# Sustainable production of Omega-3-rich fish oil from Tuna by-products using green extraction process

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**Abstract:** Omega-3 fatty acids are essential for human health, yet the increasing global demand necessitates the exploration of sustainable alternative sources, such as fishery by-products. An innovative and environmentally friendly extraction method, previously applied to small fish by-products, was employed on large fish by-products, particularly tuna soft tissues, utilising green solvents including d-limonene, 2-methyloxolane (2-MeOx), and ethanol. The results indicated that d-limonene and 2-MeOx efficiently extracted omega-3-rich oils, achieving notable concentrations of EPA (4–7%) and DHA (17–28%). However, substantial impurities were detected, attributed to the greater difficulty of purifying large fish by-products, which tend to retain blood and other fluids despite cleaning. This contrasts with previous findings from small fish such as sardines and anchovies, where extraction yielded purer oils with greater ease. These findings underscore the necessity of further optimisation of extraction protocols for larger fish waste. Nonetheless, the preliminary outcomes suggest that, with appropriate refinement, tuna by-products represent a promising and sustainable source of omega-3 fatty acids, contributing to more sustainable fishery resource management practices.

**Keywords:** Biorefinery, Fish oil, Omega3, Tuna, Solvent

## 1. Introduction

Traditionally, fish oil extraction is performed using either hydraulic pressing or thermal extraction, mainly from fresh, small-sized fish (FAO, 2022). While effective, these conventional methods often compromise oil quality and exhibit significant environmental impacts. In the context of the Blue Economy, there is a growing need to valorise fish processing by-products—such as heads, viscera, and bones—as alternative raw materials, aiming to reduce waste and enhance sustainability(Pike and Jackson, 2010). However, current industrial practices primarily utilise small fish species, leaving the potential of large fish residues largely untapped. The use of larger species, such as tuna, presents technical challenges but also opportunities for high-value lipid recovery, particularly of omega-3 fatty acids like EPA and DHA. This study explores the feasibility of extracting fish oil from large fish by-products using alternative green solvents, offering a more sustainable and efficient approach compared to traditional extraction techniques.

## 2. Materials and Methods

Fish oil was extracted from tuna by-products (viscera and heads) following a procedure adapted from Ciriminna et al. (2019). Different green solvents—d-limonene (d-Lim), ethanol (EtOH), and 2-methyloxolane (2-MeOx), —were tested to evaluate extraction efficiency and solvent suitability for subsequent anaerobic digestion.

For each extraction, 150 g of washed and dried tuna by-products were homogenised with 150 g of pre-cooled solvent at -18 °C, incubated overnight at -18 °C, then supplemented with an additional 150 g of refrigerated solvent. The mixture was agitated for 24 hours at 150 rpm using an orbital shaker in 1 L glass bottles, maintaining two-thirds headspace (Paone et al., 2021).

**Table 1.** Fish oil extraction conditions

| Code | E.C.* | Solvent | Substrate | P [mbar] | T [°C] |
|---|---|---|---|---|---|
| LIM-V1 | 1° | d-Lim | Viscera | 10 | 50 |
| LIM-V2 | 2° | d-Lim | Viscera | 10 | 45 |
| LIM-H1 | 1° | d-Lim | Heads | 10 | 45 |
| EtOH-V1 | 1° | EtOH | Viscera | 600 | 70 |
| EtOH-V2 | 2° | EtOH | Viscera | 600 | 70 |
| EtOH-H1 | 1° | EtOH | Heads | 600 | 70 |
| MEOX-V1 | 1° | MeOx | Viscera | 600 | 70 |
| MEOX-H1 | 1° | MeOx | Heads | 600 | 50 |

*Extraction Cycle

Following incubation, solid and liquid phases were separated by vacuum filtration and centrifugation. The liquid phase was subjected to rotary evaporation to recover the solvent and isolate the extracted fish oil.

Extraction conditions, including solvent type, substrate, pressure, and temperature, were adjusted according to the solvent properties (Table 1). In certain cases, extractions were performed in duplicate.

Fatty acid composition was determined by transesterification of triglycerides to fatty acid methyl esters (FAMEs), followed by analysis using GC-FID and GC-MS. Quantification and identification were conducted with reference standards and the NIST Mass Spectral Library.

## 3. Results

Eight extraction cycles were carried out using d-Lim, EtOH, and 2-MeOx. The extracts varied markedly in colour, density, and yield, with some samples containing solid residues at room temperature, indicative of fats and lipids from fish waste.

d-Lim extractions produced the most visually consistent products, with lighter colours observed at lower processing temperatures. EtOH extracts appeared highly heterogeneous, predominantly comprising dense residues such as blood. Overall yields ranged between 1–4% of the initial mass, except for LIM-H1, which reached 13%. Extraction with 2-MeOx revealed strong substrate-dependent variability: viscera-derived oils were rich in solids, whereas head-derived oils were more homogeneous but turbid. The MEOX-V1 sample fully solidified after solvent removal, likely due to lipid re-solidification.

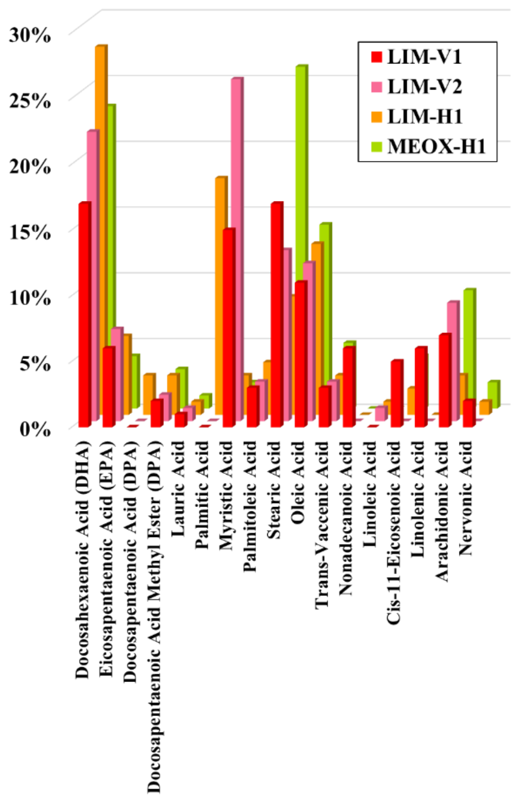


**Figure 1.** Lipid profile

Based on visual inspection and comparison with findings reported in previous studies (Ciriminna et al., 2019; Paone et al., 2021; Pizzone et al., 2024), only samples LIM-V1, LIM-V1, LIM-H1, and MEOX-H1 were selected for GC-MS analysis. The appearance of these samples, in terms of colour, density, and the absence of excessive solid residues, was consistent with characteristics typically associated with high-quality fish oil extracts in the literature.

Significant chromatographic background noise, especially in MEOX-H1, suggested incomplete removal of biological fluids, highlighting the greater suitability of small fish for extraction. The GC-MS lipid profiles are summarised in Figure 1. All analysed samples contained fatty acids of interest, with d-Lim extracts (LIM-V1, LIM-V2) showing a predominance of saturated (myristic, stearic) and polyunsaturated fatty acids (DHA). LIM-H1 exhibited the highest DHA concentration (28%), indicating tuna heads as a rich DHA source when using d-Lim. Oleic acid (11–14%) and other beneficial monounsaturated fatty acids (vaccenic and palmitoleic acids, 3–6%) were consistently detected across samples.

## 4. Conclusions

This study demonstrates that fish by-products, particularly tuna heads, represent a valuable and underutilised resource for fish oil extraction within a sustainable Blue Economy framework. Compared to viscera, head extracts exhibited a more diverse and complete lipid profile, notably with higher concentrations of health-beneficial polyunsaturated fatty acids such as DHA. Among the solvents tested, d-Lim emerged as the most effective, particularly in recovering high-value lipids from tuna heads, while 2-MeOx also showed promise, albeit with different extraction efficiencies and lipid compositions. These findings highlight the potential of using alternative green solvents to improve fish oil extraction from large fish residues, offering an environmentally friendly and efficient alternative to traditional methods. Future research should focus on optimising extraction parameters and scaling up the process to industrial applications.

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