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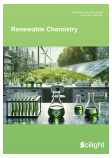

Article

Integrated Fish Oil Production from Tuna Processing Waste Using the LimoFish Process

Altea Pedullà [1], Sonia Carabetta [2], Rosaria Ciriminna [3], Mariateresa Russo [2,*], Mario Pagliaro [3,*] and Paolo Salvatore Calabrò [1,*]

[1] Dipartimento di Ingegneria Civile, dell'Energia dell'Ambiente e dei Materiali, Università degli Studi Mediterranea di Reggio Calabria, Loc. Feo di Vito, 89122 Reggio Calabria, Italy
[2] Focuss Lab, Università degli Studi Mediterranea di Reggio Calabria, Loc. Feo di Vito, 89122 Reggio Calabria, Italy
[3] Istituto per lo Studio dei Materiali Nanostrutturati, CNR, via U. La Malfa 153, 90146 Palermo, Italy
* Correspondence: mariateresa.russo@unirc.it (M.R.); mario.pagliaro@cnr.it (M.P.); paolo.calabro@unirc.it (P.S.C.)

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Abstract: The LimoFish process was applied to the by-products of the canned tuna industry under mild and safe extraction and solvent recovery conditions. The process affords a valued fish oil ("TunaOil") rich in health beneficial polyunsaturated and monounstaturated fatty acids in triglyceride form (and tocopherol from fish processing by-products (viscera, skin and head) so far primarily used to produce fishmeal. The oil yields vary between remarkably high yield of 13% for TunaOil extracted from the head, and 1–2% for oils sourced from viscera. Employed together, the LimoFish and anaerobic co-digestion processes close the material cycle for the world's most commercially valuable fish, converting abundant biological resources into valued bioproducts (fish oil, biomethane and fertilizers). Limonene readily recovered after TunaOil extraction is reused in subsequent extraction runs.

Keywords: LimoFish; fish oil; TunaOil; omega-3; tuna fillet waste

1. Introduction

Fished across all seas for thousands of years, tuna is the world's most commercially valuable fish, with commercial fisheries alone reporting $40.8 billion in revenues in 2018 [1]. Seven out of fifteen are the most caught tuna species: albacore (*Thunnus alalunga*), Atlantic bluefin (*Thunnus thynnus*), Pacific bluefin (*Thunnus orientalis*), southern bluefin (*Thunnus maccoyii*), bigeye (*Thunnus obesus*), yellowfin (*Thunnus albacares*), and skipjack (*Katsuwonus pelamis*) [1].

Chiefly commercialised as a canned product (with skipjack accounting for 47.7% revenue share, followed by yellowfin), the global canned tuna market in 2024 was valued at $ 20.4 billion [2]. Driven by affordability, long shelf life and excellent nutritional value, the market for canned tuna is expected to grow up to $26.6 billion by 2030, at an annual growth rate of 4.5% [2].

Out of 5,209,000 t of tuna fish caught in 2018, the global canned tuna industry consumed approximately 3,215,000 t (62%) [3]. Conservative estimates by Kawamoto in 2022 suggest that the demand for tuna fish will further increase by 1,100,000 t and reach 4,315,000 t by 2050 [3]. Consisting of head, viscera, gills, dark flesh and bone, fillet processing waste of the canned tuna industry amounts to about 70% of the total weight of the live fish [4]. The industry typically supplies these by-products to animal feed manufacturers, who convert it into fishmeal used in pet food and aquaculture feed.

Plentiful research has been devoted to upgrade tuna processing waste (TPW) into valued bioproducts such as fish oil rich in omega-3 lipids and fish protein hydrolysates rich in essential proteins [5,6]. Given the abundance of TPW, the extraction of fish oil rich in omega-3 lipids such as the triglycerides of eicosapentaenoic acid (EPA, 20:5 *n*-3) and docosahexaenoic acid (DHA, 22:5 *n*-3) from tuna biowaste would be particularly relevant.

Enzymatic extraction with alcalase requires relatively high temperature (55 °C) along with a substantial amount of solvent for extraction and electrical energy to separate the oil via centrifugation [6].

Extraction with supercritical carbon dioxide ($scCO_2$) at 65 °C at 40 MPa pressure or with *n*-hexane under reflux (Soxhlet method) can both be employed to source valued fish oil from tuna head, skin and viscera and acceptable free fatty acid and peroxide content [7]. Extraction using $scCO_2$, however, is both capital-intensive and is affected by high operating expenditures due to the high pressures required to produce $scCO_2$ [8]. Hexane, on the other hand, is a toxic solvent whose employment in natural product extraction should be avoided, especially for lipids that will be used for human consumption [9].

Omega-3 lipids indeed are human essential nutrients abundant in marine oils [10], whereas omega-6 lipids, are plentiful in most vegetable oils, pork, lard and turkey meat. The former polyunsaturated fatty acids (PUFAs) are key hormone precursors and moderate the propensity for arachidonic acid cascade overreactions when ω-6 lipids dominate, defending tissue against oxidative stress [10,11].

So called "western" diets raised the ω-6/ω-3 fatty acid ratio from 1:1 in 1900 to today's 20:1 (15:1 in Europe and 25:1 in the USA) creating the conditions of chronic inflammation due to continuous production of pro-inflammatory lipid mediators. Over 80% of the world's population in 2010, and still largely today [12], failed to meet the basic recommendation of 250 mg of marine omega-3s (EPA/DHA) daily.

Therefore, fish oil, which is generally highly refined and made available as a concentrate of marine omega-3 lipids in ethyl ester form, has become one of the most widely used dietary supplements in the world, being added also to food, pet food, and infant formulas (the EPA and DHA omega-3 "finished product" market reached $52.4 billion in 2023) [13].

Supplying a daily dosage of 250 mg to the world's 8 billion population would require an annual production of nearly 730,000 t of EPA and DHA. The current yearly production of EPA and DHA-enriched fish oils, however, does not exceed 85,000 t [14]. Narrowing this large nutrient gap requires shifting aquaculture production to species with the highest omega-3 content, reducing food loss throughout the supply chain, and sourcing fish oil from fish processing waste (fish processing by-products) [14].

Introduced in 2019, the "LimoFish" circular economy process based on defatting and stabilising fish processing waste with biobased *d*-limonene at low temperature converts European anchovy (*Engraulis encrasicolus*) fillet waste into a valued fish oil rich in vitamin D_3, ubiquinone and omega-3 lipids in natural (triglyceride) form [15]. Following mild drying, the solid residue of the biowaste extraction with limonene is an exceptional organic fertiliser free of antibiotics and antibiotic resistance genes [16], and rich in flavonoids, organic carbon, phosphate, sulphate, magnesium, potassium, and essential amino acids [17]. The process is generally applicable to different fish and crustacean processing by-products, including European sardine (*Sardina pilchardus*) [18] and shrimp (*Parapenaeus longirostris*) [19] processing by-products. We now extend the application of the LimoFish process to tuna filleting leftovers, a class of by-products that presents significant differences compared to small blue fish. Furthermore, we compare the extraction of *d*-limonene with biobased solvents ethanol and 2-methyloxolane (2-MeOx), due to their low toxicity and increasing relevance as green natural product extraction solvents [20].

2. Experimental

2.1. TunaOil Extraction with Different Solvents

Oil extractions were carried out from two types of tuna waste (*Thunnus albacares* and *Thunnus thynnus*), kindly donated by a tuna canned industry company based in Italy. The TPW consisted of viscera (including heart, liver, and internal organs) and heads. The heads were processed to separate the soft parts from the bones. All tissues were subjected to preliminary cleaning (washing, manual centrifugation, draining and drying at room temperature) to remove blood and body fluids. However, in large fish such as tuna, some blood remains trapped in tissues. It was therefore necessary to find a more effective cleaning method.

The optimised procedure used for the extraction of fish oil is as follows: 150 g of washed and dried TPW was blended with 150 g of solvent previously kept at −18 °C and stored overnight at the same temperature. Subsequently, a further aliquot of refrigerated solvent (150 g) was added, and the mixture stirred for 24 h at 150 rpm using an orbital incubator. The entire procedure was carried out using a 1 L glass bottle, with a headspace of approximately two-thirds of the bottle volume.

After 24 h, the contents of each bottle were poured into a beaker, allowing the solid phase and the supernatant to separate due to their different densities. The supernatant was filtered under vacuum using filter paper. The solid residue in the liquid phase was centrifuged for 5 min at 10,000 rpm to reduce its moisture levels further.

Three extraction cycles per solvent were performed on TPW by-products (two cycles on viscera and one on heads). The oil was separated by evaporation under vacuum, with temperature and pressure conditions varying according to the solvent. Following extraction, the oil was recovered and filtered to remove solid impurities.

Table 1 summarizes the extraction solvent, solvent removal conditions and tuna fish by-product employed as raw material.

Table 1. Amount of extract, extraction solvent, solvent removal conditions and tuna fish by-product employed as raw material.

Oil Sample	Solvent	Fish by-Product	P [mbar]	MG [°C]	Extract (g)	Yield (%)
LIM-V-I	Limonene	Viscera	10	50	6.0	2
LIM-V-II	Limonene	Viscera	10	45	3.2	1
LIM-H	Limonene	Head	10	45	36.2	13
EtOH-V-I	EtOH	Viscera	600	70	62.3	22
EtOH-V-II	EtOH	Viscera	600	70	9.3	3
EtOH-H	EtOH	Head	600	70	13.4	4
MEOX-V-I	2-MeOx	Viscera	600	70	7.1	3
MEOX-V-II	2-MeOx	Viscera	600	50	5.2	2
MEOX-H	2-MeOx	Head	600	50	6.9	3

2.2. GC-MS Analysis

The fatty acid composition of tuna oils was evaluated following the standard method involving the transesterification of oil triglycerides and GC-MS/GC-FID analysis of the respective fatty acid methyl esters. For fatty acid analysis, 2 mL of heptane (CHROMASOLV, for HPLC, ≥99% pure) was added to a 100 mg oil sample. Fatty acids in the form of triglycerides were trans-esterified with methanol by treating the fat residue with concentrated KOH dissolved in MeOH to obtain fatty acid methyl esters (FAMEs) required for GC-MS analysis.

Quantification of FAMEs was performed using a Shimadzu GC 2010 plus Tracera (Shimadzu, Kyoto, Japan) equipped with a flame ionisation detector (FID), while identification was done with pure standards (FAME Mix) analysed by GC-FID and GC-MS using the NIST Mass Spectral Library.

GC-MS/GC-FID Analysis

The GC-MS analysis was performed using a Shimadzu single quadrupole GC/MS QP2010 Ultra mass spectrometer. In detail, a 1 µL sample of the FAME solution was injected into the GC (split ratio 38) using an SP-2380 column (Supelco, 100 m 0.25 mm id 0.20 mm wall) with an AOC-20i autosampler, using He (6.0) as the carrier gas (flow rate of 0.59 mL/min). The temperature gradient used was the same as for GC-FID analysis. The injector temperature was set at 250 °C. After automatic tuning, the electron multiplier voltage was set to 70 eV. Data were acquired in the *m*/*z* range of 20–500 at 0.30 s, with the ion source maintained at 270 °C. Retention times and mass data of the obtained molecular fragments were processed using the instrument software. All FAME compounds were identified by critical comparison with mass spectral data from the NIST 11 Mass Spectral Library.

A 0.2 µL sample of the FAME solution was injected into the GC (split ratio 148) using a Mega-10 column (Mega, 100 m 0.25 mm id 0.20 µm film thickness) with a Shimadzu AOC-20i autosampler, using He (6.0) as carrier gas (flow rate of 0.52 mL/min). The temperature gradient used was as follows: column held for 8 min at 165 °C, after which temperature was increased at a rate of 2 °C/min to 210 °C, with a final isotherm of 45 min. The total analysis time was 75.50 min. The injector and detector temperatures were both set to 250 °C.

3. Results and Discussion

Photographs in Figure 1 show how the extraction products vary significantly in terms of colour, density, and quantity. Especially the oils extracted with EtOH and, to a lesser extent, also those sourced using 2-MeOx, contained solid parts, likely consisting of poorly soluble lipid fractions. In general, the extraction products with the most similar appearance to the expected one are the samples extracted with *d*-limonene.

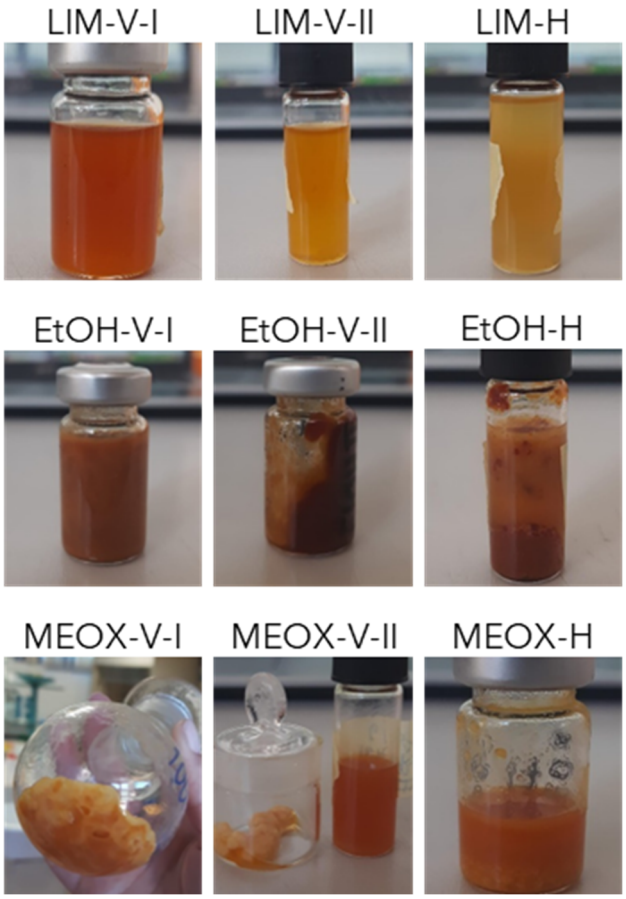

Figure 1. Extraction products obtained after different treatment of TPW with solvents limonene, EtOH and 2-MeOx. For details on the extraction conditions, see Table 1.

The lighter and brighter colours of the LIM-V-II and LIM-H samples compared to those of LIM-V-I could be attributed to the different extraction temperatures. OIls sourced with ethanol were very heterogeneous and not analyzable. From visual inspection, the EtOH-sourced samples did not show evidence of fish oil, exhibiting a prevalence of residues, such as blood and liquids with higher density.

Extractions carried out using 2-MeOx as a solvent showed particularly variable results depending on the substrate. In detail, oil extracted from the tuna viscera was rich in solid parts, fats and lipids, while oil extracted from the soft parts of the heads was more homogeneous and less dense, although more turbid in comparison to the oil extracted with *d*-limonene. Samples MEOX-V-I and MEOX-V-II, sourced from tuna viscera, were completely solidified after the solvent removal process. Likely, higher molecular fats present in the viscera, such as phospholipids, solubilised during the extraction phase and return to the solid phase at room temperature. The oily mixture MEOX-H, sourced from tuna head with 2-MeOx, was not entirely solid, though it was highly viscous. We briefly remind that besides approximately 5–8% water and 15–21% ash, the composition of the main tuna waste by-products (head, viscera, and skin) includes proteins (44–57%) and lipids (16–35%) [21]. Only the head, furthermore, comprises about 11% carbohydrates [21].

Photographs in Figure 2 show evidence that the colour of TunaOil extracted with limonene from the viscera had the deepest red color when compared to TunaOil sourced from the heads. This finding aligns with similar outcomes regarding tuna oil sourced from viscera and head byproducts using ethanol-assisted supercritical carbon dioxide [21], and is due to the higher amount of tocopherol in the viscera compared to the head.

Values in Table 2 show the outcomes of the GC-MS analyses of fatty acids for the four tuna oils obtained. All chromatograms exhibited significant background noise due to technical difficulty in thoroughly cleaning the tissues of a large fish such as tuna whose tissues retain residual blood and fluids even after thorough cleaning.

Regardless of slightly different evaporation temperatures (45 °C for LIM-V-I, and 50 °C for LIM-V-II), the yield in TunaOil extracted with limonene from viscera was twice as high (2% vs. 1%) for the oil recovered at 50 °C. Both oils contain saturated fatty acids (SFAs, such as myristic and stearic acids), monounsaturated fatty acid (MUFA, oleic acid) in similar amount (11–12%), and PUFAs (DHA and EPA). Both oils contained relatively high percentages of EPA (6% and 7% for LIM-V-I and LIM-V-II, respectively).

Figure 2. Sample of TunaOil sourced from tuna viscera via the LimoFish process.

Table 2. Lipid profile of fish oil extracted from different TPW by-products with limonene and 2-MeOx, compared to the lipid profile of AnchoisOil (Ref. [18]).

Fatty Acid	Oil LIM-V-I	Oil LIM-V-II	Oil LIM-H	Oil MeOx-H	AnchoisOil Ref. [18]
Docosahexaenoic (22:6, *n*-3) (DHA)	17%	22%	28%	23%	12%
Eicosapentenoic (20:5, *n*-3) (EPA)	6%	7%	6%	4%	5%
11-Docosenoic (22:1, *n*-11) (DPA)	0%	0%	3%	0%	5%
Oleic acid	11%	12%	13%	14%	24%
Vaccenic acid	3%	3%	3%	5%	3%
Stearic acid	17%	13%	9%	26%	1%
Myristic acid	15%	26%	3%	2%	7%
Palmitic acid	0%	0%	18%	0%	34%
Arachidonic acid	7%	9%	3%	9%	0%
Palmitoleic acid	3%	3%	4%	6%	0%
Linoleic acid	0%	1%	1%	0%	2%
Gadoleic	0%	0%	0%	0%	3%
Others	24%	7%	12%	16%	8%

The main differences between the oils are the amounts of myristic acid, nearly twice higher in LIM-V-II (26% vs. 15%), and DHA (22% vs. 17%). The oil LIM-H extracted in remarkably high yield of 13% from the head (isolated via evaporation under vacuum at 45 °C) has a very high content of DHA (28%) and a relatively good amount of EPA (6%). This outcome confirms that the tuna head is a biological matrix very rich in PUFAs, [7] and that biobased limonene is ideally suited as an extraction solvent for DHA and EPA in natural (triglyceride) form, co-extracting valued bioactive molecules such as tocopherol [18,19].

In contrast to LIM-V-I and LIM-V-II from the viscera, the oil extracted from the head contains a significant amount of palmitic acid (18%) and the highest percentage (13%) of oleic acid, along with 3% *trans*-vaccenic acid (a positional and geometric isomer of oleic acid). Abundant in human milk, palmitic [22], oleic [23], and vaccenic acid [24], provide substantial health benefits in infancy, and subsequently in adult life.

Fish oil MEOX-H obtained from tuna head using 2-MeOx as extraction solvent has a uniquely high amount of stearic acid (26%) along with a less varied composition when compared to tuna oil sourced with limonene, being similarly effective in the extraction of DHA (23%) and less effective in that of EPA (4%).

Comparing the lipid profile of TunaOil with that of AnchoisOil sourced from anchovy fillet leftovers [18] reveals a composition in tuna that is generally more enriched in omega-3 fatty acids. Another significant difference between AnchoisOil and TunaOil, regardless of the biological matrix or the solvent employed, concerns the amount of stearic acid. The latter is very low (1%) in AnchoisOil and abundant in TunaOil, especially in the oil sourced from viscera.

Both TunaOil and AnchoisOil have comparable concentrations (5–7%) of 11-docosenoic acid (22:1, *n*-11), better known as cetoleic acid. Remarkably, this long chain MUFA was recently shown to have highly beneficial effect on the omega-3 index (a validated measure of EPA and DHA in blood cells) of volunteers administered with a fish oil enriched in cetoleic acid [25].

These results demonstrate that the choice of substrate (heads or viscera) and solvent used has a significant influence on the lipid profile of the extracted fish oil.

In brief, also in the case of a large fish such as tuna, treatment of fish processing waste with limonene at room temperature affords a transparent clean oil coloured in orange (Figure 2), dubbed herein "TunaOil", with multiple potential health benefits.

Whereas the orange colour in the case of AnchoiOil is due to the presence of vitamin A (retinol) and of particularly high amounts of vitamin Q (coenzyme Q_{10}) [26], in the case of oil sourced from tuna waste by-products tocopherol (vitamin E) is predominant, with particularly high amounts of γ-tocopherol for tuna oil sourced using ethanol-assisted $scCO_2$, from the viscera, head and the skin [21].

In comparison to the LimoFish process applied to anchovy fillet leftovers in which the solvent was removed at 90 °C under 40 mbar pressure [15], in this case, the temperature was lowered to 45 or 50 °C, evaporating the solvent under 10 mbar. Limonene, furthermore, acts as an antioxidant and bactericidal agent [27]. protecting the numerous double bonds in TunaOil PUFAs from oxidation during the solvent removal. The peroxidation-prone double bonds of PUFAs are also protected from oxidation by lipophilic polyphenols such as the phlorotannins naturally present in certain fish and seafood species [28], as well as by the antioxidant tocopherol.

The fatty mixtures extracted from TPW by-products with 2-MeOx, in turn, are solid because 2-methyloxolane has a relatively high solubility for oxygenated compounds, such as phospholipids and free fatty acids [29].

Indeed, phospholipids are relatively abundant in tuna head, skin and viscera from which they can be extracted using ethanol-assisted $scCO_2$ [21].

Treatment of TPW wit with limonene in the LimoFish process, does not require expensive tuna waste freeze drying affording an oil (TunaOil) rich in omega-3 lipids, particularly in DHA with EPA varying between 6% and 7%. In contrast, extraction using ethanol-assisted $scCO_2$ affords fish oils with EPA contents between 2.8% and 4.5% depending on the TPW part (viscera, skin or head) [21], or even lower, ranging from 1.3% to 3.7% when respectively employing ethanol-assisted $scCO_2$ at 65 °C at 40 MPa [7], or at 40 °C at 30 MPa [21].

The solid residue from the LimoFish process was successfully utilised as a raw material for anaerobic co-digestion (Co-AD), yielding abundant biomethane and two fertilisers (struvite and digestate) [30]. Onion stalks were used to balance carbon and nitrogen content during co-AD. Co-AD with 30% raw fish waste increased methane yield by 25 %, integrating phosphorus sourced from fish bones into struvite precipitation. In an industrial plant, the energy required to recover limonene via distillation [31] would be readily compensated for by the AD biomethane fed into the natural gas grid.

4. Conclusions

In conclusion, applied to tuna fillet by-products under mild (room temperature) and safe conditions the LimoFish process converts processing biowaste of the tuna canning industry into a valued oil (TunaOil) rich in health-beneficial lipids in triglyceride form and tocopherol.

Closing the material cycle also for the world's most commercially valuable fish, the de-oiled residue can be used for anaerobic co-digestion affording plentiful biomethane and two fertilisers (struvite and digestate), whereas health-beneficial and renewable solvent limonene is readily recovered after the oil extraction and reused.

Author Contributions

A.P.: Conceptualization, Investigation, Methodology, Data curation; S.C.: Investigation, Methodology, Data curation; R.C.: Methodology, Writing—review & editing, Resources; M.R.: Conceptualization, Methodology, Formal analysis, Writing—review & editing, Resources; M.P.: Conceptualization, Methodology, Writing—original draft, Resources; P.S.C.: Conceptualization, Methodology, Formal analysis, Writing—review & editing, Resources, Supervision. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

All data that support the findings are available upon reasonable request to the corresponding authors.

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Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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