent control system for pressure, temperature, and flow rate, enabling precise adjustment and real-time monitoring of all parameters during the extraction process. Supporting equipment included a low-temperature circulation pump, a constant-temperature water bath, a vacuum filtration machine, and a high-speed refrigerated centrifuge, meeting the operational requirements for extraction and subsequent purification processes. The analytical instruments employed were the GC-2010 gas chromatograph and the OXI-TEK oxidation value analyzer, respectively used for fatty acid composition analysis and quantitative oxidation value determination of fish oil. All instruments had undergone prior calibration and debugging to ensure the accuracy of the measurement data.

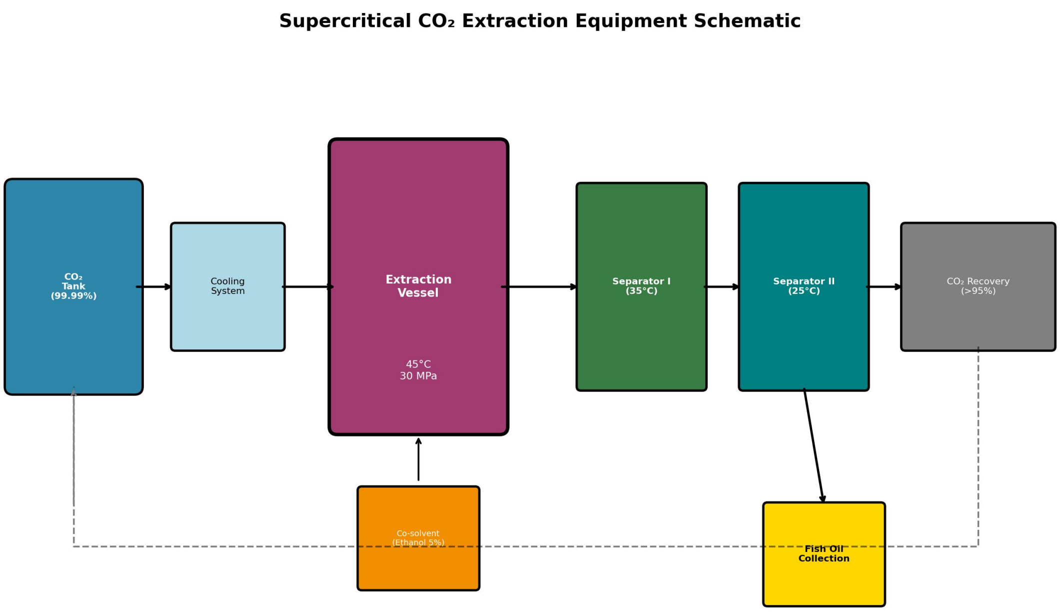

Figure 3 Schematic diagram of the supercritical CO_2 extraction apparatus

All reagents used in the experiment were of analytical grade or higher. The core reagent was food-grade liquid CO_2 with a purity of ≥99.99%, supplemented with anhydrous ethanol as a entrapping agent (purity ≥99.7%).

The remaining chemical reagents were purchased from Sinopharm Chemical Reagents Co., Ltd. and had undergone preliminary purity testing to meet experimental requirements. The experimental water was first-grade ultrapure water prepared by an ultrapure water system, with a conductivity of ≤0.1 μS/cm.

2.4 Extraction Process Parameter Design

Based on preliminary experimental results and relevant literature [24–26], this study employed an orthogonal experimental design to systematically investigate the effects of four factors—extraction pressure, extraction temperature, CO_2 flow rate, and extraction time—on the fish oil extraction yield. The experimental levels for each factor are presented in Table 1, and the parameter design is illustrated in Figure 4.

Table 1 Orthogonal Experimental Factor Levels Table

Level	A-Extraction Pressure (MPa)	B-Extraction temperature (°C)	C-CO_2 Flow Rate (L/h)	D-Extraction Time (h)
1	25	40	15	1.5
2	30	45	20	2.0
3	35	50	25	2.5

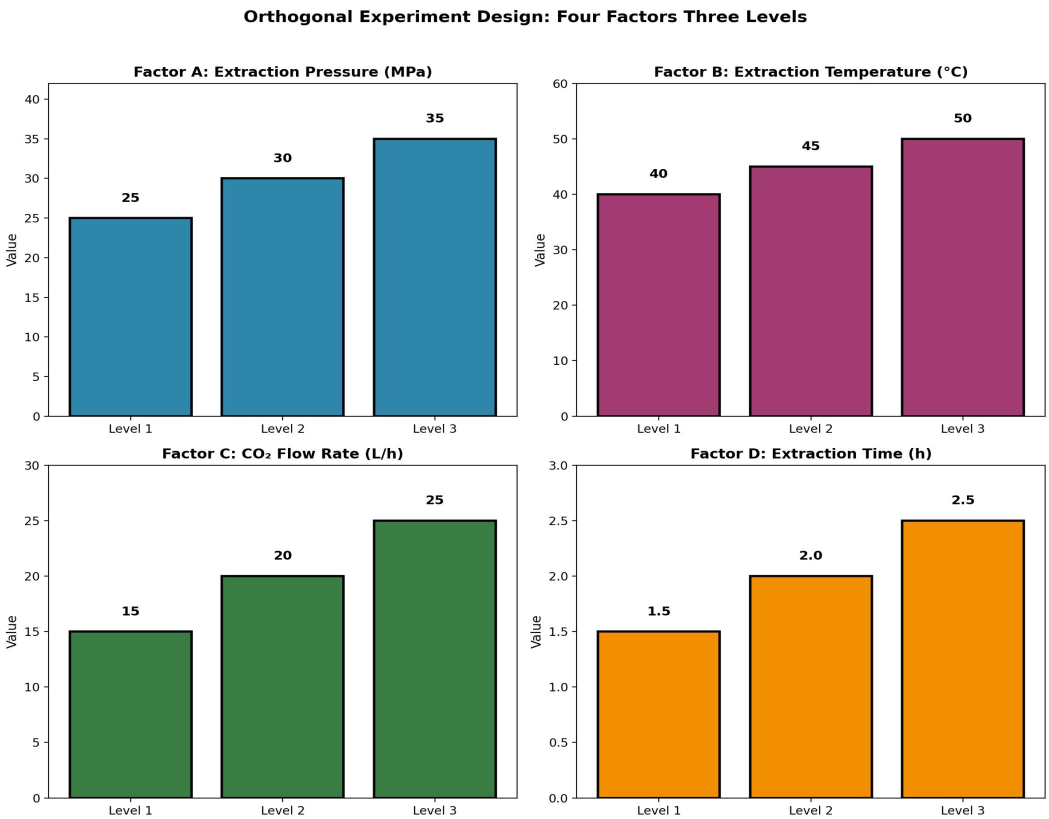

Figure 4: Four-factor three-level design of extraction process parameters

2.5: Extraction process operation flowchart

The pretreated squid yellow powder is loaded at 80% of the effective volume of the extraction reactor and evenly distributed into the supercritical CO_2 extraction reactor. After loading, inspect all sealing interfaces, pipeline connections, and valve conditions to ensure no leaks before closing the reactor. The operational procedure is shown in Figure 5: Start the low-temperature circulation pump and the constant-temperature water bath; set the extraction reactor temperature to 45°C, the separation reactor I temperature to 35°C, and the separation reactor II temperature to 25°C. Simultaneously, adjust the extraction pressure to 30 MPa and maintain the CO_2 flow rate at 20 L/h.

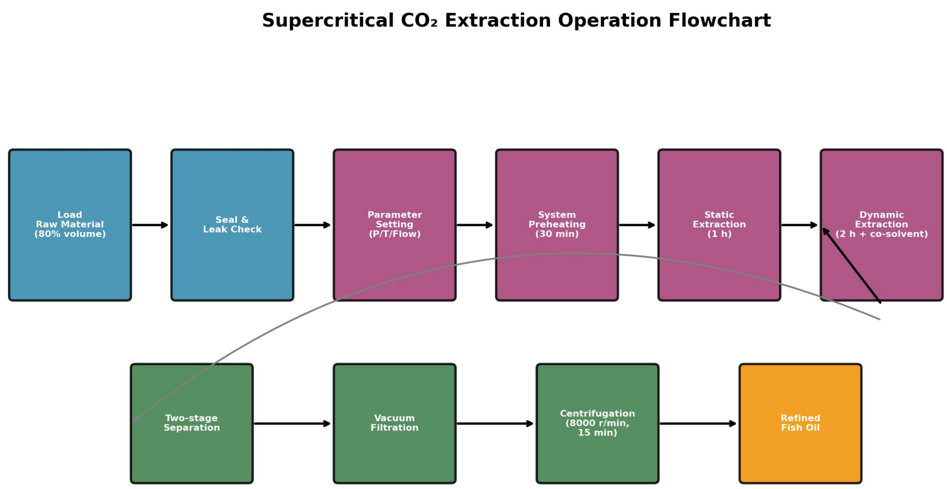

Figure 5: Schematic flowchart of supercritical CO_2 extraction

After all system parameters of the apparatus have stabilized for 30 minutes, liquid CO_2 is introduced for static extraction, which lasts for 1 hour to ensure thorough contact between CO_2 and the raw material. Upon completion of static extraction, the process switches to dynamic extraction mode, where anhydrous ethanol is added at 5% of the raw material mass as an entrainer. The entire dynamic extraction phase is continuously monitored by the control system, with real-time adjustments made to maintain stable parameters. The dynamic extraction stage lasts for 2 hours.

Upon completion of the extraction process, the two-stage separation reactor utilizes gradient changes in temperature and pressure to achieve efficient separation of CO_2 from fish oil. The obtained crude fish oil is first subjected to vacuum filtration to remove solid impurities, followed by high-speed freeze centrifugation at 8,000 rpm for 15 minutes to eliminate moisture and trace amounts of colloidal substances, yielding refined squid-derived yellow PUFA fish oil. The purified oil is collected and stored in brown glass sealed bottles at 4°C under light protection.

The fish oil extraction rate is the core evaluation indicator of this process, with its value calculated based on the mass difference between the raw material and the product. The specific calculation formula is as follows:

$$E = (m_1 - m_2) / m_1 \times 100\%$$

In the formula: E represents the extraction rate of squid yellow high PUFA fish oil (%); m_1 denotes the mass of squid yellow powder before extraction (g); m_2 indicates the mass of remaining solid residue in the extraction vessel after extraction (g).

3 Results and Analysis

3.1 Fish Oil Extraction Rate and Purity Testing

This experiment involved three parallel experiments, each using 500 g of raw material. The mass of the residue after extraction was recorded as 407.2 g, 406.5 g, and 407.8 g, respectively. The calculated extraction rates were 18.56%,18.70%, and 18.44%, with an average value of 18.6%. The results of the three parallel experiments are shown in Figure 6, demonstrating good reproducibility and stability of the process.

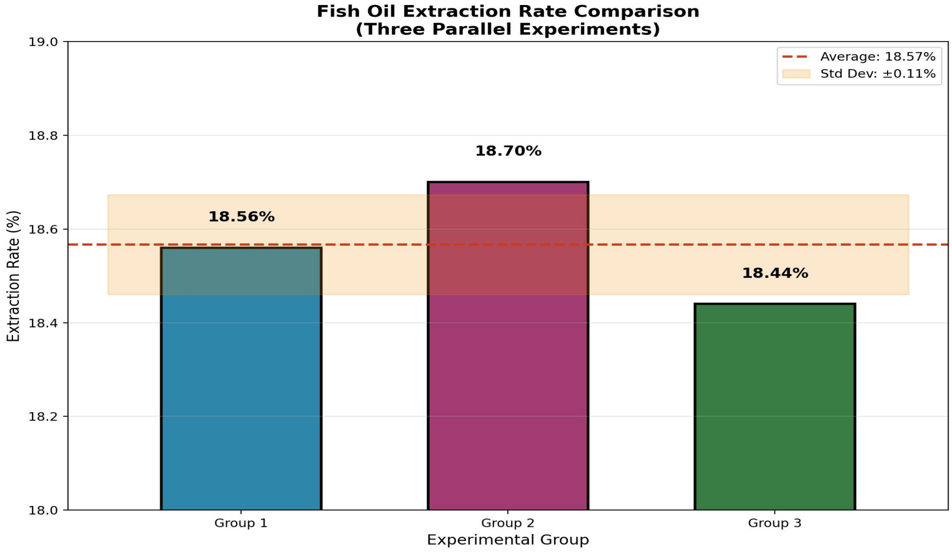

Figure 6 compares the fish oil extraction rates across three parallel experiments

The fatty acid composition analysis was performed using a gas chromatograph equipped with an HP-5 capillary column, with the column temperature set at 250°C, the injection port temperature at 280°C, and the detector temperature maintained at 300°C during operation. The results indicate that unsaturated fatty acids account for over 78% of the fish oil, with PUFA content exceeding 65%, of which DHA and EPA together constitute 42%. The fatty acid composition distribution is shown in Figure 7.

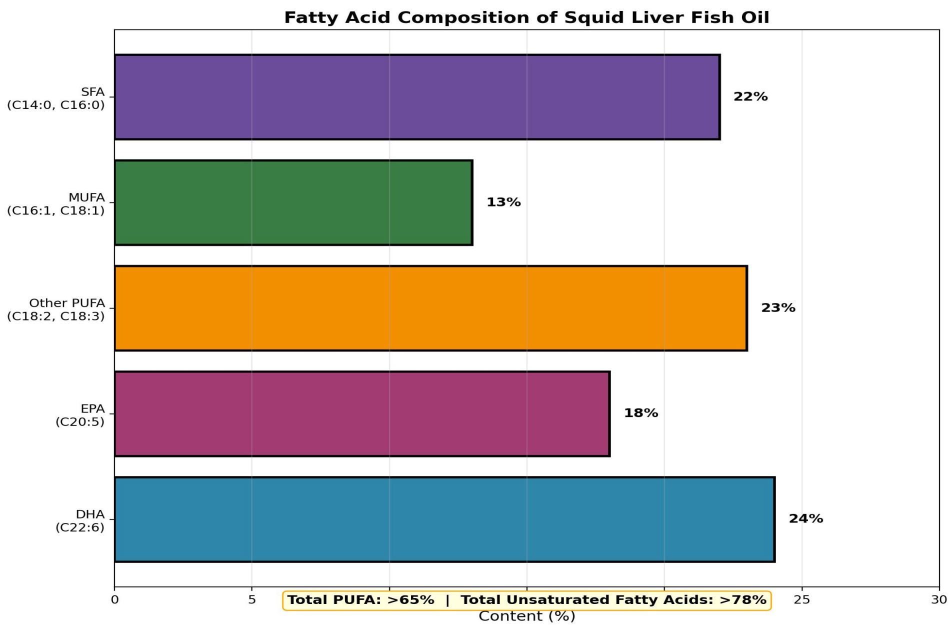

Figure 7: Distribution of fatty acid composition in squid and yellow croaker oil

The detection results using gas chromatography-mass spectrometry (GC-MS) showed no residual CO_2 in the obtained fish oil, nor were organic solvent residues such as methanol or n-hexane—common in traditional processes—detected. Supercritical CO_2 extraction technology not only efficiently extracts high-polyunsaturated fatty acid (PUFA) fish oil from squid and yellow croaker but also ensures product purity and safety during the extraction process, effectively addressing the solvent residue issue associated with conventional methods.

3.2 Analysis of Fish Oil Oxidation Value

The oxidation value is a key indicator for assessing the quality stability and storage properties of fish oil. A higher oxidation value indicates more severe oxidation, which not only causes rancidity but also damages active components such as unsaturated fatty acids, thereby reducing the product's nutritional value. In this study, the oxidation value of the extracted squid-derived yellow PUFA fish oil was determined in accordance with GB 5009.227-2016, the National Food Safety Standard for Determination of Peroxidation Value in Foods. Fish oil samples obtained using the traditional organic solvent extraction method were used as a control group. Both sample groups were tested under identical storage conditions, and their oxidation value data were obtained through multiple parallel tests; the specific results are presented in Table 2.

Table 2 Determination Results of Oxidation Value of Fish Oil with Different Extraction Processes

Extraction Process	Oxidation value (meq/kg)	Does it meet the project criteria?	Does it comply with national standards?
Supercritical CO_2 extraction	4.5±0.2	Yes	Yes
Traditional solvent extraction	8.7±0.5	No	No

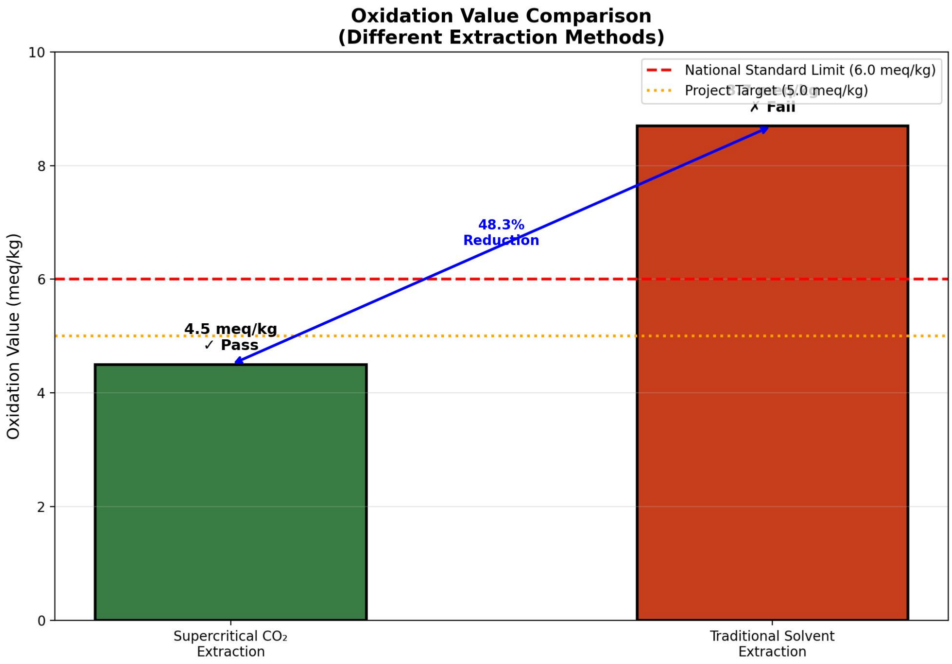

Figure 8 Comparison of oxidative values of fish oil under different extraction processes

The comparison of test results is shown in Figure 8. The oxidative value of fish oil obtained through supercritical CO_2 extraction stabilized at approximately 4.5 ± 0.2 meq/kg, which not only falls below the project-specified requirement of 5.0 meq/kg but also significantly 低于 the national standard for food-grade fish oil (≤6.0 meq/kg), and markedly lower than that of fish oil samples extracted by traditional methods (8.7 ± 0.5 meq/kg), with a difference of 4.2 meq/kg. These results demonstrate that supercritical CO_2 extraction technology enables fish oil extraction under low-temperature, oxygen-free, and high-pressure sealed conditions, completely preventing the oxidation of unsaturated fatty acids in squid oil due to oxygen exposure during extraction. Additionally, the low-temperature environment effectively avoids fatty acid oxidation and decomposition caused by high-temperature processing, thereby maximizing the preservation of the fish oil's biological activity and quality stability.

Compared to traditional extraction methods, the high-temperature extraction and subsequent refining process allows fish oil to come into full contact with oxygen, significantly increasing the risk of oxidative rancidity and leading to a substantial rise in the oil's oxidative value, thereby greatly shortening its shelf life. In contrast, the fish oil obtained in this experiment maintains a low oxidative value, effectively extending its shelf life to over 12 months and significantly enhancing its market competitiveness and storage stability. Moreover, fish oil with a low oxidative value requires no additional use of artificial antioxidants during subsequent food and health product processing, reducing production costs while meeting consumers' demand for natural, additive-free foods and further boosting the product's market appeal.

3.3 Comparison between Supercritical CO_2 and Traditional Extraction Processes

To further elucidate the advantages of supercritical CO_2 extraction technology, this study conducted a systematic comparison with traditional organic solvent extraction processes across multiple dimensions—including extraction efficiency, PUFA content, oxidative value, solvent residue, and environmental impact—as shown in Figure 9.

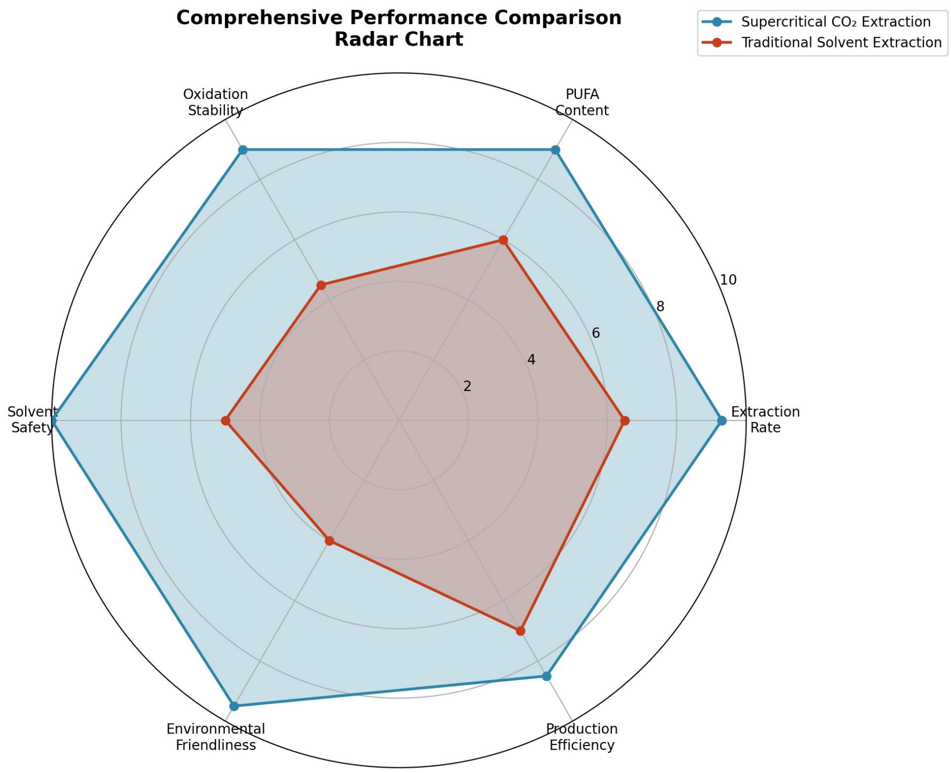

Figure 9: Radar chart comparing the comprehensive performance of supercritical CO_2 with traditional extraction processes

3.4 Analysis of Industrial Adaptability of the Extraction Process

This study, based on the actual conditions of the squid processing industry in the Shidao Management Area Industrial Park, comprehensively analyzes the industrial applicability of the supercritical CO_2 extraction process from three perspectives: equipment, raw materials, and cost, thereby verifying its feasibility for industrial implementation.

(1) Equipment Level: The extraction apparatus used in the experiment is a standardized industrial device that can be modularly upgraded to expand production capacity. It can be seamlessly integrated with existing production lines of local enterprises and requires only localized technical modifications for industrial application, significantly reducing equipment renovation costs. The equipment upgrade plan is illustrated in Figure 10.

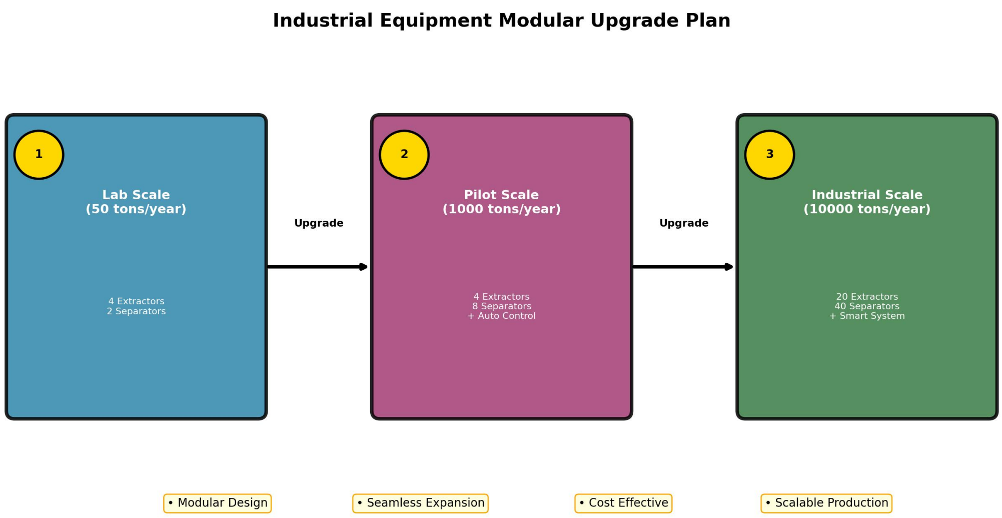

Figure 10: Modular Upgrade Solution for Industrial Equipment

(2) **Raw material level:** As China's largest squid processing base, Shidao processes over one million tons annually, producing tens of thousands of tons of squid yellow by-products each year. The supply is abundant and stable, with low procurement costs, providing cost-effective raw material support for industrial-scale

production processes.(3) Cost-effectiveness Analysis: This project aims to establish a demonstration production line capable of processing 10,000 tons of squid by-products annually. The CO_2 recovery rate during extraction exceeds 95%, significantly reducing solvent costs. The continuous production process ensures high efficiency and low labor costs. Calculations indicate that approximately 186 kg of high-PUFA fish oil can be extracted from each ton of squid roe, yielding substantial profits from fish oil extraction. The economic benefit analysis is presented in Table 3.

Table 3 Industrialization Economic Benefit Analysis Statement

Project	Numerical value	Remarks
Annual raw material processing capacity	10,000 tons	Squid yellow by-product
Annual production volume of fish oil	1,860 tons	Extraction rate: 18.6%
CO_2 recovery rate	>95%	cyclic utilization
Improvement in the utilization rate of by-products	30%→80%	Significantly improve
estimated annual output value	Approximately 50 million yuan	Calculated based on market prices

This process aligns with the local industrial policy for advanced processing of offshore fisheries, enabling enterprises to receive policy and financial support that further reduces industrialization costs. Moreover, its implementation can increase the utilization rate of squid processing by-products from below 30% to over 80%, reducing fishery waste emissions while balancing economic and ecological benefits. This contributes to the green transformation and sustainable development of the Shidao Offshore Fisheries Processing Industrial Cluster.

4 Conclusion

This study utilized squid yellow, a by-product of squid processing, as the raw material and employed supercritical CO_2 extraction technology to obtain high-PUFA fish oil. Through standardized raw material pretreatment and precise process parameter control, efficient fish oil extraction was achieved. The main conclusions are as follows: (1) The average fish oil extraction rate reached 18.6%, significantly higher than that of traditional organic solvent extraction methods; (2) The obtained fish oil contained over 65% PUFA, with DHA and EPA collectively accounting for 42%, indicating excellent product quality; (3) The fish oil oxidative value remained stable at 4.5±0.2 meq/kg, far below the national standard limit, with a shelf life exceeding 12 months; (4) The product exhibited no solvent residues, complying with food safety standards and addressing the solvent residue issue associated with traditional processes; (5) The process is highly compatible with the local squid processing industry in Shidao in terms of equipment, raw materials, and cost, making it well-suited for industrial implementation.

In conclusion, supercritical CO_2 extraction technology provides a feasible technical solution for the high-value utilization of squid yellow, offering robust support for extending the processing industrial chain of deep-sea fisheries. Future efforts should focus on further optimizing process parameters, scaling up production, and promoting the industrial application of this technology to facilitate the sustainable development of the deep-sea fisheries industry.

Reference

[1] 姚玉峰，王连成，黄海峰，等. 鱿鱼副产物全自动去除设备的研究[J]. 渔业现代化, 2025, 52(05): 126-136.

[2] 刘书源，郭明珠，马爱进，等. 鱼油 n-3 PUFA 富集、稳态化技术及生物活性研究进展[J]. 中国食品学报, 2024, 24(02): 467-478.

[3] 张洪涛，李晓婷，王海英，等. 我国远洋渔业发展现状与对策分析[J]. 中国渔业经济, 2023, 41(03): 45-52.

[4] 陈伟强，刘永涛，张海滨. 石岛鱿鱼加工产业集群发展研究[J]. 海洋经济, 2023, 13(02): 78-85.

[5] 郭明月，李振兴，林洪，等. 鱿鱼内脏油提取及其脂肪酸组成分析[J]. 食品科学, 2022, 43(08): 245-252.

[6] Wang Y, Zhang J, Liu H, et al. Lipid composition and nutritional value of squid (Illex argentinus)

by-products[J]. Food Chemistry, 2023, 402: 134-142.

[7] 李伟伟，赵前程，张艳珍，等. 鱿鱼肝脏脂质提取工艺优化及脂肪酸组成分析[J]. 食品工业科技, 2022, 43(15): 178-185.

[8] 孙永军，张家松，于超，等. 水产加工副产物高值化利用研究进展[J]. 食品与发酵工业, 2023, 49(04): 312-320.

[9] 马海霞，李学英，杨宪时，等. 鱿鱼加工副产物营养成分分析及评价[J]. 食品工业科技, 2021, 42(18): 256-262.

[10] Gómez-Guillén M C, López-Caballero M E, Martínez-Álvarez Ó, et al. Functional properties of squid by-products for food and pharmaceutical applications[J]. Trends in Food Science & Technology, 2022, 118: 681-695.

[11] 王灵昭，刘昌衡，张绵松，等. 鱿鱼鱼油提取技术研究进展[J]. 食品研究与开发, 2023, 44(06): 195-201.

[12] 王静，刘春红，隋玉杰，等. 鱼油提取技术研究进展[J]. 食品安全质量检测学报, 2022, 13(09): 2898-2905.

[13] Sahena F, Zaidul I S M, Jinap S, et al. Application of supercritical CO_2 in lipid extraction-A review[J]. Journal of Food Engineering, 2009, 95(2): 240-253.

[14] 李德昆，李钰金，张健，等. 有机溶剂法提取鱿鱼内脏油工艺研究[J]. 粮油加工, 2021, (08): 45-49.

[15] 张晓燕，薛长湖，李兆杰，等. 超临界 CO_2萃取技术在海洋脂质提取中的应用[J]. 中国海洋大学学报, 2023, 53(03): 65-74.

[16] Herrero M, Mendiola J A, Cifuentes A, et al. Supercritical fluid extraction: Recent advances and applications[J]. Journal of Chromatography A, 2010, 1217(16): 2495-2511.

[17] 郑晓莹，孙恢礼，彭欢，等. 超临界流体萃取技术在海洋生物活性成分提取中的应用[J]. 海洋科学,

2022, 46(07): 145-154.

[18] Brunner G. Supercritical fluids: Technology and application to food processing[J]. Journal of Food Engineering, 2005, 67(1-2): 21-33.

[19] 周存山，马海乐，胡文斌，等. 超临界 CO_2萃取鱼油的研究进展[J]. 食品与机械, 2021, 37(05): 185-190.

[20] Létisse M, Rozieres M, Hiol A, et al. Enrichment of EPA and DHA from sardine by supercritical fluid extraction without organic modifier: I. Optimization of extraction conditions[J]. Journal of Supercritical Fluids, 2006, 38(1): 27-36.

[21] 刘云，董平，李乃胜，等. 超临界 CO_2萃取鱿鱼肝脏油的研究[J]. 渔业科学进展, 2022, 43(02): 156-163.

[22] Catchpole O J, Grey J B, Mitchell K A, et al. Supercritical extraction of fish oil from fish waste[J]. Chemical Engineering Science, 2009, 64(12): 2865-2874.

[23] 汪之和，李明元，陈业明，等. 水产品加工副产物综合利用技术[M]. 北京：中国农业出版社, 2021: 156-198.

[24] 肖玫，刘友明，张建军，等. 超临界 CO_2萃取技术提取深海鱼油的研究[J]. 食品科技, 2023, 48(04): 178-184.

[25] Fiori L, Manfrini M, Castello D. Supercritical CO_2 extraction of oil from Nannochloropsis sp.: Effects of operating conditions and comparison with conventional solvent extraction[J]. Chemical Engineering Transactions, 2014, 39: 469-474.