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High Yields of Shrimp Oil Rich in Omega-3 and Carotenoids: Extending to Shrimp Waste the Circular Economy Approach to Fish Oil Extraction

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Abstract: A shrimp oil rich in omega-3 lipids and carotenoids is obtained in remarkably high 5 wt% yield extending to pink shrimp processing waste (head and carapace) the circular economy approach to extract fish oil from fish processing by-products using *d*-limonene. Biobased limonene, a powerful antimicrobial and antioxidant agent, is an excellent solvent for both lipids and astaxanthin-based carotenoids preventing oxidative degradation during the extraction cycle including solvent and oil separation at 85°C. A new low cost route is established to extract valued marine oil from biowaste annually made available in over 2.2 million tonnes.

Keywords: shrimp waste; astaxanthin; omega-3; limonene; bioeconomy

Driven by high and increasing demand, the global production of shrimp currently exceeds 4.5 million tonnes per year.[1] Aquaculture accounts for roughly 77 per cent of shrimp traded (in frozen form) across the world.[1] In 2018, the global farmed shrimp production increased by 6% reaching almost 3.5 million tonnes (when wild shrimp production amounted to about 1 million t).[1]

Depending on the processing conditions and on the species, the shell (body carapace) and the head of shrimp after the meat removal amount to around 50% of the overall shrimp weight.[2] Since the early 1990s, plentiful research efforts have been devoted to develop chemical[2] and biotechnological[3] (biotransformation with enzymes and microorganisms) to recover the valued chitin (coated with calcium carbonate) and carotenoids (mainly astaxanthin) comprising the shrimp waste.

Chitin-based commercial products are mostly used for wound dressing and as nutraceuticals, with industrial applications "still limited due to issues regarding optimization of mechanical and biological properties according to intended application".[4]

Shrimp waste also contains lipids originating both from the cephalothorax and eyestalk, and from the meat residues in the shell. Since 2015 one company in the port city of Cuxhaven, Germany, successfully uses also shrimp waste to obtain valued fish oil and fish protein concentrates for human consumption.[5]

In general, the current lack of effective green chemistry methods to extract the valued natural products present in shrimp waste creates the conditions for environmental hazard and rises production costs. If thrown into the sea, shrimp waste with its high protein content threatens populations of endangered species, and impacts the product quality of coastal aquaculture.[6] Current proper disposal adds to production costs because shrimp processing companies need to pay for disposal via incineration or sanitary landfill after stabilization. Finally, after processing highly perishable shrimp waste rapidly decays (within an hour in tropical climates)[6] leading to formation of toxic and bad-smelling biogenic amines.

In brief, there is a need to develop a low cost and easily scalable method to extract valued natural products from shrimp waste.

We now report the discovery of a new marine oil rich in omega-3 lipids and carotenoids -- shrimp oil – and of a green process to obtain it in remarkable high 5% yield by extending to shrimp waste the recently discovered method to extract fish oil from anchovy filleting waste using *d*-limonene as green biosolvent.[7]

A sample of deep-water pink shrimp waste (*Parapenaeus longirostris*) obtained from a fishery in Palermo, Sicily, was separated in three parts: carapace (33 g), heads (33 g) and head with carapace (33 g).

Each aliquot was added with 66 g *d*-limonene and the resulting mixture was grounded using an electric blender to obtain a pink puree (Figure 1). After grinding, the mixture was transferred to a beaker. The beaker was covered with an aluminum foil, and the mixture left under magnetic stirring for 24 h at room temperature (Figure 1).

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Figure 1. A sample of *Parapenaeus longirostris* carapace waste prior (*left*) and after homogenization with an electric blender (*right*).

The mixture was thus centrifuged at 10,000 rpm (at 4 °C) for 15 min after which the supernatant was transferred to the evaporation flask of a rotary evaporator to remove the biosolvent under reduced pressure (26 mbar) at 85 °C (Figure 2).

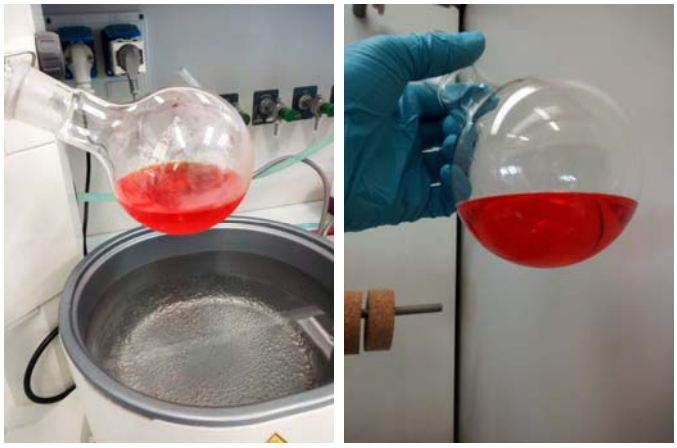

Figure 2. The extract from *Parapenaeus longirostris* carapace in limonene prior to evaporation of the biosolvent.

Rapid evaporation of limonene under reduced pressure leaves a significant amount of a deep red or red oil containing plentiful carotenoids depending on the biological sample extracted (Figure 3): carapace, 370 mg (1.12% yield); head, 1.65 g (5% yield); head with carapace, 1.38 g (4.18% yield).

The red color is due to astaxanthin and its astaxanthin monoester and astaxanthin diester, well known to impart the pink and red color to shrimp. In agreement with the fact that the highest levels of carotenoids are found in the cephalothorax,[8] the oil from the head and carapace has a much more intense color. The thin layer chromatographic separation of the carotenoids in all the oils obtained yields several bands, including the three bands correspond to astaxanthin, astaxanthin monoester and astaxanthin diester. The quantitative analysis of carotenoids will be reported shortly.

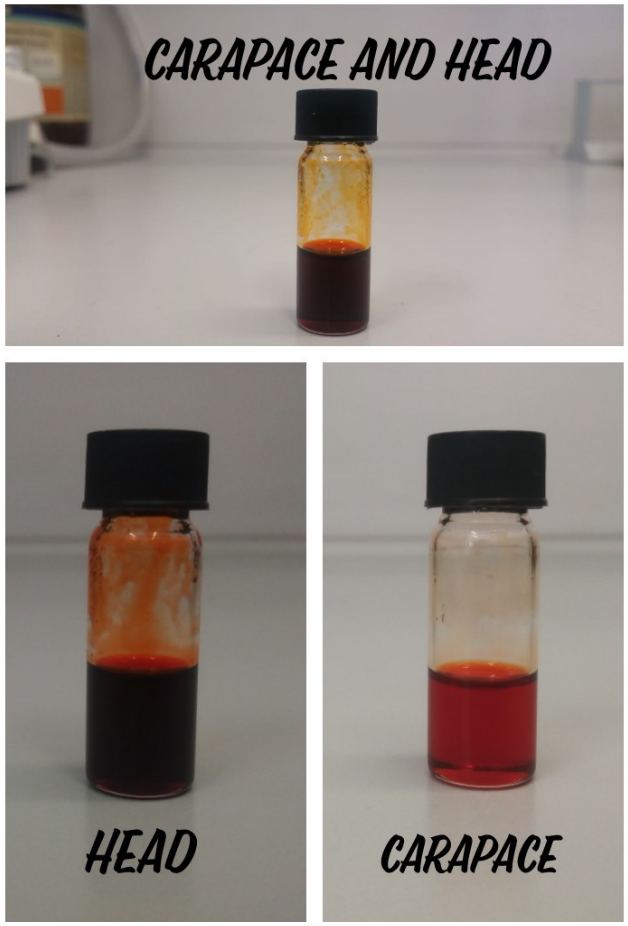

Figure 3. The different oils obtained via solid-liquid extraction with limonene from different parts of *Parapenaeus longirostris* waste.

The fatty acid analysis was carried out by via GC-MS on the fatty acid methyl esters (FAMEs) of the oils. A 100 mg sample of each shrimp oil was evaporated with a flux of nitrogen gas to remove residual traces of *d*-limonene. The oil sample was trans-esterified by adding a 50 µL aliquot of a MeOK solution (2 M) previously prepared by dissolving KOH (extrapure pellets, Merck) in methanol (≥ 99,8 %, Sigma Aldrich).

The resulting FAME was mixed with 500 µL of *n*-hexane, prior to injection into a for the GC/MS analysis employing the technique in scan mode. In detail, the analysis was carried out using a Trace1310 coupled with ISQ and Triplus autosampler (all by Thermo Fisher). The instrument was equipped with a TR FAME capillary column (100m x 0.25mm, 0.25µm) using 5.5 ultra pure helium (99.9995% purity) as gas carrier.

The injection was in split mode (1:10) and the temperature was set at 250 °C. The oven temperature started at 100 °C (held for 0.2 min) increased first until 150 °C (with a ramp temperature of 6 °C/min) and then until 240 °C (ramp temperature 2 °C/min)

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held for 5 min. Certified reference material (Supelco 37 Component FAME Mix), was used for both the qualitative and quantitative analysis.

Table 1. Fatty acids identified in the shrimp oil obtained from *Parapenaeus longirostris* heads.

Acid (in lipid numbers)	**Retention time** (min)	**Abundance** (%)
Myristic (14:0)	15.29	2.58
13-Methylmyristic	16.24	0.35
Pentadecanoic (15:0)	17.19	1.22
Palmitic (16:0)	19.39	18.92
Palmitoleic (16:1)	19.78	0.79
9-Hexadecenoic	20.40	5.29
14-Methylhexadecanoic	20.87	0.48
7-Methyl-6-hexadecenoic	21.33	0.38
Margaric (17:0)	21.52	1.25
Eptadecenoic (17:1)	22.59	0.75
Isostearic (18:0)	22.73	0.53
Stearic (18:0)	24.00	5.95
Oleic (18:1, *n*-9)	25.08	15.6
trans-13-Octadecenoic (18:1)	25.23	3.74
Linoleic (18:2, *n*-6)	26.74	1.38
cis-10-Nonadecenoic (19:1, *n*-9)	27.55	0.19
Linolenic (18:3, *n*-3)	28.93	1.14
cis-11-Eicosenoic (20:1, *n*-9)	30.03	1.72
γ-Linolenic (18:3, *n*-6)	30.24	1.40
cis-11-14-Eicosadienoic (20:2, *n*-6)	31.91	1.56
Arachidonic (20:4, *n*-6)	34.1	4.57
Eicosapentenoic (20:5, *n*-3)	36.48	10.28
8-11-14-Docosatrienoic (22:3, *n*-8)	40.25	2.18
Nervonic (24:1, *n*-9)	40.50	1.04
Docosapentaenoic (22:5, *n*-3)	41.77	1.28
Docosahexaenoic (22:6, *n*-3)	42.67	15.41
Saturated Fatty Acids	30.8%	
Unsaturated Fatty Acids	69.2%	
Mono Unsaturated Fatty Acids	30%	
Poly Unsaturated Fatty Acids	39.2%	

The retention times and molecular fragment mass data obtained were processed using the instrument software. Each measurement was repeated three times. The fatty acids were identified from the corresponding FAMEs by critical comparison with mass spectral data from NIST/EPA/NIH Mass Spectral Library 2005.

The fatty acid composition of the oil obtained from the heads is displayed in Table 1.

Table 2 shows the lipid profile of the oil obtained from the head and carapace waste mixture.

Table 2. Fatty acids identified in the shrimp oil obtained from *Parapenaeus longirostris* head and carapace waste.

Acid (in lipid numbers)	**Retention time** (min)	**Abundance** (%)
Myristic (14:0)	15.29	2.70
13-Methylmyristic	16.24	0.43
Pentadecanoic (15:0)	17.19	1.16
Palmitic (16:0)	19.35	19.44
Palmitoleic (16:1)	19.77	0.76
9-Hexadecenoic	20.38	5.30
14-Methylhexadecanoic	20.87	0.40
7-Methyl-6-hexadecenoic	21.33	0.54
Margaric (17:0)	21.52	1.26
Eptadecenoic (17:1)	22.58	0.64
Isostearic (18:0)	22.70	0.54
Stearic (18:0)	23.95	5.73
Oleic (18:1, *n*-9)	25.04	15.35
trans-13-Octadecenoic (18:1)	25.20	3.78
Linoleic (18:2, *n*-6)	26.72	1.47
cis-10-Nonadecenoic (19:1, *n*-9)	27.55	0.33
Linolenic (18:3, *n*-3)	28.91	1.35
cis-11-Eicosenoic (20:1, *n*-9)	30.01	1.90
γ-Linolenic (18:3, *n*-6)	30.24	1.53
cis-11-14-Eicosadienoic (20:2, *n*-6)	31.89	1.52
Arachidonic (20:4, *n*-6)	34.07	4.35
Eicosapentenoic (20:5, *n*-3)	36.46	10.23
8-11-14-Docosatrienoic (22:3, *n*-8)	40.22	1.82
Nervonic (24:1, *n*-9)	40.48	0.57
Docosapentaenoic (22:5, *n*-3)	41.76	1.09
Docosahexaenoic (22:6, *n*-3)	42.63	15.80
Saturated Fatty Acids	31.3%	
Unsaturated Fatty Acids	67.3%	
Mono Unsaturated Fatty Acids	29.6%	
Poly Unsaturated Fatty Acids	37.7%	

Table 3 shows the lipid profile of the oil obtained from carapace only.

The first remarkable finding is that waste shrimp oil has a high content of unsaturated acids exceeding 65% levels independent of the body part of the shrimp processing waste. Amid unsaturated acids, polyunsaturated fatty acids (PUFA) are predominant, varying between 37.7% of all fatty acids in the oil

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obtained from head and carapace waste and a maximum of 42.04% in the oil obtained from the shrimp shell waste only.

Table 3. Fatty acids identified in the shrimp oil obtained from *Parapenaeus longirostris* carapace waste.

Acid (in lipid numbers)	**Retention time** (min)	**Abundance** (%)
Myristic (14:0)	15.36	2.44
Palmitic (16:0)	19.26	21.56
Margaric (17:0)	21.54	2.52
Stearic (18:0)	23.88	7.99
Oleic (18:1, *n*-9)	24.91	16.37
trans-13-Octadecenoic (18:1)	25.14	4.91
Linoleic (18:2, *n*-6)	26.75	2.46
Linolenic (18:3; *n*-3)	28.96	2.21
cis-11-Eicosenoic (20:1, *n*-9)	30.01	2.16
γ-Linolenic (18:3, *n*-6)	30.29	1.91
cis-11-14-Eicosadienoic (20:2, *n*-6)	31.91	3.71
Arachidonic (20:4, *n*-6)	34.06	7.44
Eicosapentenoic (20:5, *n*-3)	36.38	9.84
Docosahexaenoic (22:6, *n*-3)	42.50	14.47
Saturated Fatty Acids	34.51%	
Unsaturated Fatty Acids	65.48%	
Mono Unsaturated Fatty Acids	23.44%	
Poly Unsaturated Fatty Acids	42.04%	

However, the major (and diriment) difference amid the oils is that whereas 14 fatty acids only are present in the carapace oil, almost twice as much (26) fatty acid moieties are present in the oils from the head alone (Table 1) and from head and thorax together (Table 2).

In each case, the levels of health-beneficial docosahexaenoic acid (DHA) and eicosapentenoic acid (EPA) omega-3 lipids are high, varying between a mimimum of 14.47% in the thorax oil through a maximum of 15.80% in the combined waste oil for DHA, and between 9.84% in the thorax oil and a maximum of 10.28% in the head oil for EPA.

Pointing to its presence in the head of the pink shrimp, one important omega-3 lipid is present in the head (1.28%) and in the head + thorax oils (1.09%), namely docosapentaenoic acid (DPA) which is the most abundant ω-3 long chain PUFA present in the brain and more abundant than EPA in human milk, that could be particularly beneficial for early-life development and neuroprotection in the elderly.[9]

The *trans* isomer of C18 acid 13-octadecanoic acid, found in 0.22% amount in the seed oil of *Phaleria macrocarpa* medicinal plant[10] and rapidly adsorbed by human plasma, is particularly abundant in the pink shrimp's thorax oil (4.91%) and also in the head (3.74%) and head + thorax (3.78) oils.

Rare MUFAs 11-eicosenoic acid (gondoic acid, 1.72% and 1.90%) and nervonic acid (1.04% and 0.47%) lately associated with mortality[11] are present in oils extracted from shrimp waste containing the animal heads.

The color of the present oils is chiefly due due to natural astaxanthin, a carotenoid with numerous health-beneficial properties today widely used as nutraceutical and food ingredient.[12] We decided therefore to perform a theoretical study via a computational method (COSMO-RS, software package from COSMOlogic, Germany) in order to predict and rationalize solubility of different astaxanthin derivatives present in shrimp in different solvents, including limonene.

In the simulation (Figure 4) all selected molecules are embedded into virtual conductors simulated in the first step by the COSMO model, where the molecule induces a polarization charge density (σ) on its surface (a good local descriptor of the molecular surface polarity).

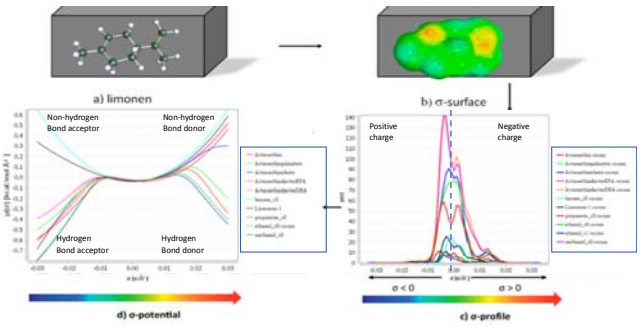

Figure 4. The σ-potential of limonene and of all astaxanthin-based solutes studied (*left*) and the three-dimensional σ-profile surface modelling (*right*).

Table 4 shows the resulting predictions of different astaxanthin-based compounds, such as astaxanthin, astaxanthin palmitate, astaxanthin oleate, astaxanthin diester of EPA, and astaxanthin diester of DHA, in *n*-hexane, limonene, propanone, methanol and ethanol.

Results of the simulations, expressed in $\log_{10}(x_{solub})$, show a much higher theoretical solubility at 25°C of the compounds as the color approaches green. The simulation shows that *d*-limonene is a better solvent than *n*-hexane for astaxanthin derivatives, with $\log_{10}(x$ solubility) values lower each time. Limonene is also a better solvent than ethanol and methanol, except for free astaxanthin. The best solvent for the selected astaxanthin-based compounds is acetone (propanone), but limonene is a good alternative in terms of solubilization power.

Table 4. Predicted solubility of astaxanthin and different mono and diesters in *d*-limonene at 25°C. Values are given as $\log_{10}(x_{solub})$.

Solute	Solvent				
	n-hexane	*d*-limonene	propanone	methanol	ethanol
Astaxanthin	-4,34	-3,08	-0,99	-2,0	-1,64
Astaxanthin palmitate	-2,96	-1,81	-0,99	-2,61	-1,92
Astaxanthin oleate	-1,13	-0,89	-0,85	-3,06	-2,06
Astaxanthin diesterEPA	-7,41	-6,18	-4,70	-9,94	-8,52
Astaxanthin diesterDHA	-7,87	-6,33	-4,09	-9,90	-8,54

Bad solubility **Good solubility**

The fact that ethanol is an excellent extraction solvent for astaxanthin (but not so for the omega-3 diesters) was recently experimentally confirmed by scholars in China who, under the optimal conditions of solid-liquid ratio 1:7, T = 50 °C, and 20 min extraction time, obtained a 50.32 μg/g astaxanthin yield from fresh *Pandalus borealis* shells, eventually obtaining an astaxanthin extract with 0.34% content.[13]

Our computational results are also in agreement with the fact that a considerably higher yield of 72.42 μg/g was obtained by extracting the carotenoid from the shells of deep-water pink shrimp (*Parapenaeus longirostris*) fished in Tunisia with acetone in the dark at 4°C.[14]

Besides exclluding light, the latter process required the use of 20 mg of synthetic (and toxic) antioxidant butylated hydroxytoluene to prevent astaxanthin oxidative degradation.[14]

The new extraction process reported in the present study does not require to exclude light neither commands the use of synthetic antioxidants. Limonene, indeed, is a terpene with antioxidant properties,[15] which intrinsically prevents the oxidative degradation of both the carotenoid as well as of the polyunsaturated omega-3 fatty acids abundant in marine lipids.

The terpene, furthermore, is a powerful antibacterial[15] inhibiting the growth of most pathogenic bacteria causing fish food poisoning.[16]

Analyzing the technical and economic feasibility of fish oil production from anchovy leftovers using *d*-limonene as the only solvent at room temperature, we have lately shown that the process provides clear economic and environmental benefits in face of relatively small capital and operational expenses.[17]

The findings reported in this account establish a new, low cost and simple route to a new marine oil rich in omega-3 and in astaxanthin directly made available from biowaste globally available in over 2.2 million tonnes per year.

The lipid- and carotenoid-free solid residue after the extraction contains plentiful chitin and proteins whose decomposition has been arrested thanks to prolonged contact with antimicrobial limone.[15,16] In a subsequent communication, we will show how to effectively process this solid (95% in weight of the original shrimp waste) separating the proteic fraction from chitin, making both available for further bioeconomy uses.

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