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Inês de Jesus Brochado Ferreira

Extração de óleo de bolota por alta pressão

Acorn oil extraction by high-pressure

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Dissertação apresentada à Universidade de Aveiro para cumprimentos dos requisitos necessários à obtenção do grau de Mestre em Bioquímica Alimentar, realizada sob a orientação científica do Professor Doutor Jorge Manuel Alexandre Saraiva, Professor Associado do Departamento de Química da Universidade de Aveiro, da Professora Doutora Maria Manuela Estevez Pintado, Professora Associada da Escola Superior de Biotecnologia da Universidade Católica Portuguesa, e da Doutora Elisabete Maria da Cruz Alexandre, Investigadora do Departamento de Química da Universidade de Aveiro.

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o júri

Presidente	Professor Doutor Carlos Pedro Fontes Oliveira Professora Auxiliar do Departamento de Química da Universidade de Aveiro
Arguente	Doutora Glenise Bierhalz Voss Investigadora do Centro de Biotecnologia e Química Fina (CBQF) da Escola Superior de Biotecnologia da Universidade Católica Portuguesa
Orientador	Professor Doutor Jorge Manuel Alexandre Saraiva Professor Associado do Departamento de Química da Universidade de Aveiro

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palavras-chave

Extração assistida por alta pressão, tecnologia emergente, óleo de bolota, ácidos gordos, atividade antioxidante

resumo

Nas últimas décadas, o aumento da preocupação do consumidor com a saúde, a procura de um estilo de vida saudável, e a preservação do meio ambiente foram os principais fatores que levaram a considerar a utilização de tecnologias verdes. A alta pressão é uma tecnologia emergente que se tem vindo a destacar para a extração de compostos bioativos. A bolota é um fruto muito abundante em Portugal, mas ainda é subvalorizada, apesar do seu perfil lipídico ser muito semelhante ao do azeite. Assim, este trabalho teve como objetivo valorizar a bolota através da otimização da extração do óleo deste fruto por alta pressão e posterior avaliação do seu perfil lipídico e da atividade antioxidante. Várias condições foram estudadas para melhorar a extração do óleo (solventes, métodos, rácios bolota:solvente, etc.), tendo-se optado por estudar através de um desenho experimental fatorial o impacto da pressão (0.1, 250 e 500 MPa), tempo de extração (5, 12.5 e 20 min) e temperatura (10, 25 e 40 °C) no rendimento de óleo, no teor de ácido oleico e de ácidos gordos saturados, monoinsaturados e polinsaturados totais presentes no óleo de bolota.

A metodologia de resposta de superfície foi empregue para analisar os resultados, tendo-se determinado depois as condições ótimas previstas pelos modelos que foram devidamente validados. Os modelos mostraram um ajuste satisfatório e adequado aos dados experimentais e as correlações dos modelos indicaram que os modelos podem ser utilizados para prever os resultados. No entanto, os resultados previstos e os experimentais diferiram mais de 45%, sendo necessário reforçar com mais ensaios de validação no futuro.

A condição ótima de extração para o rendimento foi de 394 MPa, 5 min e 40 °C, enquanto que para os outros parâmetros analisados foi de 500 MPa, 13 min e 10 °C. Estes extratos apresentaram uma atividade antioxidante por DPPH relevante até 327.65 ± 33.40 µmol TE/ g óleo. Os ácidos gordos principais foram o oleico (58-59%), linoleico (21-23%) e palmítico (14-15%). Os rendimentos obtidos por alta pressão nas condições ótimas foram 19 e 37% inferiores do que o obtido com Soxhlet. No entanto, o óleo extraído por alta pressão a 500 MPa, 13 min e 10°C apresentou uma composição de ácidos gordos similar ao óleo extraido pelo método tradicional com as vantagens adicionais de ter sido obtido 72 vezes mais rápido e apresentar uma maior estabilidade oxidativa.

Os óleos de bolota apresentam boa qualidade nutricional que, quando convenientemente recuperados, podem ter inúmeras aplicações em diversos sectores, e ao mesmo tempo promover o valor das bolotas. As otimizações obtidas neste estudo tornam a tecnologia de alta pressão num processo promissor de extração de óleo de bolota.

keywords

High-pressure assisted extraction, emerging technology, acorn oil, fatty acids and antioxidant activity

abstract

In recent decades, there has been an increasing concern for consumer health, the pursuit of a well-being-oriented lifestyle and the preservation of the environment. These factors led to considering the use of green technologies. High-pressure is an emerging technology that has been highlighted for the extraction of bioactive compounds. Acorn is a very abundant fruit in Portugal, but is still sub-valorised, despite its lipid profile being very similar to that of olive oil. Thus, in this work, it is intended to valorise the acorn by extracting the oil by high-pressure and subsequently evaluate the lipid profile and antioxidant activity of the oil. Several approaches were taken to improve oil extraction (solvents, methods, acorn: solvent ratios, etc.), having opted to study through a factorial experimental design the impact of pressure (0.1, 250 and 500 MPa), extraction time (5, 12.5 and 20 min) and temperature (10, 25 and 40 °C) on oil yield, the content of oleic acid, total saturated, monounsaturated and polyunsaturated fatty acids present in acorn oil. The surface response methodology was used to analyse the results, having then determined the optimal conditions predicted by the models that were properly validated. The models showed a satisfactory and adequate fit to the experimental data and the correlations of the models indicated that the models can be used to predict the results. However, the predicted and experimental results differed by more than 45%, which requires more validation tests in future.

The optimum extraction condition for the yield was 394 MPa, 5 min and 40 °C, while for the other analysed parameters it was 500 MPa, 13 min and 10 °C. These extracts showed a remarkable antioxidant activity by DPPH up to 327.65 ± 33.40 µmol TE/ g oil. In addition, the main fatty acids were oleic (58-59%), linoleic (21-23%) and palmitic (14-15%). The yields obtained by high-pressure in optimal conditions were 19 e 37% lower than that obtained with Soxhlet. However, the oil extracted by high-pressure at 500 MPa, 13 min e 10°C showed a fatty acid composition similar to the oil extracted by the traditional method with the additional advantages of having been obtained 72 times faster and presenting greater oxidative stability.

Acorn oils have good nutritional quality which, when conveniently recovered, can have numerous applications in different sectors, while at the same time valuing acorns. The results obtained in this study make high-pressure technology a promising process for extracting acorn oil.

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Abbreviations and Symbols

ATR	Attenuated total reflection
Cox	Calculated oxidizability value
DESs	Deep eutectic solvents
DMC	Dimethyl carbonate
DMF	N,N-dimethylformamide
DPPH	2,2-diphenyl-1-picrylhydrazyl
EAE	Enzyme-assisted extraction
EG	Ethylene glycol
EtOAc	Ethyl acetate
EtOH	Ethanol
FA	Fatty acids
FAME	Fatty acid methyl esters
FDA	Food and drug administration
FTIR	Fourier transform infrared spectroscopy
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
HP	High-pressure
HPE	High-pressure assisted extraction
Hx	Hexane
HPP	High-pressure processing
ILs	Ionic liquids
IPA	Isopropanol
IR	Infrared spectrum
IV	Iodine value

MalA	Malonic acid
MeTHF	2-Methyltetrahydrofuran
MAE	Microwave-assisted extraction
MUFA	Total monounsaturated fatty acids
NADES	Natural deep eutectic solvent
NADESs	Natural deep eutectic solvents
P	Pressure
PUFA	Total polyunsaturated fatty acids
RSM	Response surface methodology
SE	Soxhlet extraction
SFA	Total of saturated fatty acids
SubFE	Subcritical fluid extraction
SFs	Supercritical fluids
SupFE	Supercritical fluid extraction
T	Temperature
t	Time
Trolox	(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid
Tween 80	Polyoxyethylene- 20 sorbitan- monooleate
UAE	Ultrasound assisted extraction
VOCs	Volatile organic compounds

1. Literature Revision

Traditionally, fruits oils can be extracted using conventional techniques, which usually are time-consuming processes, use high temperatures and the solvents involved present high toxicity [1]. Therefore, the method used for the extraction affects the flavors, nutritional quality and oxidative stability. Emerging technologies such as high-pressure assisted extraction, supercritical and subcritical fluid extractions, ultrasound and microwave assisted extractions, as well as enzymatic assisted extraction have been developed as extraction methods. High-pressure assisted extraction operates under very high pressures, which cause cellular membranes to be less selective [2]. Supercritical and subcritical fluid extraction use the properties of solvents to help the extraction of analytes from samples [3,4]. In the ultrasound and microwave extraction, the application of energy conduce to physical modification of the matrix sample and consequently improving the extraction efficiency [5,6]. Enzyme assisted extraction use specific enzymes to improve the extraction of analytes by polysaccharides hydrolysis and cell wall breakage [7]. Thus, all of these technologies have been used to obtain oils of high quality from different fruits.

1.1. Traditional extraction techniques

These techniques are usually based on the selection of the appropriate solvent and mixture and/or heat to increase mass transfer, increasing the solubility of the compounds [8]. Therefore, the efficiency of these extraction techniques depends on the chosen solvent. Several factors can be considered in selecting a solvent, such as financial viability: environmental safety and human toxicity. However the most important fact is the polarity of the target compound [9].

1.1.1. Extraction by Soxhlet

In 1879, Franz Ritter von Soxhlet first developed Soxhlet extraction (SE) for the determination of fat in milk [10]. Currently, SE is the oldest extraction method, it is no longer limited to lipid extraction, and is the most widely used technique as a benchmark to compare the performance efficiency of other extraction methods, except in restricted fields of applications such as the extraction of thermo-sensitive compounds [11,12]. Generally, the sample is placed in a thimble-holder, which is gradually filled with condensed fresh solvent from a distillation flask. After the liquid reaches an overflow level, the thimble-holder solution is aspirated by a siphon, which discharges the solution back to the distillation flask. This solution transports the extracted analytes to bulk liquid. The solutes remain in the distillation flask and the solvent passes to the solid bed. This operation is repeated until

extraction is complete [10]. Traditional SE has some appealing advantages, such as: the sample being repeatedly brought into contact with the solvent; ensuring complete extraction; the system can maintain a relatively high temperature; no filtration is required after extraction; the low cost of basic equipment facilitates increased sample throughput through multiple parallel extractions [9]. Although SE is simple and easy to perform, there are also disadvantages to its execution such as long extraction time, which increases the cost of extraction; extraction of thermolabile compounds, which undergo thermal degradation due to high temperature; environmental concerns about the large amount of expensive organic waste, because of the use of potential harmful organic solvents such as petroleum ether and hexane (Hx) [9,13].

SE can be used for the extraction of various compounds, such as organic acids and phenolic compounds [14,15]. Besides that, SE is also used to extract oils from fruits (Table 1).

1.1.2. Extraction by hydrodistillation

Hydrodistillation is one of the most commonly used traditional methods for extracting essential oils from fruit peels and seeds [16–18]. As similar to the SE, high temperatures and long extraction times can cause modifications of the oil components and often a loss of some volatile components, which limits their application for thermolabile compound extraction [13]. The use of large amounts of samples is also a disadvantage, but the extraction by hydrodistillation is more environmental friendly because water is the main solvent used [12,19].

In order to isolate an essential oil, hydrodistillation may be performed by three different ways: water distillation, water and steam distillation and direct steam distillation. The water and steam distillation technique consists in a mix of both principals of water distillation and direct steam distillation. Whereas the first one consists in the mixture of plant samples with water in sufficient amount and then boiled, the second one consists in the injection of steam into the sample [20]. During hydrodistillation, the main factors release of bioactive compounds from plant tissues are the action of hot water and steam. Furthermore, this method permits an automatically separation of oil and bioactive compounds from the water because of the indirect cooling by water condensates the vapour mixture of water and oil, which flows from condenser to a separator [20,21].

Table 1. Extraction of oils from fruits by SE.

Source	Analyte	Solvent(s)	T(°C)/ t(h)	Yield (%)	Reference
Pomegranate seeds	Oil	Petroleum benzene and Hx	35/6	18.6 ± 0.2 18.7 ± 0.2	[22]
Goldenberry	Oil	Hx	-/8	19.3	[23]
Grape seeds	Oil	Hx	70/6	14.64 ± 0.29	[24]
Pequi	Oil	Hx and EtOH	-/6	49.58 ± 0.16 52.78± 1.75	[13]
Gardenia fruit	Oil	petroleum ether	45/12	10.89 ± 0.03	[25]
Pomegranate seeds	Oil	Hx	68/6	26.8 ± 0.0	[26]
Sweet passion fruit seeds	Oil	Hx and EtOH	Boiling point/4	28.33 ± 1.60 22.19 ± 1.65	[5]
Pistacia lentiscus fruits	Oil	bio-based solvents	-/8	Between 6.14 and 8.61	[6]
Yellow Passion Fruit seeds	Oil	Hx and EtOH	Boiling point/ 4	26.12 ± 0.74 20.46 ± 1.36	[27]

T: Temperature; t: time; Hx: hexane; EtOH: ethanol.

1.1.3. Extraction by maceration

Maceration is a conventional technique that has become a popular and inexpensive way to obtain essential oils and bioactive compounds [12,28]. This method is frequently used for a small-scale extraction that generally consists on the following steps [12]. The

samples are milled to increase the contact surface area with the appropriate solvent. Then the liquid is filtered, but the solid residue from the extraction is pressed to recover large occluded solutions. Finally, the obtained strained and press liquids are mixed and filtered [12]. As other conventional solvent extraction technologies, this method requires a long extraction period and a large quantity of solvent [28]. Although, its disadvantages are more pronounced than for SE, the organic solvents used in maceration (e.g. water and ethanol (EtOH)) are more friendly to the environment and health [29–31].

Although, high solvent consumption leads to loss of material and/or metabolites, maceration has the advantage over SE to be often done at room temperature, so it is less likely for compounds sensitive to high temperatures to degrade. On the other hand, since extraction is performed at room temperature, some compounds may not be efficiently extracted if they are poorly soluble at room temperature [32].

1.1.4. Cold pressed extraction

The demand of oil production by cold pressing is increasing, because of the increasing interest of consumers for unrefined vegetable oils with good sensory quality [33]. Cold pressing is a mechanical extraction method that can be carried out with a screw press (continuous pressing machine) or hydraulic press (batch mechanical pressing machine) [33–35]. This technology is based on pressing to obtain safe, pure and homogeneous oil bearing materials under very mild operation conditions (usually at room temperature and very low pressure) to not damage both the oil and the meal [36]. The main advantages of this technology are: solvent free; reach a low press temperature; simple to use; ecological and do not cost much investment [33,35]. Furthermore, this technology allows high levels of beneficial phytochemicals and natural antioxidants, because cold pressed do not need refining steps [37]. The major disadvantage is related with the oil yielded from raw material, which is low, although it is possible to reduce it by using pre-treatments and process parameters applied to the raw material [33]. Cold press oils are ready for direct consumption without needing to be refined and they are generally referred as a high-quality oils [33].

1.2. Innovative and emerging extraction technologies

1.2.1. High-pressure assisted extraction

High-pressure (HP) technology started to be applied as processing method at the beginning of the 20th century. Hite (1899) reported that a pressure treatment of 1 hour at 600 MPa (room temperature) could extend the raw milk shelf life for 4 days, proving the effectiveness of pressure to inactivate spoilage bacteria. Nowadays, HP processing (HPP) is recognized as an environmentally friendly and clean technology by Food and Drug Administration (FDA), being extensively used industrially to process different kinds of food products [38]. The effect of HPP is based in two principals: i) **Pascal or Isostatic Principle** that states that the transmittance of pressure is uniform and instantaneous throughout the sample, regardless of the size and geometry of the product. This means that an uniform pressure (isostatic) is applied in all directions of the samples, thus when the pressure is released it allows the product to return to its original shape [37,38]. Since air and water are compressed differently under pressure, the shape and structure of the foods containing air pockets may be altered after pressure treatment, unless the food product is perfectly elastic and have cells from which air cannot escape [39]. ii) **Le Chatelier's Principle** states that any phenomena (phase transition; chemical reaction; change in molecular configuration) which is accompanied by a decrease/increase in volume can be enhanced/counteracted by pressure [40]. Thus, HP affects phenomena when volume changes are involved, favoring reactions that cause decrease in volume and inhibiting reactions that involve an increase of volume [39]. These effects also may alter the bio accessibility of bioactive compounds and thus, the HP technology also can be used for the extraction of valuable compounds that are difficult to release from their matrix [41–43].

HP assisted extraction (HPE) consists in the application of pressures between 100 and 600 MPa, uses any type of solvent and, usually, is performed at room temperature or low-to-mild temperatures, which results in a preservation of the compounds structure, since it is generally accepted that this method mainly affects non-covalent bonds [42,44]. The application of HPE was first described in 2004 by Zhang and co-workers [45]. These authors concluded that HPE can reduce extraction time of some herbals essential components (icariin from *Epimedium;* polyphenols from green tea; flavonoids from *Propolis* and baicalin from *Scutellaria)* and increase process efficiency [45]. The extraction of icariin from *Epimedium* was done through three processes (HPE; reflux and ultrasonic) using 70% EtOH as solvent.

The best yields were obtained by HPE at 600 MPa (10%), followed by ultrasonic (7%) and reflux (7%). In addition, the extraction time was shorter for HPE (5 min), followed by ultrasonic (1 hour) and finally reflux (2 hours). The extraction of polyphenols from green tea with HPE at 380 MPa was compared with the process of boiling water, where the yields and time necessary for extraction were 28% with 10 min and 24% with 45 min by HPE with 80% EtOH and boiling water, respectively. The extraction of flavonoids from *Propolis* was done with HPE at 500 MPa and EtOH leaching and like the others extraction of herbals essential components it was observed a similar yield within a shorter time with HPE using 90% EtOH as a solvent (5% with 1 min), when compared with EtOH leaching (5% with 1.5 hours). The extraction of baicalin from *Scutellaria* was made with HPE and reflux both using methanol as solvent. The results showed better yields at shorter times with HPE (3%), which was tested at 0 MPa, 200 MPa and 600 MPa and the extraction time was 5 min for each, than with reflux (1% with 2.5 hours).

Yan and Xi [46] stated that the pressure had direct mechanical effect on the disruption of organelle and blend of cellular contents. Therefore, when the pressure is released the cell wall disrupts and releases the content, which contains the target compounds [47]. This effect probably facilitates mass transfer between cellular contents, which contains the target compounds, and the solvent that subsequently is the major reason for high efficiency of HPE [45,47]. The main HPE conditions to recovery the target compounds, like bioactive compounds, are: pressure level; temperature; time; type and concentration of solvent; and solvent-to-solid ratio [42]. The main advantages of HPE in relation to conventional extraction techniques are: the extraction of natural products can be done in shorter time; improve the extraction yields; require lower power consumption; demand less solvent consumption; higher purity of the extracts obtained; originate no negative effects on the biological function and structure of molecules of interest with low molecular weight and covalent-bond-stabilized compounds, such as the bioactive compounds [39,42]. Moreover, this extraction technique results in the retention of thermo-sensitive components [44,48]. Regarding HPE of oil, HP was already used in the direct extraction of oil from papaya seeds [2] and house cricket and yellow mealworm [49].

1.2.2. Other extraction technologies

The quality of the oil is mainly determined by the process and the raw materials [50]. To suppress the disadvantages of the traditional extraction methods, alternative oil extraction technologies, which are more environmentally friendly and provide high quality products, need to be developed [1].

Enzyme-assisted extraction

Enzyme-assisted extraction (EAE) for oil extraction is considered to be an environmentally clean technology [51]. Enzymes are nontoxic and they have the inherent ability to catalyse reactions with high specificity, disrupt or degrade cell walls and membranes and operate under mild conditions in aqueous solutions (temperature, pressure and pH) [7]. Therefore, this technology improves the extraction of compounds by polysaccharides hydrolysis and cell wall breakage, since some compounds are hardly accessible with a solvent at the conventional extractions [7]. In the extraction process, water is used as a separation medium, since oil is not dissolved in water, and the oil extraction should be performed the way in which the amount of enzyme used is reduced [33]. After the extraction, a centrifugal separation of the slurry into oil, emulsion, and the aqueous and solid phases occurs [52].

EAE has advantages when compared with traditional methods, such as lower operational costs, faster extraction and the use of an aqueous medium and mild conditions, what is important in the extraction of thermolabile compounds and results in a substantial energy saving [53-55]. However, the non-availability of enzymes on a commercial scale has limited the development of such processes [56].

Ultrasound assisted extraction

The history of scientific advances of ultrasound are rooted in the study of sound, with Sir Isaac Newton first proposing his theory of sound waves in 1687 [57]. The major difference between sound and ultrasound is the wave frequency [57]. Ultrasound waves have frequencies above the audible range (10 Hz-20 kHz) and less than microwave frequencies (up to 10 MHz) [12,58]. UAE is based on the application of the energy from sound waves that provokes compression and expansion cycles in a medium [5]. During propagation of this mechanical vibration, these pressure changes cause the production, growth, and collapse of a series of microbubbles inside the liquid phase [57,59]. As a consequence, large amounts

of energy are released generating macroturbulences, which are responsible for the plant tissue disruption and release of intracellular compounds into the solvent, resulting in an increased mass transfer from solutes to the solvent in short extraction time [11,12].

UAE can be accomplished by two types of ultrasound equipment: ultrasonic probe system fitted to horn transducer and ultrasonic water bath [11]. The major advantage of this technology is that transducers are not in direct contact with the sample. In addition, UAE is also simple to operate, the equipment is relatively inexpensive, it presents high efficiency that derives from the mechanical effects, uses a reduced solvent/solid sample ratio and reduces the extraction times and temperatures, which minimizes the loss of thermolabile compounds [11,27,60]. However, it presents some disadvantages, for example, the selectivity of the extraction is normally low; the solvent cannot be renewed during the process, and it requires not only filtration but also solvent evaporation [5].

Supercritical fluid extraction

Although the first literature report of supercritical fluid extraction (SupFE) was in 1879 by Hannay and Hogarth, it was not until around 1960 that this technique started to be investigated as an alternative to traditional extraction methods [61,62]. SupFE is characterized mainly by the extraction of analytes from the matrix with supercritical fluids (SFs), which are substances that are in an aggregated state at pressures and temperatures above their critical point [3,63]. In the supercritical region, the SFs have intermediary properties between liquids and gases, hence these have a wide capacity to dissolve analytes that are less soluble in separate liquid or gas state [64,65]. Moreover, SupFE shows high selectivity, elimination of clean-up steps, low extraction time and does not leave solvent residues in the extract, therefore it has wide applications in pharmaceutical, cosmetic and food industries [62–64].

Carbon dioxide is the most widely used SF for its advantages, such as a low-cost and non-toxic solvent [63]. In the supercritical region, CO_2 exhibits a low polarity, which makes it ideal for the extraction of lipophilic substances, but unsuitable for polar substances [12,64]. However, the use of this solvent has some drawbacks, like being matrix dependent and affected by the mass transfer rate between the fluid and the solute, heavy solutes can clog the exit restrictor and at higher pressures the solute could be pushed out the column if the density of SFE with CO_2 is bigger than the solute [11,66].

Subcritical fluid extraction

Subcritical fluid extraction (SubFE) is based on the use of solvents at a temperature between its boiling point and critical temperature and at a pressure below the critical point able to maintain the solvent in a liquid state, which improves the solvents transport properties [4,67,68]. SubFE is accomplished at temperature and pressure below the critical point contrarily to those employed in SupFE [67,69].

The advantages of SubFE extraction over conventional extraction methods are: shorter extraction time; efficiency; safety; preservation of oil quality due to low extraction temperature; reduction of the cost of utilities and total investment [67,70]. Furthermore, low residual solvent in the oil is attributed to the reduction of the subsequent refining process [70]. Among the various subcritical fluids used, n-butane and propane are the most used in oil extraction, because they have excellent dissolving power for lipophilic compounds and can be used at shorter extraction times, lower critical pressures and temperatures, leading to a high quality product with minimum damage [5,71].

Microwave assisted extraction

Although the use of microwave energy was mentioned for the first time in 1975, the first patent for extraction of natural product using microwave-assisted extraction (MAE) was filled by Pare in 1995 [72]. MAE is a green technology that establishes the direct effects of high frequency (300 MHz to 300 GHz) nonionizing electromagnetic radiation on the molecules of the sample [12,72]. The energy is converted to heat by ionic conduction and dipole rotation mechanisms [12]. The extraction mechanism of MAE involves the following steps: evaporation of the intracellular moisture under increased temperature; physical modification of the matrix generated by the HP on the cell walls and organelles; diffusion of solvent across sample matrix and release of solutes from sample matrix to solvent improving the extraction efficiency [9,12,69].

MAE has been defined as an effective tool for oil extraction due to its benefits over SE, such as a high yield in a short extraction time; less production of waste; selective heating of the sample solvent mixture; and the fact that the energy homogeneously heats the sample, which leads to rapid energy transfer, lower energy consumption and higher volume of raw material processed over a given period [11,73,74]. However, compared with other emerging extraction techniques, MAE needs an additional stage after extraction for the removal of

solid residues and the efficiency of microwaves can be poor when the target compounds are nonpolar, volatile or thermolabile [11].

Comparison of different extraction methods

Table 2 shows some examples of these emerging technologies described above for extraction of oil from fruits and seeds. Mariano and co-workers [51] compared pequi pulp oil yield when applying four methods: aqueous extraction at 80 °C; aqueous enzymatic extraction; hydraulic pressing and enzymatic hydrolysis combined with hydraulic pressing at room temperature. The extraction efficiency of pressing (40%) has presented higher efficiency and lower oxidation of unsaturated fatty acids. Therefore, pequi pulp pressing at room temperature has produced better quality oil. However, its efficiency is still smaller than the combined enzymatic treatment and pressing process, because the enzymatic treatment favours the extraction efficiency in about 20%.

Table 2. Extraction of oils from fruits by emerging technologies.

Emerging technology	Source	Analyte	Solvent(s)	Reference
EAE	Pequi pulp	Oil	Water	[51]
UAE	Yellow Passion Fruit	Oil	EtOH	[25]
SupFE	Pomegranate seeds	Oil	Carbon dioxide	[22]
SubFE	Yellow passion fruit seeds	Oil	Propane	[27]
MAE	Pomegranate seeds	Oil	Hx and Petroleum benzene	[22]
HPE	Papaya seeds	Oil	Hx	[2]

T: Temperature; t: time; EtOH: ethanol; Hx: hexane.

Cai and co-workers [25] compared UAE of yellow passion fruit oil with three conventional extraction, which used petroleum ether as a solvent, except cold pressed extraction. The SE at 45 °C was found to have the highest extraction yield (11%), followed by UAE (9%), traditional extraction at 80 °C and stirred at 400 rpm (7%) and cold pressed extraction (6%). UAE was studied with four organic solvents (Hx, cyclohexane, petroleum

ether, and ethyl acetate), from which petroleum ether proved to be preferable for a higher oil yield, while a higher proportion of unsaturated fatty acids to saturated fatty acids was achieved with ethyl acetate (EtOAc).

Pereira and co-workers [27] studied the effect of SubFE with compressed propane, UAE with EtOH and SE with two different solvents on the extraction of oil from yellow passion fruit seeds. The highest extraction yields obtained were 26% and 25% for SE with Hx and SubFE (30 °C and 8 MPa), respectively, followed by UAE with 21%. The results indicated that the compressed propane extraction could be performed to obtain a highly nutritious oil with good antioxidant and antimicrobial activities.

Abbasi and co-workers [22] studied the extraction of essential oils from pomegranate seeds using SupFE with CO_2 and organic solvents applying in four extraction methods (normal stirring, SE, microwave irradiation and ultrasonic irradiation). The results show that the extraction yields with SupFE in all different conditions tested were lower than the other extraction methods using organic solvents (petroleum benzene and Hx). The best oil yields were with SE (19%), ultrasound (16%) and microwave irradiation at 800 W (16%), followed by normal stirring (13%) and SupFE at 40 °C, 28 MPa and EtOH as modifier (3%). Although SupFE presented low oil extraction yield, the results from the fatty acids (FA) compositions of the oils obtained by this technology indicated that these kinds of extractions could be more selective than the other four extraction methods.

Briones-Labarca and co-workers [2] studied the extraction of oils from papaya seeds using two extraction methods (HPE and SE) with Hx. The highest extraction yields obtained were 41% and 32% for HPE (500 MPa, 15 min and room temperature) and SE, respectively. The results indicated that HPE could be performed quickly to achieve papaya oils with better FA compositions compared to the oil extracted by SE.

1.3. Extraction solvents

The use of organic solvents from nonrenewable resources has become a worry matter due to the rapid growth of plastic and chemical industries, since most organic solvents are volatile, flammable, and are responsible for health and environmental problems [75,76]. For example, Hx is still used as an organic solvent for extraction of vegetable oils, because of its various qualities, however it is highly volatile, causing environmental problems [75,77]. Therefore the development of green chemistry has prompted chemists to search for greener,

non-dangerous and biodegradable solvents, yet equally effective like traditional solvents [78,79].

The concept of green chemistry is governed by 12 principles (Figure 1) which are mainly aimed at designing of chemical products and processes that reduce or eliminate the generation and use of hazardous substances [80]. These principles provide a good basis to develop more environmentally acceptable experiments [80]. Developing less hazardous and more environmentally benign solvents is perhaps one of the most active areas of green chemistry. Such solvents are called green solvents [78,79].

In order to be identified as green, a solvent should fulfil the 12 principles of green chemistry (Figure 1). Some of the parameters and pre-requisites that a solvent should possess to be qualified as a green solvent are: to be from renewable raw materials, exhibit similar properties as common solvents, have a high boiling point and low vapor pressure and be deemed harmless or easily reused by the environment, etc. [81,82].

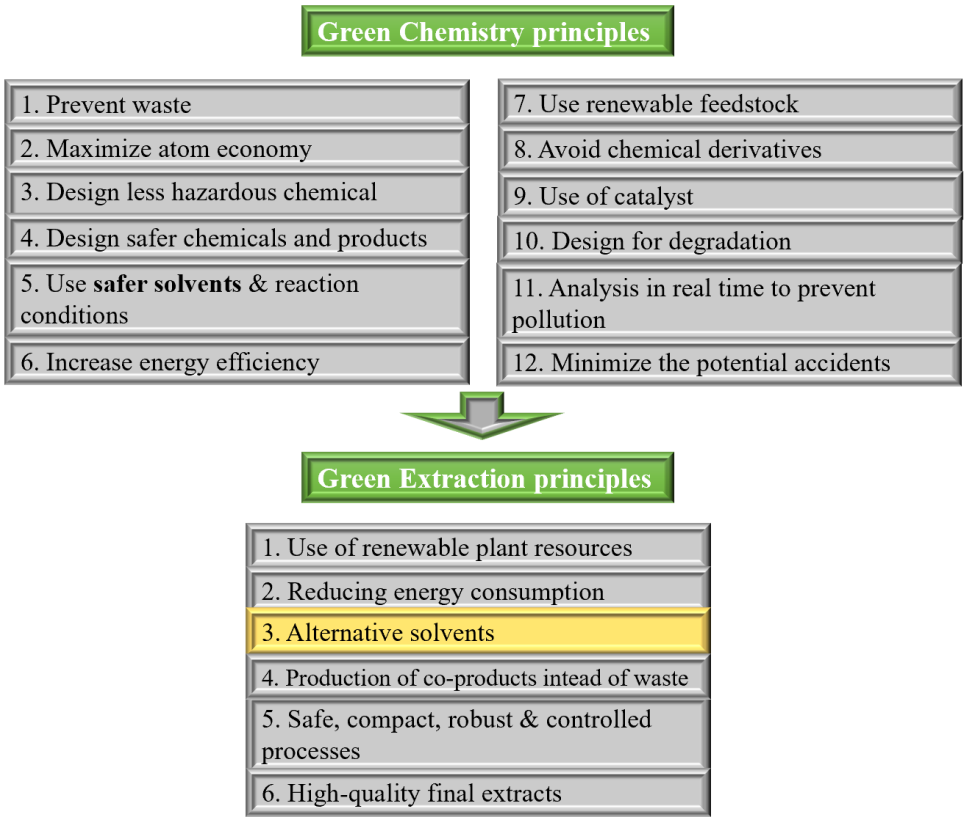

Figure 1. The evolutionary alternative solvent principle from Green Chemistry to Green Extraction, based on Zhuang and co-workers [83].

Recently, the green solvents for more sustainable and green separation processes, have been classified in six main solvent classes: a) bio-based solvents (such EtOH, ethyl lactate, D-limonene, etc.), b) ionic liquids (ILs), c) deep eutectic solvents (DESs), d) natural DESs (NADESs), e) aqueous solvent systems, and f) switchable solvent systems [84].

1.3.1. Bio-based solvents

Bio-based solvents are biodegradable solvents that are produced from agricultural biomass (renewable sources) and they often exhibit comparable properties to traditional solvents [85,86]. These solvents can be prepared from 4 different biomass sources categories (sugar and starch, lignocellulosic, protein and oil based and other forestry and food wastes) by fermentation or chemical transformation processes [82]. Furthermore, these solvents can be classified based on their functional groups (alcohols, esters, ethers and terpenes) or based on the conventional solvent they were intended to replace [82].

Bio-based solvents have many advantages for instance a high solvent power; low toxicity, non-flammable and low environmental impact [86,79]. Their limitations are due to cost; high boiling point; high viscosity and generation of off- flavors [86].

Market cost level

When assessing solvent economics it is important to notice sustainable practices, such as recycling and volume reduction [87]. Although process efficiency improvements can do these sustainable practices, there are some economic factors that move bio-based solvents to replace conventional solvents [87].

The organic solvents used for extraction processes have been predominantly volatile organic compounds (VOCs) [88]. The price of crude oil, which is the main raw material for VOCs, has experienced rising with some fluctuations. Considering the health and environmental concerns as well as the increasing price and unsteady supply of VOCs, this poses further problems to their utilization. These problems encourage the exploration of renewable alternatives, which have a more secure supply of solvent [88,89].

In search of suitable alternative solvent for lipid extraction, studies have shown that bio-based solvents could be used in these cases [90]. However, bio-based solvents are confined by a significant economic barrier: the cost. In the future, this barrier could significantly be reduced by developments in bio-based solvent production [87]. Table 3

shows a review of the solvent volume, cost, biomass and the extraction yield for extractions with Hx (reference solvent) and bio-based solvents.

Table 3. Market cost level and efficiency of oil extraction solvents. The extraction cost was based on https://www.alibaba.com/ [175].

Solvent	Solvent volume (mL)	Extraction Cost (euros)	Biomass mass (g)	Oil yield (%)	Reference
Hx	20-300	0.10-1.80	5-30	7.71–52.6	[5,6,13,22,26,27]
MeTHF	250-300	1.14-1.36	15-30	5.02-23.7	[6,91,92]
D-limonene	42- 300	0.42-2.51	25-30	7.41-44.9	[6,92,93]
EtOH	100-300	0.07-0.21	5-30	7.52-86.5	[5,6,81,83]
IPA	30-300	0.11-1.16	5-30	15.9-86.5	[6,83,94]
EtOAc	200- 300	0.16-0.24	20-30	8.30-19.2	[6,95]
DMC	100-300	1.93-5.79	5-30	8.09-95.7	[6,83]
ethyl lactate	300	0.02	30	7.54	[6]
p-cymene	300	0.02	30	6.14	[6]
α-pinene	300	0.46	30	5.67-67.2	[6,96]

Hx: hexane, MeTHF: 2-methyltetrahydrofuran, EtOH: ethanol, IPA: isopropanol, EtOAc: ethyl acetate, DMC: dimethyl carbonate.

Chaabani and co-workers [6] classified the lipid classes of *Pistacia lentiscus* oil obtained by Hx and some bio-based solvents. According to these authors, the extracts obtained with 2-methyltetrahydrofuran (MeTHF), EtOAc, dimethyl carbonate (DMC), *p*-cymene and Hx have more triacylglycerols, while the extracts of isopropanol (IPA) and EtOH are richer in phospholipids, since alcohols have a good solvating power of polar lipids. Contrarily, ethyl lactate, limonene and α-pinene have a different lipid class profile, characterized by a high monoacylglycerol and diacylglycerol content. Although these solvents present significant differences in the final constitution of the lipid extract, since limonene, ethyl lactate, α-pinene and *p*-cymene have a high boiling point (176, 154, 155,

177 ºC, respectively) and their elimination is not possible by conventional evaporation under reduced pressure [6].

D-limonene and α-pinene are the most popular terpenes used as solvents, because some of their physicochemical properties are similar to Hx properties [79]. Furthermore, ethyl lactate and *p*-cymene are cheaper that Hx, which is an important feature since most bio-based solvents are more expensive than Hx. However limonene, ethyl lactate, α-pinene and *p*-cymene are not suitable for oil extraction, since these solvents have a high boiling point, which affects their efficiency and induce lipid degradation [6].

Alcohols, such as EtOH and IPA, appear to be economically viable and environmentally safe [83]. EtOH is the most common bio-based solvent, however, it is a flammable and potentially explosive. This solvent, obtained by the fermentation of sugar-rich materials, has positive points like a low price and is completely biodegradable [86]. Considering Table 3, EtOH also provides a higher yield than Hx. However, the level of benefit equivalent to IPA is disadvantageous. Despite offering the same yields as EtOH, IPA is more expensive (Table 3).

DMC provided higher extraction yields compared with the other bio-based solvents and Hx (Table 3), however the selection of an alternative solvent has to consider some parameters, such as: financial viability, solubility, boiling point, toxicity index and energy required for the evaporation of the solvent [6].

Chaabani and co-workers [6] reported that MeTHF and EtOAc were the most suitable alternative solvents for Hx, taking into account global yield, lipid composition and statistical analysis. Furthermore, based on economic, solubility and energy parameters MeTHF was found to be the best alternative to replace Hx for the extraction of Lentisk fruit oil [6]. This solvent requires practically the same energy to evaporate as Hx and less energy to evaporate than DMC and EtOAc [6]. Given this, the most suitable solvent to use in this food matrix was MeTHF. However, despite being less toxic than Hx [6], it is not considered food grade due to its toxicity.

According to Directive 2009/32/CE of the European parliament and the 23rd of April (Directiva 2009/32/CE do Parlamento Europeu e do Conselho de 23 de Abril de 2009) [176] from all of the above mentioned bio-based solvents only EtOH and EtOAc are considered food grade.

1.3.2. Ionic liquids and deep eutectic solvents

ILs are a group of non-molecular solvents prepared by the combination of organic cations and organic or inorganic anions [82]. These solvents have attracted interest as an alternative for volatile organic solvents, because of their unique physicochemical properties [79]. However their "green" aspect has been challenged due to their poor biodegradability and biocompatibility [82]. Furthermore, ILs have other limitations such as toxicity and high cost [98]. All of these factors make their life cycle assessment very negative from the point of view of environmental sustainability [99]. To overcome the limitations of ILs, DESs have been emerging as a sustainable alternative to ILs derived from green natural and renewable components [79,82]. DESs, as a subclass of ILs, show comparable characteristics to ILs, but with additional advantages, like they are cheaper and easy to be produced due to the low cost of their starting materials, often biodegradable and less toxic [100]. Unfortunately, the high viscosity and solid state of most DESs at room temperature restricts their application as extraction solvents [79,99].

DESs belong to a group of solvents prepared by mixing halide salt or a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD), which form a eutectic mixture with a melting point lower than either of the individual components melting points [99,101]. Some DESs are also known as low transition temperature mixtures, because their melting point is not found but instead a glass transition temperature is obtained [102]. The vast hydrogen bond network between HBA and HBD is responsible for some characteristics of DESs, such as their low melting points, low vapour pressure, chemical and thermal stability, non-flammability and low volatility [103].

These solvents can be described by the general formula: $Cat^+X^- zY$, in which Cat^+ represents any ammonium, phosphonium, or sulfonium cation; X^- is a Lewis base, generally a halide anion; Y is a Lewis acid; and z is the number of molecules of Y molecules that interact with the anion [102,104]. This formula is used to classify four types of DESs depending on the nature of the complexing agent used (Table 4) [91].

The DESs from type I formed from MCl_x and quaternary ammonium salts (Table 4), can be considered to be of an analogous type to the well-studied metal halide/ imidazolium salt systems [101]. However, the range of nonhydrated metal halides that have a suitably low melting point to form type I DESs is limited. The DESs from type II entailed better applications due the use of hydrated metal halides and choline chloride. The low cost of

many hydrated metal salts and their inherent air/moisture insensitivity makes them a good option for industrial processes. The third type, formed from choline chloride and hydrogen bond donors, are the most studied due to their advantages, such as simple to prepare, relatively unreactive with water, many are biodegradable, low cost of their starting materials, etc. [102,104]. The type IV, a range of transition metals that can be incorporated into ambient temperature, rarely has been characterized. They are produced with a range of transition metals that can be incorporated into ambient temperature [101,104]. The relatively high polarity and ionic nature of DESs make them compounds with high solubility for polar compounds and not for nonpolar compounds [105]. Some applications of the DESs in separation are: extraction of bioactive compounds; extraction of sulfur compounds and nitrogen compounds from fuel oils; extraction of phenolic compounds in oils and other separations [105]. DESs are not yet used for oil extraction, however their use for extracting oil analytes has been documented, Table 5. This could be related with the ionic nature and relatively high polarity of these solvents, which makes them have a high solubility for polar compounds and not for nonpolar compounds [80,105,106]. The increased solubility for compounds, means that DESs can increase the extraction efficiency [98].

Table 4. General formula for the classification of DESs, based on Mbous and co-workers [101].

Type	Formula	Term
I	$Cat^+X^-zMCl_x^-$	**M**: Zn, Sn, Fe, Al, Ga, In
II	$Cat^+X^-zMCl_x \cdot yH_2O$	**M**: Cr, Co, Cu, Ni, Fe
III	Cat^+X^-zRZ	**Z**: $CONH_2$, COOH, OH
IV	$MCl_x + RZ = MCl^+_{x-1} \cdot RZ + MCl_{x+1}$	**M**: Al, Zn; **Z**: $CONH_2$, OH

1.3.3. Natural deep eutectic solvents

NADESs have been introduced as a green alternative to DESs and were presented by Choi and co-workers [107] who defined them as the third liquid phase naturally occurring in all living organisms and cells, that play an important role as an alternative medium for biosynthesis, transport and storage of compounds with intermediate polarity [82,107,108].

Table 5. Extraction of analytes from oils with DESs.

Sample matrix	Analytes	DESs composition (molar ratio)	References
Edible oils	Pb and Cd	ChCl:urea (1:2)	[106]
Edible oils	Plant growth regulators	Tetramethylammonium Chloride:EG (1:3)	[109]
Virgin olive oil	Phenolic compounds	ChCl:xylitol (2:1)	[110]
Crude palm oil	Tocols	ChCl:MalA (1:1)	[111]
Olive, almond, sesame, cinnamon oil	Phenolic acids	ChCl-EG (1:2) ChCl- Glycol (1:2)	[112]

DESs: deep eutectic solvents, ChCl: choline chloride, Pb: lead, Cd: cadmium, MalA: Malonic Acid, EG: ethylene glycol

NADESs can be obtained by heating a mixture of two or three components in certain molar ratios with or without the presence of water, including HBA, which are usually nontoxic quaternary ammonium salts, amines (ChCl, ammonium chloride) or amino acids (alanine, proline, glycine, betain), and HBD that are normally organic acids (oxalic acid, lactic acid, malic acid, etc.) or carbohydrates (glucose, fructose, maltose, etc.) [107–108, 113, 114]. The number of these natural compounds and the arrangements of molecules is estimated to ascend to 10^6, so NADESs have high versatility and virtually unlimited number of combinations [114]. Additionally, these solvents are generally composed of nontoxic substances occurring naturally in foods, therefore majority of this combinations are regarded as of low toxicity and may be directly incorporated in food formulations, being a major advantage over conventional solvents [107,108].

Like DESs also in NADESs, the charge delocalization decrease the melting point of the mixture relative to the melting points of individual components [115]. NADESs solvents can be classified into 4 groups depending on nature of their components: (1) derivatives of organic acids, (2) derivatives of choline chloride, (3) mixtures of sugars and (4) other combinations [82]. These solvents have been attracting great attention of the scientific community no only due to their environmental and economic benefits (including low costs,

readily available components, a low toxicity profile, sustainability, simple and ease preparation of NADESs with high purity and without waste generation) but also due to their good physicochemical properties (negligible volatility, very low melting point, adjustable viscosity, a broad polarity range, chemical and thermal stability, non-inflammability and a high solubilisation) [108, 114, 116]. Therefore, these solvents fully comply the 12 principles of green chemistry [115].

Polarity of NADESs

Polarity is one of the most important properties of NADESs, since it affects theirs solubilizing capacity [105,108]. Majority of NADESs reported are hydrophilic, while hydrophobic NADESs were reported for the first time in 2015 by Van Osch and co-workers [117] and Ribeiro and co-workers [118]. These were tested for the extraction of volatile FA and other molecules from an aquatic environment [119]. Although their utilization is yet to be explored some papers about their use were published [108,119]. These include the removal of furfural, hydroxymethylfurfural and metal ions by the use of membrane technology and pesticides from water [119].

Hydrophobic NADESs may improve the effectiveness of certain extraction procedures due to their ability to extract non-polar analytes from aqueous solutions. Additionally, these solvents can be applied in different pH environments, for example they have the ability to extract both dissociated and undissociated forms of acidic compounds [109,118]. Therefore, these solvents are promising, but they need several improvements, for example the use of more natural components [119].

So far lipid extraction has only been reported by Van Osch and co-workers (2015) [117]. These authors used hydrophobic NADESs which consisted of decanoic acid and various quaternary ammonium salts, including tetrabutylammonium chloride, methyltrioctylammonium chloride, tetraheptylammonium chloride, tetraoctylammonium chloride, methyltrioctylammonium bromide and tetraoctylammonium bromide [117]. Besides these new NADESs were successfully evaluated for the recovery of volatile FA, the use of NADESs with quaternary ammonium salts is not the best from an environmental point of view [119]. It is the idea to overcome this problem by the use of natural components, such as terpenes [108,119].

1.4. Acorn

There is a huge diversity of acorn plant species around the world, however just a few of them are actually used in human consumption [120]. Besides, the usual plant sources show interesting nutritional characteristics, so acorns have been study on a larger scale [120].

Acorn (Figure 2) is a term to identify the fruits produced by several trees belonging to the *Quercus* genus, which is comprised of around 450 species that are generally found in sub-tropical climate areas in a diversity of places around of the world, including Europe [121–123].

In Portugal, the most common *Quercus* genus species are *Quercus faginea* (Oak), *Quercus ilex, Quercus rotundifolia* (Holm Oak) and *Quercus suber* (Cork Oak) [123]. *Quercus* species are abundant in this territory occupying 1 200 000 he, an area much bigger than that is dedicated to chestnut and almond [124]. However, acorns are currently far from being as widely used as other common fruits and according to Miguel Sottomayor (2015) [128] 55% of the acorns in Portugal " are being wasted" [121]. In past times of scarcity, the acorns were used for human and animal consumption and to support the local economy [126]. Nowadays, some products made from acorns are already on the market, but oak fruits are typically perceived as animal feed and its inclusion in human nutrition is still scarce [124,127,128]. Due to the underutilization, acorns have a high potential in the processing industry for being considered a good source of cheap plant material with important biological activities. Besides that, a scientific interest for the quality attributes of acorn products emerged [129].

Figure 2. Acorns.

1.4.1. Acorn fruit structure

Acorn is characterized by the presence of an achlorophyllous embryo, where the energy reserves accumulate, and there is an absence of an endosperm (Figure 3) [121]. This fruit is of oval shape growing in an upper part that is called cupule, which is a robust, massive and often compound envelope of fruits [130]. Acorn consists of a hard and indehiscent shell surrounds a kernel. However, phylogenetic factors cause significant differences between *Quercus* species [121]. Besides these factors, soil composition and climate are also important due to minerals and chemical compounds that may produce sensory and nutritional changes [121]. The size of a fully developed acorn generally depends on its growing conditions, it is normal that the size of them differs between species, subspecies and even within the same population [130,131]. Therefore attempts have been made to correlate ecological factors, such as climate and vegetation type, with characteristics of this fruit like shape, size and moisture content [131].

For acorns to germinate, they need favourable conditions and time, though even with those some of them never germinate [132].

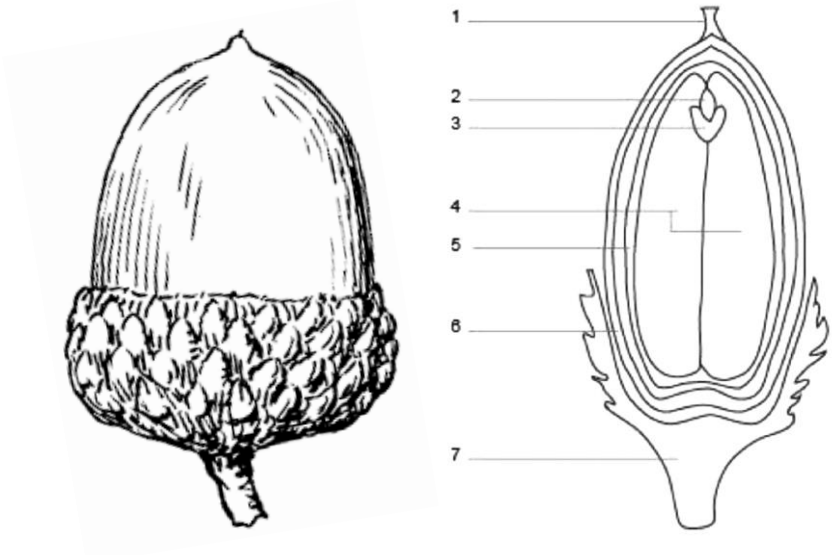

Figure 3. Schematic representation of an acorn, highlighting the main morphological characters, based on Vinha *et al.* (2016) [121]. 1 - remains of style; 2,3,4- constituents of the embryo (radicle, plumule and cotyledons, respectively); 5 – pericarp; 6 - seed coat; 7- cupule.

1.4.2. Acorn chemical composition

Acorns are considered a staple resource for wildlife [133]. They are described as being rich in water, carbohydrates (containing about 48 - 50% starch), poor in proteins and generally poor in fat content although the percentages may vary from 2% to a maximum value of 30%, of which over 80% is unsaturated [120,121,124,128]. Fibre percentages have been found to remain similar throughout the year, while protein and fat content vary with seasons, with the highest protein and fat levels tending to be in spring and autumn, respectively [121]. In addition, acorns are also a precious resource of minerals, because they can supply higher amounts of minerals (mainly Fe, Cu, Zn, and Mn) than other vegetative parts of forest-dwelling herbivores [121,133]. This content of minerals is also important, because these substances participate in several metabolic processes, such as directly involved in the digestion, absorption and energy supply processes [134].

Even though acorns are not as nutritionally rich as common nuts, they represent good alternatives to other high-starch content products and a good source of oil [124]. Furthermore, it is a gluten free source that could be used to the development of relevant gluten free products, which are a continuously expanding demand [129].

Proteins represent 4 – 8% of dry matter from acorn pulp [135]. Regarding amino acid content, the acorn is a rich source of some essential amino acids, such as valine, threonine, isoleucine, leucine, phenylalanine and lysine, that would meet the requirements for adults [135]. Moreover, the acorn contains all the essential amino acids except tryptophan [135].

The lipid fraction of acorns shows high values of monounsaturated and polyunsaturated FA [123]. In general, the acorns FA profiles are similar to those from olive, peanut and avocado oils [124].

The profile of bioactive compounds in acorn fruits might be modulated by genetic, physiological and extrinsic factors [121]. However the main bioactive compounds in acorn fruits are tocopherols and phenolic compounds, which have been reported as strong natural antioxidants [124]. From these phenolic compounds, acorn has been described as containing more than 60 individual compounds already identified, such as tannins, phenolic acids and flavonoids [121]. Tannins are responsible for the astringency of foods, because when they are presented in higher concentrations it leads to bitterness of the foods [140,141]. Even though, all acorns of all *Quercus* are edible, however in some varieties it is necessary to remove the tannins beforehand in order to reduce the astringency of the final product [135].

Phenolic compounds have been associated with biological functions, which are mainly correlated to their high antioxidant activity, therefore, it is necessary to maintain as much as possible the nutritional and phytochemical profiles of acorns [121,124].

Acorns are also considered as good source of vitamins (mostly A and E), phytosterols (among which *β*-sitosterol was the major compound) and aliphatic alcohols (especially tetracosanol) [121]. Studies have indicated that a diet high in phytosterols, which have a chemical structure similar to that of cholesterol, can inhibit cholesterol absorption and reduce serum cholesterol levels by competing for intestinal absorption [135].

1.4.3. Acorns as an alternative food

Acorn must be considered as functional food or as an alternative source of several highly valued food ingredients. Therefore, acorns are standing out for their growing relevance in the food industry, stimulating acorn valorisation, which will require additional research to develop new health-promoting and competitive market products. In human nutrition, acorn consumption falls mainly in 3 categories: acorns as fruits, as flour, or as cooking oil [121]. Researches have shown that acorn and its derived products have a huge potential as an alternative functional food, specifically considering their nutritional value and its consumer health benefits [120].

Acorn oil

In the sixties, the acorn oil was valorised as a possible raw material for the food industry [130]. The oil content of the most common *Quercus* genus species in Portugal (*Quercus faginea, Quercus ilex, Quercus rotundifolia*, and *Quercus suber*) do not exceed 12% [120,142]. In addition, this oil presents good nutritional quality with a flavour and colour comparable to that of olive oil [120,121]. Bernardo-Gil *et al.* (2007) [127] reported that the Portuguese legislation includes acorn oil in the category of alimentary oils, although according to Makhlouf *et al.* (2018) [120] no industrial oils are being produced.

Acorn oil is a good source of FA, in particular oleic, linoleic and palmitic acids with values ranging from 16 to 50%, 25 to 49% and 14 to 24%, respectively [120]. This justifies the fact that this oil must be part of the human diet for FA intake, particularly linoleic acid, which has important rules in human health (eicosanoid synthesis, decrease of blood serum triglycerides and increase of HDL-cholesterol levels) [121,127]. It has been shown that the FA composition of acorn oil from *Quercus suber* and *Quercus ilex* is similar to other edible vegetable oils, like those obtained from peanut and avocado [143]. In addition, acorn oil

contains numerous compounds with antioxidant properties, mainly flavonoids, tocopherols, and phenolic compounds [120].

The *Quercus genus* is generally characterized by elevated percentages of β-sitosterol (> 90% of sterols), which like other phytosterols is important to reduce the cholesterol levels in the blood and to prevent several diseases [121]. In addition, the values of acorn phytosterols are higher than those obtained in olive, almond and pistachio oils [121,135].

Acorn oil also has high amounts of tocopherol (vitamin E), which has an antioxidant action that can protect the oil from rancidity oxidative leading to a longer shelf-life [135,144,145]. In general, the main vitamer in this oil is γ -tocopherol (90% of total tocopherol content) and it reaches levels 5 - 9 fold higher than those detected for α-tocopherol, but there is a wide variation between species [121,145]. Acorns are also an excellent source of aliphatic alcohols, mainly tetracosanol. The presence of these alcohols in acorn oil might have industrial relevance, because these compounds contain beneficial health effects and they can be used as emulsifiers, emollients, and thickeners in food [121].

1.5. Review article

The literary review was accompanied by the writing of an article, with the following title “Revision of green emerging extraction technologies to obtain high quality oils from nuts”, which is being corrected by authors to be published in the Journal “Innovative Food Science and Emerging Technologies”.

1.6. Objectives

No literature has been reported on the high-pressure assisted extraction of oils from acorn and so, based on the present review, the objectives of this work were:

1. Valorise the acorn through the extraction of acorn oil by high-pressure;
2. Select an efficient environmentally friendly solvent to perform the extraction;
3. Study the impact of pressure (0.1, 250 and 500 MPa), extraction time (5, 12.5 and 20 min) and temperature (10, 25 and 40 °C) on the oil extraction yield, the content of main FA (oleic acid), total saturated, monounsaturated and polyunsaturated FA present in extracted acorn oil;
4. Analyse each parameter by response surface methodology and validate the models;
5. Compare HPE with SE;
6. Evaluate the antioxidant activity by DPPH of oils obtained under optimal conditions.

2. Materials and Methods

2.1. Chemicals

The chemicals purchased from Sigma-Aldrich (Missouri, USA) were 2,2-diphenyl-1-picrylhydrazyl (DPPH), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), D-(+)-glucose anhydrous, polyoxyethylene- 20 sorbitan- monooleate (Tween 80), sulphuric acid and lactic acid. The chemicals acquired from VWR (Pennsylvania, USA) were N,N-dimethylformamide (DMF), methanol, Hx and petroleum ether. The remaining chemicals used were EtOAc and EtOH absolute from Carlo Erba reagents (Barcelona, Spain), tritridecanoin from Clarodan and sodium methoxide from Acros Organics (Geel, Belgium).

2.2. Preparation of acorn powder

The dried and peeled acorns (*Quercus ilex*) were supplied by Herdade do Freixo do Meio (Montemor-o-Novo, Portugal). In the laboratory, the acorns were stored at -20 °C until use for the extractions. For that, acorns were cut into smaller portions and then crushed in a mill (Taurus aromatic).

2.3. Preliminary tests: extraction methods

2.3.1. Solid/solvent ratio selection

The preliminary experiments were done to optimize the acorn weight to a specific volume of solvent. The solvent used was EtOH and the solid/solvent ratios tested were 1:20; 1:10; 1:5 and 1:3.33 (w/v). The extractions were performed in a magnetic stirrer for 30 min at atmospheric pressure. Then, the extracts were centrifuged (10000 rpm; 10 min; 4 ºC), filtered (Whatman filter paper No. 1) and the solvent was evaporated by reduced pressure evaporation (Büchi, Rotavapor, Switzerland) at 60 °C. All analyses were performed in triplicate and the extraction yields were calculated based on the dry weight of the samples.

2.3.2. High-pressure assisted extraction with NADESs

The natural deep eutectic solvent (NADES) was prepared using a method previously described by Gomez and co-workers [146]. This solvent was developed using lactic acid, glucose and water in the following mole ratio 5:1:3. The different components were mixed and heated in a water bath below 80 °C for 60 min with agitation until a homogeneous liquid was formed.

Pressurized extracts were performed using an industrial-scale hydrostatic press equipment (Model 55, Hyperbaric, Burgos, Spain) with a pressure vessel of 55 L, 200 mm

inner diameter and 2000 mm length and a maximum operation pressure of 600 MPa. This equipment is connected to a refrigeration unit (RMA KH 40 LT, Ferroli, San Bonifacio, Italy) that allows control of the input water's temperature as a pressurizing fluid. An amount of 7.5 g of acorn powder was mixed with 150 mL of NADES in low permeability polyamide-polyethene bags, which were heat sealed and pressurized at 300 MPa during 10 min, at room temperature. The chosen pressure corresponds to an average value of the pressure range that is generally used in this type of extraction.

After processing, the solution obtained in the process was submitted to a test with Hx (solvent with very low polarity), where the HPE solution and Hx were mixed in a falcon tube to achieve a solution/solvent ratio of 1:1 (v/v) and finally was centrifuged at 5000 rpm for 10 min. This experiment was replicated four times.

In addition, the eutectic solvent was also mixed with olive oil (extra virgem Continente) to verify their solubility.

2.3.3. Extraction of oil with one surfactant and/or NADES

Two percent (w/v) of Tween 80 was prepared with distilled water being 20 mL mixed with 20.0 g of acorn powder in a falcon tube, which was centrifuged at 10000 rpm for 10 min. This experiment was repeated twice.

In addition, another experiment was carried out, where a solution was made with 98% eutectic solvent (lactic acid, glucose and water), 1% Tween 80 and 1% olive oil. Then, the mixture was submitted to turrax (IKA T80 digital ULTRA TURRAX) for 5 min. After 45 min left to stand, it was found that the mixture remained as emulsion.

2.3.4. Aqueous extraction of acorn oil

Three and twenty g of acorn powder was mixed with 15 and 10 mL, of distilled water respectively. The extracts were homogenised in a turrax (IKA T80 digital ULTRA TURRAX) for 10 min. Subsequently, mixtures were centrifuged at 10000 rpm for 15 min.

Other approach was developed, but by using the kernel acorn and shell instead (3.0 g of powder for 15 mL of distilled water). The extracts were homogenised in a turrax (IKA T80 digital ULTRA TURRAX) for 10 min and at the end centrifuged (10000 rpm; 15 min).

A third approach was performed, but by using a disc centrifuge where a solution with 300.0 g of acorn powder was mixed with 1000 mL of water to be then centrifuged. All experiments used centrifugation to separate the extracted oil from the aqueous phase.

2.3.5. Soxhlet extraction

The classical SE was performed using Hx, EtOAc and EtOH with ascending polarity of 0, 4.4 and 5.2, respectively, to select the best extraction solvent based on the extraction yields [147]. For each extraction, 4.5 g of acorn powder were packed in Soxhlet cartridge and placed inside the thimble of the SE apparatus. The solvent (90 mL) was added and the system was heated until it reached the boiling temperature of the solvent (70 ℃ for Hx, 80 ℃ for EtOAc and 60 ℃ for EtOH) though a water bath for 6 h, since it has been verified that higher extraction times do not increase the extraction yield [148]. Afterwards, the solvent was evaporated by reduced pressure evaporation (Büchi, Ropavapor, Switzerland) at 40 ℃ to constant weight. The oil samples were weighed to calculate the extraction yields, resuspended in 5 mL of Hx and stored at -20 ℃ for further analyses. All analyses were performed in triplicate.

2.3.6. High-pressure assisted extraction

Pressurized extracts were performed using the equipment described in section 2.3.2. In these experiments, 7.5 g of acorn powder was mixed with 150 mL of Hx, EtOAc or EtOH, which were pressurized at 300 MPa during 10 min, at room temperature. The extracts were centrifuged (10000 rpm; 10 min), filtered and then concentrated by rotary evaporation (Büchi, Ropavapor, Switzerland) at 40 ℃ to constant weight. Afterwards, the oil samples were weighted on the analytical balance (Mettler AE 100), resuspended in 5 mL of Hx and stored at -20 ℃ for further analyses. All analyses were performed in triplicate. Afterwards, the selected solvent was EtOH based on extraction yields efficiency.

2.4. Extraction methods used for response surface methodology and optimization

2.4.1. High-pressure assisted extraction

The extractions were performed as described in section 2.3.6. but using only the EtOH as solvent. The equipment used was the same as described in section 2.3.2.. The extraction conditions used were: pressure (0.1, 250 and 500 MPa), extraction time (5, 12.5 and 20 min) and temperature (10, 25 and 40 ºC). The extraction process was developed following a Box–Behnken design (Box and Behnken 1960) formed by an incomplete 3^3 design (Table 6). For the optimum conditions, the samples of each conditions were divided into 220 mL and 80 mL. The solvent was evaporated by reduced pressure evaporation (Büchi, Ropavapor, Switzerland) at 40 ℃ to constant weight. At the end the oil samples were weighed to calculate the extraction yields and then the aliquot with the highest initial

volume (220 mL) was resuspended in 12 mL of EtOH for the purpose of being analyzed by DPPH assay, while the other aliquot was resuspended in 5 mL of Hx to be analysed by liquid gas chromatography. All analyses were performed in triplicate. The recovery rate was determined according to Equation 1 (1).

$$\text{Recovery rate (\%)} = \left(\frac{\eta_{HPE}}{\eta_{SE}}\right) \times 100 \qquad (1)$$

where η_{HPE} is the extraction yield obtained by HPE, and η_{SE} is the extraction yield obtained by SE.

SE was performed as described in section 2.3.5. but only EtOH was used. These results were used as reference method to compare all the results.

Table 6. Experimental design including process variables.

Nomenclature	P (MPa)	t (min)	T (°C)
P0.1/t5/T25	0.1	5	25
P500/t5/T25	500	5	25
P0.1/t20/T25	0.1	20	25
P500/t20/T25	500	20	25
P0.1/t12.5/T10	0.1	12.5	10
P500/t12.5/T10	500	12.5	10
P0.1/t12.5/T40	0.1	12.5	40
P500/t12.5/T40	500	12.5	40
P250/t5/T10	250	5	10
P250/t20/T10	250	20	10
P250/t5/T40	250	5	40
P250/t20/T40	250	20	40
P250/t12.5/T25	250	12.5	25
P250/t12.5/T25	250	12.5	25
P250/t12.5/T25	250	12.5	25

P: pressure T: temperature; t: time.

2.5. Extract characterization

2.5.1. Extraction yields

The extraction efficiency was calculated as oil yield:

$$\eta\ (\%) = \left(\frac{W_{oil}}{W_{sample}}\right) \times 100 \qquad (2)$$

where η is the total extraction yield, W_{oil} is the mass of oil obtained after extraction (g), and W_{sample} is the initial acorn powder mass.

2.5.2. Identification and quantification of fatty acids profile

Quantitative and qualitative profiles of FA were carried out by gas chromatography-flame ionization detector (GLC-FID), according to the method described by Pimental and coworkers (2015) [149] with some modifications. Before GLC-FID analysis, FA were converted to more volatile and nonpolar derivatives (fatty acid methyl esters) using acid-catalysed esterification and transesterification. For that, 200 μL of sample was added to a glass falcon tube, the solvent (Hx) was evaporated with nitrogen and the weight of the oil was calculated. Then, 200 μL of tritridecanoin was added, followed by 800 μL of Hx, 2.26 mL of methanol and 240 μL of sodium methoxide (5.4 M). Samples were vortexed and incubated at 80 ºC for 10 min. After cooling in ice, 1.25 mL of DMF and 1.25 mL of sulphuric acid/methanol (3 M) were added. Samples were vortexed and incubated at 60 °C for 30 min. In the end, after cooling, they were vortexed and centrifuged (3630 rpm, 18 °C, 5 min). The upper layer pper layer containing FA methyl esters (FAME) was collected for further analysis. Samples were analysed in a gas chromatograph HP6890A (Hewlett-Packard, Avondale, PA, USA), equipped with a flame ionization detector and a BPX60 capillary column (60 m x 0.25 mm x 0.25 μm; SGE Europe Ltd, Courtaboeuf, France). Analysis conditions were as follows: injector (split 25:1; injection volume 1 μL) and detector temperatures were 250 ºC and 275 ºC, respectively; flow rate was of 1 mL/min.

Based on total extraction yields, the main FA present and the total saturated FA (SFA), monounsaturated FA (MUFA) and polyunsaturated FA (PUFA), it was performed an optimization and there were defined only two extraction combinations to continue the experiments (394 MPa; 5 min; 40 ºC and 500 MPa; 13 min; 10 ºC).

2.5.3. Iodine value

Iodine value (IV) is a very common method all over the world used to determine the degree of unsaturation of oils and fats and their fatty acid derivatives and can be determined in numerous ways [150]. IV was calculated from the multiplication of FAME percentages and assigned weighthing factors (equation 3) [150,151]. However, theoretical IV tend to be slightly higher than the IV determine by titration, because of the presence of unsaponifiables in the oils [150].

$$\mathrm{IV} = 1.001 * (\mathrm{C16{:}\,1\%}) + 0.899 * (\mathrm{C18{:}\,1\%}) + 1.814 * (\mathrm{C18{:}\,2\%}) + 2.737 * (\mathrm{C18{:}\,3\%}) \quad (3)$$

Where C16:1, 18:1, 18:2 and 18:3% represent the percentage contents of palmitoleic, oleic, linoleic and linolenic acid, respectively.

2.5.4. Determination of oxidizability value

Fatemi and Hammond [152] reported that the relative oxidation rates of methyl oleate, linoleate and linolenate in mixtures were 1, 10.3 and 21.6. Therefore, the calculated oxidizability (Cox) value of oil samples was calculated based on the percentage of the unsaturated C_{18} FA, using the following equation [152,153]:

$$\text{Cox value} = \frac{[1*(\mathrm{C18{:}1\%})+10.3*(\mathrm{C18{:}2\%})+21.6*(\mathrm{C18{:}3\%})]}{100} \quad (4)$$

where 18:1, 18:2 and 18:3% represent the percentage contents of oleic, linoleic and linolenic acid, respectively.

2.5.5. Total lipid content

Total lipid content was determined by gravimetric SE using petroleum ether as solvent. Soxhlet extractions were performed using 4.5 g of acorn powder, which were packed in Soxhlet cartridge and placed inside the thimble of the SE apparatus. A 90 mL volume of petroleum ether was added and the whole assembly was heated for 6 h using a water bath. Finally, the solvent was evaporated by reduced pressure evaporation (Büchi, Ropavapor, Switzerland) at 40 °C to constant mass. The oil samples were weighed, resuspended in 5 mL of Hx and stored at -20 °C for further analyses. Analyses were conducted in triplicate.

2.5.6. Infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) was used to compare the infrared spectrum (IR) from two oils extracted by HPE (higher extraction yield: 250 MPa; 20 min; 40 ºC and lower extraction yield: 500 MPa; 12.5 min; 10 ºC) with IR from olive oil and IR

of oil obtained by SE. FTIR analysis was performed using a FT-IR spectrometer (Spectrum 100, Perkin Elmer, USA), equipped with an attenuated total reflection (ATR) accessory with a zinc selenide crystal. The temperature of the crystal was kept at room temperature. All spectra were recorded in the wavelength of 4000 - 500 cm^{-1} with a spectral resolution of 4 cm^{-1} at 28 scans and the collection time of each spectrum being approximately 2.5 min. Each spectrum was subtracted against its corresponding air background spectrum.

The oil samples were placed directly on the ATR baseplate by pipetting a small quantity (~10 µl), avoiding air bubble formation. For each sample, 1 replicate was measured. Between samples the ATR crystal was cleaned by scrubbing with EtOH (95%) and dried with soft tissue

2.5.7. DPPH radicals scavenging assay

For this analysis, the samples were centrifuged at 14000 rpm for 5 min and then 25 µL of diluted samples were mixed with 175 µL ethanolic DPPH˙(60 µM) in the dark at room temperature. After 30 min, the absorbance of mixture was read at 515 nm using a microplate reader (Synergy H1, Vermont, USA). Controls were performed with 175 µL of DPPH reagent and 25 µL of solvent. A Trolox stock solution (0.250 mg/mL) prepared in EtOH absolute was used as a standard and a series of Trolox solutions (25-250 µmol/L) were prepared to establish the standard curve of the antioxidant activity by the concentration of the Trolox solutions. In data processing, the DPPH radical scavenging activity is expressed as percentage of reduction in absorbance regarding the control (Equation 5).

$$\text{DPPH scavenging (\%)} = \frac{A_{CTL} - A_{SPL}}{A_{CTL}} * 100 \qquad (5)$$

where $A_{CTL}$is the absorbance at 515 nm of the DPPH˙ solution and A_{SPL} is the absorbance at 515 nm of the sample. Results were expressed as micromol of Trolox equivalents per gram of acorn oil (µmol TE/ g oil).

2.6. Response surface methodology and statistical analysis

The experimental extraction methodology was developed following a Box-Behnken design formed by an incomplete 3^3 design, and data was analysed by response surface methodology (RSM). The independent variables were pressure (0, 250, 500 MPa), time (5, 12.5, 20 min) and temperature (10, 25, 40 °C), while the dependent factors were the extraction yield, the main fatty acid (oleic acid), SFA, MUFA and PUFA present in acorn oil. In this experimental design, 15 randomized experiments including three replicates of the

central point (250 MPa, 12.5 min and 25 °C) were performed, which were used for the error assessment. The optimization was done using Startgraphics Software. The relationship between independent variables and response was investigated using a second-order polynomial equation, according to the model (Equation 6).

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i \neq j=1}^{3} \beta_{ij} x_i x_j \qquad (6)$$

where Y is the predicted response, β_0 is the constant of the model, β_i, β_{ii} and β_{ij} are linear, quadratic, and interaction effects of the model, respectively and x_i and x_j represent the independent variables. To validate the models, additional extraction trials were carried out in triplicate for 2 optimal conditions and the experimental results were compared to the values predicted by the regression models regarding the amount of oil yields, oleic acid, SFA, MUFA and PUFA extracted. Antioxidant activity (DPPH) were performed only for these extraction conditions.

3. Results and Discussion

3.1. Preliminary tests: extraction methods

3.1.1. Solid/solvent ratio selection

For the solid solvent ratio selection, the solvent used was EtOH. This solvent was selected since the use of EtOH may replace the use of organic solvents from non-renewable-resources in the extraction of acorn oil [142,143,154–158] and is a bio-based and food grade solvent. The highest extraction yield (8%) was carried out using 1:20 (w/v) solid/solvent ratio, which was 12, 66 and 81% higher than the one obtained using 1:10, 1:5 and 1:3.33 (w/v), respectively. Therefore, the chosen solid/solvent ratio was 1:20 (w/v).

3.1.2. High-pressure assisted extraction with NADESs

As it says in section 1.3.3., the lipids extraction with NADESs has only been described with quaternary ammonium salts. Recently, Van Osch and co-workers (2019) [159] developed new hydrophobic NADESs based on terpenes for the removal of riboflavin (vitamin B_2), however more investigation should be addressed about their possible capability of lipid extraction, sustainability and toxicity. Therefore, these solvents were excluded, so as there were no more referenced hydrophobic NADESs tested, it was necessary to choose a hydrophilic NADES that was food grade. The NADES chosen was lactic acid, glucose and water in the following mole ratio 5:1:3, which is composed of non-toxic substances and therefore may be directly incorporated in food formulations [160]. However, the water addition of this NADES is responsible by the increased polarity [110] and therefore this NADES is generally used to extract for example anthraquinones [161] and phlorotannins [162].

In HPE experience with NADES, a change in the color of the solution obtained in the process was expected in the case of oil extraction, however colour and consistency (slimy) of the solution remained the same from the beginning to the end of the processing. Therefore, there was no oil or an insignificant amount of oil. To make sure of this conclusion, the solution obtained was submitted to a test with Hx, where it was verified that the supernatant (Hx phase) was colourless before and after centrifugation, which suggests that no oil was extracted. In addition, the eutectic solvent was mixed with olive oil (extra virgem Continente) and it was verified that it was not soluble in the oil and so it is unable to extract this analyte.

3.1.3. Extraction of oil with one surfactant and/or NADES

The attempt to use Tween 80 to recover oil from acorn failed, since the mixture remained an emulsion. In addition, another experiment was carried out to solve the problem of the solubility of oil in the NADES (lactic acid, glucose and water), it was made a mixture with the eutectic solvents, olive oil and Tween 80, which is a non-ionic surfactant that is environmentally-friendly and approved for food and pharmaceutical uses [163,164]. The resulting solution was found to remain as emulsion, which could be related with the main function of using surfactants in the oil recoveries that is to reduce the interfacial tension between the immiscible phases, facilitating oil droplets breakup and allowing the oil recovery [164].

3.1.4. Aqueous extraction of acorn oil

Aqueous extraction is a safe, environmentally friendly and economic technique for oil extraction [165]. Unlike organic solvents extraction, aqueous extraction use the insolubility of the analyte in the extraction medium (water) to remove the oil as an emulsion or free oil [166,167]. However, aqueous extraction has some disadvantages, such as formation of a stable oil-in-water emulsion; requires cell wall rupturing before extraction and the lower water usage results in a harder release of free oil [166].

After aqueous extraction, there was no separation phase between oil and water. Therefore, in future experiments, it would be necessary to optimize the extraction conditions, specially the sample pre-treatment before the extraction and the solid/water ratio.

3.1.5. HPE and SE with organic solvents

The extractions carried out by HPE and SE using EtOH provided the highest yields (8.8 ± 1.1 and 12.3 ± 2.4%, respectively). The good performance of this solvent suggests the presence of components with intermediate to high polarity due to the polarity of the solvent and consequently the low selectivity for oil extraction from *Quercus Ilex.* The feasibility of EtOH for oil extraction from *Quercus suber* L. was already described by Ferreira-Dias and co-workers (2003) [154] and they observed that EtOH was not an adequate solvent, since it has a low selectivity for oil extraction from *Quercus suber* L.

The lowest yields were obtained for EtOAc and Hx using HPE method (5.3 ± 0.2 and 5.1 ± 0.1%, respectively) and SE (6.3 ± 0.7 and 5.0 ± 0.7%, respectively). In addition, it is possible to observe a yield increase trend due to the increase in solvent polarity, which was most notable for SE, suggesting that compounds present in acorn matrix have medium to high polarity. Considering these results, the chosen solvent was EtOH even knowing its low

selectivity, but positively reinforced for being food compatible and environmentally friendly.

3.2. Effect of HPE conditions: response surface methodology and optimization

3.2.1. Response surface methodology for extraction yields

Comparing different temperatures, the highest extraction yield (15%) was obtained for the combination at P250/t5/T40 (Table 7, Figures A1 and A2 in Appendix A), which represented an increase of 35% in relation to extracts obtained in the same conditions at 10 °C (P250/t5/T10). Similar results were found by Ugur *et al.* [49] on the total extraction yields of oils from *Acheta domesticus* and *Tenebtio molitor*, where the maximum extraction yield was obtained for 40 °C. From this temperature, authors verified that by decreasing the temperature to 30 °C, the extraction yields decreased.

Concerning the extraction time, 5 min (P250/t5/T40) led to an increase in the extraction yields of 6% in relation to extraction performed in same conditions at 20 min (P250/t20/T40). However, Briones-Labarc *et al.* [2] studied the effect of HPE upon oil extraction from papaya seeds and observed that the extraction yield increased along with an increased extraction time from 5 to 15 min, and concluded that this may be related with the rapid equilibrium of pressure between the inside and outside of the cells or the diffusion speed of the solvent is very high during a very short time under HP.

In general, the pressure of 500 MPa conduced to an increase of the oil yield between 12 - 13% when compared to extraction performed at 0.1 MPa. HPE is expected to increase the yield, because it can cause enhancement of chemical reactions in the cells, resulting in more solvent that could penetrate into cells [49]. Ugur *et al.* [49] observed that the *Acheta domesticus* and *Tenebtio molitor* oil content decreased with HPE (500 MPa, 15 min and 30 or 40 °C) compared to the conventional extraction (0.1 MPa, 15 min and 30 or 40 °C) and they put the hypothesis that HPE may had disrupted the structures of triglycerides.

Temperature was the factor that showed the highest and significant impact on extraction efficiency, followed by the quadractic effect of pressure and the interaction of pressure temperature in descending order (Figure 4). As shown in Table 8, *F*-value was 64.18 for temperature linear effect, followed by 8.18 for pressure quadratic term and 6.78 for the interaction of pressure temperature for a significance level of $p < 0.05$. According to Balvardi *et al.* [167] increasing the temperature leads to an increase in solubility and consequently to a higher extraction yield.

Table 7. Experimental (Exp) and predicted (Pre) values for oil yields, oleic acid, SFA, MUFA and PUFA.

Extraction conditions	**Oil yields** (%)		**Oleic acid** (µg/mg of fat)		**SFA** (µg/mg of fat)		**MUFA** (µg/mg of fat)		**PUFA** (µg/mg of fat)	
	Exp	**Pre (%variation)[1]**	**Exp**	**Pre (%variation)[1]**	**Exp**	**Pre (%variation)[1]**	**Exp**	**Pre (%variation)[1]**	**Exp**	**Pre (%variation)[1]**
P0.1/t5/T25	9.71	10.11 (-4.16)	583.32	611.80 (-4.88)	23.18	25.22 (-8.76)	760.83	797.35 (-4.80)	240.85	249.37 (-3.54)
P500/t5/T25	11.06	10.74 (2.94)	637.88	669.87 (-5.01)	36.62	39.16 (-6.94)	842.82	872.28 (-3.49)	274.54	281.71 (-2.61)
P0.1/t20/T25	9.48	9.76 (-3.02)	547.62	505.30 (7.73)	25.47	22.93 (9.98)	705.71	653.35 (7.42)	227.48	225.23 (0.99)
P500/t20/T25	10.89	10.39 (4.65)	581.52	563.37 (3.12)	26.09	24.06 (7.78)	741.89	728.28 (1.83)	271.00	257.57 (4.96)
P0.1/t12.5/T10	10.30	9.62 (6.67)	800.69	783.03 (2.20)	34.01	32.83 (3.48)	1040.52	1014.32 (2.52)	333.51	309.50 (7.20)
P500/t12.5/T10	8.19	8.27 (-0.91)	1102.89	1071.40 (2.86)	56.65	54.96 (2.99)	1433.35	1391.32 (2.93)	423.95	406.21 (4.18)
P0.1/t12.5/T40	11.94	11.94 (0.03)	943.74	975.23 (-3.34)	41.49	43.19 (-4.09)	1217.70	1259.74 (-3.45)	334.21	351.96 (-5.31)
P500/t12.5/T40	13.77	14.53 (-5.50)	785.36	803.00 (-2.25)	34.94	36.13 (-3.39)	1006.41	1032.59 (-2.60)	295.92	319.93 (-8.11)
P250/t5/T10	9.84	10.24 (-4.06)	649.32	643.66 (0.87)	31.09	30.24 (2.72)	828.13	829.26 (-0.14)	233.57	246.61 (-5.58)
P250/t20/T10	9.68	9.89 (-2.20)	602.54	657.34 (-9.10)	28.15	31.87 (-13.24)	773.83	840.93 (-8.67)	233.37	262.09 (-12.31)
P250/t5/T40	15.15	14.53 (4.09)	780.59	725.79 (7.02)	40.06	36.33 (9.30)	995.44	928.34 (6.74)	293.06	264.34 (9.80)
P250/t20/T40	14.32	14.18 (0.94)	493.45	499.12 (-1.15)	16.47	17.32 (-5.14)	629.81	628.68 (0.18)	213.61	200.58 (6.10)
P250/t12.5/T25	12.52	11.37 (9.13)	737.92	706.76 (4.22)	35.40	33.33 (5.87)	950.01	909.79 (4.23)	268.68	257.04 (4.33)
P250/t12.5/T25	11.04	11.37 (-3.01)	791.08	706.76 (10.66)	37.54	33.33 (11.22)	1022.57	909.79 (11.03)	294.18	257.04 (12.63)
P250/t12.5/T25	10.42	11.37 (-9.15)	591.29	706.76 (-19.53)	27.04	33.33 (-23.27)	756.80	909.79 (-20.22)	208.25	257.04 (-23.43)
Variation (%)	4.03		5.60		7.88		5.35		7.41	

P: pressure (MPa); t: time (min); T: temperature (°C); SFA, MUFA and PUFA: total saturated, monounsaturated and polyunsaturated fatty acids.

1: Variation (%) $= \frac{\text{experimental} - \text{predicted}}{\text{experimental}}$ x 100; 2: average deviation from the predicted values.

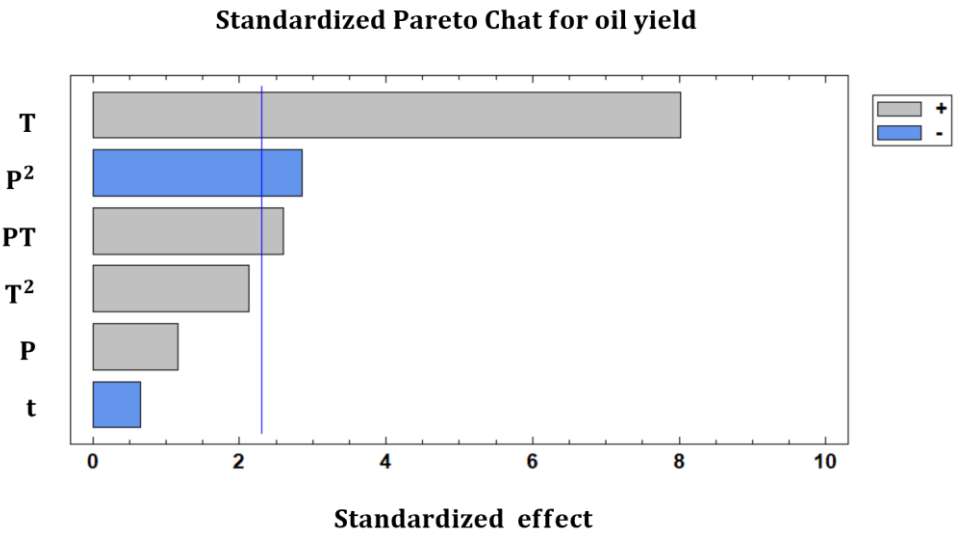

Figura 4. Standardized Pareto chart for oil yield. P: pressure (MPa); t: time (min); T: temperature (°C).

The sign of the bar of standardized pareto chart (corresponding to gray (+) or blue (-)) indicates that the factor is an increasing or decreasing effect on the oil yield. The linear effect of temperature and the interaction of pressure temperature were the variables statistically significant that had an increasing effect on the oil yield.

Table 8. Analysis of variance (ANOVA) for linear, quadratic and crossed effects of pressure, extraction time and temperature at a significance level of 95% confidence for oil yields. The significant coefficients in each case are written in bold.

Relationship		**Factor**	***F***	***p***
Main effects	Linear	P	1.35	0.278
		t	0.43	0.531
		T	64.18	**0.000**
Interactions	Pure quadratic	P^2	8.18	**0.021**
		T^2	4.53	0.066
	Cross product	PT	6.78	**0.031**
$R^2 = 0.9153$				
R^2 adjst= 0.8517				

P: pressure (MPa); t: time (min); T: temperature (°C).

The following second-order model satisfactorily explained the oil yield:

$$\eta(\%) = 10.6224 + 0.0037P - 0.0234t - 0.1088T - 0.0000P^2 + 0.0003PT + 0.0037T^2 \quad (7)$$

where η is the extraction yield (%) and P, T, and t are the pressure, temperature and extraction time, respectively.

Two- dimension (2D) contour plot (Figure 5 b) indicates that the interactions between pressure and temperature were significant, because the contour lines took an elliptical shape [168]. In addition, it is also noted that the increase in oil yield with increasing temperature became more apparent as the pressure increased, what could be explained by the interactions between the two parameters. Decreasing the influence of temperature or increasing extraction period led to a decrease in oil yield.

In general, the experimental results and the predicted values by the model (Table 7) were in good agreement, presenting a variation lower than 7%, except for two samples obtained at P250/t12.5/T25, where the variation was lower than 10%.

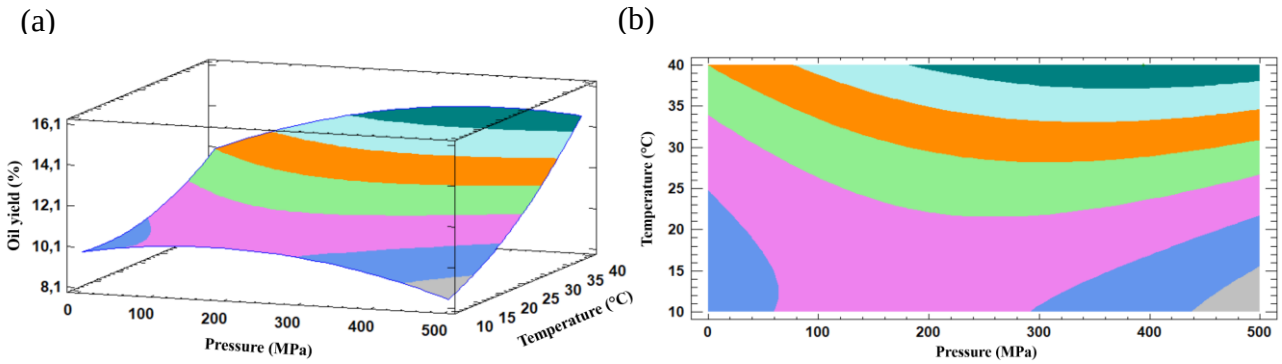

Figure 5. Interaction effects of pressure and temperature at fixed 5 min on oil yield: (a) surface plot and (b) contour plot.

3.2.2. Identification and quantification of fatty acids profile

The FA profile and total FA, which was determined by the sum of all FA in each condition, are summarized in Table 9. The main FA of acorn oil were oleic acid (about 58%), followed by linoleic acid (about 22%) and palmitic acid (about 16%). Léon-Camacho *et al.* [145] also found that the most abundant fatty acids in acorn oil were oleic (63%),

linoleic (17%) and palmitic (14%). The difference in the amount of the three main fatty acids could be related with the different origins where the acorns were collected.

The essential FA, oleic acid and linoleic acid accounted for 80% of the total amount, but the oil extracted using HPE had an essential fatty acid content of 81% at P500/t20/T25. The amount of the three main FA increased with temperature, except in one specific situation for linoleic acid when the temperature increased from 10 to 40 ℃ at 0.1 MPa and 12.5 min.

Concerning the extraction time, the amount of oleic, linoleic and palmitic acids decreased with time, however no significant differences were found between the results from the combination P250/t5/T10 and P250/t20/T10 for linoleic acid. The amount of three main FA decreased with pressure, however the situation was different at 12.5 min and 40 °C. In addition, the highest amount of oleic acid (1102.89 µg/ mg of fat), linoleic acid (405.34 µg/ mg of fat) and palmitic acid (310.08 µg/ mg of fat) were found at P500/t12.5/T10, therefore all other combinations presented a lower amount of these FA. However, the highest amount of total fatty acid content was obtained at P250/t5/T40.

3.2.2.1. Response surface methodology for oleic acid

The highest extraction yield of the oleic acid was obtained using P500/t12.5/T10 (Table 7, Figures B1 and B2 in Appendix B) achieving 1102.89 µg/mg of fat. This value was 27% higher than the one obtained in the same extraction time and temperature but at atmospheric pressure (P0.1/t12.5/T10). Briones-Labarca *et al.* [2] reported that the higher pressures allows more solvent to enter the cells, thus more compounds can permeate the cell membrane increasing the extraction yields. The disruption of cellular walls and hydrophobic bonds in the cell membrane may lead to a higher permeability of the solvent into the cells [2]. Moreover, the differential pressure between the inside and outside of the cells is large leading to a rapid permeation [2]. In addition, the highest extraction yield of the oleic acid (P500/t12.5/T10) also represented an increase of 29% in relation to extraction performed in same conditions at 40 °C (P500/t12.5/T40). However, these results are not in accordance with Ugur *et al.* [49], which observed an increase of the percentage of unsaturated FA from 30 to 40 °C for mealworm and cricket oils extracted with HPE.

Table 9. Fatty acid composition (µg/ mg of fat) of acorn oils extracted by HPE.

Extraction conditions	Myristic acid (C 14:0)	Myristoleic acid (C 14:1)	Palmitic acid (C 16:0)	C 16:1 C7	Palmitoleic acid (C 16:1 C9)	Heptadecanoic acid (C 17:0)	Stearic acid (C 18:0)	Oleic acid (C 18:1 C9)	C 18:1 C11	Linoleic acid (C 18:2 cis 9 cis 12)	α-Linolenic acid (C 18:3 C9 C12 C15)	C 18:2 C9 t11	C 18:4	Total FA
P0.1/t5/T25	1.25	ND	165.95	0.68	1.08	1.47	21.71	583.32	9.80	232.23	3.26	5.36	ND	1026.11
P500/t5/T25	1.39	ND	191.81	0.67	1.19	1.84	33.39	637.88	11.28	262.43	4.73	6.75	0.64	1153.98
P0.1/t20/T25	1.22	ND	147.87	ND	1.14	1.36	22.90	547.62	9.08	218.93	3.36	5.19	ND	958.67
P500/t20/T25	1.44	ND	150.87	ND	ND	1.34	23.30	581.52	9.51	263.48	2.91	4.61	ND	1038.98
P0.1/t12.5/T10	1.76	ND	223.95	0.86	1.52	2.03	30.22	800.69	13.50	321.45	4.39	7.67	ND	1408.04
P500/t12.5/T10	2.20	ND	310.08	ND	2.04	2.91	51.54	1102.89	18.34	405.34	7.49	11.12	ND	1913.95
P0.1/t12.5/T40	1.97	0.65	255.11	0.95	1.83	2.44	37.08	943.74	16.07	318.62	5.44	9.28	0.86	1594.05
P500/t12.5/T40	1.64	ND	206.25	0.74	1.40	1.96	31.34	785.36	12.65	283.73	4.75	7.44	ND	1337.27
P250/t5/T10	1.16	0.40	167.11	0.59	1.10	1.56	28.37	649.32	10.00	223.29	4.20	6.09	0.51	1093.70
P250/t20/T10	1.23	0.39	159.43	0.60	1.18	1.46	25.46	602.54	9.70	223.41	3.73	5.74	0.49	1035.35
P250/t5/T40	1.40	0.47	200.72	0.83	1.22	1.88	36.79	780.59	12.08	280.08	5.47	7.50	ND	1329.03
P250/t20/T40	1.15	ND	128.59	ND	0.98	1.07	16.47	493.45	7.76	208.05	1.87	3.69	ND	863.10
P250/t12.5/T25	1.37	0.49	197.97	0.69	1.27	1.91	32.12	737.91	11.68	256.44	4.74	6.89	0.61	1254.09
P250/t12.5/T25	1.51	0.56	215.73	0.81	1.46	1.97	34.05	791.08	12.94	281.73	4.95	7.50	ND	1354.29
P250/t12.5/T25	1.05	0.36	154.35	0.56	0.99	1.45	24.54	591.29	9.25	199.13	3.63	5.49	ND	992.08

P: pressure (MPa); t: time (min); T: temperature (°C); FA: fatty acids; ND: not detected.

Concerning the extraction time, the combination P500/t5/T25 conducted to a significant increase of 9% in the extraction yield of the oleic acid compared to the same extraction conditions at 20 min (P500/t20/T25). However, Briones-Labarca *et al.* [2] observed the opposite, since the oleic acid content of papaya seed oil was higher in the condition with 500 MPa, 15 min and room temperature than in the condition with same pressure and temperature but at 5 min.

The Standardized Pareto chart (Figure 6) revealed that the most significant factor on extraction efficiency was the quadractic effect of time followed by the quadractic effect of temperature and the interaction of pressure temperature in descending order. Considering the bars in the Pareto chart beyond the vertical line, the quadractic effect of temperature was the only variable statistically significant that had an increasing effect on the concentration of oleic acid.

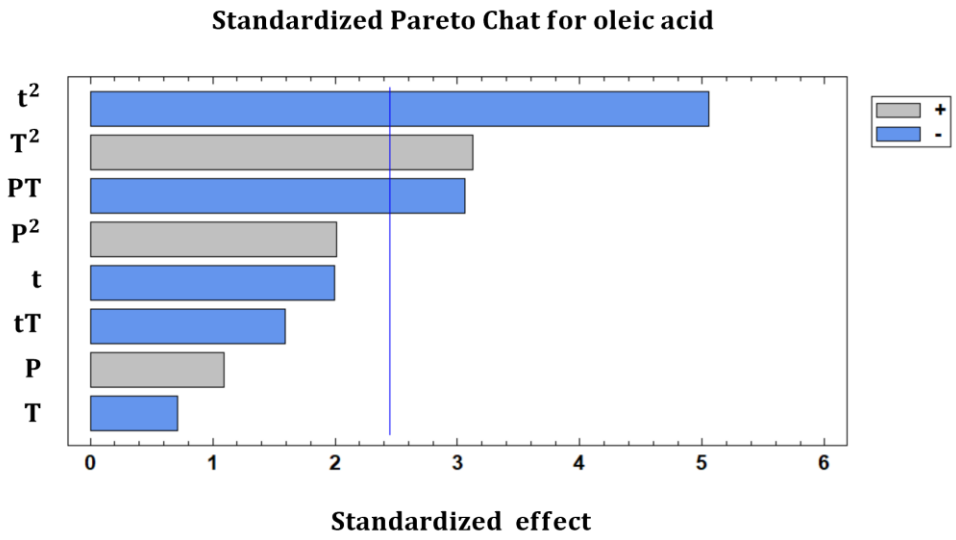

Figura 6. Standardized Pareto charts for oleic acid. P: pressure (MPa); t: time (min); T: temperature (°C).

The results of ANOVA for the regression model for the concentration of oleic acid in acorn oil obtained by HPE is shown in Table 10. The model response was mainly affected by the effect of time quadratic term since *P*-value was low ($p<0.01$) and the *F*-value was 25.49. Quadratic temperature and its interaction with time were also significant ($p<0.05$) model terms presenting an F-value of 9.79 and 2.55, respectively. The linear effects (P, t, T), quadratic pressure and the interaction of pressure with temperature were insignificant

(p>0.05) model terms with F-values between 0.51 and 4.04, except for the interaction pressure temperature (9.35).

Tabela 10. ANOVA of the regression models at a significance level of 95% confidence for the concentration of oleic acid. The significant coefficients in each case are written in bold.

Relationship		**Factor**	***F***	***p***
Main effects	Linear	P	1.19	0.318
		t	4.00	0.093
		T	0.51	0.501
Interactions	Pure quadratic	P^2	4.04	0.091
		t^2	25.49	**0.022**
		T^2	9.79	**0.002**
	Cross product	PT	9.35	0.162
		tT	2.55	**0.020**
R^2 = 0.9092				
R^2 adjst = 0.7885				

P: pressure (MPa); t: time (min); T: temperature (°C).

According to Briones-Labarca *et al.* [2], a better FA composition was achieved for papaya oil extracted with HPE, when the extraction time increased from 5 to 15 min. However, in this study the linear effect of time was an insignificant (p>0.05) model term.

The results from this study helped to frame a second order polynomial equation (Eq. 8) that relates the concentration of oleic acid, C 18:1 C9 (µg/mg of fat) to the conditions of pressure (P), extraction time (t) and temperature (T).

$$\text{C 18:1 C9 (µg/mg of fat)} = 308.9970 + 0.2535P + 94.2240t - 14.1685T - 0.0013P^2 - 0.0307PT - 3.5188t^2 - 0.5341tT + 0.5451T^2 \quad (8)$$

Figures 7 a,b show the three-dimensional (3D) response surface plot and two-dimensional (2D) contour plot based on extraction temperature and pressure at a constant extraction time (12.5 min). The results imply that the concentration of oleic acid decreased, evidently, with the decrease of temperature from 40 to 27 °C and increased again from 12 to 10 °C at low pressures. In addition, concentration of oleic acid increased with the increase of pressure and temperature decrease to 500 MPa and 10 °C, respectively, then declined with

the decrease of pressure and increase of temperature. This result demonstrate that the response surface had a maximum point for the concentration of oleic acid. In addition, Figure 7 b indicate that the interactions between extraction temperature and pressure were significant, because the contour lines took on an elliptical shape [168], which is in accordance with the *P*-value for the interaction of those parameters.

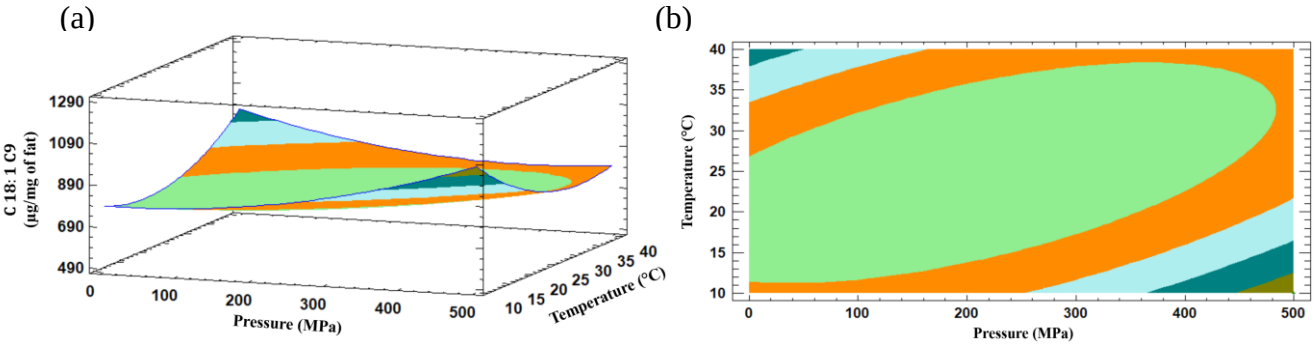

Figure 7. Interaction effects of pressure and temperature at fixed 12.5 min on oleic acid: (a) surface plot and (b) contour plot.

The experimental results and the predicted values by the model (Table 7) were in good agreement, presenting a variation lower than 8%, except for two samples obtained at P250/t12.5/T25 and one sample at P250/t20/T10, where the variation was lower than 20%.

3.2.2.2. Response surface methodology for SFA, MUFA and PUFA

The highest extraction yields of SFA, MUFA and PUFA were obtained using P500/t12.5/T10 (Table 7, Figures C1, C2, D1, D2, E1 and E2 in Appendices C, D and E) achieving 56.65 µg/mg of fat, 1433.35 µg/ mg of fat and 423.947 µg/ mg of fat for SFA, MUFA and PUFA, respectively. These values were 40, 27 and 21% higher than the ones obtained in the same extraction time and temperature but at atmospheric pressure (P0.1/t12.5/T10), respectively for SFA, MUFA and PUFA. In addition, the highest extraction yields also represented an increase of 38, 30 and 30% in relation to extraction performed in same conditions but at 40 °C (P500/t12.5/T40), respectively for SFA, MUFA and PUFA. However, these results are not in accordance with Ugur et al. [49], which observed an increase of the percentage of SFA, MUFA and PUFA from 30 to 40 °C for mealworm oil extracted with HPE. Concerning the extraction time, the combination P500/t5/T25 conducted to an increase of 29, 12 and 6% in the extraction yield of SFA,

MUFA and PUFA, respectively, compared to the same extraction conditions at 20 min (P500/t20/T25).

The ANOVA results were analysed by plotting the standardized main factors and interaction effects against each response in the form of Pareto charts (Figure 8). Effects due to the chosen variables and their interactions on the responses are represented by bars, whose length is proportional to the standardized effect. In addition, the colour of the bars indicated that the factors with significant effect of the SFA model showed a decreasing effect, while the MUFA and PUFA models had a factor with an increasing effect (temperature and pressure quadratic terms, respectively).

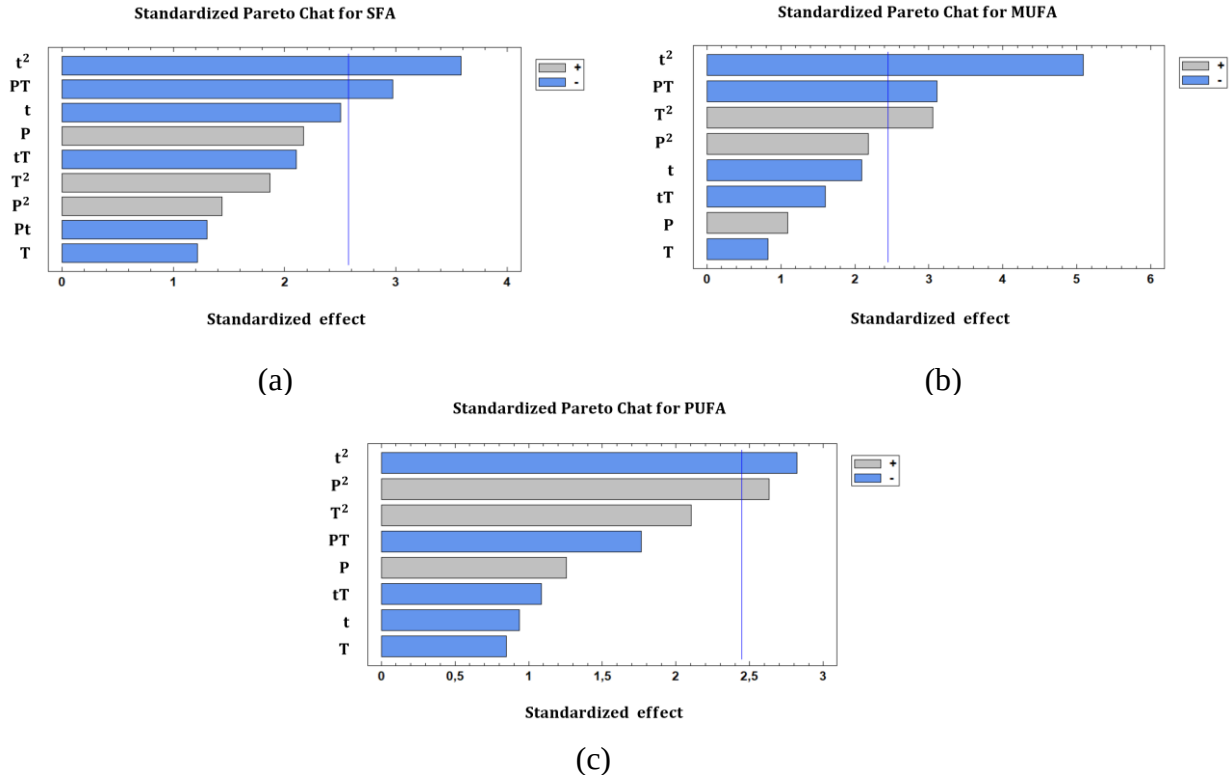

Figure 8. Pareto charts for the standardized effects: (a) total saturated fatty acids, (b) total monounsaturated fatty acids and (c) total polyunsaturated fatty acids.

ANOVA for the models of SFA, MUFA and PUFA is shown in Table 11. In these cases, SFA and PUFA models showed two effects with $p<0.05$, while the MUFA model acquired three statistically significant effects. The responses were mainly significantly affected by time since $p<0.05$ and the F-value high for quadratic effects (12.85, 25.84 and 7.96 correspond respectively to SFA, MUFA and PUFA). Other significant effects were provided by the interaction of pressure temperature with F-value of 8.84 and 9.64 for SFA

and MUFA models, temperature quadratic term with *F*-value of 9.29 for MUFA model and pressure quadractic term with *F*-value 6.93 for PUFA model. However, linear effects (P,t and T) and the interaction time temperature were not significant (p>0.05) for all models. In addition, two quadratic terms (P^2 and T^2) for SFA and MUFA models and one interaction parameter (PT) for PUFA model were found to be insignificant.

Table 11. ANOVA of the regression models at a significance level of 95% confidence for SFA, MUFA and PUFA. The significant coefficients in each case are written in bold.

Relationship		**Factor**	**SFA**		**MUFA**		**PUFA**	
			F	*p*	*F*	*p*	*F*	*p*
Main effects	Linear	P	4.71	0.082	1.19	0.318	1.57	0.256
		t	6.27	0.054	4.38	0.081	0.88	0.385
		T	1.49	0.277	0.68	0.442	0.72	0.428
Interactions	Pure quadratic	P^2	2.07	0.210	4.75	0.072	6.93	**0.039**
		t^2	12.85	**0.016**	25.84	**0.002**	7.96	**0.030**
		T^2	3.49	0.121	9.29	0.023*	4.42	0.080
	Cross product	Pt	1.7	0.249	-	-	-	-
		PT	8.84	**0.031**	9.64	**0.021**	3.12	0.128
		tT	4.42	0.090	2.56	0.161	1.18	0.319
R^2			0.9045		0.9113		0.8242	
R^2 adjst			0.7326		0.7931		0.5897	

P: pressure (MPa); t: time (min); T: temperature (°C); SFA, MUFA and PUFA: total saturated, monounsaturated and polyunsaturated fatty acids.

Effect of pressure, extraction time and temperature on the responses of the SFA, MUFA and PUFA were evaluated. And mathematical equations were obtained to calculate predicted values for SFA, MUFA and PUFA in acorn oil, Eq. 9, 10 e 11, respectively.

$$\text{SFA (µg/mg of fat)} = -0.0237 + 0.0557X_1 + 5.0658X_2 - 0.1419X_3 + 0.000X_1^2 - 0.0017X_1X_2 - 0.0019X_1X_3 - 0.1628X_2^2 - 0.0459X_2X_3 + 0.0212X_3^2 \quad (9)$$

$$\text{MUFA (µg/mg of fat)} = 395.8350 + 0.2736X_1 + 122.0690X_2 - 17.4648X_3 + 0.0018X_1^2 - 0.0403X_1X_3 - 4.5749X_2^2 - 0.6919X_2X_3 + 0.6860X_3^2 \quad (10)$$

$$\text{PUFA } (\mu g/mg \text{ of fat}) = 182.6780 - 0.1206X_1 + 26.5843X_2 - 5.2487X_3 + 0.0008X_1^2 - 0.0086X_1X_3 - 0{,}9517X_2^2 - 0.1760X_2X_3 + 0.1773X_3^2 \quad (11)$$

Surface and contour plots demonstrating the effects of extraction temperature and pressure on the SFA, MUFA and PUFA were shown in Figure 9. The results imply that the SFA, MUFA and PUFA decreased evidently with the decrease of temperature from 40 to 31°C, 23 °C or 31 °C, respectively, and increased again from 15 °C and 11 °C for MUFA and PUFA, respectively, at low pressures. In addition, SFA, MUFA and PUFA increased to the peak with the increase of pressure and temperature decrease to 500 MPa and 10°C, respectively, and then declined with the decrease of pressure and increase of temperature. Therefore, the response surface plots had a maximum point for the SFA, MUFA and PUFA. The interaction pressure temperature was found to be significant for SFA and MUFA models, which was not verified for PUFA model, because an elliptical contour plot (indicate a significant interaction between variables) is obtained when there is a perfect interaction between factors [171]. These evidences are in accordance with *p*-values from Table 11.

The experimental results and the predicted values by the model (Table 7) were in good agreement, presenting a variation lower than 8% for MUFA model and lower than 10% for other models. However, the one sample obtained at P250/t20/T10 and two samples obtained at P250/t12.5/T25 the variation was lower than 21, 23 and 24% for MUFA, SFA and PUFA models.

3.2.3. Models fit and adequacy

In general, the predicted values were in good agreement with the experimental results. Predicted and experimental values differed in average deviation less than 6%, except for the model developed for SFA and PUFA, where values differ in average 8% (Table 7), indicating that the models were satisfactory and accurate.

The coefficient of determination (R^2) and coefficient of determination adjusted (R^2 adjust) were calculated for all models by analysis of variance and reported in the Table 7, 8 and 9. The coefficient of determination gives the variation proportion of the model-predicted response, therefore according to Man *et al.* [168] a model is adequate when $R^2 > 0.75$. The concentrations of FA models (oleic acid; SFA and MUFA) and total oil yield model presented a coefficient of determination of 0.9093, 0.9045, 0.9113 and 0.9153, respectively, meaning that only 9, 10, 9 and 8% of the experimental values were not described by the model, respectively. The PUFA model presented the lowest R^2 of 0.8242, indicating that the

model only explained 82% of the obtained results. The R^2 of all models in this experiment was considered adequate, however this parameter tend to increase when the sample size is small [169]. In this study, the modelling of the models was performed with one replica, so it can be considered that R^2 increased with the sample size.

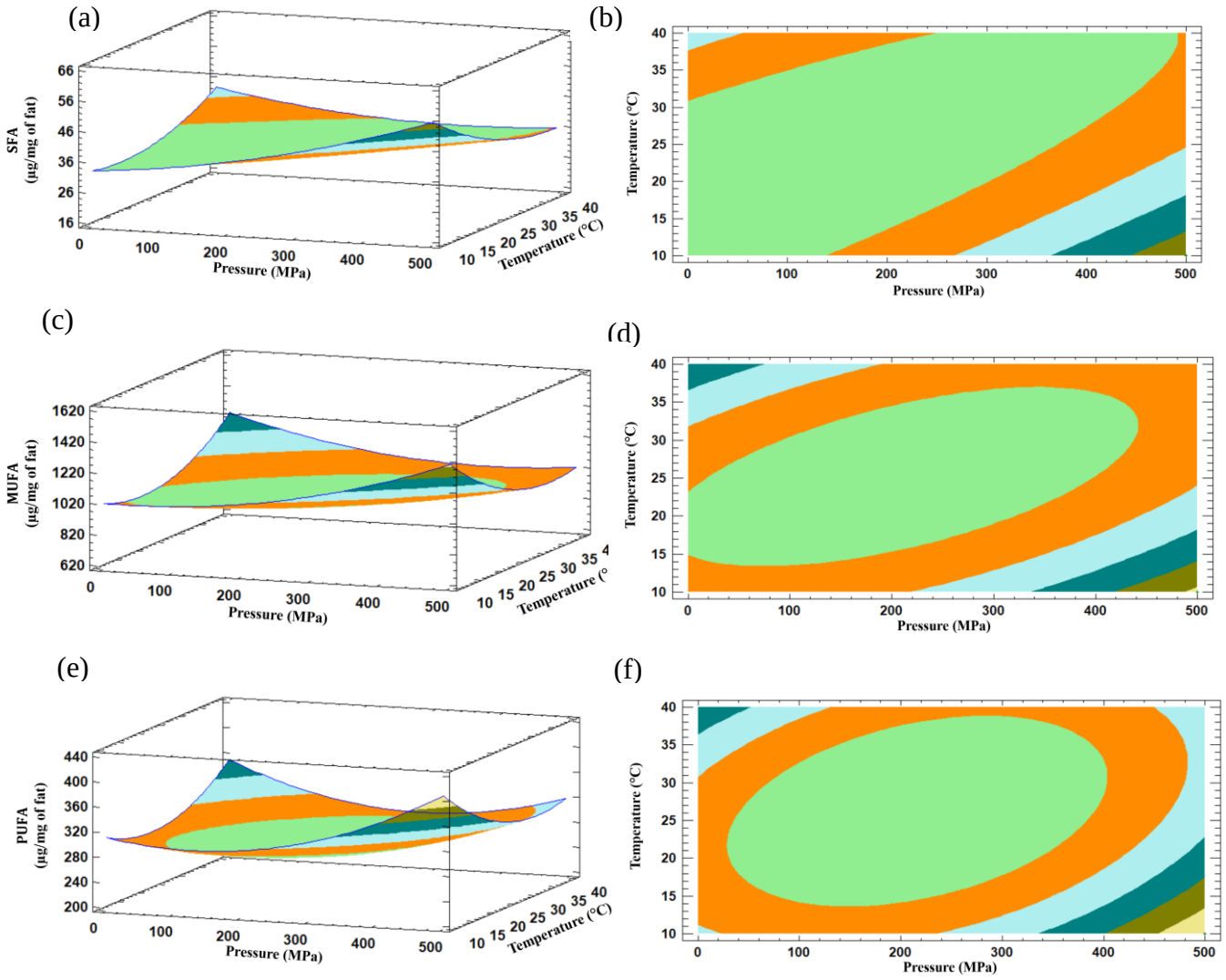

Figure 9. Interaction effects of pressure and temperature at fixed 12.5 min on SFA, MUFA and PUFA: surface plots (a,c and e) and respective contour plots (b,d and f).

The R^2 adjust is a very important parameter, as it corrects this overestimation problem by accounting of the number of the independent variables that are significant and affect the dependent variable [169]. Consequently, the difference between R^2 and R^2 adjust is usually larger when the sample size is smaller or when there are variables that were not considered in the experimental design that could have contributed to a better explanation of

the data [170]. Therefore, the greater the difference between these two parameters, the worse the performance of the model, so there will be a greater variation between the predicted and experimental results. In all models, the R^2 adjust parameter was significantly different from R^2 values, which may be related to variables that did not fit the models. However, the models presented a good fit to experimental data since all developed models could explain more than 73% of the experimental values, except the PUFA model, which just explained 59% of the results. The best model was the oil yield, because it presented higher R^2 and R^2 adjust values and the smallest difference between these two parameters indicating a better performance of the model with new data.

3.2.4. Optimization of HPE and validation of the models developed

To validate the models, experimental extractions under optimal conditions were also performed and results are in Table 12. The optimal conditions are dependent of the parameter to be analysed. Optimum pressure and temperature were the same for all models (500 MPa and 10 °C). Regarding the model of the oil yield, the optimal conditions were 394 MPa, 5 min and 40 °C. The optimal predicted extraction values for each model were not in agreement to the experimental results obtained experimentally under optimum conditions by each model. Results differ significantly (less than 135%). However, for oil yields, oleic acid and MUFA models, results differ 45, 87 and 89%, respectively. These results must be related to some experimental problem or error, which was not identified in the validation tests.

3.3. High-pressure assisted extraction versus Soxhlet extraction

The extraction yield and recovery rate of acorn oil extracted by HPE and SE were examined. Total lipids from acorn were determined by the traditional method (SE with petroleum ether) and this value was of 5.8 ± 0.6%. The results showed that the highest extraction yield obtained by HPE (394 MPa, 5 min and 40°C) was 10%, which was 44% higher than the one obtained with SE (petroleum ether). In addition, the recovery rate of acorn oil for HPE was 178% at 394 MPa, 5 min and 40°C, taking the SE (petroleum ether) yield for acorn oil to be 100%. These results indicate that the extracts obtained by HPE had polar compounds that were not extracted with SE, which is associated with the polarity of the chosen solvent (EtOH).

Regarding the SE with EtOH, it was expected that HPE would increase the oil extraction yield, because HPE causes the deprotonation and disruption of salt bridges and hydrophobic bonds, thus, the compounds are more accessible to extraction [2,49]. However,

the results showed that the extraction yield obtained by HPE at 394 MPa, 5 min and 40°C was 19% lower than the one obtained with SE (EtOH), while the extraction yield archived by HPE at 500 MPa, 13 min and 10°C was 37% lower than the one obtained with the traditional method. Moreover, HPE extraction time was significantly lower.

Table 12. Optimal extraction conditions, predicted optimum values (POV), experimental optimum values (EOV) and average variation between experimental and predicted results.

	Oil yields	**Oleic acid**	**SFA**	**MUFA**	**PUFA**
P (MPa)	394	500	500	500	500
t (min)	5	13	12	13	13
T (°C)	40	10	10	10	10
POV (µg/mg of fat)[1]	14.91	1071.46	55.11	1391.36	406.49
EOV (µg/mg of fat)[1]	10.32±0.77	509.66±12.11	23.68±2.79	739.52±41.84	212.25±5.87
Average variation of predicted values (%)	45.0	87.4	135.0	88.6	91.6

1: µg/ mg of fat except for oil yields, which are presented in %; SFA, MUFA and PUFA: total saturated, monounsaturated and polyunsaturated fatty acids.

Regarding the fatty acid composition, no significant differences were found when the *Quercus Ilex* acorn oils were extracted by Soxhlet (EtOH) or by HPE at 500 MPa, 13 min and 10°C (Table 13). Furthermore, the oils extracted by Soxhlet (petroleum ether) and HPE at 394 MPa, 13 min and 40°C presented four or one undetected FA, respectively. Therefore, the use of different solvents and the extraction conditions at high pressures revealed to interfere to the FA quality present on the final product.

The main component for acorn oils extracted by HPE at the optimal extraction conditions were oleic acid (about 58-59%), followed by linoleic acid (about 21-23%) and palmitic acid (about 14-15%).

Table 13. Fatty acid composition (% of total fatty acids), iodine and oxidizability values of acorn oils extracted by HPE and SE.

	HPE 394 MPa/5min/40°C	**HPE 500 MPa/13min/10°C**	**SE (EtOH) 360 min**	**SE (Petroleum ether) 360 min**	**Acorn oil (*Quercus Ilex* L.)** [145]
Myristic acid (C 14:0)	0.14±0.01	0.12±0.01	0.13±0.01	0.12±0.02	0.10
Palmitic acid (C 16:0)	13.98±0.12	15.23±0.13	15.50±0.01	14.98±0.01	13.57
C 16:1 C7	ND	0.04±0.03	0.06±0.00	ND	ND
Palmitoleic acid (C 16:1 C9)	0.11±0.00	0.10±0.00	0.10±0.00	0.10±0.01	0.43
Heptadecanoic acid (C 17:0)	0.12±0.00	0.13±0.01	0.14±0.00	0.14±0.01	0.13
Stearic acid (C 18:0)	2.07±0.01	2.18±0.15	1.96±0.02	2.31±0.09	2.33
C 18:1 t4	0.68±0.03	0.57±0.05	0.71±0.06	ND	ND
Oleic acid (C 18:1 C9)	58.12±0.25	58.72±0.26	57.56±0.12	59.05±0.14	62.88
C 18:1 C11	0.96±0.00	0.92±0.01	0.92±0.01	0.90±0.01	0.77
C 18:1 Cis 13	0.16±0.02	0.12±0.02	0.17±0.01	ND	ND
C 18:1 Cis 14	0.11±0.00	0.10±0.02	0.10±0.00	ND	ND
Linoleic acid (C 18:2 Cis 9 Cis 12)	22.97±0.28	21.13±0.63	22.07±0.21	21.39±0.05	17.35
α-Linolenic acid (C 18:3 C9 C12 C15)	0.24±0.01	0.28±0.03	0.31±0.05	0.34±0.01	1.03
C 18:2 C9 t11	0.34±0.02	0.37±0.01	0.28±0.14	0.67±0.01	ND
MUFA/SFA (%)	31.72±0.31	31.38±1.98	33.85±0.06	29.27±0.13	-
MUFA/PUFA (%)	3.15±0.05	3.48±0.11	3.31±0.04	3.35±0.06	-
IV	94.68±0.31	91.99±0.83	92.73±0.34	92.93±0.35	-
Cox	3.00±0.03	2.82±0.06	2.92±0.02	2.87±0.04	-

HPE: high-pressure assisted extraction; SE: soxhlet extraction; ND: not detected; SFA, MUFA and PUFA: total saturated, monounsaturated and polyunsaturated fatty acids; IV: iodine value; Cox: oxidizability value.

The oleic and linoleic acids amounts were higher for both conditions of HPE compared those from SE (EtOH), except for linoleic acid of the condition 500 MPa, 13 min and 10°C. In addition, the amount of the three main FA was lower compared to the SE (petroleum ether), except for linoleic acid of the condition 394 MPa, 13 min and 40°C. Furthermore, the composition of FA in acorn oils extracted by HPE and SE has some similarities to that found in olive oil (oleic acid, 55 - 83 %, linoleic acid, 4 - 21%, and palmitic acid, 8 - 20%) [171].

In order to evaluate the lipid quality, a set of parameters, including the IV, Cox, MUFA/PUFA ratio and MUFA/SFA ratio were calculated (Table 13). The Cox and IV of the acorn oils extracted by SE and HPE at the optimum extraction conditions were calculated (Table 13). According to COX and IV, higher oxidation stability can be predicted for acorn oil extracted by HPE at 500 MPa, 13 min and 10°C, followed by the samples extracted by SE (EtOH and petroleum ether) and HPE at 394 MPa, 13 min and 40°C (only based on the fatty acid composition). There is a direct relation between these parameters so that IV value decreases by declining the Cox value [172], as can been seen in Table 13. The IV values calculated for oils extracted by HPE and SE were 7 to 9% and 7 to 8% to, respectively, higher than those obtained experimentally by Charef *et al.* [143] for *Quercus Ilex* oil. These results were in agreement with Bart et al. [149], since the theoretical IV tends to be slightly higher (5 – 10%) than the IV determined experimentally.

The MUFA/SFA ratio was positive for both oils indicating that they have beneficial health properties [152]. In this study, the MUFA/SFA ratio was higher for the oil extracted by SE (EtOH), followed by those extracted by HPE at 394 MPa, 13 min and 40°C and 500 MPa, 13 min and 10°C and SE (petroleum ether). In addition, the MUFA/PUFA ratio was higher for the oil extracted by HPE at 500 MPa, 13 min and 10°C, followed by those extracted by SE (petroleum ether and EtOH) and HPE at 394 MPa, 13 min and 40°C. Thus, it can be expected that the oil extracted by HPE at 500 MPa, 13 min and 10°C has more MUFA, which are the most stable unsaturated FA, than the other oils [174].

3.4. Infrared spectroscopy

Since vegetable oils are mainly constituted of triglycerides, they are the major spectral contributions in acorn oil. As shown in Figure 10, the FTIR spectra of studied oil obtained by HPE showed absorption bands at different wavelengths, as follows: peaks with high intensity at 2922 and 2853 cm^{-1} (assigned to asymmetric and symmetric stretching

vibration of C-H bonds of aliphatic CH_2 groups of triglycecerides, respectively), intense peak at 1744 cm^{-1} (represents carbonyl stretching vibration of ester functional group (C=O), in this case associated with the triglyceride ester linkage or the carboxylic group of free FA), peak with medium intensity at 1463 cm^{-1} (caused by C-H deformations due the scissoring vibration of the CH_2 groups), small peak of weak intensity at 1377 cm^{-1} (assigned to the terminal CH_3 groups symmetrix bending) and sharp peaks at 1161 and 1095 cm^{-1} (stretching vibration of C-O in ester groups of triglycerides).

Additionally, IR spectra from acorn oil extracted by SE exhibited the same peaks with similar intensities, in special the characteristic band of FA (around 1744 cm^{-1}). This evidenced that fatty acid was not decomposed and the peak intensities of C-O and C=O were similar. This result indicates that no significant differences should be found on FA composition of the acorn oils extracted by SE or HPE.

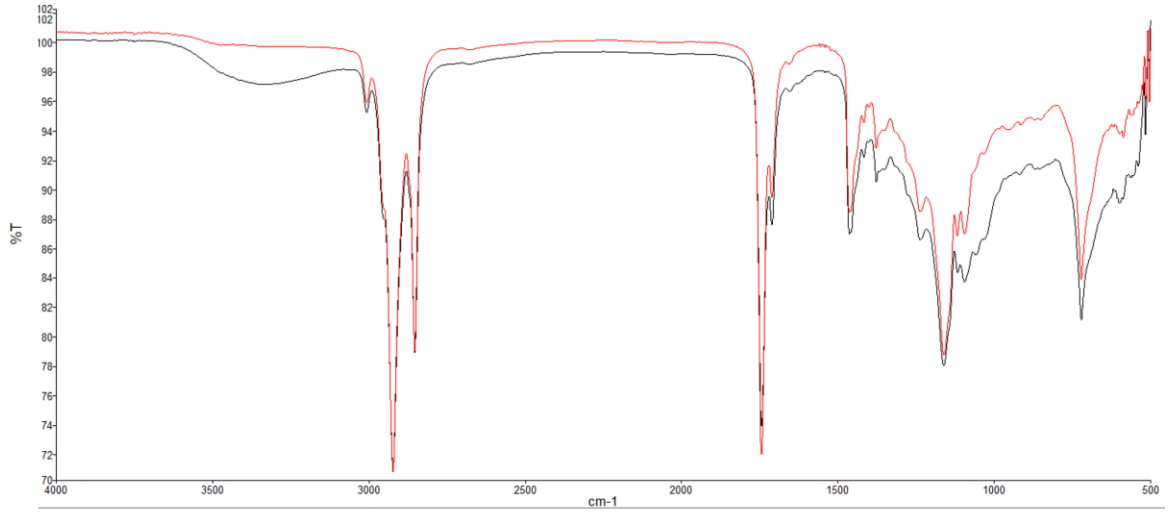

Figure 10. Infrared spectrum of acorn oil obtained by HPE in conditions with better (red) and worse (black) oil yield.

3.5. Antioxidant activity

Free radical scavenging activity (DPPH)

The DPPH radical scavenging activity of the acorn oils obtained by HPE is shown in Table 14. From the obtained results, the DPPH activity was higher in the acorn oil extracted by HPE at 394 MPa, 5 min and 40 °C (327.65 ± 33.40 µmol TE/ g oil) than in the one extracted at 500 MPa, 13 min and 10 °C (289.30 ± 23.33 µmol TE/ g oil). Therefore, these results confirmed that the antioxidant activity of acorn oils was influenced by HP conditions, when the pressure and time decreased, while the temperature of pressurisation increased.

Ugur *et al.* [49] reported that HP (500 MPa) compared to atmospheric pressure might have helped to released antioxidant compounds during oil extraction and consequently it was observed an increase in antioxidant activity for DPPH. In addition, Ugur *et al.* [49] revealed that increasing temperature from 30 °C to 40 °C increase the oil yield and the recovery of antioxidant compounds.

The antioxidant activity obtained in the present study was high when compared to those found by Makhlouf *et al.* (2018) [173] and Makhlouf *et al.* (2019) [174] for *Quercus Ilex.* These differences may be related to the extraction methodologies, since HPE is a technology that allows using lower temperatures and extraction times, leading to high oil yields when compared to SE, which was the method used by Makhlouf *et al.* (2018) [173] and Makhlouf *et al.* (2019) [174] to extract oil from *Quercus Ilex*. In addition, the high values of antioxidant activities can be attributed to the solvent ability to extract different compounds from acorn [5].

Table 14. Antioxidant activity by DPPH of the extracted acorn oils by HPE.

Extraction conditions			**DPPH**
P (MPa)	**t** (min)	**T** (°C)	**(µmol TE/ g oil)**
394	5	40	327.65 ± 33.40
500	13	10	289.30 ± 23.33

4. Conclusions and Future Work

Several approaches were taken to look for a green solvent during preliminary tests, from extraction with NADES, Tween 80% and water. However, these experiments were unsuccessful, therefore the solvent selected to study the impact of pressure, extraction time and temperature on oil yield, lipid profile and SFA, MUFA and PUFA present in acorn was EtOH for being food compatible and environmentally friendly. The three variables studied (pressure, extraction time and temperature) influenced the models, independently and interactively. Temperature was the variable that showed the highest effect on extraction yields, while the effect of time quadratic term showed the highest effect for the FA models. The optimum extraction condition for oil yield was 394 MPa, 5 min and 40 °C, while for the other analysed parameters it was 500 MPa, 13 min and 10 °C. The extraction yields obtained by HPE at both optimal conditions were lower than the one obtained with SE (EtOH), however presented lower extraction times. Furthermore, the acorn oil recovery rate at 394 MPa, 5 min and 40°C was 178%, taking the SE (petroleum ether) yield for acorn oil to be 100%. These results indicate that the extracts obtained by HPE had polar compounds that were not extracted with SE (petroleum ether), which is associated with the polarity of the chosen solvent (EtOH). In general, HP increased the FA responses up to 27%, except for SFA model (40%) and PUFA model (21%) when compared to the conditions without pressure.

The FTIR analysis indicated no significant differences on FA composition of the acorn oils extracted by SE or HPE. Regarding the FA composition, no significant differences were found when the oils were extracted by SE (EtOH) or by HPE at 500 MPa, 13 min and 10°C. In addition, the oil extracted by HPE at 500 MPa, 13 min and 10°C presented more MUFA and higher oxidation stability than the oils extracted by HPE at 394 MPa, 13 min and 40°C and SE (EtOH and petroleum ether). The main components for acorn oils extracted by HPE were oleic acid (about 58-59%), followed by linoleic acid (about 21-23%) and palmitic acid (about 14-15%).

The fitness and adequacy of models were high since the R^2 obtained were higher than 0.90 for all models, except for PUFA (0.82). In addition, the predicted values were in agreement with the experimental results, indicating a good adequacy of models. However, the optimal extraction conditions predicted and experimental results differed more than 45%, which suggests performing further validation tests in the future. The selected extracts

showed a remarkable antioxidant activity by DPPH up to 327.65 ± 33.40 μmol TE/ g oil. HPE provided lower oil yields than SE, however presented lower extraction times.

Acorn oils are not yet produced at an industrial level, however the optimizations obtained in this study make high pressure technology a promising process for scale up. Despite that, higher scale tests will be necessary to ponder the economic viability of the method.

Considering both, the literature revision and the experimental work in this thesis, there are still many areas that need further exploitation. Therefore, for further studies as future work, it would be interesting to:

- Analyse the antioxidant activity in the oil samples obtained in the SE, in order to understand the impact that the different methodologies have on the final product;
- Make an experimental design for antioxidant activity by DPPH to understand the impact of pressure, time and temperature;
- Optimization of the acorn oil quality with additional dependent variables (responses) on the Box-Behnken experimental design such as, IV, peroxide value and *p*-anisidine value;
- Characterization of the chemical properties of the studied acorn oil (IV, acid value, peroxide value, *p*-anisidine value, saponification value) should be of interest.

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6. Appendices

Appendix A. Response surface and contour plots for acorn oil yields.

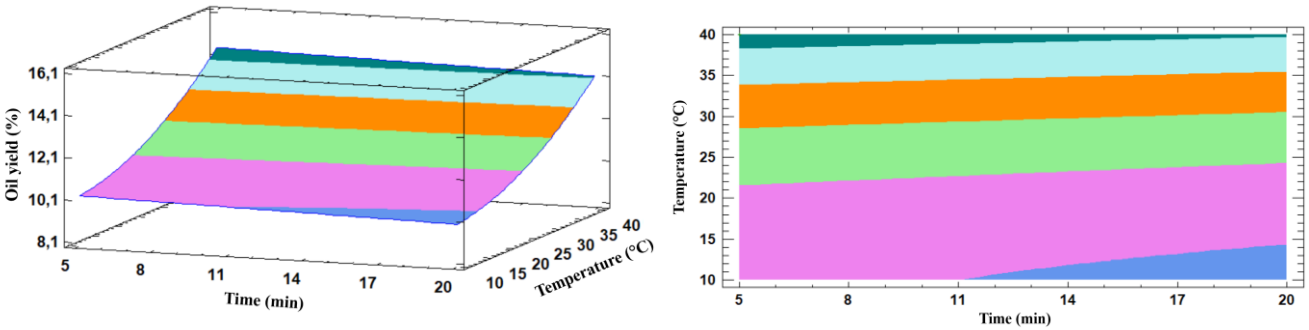

Figure A1. Response surface and contour plot of oil yields for 250 MPa.

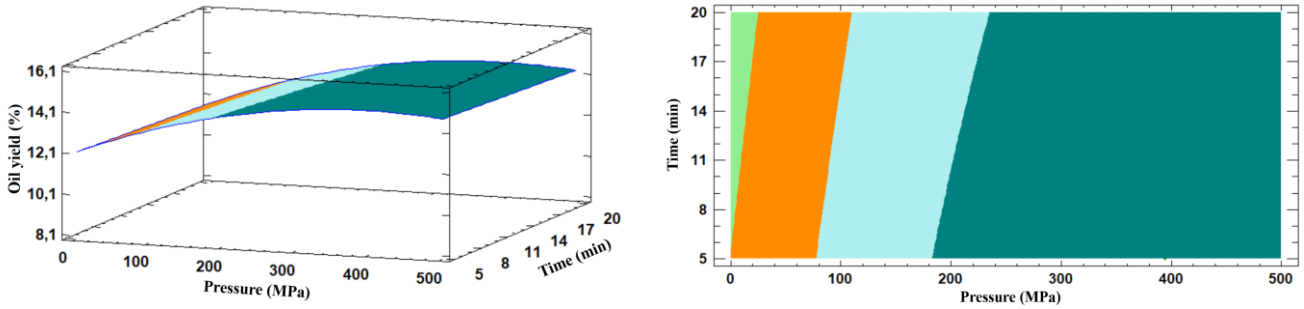

Figure A2. Response surface and contour plot of oil yields for 40 °C.

Appendix B. Response surface and contour plots for oleic acid.

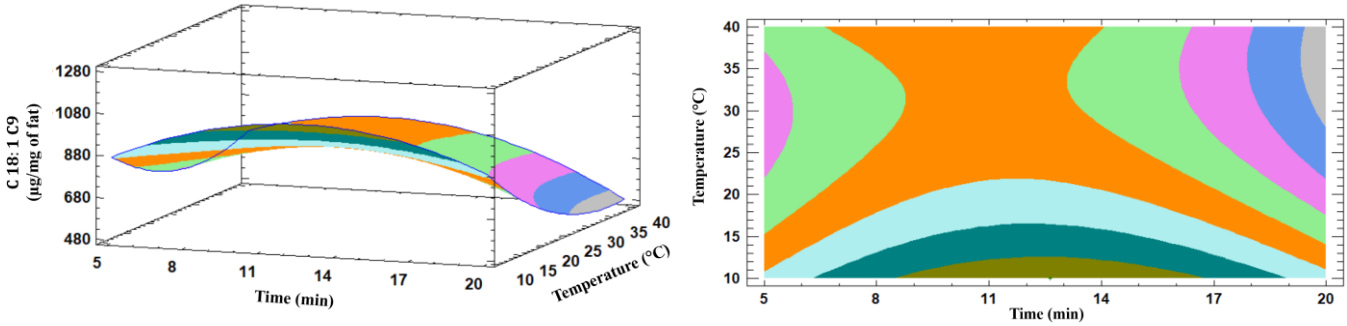

Figure B1. Response surface and contour plot of oleic acid for 500 MPa.

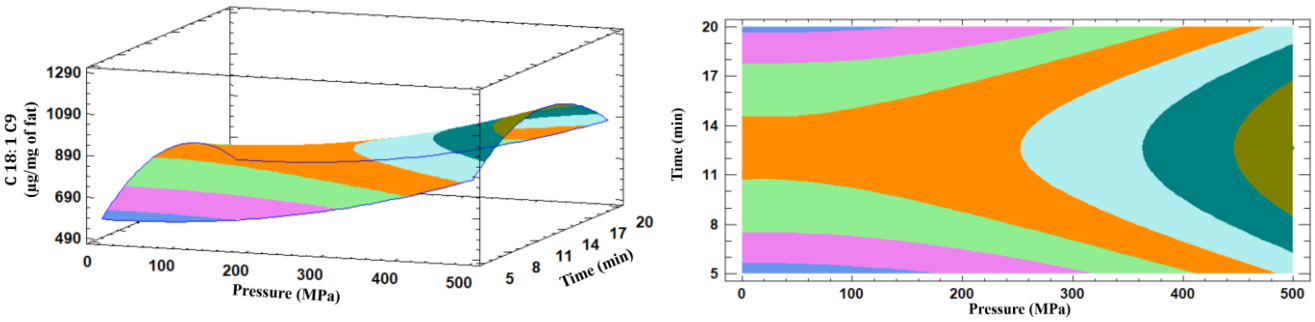

Figure B2. Response surface and contour plot of oleic acid for 10 °C.

Appendix C. Response surface and contour plots for SFA.

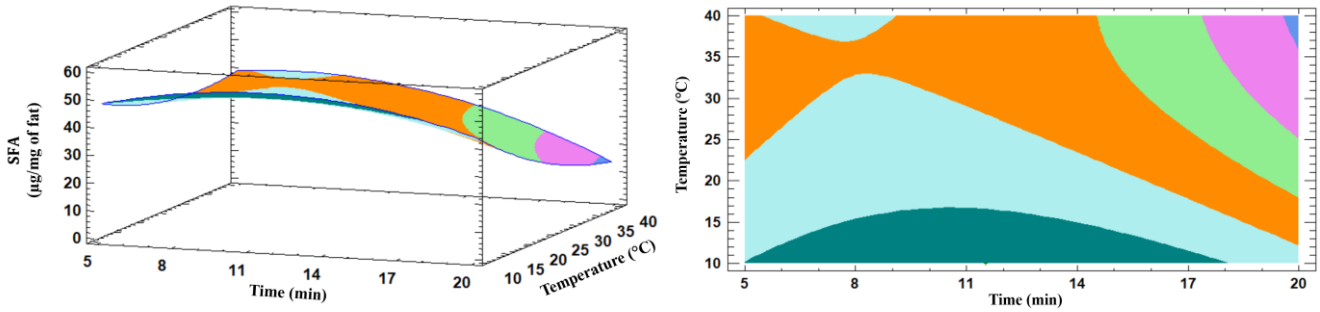

Figure C1. Response surface and contour plot of SFA for 500 MPa.

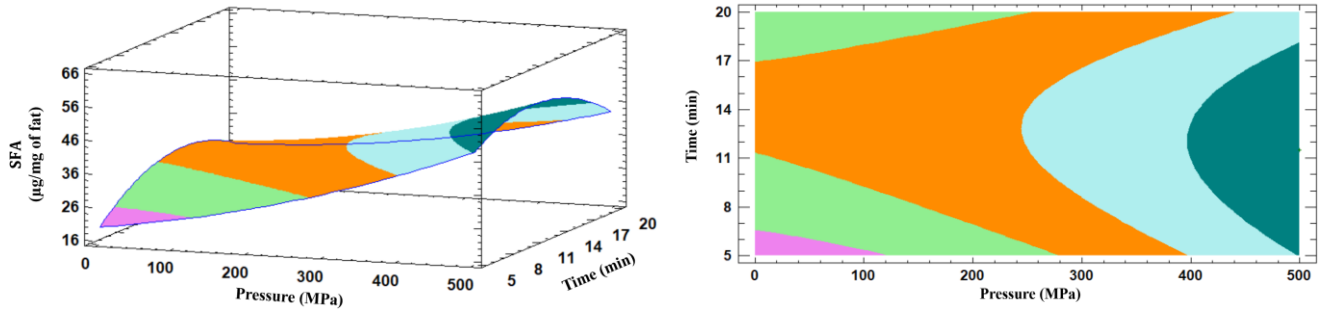

Figure C2. Response surface and contour plot of SFA for 10 °C.

Appendix D. Response surface and contour plots for MUFA.

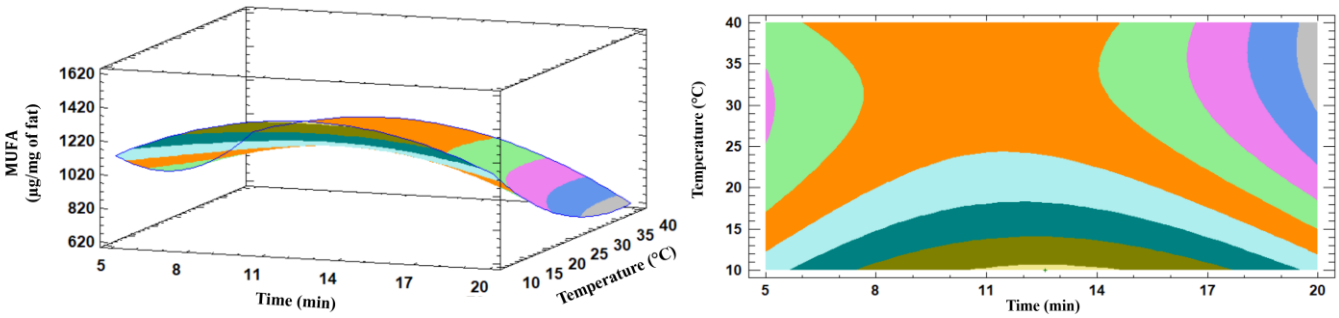

Figure D1. Response surface and contour plot of MUFA for 500 MPa.

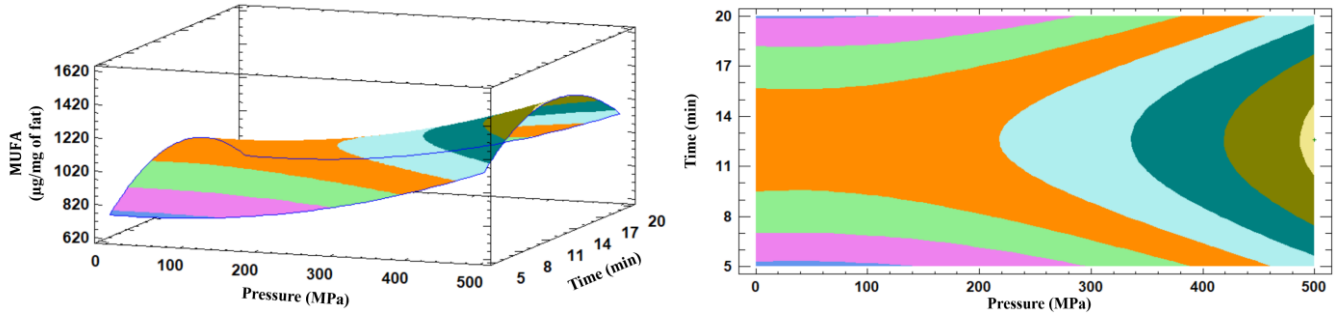

Figure D2. Response surface and contour plot of MUFA for 10 °C.

Appendix E. Response surface and contour plots for PUFA.

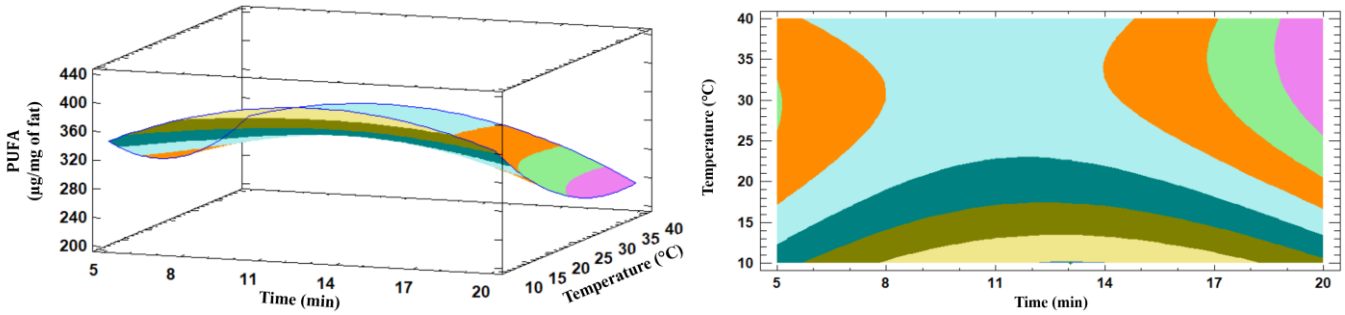

Figure E1. Response surface and contour plot of PUFA for 500 MPa.

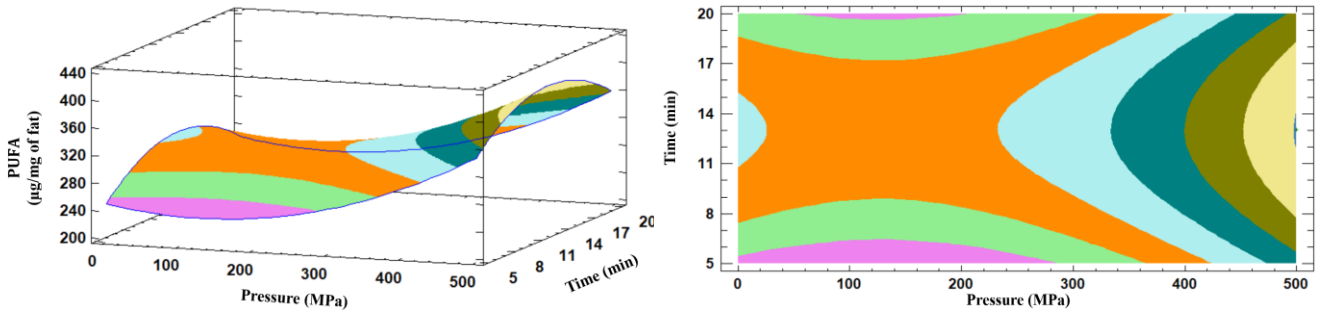

Figure E2. Response surface and contour plot of PUFA for 10 °C.