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Grasas y aceites

CONTENIDO

Investigación

CONTENTS

Research

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Review on preparation methods, mechanisms and applications for antioxidant peptides in oil

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SUMMARY: Natural antioxidants, especially those used in edible oil, are safer compared to chemically synthesized antioxidants. Therefore, research on natural antioxidants has become prevelant. Antioxidant peptides derived from food protein can effectively prevent oil oxidation. Protein hydrolyzation is widely applied for the production of antioxidant peptides in industry, and bioinformatics is employed nowadays to generate the desired peptide sequence. Furthermore, the mechanism of antioxidant peptides in the oil system is still controversial, which limits the further development of antioxidant peptides as food antioxidants. This review introduces the preparation method of antioxidant peptides and their mechanisms as well as applications in the oil. It will help to comprehensively understand the function of antioxidant peptides and promote their development in the oil field.

KEYWORDS: *Antioxidant peptide; Bioinformatics; Mechanism; Oil oxidation*

RESUMEN: ***Revisión sobre métodos de preparación, mecanismos y aplicaciones de péptidos antioxidantes en aceites.*** Los antioxidantes naturales, especialmente utilizados en aceites comestibles, son más seguros en comparación con los antioxidantes sintetizados químicamente. Por lo tanto, la investigación sobre antioxidantes naturales se convierte en un punto de interés. Los péptidos antioxidantes derivados de las proteínas alimentarias pueden prevenir eficazmente la oxidación del aceite. La hidrolización de proteínas se usa ampliamente en la industria para la producción de péptidos antioxidantes y la bioinformática se emplea hoy en día para generar la secuencia de péptidos deseada. Además, el mecanismo de los péptidos antioxidantes en el sistema oleoso sigue siendo controvertido, lo que limita el desarrollo posterior de péptidos antioxidantes como antioxidantes alimentarios. Esta revisión presenta el método de preparación de péptidos antioxidantes y su mecanismo, así como las aplicaciones en aceite, lo que ayudará a comprender de manera integral la función de los péptidos antioxidantes y promoverá su desarrollo en el campo petrolero.

PALABRAS CLAVE: *Bioinformática; Mecanismo; Oxidación de aceite; Péptido antioxidante*

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1. INTRODUCTION

Oil, which is mainly derived from plants and rich in unsaturated fatty acids (UFA), can provide essential fatty acids for humans. However, the UFA can easily form free radicals at the α-C of the C=C double bond. This will initiate free radical chain reactions and oil oxidation. The oxidation products of oil will produce peculiar smells and carcinogens that can reduce the oil's nutritional value and shorten the shelf-life of the oil (Ahn *et al.*, 2012b). Therefore, it is necessary to control oil oxidation, which is of great significance to improving the level of food safety and develop the oil industry (Kiralan *et al.*, 2021; Rathod *et al.*, 2021).

In order to safely and effectively prevent or inhibit oil oxidation without changing its sense and quality, the development and research of antioxidants have been developed rapidly (Budilarto and Kamal-Eldin, 2015; Mishra *et al.*, 2020). At present, chemically synthesized antioxidants are still predominant and mainly including propyl gallate (PG), butylated hydroxy anisole (BHA), dibutyl hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ). These antioxidants are phenolic antioxidants with cheap and stable performance (Tadesse and Emire, 2020). However, synthetic antioxidants have potential toxicity and carcinogenicity, so it is necessary to find safe and effective antioxidants (Taghvaei and Jafari, 2015). It is well known that in addition to vitamins and phenolic compounds, some polypeptides also have antioxidant activities. Most of these substances exist in plants and animals. (Pejin *et al.*, 2013; Im *et al.*, 2014; Tesanovic *et al.*, 2017; Karaman *et al.*, 2019; Jie *et al.*, 2019a). Nowadays, antioxidant peptides have become a focus in the development of natural antioxidants (Zarei *et al.*, 2012). Antioxidant peptides, as antioxidant components derived from protein, have the advantages of relatively simple structure, easy absorption, good stability and immunological unresponsiveness. Antioxidant peptides have efficient free radical scavenging ability, so they have a significant protective effect on oil peroxidation induced by free radicals (Yang *et al.*, 2018).

In recent years, the traditional preparation methods of antioxidant peptides have been well developed. In order to further optimize the preparation of antioxidant peptides to generate peptides with desired function and purpose, some bioinformatics have been applied to the research of antioxidant peptides (Chiozzi *et al.*, 2016; Jara *et al.*, 2018; Borawska-Dziadkiewicz *et al.*, 2021). The acquisition of purposeful antioxidant peptides from different biological resources is increasingly becoming the driving force of the oil industry (Tadesse and Emire, 2020). In order to promote the application of antioxidant peptides as food antioxidants, we discussed the preparation methods of antioxidant peptides, their different antioxidant mechanisms and applications in the oil. They are of great significance to furthering the use of antioxidants and oil storage.

2. PREPARATION OF ANTIOXIDANT PEPTIDES

The most well-known antioxidant peptides are carnosine (β-alanyl-L-histidine) and glutathione (r-glutamyl-cysteinyl-glycine). They are two kinds of natural antioxidant peptides existing in the organism. Their antioxidant functions are mainly reflected in their ability to scavenge free radicals and peroxides produced by oil oxidation. In order to find other antioxidant peptides, researchers hydrolyzed protein with different methods. The methods of hydrolyzing protein mainly include acid-base hydrolysis, microbial fermentation and enzymatic hydrolysis, which is the most common (Borrajo *et al.*, 2019).

2.1. Preparation of antioxidant peptides by traditional hydrolysis

Hydrolyzing protein is the most common method for preparing antioxidant peptides. Acid-base hydrolysis uses acid or alkali to hydrolyze protein. Some researchers hydrolyzed ostrich egg white protein with 0.25 mol/L NaOH at 40 ºC for eight hours. They proved that the antioxidant activity of the protein was significantly enhanced after hydrolysis (Khueychai *et al.*, 2019). This method is cheap and time-saving. Nonetheless it is difficult to control the degree of hydrolysis and product quality, because the violent reaction conditions involved are destructive to amino acids (Hall and Ahmad, 1997).

Microbial fermentation can produce enzymes and enzymatic hydrolysis simultaneously. This method also can directly isolate and purify antioxidant peptides (Chai *et al.*, 2020). After fenugreek protein was fermented with *Lactococcus lactis* for 24 hours,

the free radical scavenging activities of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ammonium salt (ABTS) increased by 42.9 and 40%, respectively (Setti *et al.*, 2017). However, the process of screening microorganisms is cumbersome and the safety of microbial enzyme production is uncontrollable (He *et al.*, 2012).

Enzymatic hydrolysis uses proteolytic enzymes to hydrolyze protein into small molecular polypeptides. It is characterized by mild conditions, strong operability, strong specificity and fewer by-products. The same protein hydrolyzed by different enzymes produces different antioxidant peptides (da Rosa *et al.*, 2018). A study has measured the antioxidant activity of camel colostrum protein hydrolyzed by pepsin, trypsin, pancreatin and another two enzymes. The results showed that the proteolytic peptide hydrolyzed by pancreatin had the highest antioxidant activity (Oussaief *et al.*, 2019). Hydrolysis conditions are also critical. Some researchers have optimized the hydrolysis conditions from egg white with chymotrypsin and pepsin through response surface methodology, and the antioxidant activity of the peptide was improved (Yuan *et al.*, 2020). Table 1 summarizes experiments related to the preparation of antioxidant peptides by enzymatic hydrolysis.

However, enzymatic hydrolysis usually obtains crude peptides, so it needs to be separated and purified. Usually, ultrafiltration or gel chromatography can be carried out according to the size of antioxidant peptides. Ion exchange chromatography and reverse chromatography can also be used (based on the charge and hydrophobicity of the antioxidant peptide, respectively) (Vanvi and Tsopmo, 2016). After that, tandem mass spectrometry is applied to identify the antioxidant peptides. One study extracted antioxidant peptide from duck plasma hydrolysate (DPH) and then separated and purified the antioxidant peptide by ultrafiltration, dimensional exclusion chromatography and reversed-phase high performance liquid chromatography. The researchers also identified the DPH fraction with the highest antioxidant activity by nanoscale liquid chromatography-tandem mass spectrometry (Yang *et al.*, 2020). In fact, DPPH, ABTS free-radical scavenging and oxygen-radical absorbance capacity (ORAC) tests are generally used to evaluate the effect of antioxidant peptides (Kim *et al.*, 2018). Other researchers have pre-treated the protein with ultrasound, microwave and irradiation to improve the antioxidant activity of the peptide (Wang *et al.*, 2019; Zhang *et al.*, 2019). However, the single use of enzymatic hydrolysis is time consuming when selecting hydrolytic enzymes. The process of iden-

Table 1. Preparation of antioxidant peptides by enzymatic hydrolysis

Protein source	Hydrolysis conditions	Enzyme	Application model	Reference
Zein	pH 9.0, 50 ºC, 1h	Alcalase	Myofibrillar protein oil-in-water emulsions	(Li *et al.*, 2017)
Porcine blood	pH 6.5, 50 ºC, 6h	Papain	Pork emulsion	(Verma *et al.*, 2018)
Oat brans	pH 2.0, 37 ºC, 3h	Pepsin	Not studied	(Vanvi and Tsopmo, 2016)
Common bean	pH 8.0, 50 ºC, 3h	Alcalase	Not studied	(Oseguera-Toledo *et al.*, 2011)
Cow milk	pH 3.0, 37 ºC, 3h	Porcine pepsin	Zebrafish larvae model	(Carrillo *et al.*, 2017)
Salmon byproduct	pH 7.0, 50 ºC, 8h/ pH 1.0, 37 ºC, 8h/ pH 8.0, 37 ºC, 8h	Alcalase/Flavourzyme/ Neutrase/Protamex/ Pepsin/Trypsin	Chang liver cells	(Ahn *et al.*, 2012a)
Palm kernel cake protein	pH 6.5, 65 °C/ pH 7.5, 55 °C/ pH 1.5, 37 °C/ pH 8.0, 37 °C/ pH 8.0, 55 °C/ pH 5.0, 55 °C/ pH 6.8, 50 °C, 6h	Papain/Alcalase/Pepsin/ Trypsin/Flavourzyme/Bromelain/ Chymotrypsin	Not studied	(Zarei *et al.*, 2014)
Cod protein	pH 8.1, 45 ºC, 6h	Protamex	Washed cod model	(Jonsdottir *et al.*, 2016)

Table 2. Bioinformatics databases used in antioxidant peptides

Classification	Name	Function	Reference
Protein database	UniProtKB	Provide protein sequence and function information	(Bechaux *et al.*, 2020)
	PIR	Provide protein sequence	(Panchal *et al.*, 2021)
Simulated enzymatic hydrolysis	BIOPEP-UWM	Predict the potential sites for protease cleavage of the target protein sequence	(Ibanez *et al.*, 2013)
	Peptide Cutter	Predict the potential sites for protease cleavage of the target protein sequence	(Fu *et al.*, 2016)
Antioxidant peptide database	Pep Bank	Provide peptide structure and classify	(Chiozzi *et al.*, 2016)
	NCBI	Provide peptide sequence	(Tian *et al.*, 2016)
Predict antioxidant peptide	Peptide Ranker	Evaluation of antioxidant peptides and their precursor proteins	(Ibanez *et al.*, 2013)
	BIOPEP-UWM	Predict the potential antioxidant activity of the input peptide sequence	(Su *et al.*, 2011)

tifying antioxidant peptides is complicated and the target antioxidant peptides may not be obtained (Wen *et al.*, 2020). Therefore, bioinformatics has attracted people's attention.

2.2. Bioinformatics guides the preparation of antioxidant peptides by enzymatic hydrolysis

Bioinformatics based on computer technology can guide the selection of precursor proteins and simulate enzymatic hydrolysis (Zhou *et al.*, 2019). Moreover, the function of the enzymatic hydrolysis products can be predicted and identified based on the sequence, structure and other parameters of proteins and peptides by bioinformatics. It is widely used in the research of hydrolyzing protein to obtain antioxidant peptides (Tejano *et al.*, 2019). This method is often used with databases such as Peptide Ranker, BIOPEP-UWM and UniProtKB (Chen *et al.*, 2017; Pearman *et al.*, 2020). Some commonly used bioinformatic databases are listed in Table 2. These databases contain the amino acid sequences of various proteins and peptides to improve the efficiency of obtaining target peptides at a low cost (Tadesse and Emire, 2020). Darewicz *et al.* (2016) retrieved the amino acid sequence, molecular weight and chain length of *Cyprinus carpio* protein from UniProtKB. After using the ClustalW2–Multiple Sequence Alignment program to eliminate the same sequence, they selected 33 carp protein amino acid sequences with less than 90% identity for further analysis. The number of amino acid residues in the analyzed carp protein amino acid sequence ranged from 62 (light myosin, Q90335) to 1938 (myosin heavy chain, Q2HX56).

These bioinformatic methods can also simulate protease hydrolysis. In 2018, researchers used Peptide Cutter to simulate 100 tripeptides from the myosin of *Mizuhopecten yessoensis* by pepsin and trypsin (Yu *et al.*, 2018). For example, the BIOPEP-UWM database can not only simulate hydrolysis, but also predict the bioactive peptides released from protein sequences and their bioactivity and physicochemical properties (Yang *et al.*, 2017). Researchers estimated the solubility of antioxidant peptides from flaxseed proteins in water by using Innovagen Peptide Solubility Calculator Proteomic. They also predicted the release of antioxidant peptides by opening the 'search for active fragments tab of BIOPEP. Thereafter, they estimated several properties and indices of the silico-derived antioxidant peptides by using the application of the 'Peptides' package in R or ProtParam of ExPASy. These properties included amino acid composition, amino acid length, molecular weight (MW), isoelectric point (pI), Boman index, net charge and hydrophobicity index (Ji *et al.*, 2019). In another research, Salim and Gan (2020) used Peptide Cutter to simulate the digestion of egg white ovalbumin, then Peptide Ranker was used to screen the obtained peptide sequences. After that, they predicted the peptide binding sites by Pepsite2 and finally used an in vitro assay to verify the bioactivity of the peptide. The *AnOxPePred*, a method for predicting the free-radical scavenging ability and chelating properties of antioxidant peptides was developed. When constructing the predictive variable,

a standard database consisting of peptides and their free-radical scavenging capabilities and chelating properties was created (Olsen *et al.*, 2020).

Although bioinformatics is an effective method to study and predict the antioxidant peptides released by proteins and their potential activities, there are some limitations to this approach. For example, there is a lack of research on the protein sequence in simulated hydrolysis and its influence on enzymatic hydrolysis (Zhou *et al.*, 2019). Bioinformatics simulates the hydrolysis of the protein sequence completely, but it is often necessary to consider the secondary and tertiary structures of protein and other factors such as inhibitors, pH and temperature when verifying in vitro (Bechaux *et al.*, 2020). Some scientists showed that in the peptides obtained by computer simulation of a porcine actin sequence, only a few could be verified through laboratory experiments (Keska and Stadnik, 2016). This proved that bioinformatics was an auxiliary method for selecting protein families and optimal hydrolyses. Bioinformatics was still theoretical, so it needed to be verified by in vitro experiments.

In order to efficiently and economically prepare antioxidant peptides, more and more researchers combine enzymatic hydrolysis and bioinformatics. The amino acid sequence of the target protein can be searched in the database to facilitate the selection of hydrolases, and it is estimated that there are more than 8 million amino acid sequences of proteins in the database (Agyei *et al.*, 2018). Some researchers used the amino acid sequence of the 11S coffee globulin (the entry Q9ZNY2_COFAR and accession number NCBI 13443) deposited in the UniProtKB database. Subsequently, they selected enzymes (pepsin (EC 3.4.23.1), trypsin (EC 3.4.23.4) and chymotrypsin (EC 3.4.21.1)) to simulate gastrointestinal digestion. They carried out the prediction of peptides generated through simulation by using the BIOPEP-UWM "enzyme action". Based on the results for the prediction of bioactivity of the 11S globulin, a series of in vitro studies was carried out, including protein extraction, enzymatic hydrolysis, protein isolation and determination of its antioxidant capacity. Finally, they proved that the hydrolyzed peptides of roasted coffee beans have higher antioxidant activity (Ribeiro *et al.*, 2021). Computer simulation of hydrolysis can also greatly improve the screening efficiency of hydrolytic enzymes. Jie *et al*. (2020) used gluten as the bioinformatics template of *Caragana korshinskii* seed protein, then carried out virtual enzymatic hydrolysis with the assistance of the BIOPEP-UWM database. They determined that papain was the main protease to hydrolyze the *Caragana korshinskii* seed protein, which improved preparation efficiency.

After simulated hydrolysis, the computer generates an amino acid sequence. By comparing the sequence with the antioxidant peptide sequences reported in databases such as Swiss-Prot, Peptide Ranker or in the literature, the activity of the antioxidant peptide is predicted, evaluated and the target peptide is selected (Tu *et al.*, 2018). For example, Sun *et al*. (2021) first prepared defatted collagen hydrolysates from yak bone, and then separated and purified the hydrolysate by ultrafiltration and reversed-phase high-performance liquid chromatography. After that, they determined the antioxidant activities of different hydrolysates and screened the best components for identification by mass spectrometry. Mass spectrometry was used to identify components with better antioxidant activity- Swiss-Prot was used to determine the amino acid sequence of the target peptide. Finally, they selected peptides with molecular weight of less than 1 kDa for synthesis according to the possible structural features of antioxidant peptides and the number of enzyme-cutting sites contained in the peptides. Other researchers isolated and purified antioxidant peptides from *Asparagus* by-products by reverse-phase chromatography, and then identified the peptides by tandem mass spectrometry. Peptide identification was carried out by using the Swiss-Prot database. PeptideRanker was used to predict the probability of the antioxidant activities of these peptides. This study only considered peptides with probability scores higher than 0.95. Therefore, only five peptides were retained and five novel peptide sequences were synthesized, and their antioxidant activity was finally evaluated in vitro (Montone *et al.*, 2019). It is also necessary to predict the toxicity of antioxidant peptides. Harvian *et al*. (2019) used ToxinPred to analyze the antioxidant peptide obtained from jack bean (*Canavalia ensiformis*) canavalin protein. ToxinPred is a *in silico* database for the prediction of the toxicity of selected peptides. The support

vector machine (SVM)-based prediction method and SVM threshold value (0.0) were used to separate toxic from non-toxic peptides. Their results showed that the antioxidant peptide was non-toxic. Of course, in vivo or in vitro tests are required to further determine the physiological activity of antioxidant peptides eventually.

In summary, the bioinformatics method can simulate protein hydrolysis, determine the most suitable hydrolase and screen out peptides with good antioxidant effects by comparing the amino acid sequences of peptides gained through experiments with those in the database for in vitro verification. The combination of the two methods will simplify the cumbersome experimental process of traditional enzymatic hydrolysis, save cost, improve preparation efficiency and provide a promising method to improve not only the availability but also the high-value utilization of antioxidant peptides (Ji *et al.*, 2019).

3. MECHANISM OF ANTIOXIDANT PEPTIDES INHIBITING OIL OXIDATION

The main ways for oil oxidation are automatic oxidation, photosensitive oxidation and enzymatic oxidation (Hammer and Schieberle, 2013; An *et al.*, 2014). Autoxidation includes three stages: chain initiation, chain transfer and chain termination (Scheme 1).

$$\text{Chain initiation: } R:H \rightarrow R\cdot + H\cdot$$

$$\text{Chain transfer: } R\cdot + O_2 \rightarrow ROO\cdot$$

$$ROO\cdot + R:H \rightarrow ROOH + R\cdot$$

$$\text{Chain termination: } R\cdot + R\cdot \rightarrow R:R$$

$$R\cdot + ROO\cdot \rightarrow ROOR$$

$$ROO\cdot + ROO\cdot \rightarrow ROOR + O_2$$

Scheme 1. Mechanism of oil automatic oxidation (R· stands for free radical).

Photosensitive oxidation and enzymatic oxidation are important factors to initiate automatic oxidation in oil. Their oxidation products decompose to produce various free radicals which make the automatic oxidation chain reaction continue to circulate (Ladikos and Lougovois, 1990). The automatic oxidation of oil can damage its color, flavor and texture to reduce the nutritional value and also generate cytotoxic and genotoxic compounds (Sohaib *et al.*, 2017). These substances cause irreversible oxidative damage to humans (Qi *et al.*, 2016). Therefore, the oxidation of oil must be inhibited. Antioxidant peptides are unique to some extent. Their uniqueness is not only reflected in their small size and high utilization rate in the body, but also in that peptides are easier to metabolize than amino acids and optimize the environment in the body. (Yang *et al.*, 2021). According to current research, antioxidant peptides can inhibit oil oxidation by scavenging free radicals, suppressing the activity of catalytic metal ions and restraining the formation and reactivity of hydroperoxide, so they are potentially multifunctional antioxidants (Agyei *et al.*, 2016). The activity of antioxidant peptides is mainly related to the composition of different amino acids, the sequence of amino acids and the molecular weight of antioxidant peptides (Wu *et al.*, 2015b). This is similar to the results of another study (Chen *et al.*, 2020b).

3.1. Scavenging free radicals

Free radicals exhibit strong reactivity, so they are initiators in oxidation reactions (Tomycz *et al.*, 2011). At present, it is generally recognized that an important cause of oil automatic oxidation is the chain reaction initiated by free radicals, such as hydroxyl radicals, peroxy free radicals and alkoxy free radicals (Sonklin *et al.*, 2018). Hydroxyl radicals can remove the hydrogen atoms in the adjacent fat chain and induce oxidation chain reactions. High-energy peroxy free radicals and alkoxy free radicals can combine hydrogen atoms from adjacent fatty chains to destroy fatty acids and deteriorate oil. Therefore, it is necessary to combine free radical scavengers with free radicals to slow down the rate of chain reactions. Many antioxidant peptides are free radical scavengers. As shown in Scheme 2, together with amino acids, they act as hydrogen donors or electron donors to terminate the chain reactions of free radicals by converting them into stable products (Yarnpakdee *et al.*, 2015).

$$\text{Hydrogen donation: } R\cdot + B:H \rightarrow R:H + B\cdot$$

$$\text{Electron donation: } R\cdot + B:H \rightarrow R^- : + B\cdot H^+ \rightarrow R:H + B\cdot$$

Scheme 2. Pathways of antioxidant peptides scavenging free radicals. B:H stands for antioxidant peptide. Hydrogen donation: the antioxidant peptide transfers H· to R· to form a stable compound R:H, while the antioxidant peptide converts to B·, which is less likely to initiate a new free radical chain reaction. Electron donation: electron transfer and proton transfer are required (Liang and Kitts, 2014).

The antioxidant capacity of peptides and their amino acid composition are closely related, and the mechanism of amino acids depends on the functional groups of their side chains (Elias *et al.*, 2008). For example, peptide sequences with hydrophobic amino acids, aromatic amino acids and sulfur-containing amino acids usually exhibit strong antioxidant activity (Wu *et al.*, 2015a). Antioxidant peptides containing hydrophobic amino acids such as alanine, valine and leucine have strong antioxidant capacity. The non-polar aliphatic hydrocarbon side chains of these amino acids can enhance the interaction between fatty acids and peptides to prevent hydrogen atoms from being attacked (as shown in Figure 1A) (Rajapakse *et al.*, 2005). Their presence can also increase the solubility of antioxidant peptides in oil, thus promoting interactions with free radicals and exhibiting higher antioxidant activity (Aguilar-Toalá and Liceaga, 2020). Some scientists proposed that hydrophobic amino acids such as valine and aspartic acid present in the peptide sequence could contribute to improving the antioxidant activity of peptides (Najafian and Babji, 2014).

Antioxidant peptides with aromatic amino acids such as tyrosine, tryptophan and phenylalanine in the sequence also have strong antioxidant properties. Aromatic amino acids can not only enhance the solubility of antioxidant peptides in oil like hydrophobic amino acids, but can also scavenge free radicals produced by oxidation by removing a H· from the phenolic hydroxyl or indole group in these amino acids. These amino acids are converted into relatively stable free radicals to terminate the chain reaction and inhibit the automatic oxidation of oil (as shown in Figure 1B) (Wang *et al.*, 2014). One study isolated two peptides containing tryptophan and tyrosine from the hydrolysate of *Sardinella aurita*, both of which have high DPPH scavenging activity (Bougatef *et al.*, 2010). A related study also showed that the antioxidant activity of the dipeptides Tyr-Leu and Phe-Tyr derived from Perilla is related to the presence of tyrosine (Yang *et al.*, 2018).

Methionine and cysteine are two kinds of sulfur-containing amino acids with active sulfhydryl in their structures. They can be used as precursors for the synthesis of glutathione. The methionine and cysteine in the antioxidant peptide sequence contribute to scavenging free radicals through the electron transfer pathway (Atmaca, 2004). The sulfhydryl group loses electrons to form free radical cations. The free radical cations then undergo a proton transfer reaction to generate stable free radicals and hydrogen ions (Scheme 3).

$$BSH + R\cdot \rightarrow BSH^{+}\cdot + R^{-}$$
$$BSH^{+}\cdot \rightarrow BS\cdot + H^{+}$$

SCHEME 3. Mechanism of scavenging free radicals by sulfur-containing amino acids.

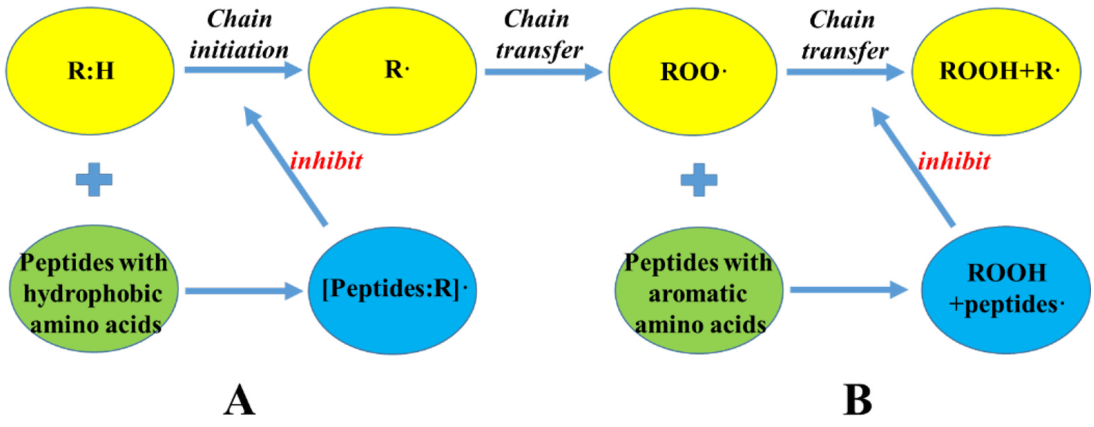

FIGURE 1. Mechanism of scavenging free radicals by hydrophobic amino acids and aromatic amino acids.

Jiang *et al*. (2014) obtained His-Asp-His-Pro-Val-Cys and His-Glu-Lys-Val-Cys from *Decapterus maruadsi*. The DPPH scavenging activity of the former was similar to the glutathione (Jiang *et al.*, 2014). In addition, the CFCTKPC synthesized by Huang *et al*. (2012) contained three cysteines, and showed strong free radical scavenging ability and inhibited linoleic acid oxidation.

Histidine is one of the most common amino acids in antioxidant peptides. Its imidazole group can act as hydrogen donors to react with free radicals, especially superoxide anion-free radicals (Jie *et al.*, 2019b). Kim *et al*. (2020) obtained histidine-containing low-molecular-weight (LMW) peptides from tuna waste meats. The histidine-containing LMW peptides exhibited a high DPPH radical scavenging effect in a dose-dependent manner.

3.2. Inhibiting the activity of catalytic metal ions

Catalytic metal can accelerate the decomposition of oil oxidation primary products to generate free radicals as shown in Scheme 4 (Muhr *et al.*, 2020).

$$M^{n+} + ROOH \rightarrow M^{(n+1)+} + OH^- + RO\cdot$$
$$M^{n+} + ROOH \rightarrow M^{(n-1)+} + OH^- + ROO\cdot$$

SCHEME 4. Transition metal ions accelerate the decomposition of oil oxidation primary products (M^{n+} represents transition metal ions).

The catalysis of catalytic metal depends on their ability to react with unoxidized oil to form hydroperoxides (Juita *et al.*, 2011). For example, Fe^{2+} catalyzes hydrogen peroxide to generate hydroxyl radicals with high activity through the Fenton reaction, thus promoting oil oxidation (Walters *et al.*, 2018). Another theory was that the presence of transition metal would significantly reduce the content of tocopherols, thereby affecting the oxidation stability of the oil (Fomuso *et al.*, 2002).

Antioxidant peptides can reduce the chemical reactivity of metals by chelating with metal ions to form stable complexes. Antioxidant peptides can also block the interaction between metals and oil in space. For example, changing the physical position of metal ions to reduce the rate of free radical reactions indirectly achieves the antioxidant effect (Feng *et al.*, 2016). Regarding antioxidant peptides, suitable amino acids and their correct position in the sequence play a crucial role in their chelating activity (Walters *et al.*, 2018). The imidazole group of histidine in the antioxidant peptide sequence can chelate with metal ions, thus inhibiting the generation of free radicals catalyzed by them (Burkitt, 2001). Basic amino acids such as glutamic acid and aspartic acid (with carboxylic acid groups) can also inhibit the catalytic oxidation of transition metal ions (Sonklin *et al.*, 2018). Besides, amino acids such as arginine and lysine with amino groups, cysteine with thiol groups and serine and threonine with hydroxyl may form complexes with antioxidant peptides by electrostatic interactions or H-bond coordination (Canabady-Rochelle *et al.*, 2018; Walters *et al.*, 2018). It has also been reported that casein-hydrolyzed peptides have the ability to inhibit the oxidation of oil. The peptides can oxidize Fe^{2+} into less reactive Fe^{3+} to delay oil oxidation (Diaz *et al.*, 2003). Some antioxidant peptides with metal chelating properties can act as potential antioxidants because of their charged amino acids in their sequences. The charged amino acids can be electrostatically attracted to metal ions to inhibit their catalytic effect. For example, the positive charge of histidine can attract negative free radicals, which is consistent with the results of Saiga *et al.* (2013). It is worth noting that antioxidant peptides with molecular weight less than 3 kDa have less chelating force on Fe^{2+} than the peptides with the formula weight of 5-10 kDa (He *et al.*, 2013). Therefore, antioxidant peptides with large molecular weight have a better effect on inhibiting oil oxidation.

Moreover, it must be noted that even though a peptide chelates metal, that does not mean it inhibits metal-promoted oil oxidation. Some chelators bind iron but do not decrease its reactivity (Durand *et al.*, 2021). Therefore, additional assays should be conducted to better assess the ability of these peptides to inhibit oil oxidation induced by prooxidant metals.

3.3. Restraining the formation and reactivity of hydroperoxide

Hydroperoxide is the primary product of oil oxidation. It can react with the ingredients in food, and reduce its quality. With the development of oil oxidation, hydroperoxide eventually decomposes into small molecules such as aldehydes

and ketones (Chen *et al.*, 2020a). Hydroperoxides may even decompose into toxic substances such as malonaldehyde (MDA) and 4-hydroxynonenal (HNE). During the chain transfer period, the free radicals generated by the decomposition of the hydroperoxides can react with unoxidized oil, continuously creating new free radicals and hydroperoxides to accelerate the oxidation of oil (Krugovov *et al.*, 2014).

The acidic amino acids in the antioxidant peptide sequence form hydrogen bonds with unsaturated fatty oil to protect the double bonds of fatty acids and decrease the cleavage of C-H bonds, thereby reducing the generation of hydroperoxides (Jie *et al.*, 2019b). A new antioxidant peptide was isolated from the *Caragana ambiguas* seed protein. The glutamine in the *Caragana ambigua* seed peptide (CSP) can form hydrogen bonds with unsaturated oil to protect the double bond of fatty acids, thus reducing the generation of hydroperoxides. In addition, CSP can delay the autoxidation of oil by protecting the phenolic compounds (Jie *et al.*, 2019a). Antioxidant peptides can also reduce the reactivity of hydroperoxides by proton or electron transfer, thus inhibiting the chain transfer period of oil oxidation (Li and Yu, 2015; Lacou *et al.*, 2016). Some researchers prepared an antioxidant peptide (APHPH) with the molecular weight of 1801 Da. The hydrophobic amino acids in its sequence can provide protons to inhibit the reaction between hydroperoxides and free radicals, and effectively inhibit oil oxidation in the linoleic acid emulsion system (Kim *et al.*, 2007). An antioxidant peptide was prepared by using Alcalase alkaline protease and ultrafiltration technology from silkworm sericin. Silk sericin peptide has an inhibitory effect on the hydroperoxides induced by 4NQO (an oral carcinogen) in liver tissue (Fan *et al.*, 2016). Other researchers believe that antioxidant peptides can also reduce hydroperoxides to relatively inactive hydroxides through non-radical reactions. The reaction mechanism is to generate sulfenic acid and sulfoxide derivatives through double electron transfer from the sulfur of cysteine or methionine (Esfandi *et al.*, 2019).

In summary, antioxidant peptides can inhibit oil oxidation by scavenging free radicals, chelating metal ions that promote oxidation and reducing hydroperoxides generation. In addition to these common mechanisms, it has been proposed that antioxidant peptides may interact with secondary oxidation products of oil, such as aldehydes. These reactions may interfere with oil oxidation and alter the rate and pathway of oil rancidity (Berton-Carabin *et al.*, 2014). However, since the secondary oxidation products of oil are bound to the antioxidant peptides and not available for determination (e.g., by GC headspace analysis), the mechanism remains to be further explored (McClements and Decker, 2018).

4. APPLICATION OF ANTIOXIDANT PEPTODES IN THE OIL INDUSTRY

The antioxidant peptides from the enzymatic hydrolysis of proteins are effective substitutes for chemically synthesized antioxidants. Antioxidant peptides can theoretically be used as food additives to delay the oxidative rancidity of oil and maintain food quality without threatening human health. As antioxidant peptides are more accessible to free radicals and have high efficiency, only a small amount of them (0.001-0.02%) can be added to food to exert a powerful antioxidant effect (Sila and Bougatef, 2016). Besides, the antioxidant peptides incorporated into food can also provide nutrition in the form of amino acids when consumed, which is also one of the advantages of antioxidant peptides (Chai *et al.*, 2017). Although antioxidant peptides have shown their potential as food antioxidants in small-scale experiments, only their real application in food or oil can support their large-scale industrial development as food additives. However, the practical application of antioxidant peptides is mainly concentrated in the fat of meat and oil at present and there are few studies on other food stuffs.

Meat is prone to fat oxidation during storage and will lead to quality degradation, so special protection for substances is needed. Adding antioxidant peptides is one of the most effective ways to prevent fat oxidation in meat and its products. Edible vegetable oil generally contains unsaturated fatty acids, so it is prone to oxidation, which leads to rancidity (Lorenzo *et al.*, 2018). Compared to other natural antioxidants, antioxidant peptides not only show good solubility and a wide range of pH values in oily or oil-rich food, but also have nutritional and functional properties (Decher

Table 3. Applications of antioxidant peptides in meat and oil

Raw material	Enzyme	Application product	Influence on product	Reference
Bovine hemoglobin or bovine cruor	Pepsin	Ground beef	The peptide reduced fat oxidation by about 60% with a concentration of 0.5% (w/w).	(Przybylski *et al.*, 2016)
Amur sturgeon skin gelatin	Alcalase	Japanese sea bass	The PAGT (Pro–Ala–Gly–Tyr) at 12.5 ppm showed the greatest effect in lowering fat oxidation.	(Nikoo *et al.*, 2015)
Rice protein	Validase® FP/Alkaline protease/ Neutral protease	Ground beef	The fat oxidation rate was decreased by 19 and 15% respectively after one or two weeks of storage	(Zhou *et al.*, 2013)
Rahu fish	Alcalase	Broiler breast meat	Dietary interventions of peptides can increase the antioxidant and shelf stability of broiler breast meat.	(Aslam *et al.*, 2020)
Amaranth protein	Alcalase	Sunflower oil and canola oil	The hydrolysate showed inhibition of the decomposition of primary to secondary oxidation products	(Tironi and Anon, 2014)
Sheep visceral	Alcalase	Soybean oil	The peroxide value of soybean oil increased slowly.	(Meshginfar *et al.*, 2017)
Bovine visceral	Alcalase	Soybean oil	The peptide at 500 and 1000 mg/kg showed the best oxidation prevention activity in soybean oil.	(Taghvaei *et al.*, 2014)
Mushroom *Ganoderma lucidum*	\	Soybean oil	The peroxide levels in soybean oil were significantly reduced.	(Sun *et al.*, 2004)
Bovine hair	\	Peanut oil	The POV value of peanut oil was significantly reduced	(Zeng *et al.*, 2013)

et al., 2005; Harnedy and FitzGerald, 2012). Table 3 lists some applications of antioxidant peptides used in meat and oil.

5. CONCLUSION AND FUTURE PROSPECTS

With more and more research on antioxidant peptides, their preparation methods are gradually being diversified. Enzymatic hydrolysis is the most common preparation method to prepare antioxidant peptides, but the selection of the optimal enzyme and the tedious enzymatic hydrolysis process make the traditional method inefficient and aimless. Therefore, bioinformatics is combined with enzymatic hydrolysis. Bioinformatics technology improves the traditional research methods for antioxidant peptides to a certain extent. It can search the target protein information on demand and prepare antioxidant peptides purposefully through virtual hydrolysis. Bioinformatics can also use the database to evaluate and predict the antioxidant peptides' function to screen out the peptides with the highest antioxidant activity. The purpose of improving the peptides' properties and the possibility of antioxidant peptides' industrial production has been achieved. However, there are still many scientific and technical problems to be solved for the combined preparation of the two methods. At present, the information on the antioxidant peptide database is still incomplete, so the preparation of some antioxidant peptides cannot be fully realized. Secondly, no unified standards and methods have been established for the assessment of antioxidant peptides' capacity, so the activity prediction system is not perfect.

Antioxidant peptides can prevent or delay oil oxidation mainly through scavenging free radicals. The non-polar aliphatic hydrocarbon side chains of hydrophobic amino acids can protect the hydrogen atoms of fatty acids in the oil and enhance the interaction between antioxidant peptides and oil. Aromatic amino acids can react with free radicals as hydrogen donors to shape into stable compounds. Sulfhydryl-containing amino acids can scavenge free radicals through the electron transfer pathway

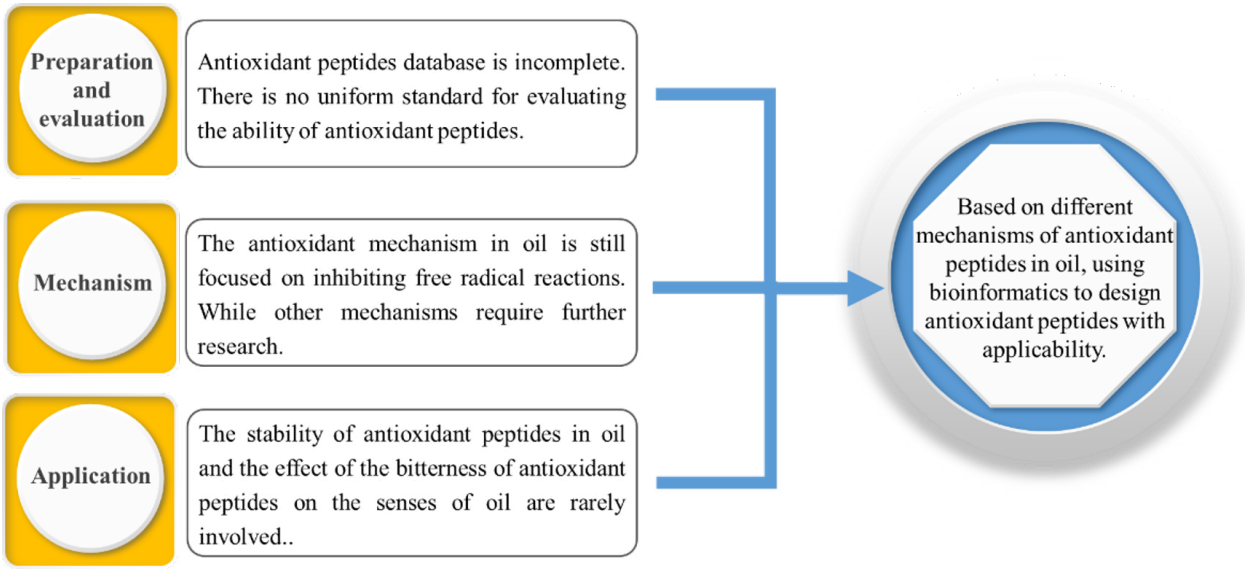

FIGURE 2. The existing problems and prospects of antioxidant peptides applied in oil.

to delay the oxidation of the oil. Antioxidant peptides can also make metal ions lose their ability to catalyze oil oxidation by chelating with metal ions, changing their valence state and electrostatic interaction. Reducing the generation of hydroperoxides and inhibiting decomposition to form new free radicals which participate in the chain reaction are also ways to delay oil oxidation. However, the current research on the antioxidant mechanism of antioxidant peptides in oil is still focused on inhibiting free radical reactions, while other mechanisms have not been fully elucidated and require further research. Moreover, although there have been relevant studies on the stability of antioxidant peptides themselves and their effect on oxidative stability in food, the stability of antioxidant peptides in oil or other food has rarely been studied at present (Jang *et al.*, 2016; Garcia-Moreno *et al.*, 2020). This is a factor which limits the further application of antioxidant peptides. In addition, although there are methods to reduce the bitterness of proteolytic peptides, there are few studies on whether the bitterness of antioxidant peptides will affect the texture of oil (Slizyte *et al.*, 2014; Tong *et al.*, 2020). Therefore, the application of antioxidant peptides in the food industry, especially in the oil industry, is limited.

It is believed that these problems will be solved with the continuous update of modern technology and in-depth research. The database on antioxidant peptides and the standard evaluation system of their activities will be gradually be improved to facilitate the screening of target protein and sequences. Moreover, the mechanism of action and safety evaluation of antioxidant peptides in oil and in vivo will be further clarified to accelerate the utilization of antioxidant peptides. Based on the different antioxidant mechanisms of antioxidant peptides in oil, it is hoped that some researchers can use bioinformatics to design antioxidant peptides with high applicability (Figure 2), so that they can exert a stable antioxidant effect and open a broader scope for the development and application of antioxidant peptides.

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REFERENCES

Aguilar-Toalá JE, Liceaga AM. 2020. Cellular antioxidant effect of bioactive peptides and molecular mechanisms underlying: beyond chemical properties. *Int. J. Food. Sci. Tech.* **56** (5)**,** 2193–2204. http://dx.doi.org/10.1111/ijfs.14855

Agyei D, Ongkudon CM, Wei CY, Chan AS, Danquah MK. 2016. Bioprocess challenges to the isolation and purification of bioactive peptides. *Food Bioprod. Process.* **98,** 244–256. http://dx.doi.org/doi:10.1016/j.fbp.2016.02.003

Agyei D, Tsopmo A, Udenigwe CC. 2018. Bioinformatics and peptidomics approaches to the discovery and analysis of food-derived bioactive peptides. *Anal. Bioanal. Chem.* **410** (15)**,** 3463–3472. https://doi.org/10.1007/s00216-018-0974-1

Ahn CB, Je JY, Cho YS. 2012a. Antioxidant and anti-inflammatory peptide fraction from salmon byproduct protein hydrolysates by peptic hydrolysis. *Food Res. Int.* **49** (1)**,** 92–98. https://doi.org/10.1016/j.foodres.2012.08.002

Ahn JH, Kim YP, Kim HS. 2012b. Effect of natural antioxidants on the lipid oxidation of microencapsulated seed oil. *Food Control* **23** (2)**,** 528–534. http://dx.doi.org/10.1016/j.foodcont.2011.08.026

An S, Lee E, Choe E. 2014. Improvement of chlorophyll-photosensitized oxidation of fish oil-enriched emulsion by sesame oil addition and antioxidant content changes. *Korean J. Food Sci. Technol.* **46** (2)**,** 127–134. https://doi.org/10.9721/KJFST.2014.46.2.127

Aslam S, Shukat R, Khan MI, Shahid M. 2020. Effect of dietary supplementation of bioactive peptides on antioxidant potential of broiler breast meat and physicochemical characteristics of nuggets. *Food Sci. Anim. Resour.* **40** (1)**,** 55–73. http://dx.doi.org/10.5851/kosfa.2019.e82

Atmaca G. 2004. Antioxidant effects of sulfur-containing amino acids. *Yonsei Med. J.* **45** (5)**,** 776–788. https://doi.org/10.3349/ymj.2004.45.5.776

Bechaux J, Ferraro V, Sayd T, Chambon C, Page JFL, Drillet Y, Gatellier P, Sante-Lhoutellier V. 2020. Workflow towards the generation of bioactive hydrolysates from porcine products by combining *in silico* and *in vitro* approaches. *Food Res. Int.* **132,** 109–123. https://doi.org/10.1016/j.foodres.2020.109123

Berton-Carabin CC, Ropers MH, Genot C. 2014. Lipid oxidation in oil-in-water emulsions: involvement of the interfacial layer. *Compr. Rev. Food Sci. Food Saf.* **13** (5)**,** 945–977. https://doi.org/10.1111/1541-4337.12097

Borawska-Dziadkiewicz J, Darewicz M, Tarczynska AS. 2021. Properties of peptides released from salmon and carp *via* simulated human-like gastrointestinal digestion described applying quantitative parameters. *PLoS One* **16** (8)**,** e0255969. https://doi.org/10.1371/journal.pone.0255969

Borrajo P, Pateiro M, Barba FJ, Mora L, Franco D, Toldra F, Lorenzo JM. 2019. Antioxidant and antimicrobial activity of peptides extracted from meat by-products: a review. *Food Anal. Methods* **12** (11)**,** 2401–2415. https://doi.org/10.1007/s12161-019-01595-4

Bougatef A, Nedjar-Arroume N, Manni L, Ravallec R, Barkia A, Guillochon D, Nasri M. 2010. Purification and identification of novel antioxidant peptides from enzymatic hydrolysates of sardinelle (*Sardinella aurita*) by-products proteins. *Food Chem.* **118** (3)**,** 559–565. https://doi.org/10.1016/j.foodchem.2009.05.021

Budilarto ES, Kamal-Eldin A. 2015. The supramolecular chemistry of lipid oxidation and antioxidation in bulk oils. *Eur. J. Lipid Sci. Technol.* **117** (8)**,** 1095–1137. http://dx.doi.org/10.1002/ejlt.201400200

Burkitt MJ. 2001. A critical overview of the chemistry of copper-dependent low density lipoprotein oxidation: roles of lipid hydroperoxides, alpha-tocopherol, thiols, and ceruloplasmin. *Arch. Biochem. Biophys.* **394** (1)**,** 117–135. https://doi.org/10.1006/abbi.2001.2509

Canabady-Rochelle LLS, Selmeczi K, Collin S, Pasc A, Muhr L, Boschi-Muller S. 2018. SPR screening of metal chelating peptides in a hydrolysate for their antioxidant properties. *Food Chem.* **239,** 478–485. https://doi.org/10.1016/j.foodchem.2017.06.116

Carrillo W, Guzman X, Vilcacundo E. 2017. Native and heated hydrolysates of milk proteins and their capacity to inhibit lipid peroxidation in the zebrafish larvae model. *Foods* **6** (9)**,** 81. https://doi.org/10.3390/foods6090081

Chai KF, Voo AYH, Chen WN. 2020. Bioactive peptides from food fermentation: a comprehensive review of their sources, bioactivities, applications, and future development. *Compr. Rev. Food Sci. Food Saf.* **19** (6)**,** 3825–3885. http://dx.doi.org/10.1111/1541-4337.12651

Chai TT, Law YC, Wong FC, Kim SK. 2017. Enzyme-assisted discovery of antioxidant peptides from edible marine invertebrates: a review. *Mar. Drugs* **15** (2)**,** 42. http://dx.doi.org/10.3390/md15020042

Chen C, Huang H, Wu CH. 2017. Protein bioinformatics databases and resources. *Methods Mol. Biol.* **1558,** 3–39. https://doi.org/10.1007/978-1-4939-6783-4_1

Chen C, Sun-Waterhouse DX, Zhang Y, Zhao MM, Sun WZ. 2020a. The chemistry behind the antioxidant actions of soy protein isolate hydrolysates in a liposomal system: their performance in aqueous solutions and liposomes. *Food Chem.* **323,** 126789. https://doi.org/10.1016/j.foodchem.2020.126789

Chen C, Sun-Waterhouse DX, Zhao MM, Sun WZ. 2020b. Beyond antioxidant actions: insights into the antioxidant activities of Tyr-containing dipeptides in aqueous solution systems and liposomal systems. *Int. J. Food. Sci. Tech.* **55** (10)**,** 3227–3234. http://dx.doi.org/10.1111/ijfs.14585

Chiozzi RZ, Capriotti AL, Cavaliere C, La Barbera G, Piovesana S, Samperi R, Lagana A. 2016. Purification and identification of endogenous antioxidant and ACE-inhibitory peptides from donkey milk by multidimensional liquid chromatography and nanoHPLC-high resolution mass spectrometry. *Anal. Bioanal. Chem.* **408** (20)**,** 5657–5666. https://doi.org/10.1007/s00216-016-9672-z

da Rosa LOL, Santana MC, Avezedo TL, Santa Brigida AI, Godoy R, Pacheco S, Mellinger-Silva C, Cabral LMC. 2018. A comparison of dual-functional whey hydrolysates by the use of commercial proteases. *Food Sci. Technol.* **38,** 31–36. https://doi.org/10.1590/fst.08417

Darewicz M, Borawska J, Pliszka M. 2016. Carp proteins as a source of bioactive peptides-an in silico approach. *Czech J. Food Sci.* **34** (2)**,** 111–117. http://dx.doi.org/10.17221/300/2015-cjfs

Decher EA, Warner K, Richards MP, Shahidi F. 2005. Measuring antioxidant effectiveness in food. *J. Agric. Food Chem.* **53** (10)**,** 4303–4310. http://dx.doi.org/10.1021/jf058012x

Diaz M, Dunn CM, McClements DJ, Decker EA. 2003. Use of caseinophosphopeptides as natural antioxidants in oil-in-water emulsions. *J. Agric. Food Chem.* **51** (8)**,** 2365–2370. https://doi.org/10.1021/jf025984l

Durand E, Beaubier S, Fine F, Villeneuve P, Kapel R. 2021. High metal chelating properties from rapeseed meal proteins to counteract lipid oxidation in foods: controlled proteolysis and characterization. *Eur. J. Lipid Sci. Technol.* **123** (6)**,** 2000380. https://doi.org/10.1002/ejlt.202000380

Elias RJ, Kellerby SS, Decker EA. 2008. Antioxidant activity of proteins and peptides. *Crit. Rev. Food Sci. Nutr.* **48** (5)**,** 430–441. http://dx.doi.org/10.1080/10408390701425615

Esfandi R, Walters ME, Tsopmo A. 2019. Antioxidant properties and potential mechanisms of hydrolyzed proteins and peptides from cereals. *Heliyon* **5** (4)**,** e01538. http://dx.doi.org/10.1016/j.heliyon.2019.e01538

Fan JB, Zhou SZ, Cai QT, Hou Y. 2016. Protective effect of sericin peptide on liver tissue injury induced by 4NQO. *J. Chin. Institute of Food Sci. Tech.* **16** (2)**,** 44–49. http://dx.doi.org/10.16429/j.1009-7848.2016.02.007

Feng JH, Cao AL, Cai LY, Li XX, Yu ZP, Zhao W, Li JR. 2016. Research progress of antioxidant peptides in fish by-products and their active mechanism. *Sci. Tech. Food Ind.* **37** (15)**,** 365–374. http://dx.doi.org/10.13386/j.issn1002-0306.2016.15.063

Fomuso LB, Corredig M, Akoh CC. 2002. Metal-catalyzed oxidation of a structured lipid model emulsion. *J. Agric. Food Chem.* **50** (24)**,** 7114–7119. https://doi.org/10.1021/jf020256j

Fu Y, Wu W, Zhu MP, Xiao ZG. 2016. *In silico* assessment of the potential of patatin as a precursor of bioactive peptides. *J. Food Biochem.* **40** (3)**,** 366–370. https://doi.org/10.1111/jfbc.12213

Garcia-Moreno PJ, Jacobsen C, Marcatili P, Gregersen S, Overgaard MT, Andersen ML, Sorensen ADM, Hansen EB. 2020. Emulsifying peptides from potato protein predicted by bioinformatics: stabilization of fish oil-in-water emulsions. *Food Hydrocolloids* **101,** 105529. https://doi.org/10.1016/j.foodhyd.2019.105529

Hall GM, Ahmad NH. 1997. Surimi and fish-mince products. *Fish Process. Technol.* 74–92. https://doi.org/10.1007/978-1-4613-1113-3_3

Hammer M, Schieberle P. 2013. Model studies on the key aroma compounds formed by an oxidative degradation of omega-3 fatty acids initiated by either copper(II) ions or lipoxygenase. *J. Agric. Food Chem.* **61** (46)**,** 10891–10900. https://doi.org/10.1021/jf403827p

Harnedy PA, FitzGerald RJ. 2012. Bioactive peptides from marine processing waste and shellfish: a review. *J. Funct. Foods* **4** (1)**,** 6–24. http://dx.doi.org/10.1016/j.jff.2011.09.001

Harvian ZA, Ningrum A, Anggrahini S, Setyaningsih W. 2019. *In silico* approach in evaluation

of Jack bean (*Canavalia ensiformis*) canavalin protein as precursors of bioactive peptides with dual antioxidant and angiotensin I-converting enzyme inhibitor. *Mater. Sci. Forum* **948,** 85–94. https://doi.org/10.4028/www.scientific.net/MSF.948.85

He R, Girgih AT, Malomo SA, Ju XR, Aluko RE. 2013. Antioxidant activities of enzymatic rapeseed protein hydrolysates and the membrane ultrafiltration fractions. *J. Funct. Foods* **5** (1)**,** 219–227. https://doi.org/10.1016/j.jff.2012.10.008

He R, Ju XR, Yuan J, Wang LF, Girgih AT. 2012. Antioxidant activities of rapeseed peptides produced by solid state fermentation. *Food Res. Int.* **49** (1)**,** 432–438. https://doi.org/10.1016/j.foodres.2012.08.023

Huang GJ, Deng JS, Chen HJ, Huang SS, Liao JC, Hou WC, Lin YH. 2012. Defensin protein from sweet potato (*Ipomoea batatas* [L.] Lam 'Tainong 57') storage roots exhibits antioxidant activities *in vitro* and *ex vivo*. *Food Chem.* **135** (3)**,** 861–867. https://doi.org/10.1016/j.foodchem.2012.05.082

Ibanez C, Simo C, Garcia-Canas V, Cifuentes A, Castro-Puyana M. 2013. Metabolomics, peptidomics and proteomics applications of capillary electrophoresis-mass spectrometry in Foodomics: A review. *Anal. Chim. Acta* **802,** 1–13. https://doi.org/10.1016/j.aca.2013.07.042

Im S, Nam TG, Lee SG, Kim YJ, Chun OK, Kim DO. 2014. Additive antioxidant capacity of vitamin C and tocopherols in combination. *Food Sci. Biotechnol.* **23**(3)**,** 693–699. https://doi.org/10.1007/s10068-014-0094-4

Jang HL, Liceaga AM, Yoon KY. 2016. Purification, characterisation and stability of an antioxidant peptide derived from sandfish (*Arctoscopus japonicus*) protein hydrolysates. *J. Funct. Foods* **20,** 433–442. https://doi.org/10.1016/j.jff.2015.11.020

Jara AMR, Liggieri CS, Bruno MA. 2018. Preparation of soy protein hydrolysates with antioxidant activity by using peptidases from latex of *Maclura pomifera* fruits. *Food Chem.* **264,** 326–333. https://doi.org/10.1016/j.foodchem.2018.05.013

Ji DW, Udenigwe CC, Agyei D. 2019. Antioxidant peptides encrypted in flaxseed proteome: an *in silico* assessment. *Food Sci. Hum. Well.* **8** (3)**,** 306–314. https://doi.org/10.1016/j.fshw.2019.08.002

Jiang H, Tong T, Sun J, Xu Y, Zhao Z, Liao D. 2014. Purification and characterization of antioxidative peptides from round scad (*Decapterus maruadsi*) muscle protein hydrolysate. *Food Chem.* **154,** 158–163. https://doi.org/10.1016/j.foodchem.2013.12.074

Jie F, Zhao HF, Sun XQ, Lv XR, Zhang Z, Zhang B. 2019a. Isolation of antioxidative peptide from the protein hydrolysate of *Caragana ambigua* seeds and its mechanism for retarding lipid auto-oxidation. *J. Sci. Food Agric.* **99** (6)**,** 3078–3085. http://dx.doi.org/10.1002/jsfa.9521

Jie Y, Hu JL, Zhao HF, Zhang BL. 2020. Optimization of the preparation of antioxidant peptides from *Caragana korshinskii* seed protein based on bioinformatics. *Food Sci.* **41** (20)**,** 278–284. http://dx.doi.org/10.7506/spkx1002-6630-20190822-224

Jie Y, Zhao HF, Zhang B. 2019b. The role of an acidic peptide in controlling the oxidation process of walnut oil. *Foods* **8** (10)**,** 499. http://dx.doi.org/10.3390/foods8100499

Jonsdottir R, Geirsdottir M, Hamaguchi PY, Jamnik P, Kristinsson HG, Undeland I. 2016. The ability of *in vitro* antioxidant assays to predict the efficiency of a cod protein hydrolysate and brown seaweed extract to prevent oxidation in marine food model systems. *J. Sci. Food Agric.* **96** (6)**,** 2125–2135. https://doi.org/10.1002/jsfa.7328

Juita, Dlugogorski BZ, Kennedy EM, Mackie JC. 2011. Oxidation reactions and spontaneous ignition of linseed oil. *P. Combust. Inst.* **33** (2)**,** 2625–2632. https://doi.org/10.1016/j.proci.2010.06.096

Karaman M, Atlagic K, Novakovic A, Sibul F, Zivic M, Stevanovic K, Pejin B. 2019. Fatty acids predominantly affect anti-hydroxyl radical activity and FRAP value: the case study of two edible mushrooms. *Antioxidants* **8** (10)**,** 480. http://dx.doi.org/10.3390/antiox8100480

Keska P, Stadnik J. 2016. Porcine myofibrillar proteins as potential precursors of bioactive peptides-an *in silico* study. *Food Funct.* **7** (6)**,** 2878–2885. https://doi.org/10.1039/C5FO01631b

Khueychai S, Jangpromma N, Daduang S, Klaynongsruang S. 2019. Effects of alkaline hydrolysis and storage conditions on the biological activity of ostrich egg white. *J. Food Process. Preserv.* **43** (4)**,** e13921. http://dx.doi.org/10.1111/jfpp.13921

Kim HK, Song HS. 2020. Antioxidant effect of histidine-containing low-molecular-weight peptides seperated from tuna meat. *J. Food Hyg. Saf.* **35** (5)**,** 513–520. https://doi.org/10.13103/JFHS.2020.35.5.513

Kim SS, Ahn CB, Moon SW, Je JY. 2018. Purification and antioxidant activities of peptides from sea squirt (*Halocynthia roretzi*) protein hydrolysates using pepsin hydrolysis. *Food Biosci.* **25,** 128–133. https://doi.org/10.1016/j.fbio.2018.08.010

Kim SY, Je JY, Kim SK. 2007. Purification and characterization of antioxidant peptide from hoki (*Johnius belengerii*) frame protein by gastrointestinal digestion. *J. Nutr. Biochem.* **18** (1)**,** 31–38. http://dx.doi.org/10.1016/j.jnutbio.2006.02.006

Kiralan SS, Karagoz SG, Ozkan G, Kiralan M, Ketenoglu O. 2021. Changes in volatile compounds of virgin olive oil flavored with essential oils during thermal and photo-oxidation. *Food Anal. Methods* **14** (5)**,** 883–896. https://doi.org/10.1007/s12161-020-01926-w

Krugovov DA, Mengele EA, Kasaikina OT. 2014. Acetylcholine as a catalyst of hydroperoxide decomposition to free radicals. *Russ.Chem.Bull.* **63** (8)**,** 1837–1842. http://dx.doi.org/10.1007/s11172-014-0673-9

Lacou L, Leonil J, Gagnaire V. 2016. Functional properties of peptides: from single peptide solutions to a mixture of peptides in food products. *Food Hydrocolloids* **57,** 187–199. https://doi.org/10.1016/j.foodhyd.2016.01.028

Ladikos D, Lougovois V. 1990. Lipid oxidation in muscle foods: a review. *Food Chem.* **35,** 295–314. https://doi.org/10.1016/0308-8146(90)90019-Z

Li Y, Yu JM. 2015. Research progress in structure-activity relationship of bioactive peptides. *J. Med. Food* **18** (2)**,** 147–156. https://doi.org/10.1089/jmf.2014.0028

Li YY, Kong BH, Liu Q, Xia XF, Chen HS. 2017. Improvement of the emulsifying and oxidative stability of myofibrillar protein prepared oil-in-water emulsions by addition of zein hydrolysates. *Process Biochem.* **53,** 116–124. https://doi.org/10.1016/j.procbio.2016.11.010

Liang NJ, Kitts DD. 2014. Antioxidant property of coffee components: assessment of methods that define mechanisms of action. *Molecules* **19** (11)**,** 19180–19208. http://dx.doi.org/10.3390/molecules191119180

Lorenzo JM, Munekata PES, Gómez B, Barba FJ, Mora L, Pérez-Santaescolástica C, Toldrá F. 2018. Bioactive peptides as natural antioxidants in food products–a review. *Trends Food Sci. Technol.* **79,** 136–147. http://dx.doi.org/10.1016/j.tifs.2018.07.003

McClements DJ, Decker E. 2018. Interfacial antioxidants: a review of natural and synthetic emulsifiers and coemulsifiers that can inhibit lipid oxidation. *J. Agric. Food Chem.* **66** (1)**,** 20–35. https://doi.org/10.1021/acs.jafc.7b05066

Meshginfar N, Sadeghi Mahoonak A, Ghorbani M, Aalami M. 2017. Effects of protein hydrolysate from sheep visceral on oxidative stability of soybean oil and chicken sausage. *J. Food Process. Preserv.* **41** (2)**,** e12875. https://doi.org/10.1111/jfpp.12875

Mishra SK, Belur PD, Iyyaswami R. 2020. Use of antioxidants for enhancing oxidative stability of bulk edible oils: a review. *Int. J. Food. Sci. Tech.* **56** (1)**,** 1–12. https://publons.com/publon/10.1111/ijfs.14716

Montone CM, Chiozzi RZ, Marchetti N, Cerrato A, Antonelli M, Capriotti AL, Cavaliere C, Piovesana S, Lagana A. 2019. Peptidomic approach for the identification of peptides with potential antioxidant and anti-hyperthensive effects derived from *Asparagus* by-products. *Molecules* **24** (19)**,** 3627. https://doi.org/10.3390/molecules24193627

Muhr L, Pontvianne S, Selmeczi K, Paris C, Boschi-Muller S, Canabady-Rochelle L. 2020. Chromatographic separation simulation of metal-chelating peptides from surface plasmon resonance binding parameters. *J. Sep. Sci.* **43** (11)**,** 2031–2041. http://dx.doi.org/10.1002/jssc.201900882

Najafian L, Babji AS. 2014. Production of bioactive peptides using enzymatic hydrolysis and identification antioxidative peptides from patin (*Pangasius sutchi*) sarcoplasmic protein hydolysate. *J. Funct. Foods* **9,** 280–289. https://doi.org/10.1016/j.jff.2014.05.003

Nikoo M, Regenstein JM, Ghomi MR, Benjakul S, Yang N, Xu XM. 2015. Study of the combined effects of a gelatin-derived cryoprotective peptide and a non-peptide antioxidant in a fish mince model system. *LWT-Food Sci. Technol.* **60** (1)**,** 358–364. http://dx.doi.org/10.1016/j.lwt.2014.07.051

Olsen TH, Yesiltas B, Marin FI, Pertseva M, Garcia-Moreno PJ, Gregersen S, Overgaard MT, Jacobsen C, Lund O, Hansen EB, Marcatili P. 2020. AnOxPePred: using deep learning for the prediction of antioxidative properties of peptides. *Sci. Rep.* **10,** 21471 . https://doi.org/10.1038/s41598-020-78319-w

Oseguera-Toledo ME, de Mejia EG, Dia VP, Amaya-Llano SL. 2011. Common bean (*Phaseolus vulgaris* L.) hydrolysates inhibit inflammation in LPS-induced macrophages through suppression of NF-κB pathways. *Food Chem.* **127** (3)**,** 1175–1185. https://doi.org/10.1016/j.foodchem.2011.01.121

Oussaief O, Jrad Z, Adt I, Dbara M, Khorchani T, El-Hatmi H. 2019. Antioxidant activities of enzymatic-hydrolysed proteins of dromedary (*Camelus dromedarius*) colostrum. *Int. J. Dairy Technol.* **73** (2)**,** 333–340. http://dx.doi.org/10.1111/1471-0307.12668

Panchal G, Sakure A, Hati S. 2021. Peptidomic profiling of fermented goat milk: considering the fermentation-time dependent proteolysis by *Lactobacillus* and characterization of novel peptides with antioxidative activity. *J. Food Sci. Technol.* **59**, 2295–2305 https://doi.org/10.1007/s13197-021-05243-w

Pearman NA, Ronander E, Smith AM, Morris GA. 2020. The identification and characterisation of novel bioactive peptides derived from porcine liver. *Curr. Res. Food Sci.* **3,** 314–321. https://doi.org/10.1016/j.crfs.2020.11.002

Pejin B, Bogdanovic-Pristov J, Pejin I, Sabovljevic M. 2013. Potential antioxidant activity of the moss *Bryum moravicum*. *Nat. Prod. Res.* **27** (10)**,** 900–902. http://dx.doi.org/10.1080/14786419.2012.665915

Przybylski R, Firdaous L, Chataigne G, Dhulster P, Nedjar N. 2016. Production of an antimicrobial peptide derived from slaughterhouse by-product and its potential application on meat as preservative. *Food Chem.* **211,** 306–313. http://dx.doi.org/10.1016/j.foodchem.2016.05.074

Qi BK, Zhang QZ, Sui XN, Wang ZJ, Li Y, Jiang LZ. 2016. Differential scanning calorimetry study-assessing the influence of composition of vegetable oils on oxidation. *Food Chem.* **194,** 601–607. http://dx.doi.org/10.1016/j.foodchem.2015.07.148

Rajapakse N, Mendis E, Jung WK, Je JY, Kim SK. 2005. Purification of a radical scavenging peptide from fermented mussel sauce and its antioxidant properties. *Food Res. Int.* **38** (2)**,** 175–182. https://doi.org/10.1016/j.foodres.2004.10.002

Rathod NB, Ranveer RC, Benjakul S, Kim SK, Pagarkar AU, Patange S, Ozogul F. 2021. Recent developments of natural antimicrobials and antioxidants on fish and fishery food products. *Compr. Rev. Food Sci. Food Saf.* **20** (4)**,** 4182–4210. https://doi.org/10.1111/1541-4337.12787

Ribeiro E, Rocha TD, Prudencio SH. 2021. Potential of green and roasted coffee beans and spent coffee grounds to provide bioactive peptides. *Food Chem.* **348,** 129061. https://doi.org/10.1016/j.foodchem.2021.129061

Saiga AE, Nishimura T. 2013. Antioxidative properties of peptides obtained from porcine myofibrillar proteins by a protease treatment in an Fe(II)-induced aqueous lipid peroxidation system. *Biosci. Biotechnol. Biochem.* **77** (11)**,** 2201–2204. https://doi.org/10.1271/bbb.130369

Salim MASM, Gan CY. 2020. Dual-function peptides derived from egg white ovalbumin: bioinformatics identification with validation using *in vitro* assay. *J. Funct. Foods* **64,** 103618. https://doi.org/10.1016/j.jff.2019.103618

Setti K, Kachouri F, Hamdi M. 2017. Improvement of the antioxidant activity of fenugreek protein isolates by *Lactococcus lactis* fermentation. *Int. J. Pept. Res. Ther.* **24** (4)**,** 499–509. http://dx.doi.org/10.1007/s10989-017-9636-y

Sila A, Bougatef A. 2016. Antioxidant peptides from marine by-products: isolation, identification and application in food systems. A review. *J. Funct. Foods* **21,** 10–26. http://dx.doi.org/10.1016/j.jff.2015.11.007

Slizyte R, Carvajal AK, Mozuraityte R, Aursand M, Storro I. 2014. Nutritionally rich marine proteins from fresh herring by-products for human consumption. *Process Biochem.* **49** (7)**,** 1205–1215. https://doi.org/10.1016/j.procbio.2014.03.012

Sohaib M, Anjum FM, Sahar A, Arshad MS, Rahman UU, Imran A, Hussain S. 2017. Antioxidant proteins and peptides to enhance the oxidative stability of meat and meat products: a comprehensive review. *Int. J. Food Prop.* **20** (11)**,** 2581–2593. http://dx.doi.org/10.1080/10942912.2016.1246456

Sonklin C, Laohakunjit N, Kerdchoechuen O. 2018. Assessment of antioxidant properties of membrane ultrafiltration peptides from mungbean meal protein hydrolysates. *PeerJ.* **6,** e5337. https://doi.org/10.7717/peerj.5337

Su GW, Ren JY, Yang B, Cui C, Zhao MM. 2011. Comparison of hydrolysis characteristics on defatted peanut meal proteins between a protease extract from *Aspergillus oryzae* and commercial proteases. *Food Chem.* **126** (3)**,** 1306–1311. https://doi.org/10.1016/j.foodchem.2010.11.083

Sun J, He H, Xie BJ. 2004. Novel antioxidant peptides from fermented mushroom *Ganoderma lucidum*. *J. Agric. Food Chem.* **52** (21)**,** 6646–6652. https://doi.org/10.1021/jf0495136

Sun XH, Wang K, Gao S, Hong H, Zhang LT, Liu HG, Feng LG, Luo YK. 2021. Purification and characterization of antioxidant peptides from yak (*Bos grunniens*) bone hydrolysates and evaluation of cellular antioxidant activity. *J. Food Sci. Technol.* **58**, 3106–3119. https://doi.org/10.1007/s13197-020-04814-7

Tadesse SA, Emire SA. 2020. Production and processing of antioxidant bioactive peptides: a driving force for the functional food market. *Heliyon* **6** (8)**,** e04765. https://doi.org/10.1016/j.heliyon.2020.e04765

Taghvaei M, Jafari SM. 2015. Application and stability of natural antioxidants in edible oils in order to substitute synthetic additives. *J. Food Sci. Technol.* **52** (3)**,** 1272-1282. https://doi.org/10.1007/s13197-013-1080-1

Taghvaei M, Jafari SM, Mahoonak AS, Nikoo AM, Rahmanian N, Hajitabar J, Meshginfar N. 2014. The effect of natural antioxidants extracted from plant and animal resources on the oxidative stability of soybean oil. *LWT-Food Sci. Technol.* **56** (1)**,** 124-130. https://doi.org/10.1016/j.lwt.2013.11.009

Tejano LA, Peralta JP, Yap EES, Panjaitan FCA, Chang YW. 2019. Prediction of bioactive peptides from *Chlorella sorokiniana* proteins using proteomic techniques in combination with bioinformatics analyses. *Int. J. Mol. Sci.* **20** (7)**,** 1786. http://dx.doi.org/10.3390/ijms20071786

Tesanovic K, Pejin B, Sibul F, Matavulj M, Raseta M, Janjusevic L, Karaman M. 2017. A comparative overview of antioxidative properties and phenolic profiles of different fungal origins: fruiting bodies and submerged cultures of *Coprinus comatus* and *Coprinellus truncorum*. *J. Food Sci. Technol.* **54** (2)**,** 430–438. http://dx.doi.org/10.1007/s13197-016-2479-2

Tian YF, Chen WL, Mo GX, Chen R, Fang MQ, Yedid G, Yan XW. 2016. An immunosuppressant peptide from the hard tick *Amblyomma variegatum*. *Toxins* **8** (5)**,** 133. https://doi.org/10.3390/toxins8050133

Tironi VA, Anon MC. 2014. Antioxidant activity of amaranth protein hydrolysate against thermal oxidation of vegetable oils. *J. Am. Oil Chem. Soc.* **91** (9)**,** 1583–1594. http://dx.doi.org/10.1007/s11746-014-2509-z

Tomycz ND, Richardson RM, Friedlander RM. 2011. Seizures and free radicals: the antioxidant may become the next anticonvulsant. *Neurosurgery* **69** (6)**,** N12–N13. https://doi.org/10.1227/01.neu.0000407917.06492.a8

Tong XH, Lian ZT, Miao LM, Qi BK, Zhang S, Li Y, Wang H, Jiang LZ. 2020. An innovative two-step enzyme-assisted aqueous extraction for the production of reduced bitterness soybean protein hydrolysates with high nutritional value. *LWT-Food Sci. Technol.* **134,** 110151. https://doi.org/10.1016/j.lwt.2020.110151

Tu ML, Cheng SZ, Lu WH, Du M. 2018. Advancement and prospects of bioinformatics analysis for studying bioactive peptides from food-derived protein: sequence, structure, and functions. *TrAC Trends Anal. Chem.* **105,** 7–17. http://dx.doi.org/10.1016/j.trac.2018.04.005

Vanvi A, Tsopmo A. 2016. Pepsin digested oat bran proteins: separation, antioxidant activity, and identification of new peptides. *J. Chem.* **2016,** 8216378. http://dx.doi.org/10.1155/2016/8216378

Verma AK, Chatli MK, Mehta N, Kumar P. 2018. Assessment of physico-chemical, antioxidant and antimicrobial activity of porcine blood protein hydrolysate in pork emulsion stored under aerobic packaging condition at 4±1ºC. *LWT-Food Sci. Technol.* **88,** 71–79. https://doi.org/10.1016/j.lwt.2017.10.002

Walters ME, Esfandi R, Tsopmo A. 2018. Potential of food hydrolyzed proteins and peptides to chelate iron or calcium and enhance their absorption. *Foods* **7** (10), 172. http://dx.doi.org/10.3390/foods7100172

Wang B, Gong YD, Li ZR, Yu D, Chi CF, Ma JY. 2014. Isolation and characterisation of five novel antioxidant peptides from ethanol-soluble proteins hydrolysate of spotless smoothhound (*Mustelus griseus*) muscle. *J. Funct. Foods* **6,** 176–185. https://doi.org/10.1016/j.jff.2013.10.004

Wang L, Li T, Sun DL, Tang MQ, Sun Z, Chen LF, Luo XH, Li YF, Wang R, Li YA, Li J, Chen ZX.

2019. Effect of electron beam irradiation on the functional properties and antioxidant activity of wheat germ protein hydrolysates. *Innov. Food Sci. Emerg. Technol.* **54,** 192–199. https://doi.org/10.1016/j.ifset.2018.09.003

Wen CT, Zhang JX, Zhang HH, Duan YQ, Ma HL. 2020. Plant protein-derived antioxidant peptides: isolation, identification, mechanism of action and application in food systems: a review. *Trends Food Sci. Technol.* **105,** 308–322. https://doi.org/10.1016/j.tifs.2020.09.019

Wu RB, Wu CL, Liu D, Yang XH, Huang JF, Zhang J, Liao BQ, He HL, Li H. 2015a. Overview of antioxidant peptides derived from marine resources: the sources, characteristic, purification, and evaluation methods. *Appl. Biochem. Biotechnol.* **176** (7)**,** 1815–833. http://dx.doi.org/10.1007/s12010-015-1689-9

Wu XS, Cai LY, Zhang YH, Mi HB, Cheng XR, Li JR. 2015b. Compositions and antioxidant properties of protein hydrolysates from the skins of four carp species. *Int. J. Food. Sci. Tech.* **50** (12)**,** 2589–2597. http://dx.doi.org/10.1111/ijfs.12927

Yang FJ, Chen X, Huang MC, Yang Q, Cai XX, Chen X, Du M, Huang JL, Wang SY. 2021. Molecular characteristics and structure-activity relationships of food-derived bioactive peptides. *J. Integr. Agric.* **20** (9)**,** 2313–2332. https://doi.org/10.1016/S2095-3119(20)63463-3

Yang J, Huang JC, Dong XL, Zhang YL, Zhou XH, Huang M, Zhou GH. 2020. Purification and identification of antioxidant peptides from duck plasma proteins. *Food Chem.* **319,** 126534. https://doi.org/10.1016/j.foodchem.2020.126534

Yang JJ, Hu L, Cai TT, Chen QL, Ma Q, Yang J, Meng C, Hong J. 2018. Purification and identification of two novel antioxidant peptides from perilla (*Perilla frutescens* L. Britton) seed protein hydrolysates. *PLoS One* **13** (7)**,** e0200021. https://doi.org/10.1371/journal.pone.0200021

Yang RW, Li XF, Lin SY, Zhang ZM, Chen F. 2017. Identification of novel peptides from 3 to 10 kDa pine nut (*Pinus koraiensis*) meal protein, with an exploration of the relationship between their antioxidant activities and secondary structure. *Food Chem.* **219,** 311–320. http://dx.doi.org/10.1016/j.foodchem.2016.09.163

Yarnpakdee S, Benjakul S, Kristinsson HG, Kishimura H. 2015. Antioxidant and sensory properties of protein hydrolysate derived from Nile tilapia (*Oreochromis niloticus*) by one-and two-step hydrolysis. *J. Food Sci. Technol.* **52** (6)**,** 3336–3349. http://dx.doi.org/10.1007/s13197-014-1394-7

Yu ZP, Wu SJ, Zhao WZ, Ding L, Shiuan D, Chen F, Li JR, Liu JB. 2018. Identification and the molecular mechanism of a novel myosin-derived ACE inhibitory peptide. *Food Funct.* **9** (1)**,** 364–370. https://doi.org/10.1039/c7fo01558e

Yuan J, Zheng Y, Wu Y, Chen HB, Tong P, Gao JY. 2020. Double enzyme hydrolysis for producing antioxidant peptide from egg white: optimization, evaluation, and potential allergenicity. *J. Food Biochem.* **44** (2)**,** e13113. https://doi.org/10.1111/jfbc.13113

Zarei M, Ebrahimpour A, Abdul-Hamid A, Anwar F, Saari N. 2012. Production of defatted palm kernel cake protein hydrolysate as a valuable source of natural antioxidants. *Int. J. Mol. Sci.* **13** (7)**,** 8097–8111. https://doi.org/10.3390/ijms13078097

Zarei M, Ebrahimpour A, Abdul-Hamid A, Anwar F, Abu Bakar F, Philip R, Saari N. 2014. Identification and characterization of papain-generated antioxidant peptides from palm kernel cake proteins. *Food Res. Int.* **62,** 726–734. https://doi.org/10.1016/j.foodres.2014.04.041

Zeng WC, Zhang WC, Zhang WH, Shi B. 2013. Antioxidant activity and characterization of bioactive polypeptides from bovine hair. *React. Funct. Polym.* **73** (3)**,** 573–578. https://doi.org/10.1016/j.reactfunctpolym.2012.12.013

Zhang M, Huang TS, Mu TH. 2019. Improvement of thermal, microwave and ultrasonication pretreatment on the production of antioxidant peptides from sweet potato protein via *in vitro* gastrointestinal digestion. *Int. J. Food Sci. Technol.* **54** (7)**,** 2338–2345. https://doi.org/10.1111/ijfs.14095

Zhou KQ, Canning C, Sun S. 2013. Effects of rice protein hydrolysates prepared by microbial proteases and ultrafiltration on free radicals and meat lipid oxidation. *LWT-Food Sci. Technol.* **50** (1)**,** 331–335. http://dx.doi.org/10.1016/j.lwt.2012.05.002

Zhou TY, Gao XC, Dang YL, Pan DD, Cao JX. 2019. Research progress of bioactive peptides based on bioinformatics. *Sci. Technol. Food Ind.* **40** (12)**,** 335–340. .http://dx.doi.org/10.13386/j.issn1002-0306.2019.12.054

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Can rice bran, sesame, and olive oils be used as substitutes for soybean oil to improve French salad dressing quality?

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SUMMARY: Soybean oil is a commonly-used vegetable oil for the industrial manufacture of French salad dressing. The effect of rice bran, sesame, olive, and soybean oils on French salad dressing's quality characteristics was investigated. After one month, the highest acidity, peroxide value (PV), and the lowest emulsion stability were observed in the control containing soybean oil ($p < 0.05$). Samples formulated with sesame (T4) and rice bran oils (T3) had the lowest PVs. Color measurement results indicated that a* of a sample containing olive oil (T2) was most influenced and declined on the 30th day ($p < 0.05$). In the rheological test, samples were solid viscoelastic. The elastic modulus and complex viscosity of T2 were slightly higher. The highest and the lowest overall sensory acceptance belonged to T3 and T2, respectively. Therefore, soybean oil could be replaced to obtain a more desirable product. Finally, T3 was selected as the superior sample.

KEYWORDS: *French salad dressing; Olive oil; Rice bran oil; Sesame oil; Soybean oil*

RESUMEN: ***¿Se pueden usar aceites de salvado de arroz, sésamo y oliva como sustitutos del aceite de soja para mejorar la calidad del aderezo de ensaladas francesas?*** El aceite de soja es un aceite vegetal de uso común para la fabricación industrial de aderezo para ensaladas francesas. Se investigó el efecto de los aceites de salvado de arroz, sésamo, oliva y soja sobre las características de calidad del aderezo para ensaladas francesas. Después de un mes, la mayor acidez, el índice de peróxido (PV) y la menor estabilidad de la emulsión se observaron en el control que contenía aceite de soja ($p < 0,05$). Las muestras formuladas con aceites de sésamo (T4) y salvado de arroz (T3) tuvieron los PV más bajos. Los resultados de la medida del color indicaron que a* de una muestra que contenía aceite de oliva (T2) fue la más influenciada y disminuyó en el día 30 ($p < 0,05$). En la prueba reológica, las muestras fueron sólidas viscoelásticas. El módulo elástico y la viscosidad compleja de T2 fueron ligeramente superiores. La aceptación sensorial general más alta y más baja correspondió a T3 y T2, respectivamente. Por lo tanto, el aceite de soja podría reemplazarse para obtener un producto más deseable. Finalmente, se seleccionó a T3 como la muestra superior.

PALABRAS CLAVE: *Aceite de oliva; Aceite de salvado de arroz; Aceite de sésamo; Aceite de soja; Aderezo para ensalada francesa*

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1. INTRODUCTION

Salad is one of the most popular and frequently-consumed food products and have been more in demand in recent years as a healthy food. Salad dressings can be used to improve the flavor and increase the acceptance of salads by consumers (de Melo *et al.*, 2015; Ma and Boye, 2013; Manshadi *et al.*, 2019). Salad dressings are oil-in-water emulsions containing different fats (Paraskevopoulou *et al.*, 2007). French salad dressing formulation includes oil, vinegar, eggs, tomato paste, flavoring agents, and stabilizers (Yaghouti Moghaddam *et al.*, 2013). Due to USFDA, the amount of vegetable oil in salad dressings should be at least 30%. As a dispersed phase of the emulsion, the oil plays a significant role in shelf-life and the product's rheological, textural, and sensory properties (Ma and Boye, 2013). According to Amin *et al.* (2014), different types of oils (sunflower, soybean, corn, sesame, and olive) have a significant effect on the quality characteristics of mayonnaise. Vegetable oils extensively used in salad dressings include soybeans, canola, and sunflower oil (Amin *et al.*, 2014).

Soybean oil is one of the most commonly utilized oils in the industry to manufacture emulsified dressing due to its abundance and low cost (Neia *et al.*, 2019). However, it contains large amounts of unsaturated fatty acids, including linolenic acid, which reduces its stability against oxidation (Shahidi, 2005). Despite the restrictions of using peroxide value as the main factor for oil's shelf-life, it is usually used as an indicator of oil oxidation. An undesirable change may have occurred in the flavor of soybean oil at the peroxide value of 5-10 meqO_2/kg oil (Rasmussen *et al.*, 2008). Some oils, such as olive, sesame, and rice bran, are rich in oleic or linoleic fatty acids, and have less linolenic acid than soybean oil.

Moreover, due to the presence of natural compounds with potent antioxidant properties, oils have high nutritional value (Shahidi, 2005). Olive oil from the fruit of the *Olea europaea* tree is one of the healthiest and most useful vegetable oils. It has large amounts of unsaturated fatty acids, and the major one is oleic acid (Karbasian *et al.*, 2015). Olive oil, especially the extra virgin type, is rich in phenolic compounds with antioxidant activity (Di Mattia *et al.*, 2015; Karbasian *et al.*, 2015). Sesame oil contains a high concentration of unsaturated fatty acids such as linoleic and oleic acid. However, its oxidation stability is high due to highly antioxidant compounds such as sesamol, sesamolin, and tocopherol (Pazhvand and Khavarpour, 2019). Rice bran oil is a byproduct of rice that has high nutritional value. Most of its fatty acids are unsaturated, and its dominant acid is oleic acid with gamma oryzanol as an antioxidant compound (Garcia *et al.*, 2009; Phan *et al.*, 2019). Due to previous studies, rice bran oil showed good potential for use as an alternative to soybean oil in mayonnaise formulation (Garcia *et al.*, 2009). In addition, according to the results of Pazhvand and Khavarpour (2019), using 20% sesame oil in mayonnaise formulation is suitable for improving its nutritional value (Pazhvand and Khavarpour, 2019). Most previous research on French salad dressings has focused on stabilizing (Yaghouti Moghaddam *et al.*, 2013) and emulsifying compounds (de Melo *et al.*, 2015), but the effect of different types of oils has not been compared. This study aimed to compare the effects of olive, sesame, rice bran, and soybean oils on the quality characteristics of French salad dressings in order to select a more desirable product.

2. MATERIALS AND METHODS

2.1. Preparation of French salad dressing

The ingredients used in the formulation of samples include eggs (10%), vinegar (8%), oil (36%), salt (2%), tomato paste (6.2%), garlic powder (0.04%), pepper powder (0.08%), citric acid (0.1%), xanthan gum (0.15%) (GRINDSTED, Danisco, Denmark), Guar gum (0.05%) (GRINDSTED, Danisco, Denmark), sodium benzoate (0.06%), potassium sorbate (0.01%), and water (31.31%). The vegetable oils included sesame oil (Pick, Iran), rice bran oil (Gaetano Giurlani, Italy), soybean oil (Olitaia, Italy), and olive oil (Villa Vinci, Italy) and were purchased from a local market in Tehran.

For sample preparation, tomato paste was mixed with spices in water, pasteurized at 90 °C for 30 minutes and then cooled. A part of pasteurized phase was mixed with powdered materials and eggs. Then, half of the oil was added gradually to form the emulsion. After that, the rest of the pasteurized phase, vinegar, and remaining oil were added and the mixture was well blended. Finally, French dressing samples

were poured into glass containers and stored at 4 °C (Yaghouti Moghaddam *et al*., 2013). Analyses were performed on the first, fifteenth, and 30th days, and a sensory test was performed on the first and 30th days of storage.

2.1.1. Edible oils' chemical analyses

The iodine (IV), peroxide (PV), anisidine (AV) values, and free fatty acids (FFA) of the samples were determined according to the AOCS official method (Firestone, 2009). Fatty acids were converted to fatty acid methyl esters through the transesterification of oils with sodium methoxide according to the AOCS method (Ce 1–62). The fatty acid methyl esters in the samples were analyzed on a Gas Chromatograph (GC) equipped with a mass spectrometry (AOCS, 2009).

2.2. Physicochemical properties

The pH was measured by Lohand-PHS-550-China. The analyses of the acidity and peroxide values in the sauces were performed according to ISO 660:2011 and ISO 3960:2010, respectively (ISO 660, 2020; ISO 3960, 2010). In the emulsion stability test, 10 g of sample (F_1) was transferred to a tube and heated in a water bath at 80 °C for 30 min. Then, the samples were centrifuged (PIT320-Universal, Iran) at 3000 rpm for 15 min, and the top layer of oil was removed. The precipitated layer was weighed (F2), and the emulsion stability was calculated as follows: $\frac{F2}{F1}$ x100 (de Melo *et al.*, 2015; Nikzade *et al.*, 2012). All the above measurements were performed in triplicate with the preparation of three treatments per sample.

2.3. Color measurement

Colorimetry was accomplished using a Hunter-Lab colorimeter (TES, Taiwan). The experiments were performed 5 times for each sample at different angles, and L*, a*, and b* parameters were measured (de Melo *et al.*, 2015).

2.4. Rheological properties

A dynamic test was performed using a modular compact rheometer (MCR302, Anton Paar, Austria) at 25 ºC with a parallel-plate configuration (diameter: 25 mm) and a gap distance of 1 mm. For the amplitude sweep test, 1 rad/s frequency was considered constant to determine the linear viscoelastic region, and the strain value applied to the samples ranged from 0.01 to 100%. The frequency sweep test was performed under the linear viscoelastic region, and the frequency range from 0.01 to 100 rad/s was applied to the samples (Di Mattia *et al.*, 2015).

2.5. Sensory evaluation

Ten semi-trained panelists performed sensory tests on days 1 and 30 after production. The taste, odor, color, texture before use, oral texture, and overall acceptance were evaluated. Before this test, the microbial characteristics of samples were confirmed according to Iran National Organization of Standardization (INSO) No. 2965 (Microbiology of mayonnaise and salad sauce- Specifications and test methods) (INSO 2965, 2017). Then the samples with different three-digit codes were given randomly to the panelists. Panelists evaluated each sample in duplicate. A comparison of sensory indices was performed with the 9-point hedonic scale from 1 (strongly disliked) to 9 (strongly liked) (de Melo *et al.*, 2015).

2.6. Statistical analysis

Data were analyzed by SPSS 24 using one-way analysis of variance (ANOVA), and the differences among means were determined by Duncan's multiple range test at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Fatty acid composition and chemical characteristics of selected oils

The results indicated that olive oil had the lowest Iodine value (86.4 ± 0.11 g I_2/100 g oil) and was more saturated in comparison with other oils because olive oil had the highest oleic acid (C18:1) and lowest linoleic (C18:2) and linolenic (C18:3) contents compared to the other oils used in this study (Table 1). In contrast with olive oil, soybean oil had the highest unsaturation or iodine value (128.25 ± 0.34 g I_2/100 g oil) among the oils and the highest polyunsaturated fatty acids (linoleic and linolenic acids). For this reason, soybean oil is more susceptible to oxidation. Primary and secondary oxidation

Table 1. Fatty acid composition and chemical characteristics of soybean, sesame, rice bran, and olive oils[a]

	Soybean	Sesame	Rice bran	Olive oil
Palmitic acid (C16:0)	11.45±0.07	8.80±0.12	21.70±0.33	15.16±0.26
Stearic acid (C18:0)	4.90±0.33	5.05±0.04	1.20±0.02	2.30±0.02
Oleic acid (C18:1)	22.88±0.61	37.01±0.55	43.05±0.78	65.05±0.81
Linoleic acid (C18:2)	52.22±0.74	47.55±0.35	29.90±0.24	13.45±0.43
Linolenic acid (C18:3)	7.51±0.05	1.34±0.07	2.10±0.06	1.05±0.02
Others	1.04±0.02	0.25±0.01	2.05+0.07	2.99±0.05
Iodine Value (g I_2/100 g oil)	128.25± 0.34	116.38± 0.05	101.34± 0.20	86.40±0.11
Peroxide value (meq/kg)	1.39± 0.01	1.02± 0.02	0.75± 0.07	1.20± 0.02
Anisidine value	3.20± 0.03	1.97± 0.02	2.49±0.05	3.20± 0.01
Free fatty acids (%)	0.41±0.00	0.42±0.02	0.70±0.14	0.30±0.00

[a] Each value in the table represents the mean value ± standard deviation of triplicate analyses.

products (PV and AV) and FFA are given in Table 1. The primary products of hydrolytic rancidity (FFA) are not toxic. However, they accelerate oil oxidation, which leads to a similar toxic effect of oxidized oils (Hosseini *et al.*, 2020).

3.2. Physicochemical properties

The results of the chemical properties in French salad dressing samples are shown in Table 2. The results showed a negligible difference among the samples' pH only on the first day of storage ($p < 0.05$). Acording to Pazhvand and Khavarpour (2019), the addition of different amounts of sesame oil to mayonnaise caused small changes in samples' pH. Chetana *et al.* (2019) also stated that the pH of egg-free mayonnaise containing sesame oil and rice bran oil was not affected by oil mixtures. According to the Commercial Item Description (CID) of The U.S. Department of Agriculture (USDA) (A-A-20140E), the pH requirement for salad dressing is not less than 3.1 or more than 4.1 (USDA, 2017). In addition, based on the Iran National Organization of Standardization (INSO) No. 2454, the pH of salad dressing should be a maximum of 4.1 (INSO 2454, 2014). In this study, the pH of all of the French salad dressing samples was in line with the two mentioned standards, except T2 (containing olive oil) on the 1st day, which was not significantly different from T4 (containing sesame oil) on the same day. The pH of French salad dressing samples containing olive, rice bran, and sesame oils significantly decreased during storage ($p < 0.05$). Besides, acidity increased after one month of storage, and T1 (containing soybean oil) had significantly higher acidity on the 30th day ($p < 0.05$), and other samples were not significantly different ($p > 0.05$). Reducing pH and increasing the acidity may

Table 2. pH, acidity, and peroxide values of French salad dressing formulations during storage (n=3, mean ± SD)

	pH			Acidity (%)			PV (meq/kg)		
Sample	**Day 1**	**Day 15**	**Day 30**	**Day 1**	**Day 15**	**Day 30**	**Day 1**	**Day 15**	**Day 30**
T_1	4.08^{bAB}±0.01	4.09^{aA}±0.00	4.06^{aB}±0.01	2.55^{bC}±0.03	2.78^{aB}±0.03	3.12^{aA}±0.07	0.19^{aC}±0.01	0.23^{aB}±0.01	0.29^{aA}±0.01
T_2	4.11^{aA}±0.00	4.09^{aAB}±0.00	4.07^{aB}±0.01	2.62^{abB}±0.02	2.68^{bAB}±0.02	2.77^{bA}±0.04	0.16^{bB}±0.01	0.22^{aA}±0.01	0.25^{bA}±0.00
T_3	4.08^{bA}±0.01	4.10^{aAB}±0.01	4.07^{aB}±0.01	2.45^{cC}±0.02	2.73^{abB}±0.03	2.87^{bA}±0.05	0.16^{bC}±0.00	0.18^{bB}±0.00	0.21^{cA}±0.01
T_4	4.10^{abA}±0.01	4.08^{aB}±0.00	4.07^{aB}±0.00	2.67^{aB}±0.03	2.80^{aA}±0.03	2.87^{bA}±0.05	0.10^{cC}±0.00	0.16^{bB}±0.00	0.20^{cA}±0.00

Each sample includes different oil: T1: soybean oil, T2: olive oil, T3: rice bran oil, T4: sesame oil. Mean values with the same superscript lowercase and uppercase letters are not significant at $p > 0.05$ in each identical column and row, respectively (according to one way ANOVA and Duncan's multiple range test).

be due to oil oxidation and the formation of hydroperoxides and the hydrolysis of triglycerides, as well as the creation of free fatty acids (de Melo *et al.*, 2015). Another reason for the decreased pH may be related to increased microbial growth, especially lactic acid bacteria (Pazhvand and Khavarpour, 2019). The results of the acidity of French salad dressing samples were in accordance with the minimum percentage of acidity of salad dressings in INSO No. 2454, which is 0.6% (INSO 2454, 2014). According to the results shown in Table 2, the highest peroxide value was obtained for the sample containing soybean oil (T1), which was significantly different from other samples on the first and 30th days and for T3 (containing rice bran oil) and T4 (containing sesame oil) on 15th day ($p < 0.05$). The peroxide value of the samples significantly increased during one month of storage, which is in line with the results of Abedinzadeh *et al.* (2016). After one month, the lowest peroxide value was observed in T4 and T3 samples, which showed no significant difference ($p > 0.05$). Oil oxidation occurs on the surface of oil droplets in oil-in-water emulsions. The reaction among hydroperoxides on the surface of oil droplets as the primary products of oxidation and metals transferred from the aqueous phase is the main reason for oxidative instability. The mechanism is the breakdown of hydroperoxides into free radicals, which react with unsaturated fatty acids inside oil droplets or at the water-oil interface, which results in the production of free radical (Paraskevopoulou *et al.*, 2007). Soybean oil is more susceptible to oxidation compared to the other three oils, as it contains about 7-8% linolenic acid (C18:3) (Kim *et al.*, 2010). Sesame oil has phenolic compounds of lignans such as sesamine, sesamolin, and sesaminol, which have strong antioxidant activity (Pazhvand and Khavarpour, 2019). The oxidative stability of rice bran oil may also be due to phenolic compounds such as gamma-oryzanol (Garcia *et al.*, 2009). Therefore, the presence of strong antioxidant compounds in sesame and rice bran oils can reduce oxidation. The presence of polyphenolic compounds in olive oil, which have antioxidant properties and high oleic acid content (monounsaturated), significantly decreased the peroxide value of T2 on day 30 compared to T1 (Di Mattia *et al.*, 2015; Karbasian *et al.*, 2015).

The results of emulsion stability are presented in Figure 1. The sample containing soybean oil had the lowest stability on all days. After one month of storage, three samples containing olive, sesame, and rice bran oils were not significantly different ($p > 0.05$). The existence of thickeners in the formulation of food emulsions is an essential factor which could affect the emulsions' stability. Adding xanthan gum can improve emulsion stability (Ma and Boye, 2013). However, since the type and amount of thickener in the samples were the same, the difference in emulsion stability is related to the type of oil used in the formulation, which is in line with the results of Chetana *et al.* (2019). The type of fatty acids in oils affects the stability of emulsions. Oils with

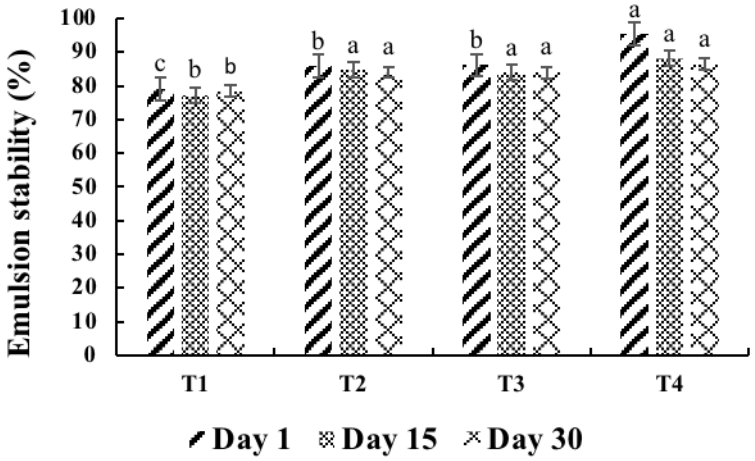

Figure 1. Emulsion stability of French dressing samples during storage. Each sample includes different oils: T1: soybean oil, T2: olive oil, T3: rice bran oil, T4: sesame oil. The same superscript letters are not significant at $p > 0.05$ on identical days (according to one-way ANOVA and Duncan's multiple range test).

large amounts of unsaturated fatty acids cannot create a strong structure (Kim *et al.*, 2010). Therefore, French salad dressing with soybean oil, which contains large amounts of unsaturated fatty acids such as linolenic acid, showed lower stability.

3.3. Color measurement

According to the results of the color analysis shown in Figure 2, L* (lightness/darkness) of T4 (containing sesame oil) was significantly lower than T3 (containing rice bran oil) and T2 (containing olive oil) on the first day of storage ($p < 0.05$). The results of Chetana *et al*. (2019) showed that the mayonnaise sample prepared with sesame oil had a lower L* than the sample formulated with rice bran oil. On day 30, the highest and lowest L* values were observed in T3 (containing rice bran oil) and T2, respectively. Moslavac *et al.* (2012) stated that olive oil also caused the lowest L* value after one month in mayonnaise. The a* value (redness) of T2 (containing olive oil) was lower than some samples such as T1 (containing soybean oil), due to the specific color and slightly greenish olive oil. Moslavac *et al.* (2012) showed that adding olive oil to the formulation of mayonnaise reduced its redness, consistent with the present study. The highest b* value was obtained in T1. The samples containing rice bran and sesame oils (T3 and T4) had the lowest b* on day 30. The results of Chetana *et al.* (2019) showed a significant difference in b* in mayonnaise samples with different ratios of sesame and rice bran oils compared to mayonnaise containing sunflower oil.

3.4. Rheological tests

The maximum strain in the linear viscoelastic region for French salad dressing samples was 1%. The trend of changes in elastic modulus (G') and viscose modulus (G") compared to frequency is shown in Figure 3. In all samples, the elastic modulus (G') was higher than the viscose modulus (G"), indicating more elastic behavior than viscose, and all samples were solid viscoelastic. Furthermore, they are classified as weak gel due to the dependence of elastic and viscose modulus on frequency

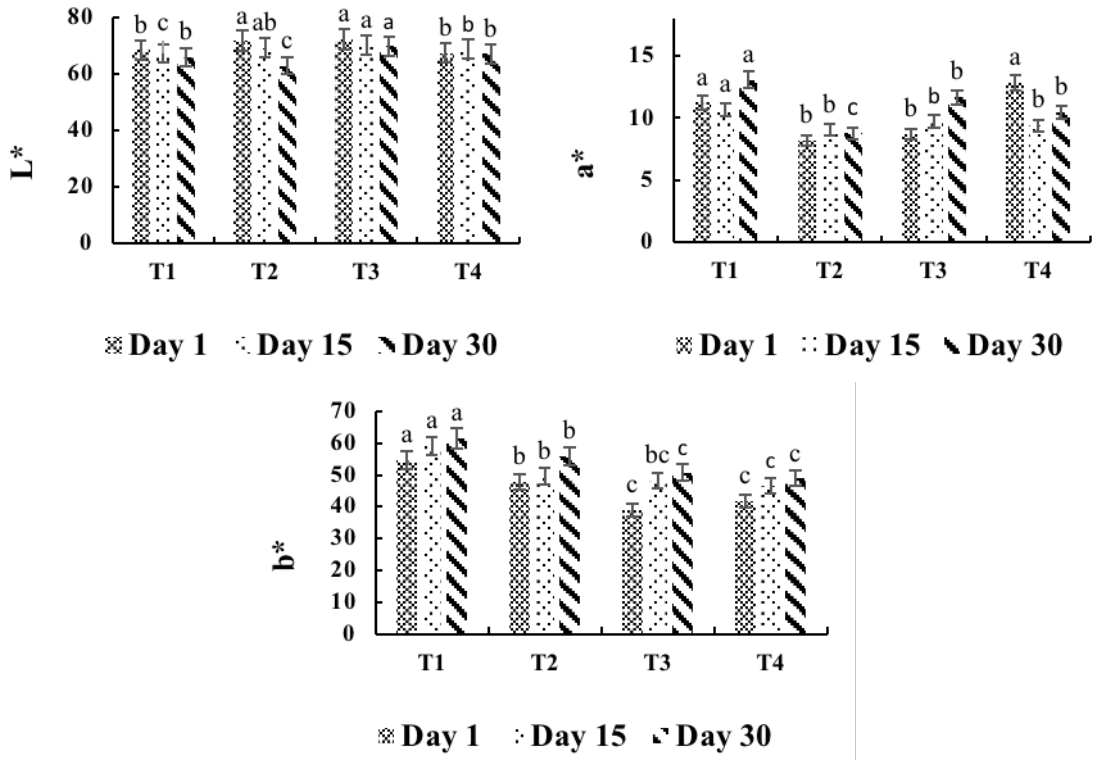

FIGURE 2. Color values for French salad dressing during storage. Each sample includes different oils: T1: soybean oil, T2: olive oil, T3: rice bran oil, T4: sesame oil. The same superscript letters are not significant at p > 0.05 on identical days (according to one- way ANOVA and Duncan's multiple range test).

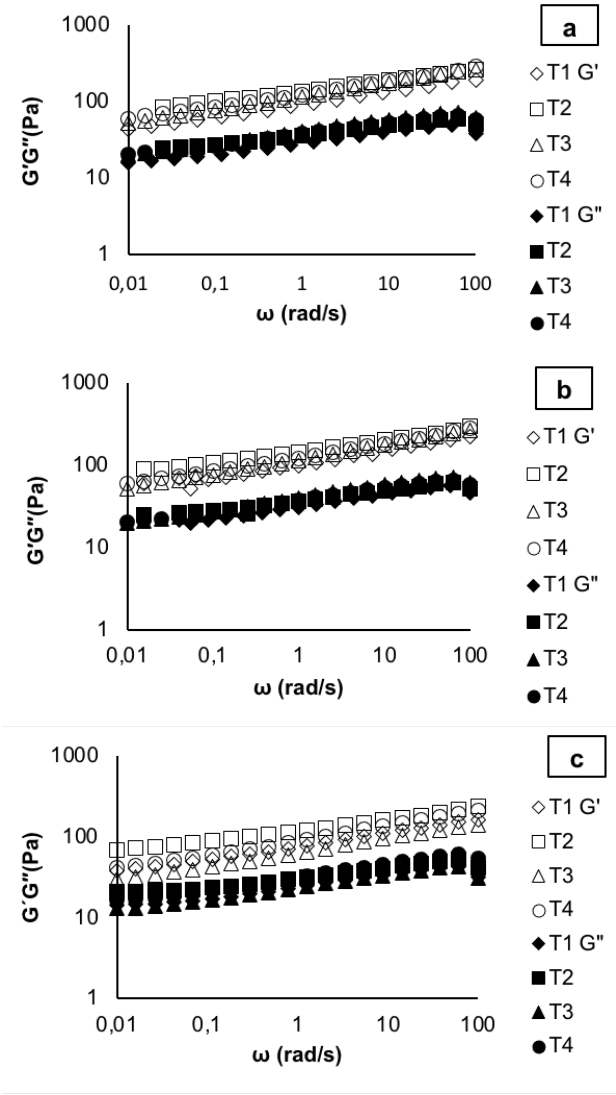

FIGURE 3. Rheogram of Storage modulus (G ‘) and loss modulus (G’’) versus frequency in French salad dressing samples after (a) 1 day (b) 15 days and (c) 30 days. Each sample includes different oils: T1: soybean oil, T2: olive oil, T3: rice bran oil, T4: sesame oil.

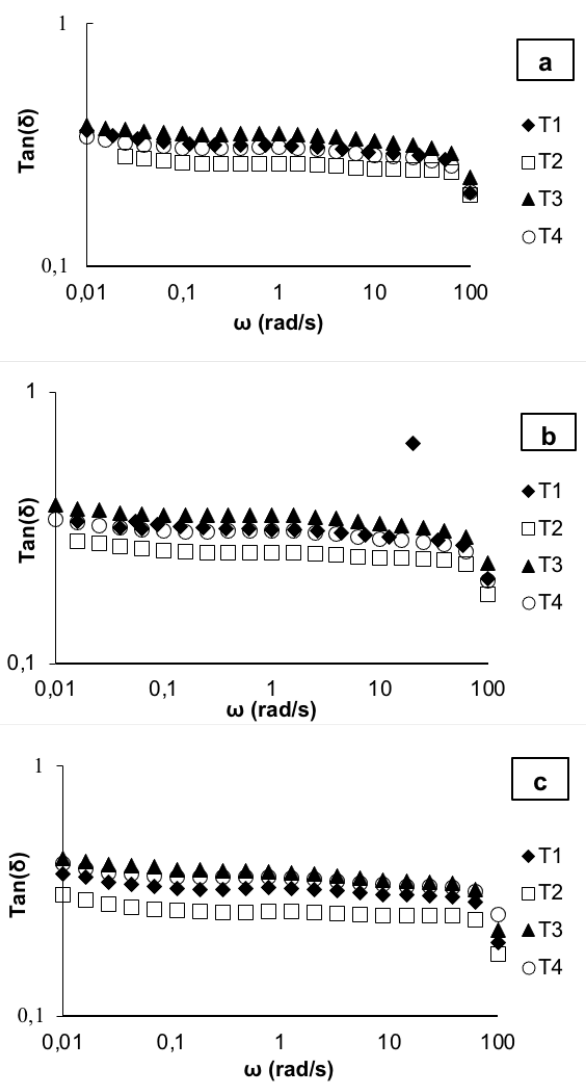

FIGURE 4. Tan (δ) of French salad dressing samples after (a) 1 day (b) 15 days and (c) 30 days. Each sample includes different oils: T1: soybean oil, T2: olive oil, T3: rice bran oil, T4: sesame oil.

as in previous studies (Bortnowska *et al.*, 2014; Mizani *et al.*, 2015). Since the type and amount of xanthan gum as stabilizer was constant, the type of oil used in the formulation affected the rheological factors. T2 (containing olive oil) had a slightly higher elastic modulus in all three days of storage. Tan (δ), which indicates the superiority of elastic or viscous properties (Bortnowska *et al.*, 2014), was obtained under 1 and indicated solid viscoelastic behavior (Figure 4). The changes in complex viscosity (*η) of samples compared to frequency are shown in Figure 5. The samples’ complex viscosity decreased by increasing frequency, indicating the pseudoplastic behavior, which is due to weak gel structure and in accordance with other studies (Bortnowska *et al.*, 2014; Mizani *et al.*, 2015). The complex viscosity was slightly higher in T2 (containing olive oil) and T4 (containing sesame oil) than in the other samples. On all days, the elastic modulus and complex viscosity of the T2 sample (with olive oil) were slightly higher than other samples. The composition of fatty acids in oils (oleic and linoleic) has an important effect on rheological behavior. The viscosity decreases in oils by increasing the ratio of linoleic to oleic acids. This phenomenon represents more double bonds in the chain. Since each

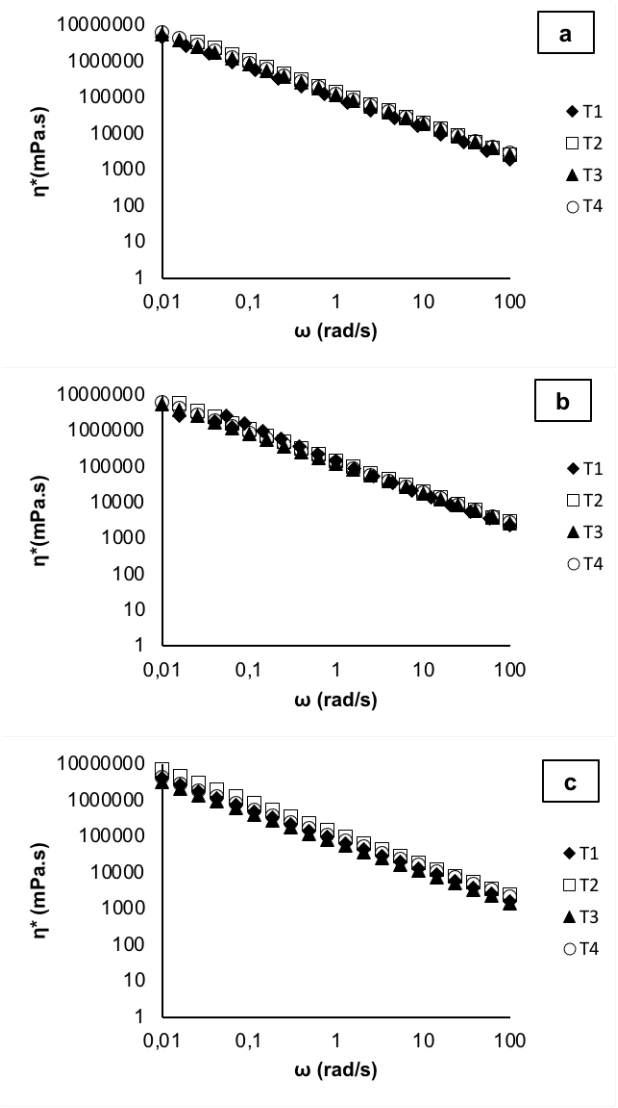

FIGURE 5. Complex viscosity (*η) of French salad dressing samples after (a) 1 day (b) 15 days and (c) 30 days. Each sample includes different oils: T1: soybean oil, T2: olive oil, T3: rice bran oil, T4: sesame oil.

double bond with cis arrangement creates a sprain in the chain, the double band's presence prevents the molecules from approaching each other (Kim *et al.*, 2010). Therefore, olive oil, which contains large amounts of oleic acid and has a high oleic to linoleic ratio, had higher elastic behavior, complex viscosity, and higher structural strength. The qualitative characteristics of the different ratios of olive, sesame, and linseed oil mixture ratios were evaluated by Hashempour-Baltork *et al.* (2018). The results showed that increasing linseed oil reduced the viscosity due to highly unsaturated fatty acids.

3.5. Sensory evaluation

The results of the sensory evaluation of French dressing samples are shown in Figure 6. The samples containing rice bran (T3) and sesame (T4) oils had higher scores for taste and odor on the 1st and 30th days after production, and a lower score belonged to T2 (olive oil). According to Di Mattia *et al.* (2015), the addition of extra virgin olive oil to mayonnaise was also associated with bitterness and astringency related to phenolic compounds (Di Mattia *et al.*, 2015). T2 had the lowest color score on the first day and after one month of storage. There was no significant difference in texture before use ($p > 0.05$), and there was little difference in oral texture among the samples after consumption ($p < 0.05$). Finally, T3 (rice bran oil) had higher overall acceptance, and the lowest acceptability was observed in T2 on days 1 and 30. Chetana *et al.* (2019) also stated that eggless mayonnaise samples formulated with rice bran oil had the highest overall acceptance compared to samples containing a mixture of rice bran and sesame oil and a sample containing sunflower oil. Amin *et al.* (2014) produced low-fat mayonnaise samples containing different oils. They stated that samples with soybean and sunflower oils were more sensorial acceptable compared to those containing corn, sesame, and olive oils. Samples containing olive and sesame oils had a lower color score.

4. CONCLUSIONS

This study's results showed that the quality characteristics of French salad dressing were influenced by the oil used in the product. Although soybean oil is one of the most widely used oils in the formulation of many commercial dressings, it causes higher peroxide value, acidity, and lower emulsion stability during storage. Since the sample containing rice bran oil had the highest sensory acceptability and other acceptable results, it was selected as the superior sample. Therefore, it is possible to use oils with higher nutritional value such as rice bran oil in French salad dressing to improve the product's quality. Future research could be conducted about the effect of oils with high nutritional value on increasing the antioxidant properties in salad dressings.

Compliance with ethical standards

The authors declare that there is no conflict of interest.

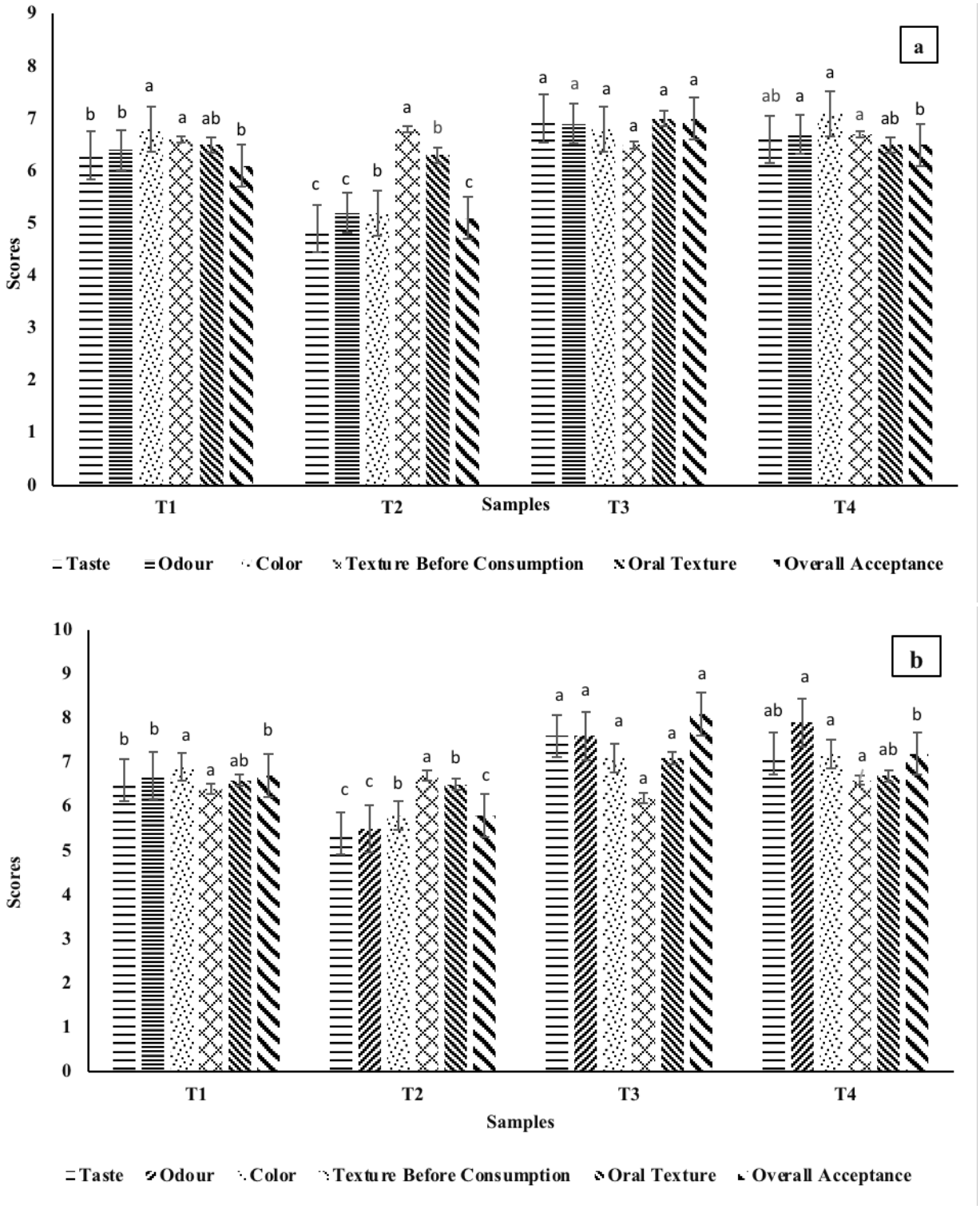

FIGURE 6. Sensory characteristics of French salad dressing samples on the days 1 (a) and 30 (b) after production. Each sample includes different oils: T1: soybean oil, T2: olive oil, T3: rice bran oil, T4: sesame oil. The same superscript letters are not significant at $p > 0.05$ for each sensory attribute on identical days (according to one- way ANOVA and Duncan's multiple range test).

REFERENCES

Abedinzadeh S, Torbati M, Azadmard-Damirchi S. 2016. Some qualitative and rheological properties of virgin olive oil-apple vinegar salad dressing stabilized with xanthan gum. *Adv. Pharm. Bull.* **6**, 597. doi:https://doi:10.15171/apb.2016.074

Amin MHH, Elbeltagy AE, Mustafa M, Khalil AH. 2014. Development of low fat mayonnaise containing different types and levels of hydrocolloid gum. *J. Agroal. Proc. Technol.* **20**, 54–63.

AOCS. 2009. *AOCS Official Method Ce 1-62 Fatty Acid Composition by Packed Column Gas Chromatography*: Champaign.

Bortnowska G, Balejko J, Schube V, Tokarczyk G, Krzemińska N, Mojka K. 2014. Stability and physicochemical properties of model salad dressings prepared with pregelatinized potato starch. *Carbohydr. Polym.* **111**, 624–632. doi:https://doi.org/10.1016/j.carbpol.2014.05.015

Chetana R, Bhavana K, Babylatha R, Geetha V, Kumar GS. 2019. Studies on eggless mayonnaise from rice bran and sesame oils. *J. Food Sci. Technol.* **56**, 3117–3125. doi:https://doi.org/10.1007/s13197-019-03819-1

de Melo ANF, de Souza EL, da Silva Araujo VB, Magnani M. 2015. Stability, nutritional and sensory characteristics of French salad dressing made with mannoprotein from spent brewer's yeast. *LWT.* **62**, 771–774. doi:https://doi.org/10.1016/j.lwt.2014.06.050

Di Mattia C, Balestra F, Sacchetti G, Neri L, Mastrocola D, Pittia P. 2015. Physical and structural properties of extra-virgin olive oil based mayonnaise. *LWT.* **62**, 764–770. doi:https://doi.org/10.1016/j.lwt.2014.09.065

Firestone D. 2009. *Official methods and recommended practices of the AOCS.* Urbana, American Oil Chemists' Society Press.

Garcia K, Sriwattana S, No HK, Corredor JAH, Prinyawiwatkul W. 2009. Sensory optimization of a mayonnaise-type spread made with rice bran oil and soy protein. *J. Food Sci.* **74**, S248–S254. doi:https://doi.org/10.1111/j.1750-3841.2009.01203.x

Hashempour-Baltork F, Torbati M, Azadmard-Damirchi S, Savage GP. 2018. Chemical, rheological and nutritional characteristics of sesame and olive oils blended with linseed oil. *Adv. Pharm. Bull.* **8**, 107. doi:https://doi:10.15171/apb.2018.013

Hosseini S, Ramezan Y, Arab S. 2020. A comparative study on physicochemical characteristics and antioxidant activity of sumac (Rhus coriaria L.), cumin (Cuminum cyminum), and caraway (Carum carvil) oils. *J. Food Meas. Charact.* **14**, 3175–3183. doi:https://doi.org/10.1007/s11694-020-00561-7

INSO 2454. 2014. *Mayonnaise & Salad dressings –Specifications and test methods*. Iran: Iran National Organization of Standardization.

INSO 2965. 2017. *Microbiology of mayonnaise and salad sauce- Specifications and test methods*. Iran: Iranian National Standardization Organization.

ISO 660. 2020. *Animal and vegetable fats and oils Determination of acid value and acidity Test method. 2011*. International Organization for Standardization.

ISO 3960. 2010. *Animal and vegetable fats and oils. Determination of peroxide value. Iodometric (visual) endpoint determination*.

Karbasian M, Givianrad MH, Ramezan Y. 2015. A rapid method for detection of refined olive oil as adulterant in extra virgin olive oil by differential scanning calorimetry. *Orient. J. Chem.* **31**, 1735–1739. doi:http://dx.doi.org/10.13005/ojc/310354

Kim J, Kim DN, Lee SH, Yoo S-H, Lee S. 2010. Correlation of fatty acid composition of vegetable oils with rheological behaviour and oil uptake. *Food Chem.* **118**, 398–402. doi:https://doi.org/10.1016/j.foodchem.2009.05.011

Ma Z, Boye JI. 2013. Advances in the design and production of reduced-fat and reduced-cholesterol salad dressing and mayonnaise: a review. *Food Bioproc. Tech.* **6**, 648–670. doi:https://doi.org/10.1007/s11947-012-1000-9

Manshadi AD, Peighambardoust SH, Azadmard-Damirchi S, Niakosari M. 2019. Oxidative and physical stability, rheological properties and sensory characteristics of 'salad dressing'samples formulated with flaxseed oil and n-OSA starch. *J. Food Meas. Charact.* **13**, 26–33. doi:https://doi.org/10.1007/s11694-018-9915-0

Mizani M, Yaghoti Moghaddam M, Alimi M, Salehifar M. 2015. Particle Size Distribution and Viscoelastic Behavior of French Dressing Containing Two Types of Commercial Waxy Maize Starches. *J. Food Biosci. Technol.* **5**, 1–10.

Moslavac T, Pozderović A, Pichler A, Benčić Đ, Vilušić M, Barulek I. 2012. The effect of extra

virgin olive oil and lemon juice on rheological properties of mayonnaise. *Meso.* **14** (2), 157-162. https://hrcak.srce.hr/file/128047.

Neia VJC, da Silva dos Santos PD, Galuch MB, dos Santos Pizzo J, Ito AAR, Santos OO, Visentainer JEL, Visentainer JV. 2019. Fatty Acid Composition and Lipid Profile of Oral/Enteral Nutrition Supplements Available on the Brazilian Market. *Eur. J. Lipid Sci. Technol.* **121**, 1800495. doi: https://doi.org/10.1002/ejlt.201800495

Nikzade V, Tehrani MM, Saadatmand-Tarzjan M. 2012. Optimization of low-cholesterol–low-fat mayonnaise formulation: Effect of using soy milk and some stabilizer by a mixture design approach. *Food Hydrocoll.* **28**, 344–352. doi:https://doi.org/10.1016/j.foodhyd.2011.12.023

Paraskevopoulou D, Boskou D, Paraskevopoulou A. 2007. Oxidative stability of olive oil–lemon juice salad dressings stabilized with polysaccharides. *Food Chem.* **101**, 1197–1204. doi:https://doi.org/10.1016/j.foodchem.2006.03.022

Pazhvand R, Khavarpour M. 2019. Rheological, Physical and Sensory Properties of Mayonnaise Formulated with Sesame Oil. *J. Food Biosci. Technol.* **9**, 35–44.

Phan VM, Junyusen T, Liplap P, Junyusen P. 2019. Effects of ultrasonication and thermal cooking pretreatments on the extractability and quality of cold press extracted rice bran oil. *J. Food Process Eng.* **42**, e12975. doi: https://doi.org/10.1111/jfpe.12975

Rasmussen CN, Wang X-H, Leung S, Andrae-Nightingale LM, Schmidt SJ, Engeseth NJ. 2008. Selection and use of honey as an antioxidant in a French salad dressing system. *J. Agric. Food Chem.* **56**, 8650–8657. doi:https://doi.org/10.1021/jf800635d

Shahidi F. 2005. *Bailey's Industrial Oil and Fat Products, Edible Oil and Fat Products: Processing Technologies* (Vol. 5): John Wiley & Sons.

USDA. 2017. Mayonnaise, salad dressing, and tartar sauce. *A-A-20140E.*

Yaghouti Moghaddam M, Mizani M, Salehifar M, Gerami A. 2013. Effect of waxy maize starch (modified, native) on physical and rheological properties of French dressing during storage. *World Appl. Sci. J.* **21**, 819–824

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Effect of different extraction methods on saffron antioxidant activity, total phenolic and crocin contents and the protective effect of saffron extract on the oxidative stability of common vegetable oils

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SUMMARY: Saffron consists of bioactive compounds with health-promoting properties and is mainly used in medicine, flavoring and coloring. In this study, we aimed to investigate the effect of extraction methods on the antioxidant activity of saffron (Crocus sativus L.) extracts (SE) and to evaluate the antioxidant performance of SE in vegetable oils. Saffron stigmas were extracted in water, ethanol, methanol, and their combinations using maceration extraction (ME), ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), and the combination of UAE with MAE. The results showed that the sample extracted by methanol/water (50:50) using the combination of UAE with MAE methods had the highest amount of total phenolic content (31.56 mg/g GAE) and antioxidant activity (83.24% inhibition). The extract with the highest antioxidant activity was freeze-dried before incorporation into oil samples. Freeze-dried SE contained trans-crocin-4 and trans-crocin-3 (most abundant constituents), kaempferol, and picrocrocin. Moreover, the addition of SE at 1000 ppm resulted in a significant increase in the oxidative stability of canola (CAO), sunflower (SO), and corn oil (COO).

KEYWORDS: *Antioxidant activity; Microwave-assisted extraction; Oxidative stability; Saffron; Ultrasound-assisted extraction.*

RESUMEN: ***Efecto de diferentes métodos de extracción sobre la actividad antioxidante del azafrán, contenido total de fenoles y crocina y efecto protector del extracto de azafrán sobre la estabilidad oxidativa de los aceites vegetales comunes.*** El azafrán contiene compuestos bioactivos con propiedades promotoras de la salud de uso destacado en medicina, saborizante y colorante. En este estudio, nuestro objetivo fue investigar el efecto de los métodos de extracción sobre la actividad antioxidante de los extractos (EA) de azafrán (Crocus sativus L.) y evaluar el rendimiento antioxidante de EA en aceites vegetales. Los estigmas de azafrán se extrajeron en agua, etanol, metanol y sus combinaciones, mediante extracción por maceración (EM), extracción asistida por ultrasonidos (EAU), extracción asistida por microondas (EAM) y la combinación de EAU con EAM. Los resultados mostraron que la muestra extraída con metanol/agua (50:50) usando la combinación de métodos EAU con EAM tuvo la mayor cantidad de fenoles totales (31.56 mg/g GAE) y actividad antioxidante (83.24 % de inhibición). El extracto que incluía la mayor actividad antioxidante se liofilizó antes de incorporarlo a las muestras de aceite. El SE liofilizado contenía *trans*-crocina-4 y *trans*-crocina-3 (los constituyentes más abundantes), kaempferol y picrocrocina. Además, la adición de 1000 ppm de EA dio como resultado un aumento significativo en la estabilidad oxidativa del aceite de canola (C), girasol (G) y maíz (M).

PALABRAS CLAVE: *Actividad antioxidante; Azafrán; Estabilidad oxidativa; Extracción asistida por microondas; Extracción asistida por ultrasonido*

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1. INTRODUCTION

The detection of natural antioxidants has recently become an attractive area of research for both food and pharmaceutical applications. Natural antioxidants can be proposed as substitutes for synthetic antioxidants which have restricted applications due to the harmful health problems like cancerogenic effects which probably occur due to their long-term consumption. Antioxidants cover a broad range of compounds that can retard the degradation of lipids during oxidation and consequently prevent diseases caused by free radicals and enhance the quality and nutritional values of food products (Ahmadian-Kouchaksaraie and Niazmand, 2017).

Saffron (*Crocus sativus L.*) is a triploid sterile plant belonging to the Iridaceae family. This plant is mainly cultivated in Iran (90% of the total annual production of saffron). The dried stigma of the flower of this plant (known as saffron) is considered to be the most expensive spice in the world. Moreover, the main by-product obtained during the harvesting of saffron is its petals. Bioactive components obtained from several parts of saffron (stigmas, petals, and corns), have shown health-promoting properties like cancer prevention, antitumor activities, neuroprotective effects against Alzheimer and Parkinson's disorders, memory enhancement, decrease in anxiety and insomnia, and antidepressant effects (Lambrianidou *et al.*, 2021).

These health-promoting effects result from the valuable nutraceuticals present in saffron, including crocins, safranal, picrocrocin, crocetin, kaempferol, quercetin, α-carotene, β-carotene, and zeaxanthin. The three main bioactive compounds in saffron stigmas are crocins, picrocrocin, and safranal. Crocins (mainly crocin-4) are the water-soluble mono- and di-glycosyl esters of crocetin (a dicarboxylic acid named, $C_{20}H_{24}O_4$). They are derived from zeaxanthin and have the ability to provide the outstanding golden-red color. Picrocrocin ($C_{16}H_{26}O_7$), the second main component of saffron, is a monoterpene glycoside which is responsible for the bitter taste of saffron resulting from the thermal and enzymatic dissociation of zeaxanthin. Safranal ($C_{10}H_{14}O$), the volatile oil that contributes to saffron's unique aroma is a product of the thermal or enzymatic degradation of picrocrocin (Ahmadian-Kouchaksaraie and Niazmand, 2017; Sarfarazi *et al*., 2019).

The extraction methods influence extraction efficiency and the quality of the bioactive constituents obtained from saffron. Maceration extraction (ME), steam distillation and Soxhlet extraction have been traditionally used for the extraction of different bioactive compounds from saffron. Furthermore, several modern procedures like microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), ohmic-assisted extraction (OHAE), supercritical fluid extraction (SFE), subcritical water extraction (SWE) and pulsed electric field have been used to obtain various bioactive constituents from saffron (Ahmadian-Kouchaksaraie and Niazmand, 2017; Hashemi Gahruie *et al.*, 2020; Manouchehri *et al.*, 2018; Pourzaki *et al.*, 2013; Sarfarazi *et al.*, 2019).

Traditional extraction approaches suffer from several disadvantages such as prolonged extraction time, use of large volumes of solvents that are not safe for the environment, high energy consumption, low selectivity, and low extraction efficiency (Garavand *et al.*, 2019; Heydari and Haghayegh, 2014).

Conventional ME, includes several steps: firstly, samples are ground to increase the surface area exposed to solvent. Then, they are placed in closed vessels and the appropriate solvent is added. Finally, the solvent containing bioactive compounds is filtered. Time, temperature, and added solvents are defined as important factors for ME (Deng *et al.*, 2017; Ozkan *et al.*, 2021)

Ultrasonication extraction involves special kinds of sound waves with high-frequency (20 kHz and 100 MHz), passing through a medium which causes the formation, developing, and collapsing of bubbles based on the cavitation phenomenon. The collapse of bubbles close to the plant cell wall leads to disruption of the cell. Then, the solvent washes out the cell contents including bioactive components. This method has some advantages such as the possibility of extraction at ambient temperature, enhancing the mass transfer, being simple and rapid, high extraction rate, and high purity of the extract (Altemimi *et al.*, 2016; Garavand *et al.*, 2019).

In the Maceration extraction process, the transfer of mass and heat occurs in opposite directions; while in MAE, it happens in the same direction from inside plant material to the solvent medium. Consequently, during microwave extraction, solute transfer is accelerated as a result of the one-pot heat-mass transfer, and the extraction rate of bioactive compounds increases. On the other hand, conventional extraction methods lead to the collection of high amounts of undesirable components in the extracted solution which cause the quality and purity of the extract to decrease (Sarfarazi *et al.*, 2020). High quality and pure extracts are obtained by using novel methods like MAE and the consumption of

solvent and energy is also optimized. Numerous studies have confirmed the superiority of MAE over ME, UAE, and supercritical CO_2 in the extraction of bioactive compounds from saffron (Garavand *et al.*, 2019).

Vegetable oils are sensitive to oxidation due to containing high levels of polyunsaturated fatty acids, and therefore the use of antioxidants is necessary to retard their oxidation (Oliveira *et al*., 2018).

Several studies have been conducted to evaluate the antioxidant properties of food products supplemented with saffron extract (SE). For example, the incorporation of saffron into wheat flour pasta and fresh ovine cheese enhanced their antioxidant activity and their sensory properties (Aktypis *et al.*, 2018; Armellini *et al.*, 2018). There are no studies regarding the implementation of saffron in vegetable oils to enhance their oxidative stability. The present study was aimed to evaluate the effect of different extraction techniques and solvents on the extraction of bioactive compounds from saffron (ME, UAE, MAE, combinations of UAE and MAE). Moreover, it was aimed to study the influence of SE on the oxidative stability of several common vegetable oils (CAO, SO and COO) containing highly unsaturated fatty acids (UFA).

2. MATERIALS AND METHODS

2.1. Materials

Dried saffron stigmas were purchased from Jamshidi Marandi producer (Khorasan-e-Razavi, Iran). Refined, bleached and deodorized (RBD), canola oil (CAO), sunflower oil (SO) and corn oil (COO), without added antioxidant, were supplied from Cargill Co., Istanbul, Turkey. Crocin-4 with 98% purity was purchased from Biopurify Phytochemicals Ltd (Sichuan, China). Gallic acid, Folin–Ciocalteu's phenol reagent, and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS)) reagent were purchased from Merck (Darmstadt, Germany). All solvents were obtained from Merck and were of HPLC-grade.

2.2. Preparation of saffron extract

2.2.1. Maceration extraction (ME), ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE)

Five grams of dried saffron stigmas were completely ground using a porcelain mortar and screened with a sieve with mesh size of 0.5 mm. Then, the dried powder from saffron stigmas (0.2 g) was extracted using 15 mL of solvents (water, ethanol, methanol, the mixture of ethanol: water, 50:50 v/v and methanol: water, 50:50 v/v) with different extraction methods including ME, UAE, MAE and combinations of UAE and MAE. Then, the extracts were filtered using a stainless-steel Buchner funnel (Sartorius AG 3400 Gottingen, Germany). Finally, the extracts were completed to 25 mL with extraction solvent, and kept at -20 °C until further analysis.

ME was carried out using a shaker incubator (IKA® KS 4000 I control, Germany). Ground saffron stigmas (0.2 g) were extracted with different previously described solvents for 24 h, at a speed of 100 rpm at room temperature (25 ±1.0 ºC).

UAE was performed using an ultrasonic probe with automatic control of time, cycles, and power (Bandelin Sonoplus HD 3100, 20 kHz frequency with an MS 73 probe). The same amount of saffron was extracted with the same extraction solvents for 3 min using 4 cycles at a power between 50-60% of the maximum power (Jalili *et al.,* 2018).

For MAE, the prepared samples were located in a conventional microwave oven (Arçelik MD 565 S, Turkey). Ground saffron stigmas (0.2 g) were extracted with extraction solvents (as mentioned above) using a microwave power level of 30% for 2 min (Sarfarazi *et al.,* 2020).

2.2.2. Combination of ultrasound-assisted extraction (UAE) with microwave-assisted extraction (MAE)

Ground saffron stigmas (0.2 g) were weighed and the extraction solvents were added. The extraction was performed using combined methods of UAE and MAE as described in the previous section. Firstly, UAE was performed, followed by MAE.

2.3. Characterization of saffron extract

2.3.1. Total phenolic content (TPC)

The TPC of all extracted solutions was determined calorimetrically at 725 nm using the Folin–Ciocalteau reagent according to the method described by Mohamed *et al.* (2018) with slight modifications. Each solution of saffron extract (100 µL) was taken in a separate test tube and completed to 3 mL with deionized water. After that, they were mixed completely by vortex with 0.5 mL Folin-Ciocalteau for 3 min. Finally, 2 mL of 20% Na_2CO_3

solution were added and left for 1 h at room temperature in the dark. Then, the absorbance of the samples was measured at 725 nm against a blank using UV-Vis spectrophotometer SP-3000 plus, OPTIMA INC. Japan. Gallic acid was used as a standard for establishing the calibration curve. A series of standard solutions with concentrations of 12.5 -75 µg/mL were prepared and their absorbance measured. TPC was calculated using the linear equation based on the calibration curve of gallic acid (y = 0.0161 x + 0.092; $R^2 = 0.98$).

2.3.2. ABTS radical scavenging activity

The antioxidant property of the extract was analyzed according to the ABTS method (Bhatt *et al.,* 2012). A mixture (1:1, v/v) of ABTS (7.0 mM) and potassium persulfate (2.45 mM) was made and allowed to stand for 16 h. A working solution was diluted with ethanol to the absorbance value of 0.7 at 734 nm. An aliquot of 100 µL of each sample was mixed with the working solution (2.9 mL) and the decrease in absorbance was measured at 734 nm after standing for 6 min at room temperature in the dark. The percentage ABTS inhibition was calculated using the following formula (1):

$$ABTS \text{ inhibition } (\%) = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100 \quad (1)$$

2.3.3. Determination of the main characteristics of saffron using the UV–Vis spectrophotometric method

Saffron samples were analyzed according to the ISO 3632 trade standard (ISO/TS 3632, 2003). This method allows the determination of the main characteristics of saffron related to picrocrocin, safranal, and crocin contents. The higher amount of these components means a higher quality of saffron. As explained before, a total of 0.2 g of saffron sample were extracted using different methods and solvents, filtrated and finally, total volume was completed to 25 mL. Then, 0.3 mL of each extract were transferred to 50-mL volumetric flask and completed to 50 mL with each solvent. The final concentration of powder saffron in the measured samples was 0.005% (w/v). The absorbance was measured for SE on a UV-Vis spectrophotometer (SP-3000 plus) at 257, 330, and 440 nm, where picrocrocin, safranal, and crocin had the maximum absorbance values, respectively. The results were obtained by direct reading of the absorbance, D, at three wavelengths, as follows:

$E^{1\%}_{1\,cm}$ 257 nm: absorbance at about 257 nm (maximum absorbance of picrocrocin); $E^{1\%}_{1\,cm}$ 330 nm: absorbance at about 330 nm (maximum absorbance of safranal); $E^{1\%}_{1\,cm}$ 440 nm**:** absorbance at about 440 nm (maximum absorbance of crocins), where:

$$E^{1\%}_{1\,cm} = (D * 10000) / [m * (100 - H)] \quad (2)$$

Where D is the specific absorbance; m is the mass of the saffron sample in grams; H is the moisture and volatile content of the sample, expressed as a mass fraction. For our sample, the H value was about 5%.

2.4. Preparation of freeze-dried saffron extract

After the characterization of the extracts obtained by different solvents and extraction methods, the extract which had the highest levels of bioactive components was selected for the second part of the study. This extract was freeze-dried to be inserted into the oils for the evaluation of their oxidative stability.

This extract was obtained using methanol/water (50:50) and combinations of UAE and MAE. Then, the obtained extract was concentrated by a rotary evaporator, filtered and kept at -20 °C for 24 h. Finally, the extract was freeze-dried using a LyoAlfa 6-50 freeze-dryer (Telstar, Terrassa, Spain) for 16 h.

2.5. HPLC and LC-MS analysis of the freeze-dried saffron extract

The Shimadzo HPLC system (Kyoto, Japan) with two pumps (LC-20AD) was applied for the detection of SE bioactive components. This system was equipped with a photodiode array detector (UV-Vis PDA, SPD-M20A). The freeze-dried SE in the concentration of 100 µg/mL was re-dissolved in 50:50 methanol/water, then filtered through a 0.2 µm (Millipore) filter and injected into the system at a volume of 20 µL at 30 °C. The column was Alltima (C18 zorbax, 250 mm × 5 m; 4.6 mm id). The mobile phase consisted of solvent A (Water containing 0.1% formic acid) and solvent B (Acetonitrile containing 0.1% formic acid). A gradient program was performed to analyse the SE components: 20% B for 5 min, then increased linearly until 80% B in 30 min, then adjusted to 98 % B and kept for 5 min and then decreased to 20 % B and kept for 15 min at a constant flow rate of 0.8 $mL \cdot min^{-1}$. The detection wavelengths were set at 440

nm and 250 nm, i.e., the maximum absorbance for crocins and picrocrocin, respectively.

Standard solutions of crocin-4 were prepared in 50% methanol/water (v/v) at concentrations of 1-20 µg/mL. (Y=156331X, R^2 = 0.999). Picrocrocin was identified by LC-MS, while its quantification was performed using the regression equation from the literature, Y = 1952830X - 3808.1 (Cossignani *et al*., 2014). The identification of other crocin compounds was carried out by the LC-MS method and the quantity of each crocin was expressed as mg crocin-4 equivalent per gram of extract.

The identification of each compound was performed with the LC system including solvent delivery pump and PDA detector. It was coupled to a LTQ Orbitrap hybrid mass spectrometer (Thermo Fisher Scientific, San Jose, USA). The HPLC conditions were the same as the conditions described above for HPLC-UV. The peaks were detected at wavelengths of 279.5-280.5 nm. Mass spectra were obtained in positive (ESI^+) and negative ion modes (ESI^-) in scan ranges of 120-2000 m/z (Larbat *et al.,* 2014).

2.6. Characterization of oils

2.6.1. Physiochemical characteristics

Peroxide value (PV) and acid value (AV) for the oil samples were determined according to the AOCS methods (Cd–8–53 and AOCS Cd–3a–63) (AOCS, 1988). The PV and AV values of the oil samples under investigation were at acceptable levels (Table 4).

2.6.2. Fatty acid profile of oils

The fatty acid composition of the oils was determined by the conversion of oil to fatty acid methyl esters (FAMEs) according to the modified method of Zahran and Tawfeuk (2019). FAMEs were analyzed on an Agilent 7890B Series gas chromatography (GC-FID) using a polar capillary column SP®-2560 (100m, 0.25mm id, 0.2µm film thickness). Helium was used as carrier gas at a flow rate of 20 $cm \cdot sec^{-1}$, and split ratio of 100:1. The column temperature was held at 100 °C for 5 min, increased to 240 °C at 4 $°C \cdot min^{-1}$; held at 240 °C for 30 min. A sample volume of 1.0 µL was injected into the GC-FID system. FAMEs were identified by comparing their relative and absolute retention times to the authentic standards of FAMEs (from C4:0 to C24:0).

2.6.3. Oxidative stability index (OSI)

Before measuring the OSI of the oil samples, the saffron extract was blended homogenously with the oil samples. At first the freeze-dried saffron extract was dissolved in an appropriate volume of 1,2-propanediol (20% w/w). Then, the solution was placed in the test tubes containing oil samples (10 mL) and subjected to the sonication (4 cycles, power of 50-60% maximum power) for 2 min using an ultrasonic probe.

The SE at concentrations of 1000 and 1500 ppm (w/w) were examined for the stabilization of oil samples. Since no significant differences were observed in the OSI values of the oil samples enriched with both concentrations of SE, 1000 ppm level was selected to be inserted into the oil samples. The OSI of control oils and oils treated with SE and BHT (200 ppm) was measured using the Metrohm Rancimat model 743 (Herisau, Switzerland) according to (AOCS, 1998). All measurements were performed at 100 °C with an air flow rate of 20 $L \cdot h^{-1}$.

2.7. Statistical analysis

All experiments were conducted in triplicate. The values of the means were statistically analyzed by IBM SPSS statistics software package (version 17.0). The results were analyzed by one-way ANOVA and followed by the TUKEY test. The cluster analysis was used to classify objects into relative groups according to Minitab® 16 Statistical Software, 2010. Data were presented as mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

Extraction techniques can be used alone or in combination with other methods for the separation and purification of different bioactive ingredients from various parts of saffron. The quality of the extracted bioactive ingredients is important for further applications in the formulation of nutraceutical and functional food products (Ozkan *et al.*, 2021). In this study, the effects of extraction methods such as ME, UAE, MAE, and both UAE and MAE on the recovery of the bioactive compounds from saffron were evaluated. Moreover, the influence of SE on the oxidative stability of common vegetable oils (CAO, SO and COO) was assessed.

3.1. Effect of different extraction methods on TPC and antioxidant activity

As can be seen from Table 1, the type of solvent and extraction method had significant effects on TPC ($p < 0.05$). In the case of ME, among the pure solvents, extraction with methanol showed the highest recovery of phenolic compounds (29.54 mg GAE /g in saffron dry basis) followed by ethanol and water (18.83 and 13.08 mg GAE/g, respectively). Similar findings were reported by other authors, who studied the extraction of phenolic compounds from rice bran and mango waste. They explained that methanol was more efficient than ethanol to extract polyphenols (Dorta *et al*., 2012; Zhou and Yu, 2004).

The TPC of the ethanol/water (50:50, v/v) extract was higher than that obtained with absolute ethanol. According to the previous studies, the blending of organic solvents with water increases the polarity of the extraction medium and may allow easier extraction of the components which are soluble in water or in organic solvents (Socaci *et al.,* 2018) .

In the case of UAE with pure solvents, the TPC of the extracts was in the range of 4.43 to 28.03 mg GAE /g in the following decreasing order: water > methanol > ethanol. Using solvent and water mixtures (50:50, v/v) for extraction led to a significant increase in TPC. The most prominent increase was observed for ethanol; the TPC of 4.43 mg GAE /g obtained with pure ethanol increased to 29.27 mg GAE/g when 50:50 (v/v)

Table **1.** Total phenolic content and ABTS radical scavenging activities of saffron extract obtained with different solvents and protocols

Treatments	Sample code	Total phenolic content (TPC) (mg GAE/g of DW*)			Antioxidant activity using ABTS+ (% inhibition)		
Maceration for 24 h							
Water	1	13.08	±	0.82^{j}	63.28	±	3.31^{f}
Ethanol	2	18.83	±	0.16^{h}	57.37	±	1.51^{i}
Methanol	3	29.54	±	0.38^{bc}	75.50	±	3.31^{bc}
Ethanol/Water (50:50)	4	24.15	±	0.22^{fg}	68.32	±	0.40^{ef}
Methanol/Water (50:50)	5	29.46	±	0.71^{bc}	78.76	±	1.91^{ab}
Ultrasonication for 3 min							
Water	6	28.03	±	0.88^{cd}	72.16	±	0.20^{cde}
Ethanol	7	4.43	±	0.44^{l}	47.03	±	1.71^{hi}
Methanol	8	27.25	±	0.44^{de}	71.66	±	0.10^{cde}
Ethanol/Water (50:50)	9	29.27	±	0.33^{bc}	72.16	±	0.20^{cde}
Methanol/Water (50:50)	10	30.86	±	0.49^{ab}	79.83	±	0.60^{ab}
Microwave at 30% / 2 min							
Water	11	11.22	±	0.82^{j}	75.64	±	0.30^{bc}
Ethanol	12	16.65	±	0.05^{i}	50.71	±	1.41^{g}
Methanol	13	27.29	±	0.49^{de}	68.75	±	1.81^{def}
Ethanol/Water (50:50)	14	25.62	±	0.22^{ef}	74.50	±	1.10^{bcd}
Methanol/water (50:50)	15	30.05	±	0.44^{ab}	79.62	±	0.30^{ab}
Ultra sonication for 3 min + Microwave at 30% / 2 min							
Water	16	22.98	±	0.33^{g}	80.54	±	1.00^{ab}
Ethanol	17	8.66	±	0.05^{k}	56.46	±	1.51^{gh}
Methanol	18	25.93	±	0.11^{ef}	74.57	±	1.41^{bcd}
Ethanol/Water (50:50)	19	24.18	±	0.16^{fg}	69.96	±	1.51^{cde}
Methanol/water (50:50)	20	31.56	±	0.38^{a}	83.24	±	0.60^{a}

Values are means ± standard deviation (n = 3); Different superscript letters in the same column represent significant difference ($p < 0.05$, one-way ANOVA with Tukey test) *DW, dry weight of saffron stigmas.

ethanol/water mixture was used. The solvent properties can affect the extraction of bioactive components from plant cells. Since ethanol has higher viscosity compared to other solvents, the mass transfer rate of these compounds reduces and moreover, the very short extraction time of the UAE method (3 min) can also result in less extraction of phenolic compounds with ethanol. Extraction with a mixture of water and ethanol enhances mass transfer and therefore accelerates the extraction of TPC due to its lower viscosity (Esmaeilzadeh Kenari *et al.,* 2014).

Kyriakoudi *et al.* (2012) used aqueous methanol to recover crocins and picrocrocin from dried saffron stigma. They concluded that the recovery of apocarotenoids was enhanced by using ultrasonic extraction.

For MAE, a similar tendency as for ME was observed. The TPC increased significantly ($p < 0.05$) when the combination of UAE and MAE was used, which ranged from 8.66 to 31.56 mg GAE /g for ethanol and methanol/water, respectively. Moreover, the combination of UAE and MAE was more efficient especially in the case of using methanol/water as the solvent.

The results of the Pearson correlation analysis shown in Figure 1 reveals that antioxidant activity is associated with TPC. The highest TPC (31.56 mg GAE/g) and the corresponding highest antioxidant activity (83.24% inhibition) were obtained by using methanol/water mixture and combined UAE and MAE techniques for extraction. Karimi *et al.* (2010) explained that saffron's bioactive components, such as phenolics, safranal, crocin, crocetin, and carotenoids contribute to its antioxidant activity. Muzaffar *et al.* (2016) evaluated the percent inhibition for two methanolic saffron extracts belonging to different ecogeographical zones (Jammu and Kashmir, India) using the ABTS radical scavenging activity method, and reported that percent inhibition of methanolic extracts of the stigma samples increased in the range of 41.34 to 92.20 % by increasing the concentrations of extract in aqueous methanol from 100 to 300 µg/mL. The percent inhibition for the methanolic extract in our study obtained by ME was 75.50, which was in the reported ranges.

3.2. Effect of extraction methods on crocin, safranal and picrocrocin contents

The saffron samples, extracted with different techniques and solvents, were analyzed by spectrophotometric analysis in order to evaluate the absorbance val-

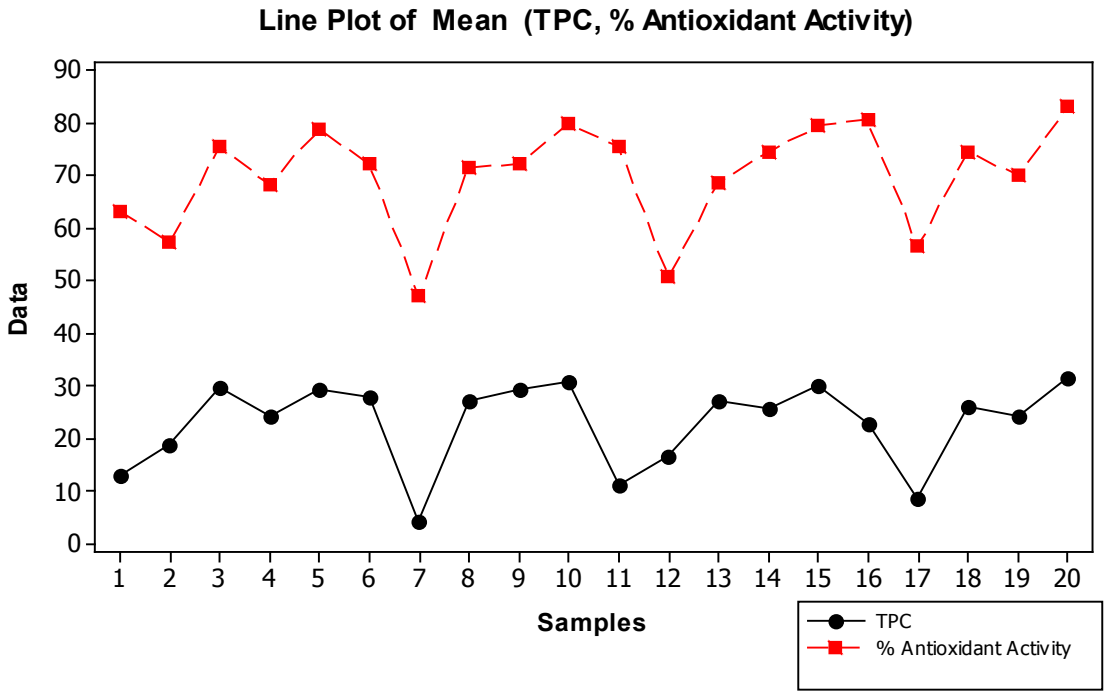

Figure 1. The correlation between antioxidant activity and total phenolic content in saffron extracts *(Pearson correlation* of TPC and antioxidant activity (%) = 0.678, p-value = 0.001

Table 2. Crocin, safranal and picocrocin contents in saffron extracts

Treatment	Sample Code	Crocin content $E^{1\%}_{1\,cm}$ 440			Safranal content $E^{1\%}_{1\,cm}$ 330			Picrocrocin content $E^{1\%}_{1\,cm}$ 257		
Maceration for 24 h										
Water	1	331.2	±	3.0^{i}	127.4	±	9.9^{def}	273.1	±	12.2^{fg}
Ethanol	2	513.4	±	41.8^{h}	89.8	±	2.3^{fg}	227.4	±	11.4^{g}
Methanol	3	1084.9	±	9.1b^{cd}	148.4	±	4.6^{de}	366.1	±	9.9^{de}
Ethanol/Water (50:50)	4	994.1	±	12.9^{def}	152.2	±	3.8^{de}	354.8	±	4.6^{de}
Methanol/water (50:50)	5	1019.4	±	21.3^{cdef}	224.2	±	6.8^{a}	467.7	±	10.6^{bc}
Ultra sonication for 3 min										
Water	6	924.7	±	53.2^{f}	199.5	±	6.8^{abc}	415.6	±	23.6^{cd}
Ethanol	7	331.2	±	9.1^{i}	39.8	±	3.0^{h}	346.8	±	29.7^{e}
Methanol	8	1191.4	±	39.5^{ab}	147.3	±	15.2^{de}	381.2	±	23.6^{de}
Ethanol/Water (50:50)	9	1178.0	±	34.2^{ab}	162.9	±	11.4^{bcde}	459.1	±	15.2^{c}
Methanol/water (50:50)	10	995.7	±	10.6^{def}	168.8	±	15.2^{bcd}	372.0	±	18.2^{de}
Microwave at 30% / 2 min										
Water	11	1031.2	±	45.6^{cdef}	204.3	±	19.8^{ab}	463.4	±	33.5^{bc}
Ethanol	12	674.7	±	9.9^{g}	72.0	±	3.0^{gh}	249.5	±	3.0^{g}
Methanol	13	1055.4	±	2.3^{cde}	125.8	±	6.1^{ef}	326.9	±	3.0^{ef}
Ethanol/Water (50:50)	14	1054.3	±	2.3^{cde}	150.5	±	7.6^{de}	525.8	±	15.2^{ab}
Methanol/water (50:50)	15	1050.5	±	1.5^{cde}	159.1	±	7.6^{cde}	369.4	±	8.4^{de}
Ultra sonication for 3 min + Microwave at 30% / 2 min										
Water	16	1124.2	±	14.4^{abc}	194.6	±	15.2^{abc}	470.4	±	16.0^{bc}
Ethanol	17	636.6	±	4.6^{g}	59.7	±	9.9^{gh}	361.8	±	34.2^{e}
Methanol	18	1137.6	±	85.2^{abc}	140.3	±	17.5^{de}	334.4	±	9.1^{ef}
Ethanol/Water (50:50)	19	959.1	±	13.7^{ef}	141.9	±	12.2^{de}	526.3	±	3.8^{ab}
Methanol/water (50:50)	20	1226.3	±	9.9^{a}	226.3	±	5.3^{a}	536.0	±	3.8^{a}

Values are means ± standard deviation (n = 3); Different superscript letters in the same column represent significant difference ($p < 0.05$, one-way ANOVA with Tukey test).

ues due to the presence of their secondary metabolites, crocins, picrocrocin, and safranal according to the ISO 3632 trade standard (ISO/TS 3632-1/2, 2003). In general, the content of these components is related to the quality of the saffron. In fact, the contents of these components differ greatly from country to country, based on several factors such as climatic conditions, drying process, harvesting and storage (Carmona *et al*., 2006) as well as extraction conditions. In particular, the techniques and solvents used in extraction directly affect the crocins, picrocrocin, and safranal contents. Table 2 shows the contents of the twenty analyzed samples under different conditions.

The absorbance values for the crocin component of the extracts were higher than picrocrocin and safranal. They ranged from 331.2 for water extract (ME) to 1226.3 for methanol/water extract using UAE in combination with MAE. The absorbance values for the picrocrocin component at 257 nm ranged from 227.4 for ethanolic extract by ME to 536.0 for methanol/water extract using combinations of UAE and MAE (Table 2). However, the absorbance values for safranal at 330 nm ranged from 39.8 for the ethanolic extract using UAE to 226.3 for the methanol/water extract using UAE together with MAE techniques.

Ethanol-water solvent mixtures were found to be the best media for the extraction of crocin and polyphenols from saffron with traditional extraction methods (Garavand *et al.*, 2019)

From the obtained data, it could be concluded that the MAE method, as well as UAE affected cro-

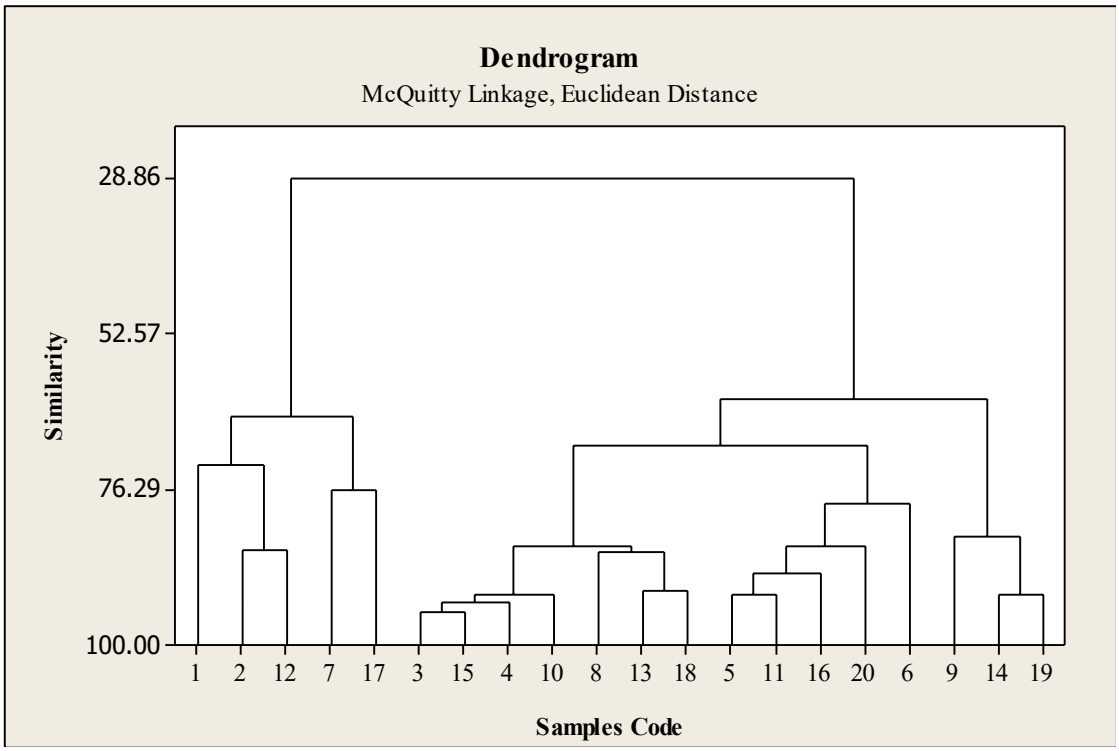

Figure 2. Dendrogram of crocin, safranal and picrocrocin content extracted by different solvents and techniques

cins, picrocrocin, and safranal contents. In addition, the extraction solvent was the most important factor in the efficiency of the extraction process. Sarfarazi *et al.* (2015) studied the effect of temperature, extraction time and ethanol concentration during maceration on the contents of crocins, picrocrocin, and safranal of SE. Based on the response surface methodology analysis, they concluded that ethanol concentration and temperature had more influence on the responses than extraction time.

A cluster analysis was carried out to classify the results of the three extraction methods and five extraction model systems into relative groups. The Dendrogram classified various crocins, picrocrocin, and safranal contents in this study into four main groups as shown in Figure 2. The cluster analysis verified the results of the model systems in which ethanol extraction showed low levels for crocins, picrocrocin, and safranal contents (First group of samples with codes of 2,7,12 and 17). the methanol (3,10 and 15) and methanol/water (8,13 and 18) showed high similarity in component contents, while the other extracts contained moderate amounts of crocins, picrocrocin, and safranal contents.

3.3. Identification of saffron extract compounds by HPLC and LC-MS analysis

Table 3 displays the quantity of main bioactive molecules of freeze-dried SE detected by LC-MS. The LC-MS analysis results were carefully discussed according to (Lech *et al.*, 2009; Cossignani *et al.*, 2014; Carmona *et al.*, 2006; Kabiri *et al*., 2017) and detailed information about retention times, fragmentation patterns and molecular weight of the mol-

Table 3. Results of LC-MS analysis of freeze-dried saffron extract

Compound	Quantity (mg/g of Extract)
Picrocrocin	3.28±0.00
Campherol diglucoside	nd[a]
Trans-crocine-4 (2 gen)	168.17±0.28
Trans-crocin-3(gen, glu)	60.55±1.99
Cis-crocin-4	10.47±0.06
Cis -crocin-3	29.49±0.47
Cis-crocin-2' (2 glu)	nd

[a] nd: not determined, value = means ± standard deviation (n = 3)

Table 4. Chemical characteristics of corn (COO), canola (CAO) and sunflower (SO) oil samples

Parameter	Oil samples		
	Corn oil	Canola oil	Sunflower oil
Acid value (mg/g)	0.12 ±0.01	0.23 ±0.02	0.17 ±0.01
Peroxide value ($meqO_2$/kg)	0.82 ±0.03	0.85 ±0.03	0.78 ±0.07
Fatty acids		Area %	
Myristic acid ($C_{14:0}$)	0.04	0.05	0.09
Palmitic acid ($C_{16:0}$)	11.46	5.1	7.9
Palmitoleic acid ($C_{16:1}$)	0.53	0.21	0.14
Margaric acid ($C_{17:0}$)	nd*	nd	0.04
Heptadecanoic acid ($C_{17:1}$)	nd	nd	0.03
Stearic acid ($C_{18:0}$)	2.14	1.7	2.7
Oleic acid ($C_{18:1}$)	33.4	60.2	35.33
Linoleic acid ($C_{18:2}$)	50.5	23.7	52.3
Linolenic acid ($C_{18:3}$)	0.93	6.6	0.16
Arachidic acid ($C_{20:0}$)	0.44	0.52	0.26
Arachidonic acid ($C_{20:1}$)	0.41	1.2	0.27
Behenic acid ($C_{22:0}$)	0.15	0.6	0.6
Erucic acid ($C_{22:1}$)	nd	0.13	nd
Lignoceric acid ($C_{24:0}$)	nd	nd	0.21
∑ SFA	14.23	7.97	11.55
∑ UFA	85.77	91.91	88.2
∑ MUFA	34.34	61.60	35.74
∑ PUFA	51.43	30.3	52.46

*nd= not detected; value = mean ± SD (n=3)

ecules can be found in our previous work (Najafi *et al.*, 2021).

The total crocin content in our freeze-dried saffron extract was 268.7 mg of crocin-4 eq/g of extract which was similar to the findings of Lahmass *et al.* (2017) in which they obtained a total crocin amount of 298 mg of crocin-4 eq/g DM of extract.

3.4. Fatty acid composition

Vegetable oils are rich in unsaturated fatty acids, particularly monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The fatty acid composition of COO, CAO and SO, expressed as saturated and unsaturated (mono and polyunsaturated) fatty acids are summarized in Table 4. The content of saturated fatty acids (SFA) amounted to 14.23 and 11.55% in COO and SO, respectively, which were both higher than CAO (7.97%). However, the unsaturated fatty acid (UFA) contents were 85.77, 88.2 and 91.91% for COO, SO and CAO, respectively. It can also be noted that the content of MUFA in canola oil (61.6%) was significantly higher than SO and COO (35.74 and 34.34%, respectively). On the contrary, the PUFA content in CAO was lower (30.3%) in comparison with COO and SO (51.43 and 52.46%, respectively). These values are in agreement with the data obtained by Kozłowska and Gruczyńska, (2018) for SO and by Carrillo *et al.* (2017) for COO.

3.5. Oxidative stability index (OSI)

The induction time for the control CAO was 17.38 h, and it increased significantly ($p < 0.05$) to 20.82 h in the case of samples treated with BHT (200 ppm), and samples treated with SE at 1000 ppm (19.41 h) (Figure 3).

There were no significant differences ($p < 0.05$) between the induction times of SO treated with BHT and SE (12.50 and 12.12 h, respectively). The difference was clear with the control (9.61 h). On the other hand, the results for COO showed a protective effect against oxidation for the samples containing BHT (21.43 h) and SE at 1000 ppm (17.55 h) compared to the control (15.28 h). The protection factor (PF) was calculated (Bandonien *et al*., 2000) and SE was found to be more effective in the protection of SO (PF=1.26) against oxidation than CAO (PF=1.12).

Some studies investigated the antioxidant activity of SE against synthetic antioxidants by the DPPH and ABTS methods (Baba *et al*., 2015; Jadouali *et al.,* 2019), but no published data were found regarding the use of SE to protect vegetable oils.

Merrill *et al*. (2008) assessed the oxidative stability of some vegetable oils with the OSI test, and they used high oleic canola oil (HOCAO), corn oil and sunflower oil purchased from Cargill. They evaluated the effect of several natural antioxidants and TBHQ (200 ppm) on the oxidative stability of some stable high-oleic vegetable oils. The Rancimat test was performed at 110 °C, and the OSI values were increased for HOCAO from 12.9 h (control) to 16 h and 36 h in oil fortified with rosemary extract and TBHQ (200 ppm), respectively (Merrill *et al.*, 2008). The difference between the OSI value for the control in our study (17.38 h) and previous study (8.4 h) can be attributed to the lower analysis temperature in the

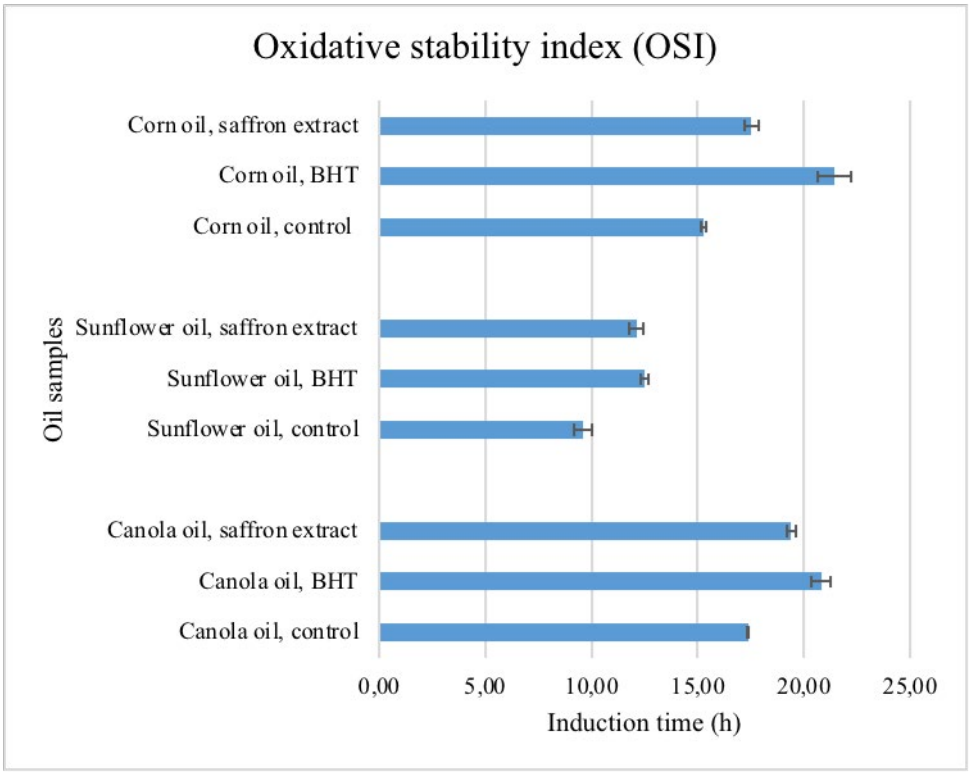

Figure 3. Oxidative stability index (OSI) of oil samples treated with 1000 ppm saffron extract, control oil and oils treated with 200 ppm BHT. The values provided in the figures are the mean values of triplicate analyses with standard deviation.

Rancimat test (100 °C) and also the difference in fatty acid profile of the oils tested. The α- linolenic content of CAO was 10% in the mentioned previous study; while the sample we used had less α-linolenic acid (6.6%).

For corn oil without antioxidant, the OSI value was reported as 9.8 h by Merrill *et al*. (2008). The difference with our result (15.28 h) might be due to the different temperature setting during OSI analysis since the fatty acid profiles were similar.

4. CONCLUSIONS

According to the results, among the different extraction methods used in this study, UAE combined with the MAE method revealed the highest TPC as well as antioxidant activity. The type of extraction solvent was found to be important to enhance the efficiency of extraction of bioactive compounds from saffron stigmas. The LC-MS analysis showed trans-crocin-4 and trans-crocin-3 as the main constituents of freeze-dried SE and this extract contained high a level of total crocin content (268 mg/g). Therefore, it exhibited significant potential in the inhibition of CAO, SO and COO oxidation in comparison with BHT as a synthetic antioxidant. Based on the oxidative stability analysis, SE (1000 ppm) had the same effect ($p < 0.05$) as BHT (200 ppm), in preventing the oxidation of sunflower oil used in the study. In the future, further studies could be conducted with different concentrations of SE on the stability of vegetable oils during accelerated storage and frying.

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REFERENCES

Ahmadian-Kouchaksaraie Z, Niazmand R. 2017. Supercritical carbon dioxide extraction of antioxidants from Crocus sativus petals of saffron industry residues: Optimization using response surface methodology. *J. Superc. Fluids* **121**, 19–31. https://doi.org/https://doi.org/10.1016/j.supflu.2016.11.008

Aktypis A, Christodoulou ED, Manolopoulou E, Georgala A, Daferera D, Polysiou M. 2018. Fresh ovine cheese supplemented with saffron (*Crocus sativus* L.): Impact on microbiological, physicochemical, antioxidant, color and sensory characteristics during storage. *Small Ruminant Res.* **167**, 32–38. https://doi.org/https://doi.org/10.1016/j.smallrumres.2018.07.016

Altemimi A, Watson DG, Choudhary R, Dasari MR, Lightfoot DA. 2016. Ultrasound assisted extraction of phenolic compounds from peaches and pumpkins. *PloS One* **11** (2), e0148758. https://doi.org/https://doi.org/10.1371/journal.pone.0148758

AOCS. 1988. *American oil chemist's society , Official and tentative methods* (Vol. 1).

AOCS. 1998. *Official Methods and Recommended Practices of the American Oil Chemists' Society* (5th Ed.). AOCS Press.

Armellini R, Peinado I, Pittia P, Scampicchio M, Heredia A, Andres A. 2018. Effect of saffron (Crocus sativus L.) enrichment on antioxidant and sensorial properties of wheat flour pasta. *Food Chem.* **254**, 55–63. https://doi.org/https://doi.org/10.1016/j.foodchem.2018.01.174

Baba SA, Malik AH, Wani ZA, Mohiuddin T, Shah Z, Abbas N, Ashraf N. 2015. Phytochemical analysis and antioxidant activity of different tissue types of Crocus sativus and oxidative stress alleviating potential of saffron extract in plants, bacteria, and yeast. *South Afr. J. Botan*. **99**, 80–87. https://doi.org/https://doi.org/10.1016/j.sajb.2015.03.194

Bandonien D, Pukalskas A, Venskutonis PR, Gruzdien D. 2000. Preliminary screening of antioxidant activity of some plant extracts in rapeseed oil. *Food Res. Int.* **33** (9), 785–791. https://doi.org/https://doi.org/10.1016/S0963-9969(00)00084-3

Bhatt ID, Dauthal P, Rawat, Gaira KS, Jugran A, Rawal RS, Dhar U. 2012. Characterization of essential oil composition, phenolic content, and antioxidant properties in wild and planted individuals of Valeriana jatamansi Jones. *Sci. Horticult.*, **136**, 61–68. https://doi.org/https://doi.org/10.1016/j.scienta.2011.12.032

Carmona M, Zalacain A, Sánchez AM, Novella JL, Alonso GL. 2006. Crocetin esters, picrocrocin and its related compounds present in Crocus sativus stigmas and Gardenia jasminoides fruits. Tentative identification of seven new compounds by LC-ESI-MS. *J. Agric. Food Chem.* **54** (3), 973–979. https://doi.org/https://doi.org/10.1021/jf052297w

Carrillo W, Carpio C, Morales D, Vilcacundo E, Alvarez M, Silva M. 2017. Content of fatty acids in corn (Zea mays L.) oil from Ecuador. *As. J. Pharm. Clinical Res.* **10**, 150–153. https://doi.org/http://dx.doi.org/10.22159/ajpcr.2017.v10i8.18786

Cossignani L, Urbani E, Simonetti MS, Maurizi A, Chiesi C, Blasi F. 2014. Characterisation of secondary metabolites in saffron from central Italy (Cascia, Umbria). *Food Chem.* **143,** 446–451. https://doi.org/10.1016/j.foodchem.2013.08.020

Deng J, Xu Z, Xiang C, Liu J, Zhou L, Li T, Yang Z, Ding C. 2017. Comparative evaluation of maceration and ultrasonic-assisted extraction of phenolic compounds from fresh olives. *Ultrason. Sonochem.* **37**, 328–334. https://doi.org/https://doi.org/10.1016/j.ultsonch.2017.01.023

Dorta E, Lobo MG, Gonzalez M. 2012. Reutilization of Mango Byproducts: Study of the Effect of Extraction Solvent and Temperature on Their Antioxidant Properties. *J.Food Sci.* **77** (1), C80–C88. https://doi.org/https://doi.org/10.1111/j.1750-3841.2011.02477.x

Esmaeilzadeh Kenari R, Mohsenzadeh F, Amiri ZR. 2014. Antioxidant activity and total phenolic compounds of Dezful sesame cake extracts obtained by classical and ultrasound-assisted extraction methods. *Food Sci. Nutrit*. **2** (4), 426–435. https://doi.org/https://doi.org/10.1002/fsn3.118

Garavand F, Rahaee S, Vahedikia N, Jafari SM. 2019. Different techniques for extraction and micro/nanoencapsulation of saffron bioactive ingredients. *Trends Food Sci. Technol.* **89,** 26–44. https://doi.org/https://doi.org/10.1016/j.tifs.2019.05.005

Hashemi Gahruie H, Parastouei K Mokhtarian M, Rostami H, Niakousari M, Mohsenpour Z. 2020. Application of innovative processing methods for the extraction of bioactive compounds from saffron (Crocus sativus) petals. *J. App. Res. Med. Arom. Plants* **19**, 100264. https://doi.org/https://doi.org/10.1016/j.jarmap.2020.100264

Heydari S, Haghayegh GH. 2014. Extraction and microextraction techniques for the determination of compounds from saffron. *Can. Chem. Trans*. **2**, 221–247.

ISO/TS 3632-1/2. 2003. *Technical Specification. Crocus sativus L. Saffron* (ISO ed.). Geneva.

Jadouali SM, Atifi H, Mamouni R, Majourhat K, Bouzoubaâ Z, Laknifli A, Faouzi A. 2019. Chemical characterization and antioxidant compounds of flower parts of Moroccan crocus sativus L. *J. Saudi Soc. Agric. Sci.* **18** (4), 476–480. https://doi.org/https://doi.org/10.1016/j.jssas.2018.03.007

Jalili F, Jafari SM, Emam-Djomeh Z, Malekjani N, Farzaneh V. 2018. Optimization of ultrasound-assisted extraction of oil from canola seeds with the use of response surface methodology. *Food Anal. Methods* **11** (2), 598–612. https://doi.org/10.1007/s12161-017-1030-z

Kabiri M, Rezadoost H, Ghassempour A. 2017. A comparative quality study of saffron constituents through HPLC and HPTLC methods followed by isolation of crocins and picrocrocin. *LWT- Food Sci. Technol*. **84,** 1–9, http://dx.doi.org/10.1016/j.lwt.2017.05.033

Karimi E, Oskoueian E, Hendra R, Jaafar HZE. 2010. Evaluation of Crocus sativus L. stigma phenolic and flavonoid compounds and its antioxidant activity. *Molecules* **15** (9), 6244–6256. https://doi.org/https://doi.org/10.3390/molecules15096244

Kozłowska M, Gruczyńska E. 2018. Comparison of the oxidative stability of soybean and sunflower oils enriched with herbal plant extracts. *Chem. Papers* **72** (10), 2607–2615. https://doi.org/10.1007/s11696-018-0516-5

Kyriakoudi A, Chrysanthou A, Mantzouridou F, Tsimidou MZ. 2012. Revisiting extraction of bioactive apocarotenoids from Crocus sativus L. dry stigmas (saffron). *Anal. Chim. Acta* **755,** 77–85. https://doi.org/10.1016/j.aca.2012.10.016

Lahmass I, Lamkami T, Delporte C, Sikdar S, Van Antwerpen P, Saalaoui E, Megalizzi V. 2017. The waste of saffron crop, a cheap source of bioactive compounds. *J. Funct. Foods* **35**, 341–351. https://doi.org/https://doi.org/10.1016/j.jff.2017.05.057

Lambrianidou A, Koutsougianni F, Papapostolou I, Dimas K 2021. Recent Advances on the Anticancer Properties of Saffron (Crocus sativus L.) and Its Major Constituents. *Molecules* **26** (1). https://doi.org/10.3390/molecules26010086

Larbat R, Paris C, Le Bot J, Adamowicz S. 2014. Phenolic characterization and variability in leaves, stems and roots of Micro-Tom and patio tomatoes, in response to nitrogen limitation. *Plant Sci.* **224**, 62–73.

Lech K, Witowska-Jarosz J, Jarosz M. 2009. Saffron yellow: characterization of carotenoids by high performance liquid chromatography with electrospray mass spectrometric detection. *J. Mass Spectrom* **44** (12), 1661–1667. https://doi.org/https://doi.org/10.1002/jms.1631

Manouchehri R, Saharkhiz MJ, Karami A, Niakousari M. 2018. Extraction of essential oils from damask rose using green and conventional techniques: Microwave and ohmic assisted hydrodistillation versus hydrodistillation. *Sustain. Chem. Pharm.* **8**, 76–81. https://doi.org/https://doi.org/10.1016/j.scp.2018.03.002

Merrill LI, Pike OA, Ogden LV, Dunn ML. 2008. Oxidative Stability of Conventional and High-Oleic Vegetable Oils with Added Antioxidants. *J. Am. Oil Chem. Soc.* **85** (8), 771–776. https://doi.org/10.1007/s11746-008-1256-4

Mohamed F, Salama HH, El-Sayed SM, El-Sayed HS, Zahran HA. 2018. Utilization of natural antimicrobial and antioxidant of Moringa oleifera leaves extract in manufacture of cream cheese. *J. Biol. Sci*. **18** (2), 92–106. https://doi.org/10.3923/jbs.2018.92.106

Muzaffar S, Rather SA, Khan KZ. 2016. In vitro bactericidal and fungicidal activities of various extracts of saffron (Crocus sativus L.) stigmas from Jammu & Kashmir, India. *Cogent Food Agric*. **2** (1), 1158999. https://doi.org/10.1080/23311932.2016.1158999

Najafi Z, Kahn CJF, Bildik F, Arab-Tehrany E, Şahin-Yeşilçubuk N. 2021. Pullulan films loading saffron extract encapsulated in nanoliposomes; preparation and characterization. *International J. Biol. Macromol.* 188, 62–71. https://doi.org/10.1016/j.ijbiomac.2021.07.175.

Oliveira AS, Ribeiro-Santos R, Ramos F, Castilho MC, Sanches-Silva A. 2018. Uhplc-dad multi-method for determination of phenolics in aromatic plants. *Food Anal. Methods* **11** (2), 440–450. https://doi.org/10.1007/s12161-017-1015-y

Ozkan K, Bayram Y, Karasu S, Karadag A, Sagdic O. 2021. Extraction of bioactive compounds from saffron species C. M. B. T.-S. Galanakis (ed.); Academic Press. *Chapter 3* pp. 99–141. https://doi.org/https://doi.org/10.1016/B978-0-12-821219-6.00003-8

Pourzaki A, Mirzaee H, Hemmati Kakhki A. 2013. Using pulsed electric field for improvement of components extraction of saffron (crocus sativus) stigma and its pomace. *J. Food Process. Preservat.* **37** (5), 1008–1013. https://doi.org/https://doi.org/10.1111/j.1745-4549.2012.00749.x

Sarfarazi M, Jafari SM, Rajabzadeh G. 2015. Extraction optimization of saffron nutraceuticals through response surface methodology. *Food Anal. Methods* **8** (9), 2273–2285. https://doi.org/10.1007/s12161-014-9995-3

Sarfarazi M, Jafari SM, Rajabzadeh G, Feizi J. 2019. Development of an environmentally-friendly solvent-free extraction of saffron bioactives using subcritical water. *LWT*, **114,** 108428. https://doi.org/https://doi.org/10.1016/j.lwt.2019.108428

Sarfarazi M, Jafari S M, Rajabzadeh G, Galanakis CM. 2020. Evaluation of microwave-assisted extraction technology for separation of bioactive components of saffron (Crocus sativus L.). *Ind. Crops Prod.* **145**, 111978. https://doi.org/https://doi.org/10.1016/j.indcrop.2019.111978

Socaci SA, Fărcaş AC, Diaconeasa ZM, Vodnar DC, Rusu B, Tofană M. 2018. Influence of the extraction solvent on phenolic content, antioxidant, antimicrobial and antimutagenic activities of brewers' spent grain. *J. Cereal Sci.* **80**, 180–187. https://doi.org/https://doi.org/10.1016/j.jcs.2018.03.006

Zahran HA, Tawfeuk HZ. 2019. Physicochemical properties of new peanut (Arachis hypogaea L.) varieties. *OCL*, **26**, 19. https://doi.org/https://doi.org/10.1051/ocl/2019018

Zhou K, Yu L. 2004. Effects of extraction solvent on wheat bran antioxidant activity estimation. *LWT-Food Sci. Technol.* **37** (7), 717–721. https://doi.org/https://doi.org/10.1016/j.lwt.2004.02.008

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Cold-pressed milk thistle seed oil: physico-chemical properties, composition and sensory analysis

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SUMMARY: Cold pressed oil was produced from milk thistle seeds, and its composition and sensorial properties were determined. The seeds were found to contain 14.98% oil, 17.31% protein and 4.14% ash. The peroxide value of the oil (11.39 meqO_2/kg oil) was within acceptable limits according to codex, but the free fatty acidity value (3.45%) exceeded the limit. The oil melted at -20.18 °C and crystallized at -3.71 °C. Linoleic acid (51.97%), β-sitosterol (67.56 mg/100 g oil) and γ-tocopherol (53.60 mg/kg oil) were determined as the main components, respectively. Six sensory descriptive terms (sweet, spicy, raw vegetable, straw, roasted and throat-catching) were described for the oil. Consumer tests proved that cold-pressed milk thistle seed oil had intermediate acceptance scores and consumer satisfaction was moderate. In conclusion, it is thought that milk thistle seeds could be used for the production of edible gourmet oil. Further studies regarding the composition of the bio-active molecules in the oil are anticipated.

KEYWORDS: *Cold press; Milk thistle seed; Oil; Quality; Sensory.*

RESUMEN: ***Aceite de semilla de cardo mariano prensado en frío: propiedades físico-químicas, composición y análisis sensorial.*** Se obtuvo aceite prensado en frío a partir de semillas de cardo mariano y se determinó su composición y propiedades sensoriales. Se encontró que las semillas contenían 14,98% de aceite, 17,31% de proteína y 4,14% de ceniza. El índice de peróxido del aceite (11,39 meqO_2/kg de aceite) se encontraba dentro del límite aceptable según el Codex, pero el índice de acidez libre (3,45 %) excedía el límite. El aceite fundió a -20,18°C y cristalizó a -3,71°C. Se determinaron como componentes principales el ácido linoleico (51,97%), β-sitosterol (67,56 mg/100 g de aceite) y γ-tocoferol (53,60 mg/kg de aceite), respectivamente. Se describieron en el aceite seis términos descriptivos sensoriales: dulce, picante, vegetal crudo, pajizo, asado y pegajoso. Las pruebas de consumo demostraron que el aceite de semilla de cardo mariano prensado en frío tenía puntuaciones de aceptación intermedias y la satisfacción del consumidor era moderada. En conclusión, se cree que las semillas de cardo mariano podrían utilizarse para la producción de aceite gourmet comestible. Se requieren más estudios sobre la composición de moléculas bioactivas del aceite.

PALABRAS CLAVE: *Aceite; Calidad; Prensado en frío; Semilla de cardo mariano; Sensorial.*

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1. INTRODUCTION

Milk thistle (*Silybum marianum* L. Gaert.) is an erect, stout, annual or biennial plant and it is a member of the *Asteraceae* family. It can grow up to 1.5-3 m in height. It has spiny leaves and stems, and purple flower heads. When the leaves and stems are broken, milky sap is released. Each stem ends with a red-purple flower head which is about 5 cm in diameter and each flower head produces approximately 190 seeds (about 6350 seeds per plant). The seeds are 5-8 mm in length, black to brown in color, and have a long white pappus (Bhattacharya, 2011; Karkanis *et al*., 2011).

Milk thistle is native to some parts of Europe, Africa and Asia and it is now widespread all over the world. It grows as a weed on roadsides or in empty fields; it is also grown specifically as a medicinal plant. For medicinal use, the seeds of milk thistle are mainly utilized. Silymarin is a biologically active compound of milk thistle. It is a mixture of flavonolignans, consisting of silybin, silydianin, and silychristine. Some pharmacological activities of this lipophilic compound are as follows: antioxidant activity, hepatoprotective activity, anti-inflammatory effects, antiviral activities, antidiabetic activities, cardio-protection, hypocholesterolaemic activity, neuroprotective activity, and anticancer activity (Bhattacharya, 2011; Karkanis *et al*., 2011; Porwal *et al*., 2019; Murray, 2021). These beneficial health effects were also observed in milk thistle seed oil. The anticancer and anti-inflammatory effects of the oil were studied by Ali *et al*. (2021). The effects of the oil on the hepatic steatosis and oxidative stress were reported by Zhu *et al*. (2018). The antioxidant and neuroprotective properties of the oil were studied by Badreddine *et al*. (2020). The effects of the oil on the cardiovascular and metabolic complications of obesity were reported by Shen *et al*. (2020).

It was reported that milk thistle seed contains 21.09% oil, 15.46% protein, 26.72% fiber, 4.72% ash, 7.64% moisture, and 24.38% total carbohydrates (Zhang *et al*., 2020). In addition, the silymarin contents in the seeds of several milk thistle populations were reported as ranging from 23.06 to 77.12 mg/g dry weight (Arampatzis *et al*., 2018). Hence, milk thistle seeds are nutritionally rich materials and when considering their health effects and nutritional value, the valorization of these seeds becomes quite important.

Cold pressing is an alternative technique for virgin oil production. It is applied under moderate conditions to keep nutritional compounds and quality safe, in the expense of yield. Clean, safe, sensorially acceptable, and high-quality oils could be produced through the application of the cold pressing technique. In this technique, after pressing, only filtration or centrifugation processes were applied to the oil, and no refining was carried out (Aydeniz *et al*., 2014; Aydeniz *et al*., 2017).

In this study, the aim was to produce oil from milk thistle seeds grown in Turkey, by the cold press technique. The physicochemical properties, thermal properties, main components and sensory properties of the oil were determined. Thus, possible uses for this oil were evaluated.

2. MATERIALS AND METHODS

2.1. Materials

Milk thistle seeds (*Silybum marianum* L. Gaert.) were purchased from Aktar Diyarı Co. (İzmir, Turkey). It was acknowledged that the seeds were harvested from cultivation plants located in the İzmir province of Turkey in the 2019 harvest season, and then cleaned and packed before marketing. The seeds were stored in deep-freeze (-18 ºC) until cold pressing in our laboratory for two months. All chemicals used in the analyses were purchased from Sigma (St. Louis, MO, USA) or Merck Co. (Darmstadt, Germany).

2.2. Analyses of the milk thistle seeds

The length, width and thickness values of the seeds were determined with a digital caliper (Leo, Nikko Ltd., China). The 1000-seed weight was determined gravimetrically by weighing (Sartorius ED224S, Sartorius, Göttingen, Germany) 25 randomly selected seeds several times, and then multiplying the weight by 40. Color values of the seeds were determined with a Minolta colorimeter CR 400 (Minolta Camera Co., Osaka, Japan). The moisture content of the seeds was measured with a OHAUS MB45 moisture analyzer (Ohaus, Pine Brook, NJ, USA). The total oil, protein, and ash contents were analyzed according to AOAC 920.39 (AOAC, 2002), AOCS Aa 5-38, and AOCS Ba 5a-49 (AOCS, 1998), respectively.

2.3. Cold pressing of the milk thistle seeds

As a result of the pre-experiments, the optimum seed moisture level, which provides maximum oil yield and ease of processing, was determined as 12%. The moisture content in the seeds was adjusted through incubation with added tap water in a closed vessel for 24 h. The amount of added water was calculated by total seed weight and initial seed moisture content. A laboratory scale cold press machine (Koçmaksan, ESM 3710, lzmir, Turkey), with a single head, 1.5 kW power and capacity for 12 kg seed/hour was used for cold-pressed oil production. The cold pressing process was carried out with a screw rotation speed of 18 rpm and a 12 mm exit die. The oil´s exit temperature did not exceed 40 ºC. After pressing, the oil was centrifuged (Sigma 2-16K, Postfach, Germany) (6797 xg, 10 min) and then filtered through Whatman no. 1 filter paper to remove suspended solid seed materials and moisture. Finally, the oil was placed in amber-colored glasses, flushed with nitrogen gas, and stored at 4 ºC during the analyses.

2.4. Physico-chemical analyses of the oil

An oil pycnometer was used to measure the specific gravity of the oil and the analysis was applied according to the AOCS Cc 10c-95 method (AOCS, 1984). Specific extinction coefficient values were measured with a spectrophotometer (Shimadzu UV-1800, Shimadzu Co., Kyoto, Japan) according to the AOCS Ch 5-91 method (AOCS, 2017). An Abbe refractometer (Bellingham and Stanley, Tunbridge Wells, UK) was used to measure refractive index values of the oil. Color values were assessed with a Minolta colorimeter CR-400 (Minolta Camera Co., Osaka, Japan). Apparent viscosity was measured with a Brookfield DV II + Pro Viscometer (Brookfield Eng. Lab., Inc., Middleborough, MA, USA). This analysis was carried out at room temperature with a LV-SC4-18 spindle and rotation speed of 50 rpm.

Free fatty acidity, peroxide value, *p*-anisidine value, iodine number, saponification number, and unsaponifiable matter in the oil samples were determined according to AOCS Ca 5a-40, AOCS Cd 8-53, AOCS Cd 18-90, AOCS Cd 1-25 (AOCS, 1998), AOCS Cd 3-25 (AOCS, 2017), and TSE 894 (TSE, 1970) methods, respectively. The total phenolic content in the oil was determined according to the Folin-Ciocalteu technique as described by Yilmaz *et al*. (2015).

2.5. Thermal analyses of the oil

Melting and crystallization parameters were determined with a Differential Scanning Calorimeter (Perkin-Elmer DSC 4000, USA) according to Dassanayake *et al*. (2009). About 10 mg oil were placed into an aluminum pan and then sealed hermetically. The analysis was carried out against an empty aluminum pan. The thermal program cycle was as follows: 20 ºC to 110 (10 ºC/min), 110 ºC to −70 ºC (10 ºC/min), held at −70 ºC for 3 min and −70 ºC to 50 ºC (5 ºC/min). Curves were calculated with Pyris 1 Manager Software.

2.6. Determination of the fatty acid, sterol, and tocopherol compositions of the oil

Fatty acid composition was determined according to AOCS Ce 2-66 (AOCS, 1998). First, 100 mg oil were weighed into a test tube and then 10 mL hexane were added and the oil was dissolved. Then, 100 µL 2 N methanolic KOH were added and mixed for 30 s. Finally, this mixture was centrifuged (Sigma 2-16K, Sartorius, Germany) (6461 xg, 10 min) and the clear phase was taken into a vial. Fatty acid composition was analyzed by GC-MS (GCMS-QP2010, Shimadzu Corporation, Nishinokyo, Japan) equipped with a Rxi-5MS column (30m x 0.25mm ID x 0.25µm film thickness, Restek Co.). The working conditions of GC were as follows: 2 µL injection volume, splitless, 0.83 mL/min flow rate, helium as carrier gas, 250 ºC injection temperature. The oven temperature program was as follows: held at 100 °C for 1 min, 100 °C to 160 °C (20 °C/min), held at 160 °C for 1 min, 160 °C to 180 °C (4 °C/min), 180 °C to 330 °C (30 °C/min), held at 330 °C for 4.70 min. The working conditions of the MS detector were as follows: 200 °C ion source temperature, 280 °C interface temperature, 5 min solvent-cutoff time. The library installed in the device was used for the evaluation of the peaks.

The sterol composition of the oil was determined according to the ISO 12228 method (ISO, 1999). First, unsaponifiable matters were extracted. Then, sterol fractions were obtained using Thin Layer Chromatography (TLC). Sterol composition was determined by using a Gas Chromatograph-FID (Agilent Technologies 7890B)

equipped with a DB5 capillary column (30 m × 0.25 mm ID × 0.1 µm film thickness, J&W Scientific Co). 1 µL injection volume, 1:100 split ratio, 0.7 mL/min flow rate, 290 ºC inlet temperature, and 300 ºC detector temperature were used during the analysis. The carrier gas was hydrogen and detector gases were hydrogen (30 mL/min) and dry air (400 mL/min). The thermal program was as follows: held at 60 ºC for 2 min, heating to 220 ºC (40 ºC/min) and held at 220 ºC for 1 min, heating to 310 ºC (5 ºC/min) and held at that temperature for 30 min. Commercial standards were used for the identification of sterols. The amount of sterols was determined from the peak area of α-cholestanol as internal standard.

The tocopherol composition of the oil was analyzed according to Grilo *et al*. (2014) with minor modifications. 200 µL oil were dissolved in 4.8 mL dichloromethane. The mixture was mixed and placed into a vial. Tocopherol composition was determined by using a HPLC (Shimadzu Corporation, Japan) equipped with Inertsil ODS-3 column (250 mm× 4.6mm× 5 µm, GL Sciences Inc., Japan) and a RF-20A fluorescent detector. The analysis was carried out with 20 µL injection volume, isocratic elution with a flow rate of 1.6 mL/min and 30 ºC oven temperature. Methanol: water (97:3, v/v) mixture was used as the mobile phase. Detector wavelengths were set to 290 and 330 nm for excitation and emission, respectively. Commercial standards were used for identification and quantification.

2.7. Sensory descriptive analysis of the oil

Sensorial properties were determined through Quantitative Descriptive Analysis (QDA) (Meilgaard *et al*., 1991; Altug and Elmacı, 2005). Five female and five male volunteer panelists, between 21 and 47 years of age, were trained for at least 15 hours. Six descriptive terms were developed under the leadership of the panel leader. The descriptive terms, their definitions and references used in the analysis are presented in Table 1. The sample was served in a glass covered with a metal lid to the panelists together with water, unsalted crackers and expectoration cups. The analyses were carried out in daylight at room temperature on different days and sessions. A 10-cm line scale (1 = minimum intensity, 10 = maximum intensity) was used. The analyses were replicated in a randomized order.

2.8. Consumer test

Appearance, smell/aroma, taste/flavor and general acceptance of the oil were evaluated by 50 volunteer consumers. A 5-point hedonic scale was used (1 = Dislike extremely, 5 = Like extremely). A sample was served in a glass covered with metal lid to consumers together with water, unsalted cracker and expectoration cups.

2.9. Statistical analysis

The same batches of seeds harvested in the 2019 season were divided into two parts for two replications of cold-press oil production. After each production, the oils obtained were designed as the replicates. For each replicate oil sample, analyses were performed in triplicate. The data presented were the mean ± SEM values.

3. RESULTS AND DISCUSSION

3.1. Physico-chemical properties of the seeds

The physico-chemical properties of the milk thistle seeds are shown in Table 2. In one study (El-Haak *et al*., 2015), length, thickness and width of wild and

Table 1. Descriptive terms, their definitions and references used in the sensory descriptive analysis

Descriptive term	Definition	Reference
Sweet	Flavor associated with sugar solution	Sugar solution (2 g sugar/100 g water)
Spicy	Flavor associated with spice mixture	Red chili pepper, black pepper and thyme mixture
Raw vegetable	Flavor associated with uncooked/raw vegetable	Green beans/green peppers
Straw	Flavor associated with dry straw	Dry straw
Roasted	Flavor associated with toasted bread	Toasted bread
Throat-catching	Burning in the throat 30 seconds after swallowing	Olive oil

Table 2. Physicochemical properties of the seeds

	Mean ± SEM
Seed dimensions (mm)	
Length	7.08 ± 0.28
Width	3.36 ± 0.16
Thickness	2.15 ± 0.15
1000-seed weight (g)	91.82 ± 0.18
Color	
L*	43.87 ± 2.18
a*	4.70 ± 0.09
b*	16.73 ± 0.51
Moisture (g/100 g seeds)	6.78 ± 0.23
Oil (g/100 g seeds)	14.98 ± 3.68
Protein (g/100 g seeds)	17.31 ± 0.06
Ash (g/100 g seeds)	4.14 ± 0.05

* Results are expressed as mean ± SEM values of six separate measurements.

Table 3. Physiochemical properties of the oil

	Mean ± SEM
Specific gravity (20 °C)	0.82 ± 0.00
Specific extinction	
E232	1.79 ± 0.07
E270	0.20 ± 0.05
Refractive index (40 °C)	1.47 ± 0.00
Viscosity (25 °C, cP)	51.20 ± 0.50
Color	
L*	28.4 ± 0.04
a*	0.5 ± 0.16
b*	3.55 ± 0.14
Free fatty acids (g linoleic acid/100 g oil)	3.45 ± 0.23
Peroxide value (meqO_2/kg oil)	11.39 ± 2.26
p-Anisidine value	4.17 ± 0.02
Iodine number (g I_2/100 g oil)	85.86 ± 0.04
Saponification value (mg KOH/g oil)	198.30 ± 5.72
Unsaponifiable matter (g/100 g oil)	1.90 ± 0.04
Total phenol content (mg GAE/100 g oil)	11.01 ± 0.1

*Milk thistle seed oil was produced twice, and each analysis for each production was done at least in triplicate. Results are expressed as mean ± SEM values of six separate measurements.

cultivated milk thistle seeds were found to be about 7 mm, 2 mm and 3 mm, respectively. Our results concur with the literature. 1000-seed weight value is an important parameter to determine seed content. In the study of El-Haak *et al*. (2015), the 1000-seed weights of wild and cultivated milk thistle seeds were found as 25.23 and 27.40 g, respectively. In another study (Arampatzis *et al*., 2018), 1000-seed weight values for the seeds in different milk thistle populations were found between 14.91 and 25.90 g. Our results are quite different from the literature, probably because of the differences in milk thistle species and cultivation conditions. L*, a* and b* values of the seeds were measured as 43.87, 4.70 and 16.73, respectively. In the study of Arampatzis *et al*. (2018), L*, C*, h values were measured as 28.12-42.79, 5.50-14.09 and 72.04-80.71, respectively. Also, in the same study, the seed color was identified as brown. Although the color spaces used in the studies were different, we can say that the colors of the seeds are similar. Moisture, oil, protein and ash contents of the seeds were found as 6.78, 14.98, 17.31 and 4.14%, respectively. In one study (El-Haak *et al*., 2015), milk thistle seeds were found to contain 28.53-29.68% oil, 22.50-27.54% protein and 3.25-4.50% ash. In another study (Zhang *et al*., 2020), the proximate composition of milk thistle seeds was determined as 7.64% moisture, 21.09% oil, 15.46% protein and 4.72% ash. There are some minor differences, but the results are generally in line with the literature.

3.2. Physico-chemical properties of the oil

The Physio-chemical properties of the oil are shown in Table 3. Specific gravity and refractive index values mainly depend on the fatty acid composition and therefore these parameters vary according to the type of oil. In one study (Meddeb *et al*., 2017), the specific gravity and refractive index values of cold-pressed milk thistle seed oils originating from different geographical areas in Tunisia were found as 0.91 and 1.46-1.47, respectively. In the study of Bahl *et al*. (2015), specific gravity of solvent-extracted milk thistle seed oil was determined as 0.885. In the study of Fathi-Achachlouei *et al*. (2019), the refractive index values of milk thistle seed oils were measured as 1.345-1.351. Our results are generally in line with the literature.

Specific extinction values are the indicators of oxidation products. In the codex, it was stated that E232 and E270 values should be 2.50 and 0.22 at the most for extra virgin olive oil, respectively (Codex, 2017). Oil types are different, but production techniques are similar (cold pressing). Therefore, these limit values could be used for comparison. As seen in Table 3, spe-

cific extinction values in the oil were lower than the limit values given in the codex. Consequently, it can be said that the oil was in accordance with the codex in terms of oxidation parameters.

Color is an important appearance property for cold-pressed oils in terms of consumer preference. In the study of Meddeb *et al*. (2017), the L*, a* and b* values of milk thistle seed oils originating from different regions were determined as 41.94-66.07, -0.85-1.53 and 5.78-14.95, respectively. The results do not match exactly with this study, but they are similar.

According to codex (Codex, 2017), free fatty acidity and peroxide value should be max. 0.8 % and 20 meqO_2/kg oil for extra virgin olive oil produced by a similar production technique (cold pressing). As seen in Table 3, the peroxide value of the oil was low and within acceptable limits according to codex, but the free fatty acidity value exceeded the limit. The results indicate that during storage of the seeds and the oil, oil hydrolysis might have occurred. Therefore, it is recommended that seeds should be processed without long storage times, and seeds and oil should be stored at low temperature and low humidity. The *p*-Anisidine value is an indicator of secondary oxidation products. In one study (Rokosik *et al*., 2020), the *p*-anisidine value of cold-pressed milk thistle seed oil was determined as 0.091. In another study (Grajzer *et al*., 2020), *p*-anisidine values for cold-pressed milk thistle seed oils were found between 0.13 and 2.19. The *p*-Anisidine value in our study is much higher than these values. Therefore, the storage conditions of the seeds and the oil should be improved.

In our study, the iodine number and the saponification value were found to be 85.86 g I_2/100 g oil and 198.30 mg KOH/g oil, respectively. In one study (Meddeb *et al*., 2017), the iodine number and saponification value for cold-pressed milk thistle seed oils originating from different geographical areas in Tunisia were found between 112.41-118.32 g I_2/100 g oil and 128.08-205.16 mg KOH/g oil, respectively. In the study of Bahl *et al*. (2015), the iodine number and saponification value for solvent-extracted milk thistle seed oil were reported as 97 g I_2/100 g oil and 199 mg KOH/g oil, respectively. There are some minor differences, but the results are generally in line with the literature.

The unsaponifiable matter content was measured as 1.90%. In the study of Meddeb *et al*. (2017), the unsaponifiable matter content of cold-pressed milk thistle seed oils were found between 1.57 and 5.84%. In another study (Harrabi *et al*., 2016), milk thistle seeds were collected at different maturity stages and oils were obtained from the seeds following the solvent extraction technique. The unsaponifiable matter contents in these oils were reported as 1.9-3.8%. Our results are in agreement with the literature.

It is known that phenolic compounds in edible oils have various health benefits (Gioxari *et al*., 2016). In this study, the total phenol content was measured as 11.01 mg GAE/100 g oil. In the study of Grajzer *et al*. (2020), the phenolic contents of cold-pressed milk thistle seed oils were found between 71.7 mg CAE/kg oil and 124.7 mg CAE/kg oil (CAE: Caffeic acid equivalent). In another study (Meddeb *et al*., 2017), the phenolic contents in cold-pressed milk thistle seed oils were determined as 3.59-8.12 mg GAE/g. It is obvious that there is a great variation between milk thistle seed oils in terms of total phenolic content, probably because of the differences in milk thistle species and cultivation conditions.

3.3. Thermal properties of the oil

The melting and crystallization parameters are presented in Table 4. Just a single peak was observed during melting and crystallization. Melting and crystallization temperatures of the oil were determined as -20.18 °C and -3.71 °C, respectively. It is stated that higher amounts of saturated fatty acids cause higher melting temperatures (Mayfield *et al*., 2015). The melting point of the oil was lower, as expected, because unsaturated fatty acids were dominant in milk thistle seed oil. In one study (Zhang *et al*., 2020), it was reported that milk thistle seed oil melts between -44 °C and 10 °C and

Table 4. Thermal properties of the oil

	Mean ± SEM
Melting	
$Onset_m$ (°C)	-22.82 ± 0.56
T_m (°C)	-20.18 ± 0.17
ΔH_m (J/g)	1.12 ± 0.24
Crystallization	
$Onset_c$ (°C)	-0.57 ± 0.04
T_c (°C)	-3.71 ± 0.75
ΔH_c (J/g)	-7.20 ± 0.61

*Milk thistle seed oil was produced twice, and each analysis for each production was done at least in triplicate. Results are expressed as mean ± SEM values of six separate measurements.

two major transitions were at -24 °C and -15 °C. In the same study, two transitions, a small peak at -2 °C and a sharp narrow peak at -55 °C, were observed during cooling. In another study (Meddeb *et al*., 2017), a major peak was observed at around -30 °C during the melting of the oils. Beyond this major peak, they also observed several small peaks from -22.92 °C to 11.62 °C. Although there are some differences, we can say that the results in our study are similar.

3.4. Fatty acid, sterol, and tocopherol compositions of the oil

The physical properties, stabilities, and usage areas of the edible oils mostly depend on their fatty acid compositions. Hence, the determination of fatty acid compositions of oils is very important. As seen in Table 5, milk thistle seed oil contained higher amounts of unsaturated fatty acids. Linoleic acid and oleic acid were the major fatty acids with 51.97 and 27.06%, respectively. The total unsaturated fatty acid content was determined as 79.48%. In one study (Zhang *et al*., 2020), linoleic acid (46.19%) and oleic acid (30.59%) were determined as major fatty acids, and total unsaturated fatty acid content was determined as 77.94% for cold-pressed milk thistle seed oil. In another study (Meddeb *et al*., 2017), the major fatty acids of milk thistle seed oils were found as linoleic acid and oleic acid with 57.00-60.30% and 15.50-22.40%, respectively. In the same study, the total unsaturated fatty acid content was determined as 82.05-83.64%. Generally, the results in this study concur with the literature.

Table 5. Fatty acid, sterol, and tocopherol compositions of the oil

	Mean ± SEM
Fatty acids (%)	
Palmitic	10.39 ± 1.35
Linoleic	51.97 ± 1.1
Oleic	27.06 ± 0.1
(E)-octadec-10-enoic	0.45 ± 0.02
Stearic	5.33 ± 0.2
Cyclopropaneoctanoic	0.59 ± 0.05
Arachidic	2.65 ± 0.2
Docosanoic	1.7 ± 0.1
Sterols (mg/100 g oil)	
β-Sitosterol	67.56 ± 4.89
Stigmasterol	9.31 ± 0.89
Campesterol	6.60 ± 0.72
Δ7-Stigmastenol	20.14 ± 3.92
Δ7-Avenasterol	4.93 ± 0.50
Tocopherols (mg/kg oil)	
δ-Tocopherol	14.91 ± 1.13
β-Tocopherol	48.87 ± 0.83
γ-Tocopherol	53.60 ± 1.74

*Milk thistle seed oil was produced twice, and each analysis for each production was done at least in triplicate. Results are expressed as mean ± SEM values of six separate measurements.

It is known that sterols have important health benefits (Berger *et al*., 2004). The sterol composition is shown in Table 5. Total sterol content was measured as 108.57 mg/100 g oil. β-Sitosterol was determined as the major sterol with 67.56 mg/100 g oil and it was followed by Δ7-stigmastenol and stigmasterol. In the study of Zhang *et al*. (2020), the total sterol content of cold-pressed milk thistle seed oil was found as 291.43 mg/100 g oil and Δ7-stigmastenol was determined as major sterol, followed by sitosterol. In another study, β-sitosterol was determined as the major sterol for cold-pressed milk thistle seed oil with a total sterol content of 1815.18 mg/kg oil (Rokosik *et al*., 2020). There are some differences in terms of total sterol content and sterol composition between different studies probably due to differences in milk thistle species and cultivation conditions.

Tocopherols are oil-soluble vitamins which have antioxidant activity. As seen in Table 5, the total tocopherol content was measured as 117.38 mg/kg oil and γ-tocopherol was determined as the major tocopherol with 53.60 mg/kg oil. In a study of Zhang *et al*. (2020), the total tocopherol content was 645.47 mg/kg oil and γ-tocopherol was determined as the major tocopherol for cold-pressed milk thistle seed oil. In another study (Meddeb *et al*., 2017), α-tocopherol was determined as the major tocopherol for cold-pressed milk thistle seed oils with a total tocopherol contents of 49.57-318.29 mg/kg oil. The results in our study do not exactly match with these previous studies probably due to differences in milk thistle species and cultivation conditions.

3.5. Sensory properties and consumer preferences of the oil

The results from the sensory descriptive analysis are shown in Table 6. Six descriptors were selected to describe the cold-pressed milk thistle seed oil, which were sweet, spicy, raw vegetable, straw, roasted and

Table 6. Sensory descriptive properties of the oil

	Mean ± SEM
Sweet	2.3 ± 1.41
Spicy	7.2 ± 2.48
Raw vegetable	1.25 ± 0.97
Straw	2.85 ± 2.80
Roasted	3.6 ± 2.31
Throat-catching	4.5 ± 2.46

*Milk thistle seed oil was produced twice, and analysis for each production was done in triplicate. Results are expressed as mean ± SEM values of six separate measurements.

throat-catching. It was observed that spicy flavor was quite dominant compared to other terms and it was followed by throat-catching. To the best of our knowledge, there is no data in the literature about the sensory descriptive terms of milk thistle seed oil. Therefore, this study presents important information to the literature for the first time. In one study (Dhouibi *et al*., 2020), volatile compounds in cold-pressed milk thistle seed oil were determined. 1,8-Cineole (minty, herbal flavor), methylpyrazine (roasted flavor), 2,5-dimethylpyrazine (roasted flavor) and hexanal (vegetative, herbal flavor) were found as the major volatile compounds in milk thistle seed oil. *p*-Cymene (spicy flavor), isocaryophyllene (woody, spicy flavor) and β-caryophyllene (woody, spicy flavor) were also detected in the oil. The descriptive terms determined for milk thistle seed oil in our study generally match the volatile compound descriptions detected in the study of Dhouibi *et al*. (2020).

The results from the consumer test are presented in Figure 1. Hedonic scores are generally higher than 3.00 points, which is the neutrality point. Therefore, it could be said that consumer satisfaction was moderate. To the best of our knowledge, there is no data in the literature regarding the consumer preferences of milk thistle seed oil. Therefore, this study presents important information to the literature for the first time.

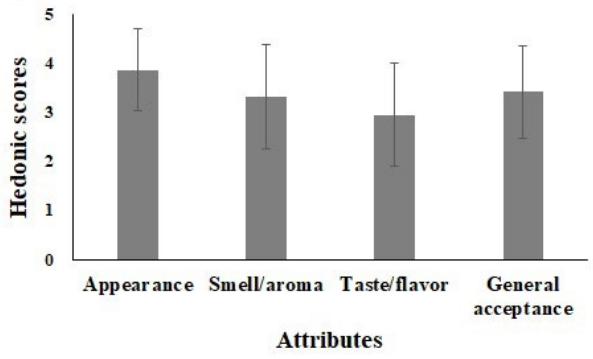

Figure 1. The consumer test results of the cold-pressed Milk Thistle seed oil (50 volunteer consumers, n = 6).

4. CONCLUSIONS

In this study, milk thistle seed oil was produced by using the cold press technique and the seeds and the oil were characterized. It was observed that cold-pressed milk thistle seed oil was rich in unsaturated fatty acids, sterols and tocopherols. Spicy flavor and throat-catching feeling were determined as dominant sensory properties of the oil. According to the consumer test, cold-pressed milk thistle seed oil had intermediate scores and consumer satisfaction was moderate. Therefore, it can be said that this oil could be preferred by consumers. In conclusion, milk thistle seeds can be used to produce high quality cold-pressed oil. Further studies regarding the composition of bio-active molecules and food applications for the oil are foreseen.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Ali SK, Abdel-Azim NS, Khalil AK, Hegazy M-EF, Mohamed TA, Hamed AR, Shams KA, Hammouda FM. 2021. The potential of cultivated milk thistle by-products as cancer chemopreventive and anti-inflammatory drugs. *Egypt. Pharma. J.* **18**, 411–418. https://doi.org/10.4103/epj.epj_34_19.

Altug T, Elmacı Y. 2005. *Gıdalarda Duyusal Değerlendirme (Sensory Evaluations of Food)*. Meta Printing Services, İzmir, Turkey.

AOAC. 2002. Association of Official Analytical Chemists (17th ed.). AOAC International.

AOCS. 1984. Official Methods and Recommended Practice of The American Oil Chemist's Society (3th ed.). American Oil Chemist's Society. AOCS Press, Champaigne, IL, USA.

AOCS. 1998. Official Methods and Recommended Practice of The American Oil Chemist's Society (5th ed.). American Oil Chemist's Society. AOCS Press, Champaigne, IL, USA.

AOCS. 2017. Official Methods and Recommended Practice of The American Oil Chemist's Society (7th ed.). American Oil Chemist's Society. AOCS Press, Champaigne, IL, USA.

Arampatzis DA, Karkanis AC, Tsiropoulos NG. 2018. Silymarin content and antioxidant activity of seeds of wild Silybum marianum populations growing in Greece. *Annl. Appl. Biol*. **174**, 61–73. https://doi.org/10.1111/aab.12470.

Aydeniz B, Güneser O, Yılmaz E. 2014. Physico-chemical, sensory and aromatic properties of cold press produced safflower oil. *J. Am. Oil Chem. Soc*. **91**, 99–110. https://doi.org/10.1007/s11746-013-2355-4.

Aydeniz B, Yılmaz E, Ok S. 2017. Cold pressed versus refined winterized corn oils: Quality, composition and aroma. *Grasas Aceites* **68**, 1–12. http://dx.doi.org/10.3989/gya.1168162.

Badreddine A, Zarrouk A, Meddeb W, Nury T, Rezig L, Debbai M, Besam FZ, Brahmi F, Vejux A, Mejri M, Nasser B, Lizard G. 2020. Antioxidant and neuroprotective properties of Mediterranean oils: Argan oil, olive oil, and milk thistle seed oil. in Martin CR, Preedy VR. (Eds.), *Oxidative Stress and Dietary Antioxidants in Neurological Diseases*. Elsevier Inc, 143–154.

Bahl JR, Bansal RP, Goel R, Kumar S. 2015. Properties of the seed oil of a dwarf cultivar of the pharmaceutical silymarin producing plant *Silybum marianum* (L.) Gaertn. developed in India. *Ind. J. Nat. Prod. Res*. **6**, 127–133.

Berger A, Jones PJH, Abumweis SS. 2004. Plant sterols: factors affecting their efficacy and safety as functional food ingredients. *Lipids Health Dis*. **3**, 1–19. http://dx.doi.org/10.1186/1476-511X-3-5.

Bhattacharya S. 2011. Chapter 90 - Milk thistle (*Silybum marianum* L. Gaert.) seeds in health. in Preedy VR, Watson RR, Patel VB (Eds.), *Nuts and Seeds in Health and Disease Prevention*, Academic Press. 759–766.

Codex. 2017. *Türk Gıda Kodeksi Zeytinyağı ve Pirina Yağı Tebliği (Turkish Olive Oil and Olive Pomace Oil Codex*), Tebliğ no: 2017/26. Resmi Gazete, Ankara, Turkey

Dassanayake LSK, Kodali DR, Ueno S, Sato K. 2009. Physical properties of rice bran wax in bulk and organogels. *J. Am. Oil Chem. Soc*. **86**, 1163–1173. https://doi.org/10.1007/s11746-009-1464-6.

Dhouibi I, Jridi M, Flamini G, Jabeur H, Masmoudi M, Bouaziz M. 2020. Volatile and phenolic contents and antioxidant and antibacterial properties of Tunisian milk thistle and mastic oils. Euro-Mediter. J. Env. Integ. **5**, 1–12. https://doi.org/10.1007/s41207-020-00200-z.

El-Haak MA, Atta BM, Abd Rabo FF. 2015. Seed yield and important seed constituents for naturally and cultivated milk thistle (*Silybum marianum*) plants. *Egypt. J. Exp. Biol. (Bot)*, **11** (2), 141–146.

Fathi-Achachlouei B, Azadmard-Damirchi S, Zahedi Y, Shaddel R. 2019. Microwave pretreatment as a promising strategy for increment of nutraceutical content and extraction yield of oil from milk thistle seed. *Indust. Crops Prod*. **128**, 527–533. https://doi.org/10.1016/j.indcrop.2018.11.034.

Gioxari A, Kogiannou DAA, Kalogeropoulos N, Kaliora AC. 2016. Phenolic compounds: Bioavailability and health effects. in Caballero B, Finglas PM, Toldra F (Eds.), *Encyclopedia of Food And Health*. Academic Press, 339–345.

Grajzer M, Szmalcel K, Kuzminski L, Witkowski M, Kulma A, Prescha A. 2020. Characteristics and antioxidant potential of cold-pressed oils-possible strategies to improve oil stability. *Foods* **9**, 1–18. https://doi.org/10.3390/foods9111630.

Grilo CE, Costa P N, Gurgel CSS, Beserra AFM, Almeida FNS, Dimenstein R. 2014. Alpha-tocopherol and gamma-tocopherol concentration in vegetable oils. *Food Sci. Technol.* **34**, 379–385. https://doi.org/10.1590/S0101-20612014005000031.

Harrabi S, Curtis S, Hayet F, Mayer PM. 2016. Changes in the sterol compositions of milk thistle oil (*Silybium marianum* L.) during seed maturation. *Grasas Aceite*s **67**, 1–6. http://dx.doi.org/10.3989/gya.0495151.

ISO. 1999. International standards official methods 12228:1999, animal and vegetable fats and oils-determination of individual and total sterols contents—Gas chromatographic method. International Organization for Standardization, Geneve, Switzerland.

Karkanis A, Bilalis D, Efthimiadou A. 2011. Cultivation of milk thistle (*Silybum marianum* L. Gaertn.), a medicinal weed. *Indust. Crops Prod*. **34**, 825–830. https://doi.org/10.1016/j.indcrop.2011.03.027.

Mayfield S, Van de Walle D, Delbaere C, Shinn SE, Proctor A, Dewettinck K, Patel A. 2015. CLA-rich chocolate bar and chocolate paste production and characterization. *J. Am. Oil Chem. Soc*. **92**, 1633–1642. http://dx.doi.org/10.1007/s11746-015-2740-2.

Meddeb W, Rezig L, Abderrabba M, Lizard G, Mejri M. 2017. Tunisian milk thistle: An investigation of the chemical composition and the characterization of its cold-pressed seed oils. *Int. J. Mol. Sci*. **18**, 1–13. https://doi.org/10.3390/ijms18122582.

Meilgaard M, Civille GV, Carr BT. 1991. *Sensory Evaluation Techniques*. CRC Press.

Murray MT. 2021. Silybum marianum (*Milk thistle*). in Pizzorno JE, Murray MT (Eds.), *Textbook of Natural Medicine* (fifth edition), Elsevier Inc, 851–855.

Porwal O, Ameen MSM, Anwer ET, Uthirapathy S, Ahamad J, Tahsin A. 2019. *Silybum marianum* (Milk thistle): Review on Its chemistry, morphology, ethno medical uses, phytochemistry and pharmacological activities. *J. Drug Del. Therap*. **9**, 199–206. http://dx.doi.org/10.22270/jddt.v9i5.3666.

Rokosik E, Dwiecki K, Siger A. 2020. Nutritional quality and phytochemical contents of cold pressed oil obtained from chia, milk thistle, nigella, and white and black poppy seeds. *Grasas Aceite*s **71**, 1-9. https://doi.org/10.3989/gya.0679191.

Shen H-H, Alex R, Bellner L, Raffaele M, Licari M, Vanella L, Stec DE, Abraham NG. 2020. Milk thistle seed cold press oil attenuates markers of the metabolic syndrome in a mouse model of dietary-induced obesity. *J. Food Bioch*. **44**, 1-11. https://doi.org/10.1111/jfbc.13522.

TSE. 1970. Yemeklik bitkisel yağlar-muayene metodlari. TSE 894. Resmi Gazete. Ankara, Turkey.

Yılmaz E, Aydeniz B, Güneşer O, Arsunar ES. 2015. Sensory and physico-chemical properties of cold press-produced tomato (*Lycopersicon esculentum* L.) seed oils. *J. Am. Oil Chem. Soc*. **92**, 833-842. https://doi.org/10.1007/s11746-015-2648-x.

Zhang Z-S, Wang S, Liu H, Li B-Z, Che L. 2020. Constituents and thermal properties of milk thistle seed oils extracted with three methods. *LWT-Food Sci. Technol*. **126**, 1–8. https://doi.org/10.1016/j.lwt.2020.109282.

Zhu SY, Jiang N, Yang J, Tu J, Zhou Y, Xiao X, Dong Y. 2018. *Silybum marianum* oil attenuates hepatic steatosis and oxidative stress in high fat diet-fed mice. *Biomed. Pharmacother*. **100**, 191–197. https://doi.org/10.1016/j.biopha.2018.01.144.

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From seeds to bioenergy: a conversion path for the valorization of castor and jatropha seeds

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SUMMARY: The world's energy matrix can be diversified with biodiesel from castor and jatropha oil. Hence, the objective of this study was to assess a conversion path for the valorization of castor and jatropha seeds. The results showed the maximum extraction of castor oil at 90 °C, 2 rpm, and 6 mm nozzle, achieving a yield of 36.97% and for jatropha oil at 100 °C, 1.5 rpm, and 10 mm nozzle, achieving a yield of 20.11%. The acid value and cloud point of castor and jatropha oil were 0.797 and 23.44 mg KOH/g, 10±1 °C and 12±0.55 °C, respectively; while the pour point was -3 °C for both. The acid value and cloud point for biodiesels ranged from 0.26–0.43 mg KOH/g, and -12.50–6.10 °C, respectively. The viscosity of oils and biodiesel ranged from 0.02–1.3 P. GC-MS indicated 66.38% of methyl ricinoleate in castor biodiesel and 31.64% of methyl oleate in jatropha biodiesel. The HHV for castor and jatropha biodiesel ranged from 32.37–40.25 MJ/kg.

KEYWORDS: *Biodiesel; Castor; Jatropha; Seeds; Valorization.*

RESUMEN: ***De semillas a bioenergía: un camino de conversión para la valorización de semillas de ricino y jatrofa.*** La matriz energética mundial puede diversificarse con biodiesel de ricino y de jatrofa. Por lo tanto, el objetivo de este trabajo fue evaluar la ruta de conversión de las semillas de ricino y jatrofa. Los resultados mostraron que la máxima extracción de aceite de ricino se dio a 90 °C, 2 rpm, y boquilla de 6 mm, alcanzando un rendimiento de 36,97% y para el aceite de jatrofa fue a 100 °C, 1,5 rpm, y boquilla de 10 mm, obteniendo un rendimiento de 20,11%. El índice de acidez y punto de nube del aceite de ricino y jatrofa fue de 0,797 y 23,44 mg de KOH/g, 10 ± 1 °C y 12 ± 0,55 °C, respectivamente, mientras que el punto de fluidez fue de -3 °C para ambos. El índice de acidez y el punto de nube del biodiésel de ricino y jatropha fueron 0,43 y 0,26 mg KOH/g, -12,50 °C y 6,10 °C, respectivamente. La viscosidad dinámica de los aceites y el biodiesel osciló entre 0,02 y 1,3 P. El análisis GC-MS indicó 66,38% de ricinoleato de metilo en biodiesel de higuerilla y 31,64% de oleato de metilo en biodiesel de jatrofa. El HHV para el biodiésel de ricino y jatrofa osciló entre 32,37 y 40,25 MJ/kg.

PALABRAS CLAVE: *Biodiesel; Higuerilla; Jatropha; Semillas; Valorización.*

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1. INTRODUCTION

The international transport industry is the biggest energy consumer. According to the International Energy Agency (IEA), in 2018, energy consumption was 416.07 EJ, with crude oil being the primary source of energy at 41%. This impact prioritizes focusing efforts on developing new technologies to improve the energy efficiency of vehicles and the diversification of energy sources (Gay y García *et al*., 2014; Álvarez *et al*., 2017).

In 2019, the production of biofuels reached 163 million·m^3 internationally, where 69% corresponded to bioethanol production, and 31% was related to biodiesel production. The raw materials used in bioethanol production were corn with at a proportion of 66%, and sugar cane with 23%, while the remaining 11% came from sugar beet, cassava, among others. For biodiesel production, vegetable oils from palm, soybean, and rape seeds were used, at a proportion of 29, 25, and 17%, respectively, and the remaining 29% corresponded to used vegetable oils, animal fats, and virgin vegetable oils (Torroba, 2020).

The bioenergy potential in Mexico has been estimated between 3,000 to 3,459 PJ per year. This potential was determined without using land dedicated to food or protected natural areas (García *et al*., 2016). Despite the bioenergetic potential, in Mexico, only firewood and bagasse are considered primary biomass energy sources, which in 2019 contributed 247.92 and 113.25 PJ to primary energy production, respectively (SENER, 2019).

In search of new energy renewable sources, one option is oilseed crops, which is why Mexico has promoted planting these bioenergy crops. Among these crops, castor (*Ricinus communis*) and jatropha (*Jatropha curcas L.*) can be found as the major groups of crops with a production increase, research, testing, and most marketed globally. This is mainly due to their high oil contents (Comité Nacional Sistema-Producto Oleaginosas, 2005).

In 2019, 304.5 ha were reported to be planted with castor and 326 ha with jatropha (SIAP-SIACON, 2019). According to SAGARPA, in Mexico, there is a planting potential of 2,174,368 ha for castor and 1,914,853 ha for jatropha (SAGARPA, 2017).

Castor belongs to the Euphorbiaceae family and is also known as castor bean, mamona, ma hong liang, wonder tree, and castor oil plant. The castor bean is typical of semi-arid regions, and due to its adaptability, is currently cultivated in tropical, and subtropical regions (Chidambaranathan *et al.,* 2020). The jatropha belongs to the Euphorbiaceae family with approximately 175-200 different varieties (Piloto *et al.,* 2011). It is a succulent perennial shrub or small tree and can reach a height of 5 m or more, depending on conditions, such as type of soil, geographical location, and weather conditions. It is a drought and extreme (cold and heat) temperature-resistant plant.

Castor oil is extracted from its seeds, with approximately 45 to 50% (Chidambaranathan *et al.,* 2020); while the oil content in jatropha seeds ranges from 28 to 42% (Patel *et al.,* 2016). Castor oil comprises 90% ricinoleic acid, 4% linoleic acid, 3% oleic acid, 1% stearic acid, and less than 1% linolenic acid (Patel *et al.,* 2016). The composition of jatropha oil is generally 43.34% linoleic acid, 35.38% oleic acid, 15.32% palmitic acid, 4.06% stearic acid, and less than 2% palmitoleic, and linolenic acids (Okullo *et al.,* 2012). Due to the high ricinoleic acid content in castor oil, it is often used as biofuels, polymeric materials, drug, cosmetics, lubricants, and others (Patel *et al.,* 2016). Jatropha oil is used for biofuel (biodiesel) production, bio-lubricant, binderless particle board, pulping, paper, medicinal, and cosmetic uses (Ahmad *et al.,* 2016).

Biodiesel is one of the most widely produced biofuels, with an approximate production of 43,138 million liters worldwide (OECD-FAO, 2020). It is made by the reaction of triglycerides to short-chain alcohols using acid, alkaline, or enzymatic catalyst to obtain the mixture of fatty acid methyl esters known as biodiesel. It can be derived from various raw materials such as edible or inedible vegetable oils, animal fats, algal oils, and waste oils (Pradhan *et al*., 2014; Chidambaranathan *et al.,* 2020).

On the other hand, more than 95% of the world's biodiesel production has been made from edible vegetable oils (Chidambaranathan *et al.,* 2020). Rapeseed, soybean, sunflower, coconut, and palm oils have been the main raw materials for the production of biodiesel (Okullo *et al.,* 2012).

Hence, the objective of the present work was to assess a conversion path for the valorization of castor and jatropha seeds. The operating conditions to achieve the highest yield of castor and jatropha oil were obtained by mechanical extraction applying an experimental

design. The physicochemical properties of the oil and biodiesel from castor and jatropha according to international standards, GC-MS Chromatography, and the estimation of HHV were determined. The importance of this work is evaluating castor and jatropha crops as raw materials in the production of biofuels, highlighting that they do not compete with food, so they do not put the country's food security and sovereignty at risk. It will contribute to the diversification of the energy matrix at the national and international level, achieving a reduction in fossil fuel dependence.

2. MATERIALS AND METHODS

2.1. Moisture content

Crucibles at a constant weight with a 5 g sample of castor and jatropha seeds were placed in a Lindberg-Blue M oven at 105 °C to determine the moisture content of castor and jatropha seeds. The samples were dried for 4 hours (Cornejo, 2012). The moisture content (% MC) was determined by equation 1.

$$\text{Equation 1} \quad \%MC = \left(\frac{M_i - M_f}{M_i}\right)(100)$$

Where: M_i is the initial mass of the wet sample (g), and M_f is the final mass of the dry sample (g).

2.2. Mechanical oil extraction and filtration

A KOMET CA59G mechanical press was used to extract oil from castor and jatropha seeds. The procedure´s conditions of the mechanic press allowed for adjustments in temperature, speed, and nozzle size. In this specific equipment, the extraction pressure or extraction force could not be modified. An experimental design was developed for each seed sample since the proper oil extraction conditions were unknown. A factorial design was selected; variables were chosen by considering the oil´s fluidity gained without generating obstruction in the nozzle cake outlet. The following parameters were chosen for castor seed: temperature of 70 °C and 90 °C, speed 1 and 2 rpm, nozzle of 6 mm and 8 mm. Each treatment was performed in duplicate. The parameters used in the factorial design for jatropha seeds were: temperature of 95 °C and 100 °C, speed of 1 and 1.5 rpm, nozzle of 8 mm and 10 mm. Castor oil has certain advantages over jatropha oil since it requires a lower temperature for extraction by mechanical pressing, which implies a lower energy demand. Due to the limited availability of jatropha seed, a test was carried out for each treatment.

The collected oil was left to settle for 24 hours, the necessary time for the seed cake to settle and separate from the oil. Subsequently, it was vacuum filtered for the removal of impurities. A Kitasato flask, Buchner funnel, vacuum pump, and Whatman #2 filter paper (8 µm) were used. The Jatropha oil filtration time was 30 min. Castor oil was centrifuged twice due to its high viscosity: 1) 4,700 rpm for 30 min and 2) 1,500 rpm for 15 min. The oil yield was calculated by equation 2 (Hernández and Mieres, 2002).

$$\text{Equation 2} \quad \%Oil\ yield = \left(\frac{M_o}{M_s}\right)(100)$$

Where M_o is the extracted oil mass (g) and M_s is the processed seed mass (g).

For castor oil, due to the presence of hydratable phospholipids, a degumming process was required. In this process the oil was heated from 50 to 70 °C using a VWR hotplate/stirrer at 250 rpm. Distilled water was added at 3% mass based on oil weight, and the mixture was left under constant stirring for 30 min, while monitoring its temperature (Hernández and Mieres, 2002).

It was necessary to ensure the elimination of impurities to carry out an adequate characterization of the oils. Therefore, a vacuum filtration was done for both oils. The percentage of impurities removed from castor oil by vacuum filtration was lower than that of jatropha oil because its high viscosity made it difficult for the oil to flow in the filtration process. For that reason, it was necessary to subject the castor oil to a centrifugation process where 10.6% of impurities were removed from the oil in the first run and 2.23% in the second one.

2.3. Castor and jatropha oil transesterification

Methanol (CH_3OH) was used for both oils, considering an oil/alcohol molar ratio of 1:3, with 31% excess CH_3OH in volume, 1% sodium hydroxide (NaOH) as the catalyst and reaction temperature of 60 °C. The reaction time for castor oil was 30 min (Ferdous *et al.,* 2013), while for jatropha oil it was 120 min (Okullo *et*

al., 2012). 150 mL of previously degummed castor oil and 100 mL of jatropha oil were used for the reaction.

The obtained biodiesel was washed with a water-jet washing method to avoid emulsions. Distilled water was used and heated to 50 °C, the biodiesel: water ratio was 1:3. The number of washes was 5, determined by visual examination of the residual water. The biodiesel was dried at 110 °C for 10 min with constant stirring and stored in a dry environment.

2.4. Oil and biodiesel acid value

The acid value was determined according to ASTM D974, using potassium hydroxide (KOH), as shown in equation 3:

$$\text{Equation 3} \quad Av = \frac{56.1\,(N)(V)}{m}$$

Where *56.1*, *N,* and *V* are the molar mass (g/mol), normality, and the volume (mL) of KOH, respectively, and *m* is the mass of the sample (g).

Due to the high acid value present in jatropha oil, it was necessary to carry out an esterification process for 60 min, at 60 °C, and with constant stirring in a VWR hotplate/stirrer. For every 100 g of jatropha oil, 60 g of methanol (CH_3OH) were added. In addition, 0.27 mL of sulfuric acid (H_2SO_4) at 0.5% w/w were added as catalyst per 100 g of oil.

After the reaction, the mixture was transferred to a separatory funnel and allowed to settle for two hours. The resulting oil was dried with vigorous stirring at 110 °C for 10 min. The CH_3OH from the reaction was recovered by a rotary evaporator DLAB RE100-PRO at 55 °C and 160 rpm.

2.5. Cloud and pour point of oils and biodiesel

The cloud point was determined according to ASTM D2500. The pour point was determined according to ASTM D97. Both determinations were made in triplicate.

2.6. Dynamic viscosity

The dynamic viscosity was determined using a BROOKFIELD CAP 2000+ viscometer. The measurements were made in triplicate, with a temperature range of 50 to 100 °C. A #6 cone was used for castor oil, a #1 cone for jatropha oil, and a #2 cone for biodiesel.

2.7. Gas chromatography

Tests were carried out using an Agilent 7890A GC chromatograph attached to a 5975C mass detector, equipped with an HP-5MS capillary column (30 m x 0.25 mm x 0.25 µm). An automatic sampler was used to inject 1 µL of solution. The ionization energy was 70 eV with a mass range of 30 to 800 m/z. The initial temperature on the column was 125 °C for up to 0.5 min, the ramp set at 25 °C/min to 150 °C for up to 2 min, then to 200 °C with a speed of 50 °C/min. The injector temperature was set at 255 °C and the detector at 270 °C. The flow rate of the carrier gas (helium) was 1.0 mL/min injected with a 1:50 gas dilution. The identification of individual components was based on comparison with the NIST98 mass spectral library. All determinations were carried out in triplicate.

2.8. Higher heating value estimation of castor and jatropha biodiesel

The HHV for castor and jatropha biodiesel was estimated using the mass fraction and the molar fraction of the fatty acid methyl esters (FAME) present in the biodiesel. Equations 4 and 5 were used to calculate the HHV.

$$\text{Equation 4} \quad HHV = \sum_{i=1}^{n} (w_i)(HHV_i)$$

$$\text{Equation 5} \quad HHV = \sum_{i=1}^{n} (x_i)(HHV_i)$$

Where w_i, x_i, and HHV_i are the mass fraction, the molar fraction, and the higher heating value of a given FAME, respectively.

The mass fraction considered was obtained by gas chromatography, while the molar fraction was determined by direct conversion from its mass fraction. To estimate the HHV_i equation 6 was applied (Ramírez *et al.,* 2012):

$$\text{Equation 6} \quad HHV_i = 46.19 - \frac{1794}{M_i} - 0.21N$$

Where: M_i and N are the molecular weight and the number of double bonds in a given FAME, respectively. For this equation, an average abso-

lute deviation of 1.92% is reported between the experimental and calculated HHV's when using the mass and molar fractions. This equation was used to estimate the HHV of beef tallow oil, soybean oil, sunflower oil, corn oil, and cottonseed oil (Ramírez *et al.,* 2012). The estimated HHV was compared to the methodology proposed by Fassinou (2012).

3. RESULTS AND DISCUSSIONS

3.1. Moisture content

The reported moisture content of castor seeds varies from 1.84–5.4% (Omari *et al.,* 2015) compared to that obtained of 3.74%. For the jatropha seeds, the reported moisture content ranges between 4.75–19.57% (Garnayak *et al.,* 2008) compared to that obtained of 4.7%. Castor and jatropha seeds have a moisture content within the reported range, making them less susceptible to deterioration by microorganisms to be stored without affecting their viability.

3.2. Mechanical extraction of oils

Table 1 shows the ANOVA corresponding to the factorial design of castor and jatropha seeds, respectively.

It was concluded that the significant factors were B and C for castor oil and A and C for jatropha oil.

The Minitab® version 18 software was used for the analysis of the results, using residuals to verify the assumptions of normality, constant variance, and independence of the model, which are characteristics that corroborate the validity of the model, indicating that the response variable is normally distributed, with the same variance in each treatment and the measurements were independent. Equations 7 and 8 are the equations obtained from the experimental designs for castor (Y_C) and jatropha (Y_J) mechanical extraction, respectively.

$$\text{Equation 7} \quad Y_C = 179.8 + 9.97S - 9.29N - 0.6T + 0.37SN - 0.127ST + 0.0718NT$$

$$\text{Equation 8} \quad Y_J = -80.8 + 150.5S - 8.49N + 1.255T - 1.6SN - 1.14ST + 0.11NT$$

Where S, N, and T are the speed (rpm), nozzle size (mm), and temperature (°C), respectively.

Based on the statistical analysis, the optimal conditions for castor oil extraction were determined: 90 °C, 2 rpm, and a nozzle of 6 mm, achieving a yield of 36.97%. For the jatropha oil, the optimal conditions were: 100 °C, 1.5 rpm, and a nozzle of 10 mm, achieving a yield of 20.11%. The castor oil yield obtained was below the range of 38–48% by warm pressing (> 70 °C) reported by Scholz and Nogueira da Silva (2008), while jatropha oil was similar to the values achieved by other researchers where similar extraction equipment was used (Yate *et al.,* 2020).

Figure 1 illustrates the influence of significant factors on the performance of castor and jatropha oil extraction processes. In the case of castor seed, the maximum oil extraction was 110.9 g from a 300 g

Table 1. Castor and Jatropha oil ANOVA

Source	Castor oil[a]					Jatropha oil[b]				
	FD	SS	MS	FV	PV	FD	SS	MS	FV	PV
Speed (rpm) A	1	0.99	0.99	2.2	0.17	1	310.01	310.01	5061.3	0.009
Nozzle (mm) B	1	58.83	58.8	130.77	0	1	0.451	0.451	7.37	0.225
Temperature (°C) C	1	33.29	33.3	74.01	0	1	33.62	33.62	548.9	0.027
AB	1	0.55	0.55	1.22	0.3	1	1.28	1.28	20.9	0.137
AC	1	1.61	1.61	3.59	0.09	1	4.061	4.061	66.31	0.078
BC	1	2.06	2.06	4.58	0.06	1	0.605	0.605	9.88	0.196
Error	8	4.05	0.51	-	-	1	0.061	0.061	-	-
Total	15	101.4	-	-	-	7	350.08	-	-	-

[a]Performed in duplicate; [b]Performed in a single analysis; FD Freedom degree; SS Square sum; MS Mean square; FV F-Value; PV P-Value.

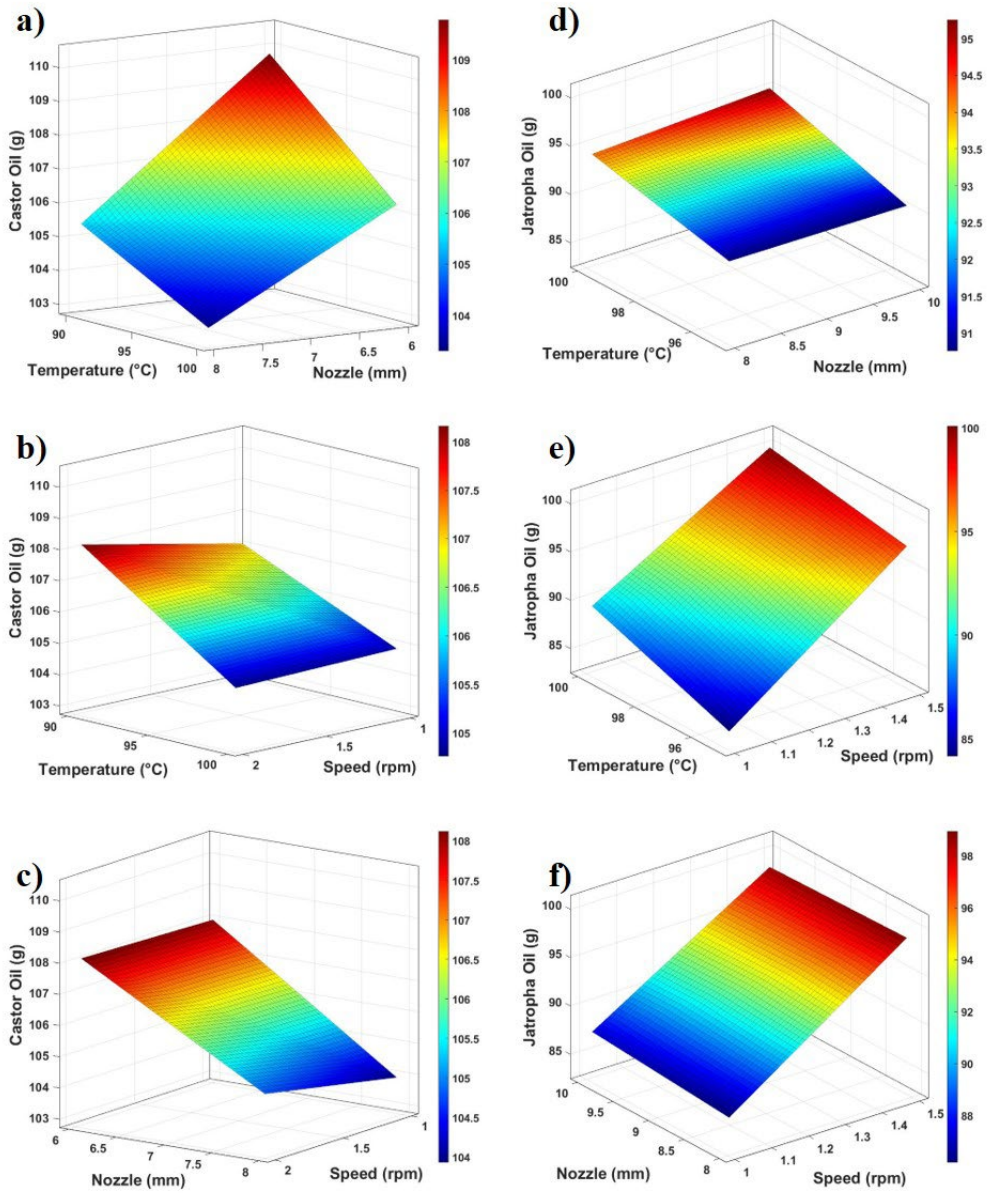

Figure 1. Response surface for castor and jatropha oil extraction. a) Temperature and nozzle vs castor oil, b) Temperature and speed vs castor oil, c) Nozzle and speed vs castor oil, d) Temperature and nozzle vs jatropha oil, e) Temperature and speed vs jatropha oil, f) Nozzle and speed vs jatropha oil. a), b) and c) performed in duplicate; d), e) and f) performed in a single analysis.

sample. For jatropha seed, the higher oil extraction was 100.55 g from a 500 g sample.

3.3. Transesterification of castor and jatropha oil

The conversion percentages of castor and jatropha oils to biodiesel were 96.66 and 98.88% respectively, which is a great advantage when used as raw material. The conversion percentage will depend mainly on the type and quality of the seeds, the pre-treatment, and the transesterification conditions applied. Other authors report conversion percentages for castor oil of 76% and jatropha oil of 91% (Okullo *et al.,* 2012). Likewise, castor oil requires a lower cost pre-treatment for its conversion into biodiesel.

3.4. Acid value, cloud point and pour point of oils and biodiesel

Table 2 displays the results of the analyzed properties for castor and jatropha oils, as well as the biodiesel obtained from them.

Table 2. Properties of castor oil, jatropha oil and biodiesel

Sample/Property	Acid value (mg KOH/g)[b]			Cloud point (°C)[c]				Pour point (°C)[c]			
	Present work	ASTM standard	Cited in Literature	Present work	(σ)	ASTM standard	Cited in Literature	Present work	(σ)	ASTM standard	Cited in Literature
Castor oil	0.797	2 (max)	0.44[d], 0.91[e]	10	-1	Report to customer	14[j]	-3	-1	Report to customer	-13[m], -15[n]
Jatropha oil	23.44 0.45[a]	2 (max)	0.1428[f], 0.7 to 1.7[g]	12	-0.6	Report to customer	14[f]	-3	-1	Report to customer	-3[o], -5[p], -6[p]
Castor biodiesel	0.43	0.5 (max)	0.25[h], 0.52[e]	-12.5	-1	-3 to -12	-18[k]	< -20	-	-15 to 16	-12[n], -30[k]
Jatropha biodiesel	0.26	0.5 (max)	0.18 to 0.29[i]	6.1	-0.6	-3 to -12	12[l]	0	-1	-15 to 16	-6 to 2[o]

[a]After esterification process; [b]Performed in a single analysis; [c]Performed in triplicate; [d]Omari *et al.*, 2015; [e]Pradhan *et al.*, 2012; [f]Ahmad *et al.*, 2016; [g]Piloto *et al.*, 2011; [h]Banerjee *et al.*, 2017; [i]Lu *et al.*, 2009; [j]Okullo *et al.*, 2012; [k]Chidambaranathan *et al.*, 2020; [l]Yate *et al.*, 2020; [m]Kumar *et al.*, 2020; [n]Tunio *et al.*, 2016; [o]Koh *et al.*, 2011; [p]De Oliveira *et al.*, 2009.

For biodiesel production, an acid value of less than 2 mg KOH/g is considered an essential condition for carrying out the transesterification reaction through alkaline catalysis, to avoid saponification (Mashad *et al.,* 2008). The acid values for castor and jatropha oils were within the ranges reported (Piloto *et al.,* 2011; Pradhan *et al.*, 2012; Omari *et al.,* 2015; Ahmad *et al.,* 2016); however, for jatropha oil, the acid value was higher than the value required to perform the transesterification reaction. Therefore, the percentage of free fatty acids was reduced by an esterification process using H_2SO_4 before transesterification (Mashad *et al.,* 2008). The high acid value for jatropha oil is attributed to the presence of oleic (C18:1) and linoleic (C18:2) acids since they are the two components with the highest proportion and present one and two unsaturations in their structure chemistry, respectively. Also, the high acid value for jatropha oil can be due to different factors such as the type of seed, the origin, and even the storage time (De Oliveira *et al*., 2009). After esterification, the free fatty acid content decreased by 98.13% at 60 °C, 60% w/w methanol, and 0.5% w/w of sulfuric acid, achieving an acid value of 0.45 mg KOH/g. The maximum acid value for biodiesel allowed by the ASTM D6751 standard is 0.50 mg KOH/g, so biodiesel from castor and jatropha oils is within the values reported in the literature (Lu *et al.,* 2009; Pradhan *et al.*, 2012; Banerjee *et al.,* 2017).

The cloud points measured for castor and jatropha oils are similar to those reported in the literature (Okullo *et al.,* 2012; Ahmad *et al.,* 2016). Castor biodiesel has a lower cloud point than jatropha biodiesel, making castor biodiesel more suitable for use in cold locations. Jatropha biodiesel may require the use of additives to improve its cloud point.

The pour points for castor and jatropha oil were reached at the same temperature, which was similar to those reported for jatropha oil (De Oliveira *et al.,* 2009; Koh *et al.,* 2011); while for castor oil, it ranges from -13 to -15 °C (Tunio *et al.,* 2016; Kumar *et al.,* 2020). However, it does not represent complications for its use in tropical areas and favors its ability to work at low temperatures. The measured pour points for jatropha oil biodiesel were within the reported ranges (Koh *et al.,* 2011). However, the pour point was not achieved for castor biodiesel due to equipment limitations, where the temperature reached was -20 °C, exceeding the measurement capacity. Temperatures of -12 to -30 °C are reported for the pour point of castor biodiesel (Tunio *et al.,* 2016; Chidambaranathan *et al.,* 2020). The difference between the values obtained and those reported in the literature may be due to factors such as the geographical area to which the seeds belong, climatic conditions, and the state of the crop.

3.5. Dynamic viscosity of oils and biodiesel

The viscosity of the oil used as raw material directly affects the viscosity of the biodiesel produced. Figure 2 exhibits the results of the tests to measure the viscosity of castor and jatropha oils and biodiesel.

In Figure 2 it can be seen that the viscosity of castor oil is much higher than that of jatropha oil. This is due to the hydroxyls present in the triglyceride molecule of castor oil, which give it this high viscosity character-

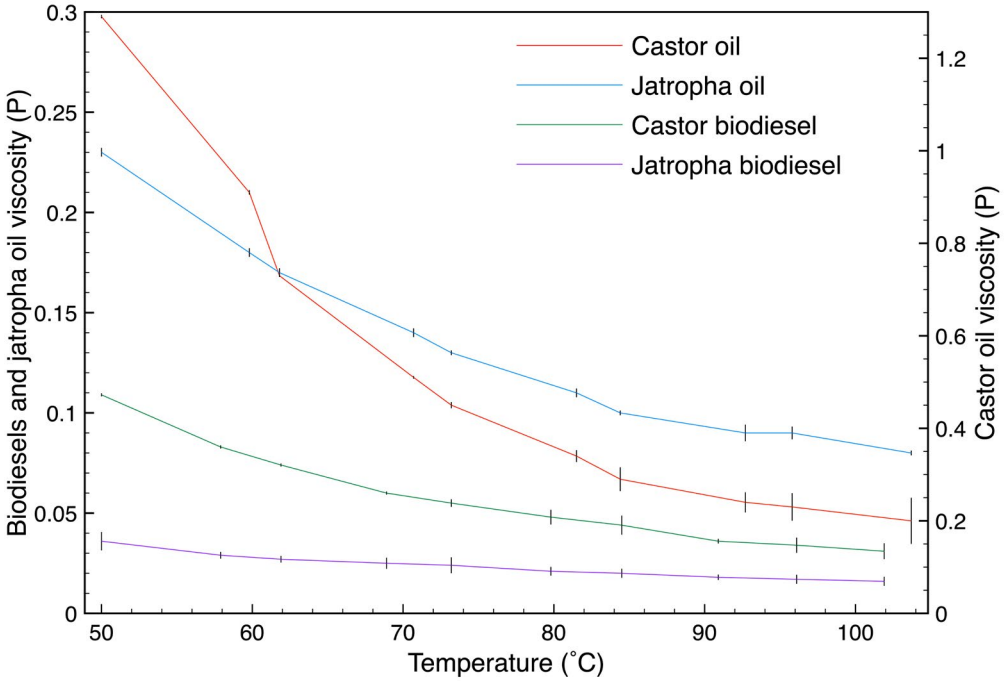

Figure 2. Dynamic viscosity at different temperatures, performed in triplicate. The standard deviation for castor oil ranges from 0.001 – 0.049 P; the standard deviation for jatropha oil ranges from 0.001 – 0.004 P; the standard deviation for castor biodiesel ranges from 0.0006 – 0.0046 P; the standard deviation for jatropha biodiesel ranges from 0.0012 – 0.0044 P.

istic. Viscosity increases with increasing chain length and decreases with the number of double bonds (level of unsaturation in the chain) (Silitonga *et al*., 2013). The presence of linoleic acid (C18:2) contributes to the decrease in the viscosity of jatropha oil since it represents the second compound with the highest proportion and presents two unsaturations in its chemical structure. The majority presence of ricinoleic acid (C18:1) in castor oil gives it a higher viscosity because this acid has one unsaturation in its chemical structure.

The viscosity of esters obtained from castor and jatropha oils through the transesterification reaction was significantly lower, achieving reductions of up to 85 and 80%, respectively, compared to the viscosity of the raw materials. Furthermore, castor biodiesel has a higher viscosity than jatropha biodiesel since castor oil has the highest viscosity of all known vegetable oils; however, its complete solubility in alcohol makes it suitable for conversion to biodiesel (Valderrama *et al.,* 1994).

3.6. Gas chromatography

The resulting chromatograms of biodiesel from castor and jatropha oil are exposed in Figures 3 and 4.

Table 3 displays the type and percentage of fatty acid methyl esters present in the extracted castor and jatropha oil.

Table 3. Methyl ester of fatty acid composition of castor and jatropha oil, expressed in percent of the total fatty acids

Component		Methyl Palmitoleate (C16:1)	Methyl Palmitate (C16:0)	Methyl Linoleate (C18:2)	Methyl Oleate (C18:1)	Methyl Elaidate (C18:1)	Methyl Stearate (C18:0)	Methyl Ricinoleate (C18:1)	Other components	ΣSFA[a]	ΣMUFA[b]	ΣPUFA[c]	TU[d]	TU/SFA index[e]
Castor oil	Mean[f]	-	6.4	7.54	13.79	1.2	3.4	66.38	1.29	9.8	81.37	7.54	88.9	9.07
	σ	-	0.006	0.008	0.01	0.002	0.003	0.024	-	-	-	-	-	-
Jatropha oil	Mean[f]	0.93	13.75	27.13	31.64	1.69	5.2	0.7	18.97	18.95	34.96	27.13	62.1	3.28
	σ	0.0003	0.0009	0.0006	0.0027	0.0033	0.0004	0.0029	-	-	-	-	-	-

[a]ΣSFA: saturated fatty acid; [b]ΣMUFA: monounsaturated fatty acid; [c]ΣPUFA: polyunsaturated fatty acid; [d]TU: total unsaturated fatty acid; [e]TU/SFA index: total unsaturated fatty acid/saturated fatty acid index; [f]Performed in triplicate.

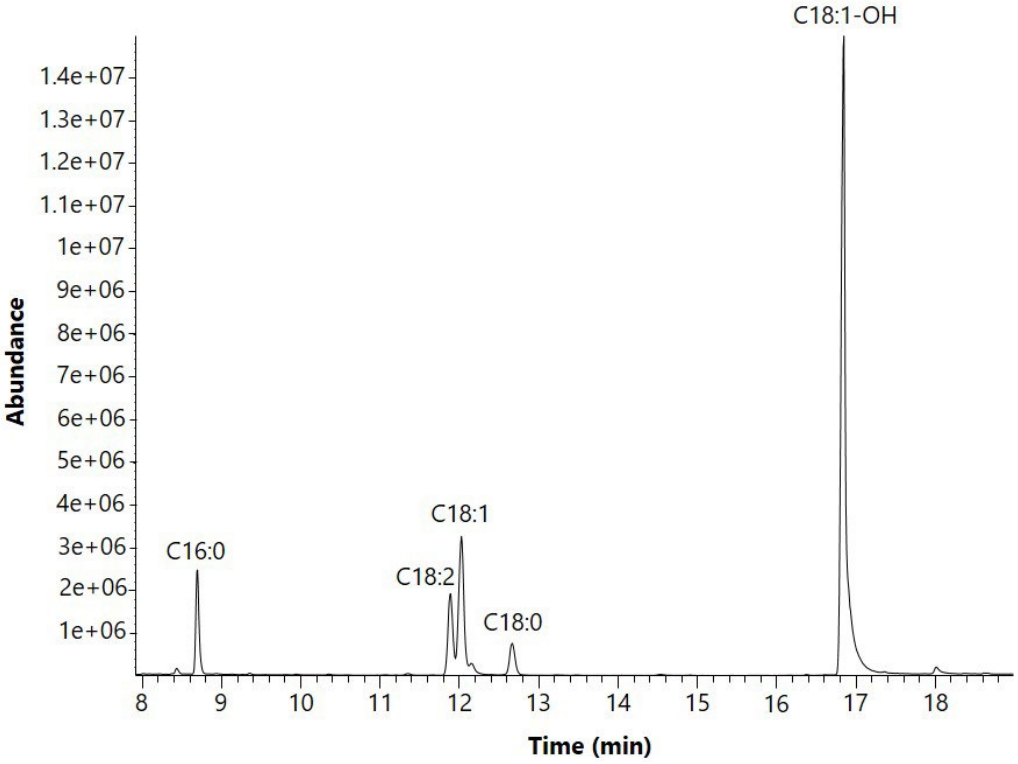

Figure 3. Chromatography of filtered castor oil.

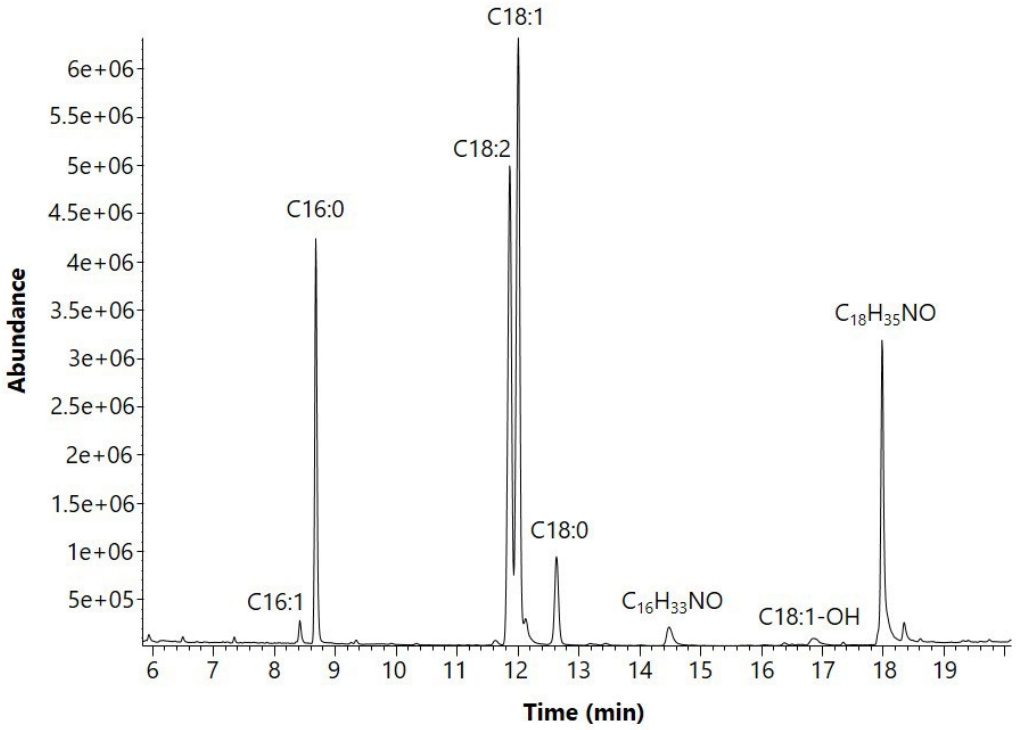

Figure 4. Chromatography of filtered jatropha oil.

The high presence of nitrogen compounds in biofuels is not desirable because when they burn, they become sources of NO_x in the exhaust gases (Kaewpengkrow *et al.,* 2013). Due to the chemical structure of FAME's, biodiesel can be more susceptible to oxidation than mineral diesel. Saturated FAME's increase cloud point, cetane number, and improve storage stability; while polyunsaturated FAME's decrease these properties (Das *et al.,* 2009). However, it has been reported that unsaturated FAME's increase fuel lubricity and improve cold flow properties (Hoekman *et al.,* 2012). Therefore, the presence of saturated and unsaturated FAME's is desirable for biodiesel production to obtain a fuel with better properties.

The central methyl esters in castor biodiesel were methyl ricinoleate (66.38%), methyl oleate (13.79%), and methyl linoleate (7.54%). These values were within the range of those in the literature (Okullo *et al.,* 2012).

The chromatographic profile of jatropha biodiesel indicates that the most representative compounds were methyl oleate (31.64%), methyl linoleate (27.13%), and methyl palmitate (13.75%), similar to literature values (Piloto *et al.,* 2011; Okullo *et al.,* 2012).

3.7. Higher heating value of castor and jatropha biodiesel

The HHV of biodiesel can be predicted by the FAME composition of biodiesel (Fassinou, 2012; Ramírez *et al.,* 2012). Table 4 reveals the values of w_i and x_i calculated with the relations taken from Table 3, and the HHV_i was calculated with equation 6.

Table 4 depicts that the compound with the highest contribution to HHV in castor biodiesel is methyl ricinoleate with 26.49–26.82 MJ/kg. The HHV reported for methyl ricinoleate is 40.37 MJ/kg (Fassinou, 2012), so comparing this value with that obtained in equation 6, there is a difference of 0.08%. For jatropha biodiesel, the two compounds with the highest contribution to HHV are methyl oleate with 12.68–15.36 MJ/kg and methyl linoleate with 10.81–13.18 MJ/kg. The reported values for the HHV of methyl oleate and methyl linoleate are 39.90 MJ/kg and 39.85 MJ/kg, respectively (Fassinou, 2012). Comparing these values with those obtained through equation 6 there is a difference of 0.45% for methyl oleate and 0.05% for methyl linoleate. Given the composition of the methyl esters, the HHV for castor biodiesel of 39.74 to 40.25 MJ/kg can be predicted; while for jatropha biodiesel, an HHV between the ranges of 32.37 to 39.94 MJ/kg can be expected. Ramírez *et al.,* (2012) report that the methodology for estimating the HHV of biodiesel should not be used for castor biodiesel, so it was compared to a methodology proposed by Fassinou, (2012). The difference between both methodologies was 0.27%.

When using blend biodiesel with diesel there is a decrease in HHV with an increasing mixing ratio. The HHV from B5 to B100 represents a range of 39.74 to 41.80 MJ/kg for castor biodiesel and 32.37 to 41.79 MJ/kg for jatropha biodiesel. The HHV of biodiesels from the current work are slightly lower than those of gasoline (40.43 MJ/kg), and diesel (41.40 MJ/kg) (SENER, 2019).

Table 4. HHV_i (MJ/kg), w_i and x_i calculated from FAME composition

FAME (Carbon chain)	M_i (g/mol)	HHV_i	Castor biodiesel		Jatropha biodiesel	
			w_i*HHV_i	x_i*HHV_i	w_i*HHV_i	x_i*HHV_i
Methyl Palmitate (C16:0)	270.46	39.72	2.54	2.91	5.46	7.25
Methyl Palmitoleate (C16:1)	268.44	39.46	-	-	0.37	0.49
Methyl Stearate (C18:0)	298.51	40.33	1.37	1.42	2.10	2.52
Methyl Oleate (C18:1)	296.50	40.08	5.53	5.77	12.68	15.36
Methyl Ricinoleate (C18:1)	313.50	40.40	26.82	26.49	0.28	0.32
Methyl Linoleate (C18:2)	294.48	39.83	3.00	3.16	10.81	13.18
Methyl Elaidate (C18:1)	296.50	40.08	0.48	0.50	0.68	0.82
Total	-	-	39.74	40.25	32.38	39.94

HHV_i Higher heating value of a given methyl ester; w_i Mass fraction of a given methyl ester; x_i Molar fraction of a given methyl ester; FAME Fatty Acid Methyl Ester; M_i Molar mass.

4. CONCLUSIONS

The physicochemical characterization of the seeds, oil, and biodiesel of castor and jatropha was carried out, and the determination of the necessary conditions for obtaining castor and jatropha oil by mechanical extraction. According to the experimental designs, the best extraction yields were at 90 °C, 2 rpm, and a nozzle of 6 mm for castor oil. For the jatropha oil, the optimal conditions were at 100 °C, 1.5 rpm, and a nozzle of 10 mm.

Castor seeds have a low energy requirement and show better performance in the oil extraction process, along with most of the properties determined for oil and biodiesel obtained from them. Castor oil presents better conditions without requiring high-cost pre-treatments, so it is more convenient to promote its use for the production of biodiesel. The conversion percentages of castor and jatropha oils to biodiesel were 96.66 and 98.88%, respectively.

Ricinoleic acid is responsible for the low acid value and high viscosity of castor oil, as it is the major compound in the oil.

Of all the measured parameters, the acid value turned out to have the most significant effect since the high acid value of the oil hinders the production and separation of methyl esters, which causes a decrease in the percentages of conversion to biodiesel, increasing losses and production costs. Linoleic acid is responsible for the high acid value and low viscosity of jatropha oil, as it is the second largest compound in the oil.

The heating value for biodiesel is closely linked to the properties of the most representative methyl esters, so it is to be expected that the heating value of biodiesel is similar or close to the heating value of esters. The HHV of castor biodiesel can be expected to be close to methyl ricinoleate HHV, as it is the major contributor with 66.38% (w/w). The HHV of jatropha biodiesel will be close to the average of the HHV of the most representative FAME.

Therefore, it is concluded that the methodology developed in the present work for castor and jatropha seeds allows for obtaining biodiesel which falls within the acceptance ranges of ASTM D6751 of the determined properties.

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REFERENCES

Ahmad KA, Abdullah ME, Hassan NA, Ambak KB, Musbah A, Usman N, Abu Bakar SKB. 2016. Extraction techniques and industrial applications of jatropha curcas. *J. Teknol.* **7**, 53–60.

Álvarez E, Menéndez J, Bravo M. 2017. Movilidad sostenible: El papel de la electricidad y el gas natural en varios países europeos. *Cuadernos Orkestra* **27**. https://dialnet.unirioja.es/servlet/libro?codigo=743961

Banerjee A, Varshney D, Kumar S, Chaudhary P, Gupta VK. 2017. Biodiesel production from castor oil: ANN modeling and kinetic parameter estimation. *Int. J. Ind. Chem*. **8**, 253–262. https://doi.org/10.1007/s40090-017-0122-3

Chidambaranathan B, Gopinath S, Aravindraj R, Devaraja A, Gokula Krishnan S, Jeevaananthan JKS. 2020. The production of biodiesel from castor oil as a potential feedstock and its usage in compression ignition Engine: A comprehensive review. *Materials Today: Proceedings* **33**, 84–92. https://doi.org/10.1016/j.matpr.2020.03.205

Comité Nacional Sistema-Producto Oleaginosas. 2005. Las oleaginosas. http://www.oleaginosas.org/cat_57.shtml

Cornejo M. 2012. Caracterización de aceite de higuerilla (Ricinus Communis) de dos variedades silvestres para la producción de biodiesel en la región del Valle de Mezquital, Hidalgo (master's thesis). Centro De Investigación en Materiales Avanzados, S. C.

Das LM, Bora DK, Pradhan S, Naik MK, Naik SN. 2009. Long-term storage stability of biodiesel produced from Karanja oil. *Fuel* **88**, 2315–2318. https://doi.org/10.1016/j.fuel.2009.05.005

De Oliveira JS, Leite PM, De Souza LB, Mello VM, Silva EC, Rubim JC, Meneghetti SMP, Suarez PAZ. 2009. Characteristics and composition of Jatropha gossypiifolia and Jatropha curcas L. oils and application for biodiesel production. *Biomass Bioenergy* **33**, 449–453. https://doi.org/10.1016/j.biombioe.2008.08.006

Fassinou WF. 2012. Higher heating value (HHV) of vegetable oils, fats and biodiesels evaluation based on their pure fatty acid's HHV. *Energy* **45**, 798–805. https://doi.org/10.1016/j.energy.2012.07.011

Ferdous K, Uddin MR, Deb A, Ferdous J, Khan MR, Islam MA. 2013. Preparation of Biodiesel from higher FFA containing Castor Oil. *Int. J. Sci. Eng. Res.* **4**, 401–406.

García CA, Riegelhaupt E, Masera O. 2016. Estado del arte de la bioenergía en México. *Red Temática de Bioenergía (RTB) del Conacyt, REMBIO*. https://rembio.org.mx/wp-content/uploads/2020/12/6d95688b94f-b96e56675c3ff6387225f-2.pdf

Garnayak DK, Pradhan RC, Naik SN, Bhatnagar N. 2008. Moisture-dependent physical properties of jatropha seed (Jatropha curcas L.). *Ind. Crop. Prod.* **27**, 123–129. https://doi.org/10.1016/j.indcrop.2007.09.001

Gay y García C, Álvarez Bejar A, Montes Delgado NL, Jazcilevich Diamant A, Manríquez García J, Delgado Ramos GC, de Diego Correa R. 2014. Biocombustibles en México: una alternativa para la reducción de la dependencia de los hidrocarburos y la mitigación de los gases efecto invernadero. Universidad Nacional Autónoma de México, México.

Hernández C, Mieres A. 2002. Rendimiento de la extracción por prensado en frío y refinación física del aceite de la almendra del fruto de la palma corozo (Acrocomia Aculeata), Universidad de Carabobo, Venezuela. http://www.ciiq.org/varios/peru_2005/Trabajos/IV/7/4.7.02.pdf

Hoekman SK, Broch A, Robbins C, Ceniceros E, Natarajan M. 2012. Review of biodiesel composition, properties, and specifications, *Renewable Sustainable Energy Rev.* **16**, 143–169. https://doi.org/10.1016/j.rser.2011.07.143

Kaewpengkrow P, Atong D, Sricharoenchaikul V. 2013. Effect of Pd, Ru, Ni and ceramic supports on selective deoxygenation and hydrogenation of fast pyrolysis Jatropha residue vapors, *Renew. Energy* **65**, 92–101. https://doi.org/10.1016/j.renene.2013.07.026

Koh MY, Mohd Ghazi TI. 2011. A review of biodiesel production from Jatropha curcas L. oil. *Renew. Sust. Energ. Rev.* **15**(5), 2240–2251. https://doi.org/10.1016/j.rser.2011.02.013

Kumar D, Das T, Shekher Giri B, Verma B. 2020. Preparation and characterization of novel hybrid bio-support material immobilized from Pseudomonas cepacia lipase and its application to enhance biodiesel production. *Renewable Energy* **147** (1), 11–24. https://doi.org/10.1016/j.renene.2019.08.110

Lu H, Liu Y, Zhou H, Yang Y, Chen M, Liang B. 2009. Production of biodiesel from Jatropha curcas L. oil. *Comput. Chem. Eng.* **33** (5), 1091–1096 https://doi.org/10.1016/j.compchemeng.2008.09.012

Mashad E, Zhang H, Bustillos A. 2008. A two step process for biodiesel production from salmon oil. *Biosyst. Eng.* **99** (2), 220–227. https://doi.org/10.1016/j.biosystemseng.2007.09.029

OECD-FAO. 2020. Table C.41.1 - Biodiesel projections: Production and use, in OECD-FAO Agricultural Outlook 2020–2029, OECD Publishing, Paris. doi:10.1787/134c730f-en

Okullo AA, Temu AK, Ogwok P, Ntalikwa JW. 2012. Physico-Chemical Properties of Biodiesel from Jatropha and Castor oil. *Int. J. Renew. Energy Res.* **2** (1), 47-52.

Omari A, Mgani Q, Mubofu E. 2015. Fatty Acid Profile and Physico-Chemical Parameters of Castor Oils in Tanzania. *Green Sust. Chem.* **5**, 154–163. https://doi.org/10.4236/gsc.2015.54019

Patel VR, Dumancas GG, Kasi Viswanath LC, Maples R, and Subong BJ. 2016. Castor Oil: Properties, Uses, and Optimization of Processing Parameters in Commercial Production. *Lipid Insights* **9**, 1–12. https://doi.org/10.4137/LPI.S40233

Piloto R, Goyos L, Alfonso M, Duarte M, Caro R, Galle J, Sierens R, Verhelst S. 2011. Characterization of Jatropha curcas oils and their derived fatty acid ethyl esters obtained from two different plantations in Cuba. *Biomass Bioen.* **35** (9), 4092–4098. https://doi.org/10.1016/j.biombioe.2011.06.003

Pradhan S, Madankar CS, Mohanty P, Naik SN. 2012. Optimization of reactive extraction of castor seed to produce biodiesel using response surface methodology. *Fuel* **97**, 848–855. https://doi.org/10.1016/j.fuel.2012.02.052

Pradhan S, Shen J , Emami S, Naik S, Reaney MJT. 2014. Fatty Acid Methyl Esters Production with Glycerol Metal Alkoxide Catalyst. *Eur. J. Lipid Sci. Technol.* **116** (11), 1590–1597. https://doi.org/10.1002/ejlt.201300477

Ramírez LF, Rodríguez JE, Jaramillo AR. 2012. Predicting cetane number, kinematic viscosity, density and higher heating value of biodies-

el from its fatty acid methyl ester composition. *Fuel* **91** (1), 102–111. https://doi.org/10.1016/j.fuel.2011.06.070

SAGARPA. 2017. Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. *Bioenergéticos: higuerilla, jatropha curcas, sorgo dulce mexicanos*. https://www.gob.mx/cms/uploads/attachment/file/257070/Potencial-Bioenerg_ticos.pdf

Scholz V, Nogueira da Silva J. 2008. Prospects and risks of the use of castor oil as a fuel. *Biomass Bioen.* **32** (2), 95–100. https://doi.org/10.1016/j.biombioe.2007.08.004

SENER 2019. *Balance Nacional de Energía 2019*. https://www.gob.mx/cms/uploads/attachment/file/618408/20210218_BNE.pdf

SIAP-SIACON. 2019. Plataforma del Sistema de Información Agroalimentaria de Consulta. Available from https://www.gob.mx/siap/prensa/sistema-de-informacion-agroalimentaria-de-consulta-siacon

Silitonga AS, Masjuki HH, Mahlia TMI, Ong HC, Atabani AE, Chong WT. 2013. A global comparative review of biodiesel production from jatropha curcas using different homogeneous acid and alkaline catalysts: Study of physical and chemical properties. *Renew. Sust. Energ. Rev.* **24**, 514–533. https://doi.org/10.1016/j.rser.2013.03.044

Torroba AM. 2020. Atlas de los biocombustibles líquidos 2019-2020. Instituto Interamericano de Cooperación para la Agricultura (IICA). https://repositorio.iica.int/bitstream/handle/11324/13974/BVE20128304e.pdf?sequence=1&isAllowed=y

Tunio MM, Samo SR, Ali ZM, Jakhrani AQ, Mukwana KC. 2016. Production and Characterization of Biodiesel from Indigenous Castor Seeds. *Int. J. Eng. Appl. Sci.* **3** (7), 28–33.

Valderrama J, Mery A, Aravena F. 1994. La higuerilla y su principal producto, el aceite de ricino. *Parte 1. Aspectos generales. Infor. Tecnol.* **5** (1), 87–91.

Yate AV, Narváez PC, Orjuela A, Hernández A, Acevedo H. 2020. A systematic evaluation of the mechanical extraction of Jatropha curcas L. oil for biofuels production. *Food Bioprod. Process.* **122**, 72–81. https://doi.org/10.1016/j.fbp.2020.04.001

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Obtaining hydrolysate from macauba oil and its application in the production of methyl esters

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SUMMARY: This work aimed to obtain a hydrolyzate rich in free fatty acids (FFA) from the hydrolysis of macauba oil for subsequent esterification and obtaining of methyl esters. To determine the conditions that maximize FFA yield in the hydrolysis step, the effects of buffer solution percentage and catalyst concentration (Lipozyme® RM IM) were determined at 55 ºC and 6 h. From the results, it was verified that both variables evaluated in the experimental range had an influence on the reaction and their increase favored the production of FFA. Additional experiments were carried out to assess the influence of reaction time with a progressive increase up to 8 h. Hydrolyzate with ~92 wt % FFA was obtained and its use in the enzymatic esterification step using Novozym® 435 as catalyst resulted in ~95 % FFA conversion. Regarding the reuse of enzymes at each stage, a ~50 % reduction in FFA yield was found and only 98 % FFA conversion.

KEYWORDS: *Enzymatic catalysis; Esterification; Hydrolysis; Macauba oil; Methyl esters*

RESUMEN: ***Obtención de hidrolizado de aceite de macauba y su aplicación en la producción de ésteres metílicos*.** Este trabajo tuvo como objetivo obtener un hidrolizado rico en ácidos grasos libres (AGL) a partir de la hidrólisis del aceite de frutos de macauba, para su posterior esterificación y obtención de ésteres metílicos. Para determinar las condiciones que maximizan el rendimiento de AGL en la etapa de hidrólisis, se determinaron los efectos del porcentaje de solución amortiguadora y la concentración de catalizador (Lipozyme® RM IM) a 55 ºC y 6 h. De los resultados se verificó que ambas variables, en el rango experimental evaluado, tienen influencia en la reacción y su incremento favorece la producción de AGL. Se llevaron a cabo experimentos adicionales para evaluar la influencia del tiempo de reacción, observándose un aumento progresivo hasta las 8 h. Se obtuvo un hidrolizado con ~92 % en peso de FFA y su uso en el paso de esterificación enzimática, usando Novozym® 435 como catalizador, resultó en ~95 % de conversión de FFA. Al investigar la reutilización de enzimas, en cada etapa, se encontró una reducción de ~50 % en el rendimiento de FFA y solo un 98 % en la conversión de FFA.

PALABRAS CLAVE: *Aceite de macauba; Catálisis enzimática; Ésteres metílicos; Esterificación; Hidrólisis*

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1. INTRODUCTION

For the past few years, advance research and development on the production of biofuels from renewable sources has been vehemently growing due to the excessive burning of fossil fuels which cause various environmental issues (Bankovicllic *et al.*, 2012). The use of biodiesel has been widely recognized due to its significant contribution to the reduction of greenhouse gas emission, specifically in the transportation sector (Lam *et al.*, 2019).

According to the Brazilian National Petroleum Agency (ANP, 2015), most of the biodiesel produced in Brazil is obtained from soybeans and since this is a crop mainly grown for human consumption, research on the exploitation of other oilseed crops with the potential to produce biodiesel has been reported. The oil from the Macauba fruit (*Acrocomia aculeata*) stands out in this sense, due to the great potential for production, which can be from 1500 to 5000 Kg of oil per hectare (Manfio *et al.*, 2011), higher than the productivity displayed by soybeans, about ~ 576 kg of oil per hectare (Tamagno *et al.*, 2020). The extraction of macauba oil can come from the kernel and the pulp, and the fatty acid composition of the oil extracted from the pulp consists of unsaturated (oleic and linoleic) and saturated (palmitic and stearic) fatty acids (Rosa *et al.*, 2020). The composition of kernel oil is mainly composed of saturated fatty acids (lauric, myristic and palmitic) (Trentini *et al.*, 2018; Rosa *et al.*, 2020) and therefore, because it contains a greater amount of these fatty acids, the use of macauba kernel oil confers the production of biofuels with greater oxidative stability (Trentini *et al.*, 2018). In addition, problems related to the supercooling of biodiesel from this oil were not observed (Menezes *et al.*, 2021).

The application of macauba oil in the synthesis of biodiesel requires its use in crude form (without refining process) in order to reduce raw material costs, which contribute with a high share in production costs. However, crude macauba oil has high acidity, with reports of 70.26% (Silva *et al.*, 2021) and 23% (Raspe *et al.*, 2013) in pulp and kernel oil, respectively. The hydrolysis of triglycerides followed by the esterification of the obtained fatty acids has stood out in obtaining fatty acid esters for substrates with high acidity (Vescovi *et al*., 2016), mainly in the conventional transesterification process with alkaline catalyst, which inevitably generates soap in the presence of these substrates, thus inactivating the catalyst, making separating biodiesel and glycerol expensive and affecting process productivity (Sousa *et al.*, 2010). However, to make this route industrially viable, in addition to the raw material, operational parameters related to the production costs of this biofuel, such as reaction time, energy demand and catalyst performance must be considered (Wancura *et al.*, 2021).

Hydrolysis and esterification reactions have been reported using enzymatic catalysts (Santos *et al.*, 2015; Zhou *et al.*, 2015; Barbosa *et al.*, 2019), mainly due to heterogeneity, the employment of soft conditions (Kabbashi *et al.*, 2015; Nguyen *et al.*, 2017), and the high degree of specificity of the desired substrates, which promotes reaction acceleration and biodegradability, making them less polluting compared to other catalysts and facilitating their reuse (Rodrigues and Ayub, 2011; Vescovi *et al.*, 2016). Although the use of these catalysts still faces problems related to low reaction rates and the need for long periods of time to achieve high yields, their use in the sequential process has stood out, demonstrating its potential to overcome these drawbacks (Wancura *et al.*, 2019).

The use of organic solvents as reaction media for enzymatic reactions provides attractive advantages over traditional systems, such as increased reaction yield over increased substrate solubility, suppression of water-dependent reactions and elimination of microbial contamination (Raspe *et al.*, 2013), besides the influence on catalytic activity and enzyme stability caused by the nature of these solvents. In contrast, other authors report that their effect causes the inactivation of enzymes, high solvent cost, limitations in mass transfer for heterogeneous systems or systems with high viscosity solvents/substrates (Doukyu and Ogino, 2010).

Therefore, the aim of this study was to evaluate the production of esters from the enzymatic hydroesterification of macauba oil in s two-step reaction: oil hydrolysis followed by esterification of the hydrolyzate obtained. The effects of the experimental variables (buffer solution percentage and catalyst concentration) were investigated in the hydrolysis step in order to maximize the free fatty acid (FFA) yield, and to determine the effect of reaction time. The hydrolyzate obtained (with maximum FFA content) was directed to the esterification step. In addition, the reuse of the enzymes used in the hydrolysis and esterification steps was evaluated.

2. MATERIALS AND METHODS

2.1. Materials

Macauba kernel oil (Cocal Brasil) was used in the reactions, and its chemical composition was previously reported by Raspe *et al.* (2013). Sodium phosphate buffer (Neon), enzyme Lipozyme® *Rhizomucor miehei* (Sigma-Aldrich) and *n*-hexane (Nuclear) were used in the hydrolysis step. In the esterification reactions, the hydrolyzate obtained from the hydrolysis step, methanol (Panreac, 99.9% purity) and enzyme Novozym® 435 (*Candida antarctica* lipase immobilized) were used. Heptane (Nuclear) and ethanol (Anidrol) were used to wash the enzymes in the catalyst reuse tests. . In titration step of the samples, a solution of ethyl ether:ethanol 2:1 (v:v) (Vetec/Nuclear), potassium hydroxide (Nuclear), and phenolphthalein as indicator (Nuclear) were used.

2.2. Experimental procedure

The hydrolysis reaction was carried out in a magnetically stirred, jacketed flask (40 mL) connected to a constant temperature bath (Marconi) for temperature monitoring. The reaction was conducted at 55 ºC, with agitation of 400 rpm and the reaction medium was composed of macauba kernel oil, sodium phosphate buffer solution (pH 8.0), *n*-hexane (oil to *n*-hexane mass ratio of 1:1) and Lipozyme® *Rhizomucor miehei* (RM IM) as catalyst (Raspe *et al.*, 2013). The enzyme was maintained at 40 ºC for 1 hour for activation before its addition to the reaction medium. After the reaction time of 6 hours, enzymes were separated by filtration and two phases (oil + solvent and water) were separated by centrifugation and the solvent in the oil phase was dried in an oven to evaporate the excess solvent.

An experimental central composite design (with axial points) was applied to evaluate the effects of process variables on FFA yield using Statistica® 8.0 software (STATSOFT™, Inc.). Buffer solution percentage (A) and catalyst concentration (B) were the variables investigated in the enzymatic hydrolysis, and these factors varied, as shown in Table 1. A total of 11 experiments with different combinations of levels of the variables were performed in duplicate, and the mean values ± standard deviation of the results were reported.

A second-order polynomial model (Barbosa *et al.*, 2019) was adjusted in relation to the responses obtained and the variables investigated. Analysis of variance (ANOVA) was used to evaluate the effects of operational variables and their interactions on the proposed model based on the values of p-value and F, where $p < 0.05$ was used as the threshold of statistical significance.

The effect of reaction time was determined from the conduction of destructive kinetics (in duplicate) in the times of 1, 2, 4, 6, 8 and 10 hours. The reactions were conducted keeping the temperature and buffer solution fixed at 55 ºC and 50 wt% (in relation to the oil mass), respectively, with the evaluation of the addition of lipase in concentrations of 10, 15 and 20 wt% (in relation to the substrate's mass).

The reactions with the macauba oil hydrolyzate were conducted keeping the temperature fixed at 65 ºC and a methanol to FFA molar ratio of 3:1. The reaction conditions were selected based on the work of Cerveró *et al.* (2014). Preliminary tests were conducted with different catalysts (Lipozyme® *Rhizomucor miehei*, Lipozyme® *Thermomyces lanuginosus* and Novozym® 435), which would indicate higher conversions with the use of Novozym® 435 with percentage in the reactions of 10 wt% (relation to substrates mass). These reactions were performed with magnetic stirrers in a batch reactor equipped with condenser and immersed in a temperature-controlled water bath. The hydrolyzate (4 g) was heated until the desired temperature was reached. At this point, methanol and the catalyst (after activation at 40 ºC for 1 hour) were added and esterification began. At the end of each reaction, the catalyst was separated by centrifugation (3000 rpm for 10 minutes), and the alcohol and water were removed from the reaction mixture using a rotary evaporator.

Table 1. Actual and coded values of the independent variables, central composite design (2^2), for enzymatic hydrolysis of macauba oil.

	Levels				
Factors	**(-1.41)**	**(-1)**	**(0)**	**(+1)**	**(+1.41)**
(A) Buffer (in relation to oil mass)	21.71	10	30	50	78.28
(B) Enzyme (in relation to substrate mass)	7.92	5	7.5	10	22.07

2.3. Analytical method

The content in free fatty acids (FFA) was determined based on the method Ca 5a-40 (AOCS, 1998), which is based on acid-base titration using an ethanol solution of potassium hydroxide (KOH) previously standardized as the titrant. Each sample was titrated in duplicate and the FFA content was calculated from Equation 1:

$$\text{FFA (wt\%)} = \frac{C \times MM \times v}{(10 \times m)} \quad (1)$$

where C is the concentration of sodium hydroxide (mol L^{-1}) used as titrant, MM corresponds to the molar mass of the predominant fatty acids in the sample, v is the volume required for the titration (mL) and m is the mass of sample (g).

The FFA yield of the hydrolysis reactions was calculated from Equation 2:

$$\text{FFA yield (\%)} = \frac{\text{FFA}}{\text{CHI}} \times 100 \quad (2)$$

where FFA corresponds to FFA content produced after the hydrolysis reaction and the CHI content in compounds present in the macauba oil that can be hydrolyzed (considering the initial content of FFA of 23.0 ± 0.4 wt%) reported by Raspe *et al.* (2013).

The macauba oil hydrolysate used was characterized in terms of the free fatty acid and water contents using the official methods recommended by the AOCS (1990): Ca 5a40 and 984.20, respectively. The glycerol content was determined by titration, using the sodium periodate method described by Cocks and Van Rede (1996).

The conversion of the esterification reaction (FFA conversion) was determined according to Equation 3:

$$\text{FFA conversion (\%)} = \frac{\text{FFA}_i - \text{FFA}_f}{\text{FFA}_i} \times 100 \quad (3)$$

where FFA_i is the initial FFA content in the hydrolyzate and FFA_f is the FFA content in the final sample of the reaction medium.

2.4. Reuse of lipase

For the reuse assays of the Lipozyme® RM IM and Novozym® 435, batches of hydrolysis and esterification were repeated for 15 cycles of 6 hours and 1 hour, respectively. After each reaction, the biocatalyst was recovered by filtration, washed with heptane and ethanol to remove adsorbed products and dried in oven at 40 ºC for 1 hour, kept in a desiccator for 24 hours and reused in another batch.

3. RESULTS

3.1. Enzymatic hydrolysis of macauba oil

Table 2 presents the results obtained for the reactions conducted in order to evaluate the effect of

Table 2. Central composite design and free fatty acid (FFA) yield obtained from enzymatic hydrolysis of macauba oil carried out at 55 ºC and 6 hours.

Run	Variables[1]		FFA yield[2] (%)
	A	B	
1	30 (-1)	10 (-1)	69.03 ± 0.38
2	30 (-1)	20 (+1)	78.09 ± 0.29
3	70 (+1)	10 (-1)	71.08 ± 0.82
4	70 (+1)	20 (+1)	80.67 ± 0.40
5	21.71 (-1.41)	15 (0)	76.67 ± 0.90
6	78.28 (+1.41)	15 (0)	80.15 ± 0.48
7	50 (0)	7.92 (-1.41)	68.56 ± 0.36
8	50 (0)	22.07 (+1.41)	80.17 ± 0.05
9.1	50 (0)	15 (0)	77.46 ± 0.05
9.2	50 (0)	15 (0)	78.39 ± 0.27
9.3	50 (0)	15 (0)	78.49 ± 0.75

[1] (A) Buffer solution percentage (in relation to oil mass) and Enzyme concentration (in relation to substrate mass); [2] calculated according FFA content produced after the hydrolysis reaction and CHI content of compounds present in the macauba oil which can be hydrolyzed (23.0 ± 0.4 wt%), mean value (2 replicates) ± standard deviation.

Table 3. Analysis of variance (ANOVA) of the quadratic model of free fatty acid (FFA) yield obtained from enzymatic hydrolysis of macauba oil.

	Sum of squares	Degree of freedom	Mean square	F	p^1
A (L)	11.38	1	11.38	35.39	0.027
A (Q)	0.40	1	0.40	1.26	0.376
B (L)	153.71	1	153.71	478.08	0.002
B (Q)	29.66	1	29.66	92.26	0.010
A*B	0.07	1	0.07	0.22	0.684
Lack of Fit	6.19	3	2.06	6.41	0.137
Pure Error	0.64	2	0.32		
Total SS	202.67	10			
$R^2 = 0.964$					
$R^2_{adjusted} = 0.949$					

[1] Statistical significance ($p < 0.05$); L - linear effect and Q - quadratic effect.

the process variables for obtaining a hydrolyzate rich in FFA.

The ANOVA of the quadratic model adjusted to the experimental data is presented in Table 3. Significant terms ($p < 0.05$) were obtained, which indicates that the experimental data can adequately describe the model proposed. The F values of 62.53 indicate that the models were significant, since these values were higher than the F_{critic} value (8.89). In addition, the values for R^2 and $R^2_{adjusted}$, calculated considering only the significant parameters, showed that the variability of the data (> 90%) is adequately explained by the regression model, which indicates good linearity between the predicted data and the observed data.

Table 3 shows that linear and quadratic terms of all variables were significant for the adjusted model, except for the buffer percentage, which showed influence only on the linear term and the binary interaction which was not significant. From the adjusted model, the linear term of the buffer percentage was the variable that presented a higher F value and a lower p value. The polynomial model for the FFA yield (%) was regressed considering the significant terms as presented in Equation 4:

$$\text{FFA yield (\%)} = 78.11 + 1.19A + 4.38B - 2.29B^2 \quad (4)$$

3.1.1. Effect of process variables

The variables evaluated in the experimental design have a greater influence on the FFA yield (based on the experimental range considered). The greater amount of buffer solution in the reaction medium caused an increase in the interfacial area of the oil-water system, providing a greater number of bonds between the substrates to be catalyzed by the lipase (Zhou *et al.*, 2015). In addition, when the percentage of the buffer solution was increased, there was less variation in the pH of the reaction medium and less aggregation (McClements and Weiss, 2005). In addition, a higher proportion of water changed the balance in favor of the products, improving the reaction rate in each of the hydrolysis steps and accelerating their completion (Wang *et al.*, 2012). This was possibly because lipase, which is a surface-active enzyme, bound with the substrates at the oil–water interface and, with the increased addition of water, the amount of water available for oil to form oil–water droplets increased, thereby increasing the available interfacial area, since the lipase catalyzes the hydrolysis reaction at the interfacial area of emulsion (Nguyen *et al.*, 2017).

Santos *et al.* (2015) observed an increase in the hydrolysis yield from ~35 to 100% FFA with the use of 50 wt% and 90 wt% buffer solution in the reaction, respectively. Zhou *et al.* (2015) evaluated the hydrolysis of unrefined jatropha oil and found that the application of the highest proportion of water (relation to oil mass) (100 wt%) resulted in obtaining ~88 wt% FFA, while using the proportion of 50 wt%, ~75 wt% FFA was obtained. Barbosa *et al.* (2019) reported a ~150% increase in the hydrolysis degree of *Moringa oleifera* Lam oil by varying the oil-to-water mass ratio from 15 to 35 wt%.

The higher catalyst concentration in the reaction medium favored the achievement of higher values for FFA yield, which is the result of increased contact between the substrate and the active sites of the lipase and the cumulative adsorption of the enzyme at the oil-water interface (Santos *et al.*, 2015), leading to increased hydrolysis rates. In general, the reaction rate increases with the greater availability of enzyme in the reaction medium. Zenevicz *et al.* (2016) found a 20% increase in the FFA content obtained from the hydrolysis of soybean oil by increasing the Lipozyme® TL IM percentage from 1 to 10 wt% (based on the total mass of substrates). For the hydrolysis of soybean oil, Corradini *et al.* (2019) obtained 1800 mM and ~500 mM of FFAs using 6 and 2 g of castor seed lipase, respectively.

3.1.2. Maximization of FFA yield

The conditions that maximized the production of FFA from the enzymatic hydrolysis of macauba oil were 50 wt% buffer solution and 20 wt% enzyme, with predicted FFA yields of 80.2%. Verification experiments were conducted (in triplicate) and provided FFA yield of 82.65 ± 0.57%. The predicted experimental values were compared and according to the t-Student test, there was agreement between these values in a significance interval of 0.05, which shows the predictive capacity of the adjusted models.

3.1.3. Effect of reaction time

Considering that for the investigated system the enzyme concentration had a greater influence on the FFA production, the kinetic of the reaction was determined keeping the temperature and buffer solution fixed at 55 ºC and 50 wt% (in relation to the oil mass), respectively, with evaluation of the addition of lipase in concentrations of 10, 15 and 20 wt% (in relation to the substrates mass).

Figure 1 presents the results obtained for the reactions carried out in the interval from 1 to 10 hours and according to this figure it can be seen that the gradual increase in the reaction time resulted in higher FFA yields, with the maximum value of ~88.90% (corresponding to FFA content of 92.08 wt%) obtained using 20 wt% catalyst and after 8 hours of reaction. For the reactions conducted with 15 and 20 wt% of catalyst, there was no increase in yield after 8 hours, indicating that the process equilibrium was reached.

Rodrigues and Ayub (2011) reported yields in the order of 95% FFA after 10 hours of reaction when investigating the hydrolysis of soybean oil using a water-to-soybean oil molar ratio of 3:1, 25 wt% (in relation to mass of oil) of the biocatalyst mixture (combination of 65% *Thermomyces lanuginosus* and 35% *Rhizomucor miehei*) at 30 °C. After 2 hours of

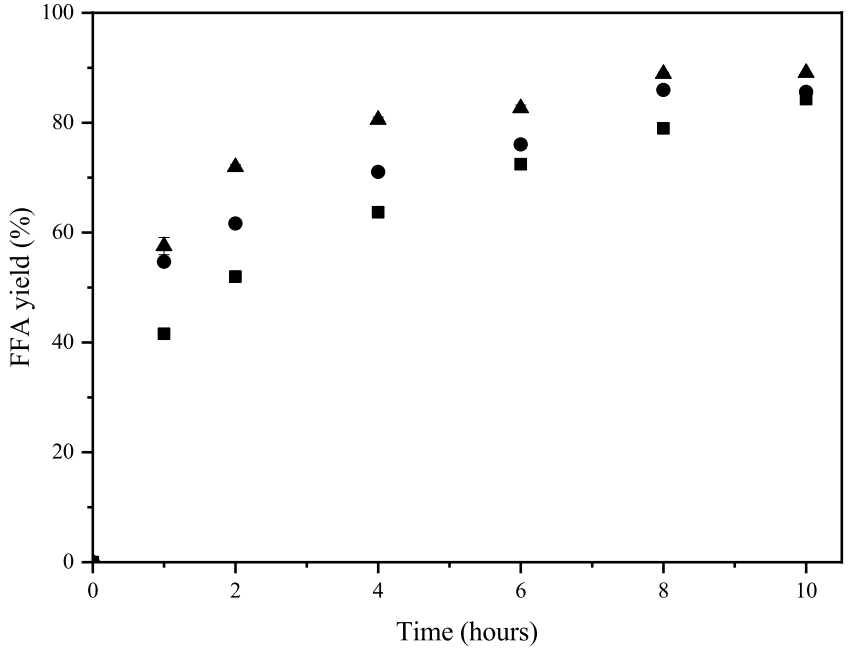

Figure 1. Kinetics of the production of free fatty acids (FFA) from enzymatic hydrolysis of macauba oil at 55 ºC, oil-to-*n*-hexane mass ratio of 1:1, 50 wt% of sodium phosphate buffer solution (pH 8.0) (in relation to oil mass) with different percentages of catalyst Lipozyme® RM IM (in relation to substrate mass): ■ 10 wt%; ● 15 wt% and ▲ 20 wt%. Mean value (2 replicates) ± standard deviation.

reaction at 40 °C, with an oil-to-water molar ratio of 1:20 and 10 wt% of Lipozyme® TL IM (in relation to the substrates mass), Zenevicz *et al.* (2016) obtained a maximum yield of 60% FFA in the hydrolysis of soybean oil. Vescovi *et al.* (2016) obtained 100% FFA yield in the hydrolysis of frying oil catalyzed by the immobilized lipase of *Thermomyces lanuginosus*, in a ratio of oil-to-water of 1:4 (v/v) and enzyme/reaction medium of 1:100 (w/v), at 30 °C and 24 h. After 40 hours of hydrolysis catalyzed by Lipozyme® RM IM, Tavares *et al.* (2018) obtained a maximum FFA yield of 74% from crambe oil, under experimental conditions of 2.7 wt% of lipase (in relation to the mass of substrates) and water-to-oil molar ratio of 10:1.

3.1.4. Characterization of hydrolyzate

The macauba oil hydrolyzate collected at 55 ºC, with 50 wt% buffer solution, 20 wt% lipase and 8 hours of reaction showed a free fatty acid content of 92.08 ± 0.28 wt%, water content of 0.865 ± 0.07 wt% and glycerol content of 0.64 ± 0.002 wt%, respectively.

3.1.5. Reuse of biocatalyst

Figure 2 shows the evaluation of the reuse of the enzyme catalyst in reactions conducted at 55 ºC, 50 wt% buffer solution and 20 wt% lipase, evaluated for 15 cycles of 6 hours each. From the data in Figure 2, it can be seen that the efficiency of the lipase declines in the course of its reuse, obtaining ~50% lower yield after 15 cycles compared to cycle 1. The loss in activity observed may be related to the saturation of the active sites of the enzyme during the reaction, since upon reaching its maximum activity, the interfacial effects and obstacles to mass transfer imply a decrease in reaction rates, preventing the enzyme from absorbing more substrate (Corradini *et al.*, 2019). Kabbashi *et al.* (2015) repored that the decrease in product yield may be attributed to desorption of the enzyme from the support and inactivation upon repeated reuse.

Rodrigues and Ayub (2011) verified a drop of ~80% in the hydrolysis yield of soybean oil catalyzed by the mixture of *Thermomyces lanuginosus* and Lipozyme® RM IM after 10 cycles of 10 hours each, relating this behavior to the lack of washing of the catalysts at the end of each process. Vescovi *et al.* (2016) when evaluating the reuse of *Thermomyces lanuginosus* lipase in the hydrolysis of residual cooking oil, determined that enzyme activity decreased during reuse in proportions similar to those reported in this work (~50%), although after only five cycles (10 hours each). According to the authors, this decrease in yield is due to the low pH of

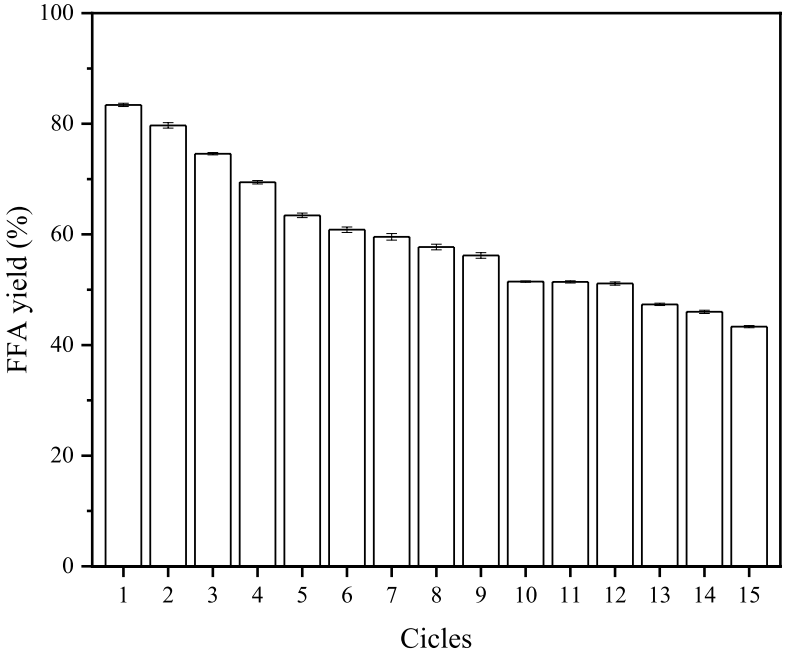

Figure 2. Evaluation of free fatty acid (FFA) yield from enzymatic hydrolysis of macauba with Lipozyme® RM IM reuse at 55 ºC, oil-to-*n*-hexane mass ratio of 1:1, 50 wt% of sodium phosphate buffer solution (pH 8.0) (in relation to oil mass) and 20 wt% (in relation to substrate mass) of catalyst and cycle of 6 hours each. Mean value (2 replicates) ± standard deviation.

the reaction medium (around pH 4.6 after 10 hours of hydrolysis), which probably caused enzyme inactivation. Assessing the reuse of Lipozyme® TL IM in the enzymatic hydrolysis of soybean oil, Zenevicz *et al.* (2016) observed that the process maintained the yield at ~60% FFA after 4 cycles (2 hours each).

3.2. Reaction carried out with macauba oil hydrolyzate

The esterification step of the FFA in solvent medium (50 wt% of *n*-hexane in relation to the hydrolysate mass) was carried out at different times, as shown in Figure 3. From the data in this figure, it appears that the reaction rate is high in short reaction times (15 min), reaching equilibrium in 60 min with ~95% conversion of FFA.

When investigating the influence of operational conditions on the use of Novozym® 435 in FFA esterification with methanol, Mulalee *et al.* (2015) reported ~ 95% conversion with 5 wt% catalyst (in relation to oleic acid), with a methanol-to-FFA molar ratio of 2:1, at 45 °C after 8 hours. ~97% FFA conversion was obtained by Teixeira *et al.* (2017) in the esterification of FFA of macauba oil, conducted at a methanol-to-FFA molar ratio of 2:1, 5 wt% of Lipozym® 435 (in relation to FFA mass), 30 °C and 60 min. Rosset *et al.* (2019) reported 94.3% conversion in the esterification of soybean oil hydrolyzate with the lipase NS 40116 (enzyme-in-liquid formulation from genetically-modified *Thermomyces lanuginosus* microorganism) at 35 °C, methanol-to-oil molar ratio of 4.5:1 and 12 hours of reaction.

3.2.1. Reuse of biocatalyst

The reuse of the Novozym® 435 in the esterification reaction was evaluated under the conditions reported in Figure 3 for the reaction time of 1 hour and during 15 cycles, as shown in Figure 4. It was verified from the results that the lipase maintained ~98% of its initial conversion capacity at the end of the evaluated cycles. The maintenance of the catalytic activity of the lipase is related to the low solubility of its support during the reactions (Shin *et al.*, 2020), promoted by operating conditions and the alcohol in the process. Adequate temperature and agitation do not weaken the enzyme support and do not promote its interfacial inactivation (Ortiz *et al.*, 2019). In addition, methanolic esterification promotes less swelling in the reuse of Novozym® 435, causing less loss in activity and degree of catalytic deactivation (Mulalee *et al.*, 2015). At the sqame time, the recovery of the enzyme by washing with heptane may also be responsible for maintaining the stability of Novozym® 435, due to the greater

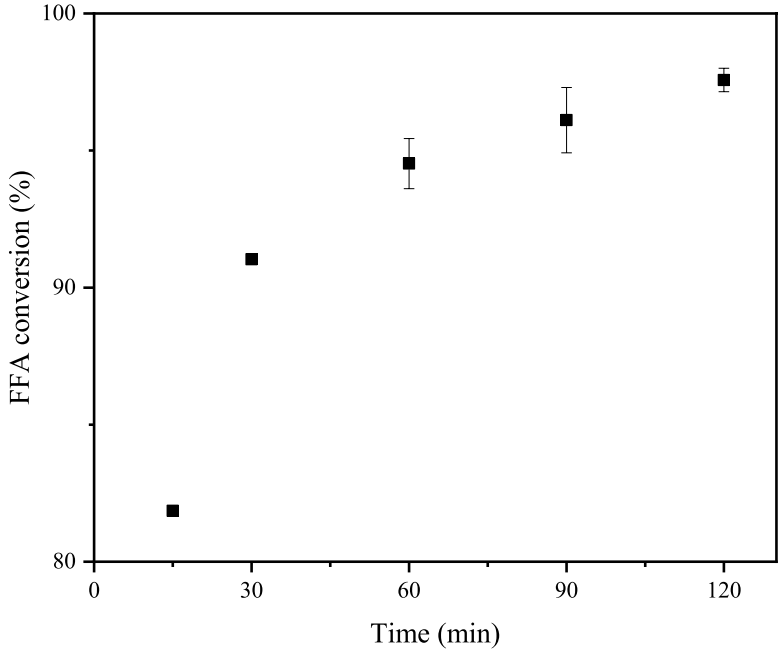

Figure 3. Free fatty acid (FFA) conversion from enzymatic esterification of macauba hydrolysate at 65 ºC, methanol-to-free fatty acid of 3:1 and 10 wt% Novozym® 435 (in relation to substrates mass). Mean value (2 replicates) ± standard deviation.

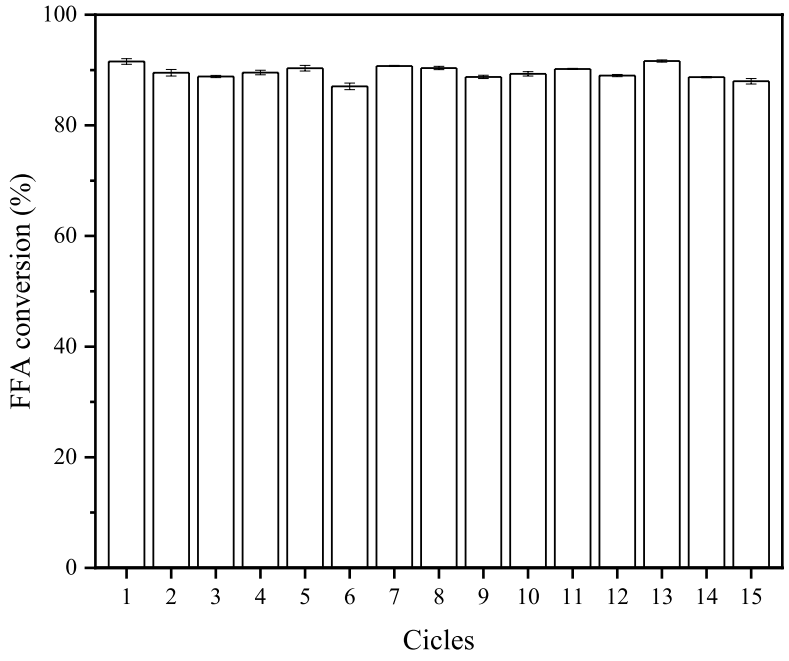

Figure 4. Evaluation of free fatty acid (FFA) conversion from enzymatic esterification of macauba hydrolysate with Novozym® 435 reuse at 65 ºC, methanol-to-free fatty acid of 3:1 and 10 wt% (in relation to substrates mass) of catalyst and cycle of 1 hour each. Mean value (2 replicates) ± standard deviation.

removal/dissolution of the constituents linked to the active sites of this enzyme (Chowdhury and Mitra, 2015), restoring the catalyst activity almost completely.

Baek *et al.* (2020) performed the enzymatic synthesis of formate ester through immobilized lipase, and observed that Novozym® 435 could be reused for 10 cycles of 1 hour, keeping the conversions at ~92%. Moreira *et al.* (2020) reported that Novozyme® 435 maintained catalytic activity at the end of 10 consecutive cycles in the enzymatic esterification of babassu FFA in reactions at 48 °C and duration of 4 hours.

4. CONCLUSIONS

The present study evaluated the hydroesterification of macauba oil using enzymatic catalysis, with evaluation of the processes of hydrolysis of macauba oil and esterification of the hydrolyzate. Evaluating the effects of the process variables, it was found a yield of ~ 80% in FFA, through positive and significant effects for the percentage of buffer solution and concentration of catalyst in the reaction medium. A hydrolyzate with ~92% FFA was obtained by evaluating the percentage of 20 wt% of catalyst (in relation to oil mass), temperature of 55 ºC, stirring 400 rpm and 50 wt% of buffer solution (in relation to mass of substrates) in the hydrolysis kinetics after 8 hours of reaction. The conversion of the hydrolyzate in the esterification step was evaluated at 65 ºC, methanol to FFA of 3:1 and 10 wt% (in relation to substrates mass) of catalyst, where 95% conversion of the FFA was achieved. In the reuse of the catalysts, the efficiency of the Lipozyme® RM IM lipase decreased ~50% the FFA yield in hydrolysis after 90 hours of the process, while Novozym® 435 maintained ~98% of its initial conversion capacity, at the end of the 15 cycles investigated (15 hours).

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REFERENCES

ANP, Agência Nacional de Petróleo, Gás Natural e Biocombustíveis. 2015. *Boletim mensal de biodiesel*.

AOCS, American Oil Chemists' Society. 1998. *Official methods and recommended practices.* 2, 4 (Ed.) Champaign.

Baek Y, Lee J, Son J, Leem T, Sobhan A, Lee J, Koo SM, Shin WH, Oh JM, Park C. 2020. Enzymatic Synthesis of Formate Ester through Immobilized Lipase and Its Reuse. *Polym.* **12**, 1–10. https://doi.org/10.3390/polym12081802

Bankovic-Llic O, Stamenkovic OS, Velikovic VB. 2012. Biodiesel production from non-edible plant oils. *Renew. Sust. Ener. Ver.* **16**, 3621–3647. http://dx.doi.org/10.1016/j.rser.2012.03.002

Barbosa MS, Freire CCC, Almeida LC, Freitas LS, Souza RL, Pereira EB, Mendes AA, Pereira MM, Lima AS, Soares CMF. 2019. Optimization of the enzymatic hydrolysis of *Moringa oleifera* Lam oil using molecular docking analysis for fatty acid specificity. *Biotechnol. Appl. Biochem.* **66**, 823–832. https://doi.org/10.1002/bab.1793

Cerveró JM, Álvarez JR, Luque S. 2014. Novozym 435-catalyzed synthesis of fatty acid ethyl esters from soybean oil for biodiesel production. *Biomass Bioenerg.* **61**, 131–137. https://doi.org/10.1016/j.biombioe.2013.12.005

Chowdhury A, Mitra D. 2015. A kinetic study on the Novozyme 435-catalyzed esterification of free fatty acids with octanol to produce octyl esters. *Biotechnol. Prog.* **31**, 1494–1499. https://doi.org/10.1002/btpr.2165

Cocks LV, Van Rede C. 1966. *Laboratory Handbook for Oil and Fat Analysis.* Londres. Acad. Press.

Corradini FAS, Alves ES, Kopp W, Ribeiro MPA, Mendes AA, Tardioli PW, Giordano RC, Giordano RLC. 2019. Kinetic study of soybean oil hydrolysis catalyzed by lipase from solid castor bean seeds. *Chem. Eng. Res. Des.* **144**, 115–122. https://doi.org/10.1016/j.cherd.2019.02.008

Doukyu N, Ogino H. 2010. Organic solvent-tolerant enzymes. *Biochem. Eng. J.* **48**, 270–282. https://doi.org/10.1016/j.bej.2009.09.009

Kabbashi NA, Mohammed NI, Alam MZ, Mirghani MES. 2015. Hydrolysis of *Jatropha curcas* oil for biodiesel synthesis using immobilized *Candida cylindracea* lipase. *J. Mol. Catal. B. Enzym.* **116**, 95–100. https://doi.org/10.1016/j.molcatb.2015.03.009

Lam MK, Jamalluddin NA, Lee KT. 2019. Production of Biodiesel Using Palm Oil. Biofuels: Alternative Feedstocks and Conversion Processes for the Production of Liquid and Gaseous Biofuels. *Biomass Biofuels Biochem.* **2**, 539–574. https://doi.org/10.1016/B978-0-12-816856-1.00023-3

Manfio CE, Motoike SY, Pimentel LD, de Queiroz V, Sato AY. 2011. Repetibilidade em características biométricas do fruto de macaúba. *Cienc. Rural* **41**, 70–76. https://doi.org/10.1590/S0103-84782011000100012

McClements DJ, Weiss J. 2005. Em Bailey's industrial oil and fat products. John Wiley & Sons Inc.: New York.

Menezes FAF, Rangel AB, Cordeiro TC, Vargas H, Silva EC. 2021. Investigation of phase transitions in vegetable oils through temperaturedependent optical measurements: supercooling efect. *J. Therm. Anal. Calorim.* **143**, 27–33. https://doi.org/10.1007/s10973-019-09168-7

Moreira KS, Moura Junior LS, Monteiro RRC, Oliveira ALB, Valle CP, Freire TM, Fechine PBA, Souza MCM, Fernandez-Lorente G, Guisan JM, Santos JCS. 2020. Optimization of the Production of Enzymatic Biodiesel from Residual Babassu Oil (*Orbignya* sp.) via RSM. *Catal.* **10**, 1–20. https://doi.org/10.3390/catal10040414

Mulalee S, Srisuwan P, Phisalaphong M. 2015. Influences of operating conditions on biocatalytic activity and reusability of Novozym 435 for esterification of free fatty acids with short-chainalcohols: A case study of palm fatty acid distillate. *Chin. J. Chem. Eng.* **23**, 1851–1856. https://doi.org/10.1016/j.cjche.2015.08.016

Nguyen VTA, Le TD, Phan HN, Tran LB. 2017. Antibacterial Activity of Free Fatty Acids from Hydrolyzed Virgin Coconut Oil Using Lipase from *Candida rugosa*. *J. Lipids* **2017**, 1–7. https://doi.org/10.1155/2017/7170162

Ortiz C, Ferreira ML, Barbosa O, Dos Santos JCS, Rodrigues R.C., Berenguer-Murcia, A., Briand, L.E., Fernandez-Lafuente, R. 2019. Novozym 435: the "perfect" lipase immobilized biocatalyst? *Catal. Sci. Technol.* **9**, 2380–2420. https://doi.org/10.1039/C9CY00415G

Raspe DT, Silva C, Cardozo-Filho L. 2013. Effect of additives and process variables on enzymatic hydrolysis of macauba kernel oil (*Acrocomia aculeata*). *Chem. Eng. J.* **2013**, 1–8. https://doi.org/10.1155/2013/438270

Rodrigues RC, Ayub MAZ. 2011. Effects of the combined use of *Thermomyces lanuginosus* and *Rhizomucor miehei* lipases for the transesterification and hydrolysis of soybean oil. *Process*

Biochem **46**, 682–688. https://doi.org/10.1016/j.procbio.2010.11.013

Rosa ACS, Stevanato N, Garcia VAS, Silva C. 2020. Simultaneous extraction of the oil from the kernel and pulp of macauba fruit using a green solvent. *J. Food Process Pres.* **44**, 1–14. https://doi.org/10.1111/jfpp.14855

Rosset DV, Wancura JHC, Ugalde GA, Oliveira JV, Tres MV, Kuhn RC. Jahn SL. 2019. Enzyme-Catalyzed Production of FAME by Hydroesterification of Soybean Oil Using the Novel Soluble Lipase NS 40116. *Biotechnol. Appl. Biochem.* **188**, 914–926. https://doi.org/10.1007/s12010-019-02966-7

Santos LDF, Coutinho JAP, Ventura SPM. 2015. From Water-in-Oil to Oil-in-Water Emulsions to Optimize the Production of Fatty Acids Using Ionic Liquids in Micellar Systems. *Biotechnol. Prog.* **31**, 1473–1480. https://doi.org/10.1002/btpr.2156

Shin M, Seo J, Baek Y, Lee T, Jang M, Park C. 2020. Novel and Efficient Synthesis of Phenethyl Formate via Enzymatic Esterification of Formic Acid. *Biomolecules* **10**, 1–15. https://doi.org/10.3390/biom10010070

Silva C, Colonelli TAS, Postaue N, Zempulski D, Trentini CP, Silva EA, Cardozo Filho L. 2021. Catalyst-free production of fatty acid ethyl esters (FAEE) from macauba pulp oil. *Grasas Aceites* **72** (1), e398. https://doi.org/10.3989/gya.0103201

Sousa JS, Cavalcanti-Oliveira EA, Arandab DAG, Freire DMG. 2010. Application of lipase from the physic nut (*Jatropha curcas* L.) to a new hybrid (enzyme/chemical) hydroesterification process for biodiesel production. *J. Mol. Catal. B. Enzym.* **65**, 133–137. https://doi.org/10.1016/j.molcatb.2010.01.003

Tamagno S, Aznar-Moreno JA, Durrett TP, Prasad PVV, Rotundo JL, Ciampitti IA. 2020. Dynamics of oil and fatty acid accumulation during seed development in historical soybean varieties. *Field Crops Res.* **248**, 1–10, 2020. https://doi.org/10.1016/j.fcr.2020.107719

Tavares F, Silva EAD, Pinzan F, Canevesi RS, Milinsk MC, Scheufele FB, Borba CE. 2018. Hydrolysis of crambe oil by enzymatic catalysis: An evaluation of the operational conditions. *Biocat. Biotransf.* **36**, 1–14. https://doi.org/10.1080/10242422.2018.1430786

Teixeira DA, Motta CR, Ribeiro CMS, Castro AM. 2017. A rapid enzyme-catalyzed pretreatment of the acidic oil of macauba (*Acrocomia aculeata*) for chemoenzymatic biodiesel production. *Process Biochem.* **53**, 188–193. https://doi.org/10.1016/j.procbio.2016.12.011

Trentini CP, Cuco RP, Cardozo-Filho L, Silva C. 2018. Extraction of macauba kernel oil using supercritical carbon dioxide and compressed propane. *Can. J. Chem. Eng.* **97**, 785–792. https://doi.org/10.1002/cjce.23236

Vescovi V, Rojas MJ, Baraldo A, Botta DC, Santana FAM, Costa JP, Machado MS, Honda VK, Giordano RLC, Tardioli PW. 2016. LipaseCatalyzed Production of Biodiesel by Hydrolysis of Waste Cooking Oil Followed by Esterification of Free Fatty Acids. *J. Am. Oil Chem. Soc.* **93**, 1615–1624. https://doi.org/10.1007/s11746-016-2901-y

Wancura, JHC, Rosset DV, Mazutti MA, Ugalde GA, Oliveira V, Tres MV, Jahn SL. 2019. Improving the soluble lipase–catalyzed biodiesel production through a two-step hydroesterification reaction system. *Appl. Microbiol. Biotechnol.* **103**, 7805–7817. https://doi.org/10.1007/s00253-019-10075-y

Wancura JHC, Fantinel AL, Ugalde GA, Donato FF, Oliveira JV, Tres MV, Jahn SL. (2021). Semi-continuous production of biodiesel on pilot scale via enzymatic hydroesterification of waste material: Process and economics considerations. *J. Clean Prod.* **285**, 124838. https://doi.org/10.1016/j.jclepro.2020.124838

Wang WC, Turner TL, Stikeleather LF, Roberts WL. 2012. Exploration of process parameters for continuous hydrolysis of canola oil, camelina oil and algal oil. *Chem. Eng. Process* **57–58**, 51–58. https://doi.org/10.1016/j.cep.2012.04.001

Zenevicz MCP, Jacques A, Furigo A, Oliveira V, Oliveira D. 2016. Enzymatic hydrolysis of soybean and waste cooking oils under ultrasound system. *Ind. Crops Prod.* **80**, 235–241. https://doi.org/10.1016/j.indcrop.2015.11.031

Zhou GX, Chen GV, Yan BB. 2015. Two-step biocatalytic process using lipase and whole cell catalysts for biodiesel production from unrefined jatropha oil. *Biotechnol. Lett.* **37**, 1959–1963. https://doi.org/10.1007/s10529-015-1883-4

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Effect of vacuum impregnation on physical changes during table olive processing

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SUMMARY: Among the benefits which vacuum impregnation (VI) may provide to fruits and vegetables, this study focused on weight and texture changes during the processing of table olives. VI applied to Manzanilla olives led to around 10% weight gain, which was maintained after their packing as black olives. However, this weight gain was only around 4 % for Hojiblanca olives. Likewise, the use of calcium chloride was recommended to maintain the firmness of the olives, in particular those of the softer Manzanilla cultivar. With regard to the Spanish-style, the Hojiblanca cultivar achieved around 4% weight gain during processing but the use of VI for Manzanilla olives was ruled out due to softening of the fruit. In addition. the black and green color of olives and their flavor were not modified by the application of VI. This technology could be very useful to reduce weight loss during table olive processing.

KEYWORDS: *Calcium; Firmness; Table olive; Vacuum; Weight.*

RESUMEN: ***Efecto de la impregnación al vacío sobre los cambios físicos durante la elaboración de aceitunas de mesa***. Entre los beneficios que la aplicación de la impregnación al vacío puede originar en frutas y verduras, este estudio se ha centrado sobre los cambios en la textura y el peso de las aceitunas de mesa durante su procesamiento. La impregnación al vacío de aceitunas Manzanilla dio lugar a un aumento cercano al 10 % de peso y ello se mantuvo después de su envasado como aceitunas negras, mientras que esta ganancia fue de sólo el 4& para aceitunas Hojiblanca. Asimismo, se vio necesario el empleo de cloruro cálcico para el mantenimiento de la textura de los frutos, en particular para aquellos de la variedad Manzanilla. Con respecto al procesamiento como verdes estilo español, la variedad Hojiblanca consiguió un aumento del 4% en peso debido al empleo de impregnación al vacío y este tratamiento se descartó para la Manzanilla debido al ablandamiento de la aceituna. Además, se debe indicar que el color verde y negro de las aceitunas no se vio afectado por el hecho de emplear impregnación al vacío. Esta tecnología podría ser muy útil para reducir las pérdidas de peso que se producen durante la elaboración de aceitunas de mesa.

PALABRAS CLAVE: *Aceitunas de mesa; Calcio; Firmeza; Peso; Vacío.*

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1. INTRODUCTION

Vacuum impregnation (VI) is a well-known technique which has been investigated in a wide range of fruits and vegetables (Fito *et al*., 1996; Zhao and Xie, 2004; Blanda *et al*., 2008; López and Moreira, 2019). Among the different applications of VI, it has been studied extensively in recent years for the elaboration of minimally processed and dehydrated fruits enriched with health-promoting substances including phenolic compounds (Luo *et al*., 2019; Tylewicz *et al*., 2019). In addition, the infusion of calcium and the enzyme pectin methylesterase into fruits via VI is carried out to increase their firmness (Banjongsinsiri *et al*., 2004; Quintanilla *et al*., 2018), although the use of VI without firming agents may induce fruit softening due to structural deformations (Tylewicz *et al*., 2019). The rapid acidification of vegetables is another interesting application of VI to control microbial growth (Derossi *et al*., 2011). With regard to table olives, VI has been studied for the enrichment of green olives with iron (Zunin *et al*., 2017) and to accelerate the debittering stage with NaOH (Tamer *et al*., 2013).

A consequence of the use of VI on fruits and vegetables is often an increase in their weight (Derossi *et al*., 2010; Sirijariyawat *et al*., 2012; Parreidt *et al*., 2018). The application of a high vacuum level to plant tissue leads to the expansion of the gas trapped inside the intercellular spaces and capillaries, so an increase in the porosity volume of the tissue occurs. Hence, the penetration of the liquid inside the pores during the relaxation step of VI gives rise to an increase in the weight of the fruit (Schulze *et al*., 2012; Tylewicz *et al*., 2019). Among the many variables which can affect the VI phenomenon, the porosity fraction of the vegetable and fruit tissue is one of the most important. This parameter may range from 1.6 to 35.9% (Derossi *et al*., 2012), and it has been estimated at around 4% in raw olives of the Hojiblanca cultivar (Romero *et al*., 1996).

Changes in the weight of table olives currently occur during processing and this is of economic importance for industries. Although no reports are available on the changes in the weight of Spanish-style green olives, small weight increases occasionally occur during the debittering and fermentation stages of this product (private industrial communication). By contrast, weight loss has been reported during the storage of olives of the Hojiblanca cultivar intended for processing as black ripe olives; whereas increase in weight takes place during the darkening stage of the elaboration of this commercial preparation (García *et al*., 2014).

The aim of this work was to investigate the application of VI on raw olives which are then processed as Spanish-style green olives and black ripe olives, in particular its effect on weight changes during elaboration and its influence on the quality of the final product. The influence of calcium addition to prevent firmness loss was also assessed.

2. MATERIALS AND METHODS

2.1. Olive material

Olives of the Manzanilla and Hojiblanca cultivars were supplied at the end of September during the 2016/2017 olive season from local farmers located in the province of Seville (Spain). Three and four independent lots of olives of the Manzanilla and Hojiblanca cultivars were purchased, respectively. The fruits were harvested with green-yellow color on the surface and, on arrival at the laboratory, leaves and small branches were removed, and the olives were sorted in order to use those with uniform size (280-320 fruit/kg).

2.2. Assays of VI

The VI equipment consisted of a modified pressure cooker (10 L volume) with a pressure gauge connected to with a vacuum pump with a rubber tube (Millipore Corp.), which allowed adjustment of the level of vacuum. Before vacuum assays, the olives were submerged in tap water and the liquid was drained for five minutes. Subsequently, lots of 4 kg of wet olives were put inside the pressure cooker and immersed in 5 L tap water at room temperature. Except for the experiments aimed at studying the effect of the vacuum level, 0.8 bar vacuum was maintained during all assays for 15 minutes inside the chamber before the atmospheric pressure was restored. All samples were kept in tap water for a relaxation time of 15 minutes at atmospheric pressure. Afterwards, VI olives were processed as table olives.

Olives of the Manzanilla cultivar with different sizes ranging from 15 to 19 mm equatorial diameter were also vacuum impregnated to test the effect of olive size on weight changes.

2.3. Spanish-style green olive processing

For each of the four Manzanilla lots, 5 kg of vacuum-impregnated olives were put into 8.5 L cylindrical polyethylene vessels and covered with 3.4 L of 0.47 M NaOH solution until it penetrated two-thirds of the way to the pit of the olives (Figure 1). Subsequently, the olives were washed with tap water for seven hours and then covered with 10% (w/v) NaCl solution or the same brine spiked with 2 g/L $CaCl_2$. Olives that were not submitted to VI were similarly debittered, washed and put into brine with and without calcium to use as controls. Finally, the fruits were left to spontaneous fermentation for eight months and the weight change was recorded (Figure 1). All assays were run in duplicate.

The four lots of Hojiblanca olives were processed as described above, except for the NaOH concentration, which was 0.52 M.

2.4. Black ripe olive processing

Vacuum-impregnated olives (5 kg) of the three lots of Manzanilla cultivar were put into the 8.5-L vessels and covered with 3.4 L of 1.5% acetic acid with and without 3 g/L $CaCl_2$ (De Castro *et al.,* 2007). The olives which were not submitted to vacuum impregnation were also stored in similar acetic acid solutions (controls). All assays were run in duplicate. After eight months of storage, the fruits were darkened following the recently developed single lye treatment (Brenes *et al*., 2017). Three kilograms of olives were put into four methacrylate horizontal cylindrical containers and covered with 3 L of a 0.63 M NaOH solution (lye) until the lye reached the pit (4h). Then the alkaline solution was removed and the olives were submerged in tap water for 24 h with aeration. This first washing water was drained and the fruits were covered with fresh tap water for another 24 h with aeration as well as the pH being controlled in the liquid below 8.0 units with the addition of CO_2. Subsequently, they were covered with a 0.1% (w/v) ferrous gluconate solution for five hours to fix the black color which had formed. Finally, the stones were removed from the olives with an industrial pitting machine (OFM, Seville, Spain), and 145 g of pitted olives were bottled in A314 jars (Juvasa, Dos Hermanas, Spain) and covered with 175 mL of a solution containing 32 g/L NaCl and 0.2 g/L ferrous gluconate. The jars were sterilized and maintained at ambient temperature for two months before analyses (Figure 1).

The four lots of Hojiblanca olives were processed as described above, except the NaOH concentration, which was 0.70 M.

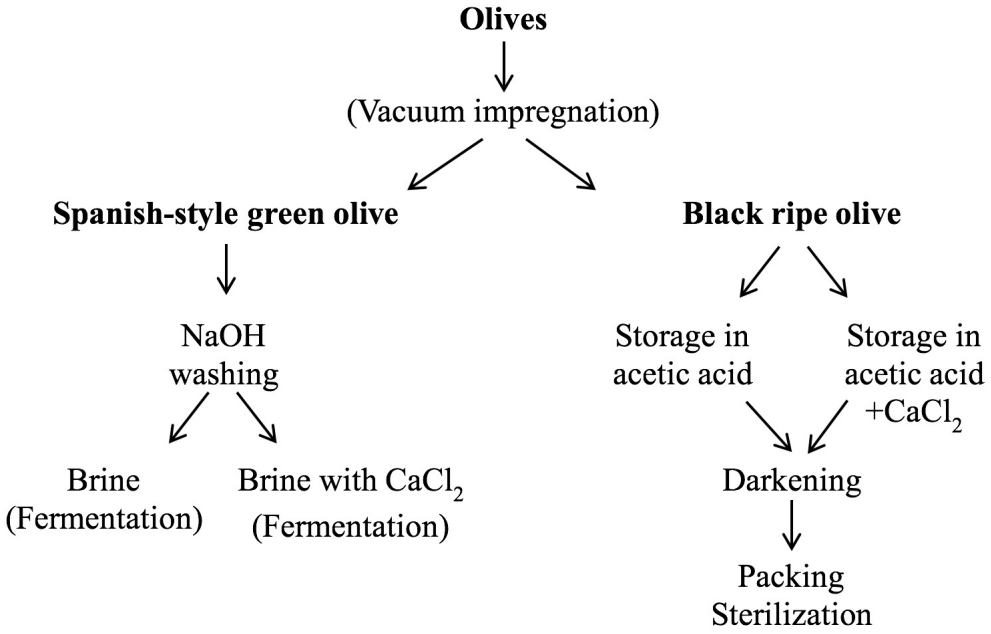

Figure 1. VI experimental design for olives of the Manzanilla and Hojiblanca cultivars.

2.5. Weight variation analysis

The percentage of weight change (DW) was calculated for every step of the olive processing as:

$$\Delta W = (W_i - W_f / W_i) \times 100$$

where W_i is the initial weight of the wet olives before the impregnation of processing (controls) and W_f is the final olive weight after each processing step.

Weight change was measured in duplicate for each of the three and four lots of Manzanilla and Hojiblanca cultivars, respectively.

2.6. Firmness analysis

The firmness of the fruits was determined using a Kramer shear compression cell coupled to a Texture Analyzer TA.TX plus (Stable Microsystems, Godalming, UK). The crosshead speed was 200 mm/min. Firmness was the mean of 10 replicate measurements, each of which was performed on three pitted olives, and expressed as Newton/100 g pitted olives.

2.7. Chemical analysis

The concentration of NaCl was analyzed by titration with a 0.1 N silver nitrate solution. The free acidity and pH of the brine were measured with a Metrohm 670 Titro processor (Herisau, Switzerland) (De Castro *et al*., 2007). Titratable acidity was determined by titrating to pH 8.3 with 0.2 M NaOH and expressed as lactic acid.

2.8. Statistical analyses

Statistical comparisons of the mean values for each experiment were carried out by one-way analysis of variance (ANOVA), followed by the Duncan´s multiple range test ($p < 0.05$) using SPSS software v. 23.0 (IBM Corp., Armonk, NY).

3. RESULTS AND DISCUSSION

3.1. Effect of vacuum degree and olive size on weight changes

Figure 2 shows the effect of the vacuum level on the weight changes in Manzanilla olives during the application of VI at room temperature. A rather linear relationship between weight gain and the vacuum applied was found from 0.2 to 0.7 bars. Thereafter the rate of weight gain decreased up to 0.8 bars. Hence, 0.8 bars was the vacuum applied thereafter for all assays. In addition, preliminary experiments demonstrated that 15 min of vacuum application and relaxation were sufficient to reach the maximum effect. In the end, more than 10% weight gain was achieved in these olives of the Manzanilla cultivar impregnated with tap water. These results are in accordance with many others reported for fruit

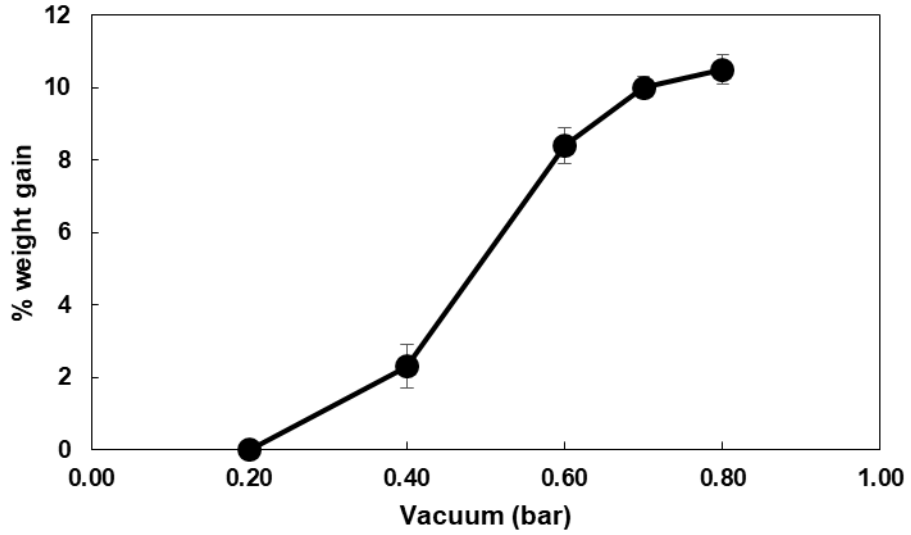

Figure 2. Influence of the degree of vacuum (bar) on the weight gain of olives of the Manzanilla cultivar (17 mm equatorial diameter).

and vegetables including apple slices (8-7% gain) (Mújica-Paz *et al*., 2003; Parlawska *et al*., 2019), peppers (4-11% gain) (Derossi *et al*., 2010), mango (10-15% gain) (Sirijariyawat *et al*., 2012), melon (4-8 % gain) and potatoes (8-11% gain) (López and Moreira, 2019; Parreidt *et al*., 2019; Luo *et al*., 2019). It is known that a high vacuum level increases the porosity of fruit tissue as a result of high expansion and release of the gas inside the pores of fruit but there is only one record regarding the intercellular volume of raw fruit of the Hojiblanca cultivar (4%) (Romero *et al*., 1996). Hence, it could be assumed that VI led to deformation and expansion of the Manzanilla olive capillary with an increase in its volume and fruit weight (Figure 2). Because the effectiveness of VI could be influenced by olive size, the influence of this parameter was studied on olives of the Manzanilla cultivar, and the results obtained are depicted in Table 1: the greater the size of the olive, the higher the weight gain. These findings also demonstrated that the volume of the fruit increased as a consequence of the VI treatment, so the caliber of the olives decreased to a large extent. Consequently, VI assays were run thereafter with olives of the same size.

3.2. Black ripe olives

First, it must be highlighted that olives of the Manzanilla and Hojiblanca cultivars which were vacuum-impregnated fermented during the storage period before the darkening stage in a similar manner to those that were not treated. In addition, the lye treatment lasted the same amount of time for both treated and non-treated olives.

The weight changes during the elaboration of the Manzanilla cultivar is depicted are Figure 3. All changes referred to the initial weight as indicated

Table 1. Influence of olive size on the physical characteristics of Manzanilla fruit submitted to VI (0.8 bar) for 15 minutes.

Equatorial diameter (mm)	Weight gain (%)	Volume gain (mL·kg^{-1})	Initial caliber (fruit·kg^{-1})	Caliber after VI (fruit·kg^{-1})
15	9.0	70	402	375
17	11.1	85	307	274
19	11.6	120	220	195

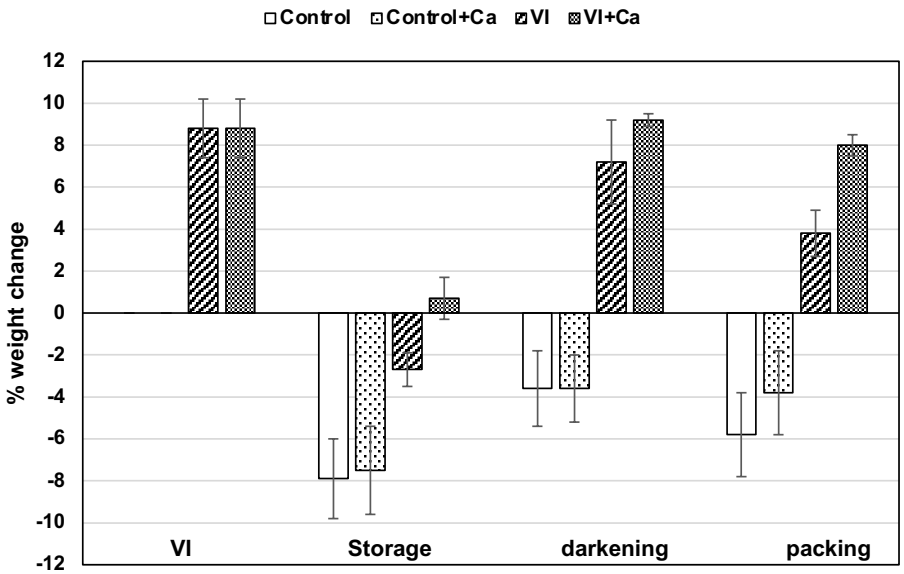

Figure 3. Effect of VI on the changes in olive weight of the Manzanilla cultivar during its processing as black ripe olives. Calcium chloride was employed during the storage stage. Bars are the mean value of three independent olive lots elaborated in duplicate, and they represent the accumulated gain or lost olive weight throughout the whole process. Standard deviation is depicted on the bars.

in section 2.5. The non-VI Manzanilla olives lost around 8% of their weight during their preservation stage in the acidified medium for eight months, which is a higher weight loss than previously reported for the Hojiblanca cultivar (García *et al*., 2014). By contrast, VI olives reduced their weight by only around 2.8%, although the weight gain achieved during the VI application must be taken into account, thereby a difference of about 5.5% weight between control and VI olives after this preservation period occurred. Subsequently, stored fruits were submitted to the darkening stage and the olives gained weight, as expected from previous studies (García-Serrano *et al*., 2020). However, weight gain was not the same for non-VI and VI olives. The former olives reduced their 8% loss obtained during the preservation stage to only 4% after the darkening stage; while the VI olives resulted in weight gain of around 7% after darkening so that the difference in weight between untreated and VI olives increased by up to 11%. Subsequently, the olives were packed, sterilized and analyzed after two months at room temperature and the difference was reduced to 9.8%.

It is well-known that the application of VI to fruit and vegetables may promote loss in firmness, in particular when using hypertonic solutions (Zhao and Xie, 2004). On the contrary, this technique has been widely tested to improve the texture of foods by introducing either calcium or the enzyme pectin methylesterase into them (Quintanilla *et al*., 2018; Servillo *et al*., 2018). We tried to perform VI on olives with calcium chloride solution but the cation did not penetrate inside the fruit nor was any weight change noted (data not shown), so all our VI assays were run with only tap water. It must be noted that olive skin is a strong barrier against the diffusion of substances into the pulp of the fruit which counteracts the effectiveness of VI, which was observed for the application of VI during the alkaline treatment of the olives (Tamer *et al*., 2013). Hence, taking into consideration previous studies on the improvement in texture with the addition of calcium to the liquid at the preservation stage (Brenes *et al*., 1994; García-Serrano *et al*., 2020), the storage solutions of both cultivars (Manzanilla and Hojiblanca) were also spiked with 3 g/L of calcium chloride (Figure 1). The influence of calcium on the weight changes in Manzanilla olives which were not treated with VI was not relevant (Figure 3). However, it was observed that the cation gave rise to higher weight gain in VI olives due to an almost 4% higher weight gain in VI packed olives with calcium added to the preservation liquid than in VI olives without calcium added (Figure 3). Additionally, calcium led to better texture in packed Manzanilla and Hojiblanca olives, regardless of the VI treatment (Figure 4).

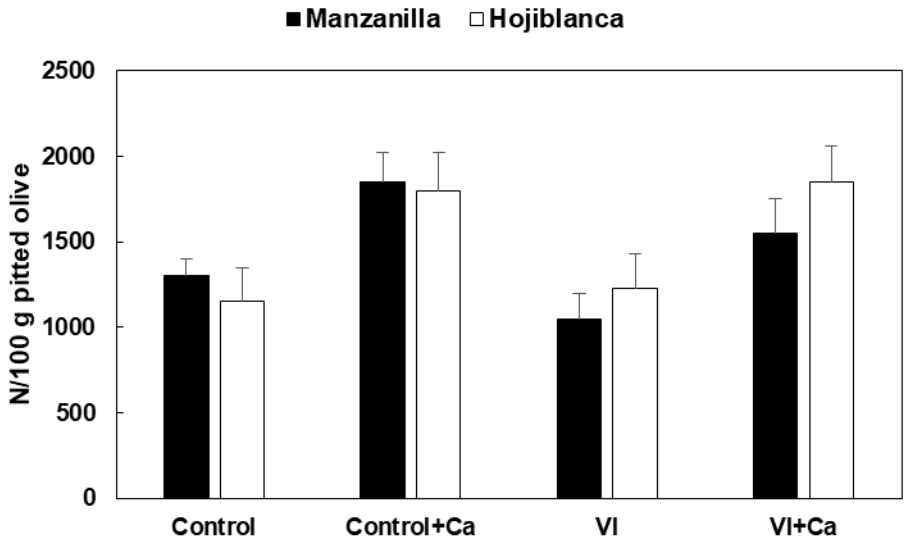

Figure 4. Influence of VI on the firmness of processed black ripe olives of the Manzanilla and Hojiblanca cultivars. Calcium chloride was employed during the storage stage. Standard deviation is depicted on the bars. Bars followed by a different letter indicate significant difference according to Duncan´s test ($p < 0.05$).

In fact, slightly lower firmness in VI olives than untreated olives of the Manzanilla cultivar was achieved although it was not statistically significant; while the texture of packed Hojiblanca olives was not affected by the VI treatment (Figure 4).

The cell wall composition of the Manzanilla and Hojiblanca cultivars is very different (Jiménez, *et al*., 1994), and the texture of the later cultivar is currently higher than the former (García-Serrano *et al*., 2020), which could explain the lower weight changes found in the VI Hojiblanca cultivar during processing as black ripe olives (Figure 5) than in the VI Manzanilla (Figure 3). The application of VI to raw Hojiblanca olives only led to 4% weight gain in comparison to around 9% in Manzanilla fruit. In addition, weight changes were similar in all Hojiblanca olives after the storage period, regardless of VI treatment or calcium addition to the preservation solution (Figure 5). However, a weight gain of around 1% was detected after darkening in VI olives in comparison to around 2% loss in untreated olives, and these differences in weight even increased after packing, in particular when calcium was added to the preservation liquid so that around 4% higher weight was achieved in the VI Hojiblanca olives with calcium added than in untreated and non-added calcium olives. Any conclusion arising from these results can be explained by the importance of the olive cultivar in weight gain in the fruit after VI application and table olive elaboration.

It must also be noted that the color and flavor of the black olives were not affected by the use of VI on either the Manzanilla or Hojiblanca cultivar (data not shown). These quality parameters were evaluated by a sensory analysis carried out by 5 trained panelists.

3.3. Spanish-style green olives

The VI technique was also investigated for processing this commercial preparation (Figure 6). Unfortunately, the application of VI to Manzanilla olives softened the fruit to such a large extent after the alkaline treatment that it made them unmarketable (data not shown). In contrast, this softening effect was not observed for the Hojiblanca cultivar, which currently has a stronger texture than the Manzanilla, as mentioned above. In this case, VI Hojiblanca olives after fermentation presented around 6-7% weight gain while non-VI olives showed only around 2-3% weight gain. Again, the alkaline treatment led to an increase in the weight in both the control and VI olives which was reduced during the eight-month fermentation period. Thus, VI olives of the Hojiblanca cultivar had around 4% higher weight than

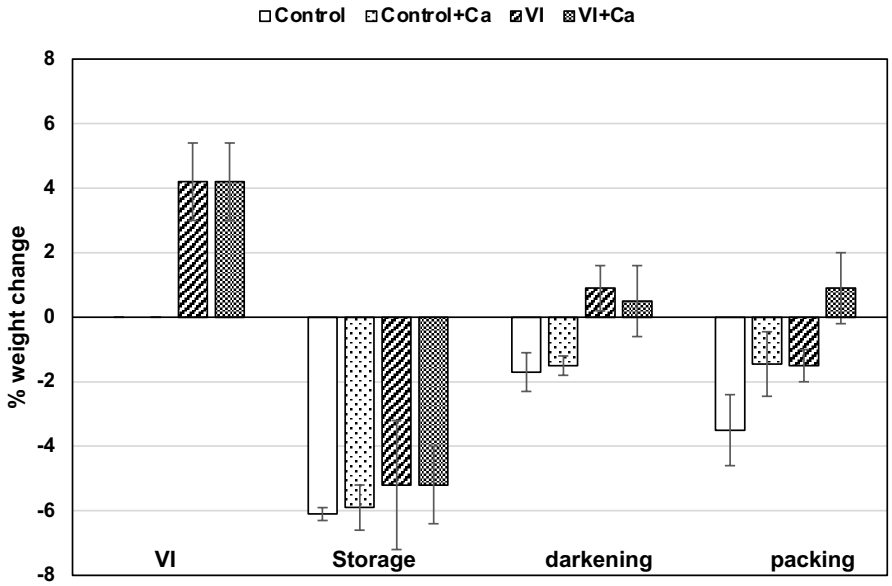

Figure 5. Effect of VI on the changes in olive weight of the Hojiblanca cultivar during its processing as black ripe olives. Calcium chloride was employed during the storage stage. Bars are the mean value of four independent olive lots elaborated in duplicate, and they represent the accumulated gain or lost olive weight throughout the whole process. Standard deviation is depicted on the bars.

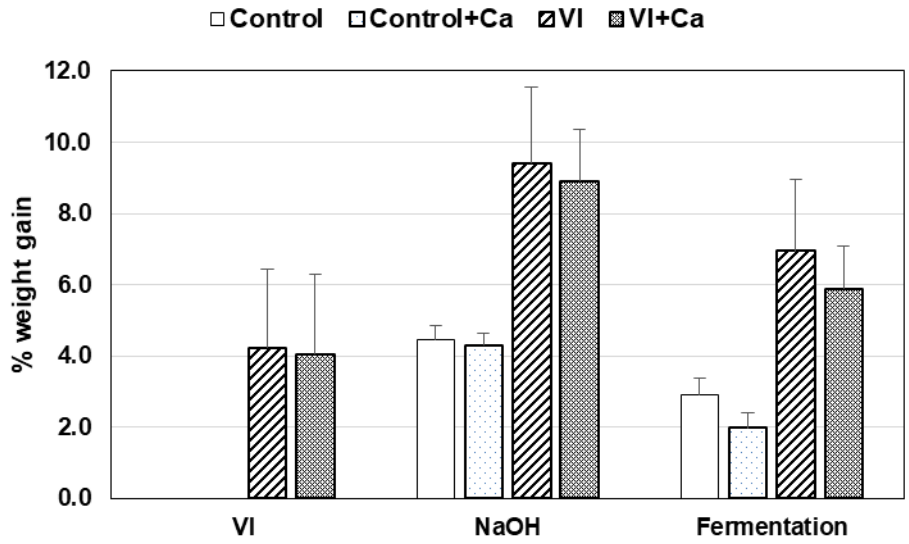

Figure 6. Influence of VI on the changes in olive weight of the Hojiblanca cultivar during its processing as Spanish-style green olives. Calcium chloride was added into the fermentation brine. Bars are the mean value of three independent olive lots elaborated in duplicate, and they represent the accumulated gain or lost olive weight throughout the whole process. Standard deviation is depicted on the bars. Bars followed by a different letter indicate significant difference according to Duncan´s test ($p < 0.05$).

untreated olives after fermentation, regardless of the calcium addition.

Finally, it is worth noting that the lactic acid fermentation was similar in the VI olives and control and the final flavor and color was not affected by the use of this technique to the raw olives (data not shown). These quality parameters were also evaluated by sensory analysis carried out by 5 trained panelists.

4. CONCLUSIONS

It has been determined that the application of VI to raw olives can contribute to the reduction in weight loss or even increase in weight during the processing of table olives. This increase in weight was cultivar dependent, higher for the Manzanilla cultivar than Hojiblanca, which could probably be attributed to the different texture and cell wall composition of these two cultivars. It must be pointed out that olives gained weight during the VI treatment, but the application of a vacuum also favored a higher weight increase in olives during alkaline treatment than in untreated olives. Consequently, VI olives of the Manzanilla and Hojiblanca cultivars gained around 10 and 4% weight, respectively, in comparison to untreated olives processed as black ripe olives. In the case of Spanish-style green olives, VI was ruled out for the Manzanilla cultivar because the firmness of the olives was too soft after the alkaline treatment. In contrast, the Hojiblanca cultivar could be processed following this elaboration method, and around 4% weight gain was found after the fermentation stage in comparison to untreated olives. In addition, the color and flavor of the olives were not affected by the use of VI. Therefore, VI seems a promising technique for processing table olives in order to reduce weight loss or even gain weight without loss in firmness in the final product, particularly with calcium addition.

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DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request

REFERENCES

Banjongsinsiri P, Shields J, Wicker L. 2004. Vacuum infusion of plant or fungal pectinmethylesterase

and calcium affects the texture and the structure of eggplants. *J. Agric. Food Chem.* **52**, 8214–8223. https://doi.org/10.1021/jf0487405.

Blanda J, Cerretani L, Bendini A, Cardinali A, Scarpellini A, Lercker G. 2008. Effect of vacuum impregnation on the phenolic content of Granny Smith and Stark Delicous frozen apple cvv. *Eur. Food Res. Technol.* **226**, 1239. https://doi.org/10.1007/s00217-007-0624-x.

Brenes M, García P, Garrido A. 1994. Influence of salts and pH on the firmness of olives in acidic conditions. *J. Food Quality* **17**, 335–346. https://doi.org/10.1111/j.1745-4557.1994.tb00155.x.

Brenes M, Romero C, García P. 2017. Optimization of ripe olive processing with a single lye treatment. *J. Food Sci.* **82**, 2078–2084. https://doi.org/10.1111/1750-3841.13827.

De Castro A, García P, Romero C, Brenes M, Garrido A. 2007. Industrial implementation of black ripe olive storage under acidic conditions. *J. Food Eng.* **80**, 1206–1212 (2007). https://doi.org/10.1016/j.jfoodeng.2006.09.010.

Derossi A, De Pilli T, Severini C. 2010. Reduction in the pH of vegetables by vacuum impregnation: A study on pepper. *J. Food Eng.* **99**, 9–15. https://doi.org/10.1016/j.jfoodeng.2010.01.019.

Derossi A, De Pilli T, La Penna MP, Severini C. 2011. pH reduction and vegetable tissue structure changes of zucchini slices during pulsed vacuum acidification. *LWT-Food Sci. Technol.* **44**, 1901–1907. https://doi.org/10.1016/j.lwt.2011.01.011.

Derossi A, De Pilli T, Severini C. 2012. The application of vacuum impregnation techniques in the food industry. In: Valdez B (ed), *Scientific, health and social aspects of the food industry*, pp 25-55, In Tech Europe.

Fito P, Andrés A, Chiralt A, Pardo P. 1996. Coupling of hydrodynamic mechanism and deformation relaxation phenomena during vacuum treatments on solid porous-liquid systems. *J. Food Eng.* **27**, 229–240. https://doi.org/10.1016/0260-8774(95)00005-4.

García P, Romero C, Brenes M. 2014. Influence of olive tree irrigation and the preservation system on the fruit characteristics of Hojiblanca black ripe olives. *LWT-Food Sci. Technol.* **55**, 403–407. https://doi.org/10.1016/j.lwt.2013.06.015.

García-Serrano P, Romero C, Medina E, García-García P, de Castro A, Brenes M. 2020. Effect of calcium on the preservation of green olives intended for black ripe olives under free sodium chloride conditions *LWT-Food Sci. Technol.* **118**, 108870. https://doi.org/10.1016/j.lwt.2019.108870.

Jiménez A, Guillén R, Fernández-Bolaños J, Heredia A. 1994. Cell wall composition of olives. *J. Food Sci.* **59**, 1192–1196. https://doi.org/10.1111/j.1365-2621.1994.tb14674.x.

López SV, Moreira RG. 2019. Increased phenolic compounds in potato chips vacuum impregnated with green tea. *J. Food Sci.* **84**, 807–817. https://doi.org/10.1111/1750-3841.14492.

Luo W, Tappi S, Patrignani F, Romani S, Lanciotti R, Rocculi P. 2019. Essential rosemary oil enrichment minimally processed potatoes by vacuum-impregnation. *J. Food Sci. Technol.* **56**, 4404–4416. https://doi.org/10.1007/s13197-019-03935-y.

Mújica-Paz H, Valdez-Fragoso A, Lopez-Malo A, Paloub E, Welti-Chanes J. 2003. Impregnation and osmotic dehydration of some fruits: Effect of the vacuum pressure and syrup concentration. *J. Food Eng.* **57**, 305–314. https://doi.org/10.1016/S0260-8774(02)00344-8.

Parlawska M, Stepién B, Nawirska-Olszanska A, Sala K. 2019. Studies on the effect of mass transfer in vacuum impregnation on the bioactive potential of apples. *Molecules* **24**, 3533. https://doi:10.3390/molecules24193533.

Parreidt TS, Schmid M, Müller K. 2018. Effect of dipping and vacuum impregnation coating techniques with alginate based coating on physical quality parameters of cantaloupe melon. *J. Food Sci.* **83**, 929-936. https://doi.org/10.1111/1750-3841.14091.

Quintanilla A, Mencía A, Powers J, Rasco B, Tang J, Sablani SS. 2018. Vacuum impregnation of firming agents in red raspberries. *J. Sci. Food Agric.* **98**, 3706–3714. https://doi.org/10.1002/jsfa.8878.

Romero C, Brenes M, García P, Garrido A. 1996. Respiration of olives stored in sterile water. *J. Hortic. Sci.* **71**, 739–745. https://doi.org/10.1080/14620316.1996.11515454.

Schulze B, Peth S, Hubbermannn EM, Schwarz K. 2012. The influence of vacuum impregnation on the fortification of apple parenchyma with quercetin derivatives in combination with pore structures X-ray analysis. *J. Food Eng.* **108**, 380–387. https://doi:10.1016/j.jfoodeng.2011.11.015.

Servillo L, Balestrieri ML, Giovane A, De Sio F, Cannavacciuolo M, Squitieri G, Ferrari G, Cautela D, Castaldo D. 2018. Improving diced tomato firmness by pulsed vacuum calcification. *LWT-Food Sci. Technol.* **92**, 451–457. https://doi.org/10.1016/j.lwt.2018.02.069.

Sirijariyawat A, Charoenrein S, Barret D. 2012. Texture improvement of fresh and frozen mangoes with pectin methylesterase and calcium infusion. *J. Sci. Food Agric.* **92**, 2581–2586. https://doi.org/10.1002/jsfa.5791.

Tamer CE, Incedayi B, Yildiz B, Çopur OU. 2013. The use of vacuum impregnation for debittering of green olives. *Food Bioprocess Technol.* **6**, 3604–3612. https://DOI 10.1007/s11947-012-0971-x.

Tylewicz U, Mannozzi C, Romani S, Castagnini JM, Samborska K, Rocculi P, Dalla Rosa M. 2019. Chemical and physicochemical properties of semi-dried organic strawberries enriched with bilberry juice-based solution. *LWT-Food Sci. Technol.* **114**, 108377. https://doi.org/10.1016/j.lwt.2019.108377.

Zhao Y, Xie J. 2004. Practical application of vacuum impregnation in fruit and vegetable processing. *Trends Food Sci. Technol.* **15**, 434–451. https://doi.org/10.1016/j.tifs.2004.01.008.

Zunin P, Turrini F, Leardi R, Boggia R. 2017. Olive fruits and vacuum impregnation, an interesting combination for dietetic iron enrichment. *J. Food Sci. Technol.* **54**, 481–487. https://doi 10.1007/s13197-017-2489-8.