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A Circular Economy Approach to Fish Oil Extraction

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Fish oil rich in polyunsaturated omega-3 fatty acids is extracted in high yield from anchovy filleting waste using *d*-limonene as green biosolvent in a simple solid-liquid extraction performed by mechanically stirring and maceration followed by limonene removal via evaporation under reduced pressure. As limonene is renewably obtained from waste orange peel, this method closes the materials cycle and establishes a circular economy process to obtain high quality fish oil from biowaste available worldwide in several million t/year amount. Significant economic, social and environmental benefits are anticipated.

1. Introduction

Consumption of omega-3 long chain polyunsaturated fatty acids (PUFA) abundant in oily fish is critical for both physical and mental health of adults and children.[1] Health authorities across the world generally recommend healthy adults increase their intake of omega-3 fats by regularly eating fish twice a week or, in case of lack of regular consumption of fish, by assuming a 2 g fish oil supplement several times a week. Fish oil, indeed, is a primary source of omega-3 PUFA.[2] Depending on the position of the first double bond from the methyl end group (ω end) of the fatty acid, the main long-chain PUFA belong to ω-6 (*n*-6) or to ω-3 (*n*-3) families.[3] In order to reestablish a better balance between ω-3 and ω-6 fats, the World Health Organization, recommends a daily intake of eicosapentaenoic acid (EPA, 20:5 ω-3) plus docosahexaenoic acid (DHA, 22:5 ω-3) of 250 mg in primary prevention of coronary hearth disease and 2 g in secondary prevention.[4] The European Food Safety Authority recommends a daily intake of 250 mg for EPA plus DHA.[5]

Omega-3 nutrients are fundamental hormone precursors which moderate the inherent propensity for arachidonic acid cascade overreactions when *n*-6 mediators dominate.[6] The efficiency of the tissue to defend itself against oxidative stress depends upon its ω-6:ω-3 composition. High percentage of omega-6 leads to persistent inflammation reinforced by the action of free radical continuously generated. Omega-3 fats reduce the concentrations of prostaglandins 2-series PG, a potent mediator of inflammation and cell proliferation, and increase the synthesis of much less inflammatory 3-series PG.

In a recent study devoted to enhanced methods for the extraction omega-3 fats from fish oil, we concluded that practically useful advances in sustainable extraction and sourcing of marine omega-3 nutrients are urgent,[7] since new health benefits of omega-3 assumption extending to the prevention of many pathologies are continuously reported,[8] while non sustainable fishing of anchovy (a preferred source of omega-3 fats) has, for example, led the Peruvian government to first suspend in 2014 one of two annual fishing seasons due to concerns about the number and size of the available anchovy, and in 2017 to set quota for first anchovy fishing season, while the previous one was still underway.[9]

Driven by rapidly growing demand in Asia, the global EPA/DHA ingredient volume has gone from 87,925 tonnes in 2015 to 91,321 tonnes in 2016.[10] Most of the oil used to produce dietary supplements originates from Peruvian anchovies, even though only about 5% of world's fish oil production is used to extract omega-3 nutrients for use in food and dietary supplement products (the remainder fish oil is employed as fishmeal for fish farming).[7]

In the conventional fish oil omega-3 concentrate production process, once caught anchovies are cooked and pressed still on board the shipping vessel. The omega-3 extraction process is carried out at industrial sites where the water-oil mixture obtained onboard is freed from water with a three-phase centrifuge, undergoing refinement in several consecutive steps including neutralization with alkalis, bleaching, deodorization, and degumming.

The refined fish oil thereby obtained typically contains about 30% omega-3 fatty acids (18% in EPA and 12% in DHA). Omega-3 supplements use either 55% omega-3 ethyl ester oil in which the triglycerides comprising natural fish oil are transesterified with ethanol followed by molecular distillation or, even better, highly concentrated fish oil extracts providing 70% active ingredients.[7]

The latter are obtained either via supercritical fluid extraction combined with supercritical fluid chromatography or via enzyme-assisted concentration for the conversion of the omega-3 ethyl esters back into triglyceride form. Today's best omega-3 dietary supplements indeed contain EPA and DHA in triglyceride form as this leads to a 70% higher increase in the omega-3 index when the ingredients are consumed as triglycerides rather than as ethyl esters.[11]

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Extending the production of omega-3 from blue fish to fishery by-products so far mostly discarded as waste, would enable to recover and transfer key essential nutrients from the sea to the human food chain with significant economic, environmental and health benefits. Only in the process of fish filleting up to 60% of the fresh fish is cut off and generally treated as waste. A large amount of blue fish and seafood industry leftovers such as head, skin, trimmings and bones is mostly thrown away back into the sea, even though the huge potential of marine processing byproducts for the production of omega-3 has been long recognized.[12]

In the following, we describe how to obtain fish oil with abundant omega-3 nutrients directly from anchovy processing waste using bio-based *d*-limonene as extraction solvent.

2. Extraction and analysis

Anchovies are amid the world's largest fish catches with overfishing threatening their overall population.[13] The European anchovy (*Engraulis encrasicolus*) is particularly abundant in the Sicilian Channel, and its capture to produce anchovies filleted, salt-cured, and stored in olive oil is a key economic asset of southern Sicily's urban centres, including the city of Sciacca from where the anchovy fillet leftovers (Figure 1) used

Figure 1. Frozen leftovers of anchovy fillets used throughout this study.

in this study were kindly made availably by a company selling anchovy fillets worldwide.

The complete experimental work is detailed in the Supporting Information. In brief, an electric blender was used to mix and homogenize the frozen leftovers along with an aliquot of *d*-limonene (Figure 2). A sample of frozen anchovy waste in the blender jar of the electric blender was added with a first aliquot of *d*-limonene refrigerated at 4 °C. After grinding, a semi-solid grey purées was obtained (see video in the Supporting Information) which was extracted with limonene.

A portion of this mixture was transferred in a glass beaker and added with another aliquot of cold *d*-limonene. A simple solid-liquid extraction was performed by magnetically stirring

Figure 2. The anchovy leftover oil obtained after evaporating limonene under reduced pressure.

the mixture kept in the beaker sealed with aluminum further coated with parafilm and left at room temperature under stirring at 700 rpm for 21 h. The yellow supernatant thereby obtained was transferred to the evaporating balloon of a rotary evaporator equipped with a vacuum pump to remove the solvent under reduced pressure (40 mbar) at 90 °C (see video in the Supporting Information).

Pure limonene was almost entirely recovered via evaporation under reduced pressure, ready for use in subsequent extraction runs.

After evaporating limonene, we obtained 3.0 g of fish oil colored in orange (Figure 2) with a pleasant and delicate odor.

For the fatty acid analysis, a 100 mg sample of the latter oil was added with three consecutive aliquots of MeOH. Most residual limonene dissolved in the methanol liquid phase. The pale yellow fat precipitate was dried with a flux of nitrogen at room temperature obtaining 18 mg of solid fat. The fatty acids in triglyceride form were trans-esterified to obtain the fatty acid methyl esters (FAME) required for the GC-MS analysis by treating the fat residue with concentrated KOH dissolved in MeOH. All FAME compounds in the chromatogram (Figure 3) were identified by critical comparison with mass spectral data from NIST/EPA/NIH Mass Spectral Library 2005.

Besides omega-3 PUFA, anchovies are a rich source of nutrients including saturated fatty acids (SFA), monounsaturated fatty acid (MUFA), vitamins including vitamin E (in α-tocopherol form), retinol (vitamin A), Vitamin D and D3 cholecalciferol, protein amino acids and minerals.[14]

Results in Table 1 show that in the leftovers of European anchovies catched in Sicily in early July, the major SFA is palmitic acid (33.55%), followed by myristic (6.98%) and pentadecanoic (1.2%) acid. Oleic acid is the most abundant MUFA with 23.97% relative abundance, whereas DHA (12.38%) and EPA (5.4%) are the most abundant omega-3 PUFAs, followed by valued stearidonic acid (1.04%) and alpha-linolenic acid (0.96%).

The 1.80% fat content in the anchovy fillet by-products oil is not far from the average 2.27% fat content found in the

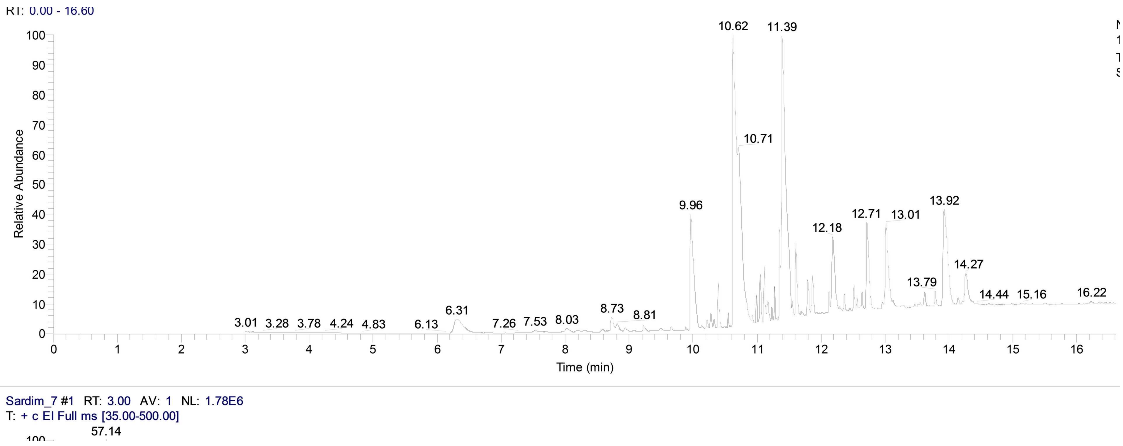

Figure 3. Chromatogram of anchovy leftover oil upon trans-esterification of the oil with MeOK.

Table 1. Main fatty acids relative abundance in the anchovy leftover oil.[a]

Acid (in lipid numbers)	Retention time (min)	Abundance (%)
Myristic acid (14:0)	9.95	6.98
Pentadecanoic (15:0)	10.38	1.2
Palmitic (16:0)	10.61	33.55
(6,Z)-7 methyl-6-Hexadecenoic	11.04	1.19
Margaric (17:0)	11.1	0.94
Stearic (18:0)	11.34	0.53
Oleic (18:1, *n*-9)	11.39	23.97
Linoleic (18:2, *n*-6)	11.6	1.97
alpha-Linolenic (18:3, *n*-3)	11.78	0.96
Stearidonic (18:4, *n*-3)	11.86	1.04
Gadoleic (20:1, *n*-11)	12.18	3.09
Eicosapentenoic (20:5, *n*-3)	12.07	5.4
11-Docosenoic (22:1, *n*-11)	13.02	4.66
Docosahexaenoic (22:6, *n*-3)	13.90	12.39

[a]Values include the retention time using a ZB-5MS column, obtained upon trans-esterification reaction with potassium methylate.

whole-body of anchovy caught in the Adriatic Sea.[15] Remarkably, scholars in Croatia discovered that the fat content in anchovy showed significant seasonal changes, being inversely correlated to water content, and varying between 0.86% in February and 4.47% in October.[15]

The *n*-3/*n*-6 ratio found for the anchovy by-product oil (10.04) is considerably higher than the *n*-3/*n*-6 ratio (6.29, 6.17 and 6.70 in November, December and January, respectively) found for anchovies catched in Turkey.[16]

The use of *d*-limonene as a green solvent for the lipid extraction as an alternative to *n*-hexane has been extensively studied by Chemat's team.[17] It is now well established that it offers several technical, environmental and health advantages over the use of *n*-hexane.[18]

For example in the extraction of rice bran oil conducted with *d*-limonene at the bio-based solvent boiling point (176 °C), regardless of the absence of antioxidants during the limonene recovery step via vacuum evaporation at 90 °C, the amount of the oxidation products in the recovered limonene is <1 wt% of the original biosolvent, and limonene could be easily reused.[19]

In our case, the extraction is conducted overnight at room temperature adding the anchovy leftovers with cold *d*-limonene kept at 4 °C in a refrigerator, which leads to even lesser degree of oxidation of the valued terpene. The recovered biosolvent could be reused at length, even for the extraction of the anchovy leftovers stored at -20 °C for several months. A study detailing these and related technical aspects will be reported in the near future.

Finally, the orange color of the anchovy leftover oily extract is likely due to retinol and α-tocopherol, both abundant in anchovies.[20] The latter is the vitamin E form (labeled with the E307 food ingredient E number in Europe) preferentially absorbed and accumulated in the human body, where it is responsible for several health beneficial effects.[21]

Remarkably, upon trans-esterification of the solid fat residue with potassium methylate and extraction of FAME with *n*-hexane for the GC-MS analysis, retinol and α-tocopherol accumulate in the glycerol/methanol layer at the bottom (Figure 4) from which they can be readily recovered and

Figure 4. The glycerol/methanol layer formed at the bottom upon trans-esterification of the solid fat residue and extraction of FAME with *n*-hexane prior to the GC-MS analysis.

analyzed. A forthcoming study will report the detailed analysis of these compounds as well as of ergosterol, the D_2 provitamin, also abundant in anchovy.[20]

3. Conclusions

In conclusion, we have discovered that high quality fish oil rich in omega-3 nutrients can be obtained in significant yield from the discards of anchovy fillets using *d*-limonene as green extraction solvent via simple solid-liquid extraction at room temperature, followed by limonene removal via evaporation under reduced pressure.

Being the main component of orange essential oil widely used in the food industry, limonene is ideally suited to produce omega-3 extracts from fish and seafood processing waste.

First demonstrated with anchovies, *i.e.* one of the world's largest fish catches,[13] the method can be extended to all other fish processing waste, namely a resource available worldwide in several million t/year amount.[22]

Besides European anchovy (*Engraulis encrasicolus*), widely catched examples of anchovies include Peruvian anchovy (*Engraulis ringens*), Japanese anchovy (*Engraulis japonicus*) and southern African anchovy (*Engraulis capensis*).

A potential new use of *d*-limonene in fish processing was discovered in 2015 reporting the significant activity of the terpene against *Anisakis* larvae when tested *in vitro,* suggesting its potential use in the industrial marinating process.[23] Its use to mask the unpleasant fishy odor co-encapsulated with fish oil in spray-dried and freeze-dried by milk protein microcapsules has been investigated by New Zealand's scholars who found that the limonene-containing microcapsules had much better flavor and odour profile than the fish oil microcapsules.[24]

Limonene indeed has antimicrobial, antifungal, anti-oxidant, anti-inflammatory and anti-carcinogenic properties, for which it is emerging as a key resource of the bioeconomy.[25]

Limonene is renewably obtained from waste orange peel. Hence, as it happens with circular economy bio-based production processes,[26] this method closes the materials cycle and establishes a circular economy process to obtain high quality fish oil from bio-based waste available worldwide in several million t/year amount. Significant economic, social and environmental benefits are anticipated.

Supporting Information

Detailed experimental extraction and analysis procedures. Videos of the extraction procedure.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: Anchovy · Circular economy · Fish oil · Fishery discards · Limonene · Omega-3

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