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Extracção verde de composto marinhos bioactivos para aplicação industrial

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GREEN EXTRACTION OF MARINE BIOACTIVE COMPOUNDS FOR INDUSTRIAL APPLICATION

by

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Abstract

A comparative life cycle assessment was performed to investigate four different scenarios for lipid extraction.

The extraction technologies studied were ultrasound-assisted extraction (UAE) and microwave assisted extraction (MAE), supercritical fluid extraction (SFE) with CO_2 as solvent and subcritical water extraction (SWE). Target lipids were ω3 polyunsaturated fatty acids, such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids.

SFE was the most energy consuming process, while MAE and SWE were the methods with the lowest environmental impact. To produce 1 kg of lipids the UAE process would need more than a ton of microalgae, which means that this process in terms of efficiency is the worst and consequently not indicated to extract lipid with the conditions used.

The life cycle assessment presented here doesn't include the cultivating and harvesting phases. Further optimizations including these steps would be required in order to reduce the environmental impact of the whole process. In this sense, the results obtained may indicate the direction to achieve this goal.

Keywords: ω3 polyunsaturated fatty acids, LCA, lipids, SFE, MAE, UAE, SWE

Resumo

Uma avaliação comparativa do ciclo de vida foi realizada para comparar quatro métodos diferentes de extração de lípidos.

As tecnologias de extração estudadas foram extração assistida por ultrassons (UAE), extração assistida por micro-ondas (MAE), extração por fluidos supercríticos (SFE) usando o CO_2 como solvente, e extração com água subcrítica (SWE). Os lípidos alvo foram os ácidos gordos polinsaturados do tipo ω3, mais concretamente os ácidos eicosapentaenóico (EPA) e docosahexaenóico (DHA).

SFE foi o processo de maior consumo de energia, enquanto MAE e SWE foram os métodos com o menor impacto ambiental. Para produzir 1 kg de lipídios, o método UAE necessita de mais de uma tonelada de microalgas, o que significa que este processo em termos de eficiência é o pior, e consequentemente o método menos indicado para extrair lípidos nas condições utilizadas.

A avaliação do ciclo de vida aqui apresentada não engloba as fases de cultivo e colheita das microalgas. De modo a reduzir o impacto ambiental da totalidade do processo seriam necessárias otimizações adicionais, incluindo estas fases. Nesse sentido, os resultados obtidos podem indicar a direção para atingir esse objetivo.

Palavras-chave: ácidos gordos polinsaturados ω3, avaliação de ciclo de vida, lípidos, SFE, MAE, UAE, SWE

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List of abbreviations and symbols used

B&D - Bligh and Dyer

CxMeOH - CO2-expanded methanol

CH_3OH - Methanol

$CHCL_3$ - Chloroform

C_6H_{14} - Hexane

C_2H_6O - Ethanol

DHA - Docasahexaenoic acid

EPA - Eicosapentaenoic acid

FAME - Fatty acid methyl esters

LCA - Life cycle assessment

LCO_2 - Liquid Carbon Dioxide

LC-PUFA - Long chain polyunsaturated fatty acids

MAE - Microwave assisted extraction

PUFA - Polyunsaturated fatty acid

$ScCO_2$ - Supercritical carbon dioxide extraction

SEE - Steam Enhanced Extraction

SF - Supercritical fluid

SFE - Supercritical fluid extraction

SHWE - Superheated water extraction

SWE – Subcritical water extraction

UAE – Ultrasound assisted extraction

Wt – percentage by weight

1. Introduction

In a changing world, with an enormous impact of human presence, the need for sustainability of all our activities has accelerated the research activity on greening all the techniques to analyze any kind of sample.

In 1998, Paul Anastas and John Warner contributed with a definition for the term *green chemistry*: *"Green Chemistry is the utilization of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture and applications of chemical products"* [1]. Green chemistry applies across the life cycle of chemical product since the design and manufacture, till the ultimate disposal. It supports the invention of more environmentally friendly chemical processes and products, which reduce or eliminate the use or generation of hazardous substances.

Regarding the manufacture, some new techniques are appearing to substitute the older ones. Green extraction techniques are one of such examples, as they improve the sensitivity and the selectivity of analytical methods in alternative to classical sample-preparation procedures used in the past [2].

One of the applications of these techniques, that brings enormous advantages, are in the extraction of lipids from marine microorganisms and animals, like microalgae and fish. The major difference from conventional processes resides in the use of alternative solvents and renewable natural products that reduce energy consumption and endure the high quality of the extract. Furthermore, green extraction processes may also use physical methods to enhance extraction, thus decreasing the necessity to employ large volumes of solvents.

1.1. Lipids

Lipids are a broad class of biomolecules, commonly known as oils and fats, which include a wide variety of compounds used in many different biological processes like store energy and contribute to cell structure. They are hydrophobic molecules soluble in organic solvents made of carbon, hydrogen and oxygen.

The fatty acids, which are the main constituents of both neutral and polar lipid molecules, are from saturated and unsaturated types [3]. Neutral lipids include triglycerides, pigments, and trace amounts of hydrocarbons, while polar lipids include phospholipids, phosphatidylcholine, sterols, as well as prenyl derivatives [4].

Lipids occur throughout the living world, and can be extracted from microorganisms, plants and animals. They have a large camp of applications like in the cosmetic and food industries.

1.2. Importance of lipids

Humans can synthesize saturated and monounsaturated fatty acids but cannot synthesize some polyunsaturated omega-3 and omega-6 fatty acids, which are indispensable nutrients for the human body and need to be supplemented in diet [5]. This is because humans like other animals, lack the desaturase enzymes essential to produce the simplest members of these families [6].

In general, lipids are strongly associated with approximately all the cells of the nervous system, and therefore play a significant role in every coordinated movement we make(ref). The membrane of every single cell in our body is primarily composed of lipids. They are a rich source of vitamins A, D, E and K and necessary for maintaining a proper functioning of the nervous system.

Biopharmaceutical, nutraceutical and food sectors are experiencing an increasing market interest in functional lipids like omega-3 fatty acids. Microalgae, fish and processing by-products represent the major source of lipids rich in omega-3 [7].

1.2.1. Omega-3 fatty acids

Omega-3 fatty acids are a specific group of long-chain polyunsaturated fatty acids (PUFA) commonly having 18, 20, or 22 carbon atoms in chain length with the first double bond of the 3-6 located between the third and fourth carbon atom counting from the methyl carbon end of the fatty acid [8].

The three types of ω3 fatty acids who play a significant role in human body are alpha-linolenic acid (found in plants), eicosapentaenoic acid (20:5, ω3 EPA) and docosahexaenoic acid (22:6, ω3 DHA), both found in aquatic environments, especially oily fish and microalgae. Alpha linolenic acid is an essential ω3 fatty acid and is converted into EPA and DHA in the body, but the percent of this conversion is inefficient (less than 1%) [9], and so it's necessary to uptake it from external sources, as mentioned above, like microalgae and fish. The figure 1 shows PUFA formation pathway.

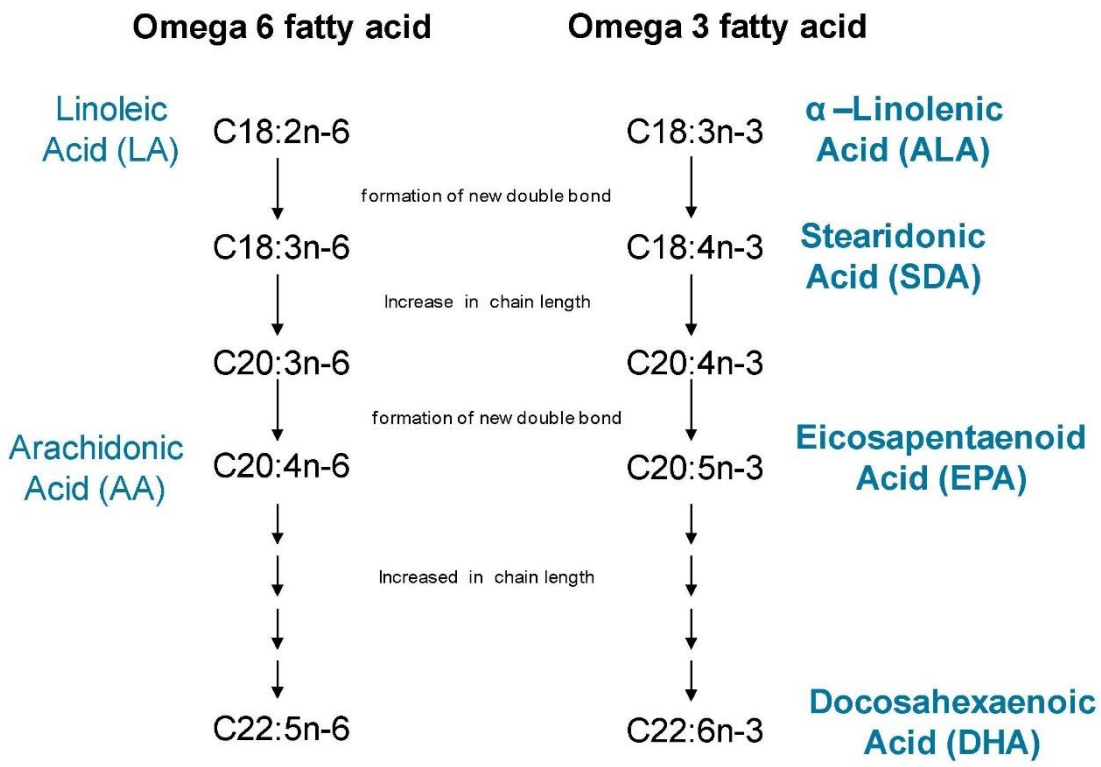

Figure 1- PUFA formation pathway *[5]*.

These fatty acids have roles in the prevention of coronary heart disease, treatment of hypertension arthritis, abnormal cholesterol levels, blood platelet aggregation, mental illness and autoimmune disorders. Studies have shown that EPA and DHA play a key role for proper fetal development, including neuronal, retinal, and immune function. EPA and DHA have also been linked to promising results in prevention and treatment of many diseases, weight management, cognitive function in people with very mild Alzheimer's disease, and could be important in treating inflammatory diseases [5][10][11].

1.3. Lipid content of microalgae

Microalgae are microscopic single-cell photosynthetic organisms, and the basis of the marine food chain. Some species provide an excellent source of lipids. Indeed, the lipid accumulation in microalgal cells can exceed 70% of their dry weight, although algae with lipid content of around 30% are more common [12]. Chemically, algal biomass is

constituted for approximately 60% of natural stored lipids, being rest protein, carbohydrate and other nutrients [13].

The composition and fatty acid profile of lipids extracted from a species is affected by the cultivation conditions, such as medium composition, temperature, light intensity, ratio of light/dark cycle, among others [3]. Table 1 shows the lipid content of some species of microalgae.

Table 1 - Oil content of some microalgae [14][15].

Microalgae specie	Lipid content (% dry wt)
Botryococcus braunii	25-75
Chlorella sp.	28-32
Crypthecodinium cohnii	20
Dunaliella primolectra	23
Nannochloropsis sp.	31-68
Scenedesmus obliquus	12-14
Schizochytrium sp.	50-77
Spirulina platensis	4-9

1.4. Lipid composition of fish

Fish processing generates considerable quantities of edible and inedible by-products. About 50% of the whole fish weight is by-products [16], depending on which components are further used for production of fishmeal, fish oils or other products. A percentage of the total catch of fish is discarded as processing leftovers, such as heads, frames, trimmings, fins, skin and viscera. Proper utilization of fishery by-products has many advantages; principally, it increases the overall value of the catch, it reduces cost of processing waste disposal or treatment, and, ultimately, lowers environmental pollution.

It has been well established in the current literature that marine oils are the most important source of PUFA [11]. In marine species, lipids are deposited beneath the skin, in the muscle, head and in the viscera, which permits the use of fish by-products for extraction of lipids. The oil content of fish by-products is highly variable and may range from 1.4 to 40.1% [17], depending on the species, food habit, geographical location, catch season and tissue type [18].

1.5. Lipid extraction

The aim of all lipid extraction procedures is to quantitatively isolate all lipids and separate then from the other constituents; such procedures can be categorized as mechanical or chemical methods, as shown in figure 2.

Lipid extraction from marine sources is traditionally carried out with organic solvents at room temperature, accordingly to the procedures developed by Folch et al. [19] or Bligh and Dyer [20], followed by lipid quantification using gravimetric estimation of lipid content. While the conventional extraction process is effective for analysis, it is also non-selective, time consuming, wasteful, uses toxic substances and is inefficient [21]. This process usually involves dewatering before extracting (since the remaining water in wet microalgal biomass hindered mass transfer of the lipids from the cell, provoking a decrease in the efficiency of lipid extraction [22]), which is time consuming and energy intensive [23]. These limitations hinder the application of conventional methods in industrial lipid extraction, despite the high extraction efficiency. Additionally, the pressure to replace organic solvents used in the conventional methods, which are highly-toxic [24], triggered the development of alternative green solvents and adopt newer technologies and eco-friendly processes to improve extraction efficiency.

Current trends in extraction techniques have largely focused on finding solutions that minimize the use of solvent and energy, such as ultrasound extraction (UAE), microwave assisted extraction (MAE), supercritical fluid extraction (SFE), and subcritical water extraction (SWE).

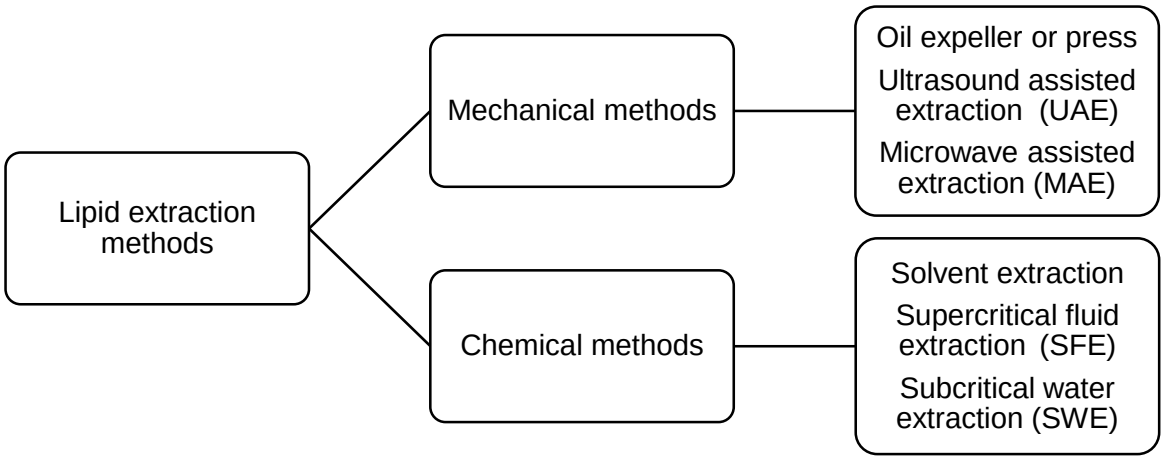

Figure 2 - Flow chart of lipid extraction methods, according to Mubarak et al. [3].

So far, current literature review has mainly focused on comprehensive lipid extraction methods, and the difference between the conventional methods and the new green methods but don't do a detailed study of the process chain. A tool for this kind of comparative studies is life cycle assessment (LCA). LCA is a standardized methodology for assessing the environmental impacts associated with the entire life cycle of the process. The main objective of this study is to fill this gap with an analysis of the cycle energy requirements and greenhouse emissions of lipid extraction from microalgae and fish by- products via UAE, MAE, SWE, SFE.

1.6. Life cycle assessment (LCA)

According to ISO 14044 [25], LCA is a standardized methodology, for determine environmental impacts associated with a product, process or service, over its entire life cycle (production, usage and disposal). It is a suitable tool to quantify and characterize flows of materials, energy and different environmental effects connected to, for example with new extraction processes [26].
There are four phases in an LCA study:

I. The goal definition and scope phase;
II. Life-cycle inventory phase;
III. The impact assessment phase;
IV. The interpretation phase.

Identifying the purpose and the expected products, including system boundary and level of detail, of an LCA depends on the subject and the intended use of the study and is presented in the scope. The structure of LCA can differ considerably depending on the goal of a particular LCA.

The life cycle inventory phase is the second phase of LCA. It is an inventory of input/output data about the system being studied. It quantifies the energy and raw material inputs and environmental releases associated with each stage of production to meet the goals of the defined study.

The third phase is the impact assessment. The purpose of this phase is to provide additional information to help assess the impacts on human health and the

environmental associated with energy and raw material inputs and environmental releases quantified by the inventory.

Life cycle interpretation is the final phase of the LCA procedure, in which the results of the above phases are, are summarized and discussed as a basis for conclusions, evaluating opportunities to reduce energy, material inputs, or environmental impacts at each stage of the product life-cycle in accordance with the goal and scope definition [25] [26][27].

2. State of Art

The process of lipid extraction from microalgae cells is an energy intensive and costly procedure because the use of solvents for the extraction of lipids requires extra energy in separating and/or recovering the same solvents from lipids after extraction.

Among the assortment of both emerging and mature extraction technologies, solvent-based extraction is still the most prevalently utilized technique for isolating lipids. The extraction method published by Bligh and Dyer (B&D) which utilizes a chloroform-methanol-water solvent system [28], is a rapid method to determine the lipid composition of frozen fish tissue; however, while B&D is considered a standard method for total lipid determination, it is not used at commercial scale due to the enormous amounts of solvent. Chloroform and methanol are toxic and flammable, which detrimentally affect health and the environment. These solvents affect the quality of the product by dissolving undesired products (chlorophyll) during the extraction process [28]. Hence, suitable solvent systems for lipid extraction that are sustainable, non-toxic and yield higher lipid content without interference of non-lipid compound should be considered.

Another standard method for lipid extraction is Soxhlet. This extraction method is assumed to completely extract all the lipids presented in microalgae, resulting in 100% recovery [29], but is extremely time-consuming and it also could cause thermo-degradation of Long chain polyunsaturated fatty acids (LC-PUFAs) [30]. Since the amounts of lipid extracted from other methods are compared based on the one extracted by this method [29], the Soxhlet extraction method was included in the lipid extraction comparison in this study.

To circumvent the problem of the conventional methods, green solvents and process intensification methods/techniques (green extraction technologies) have been studied to potentially improve the characteristics of energy reduction, eco-friendliness, non-toxicity and efficient lipid extraction.

2.1. Soxhlet

Soxhlet extraction was first created in 1879 by Franz von Soxhlet [31]. The Soxhlet procedure is based on solid-liquid extraction (leaching). The oil is extracted from the matrix using and non-polar organic solvent, usually hexane, ethyl acetate or petroleum ether [11][31] through repeated washing or percolation of fresh

organic solvent under reflux from a distillation flask. Figure 3 shows an apparatus used for Soxhlet extraction method.

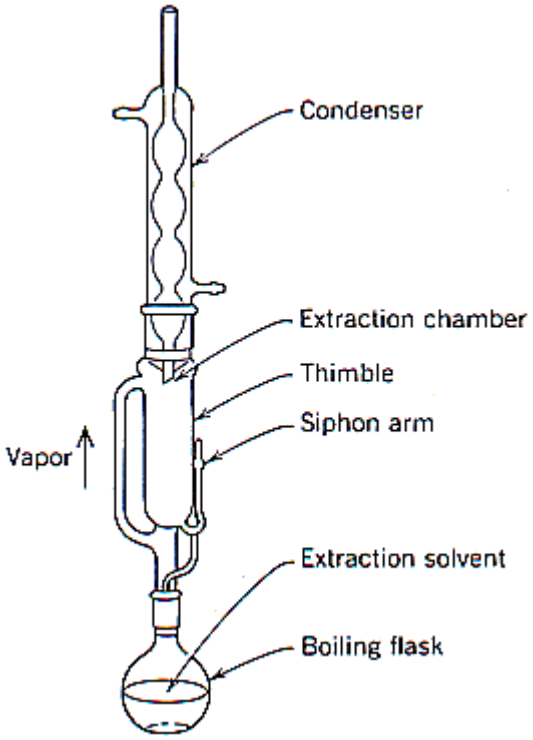

Figure 3 – Apparatus for Soxhlet extraction method.

Some advantages of Soxhlet extraction are ensuring a complete extraction since the sample phase is continually in contact with solvent, and does not require a filtration process after extraction. However, downsides include a long time required for extraction, and the large amount of organic solvent waste, which is not only costly to dispose of, but a preoccupation for the environmental [11][32].

2.2. Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE), leads a wide range of applications since the past decade.

SFE is based on the solvating properties of supercritical fluid (SF), which can be obtained by employing pressure and temperature above the critical point of a compound, mixture or element [33].

Supercritical fluids are especially useful not only to extract valuable bioactive compounds such as colorants, flavors and other biomolecules, but also to remove

undesirable compounds such as organic pollutants, toxins and pesticides. In both cases, the solid substratum can be treated as cellulosic matrix that is generally inert to the solvent and the solute or the mixtures of solute that will form the extract.

Several supercritical fluids have been investigated for lipid extraction, such as CO_2, methanol and ethanol. Supercritical CO_2 extraction $ScCO_2$ is considered as an important alternative for lipid extraction with organic solvents, since CO_2 is non-flammable, non-toxic, non-corrosive, relatively chemically inert and permits a final product free of solvent [34].

Extraction by supercritical fluid depends on some aspects like temperature, pressure and characteristics of the sample matrix, between others [33].

2.3. Microwave assisted extraction (MAE)

Microwave assisted extraction (MAE) is a extraction method that combines microwave and traditional solvent extraction [35].

This technique utilizes microwave energy to heat the whole sample volume simultaneously and homogenous, which enhances the rate and extent of mass transfer, to extract compounds of interest from sample into solvent. After being reported for extraction of chemicals from environmental matrices [36], MAE has been used for efficient extraction of lipids using conventional solvents.

MAE is based on the principle that the microwave heating system is very selective, and loses very little heat to the surroundings [11], and the heat is dissipated volumetrically inside the irradiated medium [37].

The higher extraction rates with superior quality, lower costs, less solvent used and reduced extraction time are the main advantages of MAE [3].

2.4. Ultrasound assisted extraction (UAE)

Ultrasound assisted extraction (UAE) is recognized as a simple, economical, efficient and eco-friendly technique, that increases yields and often the quality of the extract [11].

UAE involves the use of ultrasound ranging from 20 kHz to 2000 kHz, which increases the permeability of cell walls and produces cavitation [38]. The mechanic effect of acoustic cavitation from the ultrasound improve the surface contact between solvents and samples, helps disrupt cell walls and enhance mass transfer across cell membrane [12] [38].

UAE is inexpensive in comparison, for example, with SFE which needs a lot of investment in for the equipment used on the extraction [39], so it is a suitable alternative to conventional extraction techniques. Moreover, the process can be completed in a few minutes with less amount of solvent.

2.5. Subcritical water extraction (SWE)

Recently, subcritical water extraction (SWE) has been successfully employed to improve the lipid extraction from wet biomass [40].

SWE, which is also known as pressurized hot water extraction (PHWE) and superheated water extraction (SHWE) [41], is an environmentally friendly approach that uses water as the solvent, and elevated pressures and temperatures to achieve a rapid and efficient extraction of the required compounds from the matrices. The temperature applied during extraction process has an important impact on the extraction efficiency and selectivity, being normally used above the normal boiling point of water [41].

Water that maintains its liquid state in the temperature range of 100 ºC to 374 ºC under pressurized conditions is called subcritical water [42]. The water can act as an acid or base catalyst, and has a low relative dielectric constant [42], which makes it a suitable solvent for small organic compounds.

SWE does not require dry algae feedstock, which saves considerably energy and time spent on the drying process; another advantage is a reduction (or total elimination) of the amount of organic solvents used, maintaining a high quality of the extracts and efficiency of the process [41]. Also, in this process, SWE could disrupt cell wall, then allowing easier extraction of neutral lipids [43].

Additionally, the use of water instead of organic solvents, permits this process to be a good choice for extract functional food or pharmaceutical ingredients that are beneficial to health [41].

3. Methodology

In this work a research was carried out in the literature regarding the various lipid extraction methods more commonly used for microalgae, fish and its by-products matrices. Results are summarized in Table 2. The effects on the yield and composition of the extracted lipid were registered, as well as the procedures and the conditions provided for each method. This research was performed in the scientific databases from web of science, b-on and science direct.

Table 2 – Methods and results of extractions from microalgae and fish using several extraction processes.

Method	Solvent	Operating conditions	Matrix	Lipid yield	%ω3	Initial fraction	Ref.
Bligh and Dyer	$CH_3OH:CHCl_3:H_2O$ (2:1:0,8)	52 min	*Sardinella aurita*	9,94±0,37% wet basis	5,68±0,17 C22:6 ω3 17,55±0,18 % C20:5 ω3	10 g fish sample	[24]
Bligh and Dyer	$CH_3OH:CHCl_3:H_2O$ (2:1:0,8)	52 min	*Scomber japonicus*	2,24±0,05% wet basis	34,02±3,19 C22:6 ω3 6,44±0,31 % C20:5 ω3	10 g fish sample	[24]
Soxhlet	C_6H_{14}	40°C 0,1 MPa 18 h	*Schizochytrium limacinum*	45% dry weight	15,4 % C22:6 ω3	5 g microalgae powder	[44]
Soxhlet	$CHCl_3$: CH_3OH (2:1, v/v)	80°C 24 h	*Botryococcus braunii*	50 wt % dry algae	-	250 mg dry microalgae	[45]
Soxhlet	C_6H_{14}	6 h	*Thunnus tonggol* Head	36,2±1,8% dry weight	19,0±0,8 C22:6 ω3 1.6±0,1 % C20:5 ω3	5 g dry weight of sample	[46]
Soxhlet	C_6H_{14}	6 h	*Thunnus tonggol* Skin	22,4±1,4% dry weight	17,0±07 C22:6 ω3 1,4±0,1 % C20:5 ω3	5 g dry weight of sample	[46]
Soxhlet	C_6H_{14}	6 h	*Thunnus tonggol* Viscera	13,6±0,4% Dry weight	16,1±0,8 C22:6 ω3 2,5±0,1 % C20:5 ω3	5 g dry weight of sample	[46]
Solvent extraction	$CH_3OH:CHCl_3:H_2O$ (1:2:1)	150 s	*Sardinella lemuru* Head	-	15.95±1.17 C22:6 ω3% 1.84±0.15EPA%	50 g of sample	[47]
Solvent extraction	$CH_3OH:CHCl_3:H_2O$ (1:2:1)	150 s	*Sardinella lemuru* Liver	-	12.97±0.73 % C22:6 ω3 2.76±0.14 % C20:5 ω3	50 g of sample	[47]

Solvent extraction	$CH_3OH:CHCl_3:H_2O$ (1:2:1)	150 s	*Sardinella lemuru* Intestine	-	11.87±0.10 % C22:6 ω3 1.73±0.10 % C20:5 ω3	50 g of sample	[47]
Solvent Extraction	C_2H_6O	37 min Room temperature	*Picochlorum* sp.	33,04% dry weight	-	1gr dry weight of microalgae	[24]
SFE	CO_2	50 MPa 60 °C 12 g/min	Trout waste spine	0,41% dry weight	8,69 ±0,57 % C22:6 ω3 2,81±0,20 % C20:5 ω3	50 g dried freeze trout waste	[48]
SFE	CO_2	500 bar 60 °C 12 g/min	Trout waste head	0,40% dry weight	8,82 ± 0,75 % C22:6 ω3 2,96± 0,31 % C20:5 ω3	50 g dried freeze trout waste	[48]
SFE	CO_2	500 bar 60 °C 12 g/min	Trout waste viscera	0,78% dry weight	8,61 ± 0,64 % C22:6 ω3 2,89± 0,23 % C20:5 ω3	50 g dried freeze trout waste	[48]
SFE	CO_2	35 MPa 50 °C 4 h 0,434 kg/h	*Jasus edwardsii* (liver of Australian rock lobster)	24.3% dry weight	8,1 % C22:6 ω3 7,1 % C20:5 ω3	10 g of freeze-dried lobster liver	[49]
SFE	CO_2: C_2H_6O (1:1)	35 MPa, 40 ◦C 2 h	*Schizochytrium limacinum*	33,9% dry weight	27,5% C22:6 ω3	5 g microalgae powder	[44]
SFE	CO_2	37,9 MPa 50 °C 1 mL/min 1 h	*Sargassum hemiphyllum*	55,8±3,56% dry weight	1,93% C22:6 ω3 8,55 % C20:5 ω3	2 g freeze dried	[50]
SFE	CO_2	37,9 MPa 40 °C 1 mL/min 1 h	*Sargassum hemiphyllum*	50.1±3,63% dry weight	2,81% C22:6 ω3 11,2 % C20:5 ω3	2 g freeze dried	[50]
SFE	CO_2	43 °C 370 Mpa 200 min Flow rate 1,5 L/min	*Farfantepenaeus paulensis*	39% dry weight	4,72% C22:6 ω3 6,25 % C20:5 ω3	7,00±0,02 g freeze dried waste	[51]

SFE	CO_2	53°C, 50 MPa Flow rate 1,9 g/min	*Scenedesmus* sp	7,41% dry weight	-	3,0± gr of freeze dried microalgae	[52]
cxMeOH[a]	CO2:CH_3OH	35 °C 7.2 MPa	*Botryococcus braunii*	25 wt % dry algae	-	300 mg of microalgae (%dw)	[45]
LCO2[b]	CO_2	35 °C 7.2 MPa	*Botryococcus braunii*	19 wt % dry algae	-	300 mg of microalgae (%dw)	[45]
MAE	$C_4H_8O_2$: CH_3OH (2:1, v/v)	120 rpm 400 W 71 min	Tilapia fillets (*Oreochromis niloticus*)	2,3 ±0,1g/100g wet weight	15,9± 0,8 % C22:6 ω3 3,0±0,1 % C20:5 ω3 (wet algae)	50 g of sample	[53]
MAE	Petroleum ether: C_3H_6O (2:1, v/v)	-	*Trematomus bernacchii*	1,1±0,2 % w/w dry weight	10,9± 1,3% C22:6 ω3 10,3± 0,4 % C20:5 ω3	0,2 mg dried sample	[54]
MAE	CH_3OH	100 W 2,45 GHz 20 min 60 °C	*Nannochloropsis gaditana*	39,6% dry weight	1,055 % C20:5 ω3	50 g dried microalgae	[55]
MAE	20% biodiesel in C_2H_6O	80°C 50 min	*Nannochloropsis sp.*	5,4±0,6 % dry weight	-	3,3 g wet microalgae	[56]
MAE	CH_3OH: C_6H_{14} 1:2 (% v/v)	10 min 65°C Normal pressure	*Nannochloropsis* sp.	38,31% dry weight	-	10 ml wet microalgae	[57]
SWE	C_6H_{14}	200°C 0,5 h 1,38 MPa	*Nannochloropsis gaditana*	14,89±1,41% dry weight	1,19 % C22:6 ω3	100 g wet algae	[58]
SEE[c]	C_2H_6O	10% moisture 40:1 ratio 135°C 1,5 MPa 50 min	*Nannochloropsis sp.*	90,21% of the total lipids	-	9 g wet microalgae	[59]

UAE	-	1000 W 30 min Biomass dry weight content 5%	*Nannochloropsis oculata*	0,2% dry weight	29,6 % C22:6 ω3	100 g microalgae (%dw)	[60]
Bligh and Dyer assisted with UAE	$CH_3OH:CHCl_3:H_2O$ (2:1:0,8)	40 kHz Ultrasonic intensity of 2,68 W/m^2 40 min	*Chlorella vulgaris*	52,5 ± 2,3 (%w/w)	-	5 g dried microalgae	[61]

[a] Liquid Carbon Dioxide, [b] CO2-expanded methanol, [c] Steam Enhanced Extraction

The analysis had a higher incidence on the matrix microalgae, due to the largest number of manuscripts found; additionally, the extraction with conventional methods as Bligh and Dyer and Soxhlet appear in the table just as comparative means.

Analyzing the table, we observe that from the methods above mentioned, the extraction technique with more references is SFE [35, 36, 39, 40, 42, 42], followed by MAE [43, 44, 45, 46], UAE [49, 50] and SWE [47].

Regarding SFE, authors claim that it is a good method to extract lipids from microalgae and fish. Comparing with the other methods, the main difference is a need of pre-treatment of the matrix. The matrix needs to be dried [35, 36, 39, 40, 42, 42], because in most cases, the water present in the matrix competes with the solute to interact with the solvent, decreasing the yield of the process [62]. In fact, the studies abovementioned use lyophilized algae or dry algae powder as starting material. However, a few studies reported that it is possible to extract lipids using wet algae and SFE: Soh and Zimmermman [63] studied the impact of water content on extraction efficiency, by the addition of water to lyophilized algae. The results showed that water content does not changed the profile and quantity of FAME produced, which can lead to a more convenient and economical process.

In relation of the use of solvents, the most applied is CO_2 [35, 36, 39, 40, 42, 42]. To improve the lipid yield extracted, some studies use a co-solvent as ethanol or methanol. In the study of Tang et. al., [44] the use of the co-solvent ethanol was studied. They concluded that the influence of ethanol on the lipid yield and DHA was notable: both lipid yield and DHA content increase significantly with the increase in the mass ratio of co-solvent to matrix.

In general, the most fast process is SWE, with a 30 min of extraction time [58]. Despite SFE is the most used process, it's also the most consuming time, but even so is less time consuming than the conventional methods [35, 36].

When we talk about the operating conditions, we can conclude that there are many similarities inside the same method. For example, on SFE the temperature varies between 35 a 60°C and the pressure varies among 35 e 50 MPa [35, 36, 39, 40, 42, 42]. Some studies have explored the optimization conditions for the extraction. Taher et. al. [52] shows that increasing extraction temperature from 35 to 50°C increased the extraction yield, but higher temperatures than 50°C decreases the yield. The study mentions too that an increase in pressure leads to significantly higher yields.

For MAE, the operating conditions are similar too, but in relation of the other two methods, it's not conclusive what are the most used operating conditions due to the lack of available information.

Nannochloropsis has traditionally been studied as a source of PUFA, which is corroborated for this study, since it is the microalgal matrix more used. This may be because of its lipid content (31-68 % dry weight [14]), and due to its high content in EPA [48].

These techniques in general have good efficiencies. For the marine sources presented in this review, SFE was the most efficient. In optimum conditions SFE can extract around 50% lipid dry weight of a matrix of 2 gr freeze dried microalgae [52].

The use of solvents has a considerable influence in the efficiency of the extraction. About UAE, are shown two references, one uses a mixture of solvents (methanol, chloroform, water) [60] and the other don't require any solvent [60] . If we compare and extrapolate both studies for a matrix of 100 gr microalgae, the efficiency would be 10,5% and 0,2% respectively. This means that the use of organic solvents permits a higher efficiency on the extraction. However, the toxicity of these solvents leads to a search of new green solvents and studies to evaluate their potential to obtain higher extractions.

In order to evaluate the abovementioned extraction processes in terms of economic and environmental impact, a comparative life cycle analysis (LCA) was carried out, using proper databases and software.

4. Life Cycle Assessment

According to ISO 14044 [25], LCA is a methodology used to assess the environmental impacts associated with all stages of a product's life, from-cradle-to-grave. It involves four stages of analysis that are described below.

4.1. Goal and scope

The goal of this study is to perform a comparative life cycle assessment of four lipid extraction methods, supercritical fluid extraction, microwave assisted extraction, ultrasound assisted extraction and subcritical water extraction, to evaluate the overall environmental performances. It is believed that a more efficient lipid extraction setup, in terms of environmental and economic impact, would provide a better economic viability for an industrial scale production [64].

The functional unit for this study is the production of 1 kg of lipids. The system boundaries adopted include only the extraction process (in case of SFE will include too a pre-treatment), since the cultivating and harvesting phase is out of scope of this study, considering a gate to gate approach.

4.2. Life cycle inventory

In this section, the process schemes, the description of the different extraction methods and the tables with the inputs and outputs are briefly presented below. The design of these process is mainly based on experimental data published by Taher et al. [52], Wahidin et al. [57], Adam et al. [60] and Sitthithanaboon et al [58], this data was collected from scientific articles from databases web of science and science direct platforms.

4.2.1. Supercritical fluid extraction (SFE)

The first method is focused on the use of supercritical fluids, where lipids are extracted using supercritical CO_2 as solvent.

The extraction occurs in an extraction cell using fluid supercritical extraction apparatus for 1h at 53°C and 50 MPa [52]. The wet microalgae before entering the extraction cell, passes first to a pre-treatment (lyophilization) to reduce the amount of

water present. Figure 4 shows the input and output flows considered in this analysis and Table 3 summarizes the inventory.

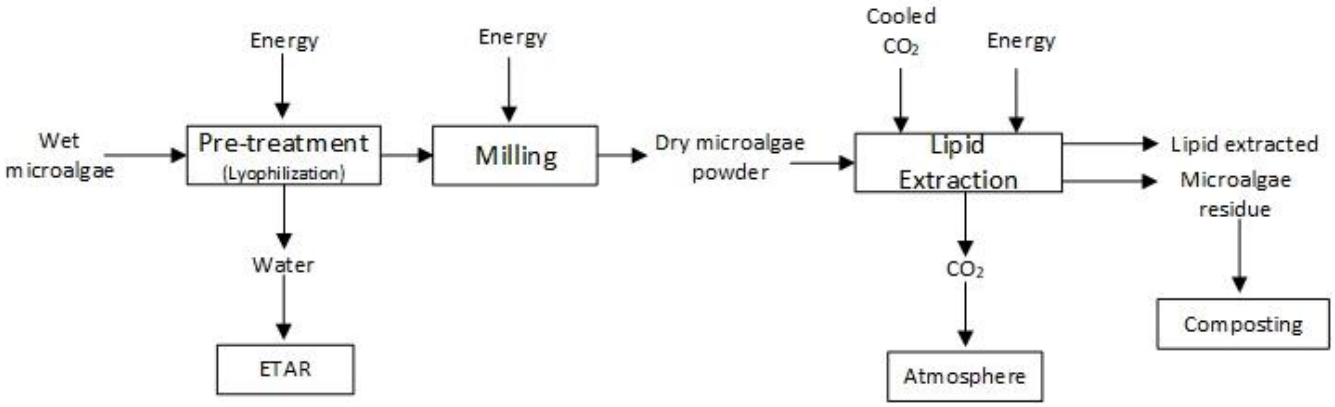

Figure 4 – Flow diagram of lipid extraction by SFE.

Table 3 – Key inventory data for SFE.

	SFE
Inputs	
Wet microalgae (g)	100
Carbon dioxide (kg)	0,9
Electricity (MJ)	117,21
Outputs	
Carbon dioxide, fossil (kg)	0,9
Lipid extracted (g)	1,32
Waste to treatment	
Municipal solid waste landfill or composting (g)	18,68

4.2.2. Microwave assisted extraction (MAE)

MAE involves the use of microwaves as a heat source, which can penetrate the biomaterial, interact with polar molecules and heat the whole sample. The wet microalgae is subjected to the microwave radiation and operated for 5 min at 65°C under normal pressure, and extracted using a mixture of methanol-hexane. After the lipid extraction the solvent phase that contained the extracted lipids was centrifuged for 5 min and separated using a separating funnel, then the solvent phase is evaporated, and 95% of the hexane was recovered and recirculated [57]. Figure 5 shows the input and output flows considered in this analysis and Table 4 resume the inventory.

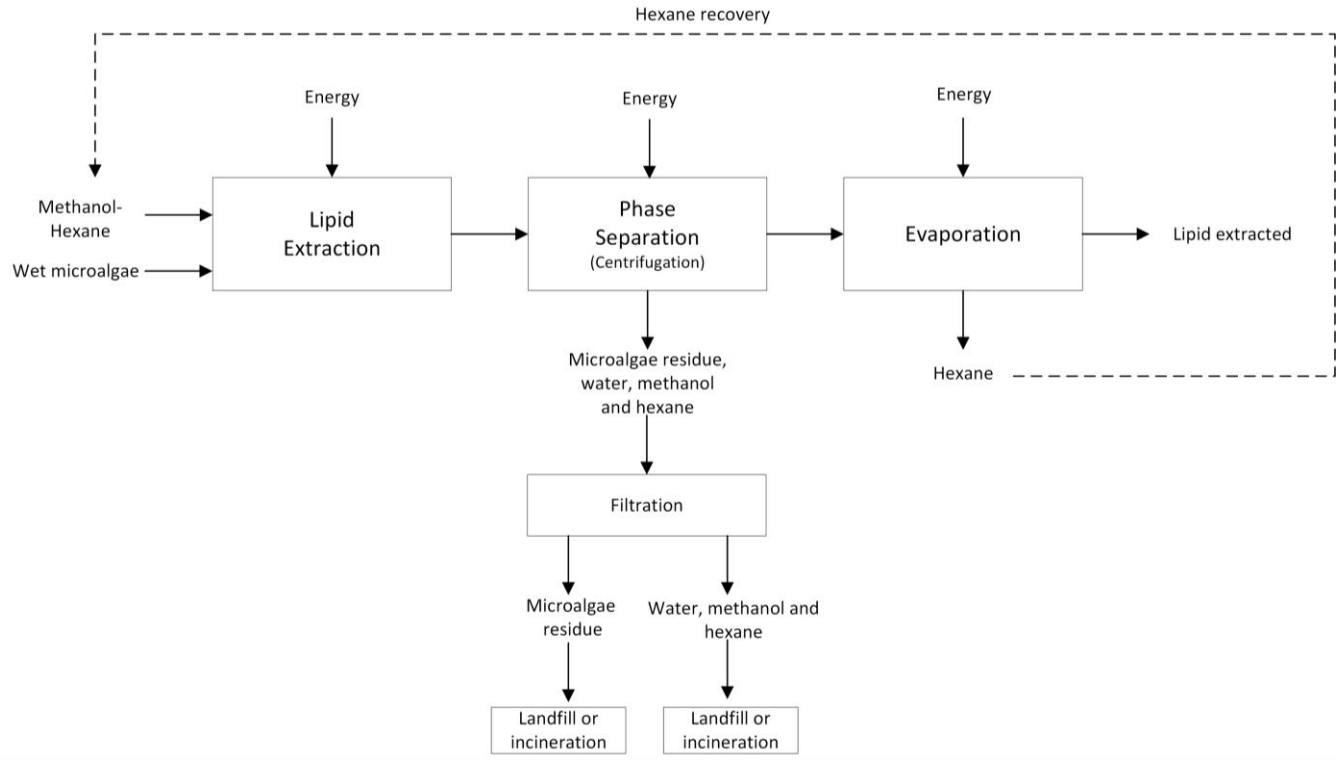

Figure 5 - Flow diagram of lipid extraction by MAE.

Table 4 - Key inventory data for MAE.

	MAE
Inputs	
Wet microalgae (g)	100
Methanol (g)	79,2
Hexane (g)	6,59
Electricity (MJ)	3,28
Outputs	
Hexane (g)	
Lipid extracted (g)	7,66
Waste to treatment	
Landfill or incineration (g)	178,09

4.2.3. Ultrasound assisted extraction (UAE)

In this scenario UAE was processed in a double glass reactor. The operating conditions was 30 min with 1000 W ultrasonic power. This process doesn't use any solvent for the extraction, but it's necessary to add in a post-treatment phase (centrifugation), a few milliliters of a hexane/isopropanol solvent mixture to the crude extracted to separate the lipid phase. After that the mixture was filtered and dried [60] and 95% of the hexane used was recovered and recirculated.

Figure 6 shows the input and output flows considered in this analysis. Table 5

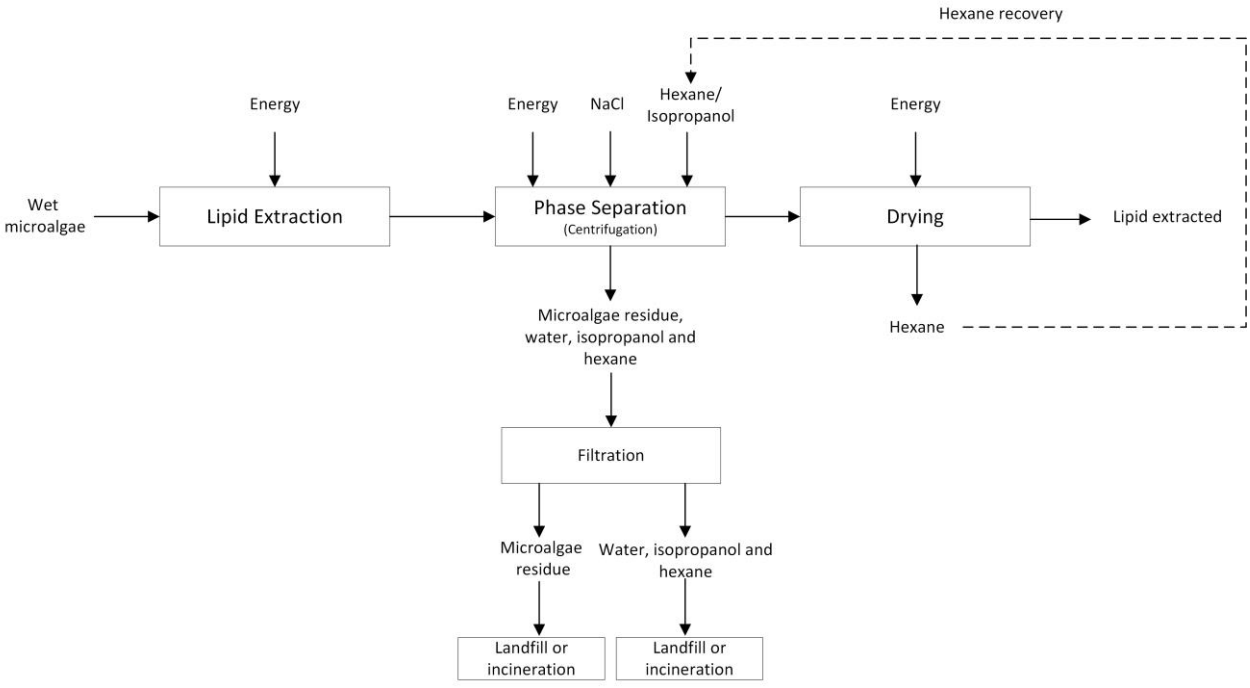

Figure 6 - Flow diagram of lipid extraction by UAE.

gather the data of the inventory.

Table 5 - Key inventory data for UAE.

	UAE
Inputs	
Wet microalgae (g)	100
Isopropanol (g)	1,57
Hexane (g)	0,099
Sodium chloride poder (g)	0,9
Tap water (g)	99,1
Electricity (MJ)	3,47
Outputs	
Hexane (g)	
Lipid extracted (g)	0,063
Waste to treatment	
Landfill or incineration (g)	201,94

4.2.4. Subcritical water extraction (SWE)

In SWE, the wet microalgae was loaded into a reactor at a controlled temperature at 200 °C for 30 min, whereas the pressure was maintained at 1.38 MPa. At the end of the reaction, the product mixture was transferred into a centrifuge when the lipid phase was separated from the microalgae residue. The hexane phase was further evaporated by rotary evaporator, and then 95% of the phase was recovered. Figure 7 shows the

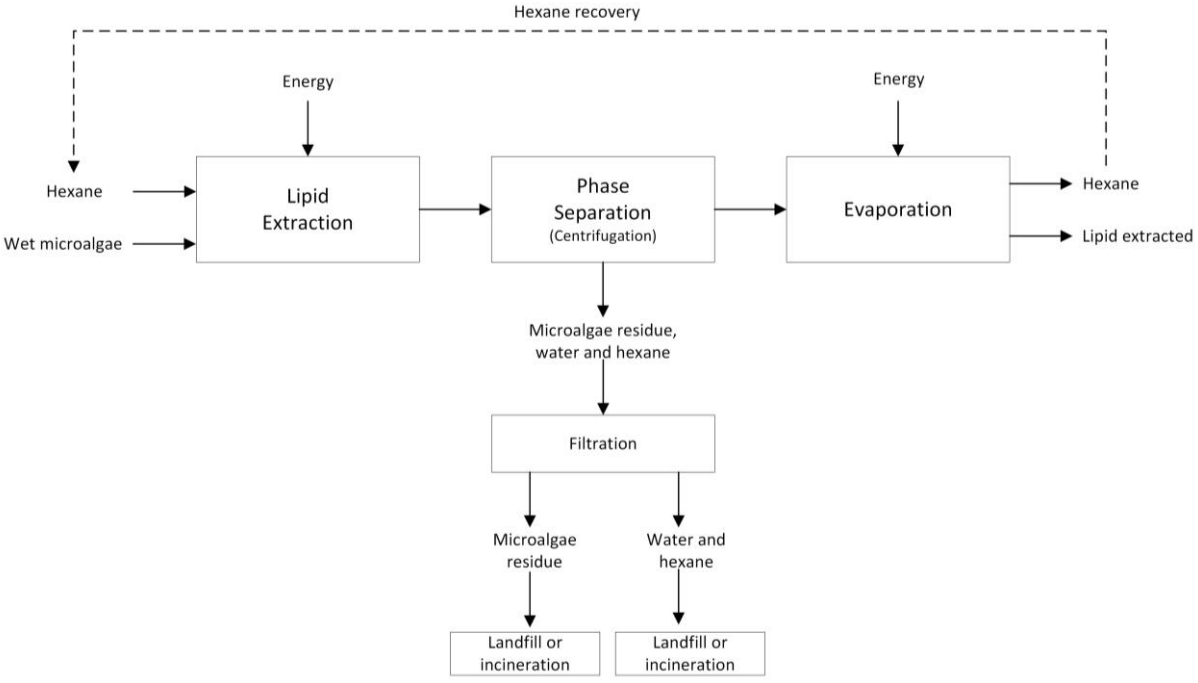

Figure 7 - Flow diagram of lipid extraction by SWE.

input and output flows considered in this analysis.

Table 6 - Key inventory data for SWE.

	SWE
Inputs	
Wet microalgae (g)	100
Hexane (g)	0,66
Electricity (MJ)	1,48
Outputs	
Hexane (g)	
Lipid extracted (g)	2,98
Waste to treatment	
Landfill or incineration (g)	97,02

4.3. Impact assessment

Impact assessment expresses the inventory in terms of their contribution to the environmental impact categories. The magnitude and significance of environmental or social costs associated with specific life cycle activities are identified during this phase.

The calculations can be undertaken manually, but are commonly facilitated with the use of LCA specific software. Here, it was used SimaPro (version 8.4.0.0). The method used was of the IMPACT 2002+ and categories of damages studied were human health, ecosystem quality, climate change and resources. The unit used is called Points.

The Figure 8 introduces a resume with all the methods and all the possible strands. The values are normalized to be possible to compare the impacts. In a first analyze, the discrepancies in impacts are significant, so it's indispensable a study in detail to understand the values.

This chapter will be divide in four subcategories each one will present the impact of each method in detail. The hexane emissions to air were considered negligible in all processes.

The names of the graphic presented are abbreviated due to the existing space. The abbreviations used means:

LAND - landfill

INC - incineration

COMP - composting

L+I - landfill + incineration

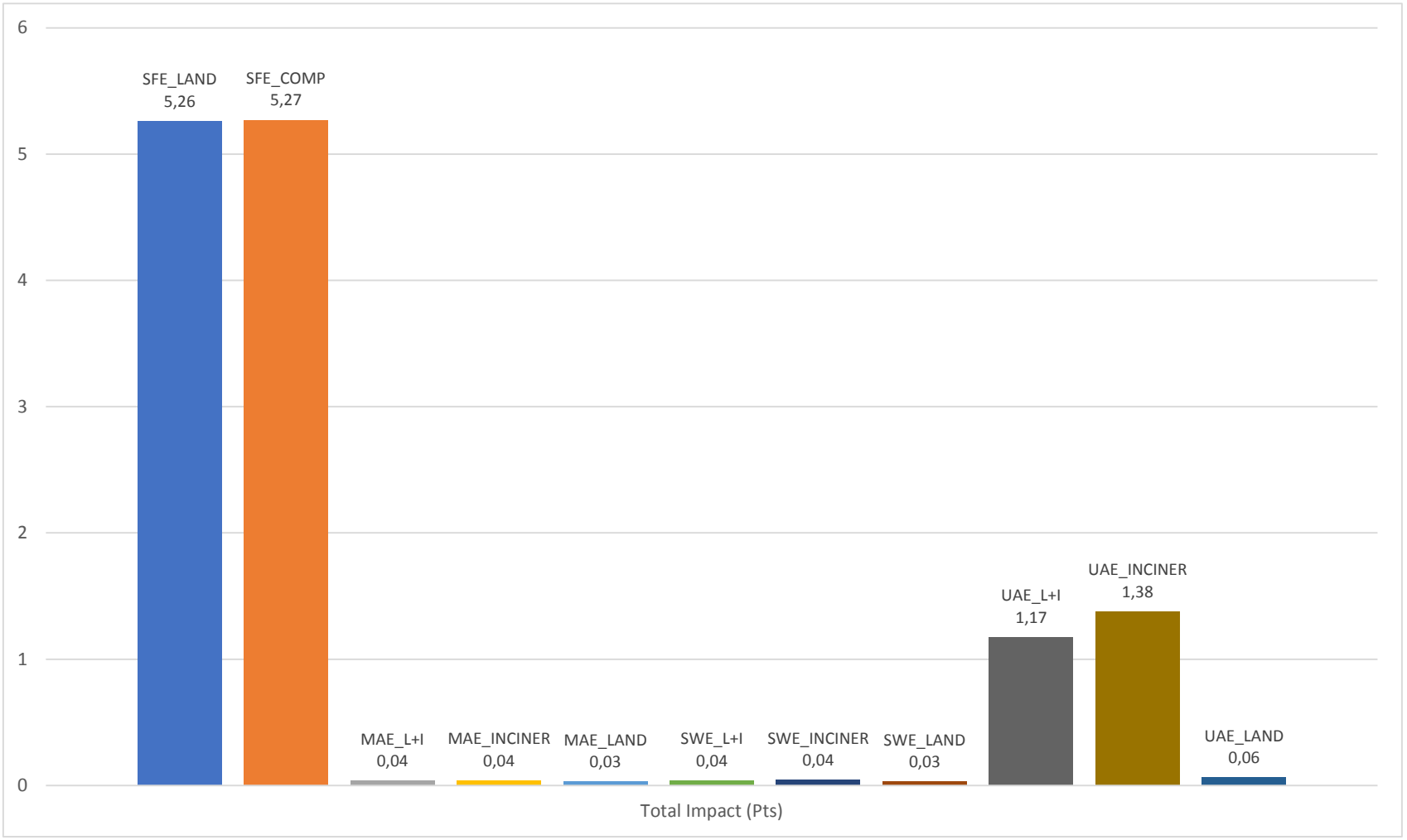

Figure 8 – Resume of the all methods and the total impact in Pts.

4.3.1. Supercritical CO_2 extraction

The Figure 9 shows the categories of damage for SFE with CO_2 as solvent. Here are compared two processes with two ways to waste treatment, landfill and composting, since the residues produced by the extraction don't contain hazardous compounds.

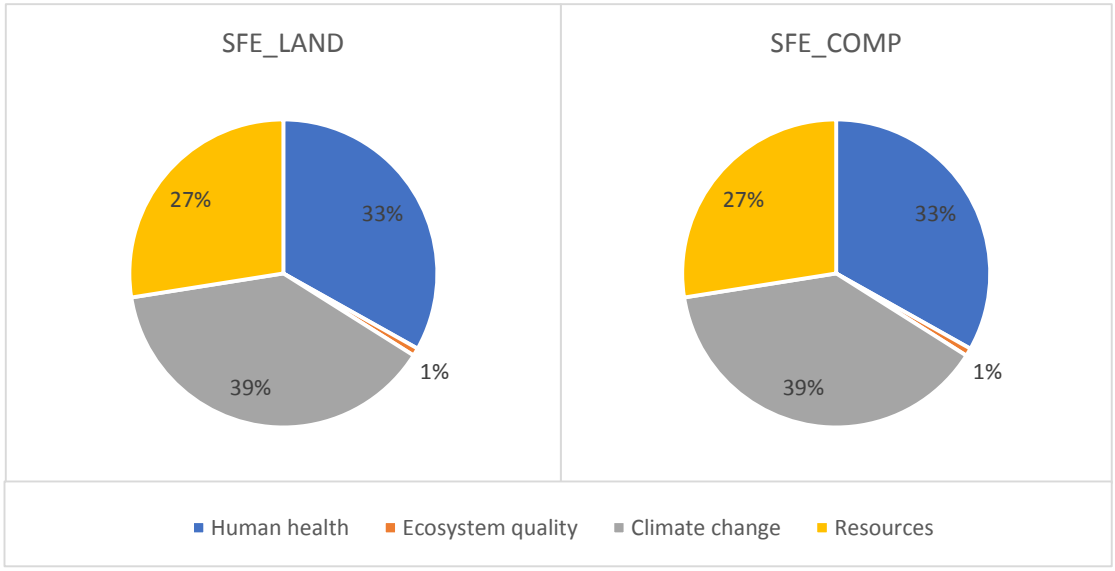

Figure 9 – Environmental impact in the four categories of damage in SFE.

The steps analyzed in this assessment encompass the phase of the extraction and the treatment of waste, as already referred. The Figure 10 shows the comparison of percental weight of each step.

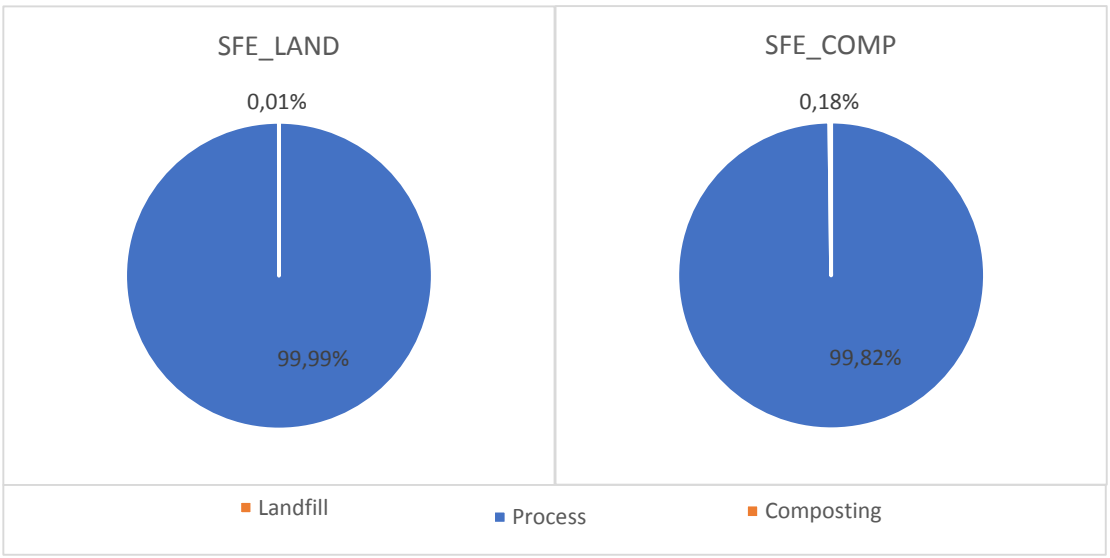

Figure 10 - Environmental impact of the waste treatment use by SFE.

4.3.2. Microwave assisted extraction

The residues and the effluents obtained by MAE, contain organic solvents, as hexane and methanol.

The legislation in vigor in Porto says that the limit of hydrocarbons in residual water is 50 mg/L [65]. In this process the hydrocarbons value overcome the legislation limit, because of this, the resultant effluents can't go to a water treatment station. The possibilities of treatment are inciner and landflill. In relation of the solid residues, they can have the same destiny as the effluents.

The Figure 11 presents the conjugation of possibilities for this process.

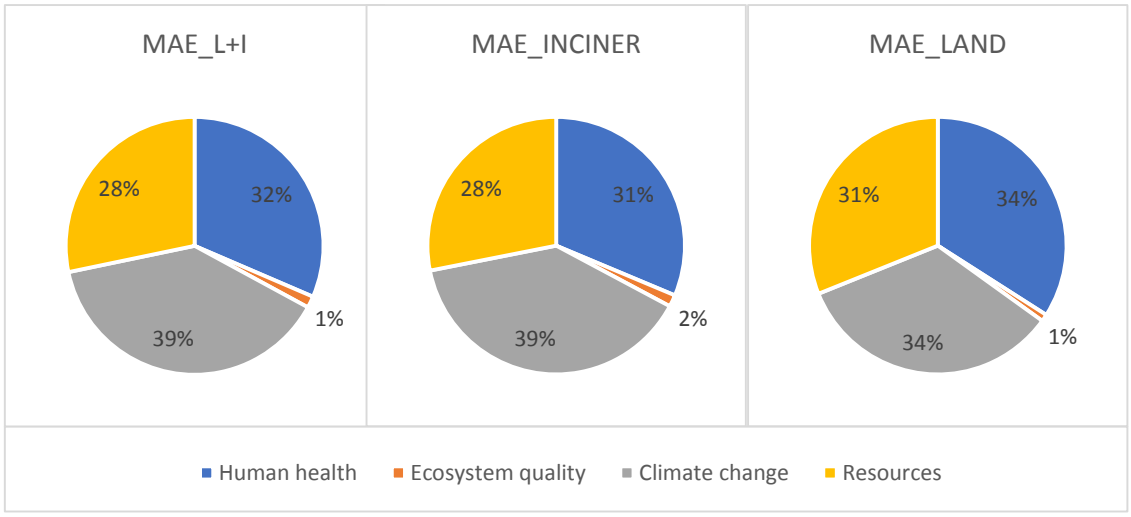

Figure 11 - Environmental impact in the four categories of damage in MAE.

In Figure 12 is compared the impact of each step of the waste treatment.

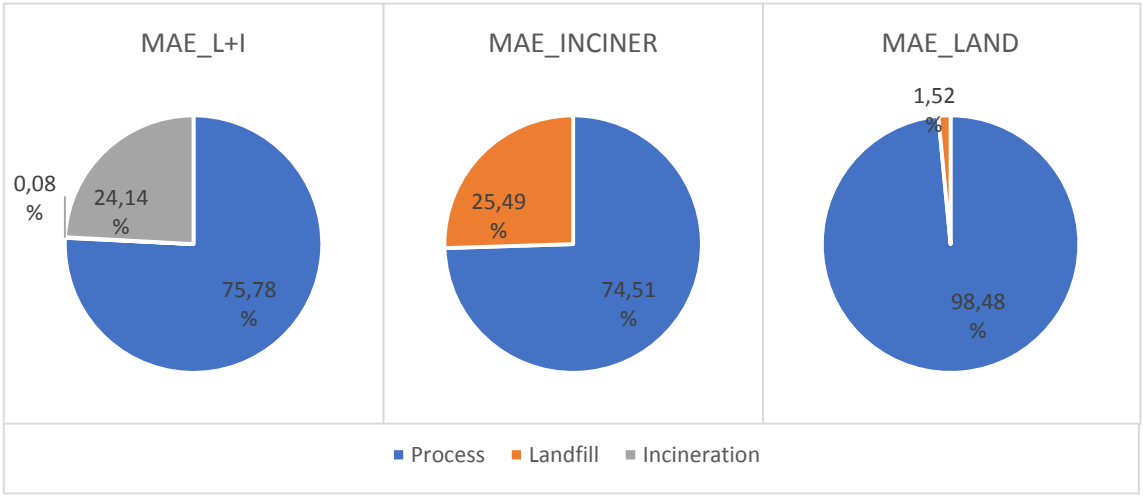

Figure 12 - Environmental impact of the waste treatment use by MAE.

4.3.3. Subcritical water extraction

As the MAE, the SWE has the same problem with the effluents, so the residues treatment solution is the same mentioned above, as see in Figure 13.

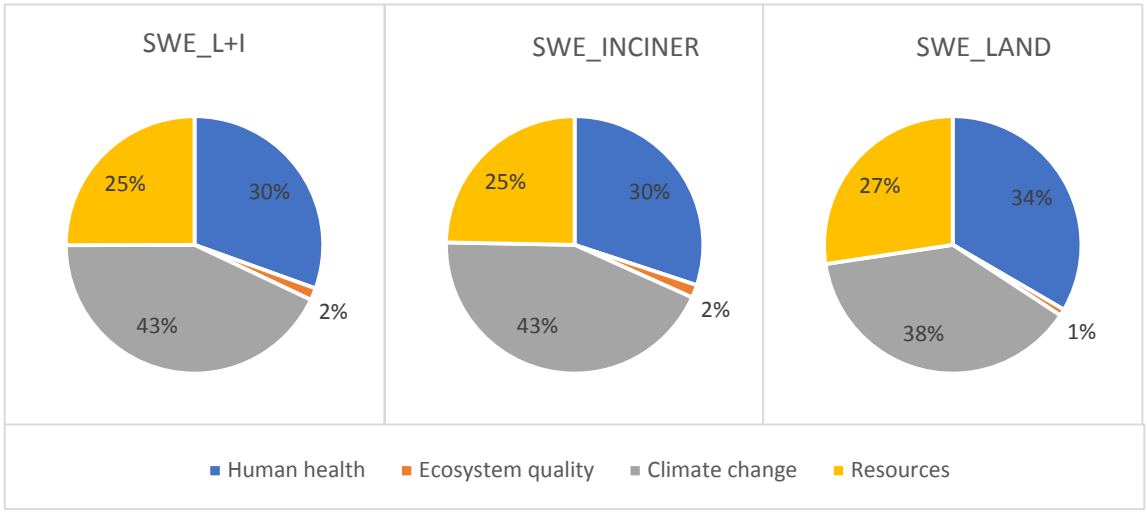

Figure 13 - Environmental impact in the four categories of damage in SFE.

In Figure 14 it's possible to observe the percentages of each step in the global impact.

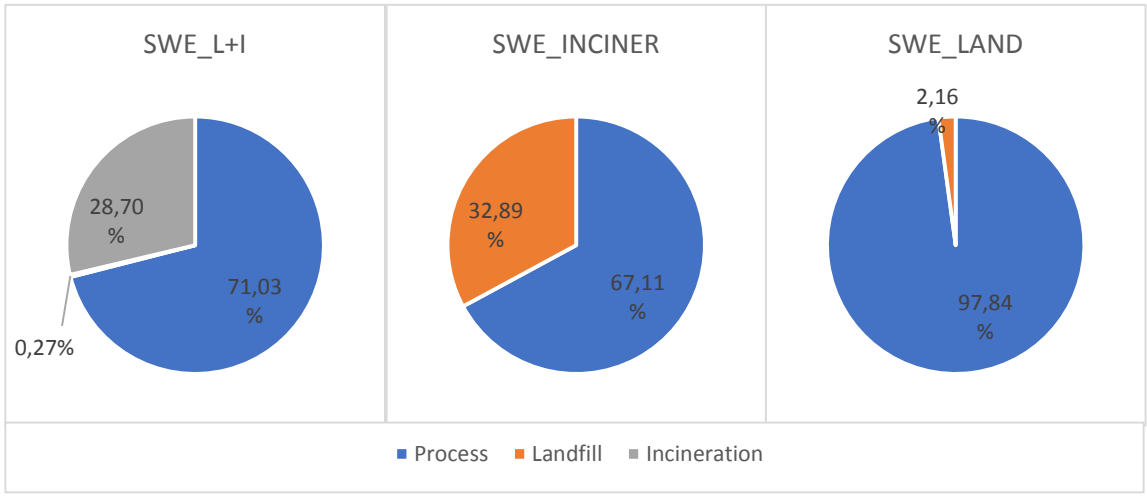

Figure 14 - Environmental impact of the waste treatment use by SWE.

4.3.4. Ultrasound assisted extraction

The ultrasound assisted extraction studied doesn't require solvents in the extraction phase, however it's necessary to add a post-treatment phase to isolate the lipids of the matrix. This fact, unfeasible the use of composting or a water treatment station. The steps studied to treat the waste were incineration and landfill. The Figure 15 shows the categories of damage of each process.

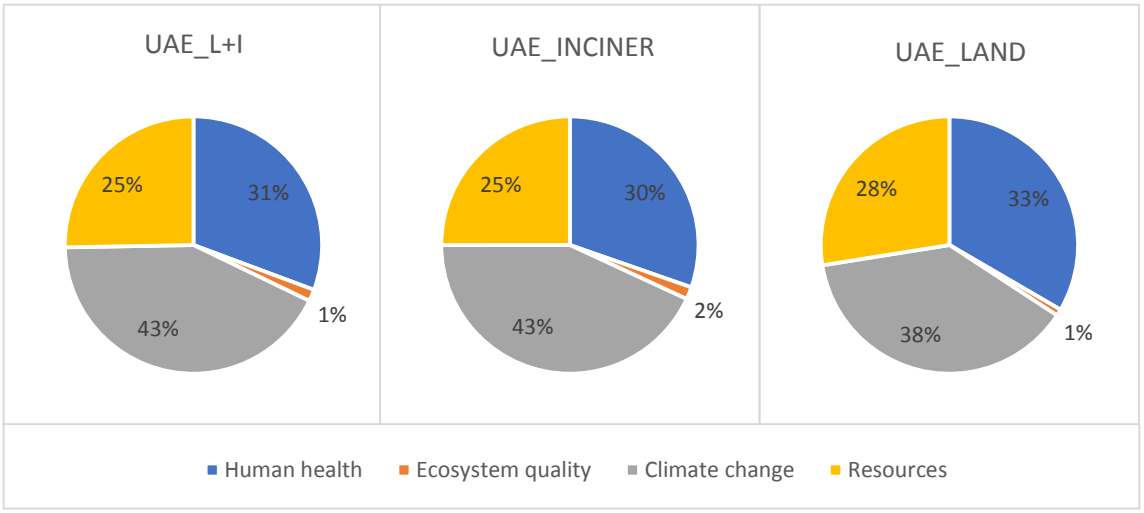

Figure 15 - Environmental impact in the four categories of damage in UAE.

The relative percentages of the extraction and the waste treatment are presented in Figure 16.

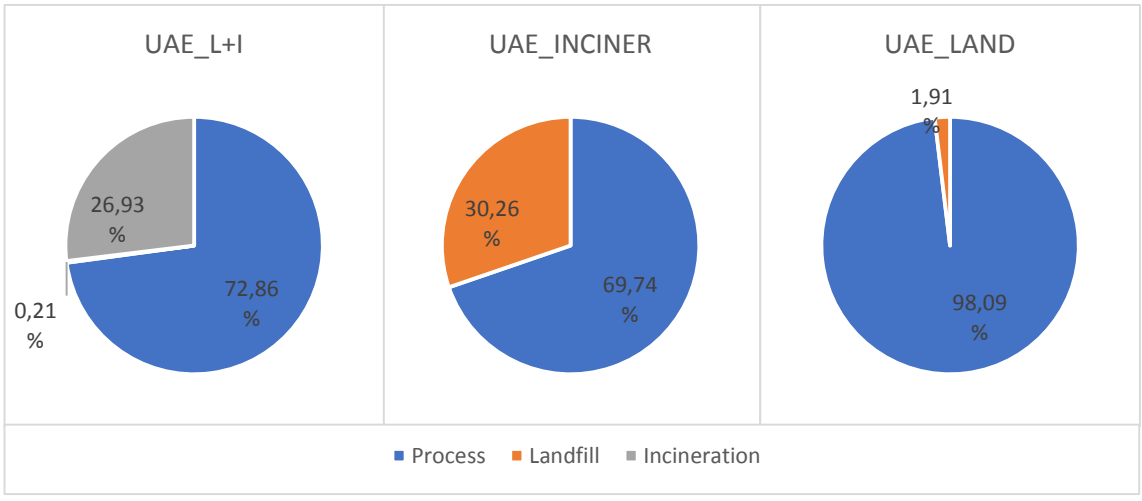

Figure 16 - Environmental impact of the waste treatment use by UAE.

4.4. Interpretation

In this study all the four methods were divided in two stages: the process and the waste treatment.

Analyzing the SFE method first, by the Figure 9 and the Tables A.7 and A.8 from the Appendix B, it is verified that the process stage has the highest environmental impact with, 5,26 Pt, corresponding to a percentage of almost 100 % to the method of extraction. The waste can go to landfill or to composting. The SFE_LAND waste treatment has an impact of 0,00027 Pt (0,01 %), and 0,00958 Pt for SFE_COMP waste treatment (0,18 %). With this, it's possible to affirm that in this method, the choice of the waste treatment process doesn't have a big environmental impact, but the use of composting avoids the production of ammonium nitrate.

In Figure 8 are discriminated the categories of damage of this method. Since the process as the main impact, it's observed that the values of these categories are the same for the both waste treatment methods. The category of damage with most impact is climate change with 39 % of the total impact (2,02 Pt), followed by human health and resources with 33 % and 27%, respectively. Finally, the ecosystem quality has the minor impact with just 1 % (0,04 Pt).

Regarding the MAE, it was tested three different waste treatments methods and the results are displayed in Figure 11 and in Table A.9, A.10 and A.11 of the Appendix C. As the method above the process has the most environmental impact but on the contrary, the value differs according to the waste treatment. For the MAE_L+I method the environmental impact is 38,55 mPt, in the MAE_INCINER the impact is 39,21 mPt, and for the MAE_LAND the value is 29,67 mPt. It is shown that the waste treatment with minor impact is to put the wastes in a landfill. The contribute of the waste treatment process is 1,52 % (0,45 mPt). For the two other the value of the impact is 9,314 mPt (24,22 %) and 10,00 mPt (25,49 %), respectively.

Considering the similar values in MAE_L+I and MAE_INCINER, in Figure 10 it is confirmed that the environmental impact on the categories of damage is almost the same. The categories with the biggest impact is climate change with 39% for MAE_L+I and MAE_INCINER, for MAE_LAND the major impact goes to climate change and human health with 34%, while the ecosystem quality is the lower with only 1% for MAE_L+I and MAE_LAND, and 2% for MAE_INCINER.

In the SWE the same methodology of waste treatment was applied with three types of treatment. According to Figure 13 and Appendix D the method with biggest environmental impact is SWE_INCIDER with 42,61 mPt, followed by SWE_L+I with 40,26 mPt and the last is SWE_LAND with 29,22 mPt. These results are due to the

type of waste treatment used. The impact for the process stage is the same with 28,59 mPt, this means that for the SWE_LAND the waste treatment as a relative weight of 2,16% of total, the incineration in the SWE_INCINER as the biggest value with 32,89%, and for SWE_L+I the percentage for waste treatment was 28,97%. It is observed that use of incineration as a treatment has a high environmental impact.

In relation to the categories of damage, it is verified once more that the climate change is the most affected category getting 17,29 mPt (43%), 18,58 mPt (43%) and 11, 22 mPt (38%) for SWE L+I, SWE_INCINER and SWE_LAND, respectively. The next one is the human health with 12,28 mPt (30%) for SWE_L+I, 12,81 mPt (30%) for SWE_INCINER and 9,76 mPt (34%) for SWE_LAND. In the same way of the other methods, the ecosystem quality as the lower impact with 2% for SWE_L+I and SWE_INCIDER and 1% for SWE_LAND.

Passing to the fourth method studied, the UAE, it is observed in the Figure 15 and the Table A.15, A.16 and AA.17 of Appendix E, that the use of incineration contributes significantly to the environmental impact. The impact of the waste treatment in UAE L+I is 1,18 Pt, in the UAE_INCINER is 1,38 Pt and in the UAE_LAND is 0,06 Pt. Knowing that the environmental impact of the process stage is 3,18 Pt, concludes that the percentages is 27,14%, 30,26% e 1,91%, respectively.

About the damage categories presented in Figure 15, for the three waste methods treatments, the climate change is the most affected category with a range between 38% and 43%, followed by human health, resources and ecosystem quality.

The Figure 8 englobes all the methods with all the different waste treatments possibilities. With a first look, it's possible to conclude that the worst method is SFE, and the better is MAE_LAND and SWE_LAND. This conclusion is just based on the impact total (Pts) not counting the quantity of the microalgae used. Taking into consideration the content in the matrix in dry matter used for the four methods, in order to recover up to 1 kg of lipids extract either 75,53 kg of wet microalgae to SFE, 13,05 kg of wet microalgae to MAE, 33,58 kg of wet microalgae to SWE either 1587,30 kg of wet microalgae to UAE is needed (values in Appendix F, Table A.18). Using different extraction methods leads to the recovery of different amounts of lipids, therefore in order to achieve the yield of 1 kg there are needed different masses. In terms of the quantity of microalgae needed, the worst method is UAE, since uses a lot more microalgae than the other methods, and that could increase considerably the impact associated with the harvesting and collecting process, which means that despite the SFE have the biggest impact, with this data the worst method for lipid extraction of the four presented is ultrasound assisted extraction (UAE).

In relation of the best method, is not possible to conclude if is MAE or SWE because the total impact is very similar in both and the amount of microalgae needed is comparable too. To conclude which method is the best to extract lipids from microalgae, it would be necessary to do further analyses and do a LCA including the harvesting and collecting phase, which was not performed on this study due to the lack of information available. Ponnusamy et. al., [43] stated that, to produce 1 kg of biodiesel without any co-products management, 36% of the energy required would be spend on cultivation and 56% on lipid extraction. With this information it can be concluded that lipid extraction is the most important step in energetic terms, and therefore the most important phase to analyze. However, in order to accurately conclude which process was the best option, it would be essential a deeper analysis, including the microalgae cultivation and harvesting steps, since the two best extraction processes from an environmental point of view present very close values to the environmental impact.

In a general way, the category most affected is the climate change and minus is ecosystem quality; this is verified in all methods and supported by the use of organic solvents and the type of waste treatment used.

Conclusion

The purpose of the thesis presented was to perform a comparative evaluation of four different scenarios for lipid extraction. Such evaluation was done in two steps: a bibliographical research, in order to ascertain the conditions and yields obtained by such technologies, and a life cycle assessment (LCA) for each technology, so as to determine their individual effectiveness in terms of environmental impact. The extraction technologies studied include processes of energy transfer generated by waves, as ultrasound assisted extraction (UAE) and microwave assisted extraction (MAE), and the use of compressed fluids in supercritical fluid extraction (SFE) and subcritical water extraction (SFE). Target lipids were ω3 polyunsaturated fatty acids, such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. Marine sources under study were microalgae.

The supercritical fluid extraction with CO_2 as solvent, appeared to be the worst alternative from an environmental impact point of view, both with landfill or composting as waste treatment with 5,26 and 5,27 total points, respectively. In relation of the best method, is not possible to conclude if is MAE or SWE because the total impact is very similar in both and the amount of microalgae needed is comparable too. To conclude which methods is the best to extract lipids from microalgae would necessary to do analyses and do a LCA including the harvesting and collecting phase.

Using different extraction methods leads to the recovery of different amounts of lipids, therefore in order to achieve the yield of 1 kg it is necessary different masses of microalgae. In terms of the quantity of microalgae needed, the worst method is UAE, since uses a lot more microalgae than the other methods, and that could increase considerably the impact associated with the harvesting and collecting process.

In global, the results are particularly significant, as it demonstrated that it is possible to reduce the environmental impact of the extraction process, which is the most impactful phase of the production of lipids. This analysis also had limitations, which is related to the lack of the information with cultivation and harvesting phase. For this reason, based on laboratory data, the inventory used for the life cycle assessment were derived from a reliable estimation of the inventory data from other studies. In particular, the effectiveness and efficiency of the lipid extraction processes, which also affects their environmental impact, depended on the combination of several factors, such as the selectivity of the solvents employed and the temperature to which the process takes place, for example. Further optimizations would be required in order to reduce the environmental impact of the process. In this sense, the results obtained may indicate the direction to achieve this goal.

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Appendix

Appendix A

To calculate the energy spent with each equipment and therefore with each process the following calculation was used. The calculation presented below is an example made with the MAE centrifuge data.

$$\text{Energy (J)} = \text{Power (W)} * \text{Time (s)}$$

Data used:

Power = 240 W

Time = 300 s

$$\text{Energy (J)} = 240 * 300 = 72\,000\ \text{J}$$

Appendix B

The values obtained through SimaPro for method SFE and used to elaborate the graphics in the chapter 4.3.2 are presented below. The Table A.7 contains the values with Landfill and the Table A.8 the values to Composting.

Table A. 7- Data used to supercritical fluid extraction with landfill.

Category of damage	Unity	Total	Process	Landfill
Total	Pt	5,260878	5,260605	0,000273
Human health	Pt	1,74248	1,742432	4,75E-05
Ecosystem quality	Pt	0,043367	0,043359	8,26E-06
Climate change	Pt	2,028381	2,028198	0,000183
Resources	Pt	1,44665	1,446616	3,38E-05

Table A.8 - Data used to supercritical fluid extraction with composting.

Category of damage	Unity	Total	Process	Composting
Totalt	Pt	5,270182	5,260605	0,009577
Human health	Pt	1,746101	1,742432	0,003668
Ecosystem quality	Pt	0,043845	0,043359	0,000486
Climate change	Pt	2,031066	2,028198	0,002869
Resources	Pt	1,44917	1,446616	0,002554

Appendix C

The values obtained through SimaPro for method MAE and used to elaborate the graphics in the chapter 4.3.2 are presented below. The Table A.9 contains the values of impact with landfill for the solid residues and incineration for effluents, the Table A.10 the values to incineration both residues and Table 11 present the values with landfill.

Table A.9 - Data used to microwave assisted extraction with landfill.

Category of damage	Unity	Total	Process	Landfill	Incineration
Totalt	mPt	38,55409	29,21641	0,031061	9,306616998
Human health	mPt	12,12769	10,02141	0,005409	2,100869025
Ecosystem quality	mPt	0,561188	0,249092	0,000941	0,311154933
Climate change	mPt	14,96733	9,776464	0,020869	5,169999176
Resources	mPt	10,89788	9,169445	0,003842	1,724593863

Table A.10 - Data used to microwave assisted extraction with incineration.

Category of damage	Unity	Total	Process	Incineration
Totalt	mPt	39,21349	29,21641	9,997077
Human health	mPt	12,27814	10,02141	2,256733
Ecosystem quality	mPt	0,583332	0,249092	0,33424
Climate change	mPt	15,33003	9,776464	5,553563
Resources	mPt	11,02199	9,169445	1,852542

Table A.11 - Data used microwave assisted extraction with landfill.

Category of damage	Unity	Total	Process	Landfill
Totalt	mPt	29,66614	29,21641	0,449725
Human health	mPt	10,09973	10,02141	0,078322
Ecosystem quality	mPt	0,26271	0,249092	0,013618
Climate change	mPt	10,07862	9,776464	0,302152
Resources	mPt	9,225078	9,169445	0,055632

Appendix D

The values obtained through SimaPro to the method SWE and used to elaborate the graphics in the chapter 4.3.3 are presented below. The Table A.12 contains the values with landfill and incineration for the waste treatment, the Table A.13 the values to incineration and Table A.14 the data of landfill.

Table A.12 - Data used to subcritical water extraction with landfill and incineration.

Category of damage	Unity	Total	Process	Landfill	Incineration
Totalt	mPt	40,25882	28,59401	0,110582	11,55422844
Human health	mPt	12,27757	9,650069	0,019258	2,608243219
Ecosystem quality	mPt	0,614698	0,225049	0,003349	0,386300971
Climate change	mPt	17,28675	10,79387	0,074296	6,418589219
Resources	mPt	10,07979	7,92502	0,013679	2,141095035

Table A.13 - Data used to subcritical water extraction with incineration.

Category of damage	Unity	Total	Process	Incineration
Totalt	mPt	42,6064	28,59401	14,01239
Human health	mPt	12,81322	9,650069	3,163147
Ecosystem quality	mPt	0,693535	0,225049	0,468487
Climate change	mPt	18,57801	10,79387	7,784144
Resources	mPt	10,52163	7,92502	2,596613

Table A.14 - Data used to subcritical water extraction with landfill.

Category of damage	Unity	Total	Process	Landfill
Totalt	mPt	29,22436	28,59401	0,630357
Human health	mPt	9,759849	9,650069	0,10978
Ecosystem quality	mPt	0,244137	0,225049	0,019088
Climate change	mPt	11,21738	10,79387	0,423512
Resources	mPt	8,002997	7,92502	0,077977

Appendix E

The values obtained through SimaPro to the method UAE and used to elaborate the graphics in the chapter 4.3.4 are presented below. The Table A.15 contains the values to landfill and incineration, the Table A.16 the values to incineration and the Table A.17 the values to landfill.

Table A.15 - Data used to ultrasound assisted extraction with landfill and incineration.

Category of damage	Unity	Total	Process	Landfill	Category of damage
Totalt	Pt	4,361002	3,177549	0,009195	1,174258076
Human health	Pt	1,337463	1,070786	0,001601	0,265076173
Ecosystem quality	Pt	0,064567	0,025029	0,000278	0,039259829
Climate change	Pt	1,85632	1,19782	0,006178	0,652322244
Resources	Pt	1,102652	0,883914	0,001137	0,21759983

Table A.16 - Data used to ultrasound assisted extraction with landfill.

Category of damage	Unity	Total	Process	Landfill
Totalt	Pt	4,55621	3,177549	1,378661
Human health	Pt	1,382004	1,070786	0,311218
Ecosystem quality	Pt	0,071122	0,025029	0,046094
Climate change	Pt	1,963692	1,19782	0,765872
Resources	Pt	1,139392	0,883914	0,255477

Table A.17 Data used to ultrasound assisted extraction with incineration.

Category of damage	Unity	Total	Process	Incineration
Totalt	Pt	3,239569	3,177549	0,06202
Human health	Pt	1,081587	1,070786	0,010801
Ecosystem quality	Pt	0,026907	0,025029	0,001878
Climate change	Pt	1,239488	1,19782	0,041669
Resources	Pt	0,891587	0,883914	0,007672

Appendix F

In this appendix the quantities of microalgae needed to produce 1 kg of lipids with each studied considering the efficiency are presented in Table A.18.

Table A.18 - Quantity of microalgae necessary to produce 1 kg of lipids to each method.

Methods	***Quantity of microalgae***
SFE	75.5287
MAE	13.05142
SWE	33.57958
UAE	1587.302