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Oil extraction and derivatization method: a review

Abstract

The objective of this work is to analyze and present the main methods of oil extraction. The present study suggests that different methods could be used to extract oil for food and feed purposes. The method to be used for the extraction depends on several factors, among which its cost and the materials to be used stand out. This work has reviewed well-known and widely practiced methods of oil extraction namely and conventional methods (solvent extraction), as well as new innovative methods aimed at raising and optimizing oil yield and improving oil quality. The main derivatization methods are also reviewed since among edible oils the determination of fatty acids is one of the quality parameters most studied and disseminated in the scientific literature. Major shortcomings associated with the conventional methods are solvent consumption, extraction time lag and adverse thermal effects at high temperatures that can produce oxidative processes of lipids. New techniques such as microwave-assisted extraction, ultrasonic-assisted extraction, and supercritical fluid extraction have been developed, and are being used to effectively reduce these shortcomings. Although, as previously stated, the researchers apply the most feasible, least-cost method that suits their research purposes.

Keywords: solvent, derivatization, parameters, methods, oil

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Introduction

Within marine species, the shark has resurfaced internationally, not only for the value of its fins, but also for the importance of oil extracted from its liver for health.[1] The main constituents and nutrients that have been identified in shark liver oil are alkylglycerols,[2] triglycerides[3,4] free fattyacids,[3] fatty acids ω-3, ω-6 andω-9,[5] vitamin E and A[6] and squalene.[7] Shark liver oil is promoted as a dietary supplement used to boost the immune system, fight infection, and heal wounds.

In recent decades, scientists have conducted studies on the positive effects of nutrients available to different marine organisms on health, which may be the key to new treatments for diseases such as cancer[8,9] neurodegenerative diseases,[10] human immunodeficiency virus,[11] or cardiovascular disease.[12,13] In addition, it provides other valuable components such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), both ω-3, important for optimal neurological development in children,[14,15] diabetes[16] and epilepsy.[17] The recent use of shark liver oil in the development of swine flu vaccines and in certain cosmetic soaps and lotions addstoits invaluable use.

The world production of fish oils reaches millions of tons, of which 88.5% is destined for aquaculture, and remains are destined to industrial production, human consumption and development of pharmaceutical products and dietary supplements.[18,19] According Garcia, 2005[20] the low levels of use of fish oil in human nutrition are mainly due to their characteristic odor and taste, as well as the high degree of un saturation of their fatty acids that make them susceptible to oxidation, which has required the application of technological processes that improve their organoleptic properties and guarantee greater stability. These difficulties, together with the need to supplement human nutrition with oils of marine origin, have motivated the food industry to apply procedures that increase the nutritional intake of these fatty acids through the development of functional foods.

The deposit of fat in most cartilaginous fish is located mainly in the liver, some species such as shark and cod accumulate a considerable amount of oil in this organ.[21] According to the literature it has been reported that the size and weight of the liver in sharks varies depending on the species and time of year and that in some sharks the liver can be up to 20% of the total weight of the animal.[22,23] There are different methods for extracting oils from marine species that can be classified into three categories: physical, biological and chemical.[24–27] Studies carried out express that the application of different extraction methods causes variation in the chemical properties of oils of marine origin.[28,29]

The physical methods of extraction are some of the most used; these include cooking/heating or processing, pressing, drying and grinding. The cooking step is designed to break the fat cells to release oil and press or centrifuge to separate the liquids from the dough.[30–33] The cooking coagulates the protein, breaks down the fat deposits and releases oil and physicochemical bound water. The cooking step also prepares materials for subsequent operations in several processing units. The coagulation of fish protein occurs at approximately 75 °C, but cooking is typically in the temperature range of 95 °C to 100 °C for the duration of (15 to 20) minutes.[32] Direct and indirect cookers are also used.[34] The extraction by heat is the most used;[35] this physical agent favors the release of the oil from the liver cells, at the same time that it inactivates the lipolytic enzymes that cause the hydrolysis of the lipids (reaction that favors the oxidation of the fatty acids).[36] During the heating process, oxidative processes of the lipids can also be produced; the nature of these alterations depends on factors such as the temperature, the heating time, the degree of unsaturation of the oil, among others,[37] therefore, the objective of this article is to summarize the sample preparation and storage, and the different shark liver oil extraction techniques, based on the basis of the method, and a flash review of the main methods of derivatization of fatty acids is carried.

Preparation and storage

When sharks are captured, they must be gutted, washed to avoid microbial contamination, placed in polyethylene bags, marked with the date of capture and provenance, then kept in an ice bath at a temperature between 1-5 °C until fishing boats arrive at the port,[20] then quickly freeze and store at a temperature below -18 ^{0}C until use.[38–40]

As soon as possible, the livers should be homogenized and removed at the lowest possible temperature, it is recommended not to exceed 20 ^{0}C.[39] In this circumstance, the main danger is a loss of components of unsaturated fatty acids through the autoxidation of these. Although salt allows for prolonged storage, contact with marine products has been reported to improve oxidation of highly unsaturated lipids directly related to the production of unpleasant tastes and odors, protein denaturation, and texture changes. Therefore, it is clear that the salty dry step involved in the preparation and storage of marine samples could result in oxidized lipids.

All tissues, regardless of their origin, should ideally be removed immediately after removal from the living organism, so that there is little opportunity for changes in lipid components. Of course, it is essential that plasma or tissue samples are taken with the minimum of stress or trauma; otherwise lipolysis will occur in *vivo*. Lipid peroxidation can also be troublesome in tissues stored at −20 ºC and even at −70 ºC, and it has been recommended that samples for free radical assays be stored at −196 ºC.[41]

Enzymatic oxidation has also been shown to cause losses not only of unsaturated fatty acids, but also of intact lipids.[42] The hydroperoxide groups of the oxidized lipids apparently reacted to form covalent bonds with the membrane proteins, from which they were released only in treatment with bacterial proteases. The different characteristics of the various fatty materials have led to extraction processes as varied as processing, pressing and solvent extraction. However, all extraction processes have certain objects in common. These are, first, to obtain the oil unscathed and as free as possible of undesirable impurities; second, obtain the oil in a performance as high as is consistent with the economy, to produce oil or a residue of the highest possible value.

Extraction methods

Recent technological advances and the development of new methods to improve production and separation have revolutionized the selection of biomolecules and provided the opportunity to obtain natural extracts that could potentially be used in the manufacture of nutraceutical products. The sin oil extraction processes can be classified into three categories: physical, chemical and biological, but for shark liver oil the most used methods are shown in Table 1.

Physical extraction methods

The different temperatures of extraction are presented in the Table 2.

Chemical extraction methods

Solvent extraction involved different methods that discussed in the Table 3.

Table 1 Most used extraction methods for the analysis of shark liver oil

Classification	Extraction methods	Brief introduction	References
Physicists	By temperature	The basic processing steps involved in shark liver oil extraction include grinding, cooking / heating or processing, and pressing or centrifuging. The cooking step is designed to break the fat cells to release oils and press or centrifuge to separate the liquids (water, soluble protein, and oil) from the dough (solid cake). Cooking coagulates protein, breaks down fat deposits, and releases oil and physicochemical bound water.	43
Chemicals	Folch et al.; Bligh & Dyer; Mc Gill & Moffatt.	Solvent methods in general are an adaptation (in terms of solvent and solvent volumes) of the procedures of Folch et al., 1957 and procedures for optimal lipid extraction as reported in the literature.	
	Using soxhlet	The principle is based on solid-liquid extraction (leaching) and has been the standard method for more than a century. Nonpolar solvents such as n-hexane, ethyl acetate, or petroleum ether are used for lipid extraction. Extraction is by repeated washing or percolation of fresh organic solvent at reflux from a distillation flask. The extraction efficiency for different classes of compounds depends largely on the properties of the organic solvent.	43
	Enzyme-assisted extraction	The basic principle of enzyme-assisted extraction is that enzymes hydrolyze the cell and completely break it down under optimal experimental conditions, to release the intracellular components. Enzymes act on the cell by binding to its active site; this causes the enzyme to change its shape to fit into the active site of the substrate, causing maximum interaction between the enzyme and the substrate. The change in the form of the enzyme leads to the breakdown of the cell wall bonds, thus releasing the active constituents of the cell wall. The extraction efficiency depends on the temperature of the system, the mode of action of the enzyme, the duration of the extraction, the enzyme load, and the availability of the substrate and the pH of the system.	44 45

Table 2 Temperatures of extraction of shark liver oils discussed by different authors

Species (s)	TAL (^{0}C)	TE (^{0}C)	Te (min)	Observations	Reference
Centrophorus squamosus, Centroscymnus coelolepis, Centroscyllium fabricii, Centroscymnus crepidater and Deania calcea	-30	70	-	They do not analyze the quality or the total oil extracted, but they refer to the percentage of extraction of Centroscymnuscoelolepis and Centrophorussquamosus which is 77.6% and 77.2% respectively.	1
Ginglimostoma cirratun, Carcharhinus longimanus and Carcharhinus falciformes	-18	70	10	42.0 ± 3.8% extraction yield Quality within standard values	46

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Table Continued...

Species (s)	TAL (^{0}C)	TE (^{0}C)	Te (min)	Observations	Reference
Squalus acanthias	-60	70-80	30	22 ± 2% extraction yield, worse than Bligh and Dyer and Soxhlet methods. Better quality of extraction at 5 ^{0}C and excluded from light for up to 31 days.	47
Carcharhinus falciformes	-	95	30	Yield of 41.7 ± 0.8%, although the Bligh and Dyer method presents 63.3 ± 1.3%. Quality within standard values.	48

Table 3 Solvent extraction methods

Method	Species (s)	Solvents	Storage temp. (^{0}C)	Reference
Folch et al	Echinorhinus brucus	Chloroform and methanol (2: 1)	-20	40
Bligh and Dyer	Carcharhinus falciformes	Chloroform, methanol and water	-	48
	19 species from Northern Australia	Chloroform, methanol and water (2:1:0,8)	-20	22
	Carcharhinus falci form is, Carcharhinus longimanus, Alopias superciliosus, Prionace glauca, Sphyrna lewini and Lamna ditropis.	Chloroform, methanol, and water (2: 1: 1.8)	-20	33
	Lamna ditropis	Chloroform, methanol, and water (2: 1: 1.8)	-40	21
	Squalus acanthias	Chloroform, methanol, and water (2: 4: 1)	-60	47
	Galeorhinus galeus and Mustelus antarcticus	Chloroform, methanol, and water (1: 2: 0.8)	-80	38
Bligh and Dyer and Mc Gill & Moffatt	Carcharhinus falci form is and Galeocerdocuvier	-	-20	39
	Carcharhinus plumbeus, Hexanchus Griseus and Squalus acanthias	Ether	-80	49
Soxhlet extraction	Squalus acanthias	Petroleum ether	-60	47

Bligh and Dyer method

This method uses chloroform and methanol (1: 2 v/v), which ensures that for practical purposes the total extraction of lipids from the muscle and that it is reproducible within with a 2% standard deviation.[50] This method has been applied and has also been modified by various researchers[51–53] and it has advantages such as that it is a simple method where the total extraction of lipids is achieved and it is also direct; however, the toxicity of the reagents used means that it is often replaced by other methods.[34]

Castanha et al.,[54] it achieves a total extraction of the lipids of *Cryptococcus laurentii*, with a high content of fatty acids with 16 and 18 carbon atoms, predominantly oleic, stearic, palmitic, linoleic and lignoceric acids. Aryee & Simpson,[55] also makes a comparison of different methods of oil extraction from salmon skin, and shows that by this method and by Folch et al.,[56] the yields are low while the Soxhlet technique with adequate extraction times offers high oil values, however, too long times do not offer better results. Another of the methods used in the comparison was that of Soxtec, which offers a significant reduction in the extraction time and in the volumes of solvents used, obtaining comparable oil contents with the Soxhlet method, it is also shown that approximately 85 are recovered by this % of solvent used. This method has been widely used[57]and modifications have been made to improve its performance and extractive processes.[51] Its use has also been proposed for the solid-liquid extraction of lipids extracted from microorganisms. Meullemiestre et al.,[58] and Breil et al.,[59,60] studying its solvation mechanism, substitution with alternative solvents.

Folch method

Folch et al.,[56] was one of the first to develop the chloroform/methanol/water phase system (the so-called "Folch" method); which, under various modifications, continues to be considered the classic and most reliable means of quantitative control for lipid extraction.[61] According Folch et al.,[56] the only advantage of using the Bligh and Dyer method[50] is the reduction in the solvent/sample ratio; 1 part tissue to 3 parts in the chloroform/methanol method instead of 1 part tissue to 20 parts chloroform/methanol. This procedure has been widely used[62,63]; Bell et al.,[64] extractedlipids from Atlantics almon (*Salmo salar*) using the procedure described by Folch,[56] like wise Gigliotti et al.,[65] reported on lipid extraction from the Atlantic Krill (*Euphausia superba*), Ramanathan and Das,[66] also extracted lipids from *Scomberomorus commerson* using the method described to study the oxidation of lipids from ground fish in the presence of

antioxidants. This method has also been used for the extraction of lipids from Atlantic mackerel (*Scombers combrus*),[67] in the shark species *Eusphyra blochii* and *Carcharhinus bleekeri*.[68]

Radin's method

Due to problems of toxicity by chloroform and removal of more aggressive solvents with health,[69] proposed a relatively safer organic solvent system, using a hexane-isopropanol solvent system and extracting both the simple lipid classes and the more complex polar lipids bound to cell constituents and membrane proteins. Aryee & Simpson,[55] performed a comparison between various salmon oil extraction processes, in which the Radin method produced approximately 32% salmon oil based on dry weight compared to 35% of the Bligh and Dyer method[50] and 43% of the Folch method.[56] The low performance of this method has been attributed to the low polarity of isopropanol compared to methanol and the limited efficiency of hexane in the extraction of polar lipids compared to chloroform.[70,71] According Christie,[72] extraction solvents or solvent mixtures must be sufficiently polar to eliminate lipid association with other cellular constituents, but must not be very polar so that the solvents do not dissolve all triacylglycerol and other non-polar lipids.

Soxhlet extraction

This is a simple procedure, which does not require intensive work and also does not use chlorinated solvents, but it has disadvantages since the lipids are not fully extracted, large volumes of solvents are required, special equipment is required and they consume long periods of time.[34] This method is generally automated in which the solid-liquid extraction is carried out for 90 minutes using apolar solvents such as diethyl ether, hexane, and ethyl acetate, to then carry out a recovery phase for 15 min and evaporation/drying by another 15 minutes.[73] However, the relative polarity of chloroform/methanol (2: 1 v/v)[56] it is higher than that of diethyl ether. Polyunsaturated fatty acids have more polarity, when they have two or more insaturations in their structure, considering that they have a linear form.[74] Therefore, the Folch method[56] is more effective than Soxhlet's method for extracting polyunsaturated fatty acids.[73]

A comparison between this method and that of Bligh & Dyer[50] to study the contents of fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fats of marine origin, it was carried out by Ozogul et al.[28] n-heptane and isooctane are used as solvents and you obtain that generally, the differences in the number and volume of fatty acids were much higher in the Soxhlet method with n-heptane and isooctane than Bligh and Dyer method,[50] although they also observed that when isooctane was used, fatty acid levels decreased, compared to n-heptane, which could be due to heating during the transmethylation procedure, so the use of n-heptane shows to be superior for the recovery of unsaturated fatty acids, especially EPA and DHA. This method has also been integrated into microwave extraction,[75] by means of the use of experimental designs, the central compound was optimized obtaining that at 32 min the oil obtained was quantitatively and qualitatively (composition of fatty acids) similar to that obtained by conventional Soxhlet for 8 h; furthermore, this is a green technology and appears as a good alternative for the extraction of fats and oils from food products; other authors have also reported the use of this technique.[76,77]

A suitable solvent extraction method can be selected based on a number of criteria.[78] For example, in a comparative study,[79] found that the Soxhlet method with methylene chloride: methanol (2: 1) is the most selective method for the extraction of neutral lipids, among others. However, the Soxhlet method is not suitable for the extraction of unsaturated lipids due to the instability of these lipids at elevated temperatures during reflux. High temperatures can also accelerate transesterification of lipids in the presence of methanol, changing their natural shape. In this sense, the Bligh and Dyer method[50] with chloroform and methanol it causes less artifacts, therefore; the results obtained with this method are considerably more reliable and reproducible.[79] However, the Bligh and Dyer method.[50] It is practically not suitable for large-scale extractions due to the high amounts of toxic waste generated, which requires expensive recycling methods and compromises user safety.[80]

Association of official analytical chemists (AOAC) method

The conventional AOAC method[81] uses acid digestion to degrade proteins that interact with fatty acids before extraction with petroleum ether/diethyl ether (1: 1 v/v). Taha et al.,[82] performs a comparison of lipid extraction methods for the determination of fatty acids. Gas chromatographic analysis revealed that total fatty acid recoveries were not statistically different in the methods used (P> 0.05), in this study. This method has also been compared with that of Soxhlet for the determination of fats in bakery products. Shin and Park,[83] obtaining that the volume of (total) fat extracted by the AOAC 996.06 method was less extracted by the automated Soxhlet and Folch methods[56] for most samples. In addition, the volumes of saturated fat, monounsaturated fat, and polyunsaturated fat determined by the AOAC 996.06 method. AOAC,[81] it was also lower than those obtained by Folch[56] and automated Soxhlet.

Solvent accelerated extraction

Solvent Accelerated Extraction (EAS) does not require the manual steps involved in preparing samples for analysis, this technique presents increased reproducibility and significant time savings. This procedure was developed for the extraction of aromatic hydrocarbons (PAHs) such as polychlorinated biphenyls (PCBs) and polycyclic[34,84] It has been used in the isolation of lipids from plants and animal tissue.[85] EAS provides a flow per system that increases productivity while cost is decreasing, while maintaining automation platform. Although it has the aforementioned advantages, this method does not achieve total lipid extraction, solvent mixtures are required as well as high temperatures and pressures in the system, in addition to the fact that the sample must be dry.[34,86] EAS has higher extraction power in routine lipid/fatty acid analysis in biological samples.[84,87]

Microwave assisted extraction

This method uses microwave radiation that causes the movement of the polar molecules and rotation of the dipoles to heat the solvents in contact with the solid matrix and extract the oil volumes,[25] in recent years it has been widely developed due to a series of factors such as the reduction of extraction time and the consumption of solvents, it can be automated,[34] a greater penetration of the solvent in the cellular tissues that causes a higher yield, higher extraction rates at low temperatures,[88,89] in addition to offering the possibility of simultaneously drawing different types of samples.[88] However, it has disadvantages such as high energy consumption, heating affects only polar solvents and/or materials, difficult technological scaling, as a result of heat generation, the oxidation of unsaturated fatty acids can be caused and the use of volatile solvents causes low efficacy.[5,90-93] By means of this extraction, high oleic acid values are obtained, according to a comparison of different lipid extraction methods in *Botalo coccus* sp.[94]

Extractionby supercritical fluids (SCFE)

Supercritical fluids extraction has received increasing attention as an alternative to conventional extraction methods.[95] Not only is there a significant reduction in the use of organic solvents, but waste concerns are also avoided. Although the time reduction is not as great as in some microwave procedures, SCFE has gained much wider acceptance. Potentially causes fewer adverse conditions for fatty acids due to the low temperature of the extracting fluid, usually supercritical carbon dioxide (S-CO_2).Extractions using only S-CO_2 generally produce good recoveries of nonpolar lipids.[96] However, polar lipids may remain unextracted due to their lower solubility in S-CO_2, and therefore samples containing a certain amount of these types of lipids (eg milk) may present extraction difficulties. To improve the extraction of non-polar lipids, the polarity of S-CO_2 can be varied using solvents such as methanol, ethanol or even water. Several researchers have reported that the solubility of lipids in S-CO_2 is greatly increased by adding ethanol, and some phospholipids are extracted at levels directly proportional to the added ethanol.[6,97]

The presence of dissolved water in the supercritical the fluid also increases the solubility of polar compounds, and has been used successfully to analyze various dairy products.[98] Sample preparation should also be considered. Particle size affects lipid recovery by influencing the surface area of the exposed sample to S-CO_2.[85] The moisture content of the samples also affects the extraction efficiency by conditioning the surface structure.[99] The high moisture content reduces the contact of the S-CO_2 sample due to the pasty consistency of the samples, and the moisture acts as a barrier to the diffusion of S-CO_2 in the sample, as well as the diffusion of lipids out of the sample.[100] In this way, an increase in lipid recovery with lower moisture content has been demonstrated in wet samples such as meat[101] and fish,[102] although humidity does not affect the extraction capacity in a low content.[102,103] Therefore, samples with a high moisture content are usually lyophilized prior to S-CO_2 extraction to improve efficiency.[101–104]

This method offers advantages such as being fast, does not require the use of toxic solvents and the extractshave a high purity, free of heavy metals and inorganic salts, there is also no presence of polar substances that can form polymers, and low temperatures of extraction (40-80 ° C). However, the equipment is expensive, and complex since it has to work at high pressures, high purity CO_2 must be used and it needs large amounts of energy for its use.[25,34,97,105]

Ultrasonic extraction

This method uses ultrasound to penetrate the solvents in contact with the solid matrix to extract the contents of the sample in solution. It presents advances with respect to other techniques such as the shorter extraction time and solvent consumption, high penetration of the solvent into the cellular material, which causes it to release its content into the medium, although these represent clear advances, its difficult scalding at an industrial level and the high energy consumption make this technique still little used.Ultrasonic assisted solvent extraction has also been used for accelerated extraction of phenolic compounds from plant matrices. This process is considered effective compared to other conventional extraction methods, as it allows cell disruption to allow interactions of phenolic molecules with solvents at a reasonably low temperature.[89]

UAE significantly reduces extraction time and increases extraction yields of many natural matrices, due to the production of cavitation bubbles in the solvent.[106] Cavitation bubbles are produced in the liquid during the expansion phase. The negative pressure exerted by the expansion cycle exceeds the local tensile strength of the liquid.[107,108] This ability to cause cavitation depends on the characteristics of the ultrasound wave, the properties of the solvent, and the environmental conditions.[108,109] After a cavitation bubble is produced, it collapses during the compression cycle, which pushes the liquid molecules together, and a high-speed micro-jet is created toward the matrix particle, promoting mixing of the solvent with the matrix. The high pressure and temperature involved in this process, which can reach up to 1000 bar and up to 4726.85 ° C, respectively, are responsible for the increase in mass transfer, since the shock wave breaks cell walls and membranes.[108,109] After cell disruption, the solvent can easily penetrate the solid particle, releasing the intracellular compounds to the bulk solvent.[109–112]

The application of ultrasound can be divided into two different categories: low intensity-high frequency ultrasound (100 kHz - 1 MHz) and high intensity-low frequency (between 20 and 100 kHz), the latter being the only case that leads to disruption of cell walls and membranes[109–114] More recent studies have shown that ultrasonic assisted extraction using acoustic cavitation and mechanical impact can improve extraction efficiency. Acoustic cavitation can break the cell wall facilitating the penetration of the solvent into the plant material and allowing the cell to release the product. The ultrasonic mechanical impact offers a greater penetration of solvents into the sample matrix because it increases the contact area between the solvent and the extractable compounds. UAE requires less extraction time and less solvent consumption and can be carried out at low temperatures, which can reduce damage caused by temperature and minimize the loss of bioactive substances.[25,115]

Abdullah et al.,[116] they used UAE in ethanol to extract the oil from the *Monopterusalbus* fillets. Before extraction, the material had to be dried (60 °C) and homogenized in a blender. The optimal extraction parameters are 25 kHz, 200 W, 25 kHz, 200 W, 60 min of sonication time and 500 ml of ethanol. Final production - 7.2% of dry fillet material. In another job, Xiao et al.,[117] extracted 94.82% of the total lipids using cyclohexane medium, optimal extraction parameters liquid/solid ratio of 4:1 to 50 ℃ in 57 min and 400 W of extraction power. Khoei & Chekin,[118] performed a comparison of this method with the Soxhlet extraction and the results showed that the oil performance in the aqueous extraction assisted by ultrasound was close to the performance of the oil extracted with hexane by Soxhlet, which implies that the performance was significantly influenced by ultrasound . Regarding quality, the oil extracted by an ultrasound-assisted aqueous process had a lower content of free fatty acids and pigments than that extracted in hexane.

Other extraction methods

Gigliotti et al.,[65] proposes an extraction method that uses a solvent system, acetone-ethanol (1: 1, v/v), which is compared to the Folch method,[56] Soxhlet,[119] and conventional two-stage extraction,[120,121] in *Euphausiasuperba*. This study demonstrated that the proposed method produced the highest yield efficiency, with phospholipids being the main compounds in the oil obtained, while this also contains ω-3 such as EPA and DHA. In addition, the extracted oil had low cholesterol contents and a high antioxidant capacity compared to other treatments.

Ghaly & Ramakrishnan,[122] an enzymatic extraction method was used in which the enzyme alcalase was used at three concentrations

of the enzyme (0.5, 1 and 2%) and four time intervals (1, 2, 3 and 4 h). The oil obtained after enzymatic hydrolysis was dark due to the formation of pigments resulting from the reaction of carbonyls of the oxidation of polyunsaturated AGs with amino acids and proteins. The highest oil yield (76.26% of the head and 75.71% of the whole fish) was obtained using 2.0% concentration of the enzyme after 4 h of hydrolysis. In summary, these methods have advantages such as the non-use of organic solvents and the low cost of protease enzymes, as well as the disadvantage of being difficult to scale technologically.[25]

Main derivatization method used

HCl-Methanol: This method is described by Antolín et al.,[123] which makes a comparison between different derivatization methods, this being the most time consuming, around 90 minutes to complete the process, while the other methods only require 10 minutes. This method has been used by several authors.[124]

Martins et al.,[124] makes a comparison between this method and the one that uses Bf3/MeOH, to later determine the fatty acids by GC in three species of Brazilian algae. When comparing the fatty acid contents in the species; when the transesterification was performed by the B&D-BF_3 method, S. *cymosum* and H. *musciformis* showed the highest and lowest fatty acid contents, respectively. S. *cymosum* also showed higher concentrations of palmitoleic, oleic and arachidonic acid methyl esters when using the B&D-Bf3 method. However, when the AOM-HCl method was used, H. *musciformis* showed higher concentrations of palmitic, myristic and arachidonic methyl esters than the other species and significant differences between palmitoleic and oleic acid species. The fatty acid contents of the three species of seaweed were significantly different when they were extracted and transesterified by the different methods. Furthermore, the best method for one species was not the same for the others, which points to a matrix effect and that the method used for the analysis of the fatty acid content of different organisms must be carefully selected. Tang et al.,[125] performs an optimization of the method for the determination of fatty acids in *Nannochloro psisgaditana*. Total yields of methylated fatty acids and EPA were 1.58 and 1.23 times higher separately than those obtained by the conventional two-step method (solvent system: methanol and chloroform). This one-step, on-site method is quick and simple for measuring fatty acid methyl ester yields and could serve as a promising method for generating EPA methyl esters from microalgae. This method has also been used by Meier et al.,[126] in which a one-step extraction/methylation procedure is validated.

H_2SO_4 – Methanol: According Antolín et al.,[123] the cost, speed, safety and response by gas chromatographic, of the method that uses sulfuric acid-methanol (H_2SO_4-MeOH) as a deriving agent turned out to be the most appropriate to determine fatty acids. Validation of this method demonstrated: linearity in a range 40–160%, accuracy was assessed through a recovery study, day-to-day and day-to-day precision, and specificity. Gu et al.,[127] although it does not use the method described by Christie,[128] in which the methylation and hydrolysis of lipids occurs in a single stage, by adding 700 µL of methanol with 2.5% H_2SO_4 and homogenizing it for 1 min, and then incubating it at 80 ° C for 90 min and practicing several extractions successive with n-hexane. The procedure has also been described by Figueiredo et al.[129]

BF_3 – Methanol: This method has been described by different authors. Antolín et al.,[123] Moss et al.,[130] and Ackman,[131] performs a review of the official methods that BF_3 is used for the preparation of fatty acid methyl esters. Zhang et al.,[132] makes a comparison between the conventional method that uses KOH - MeOH according to ISO 12966-2,[133] with some modifications and the method using BF_3–MeOH,[134] also with some modifications; in vegetable oil matrices. Lewis acid BF_3 forms a coordination complex with methanol, a powerful catalyst for the esterification of fatty acids. But the result of this study showed that the level of fatty acid esterification was very low, the chromatographic peaks were not identified and the composition of the isomers also changed when the BF_3/MeOH method was carried out. It should be noted that the reaction temperature is usually 100 ° C (the boiling point of MeOH was 60 °C), so high temperatures can cause solvent loss and as a consequence the reaction cannot be completed, in addition to there may be transfer of double bonds. Methoxides were produced from the unsaturated fatty acids by the addition of methanol to the double bond even when normal concentrations of BF_3/MeOH were used (not more than 50%), so this is not a reliable catalyst resulting in many unwanted products.[135] Side reactions have also been reported to be increased by the presence of oxidized lipids, and sample size is also critical with substantial losses sometimes occurring with samples less than 200 mg.[136] The risk of side reactions increases with longer reaction times, so BF_3/MeOH has serious disadvantages, if it is also considered that this reagent is expensive and not stable, although it is refrigerated compared to KOH which is stable during several months at low temperatures.[123,132] Compared to the BF_3/MeOH method, with the KOH/MeOH method the latter is more effective, simpler, less laborious, and easier to handle.[132] According to Martins et al.,[124] this method produces false results due to the high matrix effect it presents.

NaOH-Methanol: Guil-Guerrero et al.,[137] used a method in which the fish oil samples were mixed with a solution of methanol and acetyl chloride (20: 1, v/v) and 20 mL of hexane, previously described by Rodríguez-Ruiz et al.[138] An alternative to this method is a mixture of NaOH-methanol and hexane, for the preparation of the methyl esters, however the BF_3/MeOHreagent is used as a catalyst,[139] although this procedure has been used by several authors.[140,141] Figueiredo et al.[129] proposes and compares a new method of transesterification with conventional methods and widely described in the literature.[142] In this, 2 mL of sodium hydroxide (NaOH) (1.5 mol. L^{-1} in methanol) are added to the samples, to then be placed in an ultrasonic bath. After the alkaline reaction described above, 2 mL of H_2SO_4 or HCl (1.5 mol. L^{-1} in methanol for both cases), to be placed again in the ultrasonic bath for a time simultaneously studied by means of an experimental design. I know that H_2SO_4 was more effective than HCl.[129] Guil-Guerrero et al.,[143] using this method for the simultaneous extraction and saponification of *Isurus oxyrinchus* liver oil, this method was optimized by means of an experimental design.

Other methods: These procedures, although less described in the literature, have been developed and discussed in important investigations. Xu et al.,[144] compares three methods that use methanol/benzene (4: l, v/v) and then 200µL of acetyl chloride are added to proceed with the transesterification for 1h at 100 ° C. One-stage digestion, extract and sample esterification is known as 'direct transesterification' and is widely used due to its simplicity, speed and high accuracy.[145–147] This method presents some complications, such as: the addition of acetyl chloride generates an exothermic reaction that sometimes results in sample loss and possible injury to the analyst; adding acetyl chloride slowly and with stirring is difficult for large numbers of samples; in addition, certain polyunsaturated fatty acids are not very stable at 100°C during the transesterification process; and the generation of molecular species that can contribute to fatty acids degradation.[148] This method has also been used by Guil-Guerrero et al.,[143] and Masoodet al.[147]

Tang et al.,[125] optimizes a transesterification process in situ for the quantification of EPA in *Nannochloropsis gaditana*. This method was previously described by Laurens et al.,[149] and it provides advantages over the conventional two-stage method, such as speed, which is simple and reliable. Laffargue et al. [150] describes a method described in the literature and which has been widely described in studies of plant physiology[151,152] in which the fatty acids are separated by thin layer chromatography. The researchers use an alternative method consisting of the selective derivatization of fatty acids without their previous purification, which uses diazomethane that is ineffective for trans-methylation of fatty acids. Diazomethane is potentially explosive, it is only stable for very short periods and has to be prepared on site, in addition to being carcinogenic.[153] A method using diethoxymethane-assisted methanol is described by Zeng et al.,[154] which offers advantages such as shorter reaction time, and also requires small amounts of the catalyst and methanol. Another versatile technique for the derivatization of fatty acids is ultrasound irradiation, which has been compared to classical methylation.[135]

Conclusions

According to the estimates of the present study it is suggested that different methods could be used to extract oil for food and feed purposes. The method to be used for the extraction depends on several factors, among which its cost and the materials to be used stand out. This work has reviewed well-known and widely practiced methods of oil extraction namely and conventional methods (solvent extraction), as well as new innovative methods aimed at raising and optimizing oil yield and improving oil quality. The main derivatization methods are also reviewed since among edible oils the determination of fatty acids is one of the quality parameters most studied and disseminated in the scientific literature. Major shortcomings associated with the conventional methods are solvent consumption, extraction time lag and adverse thermal effects at high temperatures that can produce oxidative processes of lipids. New techniques such as microwave-assisted extraction, ultrasonic-assisted extraction, and supercritical fluid extraction have been developed, and are being used to effectively reduce these shortcomings. The most suitable process based on cost, environmental friendliness, and oil yieldnot is specified because depend of factors such as the conditions of the laboratory, the level of development of the industry and the objective of the research. Although, as previously stated, the researchers apply the most feasible, least-cost method that suits their research purposes.

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Conflicts of interest

Authors declare that there are no conflicts of interest.

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