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Phenolic antioxidants in coconut oil: Factors affecting the quantity and quality. A review

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SUMMARY: The total phenol content (TPC) in coconut oil varies with extraction method, variety, nature of coconut kernel components and geographical origin. Commonly reported TPCs of coconut oils extracted by dry methods and wet methods are in the range of 70-300 mg/kg and 250-650 mg/kg, respectively. Based on the commonly reported data, the TPC of coconut oil varies by up to 527 mg/kg oil, 180 mg/kg oil, and 172 mg/kg oil due to the influence of the extraction method, coconut variety and the nature of kernel components, respectively. The identity of the phenolic compounds also varies with the extraction method. Caffeic acid, catechin, *p*-coumaric acid, ferulic acid, and syringic acid are present in different quantities in coconut oil when extracted by all methods. However, chlorogenic acid, cinnamic acid, epigallocatechin, gallic acid, vanillic and epicatechin are present only in some coconut oils. Many free phenolic compounds present in olive oil are also present in coconut oil.

KEYWORDS: *Copra oil; Olive oil; Phenolic antioxidants; Virgin coconut oil.*

RESUMEN: ***Antioxidantes fenólicos en el aceite de coco: factores que afectan la cantidad y la calidad. Revisión.*** El contenido total de fenoles (CTF) del aceite de coco varía según el método de extracción, la variedad, la naturaleza de los componentes del grano de coco y el origen geográfico. Los CTF comúnmente reportados de aceites de coco extraídos por métodos secos y métodos húmedos están en el rango de 70-300 mg/kg y 250-650 mg/kg respectivamente. En base a estos datos comúnmente reportados, el CTF de los aceites de coco varía hasta 527 mg/kg de aceite, 180 mg/kg de aceite y 172 mg/kg de aceite debido a la influencia del método de extracción, la variedad del coco y la naturaleza de los componentes del grano, respectivamente. La identidad de los compuestos fenólicos también varía con el método de extracción. El ácido caféico, la catequina, el ácido *p*-cumárico, el ácido ferúlico y el ácido siríngico están presentes en diferentes cantidades en los aceites de coco extraídos por todos los métodos. Sin embargo, el ácido clorogénico, el ácido cinámico, la epigalocatequina, el ácido gálico, la vainillina y la epicatequina están presentes solo en algunos aceites de coco. Muchos compuestos fenólicos libres que están presentes en el aceite de oliva también están presentes en el aceite de coco.

PALABRAS CLAVE: *Aceite de copra; Aceite de oliva; Aceite virgen de coco; Antioxidantes fenólicos.*

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1. INTRODUCTION

Natural phenolic substances are ubiquitously-distributed secondary metabolites in berries, fruits, vegetables, coffee, herbs and edible oils. Main phenolic substances include phenolic acids, phenolic alcohols and flavonoids. Plant phenolic compounds have drawn the attention of researchers due to their antioxidant properties. The antioxidant mechanisms of the phenolic compounds in plants have been well established (Michalak, 2006; Zeb, 2020). The antioxidant properties of the phenolic compounds in plants are connected to beneficial health effects such as conferring protection against the development of cancer, diabetes, cardiovascular diseases and neurodegenerative diseases (Pandey and Rizvi, 2009). The phenolic compounds in plants can also act as food preservatives that can inhibit the lipid oxidation in edible oils and fish oils owing to their high polyunsaturated fat contents (Maqsood *et al.,* 2014). The color and flavor properties of natural foods as well as processed foods and beverages are also controlled by plants' phenolic compounds (Cheynier, 2012).

During the extraction of edible oils, the phenolic compounds of kernel materials are incorporated into the oils. Edible oils have saponifiable and unsaponifiable compounds. The saponifiable fraction may account for 90–98% of the total mass of oil while the unsaponifiable content varies from 2–10% (Narasinga Rao, 2001). The saponifiable fraction includes triglycerides, diglycerides, monoglycerides, fatty acids and other saponifiable lipids. The minor unsaponifiable fraction includes phenolic compounds, flavonoids, vitamin E, sterols, hydrocarbons, etc. (Moura Fe *et al.,* 1975). In coconut oil, the saponifiable fraction may amount to up to 99.5% of the weight of the oil (Gutfinger and Letan, 1974). In edible oils with no sedimentations or insoluble materials, the saponifiable fraction can be observed as a clear solution and unsaponifiable compounds are dissolved in the saponifiable fraction.

Phenolic compounds in several edible oils have been reported and most of these studies are limited to assessing phenolic compounds in a particular oil (Mannino *et al.,* 1999; Tripoli *et al.,* 2005; Siger *et al.,* 2008; Janu *et al.,* 2013). Among them, a vast majority of research has been focused on the phenolic fraction of olive oil (Kiritsakis, 1998; Ryan and Robards, 1998; Boskou *et al.,* 2005; Galvano *et al.,* 2007; Servili *et al.,* 2009). In addition to the studies on the phenolic compounds in specific edible oils, a recent report has comprehensively reviewed the different classes of phenolic compounds in several edible oils (Zeb, 2021). As phenolic compounds are polar molecules, their solubility in the oils is low. However, these minor components improve the quality of edible oils by enhancing health benefits and sensory properties (Visioli and Galli, 1998). The interest in the phenolic substances in coconut oil is also due to their antioxidant activity and related health benefits (Seneviratne *et al.,* 2011; Lima *et al.,* 2015; Narayanankutty *et al.,* 2018; Rohman *et al.,* 2021; Senanayake *et al.,* 2021). In addition, the phenolic compounds present in coconut oil and coconut oil meal improve the shelf-life of coconut oil as well as other edible oils and baked food (Senanayake *et al.,* 2019). Even though there is a wealth of literature regarding research conducted on the phenolic substances of olive oil and other common edible oils, the first report on the phenolic substances in coconut oil was published in 2008 (Seneviratne and Dissanayake, 2008). Since then, there has been a growing number of studies on the phenolic fraction of coconut oil. Emulsions of virgin coconut oil (VCO) containing ferulic acid and *p*-coumaric acid have been prepared with various sweeteners to improve the palatability of VCO (Wiyani *et al.,* 2020). The addition of VCO to dark chocolate formulations has shown that the nutritional properties of dark chocolate are improved by the phenolic compounds in VCO (Rashid *et al.,* 2017). New studies indicate that coconut oil improves the absorption of phenolic compounds in rats and humans, suggesting that the naturally-present phenolic compounds in coconut oil are more bioavailable than the phenolic compounds in aqueous foods (Prasadani *et al.,* 2017; Weerakoon *et al.,* 2021). Despite several health and nutritional advantages and applications as food preservatives, the quality and quantity of the phenolic compounds in coconut oil have not been reviewed. Therefore, the present review aimed to comprehensively review the up-to-date knowledge on the effect of extraction conditions, variety, country of origin and nature of kernel material on the phenolic composition of coconut oil.

2. EXTRACTION METHODS AND PHENOLIC CONTENTS

The most important factor affecting the phenolic content in coconut oil is the method of extraction (Seneviratne and Jayathilaka, 2015; Seneviratne and Jayathilaka, 2016). The coconut kernel is different from all other seed kernels due to its bulkiness and high percentage (59%) of coconut oil in the dried kernel (Gutfinger and Letan, 1974). Therefore, the extraction of coconut oil is less cumbersome compared to the extraction of other edible oils. Different methods of coconut oil extraction are summarized in Figure 1. Coconut oil prepared by the wet extraction methods under cold conditions is called VCO. In addition, VCO is also produced by dry extraction by pressing high quality dried coconut kernels under cold conditions.

2.1. Dry methods of coconut oil extraction

Dry methods involve a drying step of coconut kernels before the extraction of coconut oil. Fresh coconut kernels can be dried by sun drying or by any suitable mechanical drying method. Fresh coconut kernel contains carbohydrates (13.0 g/100 g), water (36.3 mL/100 g), proteins (4.5 g/100 g), lipids (41.6 g/100 g), fiber (3.6 g/100 g) and minerals (1 g/100 g) (Lal *et al.,* 2003). Different quantities of water and lipids up to 50 and 34%, respectively, have been reported in coconut kernels, (Withana-Gamage *et al.,* 2005). In the dry methods, coconut oil is extracted by pressing dried coconut kernels to squeeze out coconut oil. For this purpose, the moisture content of the coconut kernel should not exceed 6% (Seneviratne and Jayathilaka, 2016). Coconut oil extracted in this way is also called copra oil. VCO is also produced by this dry method by pressing dried coconut kernels under cold conditions without letting the temperature rise above 60–70 ºC during the pressing process. While pressing dried coconut kernels, a part of the phenolic substances in the coconut kernel is incorporated into the coconut oil. The TPC of the coconut oil extracted by dry methods is found to be relatively lower compared to that of coconut oil extracted by wet methods (Seneviratne *et al.,* 2009; Srivastava *et al.,* 2016).

2.2. Wet methods of coconut oil extraction

In addition to the dry method, coconut oil is extracted by wet extraction methods where an aqueous emulsion of coconut kernel (coconut milk) is processed for the extraction of coconut oil. Coconut milk contains fat, sugars, proteins and phenolic compounds (Alyaqoubi *et al.,* 2015; Nadeeshani *et al.,* 2015). Gallic acid, chlorogenic acid, *p*-hydroxybenzoic acid, caffeic acid, vanillic acid, syringic acid and ferulic acid are the major phenolic compounds in coconut milk (Karunasiri *et al.,* 2020a).

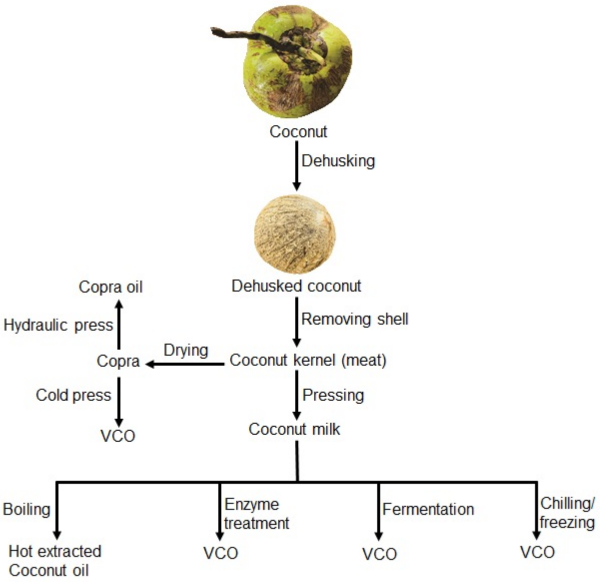

Figure 1. Different extraction methods of coconut oil

2.2.1. Boiling method (Hot extraction)

Coconut milk is boiled to evaporate water. In this process, proteins denature and carbohydrates and proteins are deposited at the bottom of the container. When the aqueous layer evaporates, the oil layer can easily be separated by decanting. During this boiling process, the temperature of the mixture may reach up to 125 ºC. Due to high temperatures, the solubility of the phenolic substances in coconut oil increases. While the oil layer is in contact with the solid kernel material at the bottom of the container, phenolic substances are easily dissolved in the oil layer. Therefore, coconut oil prepared by boiling coconut milk has a high TPC and contains a larger number of phenolic substances compared to coconut oil prepared by dry extraction methods (Seneviratne *et al.,* 2009).

2.2.2. Chilling, freezing, centrifugation methods

After chilling or freezing coconut milk, the water can be removed by decanting followed by centrifugation of the resultant mixture in the liquid form to further remove water. Direct centrifugation of coconut milk emulsion without chilling or freezing to separate coconut oil can also be done. Heating to about 60 ºC prior to centrifugation is effective in isolating oil. However, this direct centrifugation needs stronger and laborious centrifugation steps (Gopala Krishna *et al.,* 2010; Ngampeerapong *et al.,* 2018). Chilling to about 10 ºC or freezing to about –4 ºC for 6 hours, followed by centrifugation is more effective in producing VCO.

2.2.3. Fermentation

Pure cultures of *Lactobacillus* strains have been used in some cases for the fermentation of coconut milk to separate coconut oil (Che Man *et al.,* 1997; Satheesh and Prasad, 2014). However, fermentation also occurs without any addition of microorganisms if coconut milk is exposed to air for over 8–12 hours or longer (Marina *et al.,* 2009a). Due to enzyme and microbial action, coconut oil separates from the coconut milk emulsion (Seneviratne and Jayathilaka, 2015). The coconut oil layer can then be siphoned out after the separation of layers.

Copra oil is refined to remove any free fatty acids and peroxides and then bleached and deodorized. Refined, bleached and deodorized coconut oil is called RBD coconut oil. The TPCs reported as gallic acid equivalents (GAE) in coconut oils prepared by different extraction methods are given in Table 1.

3. PHENOLIC CONTENT AND COCONUT VARIETY

Most studies on the phenolic composition of coconut varieties are limited to those done on a particular variety and there are limited studies that compare the differences in composition among the different varieties. The TPC of the VCO extracted by freezing coconut milk from West African Tall variety is 160.2 mg/kg. Methanol (60%) was used to extract phenolic compounds in this study (Ahmad *et al.,* 2015). Coconut kernels of Dwarf x Tall variety from India indicate the presence of coumaric acid, caffeic acid and chlorogenic acid. (Dhanyakrishnan *et al.,* 2018). The TPC of coconut water from the Malayan green coconut variety is 95.15 mg/L; while that of the Malayan yellow coconut variety is 46.85 mg/L. (Adubofuor *et al.,* 2016). Even though there is a limited number of reports on the phenolic composition of coconut oil from different coconut cultivars, most of such data cannot be compared because of the different conditions used for the extraction of coconut oil or different extraction conditions used for the extraction of the phenolic substances from the coconut oil. A systematic comparison of the TPC in coconut oil extracted from three coconut varieties and three hybrids of these varieties grown in Thailand has been reported (Arlee *et al.,* 2013). According to this study, the TPC of VCO extracted by natural fermentation may vary significantly among cultivars and hybrids. However, the range of TPC in coconut oil extracted by the fermentation method for three cultivars and three hybrids is 481–554 mg/kg. The TPC in VCO prepared by cold pressed dry method may also vary significantly ($p \leq 0.05$) among varieties and cultivars. Nevertheless, the range of TPC is 486–579 mg/kg. The TPC in VCO from two cultivars grown in Mexico also shows that there is a significant difference in TPC with cultivar. The TPC in this study varies in the range 600–780 mg/kg (Elodio-Policarpo *et al.,* 2019).

4. EFFECT OF GEOGRAPHICAL ORIGIN ON PHENOLIC CONTENT

VCO has become popular as a functional oil due to its health benefits. The major contributor to the numerous health benefits is phenolic compounds (Srivastava *et al.,* 2018). Thailand, Indonesia, India, Malaysia, Sri Lanka and Vietnam are the main producers of VCO in the world. Even though phenolic compounds play a major role in providing health benefits to consumers, phenolic quality and quantity are not included in coconut oil standards. Detailed studies on the influence of geographical origin on the TPC in coconut oil have not been carried out. However, VCO samples obtained from the markets of Malaysia and Indonesia have been compared (Marina *et al.,* 2009b). The results indicated that the TPCs in the Malaysian VCO samples were 118–292 mg/kg; while those of the Indonesian VCO samples were 78–251 mg/kg.

5. NATURE OF COCONUT KERNEL COMPONENTS

There are two components in the coconut kernel: white coconut meat and coconut testa. The interior part of the coconut kernel is the thick, fleshy, white

TABLE 1. TPCs in coconut oils prepared by different extraction methods expressed as gallic acid equivalents (GAE)

	TPC (Reported Units)	TPC (mg/kg oil)	Reference
Dry methods			
Dry method (copra oil)	72.1 ± 5.6 mg/kg	72 ± 6	Seneviratne and Dissanayake, 2005
	91 ± 11 mg/kg	91 ± 11	Seneviratne and Dissanayake, 2008
	64.4 mg/100g	644	Arunima and Rajamohan, 2013; Nevin and Rajamohan, 2004
	292.06 ± 10.04 mg/kg	292 ± 10	Azevedo *et al.*, 2020
	1.56-8.57 mg/g	1560-8570	Ghani *et al.*, 2018
	182.82 ± 15.24 µg/g	183 ± 15	Srivastava *et al.*, 2016
	18.1 ± 2.01 mg/100g	181 ± 20	Narayanankutty *et al.*, 2016
Dry method (VCO)	1.18 mg/g	1180	Perera *et al.*, 2019
	49.82 mg/kg	49.82	Khalil *et al.*, 2020
Wet methods			
Wet method (Boiling)	506 ± 20 mg/kg	506 ± 20	Seneviratne and Dissanayake, 2005
	618 ± 46 mg/kg	618 ± 46	Seneviratne and Dissanayake, 2008
	650.35 ± 25.11 µg/g	650 ± 25	Srivastava *et al.*, 2016
Wet method (Fermentation) VCO	59.30 ± 0.39 mg/g	59,300 ± 390	Prapun *et al.*, 2016
	59.44 ± 13.40 mg/100g	594 ± 134	Ngampeerapong *et al.*, 2018
	57.11 ± 0.05 mg/100g	571 ± 1	Famurewa *et al.*, 2018
	12.54 ± 0.96 mg/g	12,540 ± 960	Ghani *et al.*, 2018
	~25 mg/100g	~250	Marina *et al.*, 2009a
	401.23±20.11µg/g	401 ± 20	Srivastava *et al.*, 2016
Chilling/freezing and Centrifugation	~18 mg/100g	~180	Marina *et al.*, 2009a
	1.16 ± 0.05 mg/g	1160 ± 50	Ghani *et al.*, 2018
	16.02 ± 0.44 mg/100g	160 ± 4	Ahmad *et al.*, 2015
	84 mg/100g	840	Arunima and Rajamohan, 2013; Nevin and Rajamohan, 2004
	32.24 ± 1.2 mg/100 g	322 ± 12	Narayanankutty *et al.*, 2016
Centrifugation	Not detected		Ngampeerapong *et al.*, 2018
Enzyme-assisted	35.02 ± 0.10 mg/g	35,020 ± 100	Prapun *et al.*, 2016
RBD coconut oil	~12 mg/100g	~120	Marina *et al.*, 2009a
	6.14 mg/100g	61	Marina *et al.*, 2009b
	2.1 ± 0.19 mg/100g	21 ± 2	Prasanth Kumar *et al.*, 2015

coconut kernel, which is known as white coconut meat. The thin brown outer skin of the white coconut kernel between the white coconut meat and the coconut shell is known as coconut testa. When water evaporates from the coconut meat during the drying of coconut halves, white coconut meat and testa separates from the shell (Figure 2). Both testa and coconut meat are taken for dry or wet extraction of coconut oil unless coconut testa is purposely removed. The TPCs in coconut meat from three coconut types from Indonesia, Vietnam and Thailand have been compared (Ngampeerapong and Chavasit, 2019). Shredded coconut meat was extracted with 80% methanol. This study reported that the TPCs of coconut meat were 72,122 mg/kg (Thailand variety), 63,910 mg/kg (Indonesian variety) and 103,421 mg/kg (Vietnam variety). However, the same paper indicated that the percentages of moisture + protein + fat + carbohydrates of coconut meat added up to 99.09% (Thailand variety), 99.01% (Indonesian variety) and 99.06% (Vietnam variety). Therefore, the phenolic contents of ~7% (Thailand variety), ~6% (Indonesian variety) and 10% (Vietnam variety) may not be realistic. The moisture content in the coconut meat was about

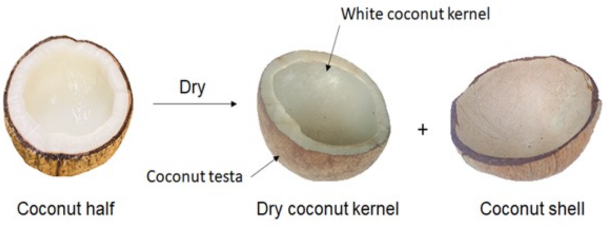

FIGURE 2. Coconut kernel components and coconut shell

50% according to the analysis of this research. Coconut kernels contain free amino acids (Baptist, 1963). These amino acids interfere with the determination of TPC by the Folin-Ciocalteu method (Everette *et al.,* 2010; Bastola *et al.,* 2017). Sugars also interfere with the Folin-Ciocalteu method (Sanchez-Rangel *et al.,* 2013; Muñoz-Bernal *et al.,* 2017). Proteins in coconut meat can be precipitated prior to the determination of TPC in coconut milk to avoid interference (Kwon *et al.,* 1996; Nadeeshani *et al.,* 2015). Interference by amino acids, proteins and sugars in the coconut meat may contribute to the high readings of TPCs in some studies. However, the reported phenolic contents of 98–100 mg/kg for coconut meat may be more reasonable (Mahayothee *et al.,* 2016).

6. COCONUT TESTA

Coconut testa is a very minor component of coconut meat. It is difficult to give an exact estimate of the phenolic contents in pure coconut testa since part of white coconut meat also combines with coconut testa during the paring of coconut meat to separate testa. Coconut testa is the richer source of phenolic compounds compared to white coconut meat (Seneviratne *et al.,* 2009; Seneviratne *et al.,* 2016). A study indicates that the TPC in the coconut oil extracted from the whole copra with both white kernel and brown testa is 1.4 ± 0.19 mg/100 g (14 ± 2 mg/kg); while coconut oils extracted from white coconut kernel alone and coconut testa alone contain 1.1 ± 0.11 mg/100 g (11 ± 1 mg/kg) and 1.9 ± 0.12 mg/100 g (19 ± 1 mg/kg) of phenolic substances, respectively (Appaiah *et al.,* 2014). Reports indicate that the extraction efficiency of phenolic compounds from coconut testa significantly ($P \leq 0.05$) varies with the solvent system, while the acidification of the solvent increases the extraction efficiency. For example, the extraction of phenolic compounds from dry and defatted testa by 80% ethanol gives TPC of 72.7 ± 1.9 mg/g (72,700 ± 1900 mg/kg); while the extraction of dry and defatted testa with acidified 80% ethanol gives TPC of 93.9 ± 5.3 mg/g (93,900 ± 5,300 mg/kg) (Arivalagan *et al.,* 2018). Phenolic compounds were extracted from powdered coconut testa with methanol using Soxhlet extraction. The reported phenolic quantities were unusually high with over 80% (822 mg/g) of the weight of the plant extract being phenolic compounds (Ojha *et al.,* 2019). If these phenolic quantities were expressed for coconut testa powder instead of plant extract, a better comparison could have been made with other reported values. The phenolic composition of coconut testa also varied with the coconut cultivar. The TPCs in coconut testa powder produced from four different coconut cultivars grown in Sri Lanka, namely San Raman, Gon Thembili, Ranthembili and Tall x Tall varied in the range of 27,530–62,580 mg/kg due to cultivar (Marasinghe *et al.,* 2019).

In both the wet and dry processing of coconut kernels for oil extraction, both coconut meat and testa can be used. Even though coconut testa is the main source of phenolic substances in coconut kernels, due to its brown color, coconut testa adds a light-yellow color to the coconut oil. Colorless coconut oil is commercially preferred. Therefore, coconut testa is removed during the preparation of desiccated coconut as well as in the large-scale preparation of VCO by both wet and dry methods.

The TPCs in coconut oil extracted from only white coconut kernels and white coconut kernels with coconut testa under wet hot extraction conditions were 78 ± 2 and 250 ± 4 mg/kg, respectively (Seneviratne *et al.,* 2009). Higher phenolic contents in the coconut oil extracted by using white kernels with coconut testa was due to the richer phenolic contents in testa compared to white coconut kernels.

The phenolic contents in coconut kernel components (white coconut meat and testa) are not fully incorporated into coconut oil during wet or dry extraction methods. Therefore, the residue after the expulsion of coconut oil from coconut kernel components is also rich in phenolic antioxidants (Illam *et al.,* 2017; Karunasiri *et al.,* 2020b).

7. VARIATION OF REPORTED TPC VALUES

Table 1 shows that the TPC clearly depends on the method of extraction of coconut oil. According to Table 1, there is a wide range of TPC reported by different authors for copra oil. The range of TPC in copra oil is 91–644 mg/kg, based on the reported studies (Nevin and Rajamohan, 2004; Seneviratne and Dissanayake, 2005; Seneviratne and Dissanayake, 2008; Arunima and Rajamohan, 2013; de Azevedo *et al.,* 2020). However, the TPC values reported by Ghani *et al.* (2018) for dry extracted coconut oil, the TPC values reported by Prapun *et al.* (2016) and by Ghani *et al.* (2018) for VCO extracted by fermentation methods and the TPC value reported for VCO prepared by the enzymatic extraction by Prapun *et al.* (2016) seem unusually higher than the values reported by other researchers. Even though the phenolic contents depend on many factors other than the extraction method, these extremely high phenolic contents compared to the other reported values may not be reasonably explained. The TPC in coconut oil prepared by oven-dried grated coconut meat and sun-dried grated coconut meat were reported in one study as 1.56 ± 0.24 mg/g (1560 ± 240 mg/kg) and 8.57 ± 0.36 mg/g (8570 ± 360 mg/kg), respectively (Ghani *et al.,* 2018). The values are higher than any other reported values for coconut oil prepared by dry methods (Table 1). The difference in phenolic contents between coconut oils prepared by oven-dried and sun-dried coconut meat was attributed to the possibility of destroying phenolic substances during the drying process. However, the temperature used in the drying process was 40 ºC in this study. Even higher temperatures up to 60–70 ºC were used in the production of VCO and thermally unstable compounds were not affected by 60–70 ºC temperatures. The TPC in coconut oil varied in the order: fermentation > chilling > RBD, suggesting that the steps involved in the RBD process removed some phenolic substances (Marina *et al.,* 2009a).

If unusually high values for TPC were omitted and if extraction method was considered the only variable, the maximum additional phenolic content that could be incorporated into coconut oil by changing the extraction method compared to the extraction method that gave the lowest phenolic content is about 527 mg/kg (Seneviratne and Dissanayake, 2008). Therefore, up to 527 mg/kg phenolic compounds can be incorporated into coconut oil by changing the extraction method. Not many studies have been conducted to evaluate how phenolic compounds in coconut oil vary with the variety of coconut. The limited data available for the variation of TPC with variety is up to 180 mg/kg. The influence of geographical origin on the TPC cannot be computed due to a lack of sufficient relevant data. TPC may change by up to 172 mg/kg due to the influence of the nature of coconut kernel components based on the reported values (Seneviratne *et al.,* 2009).

8. COMPOSITION OF PHENOLIC COMPOUNDS IN COCONUT OIL ACCORDING TO EXTRACTION METHOD

The individual phenolic antioxidants in coconut oil have been identified and quantified. Caffeic acid, *p*-coumaric acid, ferulic acid, catechin, dihydrokaempferol, rosmarinic acid and quercetin were identified by mass spectroscopy coupled with liquid chromatography (LC-MS) (Seneviratne and Dissanayake, 2008; Illam *et al.,* 2017). The structures of commonly reported phenolic compounds present in coconut oil are given in Figure 3. The quantities of the individual phenolic compounds were higher in coconut oil prepared by boiling coconut milk (hot extracted coconut oil) compared to copra oil prepared by pressing coconut copra (Seneviratne *et al.,* 2009; Srivastava *et al.,* 2016). The TPC was also higher in hot extracted coconut oil compared to VCO prepared by fermentation (Table 1) (Seneviratne *et al.,* 2009; Srivastava *et al.,* 2016). In the hot extraction method, coconut milk emulsion is heated for a long time till water in the emulsion evaporates. During this evaporation, water and oil layers separate and with the evaporation of water, hydrophilic phenolic compounds are concentrated in the aqueous phase. This allows higher partitioning of phenolic antioxidants in the oil layer. The high temperature used in the hot extraction method favored the dissolving of phenolic compounds in the oil layer (Seneviratne *et al.,* 2009). Consistent with the higher

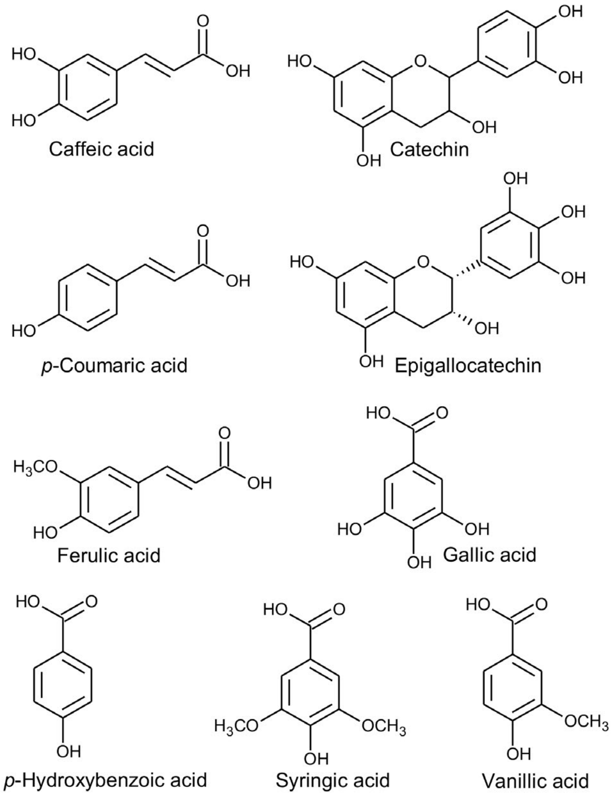

FIGURE 3. Commonly found phenolic compounds in coconut oil

TABLE 2. Phenolic compounds in coconut oil extracted by different methods

	Phenolic content mg/kg*			
Phenolic compound	Copra oil	Boiling	Fermentation	Chilling/centrifugation
Caffeic acid	0.0052 ± 0.0004 (Arunima and Rajamohan, 2013)	4.6 ± 1.5 (Seneviratne *et al.,* 2009) 1.59 ± 0.12 (Srivastava *et al.,* 2016)	0.12 ± 0.1 (Marina *et al.,* 2009a)	0.0083 ± 0.0007 (Arunima and Rajamohan, 2013)
Catechin	0.0062 ± 0.0006 (Arunima and Rajamohan, 2013) 0.21 ± 0.02 (Srivastava *et al.,* 2016)	81.7 ± 22.7 (Seneviratne *et al.,* 2009) 18.15 ± 0.93 (Srivastava *et al.,* 2016)	12.35 ± 1.03 (Srivastava *et al.,* 2016)	0.0098 ± 0.0007 (Arunima and Rajamohan, 2013)
Chlorogenic acid		1.55 ± 0.11 (Srivastava *et al.,* 2016)		
Cinnamic acid	0.024 ± 0.009 (Appaiah *et al.,* 2014)	-		
p-Coumaric acid	0.100 ± 0.007 (Appaiah *et al.,* 2014) 0.049 ± 005 (Arunima and Rajamohan, 2013)	0.53 ± 0.06 (Srivastava *et al.,* 2016)	0.75 ± 0.1 (Marina *et al.,* 2009a)	0.55±0.3 (Marina *et al.,* 2009a) 0.054 ± 0.005 (Arunima and Rajamohan, 2013)
Epigallocatechin		26.7 ± 1.7 (Seneviratne *et al.*, 2009)		
Epicatechin		1.4 ± 0.6 (Seneviratne *et al.,* 2009) 2.62 ± 0.24 (Srivastava *et al.,* 2016)		
Ferulic acid	0.017 ± 0.005 (Appaiah *et al.,* 2014) 0.020 ± 0.002 (Arunima and Rajamohan, 2013)	22.1 ± 8.9 (Seneviratne *et al.,* 2009) 12.83 ± 0.94 (Srivastava *et al.,* 2016)	5.09 ± 2.3 (Marina *et al.,* 2009a) 2.36 ± 0.32 (Srivastava *et al.,* 2016)	5.04±3.0 (Marina *et al.,* 2009a) 0.099 ± 0.009 (Arunima and Rajamohan, 2013)
Gallic acid	0.247 ± 0.012 (Appaiah *et al.,* 2014) 1.06 ± 0.05 (Srivastava *et al.,* 2016)	20.2 ± 10.1 (Seneviratne *et al.,* 2009) 25.29 ± 1.11 (Srivastava *et al.,* 2016)	18.01 ± 1.16 (Srivastava *et al.,* 2016)	
p-Hydroxybenzoic acid	0.076 ± 0.001 (Appaiah *et al.,* 2014)	4.8 ± 1.0 (Seneviratne *et al.,* 2009)		
Syringic acid	0.179 ± 0.004 (Appaiah *et al.,* 2014) 2.45 ± 0.2 (Arunima and Rajamohan, 2013)	4.1 ± 0.9 (Seneviratne *et al.,* 2009)	0.45±0.3 (Marina *et al.,* 2009a)	0.90±0.1 (Marina *et al.,* 2009a) 25.1 ± 2.3 (Arunima and Rajamohan, 2013)
Vanillic acid	0.638 ± 0.003 (Appaiah *et al.,* 2014)	-	2.08 ±1.4 (Marina *et al.,* 2009a) 1.03 ± 0.06 (Srivastava *et al.,* 2016)	

*Units were converted where appropriate

TPC of hot extracted coconut oil, the number and the quantity of the phenolic compounds were also higher in hot extracted coconut oil (Table 2) (Seneviratne *et al.,* 2009; Srivastava *et al.,* 2016). In addition to the results in Table 2, another author also reported 4.04 x10^{-2} ppm gallic acid in the phenolic extracts of VCO with no information on the extraction method (Librado and Von Luigi, 2013). Other reports have also indicated that the quantities of individual phenolic acids in coconut oil vary with extraction method (Marina *et al.,* 2009a). For example, Table 2 shows that the quantities (mg/kg) of phenolic compounds in coconut oils prepared by fermentation method and by chilling/centrifugation method contain different amounts of individual phenolic compounds. Vanillic acid and caffeic acid were not detected in VCO prepared by chilling and centrifugation; while those two phenolic compounds were detected in coconut oil prepared by fermentation. Catechin and epigallocatechin are the common flavonoids present in coconut oil (Table 2). In addition, quercetin (1.62 ± 0.09 mg/kg) has been reported in hot extracted coconut oil (Srivastava *et al.,* 2016). Myricetin-3-*o*-glucoside has also been reported to be present in VCO with no clear origin (Illam *et al.,* 2017).

9. REFINING AND PHENOLIC CONTENT

RBD coconut oil is prepared by procedures involving chemical and physical steps. In physical refining, degumming is done in the first step by treating the oil with phosphoric acid to remove phospholipids. Then the oil is heated to 80–90 °C and bleached by passing through a mixture of bleaching earth and activated carbon. Finally, the deodorizing and removal of free fatty acids are done by exposing the oil to a vacuum at 220–240 °C. The main difference in chemical refining is that free fatty acids are removed by treating the oil with a pre-determined amount of NaOH to neutralize fatty acids instead of removing free fatty acids under vacuum distillation. The resultant sodium salts in fatty acids (soap) are washed away with water. Refining, bleaching and deodorization remove most of the phenolic substances, tocopherols and sterols from coconut oil (Liu *et al.,* 2019a; Deen *et al.,* 2021). One study indicated that the TPC in crude copra oil was 618 mg/kg. However, the remaining TPC of this oil after the RBD process was 20 mg/kg (Pavan Kumar *et al.,* 2018). The TPCs in commercial RBD coconut oil purchased from the Indian market were 21 ± 2 mg/kg; while those in crude oil samples were in the range of 27–191 mg/kg. The maximum total phenol content reported for RBD oil is 120 mg/kg, according to Table 1. Some studies also reported almost zero (0.1 ± 0.0 mg/kg) TPC for RBD coconut oil (Liu *et al.,* 2019b). Individual phenolic compounds are also different in RBD coconut oil compared to other coconut oils. For example, the vanillic and syringic acids present in coconut oil extracted by fermentation are absent in RBD coconut oil. The ferulic acid content present in coconut oil extracted by the fermentation method is 5.09 ± 2.3 mg/kg; while RBD coconut oil contained only 1.39 ± 0.2 mg/kg (Marina *et al.,* 2009a). Therefore, the nutritional properties expected from the phenolic antioxidants present in crude or virgin oils may not be fully expected from RBD oils. Due to the removal of phenolic compounds, RBD coconut oil is less stable against oxidation compared to VCO (Koh and Long, 2012). RBD also has different sensory properties compared to crude coconut oil or VCO due to the removal of phenolic compounds and different volatile organic matter (Chang *et al.,* 2020).

10. COMPARISON WITH OLIVE OIL PHENOLICS

The TPCs in olive oil have been reported in numerous publications. Commonly reported ranges include 45-532 mg/kg (Mannino *et al.,* 1999; Bayram *et al.,* 2012). The TPC in the olive oil of three Italian varieties show significant ($p \leq 0.05$) differences and changes in the range of 367–530 mg/kg (Sicari, 2017); while the TPC of some traditionally prepared Turkish olive oils from different olive varieties ranges from 22.5 to 97.1 mg/kg (Tanilgana *et al.*, 2007). The TPCs in the olive oils from six Italian olive varieties prepared by a novel cooling treatment of olive paste varied in the range of 410–1005 mg/kg (Veneziani *et al.,* 2017; Veneziani *et al.,* 2018). Olive oil from two Croatian olive varieties, Bianchera and Busa, contained 312 and 248 mg/kg, respectively (Skevin *et al.,* 2003). Mean TPC and maximum TPC in olive oil from olive varieties in Greece were 483 mg/kg and 4003 mg/kg, respectively (Diamantakos *et al.,* 2021). Due to the differences in phenolic profiles, the fingerprinting of phenolic composition with suitable chemometric methods can be used to differentiate the varietal origin of olive oil (Bajoub *et al.,* 2017).

Geographical conditions also play an important role in the TPC of olive oil. The TPC in the same olive variety drastically changed from 513–1084 with the geographical area (Mansour *et al.,* 2017). European olive varieties grown in Tunisia produced different phenolic profiles compared to the traditional growing areas and the most important factor affecting the phenolic composition was suggested to be altitude (Dabbou *et al.,* 2009). In addition to the olive variety and geographical origin, the ripening stage of the olives and the crushing method also affect the phenolic composition according to studies done in Italy and Spain (Giovacchino *et al.,* 2002; Navajas-Porras *et al.,* 2020).

Several phenolic compounds present in coconut oil such as caffeic acid, ferulic acid, gallic acid, *p*-hydroxybenzoic acid, syringic acid, vanillic acid, *p*-coumaric and cinnamic acid have been reported to be present in olive oil as well (Ryan and Robards, 1998; Boskou *et al*., 2005). In addition to free phenolics and flavonoids, several bound phenolic compounds are also present in olive oil (Servili and Montedoro, 2002; Jimenez-Lopez *et al.,* 2020). However, only three bound phenolic compounds have been reported so far for coconut oil (Seneviratne *et al.,* 2009; Illam *et al*., 2017). The phenolic compounds in both olive oil and coconut oil are responsible for beneficial health effects (Covas *et al.,* 2006; Seneviratne and Jayathilaka, 2015; Narayanankutty *et al.,* 2018; Deen *et al.,* 2021). The aroma and flavor of olive oil correlate with the phenol content (Kiritsakis, 1998; Genovese *et al.,* 2018; Pedan *et al.,* 2019). Even though the sensory properties of virgin coconut oil have been studied (Villarino, Dy and Lizada, 2007, Lukic *et al.,* 2017; Fiorini *et al.,* 2018), the correlation between phenolic compounds and sensory properties in coconut oil needs further research.

11. CONCLUSIONS

The common range of TPC for coconut oil extracted with any variable such as extraction method, variety, coconut kernel material, etc. is 72–650 mg/kg according to reported values. However, unusually high values for TPC have also been reported for coconut oil and coconut meat. Even though the extraction method, variety, hybrids, refining, etc. affect the TPC in coconut oil, these factors may not account for some unusually high TPC values reported. Interference by other compounds such as amino acids and sugars during the determination of phenolic content using the Folin-Ciocalteu assay and possible calculation errors during converting colorimetric readings to given quantities in (mg/g, mg/100 g or mg/kg) units may be possible causes for the large deviation of TPC values in some reports. The RBD process removes phenolic compounds from coconut oil. Depending on the efficiency of the RBD process, the TPC in RBD coconut oil varies between 0–120 mg/kg. The antioxidant activity and health benefits of the phenolic compounds in coconut oil have been sufficiently reported. Even though there has been substantial work done on the extraction method and phenolic composition, sufficient studies have not been conducted to assess the effect of the wide range of coconut varieties available in different geographical origins on the phenolic composition. Therefore, information on the variation in phenolic composition with coconut variety and geographical origin presented in the present review was based on the limited literature available. Careful review of the literature also indicates that there are very limited studies on the identification of phenolic compounds in coconut oil by HPLC methods. Most of the reported phenolic compounds in coconut oils are free phenolic acids and flavonoids. Several of these free phenolic compounds present in coconut oil are also present in olive oil. However, the presence of glycosides, aglycones, secoiridoid derivates or any other bound forms of phenolic compounds in coconut oil have not been reported. Therefore, further studies are necessary to elucidate the complete phenolic profile of coconut oil. In addition, further studies are necessary to investigate how the phenolic compounds in coconut oil are related to the sensory properties of coconut oil.

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DATA AVAILABILITY

All the data presented in the paper are included in the manuscript and references.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

REFERENCES

Adubofuor J, Amoah I, Osei-Bonsu I. 2016. Sensory and physicochemical properties of pasteurized coconut water from two varieties of coconut. *Food Sci. Qual. Manag*. **54**, ISSN 2225–0557.

Ahmad Z, Hasham R, Aman Nor NF, Sarmidi MR. 2015. Physico-chemical and antioxidant analysis of virgin coconut oil using West African Tall variety. *J. Adv. Res. Mater. Sci.* **13**, 1–10. ISSN (online): 2289–7992.

Alyaqoubi S, Abdullah A, Samudi M, Abdullah N, Addai ZR, Musa KH. 2015. Study of antioxidant activity and physicochemical properties of coconut milk (*Pati santan*) in Malaysia. *J. Chem. Pharm. Res.* **7**, 967–973.

Appaiah P, Sunil L, Prasanth Kumar PK, Gopala Krishna AG. 2014. Composition of coconut testa, coconut kernel and its oil. *J. Am. Oil Chem. Soc.* **91**, 917–924. http://doi.org/10.1007/s11746-014-2447-9.

Arivalagan M, Roy TK, Yasmeen AM, Pavithra KC, Jwala PN, Shivasankara KS, Manikantan MR, Hebbar KB, Kanade SR. 2018. Extraction of phenolic compounds with antioxidant potential from coconut (*Cocos nucifera* L.) testa and identification of phenolic acids and flavonoids using UPLC coupled with TQD-MS/MS. *LWT* **92**, 116–126. http://doi.org/10.1016/j.lwt.2018.02.024.

Arlee R, Suanphairoch S, Pakdeechanuan P. 2013. Differences in chemical components and antioxidant-related substances in virgin coconut oil from coconut hybrids and their parents. *Int. Food Res. J.* **20**, 2103–2109.

Arunima S, Rajamohan T. 2013. Effect of virgin coconut oil enriched diet on the antioxidant status and paraoxonase 1 activity in ameliorating the oxidative stress in rats - a comparative study. *Food Funct.* **4**, 1402–1409. http://doi.org/10.1039/c3fo60085h.

Bajoub A, Medina-Rodríguez S, Gómez-Romero M, Ajal EA, Bagur-González MG, Fernández-Gutiérrez A, Carrasco-Pancorbo A. 2017. Assessing the varietal origin of extra-virgin olive oil using liquid chromatography fingerprints of phenolic compound, data fusion and chemometrics. *Food Chem.* **215**, 245–255. http://dx.doi.org/10.1016/j.foodchem.2016.07.140.

Baptist NG. 1963. Free amino-acids in the endosperm of the developing coconut (*Cocos nucifera*). *J. Exp. Bot.* **14**, 29–41. http://doi.org/10.1093/jxb/14.1.29.

Bastola KP, Guragain YN, Bhadriraju Vadlani PV. 2017. Evaluation of standards and interfering compounds in the determination of phenolics by Folin-Ciocalteu assay method for effective bioprocessing of biomass. *Am. J. Analyt. Chem.* **8**, 416–431. http://doi.org/10.4236/ajac.2017.86032.

Bayram B, Esatbeyoglu T, Schulze N, Özçelik B, Frank J, Rimbach G. 2012. Comprehensive analysis of polyphenols in 55 extra virgin olive oils by HPLC-ECD and their correlation with antioxidant activities. *Plant Food Hum. Nutr*. **67**, 326–336. http://doi.org/10.1007/s11130-012-0315-z.

Boskou D, Blekas G, Tsimidou M. 2005. Phenolic compounds in olive oil and olives. *Curr. Top. Nutraceutical Res*. **3**, 125–136.

Chang M, Zhao P, Zhang T, Wang Y, Guo X, Liu R, Jin Q, Wang X. 2020. Characteristic volatiles fingerprints and profiles determination in different grades of coconut oil by HS-GC-IMS and HS-SPME-GC-MS. *Int. J. Food. Sci. Tech*. **55**, 3670–3679. https://doi.org/10.1111/ijfs.14664.

Che Man YB, Abdul Karim MIB, Ten CT. 1997. Extraction of coconut oil with *Lactobacillus plantarum* 1041 IAM. *J. Am. Oil Chem. Soc.* **74**, 1115–1119. http://doi.org/10.1007/s11746-997-0033-0.

Cheynier V. 2012. Phenolic compounds: from plants to foods. *Phytochem. Rev.* **11,** 153–177. http://doi.org/10.1007/s11101-012-9242-8.

Covas MI, Ruiz-Gutierrez V, de la Torre R, Kafatos A, Lamuela-Raventos RM, Osada J, Owen RW, Visioli F. 2006. Minor components of olive oil: Evidence to date of health benefits in humans. *Nutr. Rev.* **64**, S20–S30. http://doi.org/10.1111/j.1753-4887.2006.tb00260.x.

Dhanyakrishnan R, Sunithaa MC, Prakash Kumara BP, Sandyab S, Nevin KG. 2018. Morphological and molecular effects of phenolic extract from coconut kernel on human prostate cancer cell growth in vitro *Mediterr. J. Nutr. Metab*. **11**, 21–36. http://doi.org/10.3233/MNM-17174.

de Azevedo WM, de Oliveira LFR, Alcantara MA, Cordeiro AMTDM, Damasceno KSFDSC, Araujo NKD, de Assis CF, de Sousa Jr FC. 2020. Physicochemical characterization, fatty acid profile, antioxidant activity and antibacterial potential of cacay oil, coconut oil and cacay butter. *PLoS ONE* **15**. e0232224. http://doi.org/10.1371/journal.pone.0232224.

Deen A, Visvanathan R, Wickramarachchi D, Marikkar N, Nammi S. Jayawardana BC, Liyanage R. 2021. Chemical composition and health benefits of coconut oil: an overview. *J. Sci. Food Agric*. **101**, 2182–2193. http://doi.org/10.1002/jsfa.10870.

Diamantakos P, Ioannidis K, Papanikolaou C, Tsolakou A, Rigakou A, Melliou E, Magiatis P. 2021. A new definition of the term "High-Phenolic Olive Oil" based on large scale statistical data of Greek olive oils analyzed by qNMR. *Molecules* **26**, 1115. https://doi.org/10.3390/molecules26041115.

Dabbou S, Issaoui M, Esposto S, Sifi S, Taticchi A, Servili M, Montedoro, GF, Hammami M. 2009. Cultivar and growing area effects on minor compounds of olive oil from autochthonous and European introduced cultivars in Tunisia. *J. Sci. Food Agric.* **89** 1314–1325. http://doi.org/10.1002/jsfa.3588.

Elodio-Policarpo F, Peñaloza-Herrera B, Maldonado-Astudillo YI, Jimenez-Hernandez J, Flores-Casamayor V, Arámbula-Villa G, Salazar R. 2019. Thermal stability of virgin coconut oil obtained from two cultivars grown in Guerrero, Mexico. *Rev. Fitotec. Mex*. **42**, 101–109.

Everette JD, Bryant QM, Green AM, Abbey YA, Wangila GW, Walker RB. 2010. Thorough study of reactivity of various compound classes towards the Folin-Ciocalteu Reagent. *J. Agric. Food Chem.* **58**, 8139–8144. http://doi.org/10.1021/jf1005935.

Famurewaa AC, Ejezieb AJ, Ugwu-Ejeziec CS, Ikekpeazud EJ, Ejezie FE. 2018. Antioxidant and anti-inflammatory mechanisms of polyphenols isolated from virgin coconut oil attenuate cadmium-induced oxidative stress-mediated nephrotoxicity and inflammation in rats. *J. Appl. Biomed.* **16**, 281–288. http://doi.org/10.1016/j.jab.2018.02.003.

Fiorini D, Boarelli MC, Conti P, Alfei B, Caprioli G, Ricciutelli M, Sagratini, G, Fedeli, D, Gabbianelli R, Pacetti D. 2018. Chemical and sensory differences between high price and low price extra virgin olive oils. *Food Res. Int.* **105**, 65–75. https://doi.org/10.1016/j.foodres.2017.11.005.

Galvano F, Fauci LL, Graziani G, Ferracane R, Masella R, Di Giacomo C, Scacco A, D'Archivio M, Vanella L, Galvano G. 2007. Phenolic compounds and antioxidant activity of Italian extra virgin olive oil Monti Iblei. *J. Med. Food* **10**, 650–656. http://doi.org/10.1089/jmf.2007.409.

Genovese A, Yang N, Linforth R, Sacchi R, Ian Fisk I. 2018. The role of phenolic compounds on olive oil aroma release. *Food Res. Int*. **112**, 319–327. http://doi.org/10.1016/j.foodres.2018.06.054.

Ghani NAA, Channip A-A, Hwa PCH, Ja'afar F, Yasin HM, Usman A. 2018. Physicochemical properties, antioxidant capacities, and metal contents of virgin coconut oil produced by wet and dry processes. *Food Sci. Nutr.* **6**, 1298–1306. http://doi.org/10.1002/fsn3.671.

Giovacchino LD, Sestili S, Vincenzo DD. 2002. Influence of olive processing on virgin olive oil quality. *Eur. J. Lipid Sci. Technol.* **104**, 587–601. https://doi.org/10.1002/1438-9312(200210)104:9/10<587::AID-EJLT587>3.0.CO;2-M.

Gopala Krishna AG, Raj G, Bhatnagar AS, Prasanth Kumar PK, Chandrashekar P. 2010. Coconut oil: Chemistry, production and Its applications—A Review. *Indian Coconut J.* **73**, 15–27.

Gutfinger T, Letan A. 1974. Studies of unsaponifiables in several vegetable oils. *Lipids* **9**, 658-663. http://doi.org/10.1007/BF02532171.

Illam SP, Narayanankutty A, Raghavamenon AC. 2017. Polyphenols of virgin coconut oil prevent pro-oxidant mediated cell death. *Toxicol. Mech. Methods* **27**, 442–450. http://doi.org/10.1080/15376516.2017.1320458.

Janu C, Soban Kumar DR, Reshma MV, Jayamurthy P, Sundaresan A, Nisha P. 2013. Comparative study on the total phenolic content and radical scavenging activity of common edible vegetable oils. *J. Food Biochem.* **38**, 38–49. http://doi.org/10.1111/jfbc.12023.

Jimenez-Lopez C, Carpena M, Lourenço-Lopes C, Gallardo-Gomez M, Lorenzo JM, Barba FJ, Prieto MA, Simal-Gandara J. 2020. Bioactive compounds and quality of extra virgin olive oil. *Foods* **9**, 1014. http://doi.org/10.3390/foods9081014.

Karunasiri AN, Gunawardane M, Senanayake CM, Jayathilaka N, Seneviratne KN. 2020a. Antioxidant and nutritional properties of domestic and commercial coconut milk preparations, *Int. J. Food Sci.* Article ID 3489605. http://doi.org/10.1155/2020/3489605.

Karunasiri AN, Senanayake CM, Hapugaswatta H, Jayathilaka N, Seneviratne KN. 2020b. Protective effect of coconut oil meal phenolic antioxidants against macromolecular damage. *In Vitro* and *In Vivo* study. *J. Chem.* http://doi.org/10.1155/2020/3503165.

Khalil HMA, Salama HH, Al-Mokaddem AK, Aljuaydi SH, Edris AE. 2020. Edible dairy formula fortified with coconut oil for neuroprotection against aluminium chloride-induced Alzheimer's disease in rats, *J. Funct. Foods* **75**, 104296. http://doi.org/10.1016/j.jff.2020.104296.

Kiritsakis AK. 1998. Flavor components of olive oil—A Review. *J. Am. Oil Chem. Soc.* **75**, 673–681. http://doi.org/10.1007/s11746-998-0205-6.

Koh SP, Long K. 2012. Oxidative stability study of virgin coconut oil during deep frying. *J. Trop. Agric. and Food Sc.* **40**, 35–44.

Kwon K, Park KH, Rhee KC. 1996. Fractionation and characterization of proteins from Coconut (*Cocos nucifera* L.). *J. Agric. Food Chem.* **44**, 1741–1745. http://doi.org/10.1021/jf9504273.

Lal JJ, Sreeranjit Kumar CV, Indira M. 2003. Coconut palm, in *Encyclopedia of Food Sciences and Nutrition,* 2nd ed, Academic Press, pp 1464–1475.

Librado AS, Von Luigi MV. 2013. Phenolic-dependent anti-lipid peroxidative, antimodulatory and antioxidant activity of virgin coconut oil *in vitro. Int. Food Res.* **20**, 1683–1689.

Lima EBC, Sousa CNS, Meneses LN, Ximenes NC, Santos Júnior MA, Vasconcelos GS, Lima NBC, Patrocínio MCA, Macedo D, Vasconcelos SMM. 2015. *Cocos nucifera* (L.) (Arecaceae): A phytochemical and pharmacological review. *Braz. J. Med. Biol. Res.* **48**, 953–964. http://doi.org/10.1590/1414-431X20154773.

Liu R, Lu M, Zhang T, Zhang Z, Jin Q, Chang M, Wang X. 2019a. Evaluation of the antioxidant properties of micronutrients in different vegetable oils. *Eur. J. Lipid Sci. Technol.* 1900079. http://doi.org/10.1002/ejlt.201900079.

Liu R, Guo X, Cheng, M, Zheng L, Gong M, Chang, M, Jin Q, Wang X. 2019b. Effects of chemical refinement on the quality of coconut oil. *J. Food Sci. Technol.* **56**, 3109–3116. https://doi.org/10.1007/s13197-019-03810-w.

Lukic I, Zanetic M, Spika MJ, Lukic M, Koprivnjak O, Bubola KB. 2017. Complex interactive effects of ripening degree, malaxation duration and temperature on Oblica cv. virgin olive oil phenols, volatiles and sensory quality. *Food Chem.* **232***,* 610-620. http://dx.doi.org/10.1016/j.foodchem.2017.04.047.

Mahayothee B, Koomyart I, Khuwijitjaru, P, Siriwongwilaichat P, Nagle M, Müller J. 2016. Phenolic compounds, antioxidant activity, and medium chain fatty acids profiles of coconut water and meat at different maturity stages, *Int. J. Food Prop.* **19**, 2041-2051. http://doi.org/10.1080/10942912.2015.1099042.

Mannino S, Buratti S, Cosio MS, Pellegrini N. 1999. Evaluation of the 'antioxidant power' of olive oils based on a FIA system with amperometric detection. *Analyst* **124**, 1115-1118. http://doi.org/10.1039/a902007a.

Mansour AB, Chtourou F, Khbou W, Flamini G, Bouaziz M. 2017. Phenolic and volatile compounds of Neb Jmel olive oil cultivar according to their geographical origin using chemometrics. *Eur. Food Res. Technol.* **243,** 403-418. https://doi.org/10.1007/s00217-016-2754-5.

Marasinghe S, Marikkar N, Wimalasiri S, Jayasinghe L, Liyanage, R. 2019. Comparison of antioxidant properties of coconut testa flour of selected local coconut cultivars of Sri Lanka. *Proceedings,* **36**, 124. http://doi.org/10.3390/proceedings2019036124.

Marina AM, Che Man YB, Nazimah SAH, Amin I. 2009a. Antioxidant capacity and phenolic acids of virgin coconut oil. *Int. J. Food Sci. Nutr*. **60**, 114123. http://doi.org/10.1080/09637480802549127.

Marina AM, Che Man YB, Nazimah SAH, Amin I. 2009b. Chemical properties of virgin coconut oil. *J. Am. Oil Chem. Soc.* **86**, 301-307. http://doi.org/10.1007/s11746-009-1351-1.

Maqsood S, Benjakul S, Abushelaibi A, Alam A. 2014. Phenolic compounds and plant phenolic extracts as natural antioxidants in prevention of lipid oxidation in seafood: A detailed review. *Compr. Rev. Food Sci. F.* **13**, 1125-1140. http://doi.org/10.1111/1541-4337.12106.

Michalak A. 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Pol. J. Environ. Stud*. **15**, 523-530.

Moura Fe JA, Brown WH, Whiting FM, Stull JW. 1975. Unsaponifiable matter of crude and processed coconut oil. *J. Sci. Food. Agric.* **26**, 523-531. http://doi.org/10.1002/jsfa.2740260417.

Muñoz-Bernal OA, Torres-Aguirre GA, Núñez-Gastélum JA, de la Rosa LA, Rodrigo-García J, Fernando Ayala-Zavala J, Álvarez-Parrilla E. 2017. New approach to the interaction between Folin-Ciocalteu reactive and sugars during the quantification of total phenols. *TIP Revista Especializada en Ciencias Químico-Biológicas* **20**, 23-28. http://doi.org/10.1016/j.recqb.2017.04.003.

Nadeeshani R, Wijayaratna UN, Prasadani WC, Ekanayake S, Seneviratne KN, Jayathilaka N. 2015. Comparison of the basic nutritional characteristics of the first extract and second extract of coconut milk. *Int. J. Innov. Res. Sci. Eng. Technol.* **4.** http://doi.org/10.15680/IJIRSET.2015.0410003.

Narasinga Rao BS. 2001. Nonglyceride components of edible oils and fats. 1. Chemistry and distribution. *Food Nutr. Bull.* **22**, 81-86. http://doi.org/10.1177/156482650102200113.

Narayanankutty A, Mukesh RK, Ayoob SK, Ramavarma SK, Suseela IM, Manalil JK, Kuzhivelil BT, Raghavamenon AC. 2016. Virgin coconut oil maintains redox status and improves glycemic conditions in high fructose fed rats. *J. Food. Sci. Technol.* **53**, 895-901. http://doi.org/10.1007/s13197-015-2040-8.

Narayanankutty A, Illam SP, Raghavamenon AC. 2018. Health impacts of different edible oils prepared from coconut (*Cocos nucifera*): A comprehensive review. *Trends Food Sci. Technol.* **80**, 1-7. http://doi.org/10.1016/j.tifs.2018.07.025.

Navajas-Porras B, Pérez-Burilloa S, Morales-Péreza J, Rufán-Henaresa J.A, Pastoriza S. 2020. Relationship of quality parameters, antioxidant capacity and total phenolic content of EVOO with ripening state and olive variety. *Food Chem.* **325**, 126926. https://doi.org/10.1016/j.foodchem.2020.126926.

Nevin KG, Rajamohan T. 2004. Beneficial effects of virgin coconut oil on lipid parameters and in vitro LDL oxidation. *Clin. Biochem.* **37**, 830-835. http://doi.org/10.1016/j.clinbiochem.2004.04.010.

Ngampeerapong C, Chavasit V, Durst RW. 2018. Bioactive and nutritional compounds in virgin coconut oils. *Malays. J. Nutr.* **24**, 257-267.

Ngampeerapong C, Chavasit V. 2019. Nutritional and bioactive compounds in coconut meat of different sources: Thailand, Indonesia and Vietnam. *CMU J. Nat. Sci.* **18**, 562-573.

Ojha SB, Roy S, Das S, Dhangadamajhi G. 2019. Phytochemicals screening, phenolic estimation and evaluation for anti-oxidant, anti-inflammatory and anti-microbial activities of sequentially Soxhlet extracted coconut testa. *Food Nutr. Sci.* **10**, 900-922. http://doi.org/10.4236/fns.2019.108065.

Pandey KB, Rizvi SI. 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cell. Longev*. **2**, 270-278. http://doi.org/10.4161/oxim.2.5.9498.

Pavan Kumar GV, Lakshmi NVVSS, Deena C, Bhavani B, Rajendra Kumar P. 2018. Copra oil: chemistry, production. An extensive review on Indian specifications and functional aspects. *Ukr. J. Food Sci.* **6**, 32-45. http://doi.org/10.24263/2310-1008- 2018-6-1-6.

Pedan V, Popp M, Rohn S, Nyfelern M, Bongartz A. 2019. Characterization of phenolic compounds and their contribution to sensory properties of olive oil. *Molecules* **24,** 2041. http://doi.org/10.3390/molecules24112041.

Perera DN, Ranaweera KKDS, Marapana RAUJ, Hewavitharana GG. 2019. Development of spicy flavored virgin coconut oil by incorporating a mixture of spices oleoresins. *Oilseeds & fats Crops and Lipids* **27**, 55. http://doi.org/10.1051/ocl/2020050.

Prapun R, Cheetangdee N, Udomrati S. 2016. Characterization of virgin coconut oil (VCO) recovered by different techniques and fruit maturities. *Int. Food Res.* **23**, 2117-2124.

Prasadani WC, Senanayake CM, Jayathilaka N, Ekanayake S, Seneviratne KN. 2017. Effect of three edible oils on the intestinal absorption of caffeic acid: An in vivo and in vitro study. *PLoS ONE* **12,** e0179292. https://doi.org/ 10.1371/journal.pone.0179292.

Prasanth Kumar PK, Gopala Krishna AG. 2015. Physicochemical characteristics of commercial coconut oils produced in India. *Grasas Aceites* **66**, e062. http://doi.org/10.3989/gya.0228141.

Rashid ANAD, Misson M, Yaakob H, Latiff NA, Sarmidi MR. 2017. Addition of virgin coconut oil: Influence on the nutritional value and consumer acceptance of dark chocolate. *Transactions on Science and Technology* **4**, No. 426-431.

Rohman A, Irnawati, Erwanto Y, Lukitaningsih E, Rafi M, Fadzilah NA, Windarsih A, Sulaiman A, Zakaria Z. 2019. Virgin coconut oil: Extraction, physicochemical properties, biological activities and its authentication analysis. *Food Rev. Int.* **37**, 46-66. http://doi.org/10.1080/87559129.2019.1687515.

Ryan D, Robards K. 1998. Phenolic compounds in olives. *Analyst* **123,** 31R–44R.

Sanchez-Rangel JC, Benavides J, Heredia JB, Cisnero-Zevallos L, Jacobo-Velazquez D. A. 2013. The

Folin–Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. *Anal. Methods* **5**, 5990-5999. http://doi.org/10.1039/c3ay41125g.

Satheesh N, Prasad NBL. 2014. Production of virgin coconut oil by induced fermentation with *Lactobacillus plantarum* NDRI strain 184. *Croatian Journal of Food Technology, Biotechnology and Nutrition* **9**, 37-42.

Senanayake CM, Algama CH, Wimalasekara RL, Weerakoon WNMTDN, Jayathilaka N, Seneviratne KN. 2019. Improvement of oxidative stability and microbial shelf life of vanilla cake by coconut oil meal and sesame oil meal phenolic extracts. *J. Food Qual.* 1263629. https://doi.org/10.1155/2019/1263629.

Senanayake CM, Hapugaswatta H, Samarawickrama GR, Jayathilaka N, Seneviratne KN. 2021. Effect of chain length and saturation of the fatty acids in dietary triglycerides on lipid metabolism in Wistar rats. *J. Food Biochem.* 00:e13664. https://doi.org/10.1111/jfbc.13664.

Seneviratne KN, Dissanayake DMS. 2005. Effect of method of extraction on the quality of coconut oil. *Journal of Science of the University of Kelaniya Sri Lanka*. **2**, 63-72.

Seneviratne KN, Dissanayake DMS. 2008. Variation of phenolic content in coconut oil extracted by two conventional methods. *Int. J. Food Sci. Technol.* **43**, 597-602. http://doi.org/10.1111/j.1365-2621.2006.01493.x.

Seneviratne KN, Hapuarachchi CD, Ekanayake S. 2009. Comparison of the phenolic-dependent antioxidant properties of coconut oil extracted under cold and hot conditions. *Food Chem.* **114**, 1444-1449. http://doi.org/10.1016/j.foodchem.2008.11.038.

Seneviratne KN, Kotuwegedara RT, Ekanayake S. 2011. Serum cholesterol and triglyceride levels of rats fed with consumer selected coconut oil blends. *Int. Food Res. J.* **18**, 1303-1308.

Seneviratne KN, Jayathilaka N. 2015. Production method and coconut oil quality, in Apetrei C. (Ed.) *Corn and Coconut Oil: Antioxidant Properties, Uses and Health Benefits*; Nova Publishers: New York, pp 103-130.

Seneviratne K, Jayathilaka N. 2016. *Coconut oil: chemistry and nutrition*. Lakva Publishers: Battaramulla, Sri Lanka.

Seneviratne KN, Prasadani WC, Jayawardena B. 2016. Phenolic extracts of coconut oil cake: a potential alternative for synthetic antioxidants. *Food Sci. Technol. (Campinas)* **36**, 591-597. http://doi.org/10.1590/1678-457X.07316.

Servili M, Montedoro GF. 2002. Contribution of phenolic compounds to virgin olive oil quality. *Eur. J. Lipid Sci. Technol.* **104**, 602-613. http://doi.org/10.1002/1438-9312(200210)104:9/10<602::AID-EJLT602>3.0.CO;2-X

Servili M, Esposto S, Fabiani R, Urbani S, Taticchi A, Mariucci FR, Selvaggini R, Montedoro GF. 2009. Phenolic compounds in olive oil: antioxidant, health and organoleptic activities according to their chemical structure. *Inflammopharmacology* **17**, 76-84. http://doi.org/10.1007/s10787-008-8014-y.

Sicari V. 2017. Antioxidant potential of extra virgin olive oils extracted from three different varieties cultivated in the Italian province of Reggio Calabria. *J. Appl. Bot. Food Qual.* **90**, 76-82. http://doi.org/10.5073/JABFQ.2017.090.011.

Siger A, Nogala-kalucka M. Lampart-szczapa E. 2008. The content and antioxidant activity of phenolic compounds in cold-pressed plant oil. *J. Food Lipids* **15**, 137-149. http://doi.org/10.1111/j.1745-4522.2007.00107.x.

Skevin D, Rade D, Strucelj D, Mokrovcak Z, Nederal S, Bencic D. 2003. The influence of variety and harvest time on the bitterness and phenolic compounds of olive oil. *Eur. J. Lipid Sci. Technol*. **105**, 536-541. http://doi.org/10.1002/ejlt.200300782.

Srivastava Y, Semwal AD, Majumdar A. 2016. Quantitative and qualitative analysis of bioactive components present in virgin coconut oil. *Cogent Food & Agric.* **2**, 1164929. http://doi.org/10.1080/23311932.2016.1164929.

Srivastava Y, Semwal AD, Sharma GK. 2018. Virgin Coconut Oil as Functional Oil, in Grumezescu AM, Holban AM. (Ed.) Therapeutic, Probiotic and Unconventional Foods; Academic Press. pp 291-301. https://doi.org/10.1016/B978-0-12-814625-5.00015-7.

Tanilgana K, Özcanb MM, Ünver A. 2007. Physical and chemical characteristics of five Turkish olive *(Olea europea L.)* varieties and their oils. *Grasas Aceites* **58**, 142-147. https://doi.org/10.3989/gya.2007.v58.i2.78.

Tripoli E, Giammanco M, Tabacchi G, Majo DD, Giammanco S, Guardia ML. 2005. The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. *Nutr. Res. Rev.* **18**, 98-112. http://doi.org/10.1079/NRR200495.

Veneziani G, Esposto S, Taticchi A, Urbani S, Selvaggini R, Di Maio I, Sordini, Servili M. 2017. Cooling treatment of olive paste during the oil processing: impact on the yield and extra virgin olive oil quality. *Food Chem*. **221**, 107-113. https://doi.org/10.1016/j.foodchem.2016.10.067.

Veneziani G, Esposto S, Taticchi A, Urbani S, Selvaggini R, Sordini B, Servili M. 2018. Characterization of phenolic and volatile composition of extra virgin olive oil extracted from six Italian cultivars using a cooling treatment of olive paste. *LWT* **87**, 523-528. https://doi.org/10.1016/j.lwt.2017.09.034.

Villarino BJ, Dy LM, Lizada MCC. 2007. Descriptive sensory evaluation of virgin coconut oil and refined, bleached and deodorized coconut oil. *LWT* **40**, 193-199. http://doi.org/10.1016/j.lwt.2005.11.007.

Visioli F, Galli C. 1998. Olive oil phenols and their potential effects on human health. *J. Agric. Food Chem.* **46***,* 4292-4296. http://doi.org/10.1021/jf980049c.

Weerakoon WNMTDN, Anjali NVP, Jayathilaka N, Seneviratne KN. 2021. Soybean oil and coconut oil enhance the absorption of chlorogenic acid in humans. *J. Food Biochem.* e13823. http://doi.org/10.1111/jfbc.13823.

Withana-Gamage TS, Perera SP, Wanasundara UN. 2005. Edible oil and fat products: Edible oils, in *Bailey's Industrial Oil and Fat Products*, *7th ed.* http://doi.org/10.1002/047167849X.bio054.pub2.

Wiyani L, Rahmawati, Aminah, Aladin A, Mustafiah, Juniar, ME. 2020. Antioxidant activity of virgin coconut oil and virgin coconut oil emulsion. *Sys. Rev. Pharm*, **11**, 973-979. http://doi.org/10.31838/srp.2020.5.139.

Zeb A. 2020. Concept, mechanism, and applications of phenolic antioxidants in foods. *J. Food Biochem*. **44**, e13394. https://doi.org/10.1111/jfbc.13394.

Zeb A. 2021. A comprehensive review on different classes of polyphenolic compounds present in edible oils. *Food Res. Inter. 143*, 110312. https://doi.org/10.1016/j.foodres.2021.110312.

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Pre-cooling and cold storage of olives (cv Picual) in containers with a capacity of 400 kg

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SUMMARY: The cooling of olives stored in containers with a capacity of 400 kg risk accumulation of respiration heat and subsequent fruit deterioration. Pre-cooling the fruit to 5 °C before cold storage was studied as a possible solution to overcome this obstacle. The fruit temperature within the containers was recorded daily for 14 days and oil was extracted at days 0, 4, 8, and 14. A second experiment evaluated a rapid pre-cooling procedure at -18 °C for 3 min. No significant alterations at the level of the examined parameters were recorded. The internal temperature of the control container declined and stabilized at around 12 °C. The temperature of the pre-cooled fruit increased to up to 8 °C. The examined parameters showed no significant alterations in either experiment and the rapid pre-cooling treatment did not lead to any visible 'chill injuries'. A pre-cooling treatment at 5 °C was successfully introduced at the farm of a small producer.

KEYWORDS: *Conservation; Harvesting system; Olea europaea; Quality; Small producers*

RESUMEN: ***Preenfriamiento y conservación en frío de aceitunas (cv Picual) almacenadas en contenedores con una capacidad de 400 kg.*** El enfriamiento de aceitunas almacenadas en contenedores (400 kg) corre el riesgo de acumulación de calor respiratorio y posterior deterioro de la fruta. Se estudió el preenfriamiento de la fruta a 5 °C antes del almacenamiento en frío como una posible solución para superar este obstáculo. La temperatura de la fruta dentro de los contenedores se registró diariamente durante 14 días y el aceite se extrajo los días 0, 4, 8 y 14. Un segundo experimento evaluó un procedimiento de preenfriamiento rápido a -18 °C durante 3 min. No se registraron alteraciones significativas a nivel de los parámetros examinados. La temperatura interna del recipiente de control disminuyó y se estabilizó alrededor de 12 °C. La fruta pre-enfriada aumentó hasta 8 °C. Los parámetros examinados no mostraron alteraciones significativas en ambos experimentos y el preenfriamiento rápido no provocó "lesiones por frío" visibles. Se introdujo con éxito un tratamiento de pre-enfriamiento a 5 °C en la finca de un pequeño productor.

PALABRAS CLAVE: *Calidad; Conservación; Olea europaea; Pequeños productores; Sistema de recolección*

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1. INTRODUCTION

The production of 'premium' olive oil depends to a large extent on the quality of the harvested fruit (García and Yousfi, 2006; Ferguson, 2006; Rallo *et al.*, 2018; Faminiani, 2020). The mechanical harvesting of olives on an industrial scale differs when compared to manual picking with nets and sticks, as thousands of cooperatives in Spain still do (Junta de Andalucía, 2015; Sola-Guirado *et al.*, 2014). The degree of damage caused and the time elapsed from harvesting to storage prior to grinding stands out as crucial factors (Yousfi *et al.*, 2012; Dag *et al.*, 2012; Morales-Silero and García, 2015; Faminiani *et al.*, 2020). Moreover, the post-harvest conditions do vary along with the kind of harvesting employed as well as the processing and storing conditions at the mill (García and Yousfi, 2006; García and Yousfi, 2011).

To obtain 'premium' olive oils, small producers face additional challenges in this respect, especially when they do not possess a proper mill. Besides the fact that they need to look for an efficient and affordable harvesting method that guarantees the least damaged fruit possible, such as manual inverted umbrellas, they ought to take into account the particular conditions that are imposed by the mill they are working with and especially the minimum quantity per batch requested (Plasquy *et al.*, 2019). The characteristics of the mill, and especially its production capacity, define the time and the way olives are piled up, risking fermentation processes that compromise the final quality of the extracted oil (García and Yousfi, 2006, García and Yousfi, 2011). When this amount cannot be picked in one day, storage becomes inevitable.

Temporary cold storage at the farm could be a possible solution for maintaining a high product quality but comes along with specific problems. Several works clarified that the storage of olives at 5 °C for up to 4 weeks does significantly reduce the risk of fruit deterioration and subsequently olive oil defects (García *et al.*, 1996; Kiritsakis *et al.*, 1998; Canet and García, 1999; Clodoveo *et al.*, 2006; Kalua *et al.*, 2008). However, cold storage procedures imply the use of small boxes that can contain 40 kg at the most and ideally 20 kg of olives, instead of agricultural containers, or bins, with a capacity of 400 kg.

Empirical studies and simulations of the cooling process of fresh fruits and vegetables revealed complex interactions between the thermophysical properties of the commodity (heat generation due to respiration, specific heat and thermal conductivity), the kind of packaging and palletization, flow field parameters such as airflow rate and cooling temperature, and the accessibility of the cooling air to the produce (Becker *et al.*, 1996; Brosnan and Sun, 2001; Reading *et al.*, 2016; Mercier *et al.*, 2017; Plasquy *et al.*, 2021). It is well documented that fruit respiration involves the oxidation of sugars to produce carbon dioxide, water, and heat while at the same time, the respiration rate is itself a function of the commodity's temperature (Kader, 1985; Becker *et al.*, 1996, García *et al.*, 1995). García *et al.* (1994) and García and Yousfi (2006) reported that the inner part of a container of olives maintained its initial temperature while stored at 5 °C. As a consequence, the fruit respiration and accompanying heat production induced a self-reinforcing process that rapidly led to a severe deterioration process and the growth of decay-producing microorganisms.

Although most manual or semi-mechanical methods make use of small boxes to collect the fruit after harvesting, the further handling of a large number of boxes leads to large logistic problems once dealing with several tons of olives and especially when cold storage forms a crucial step in maintaining fruit quality. By applying a pre-cooling treatment to the olives manually harvested in the boxes before dumping them into a container for conservation at 5 °C, high-quality oils could be obtained. To explore this possibility, two experiments were set up. The first one monitored the evolution of the internal temperature of a container of 400 kg with or without a pre-cooling treatment at 5 °C and compared the effect of bulk storage at 5 °C on the quality parameters of the extracted oil up to 14 days. A second experiment probed the effects of a short cooling treatment at -18 °C to lower the fruit temperature rapidly to 5 °C. During the following 14 days' storage period at 5 °C, the presence of chilling injuries and quality defects were assessed. The results of the first experiment were used to implement concrete modifications in a family-run olive farm.

2. MATERIALS AND METHODS

2.1. Fruit and storing

Olives of the 'Picual' cultivar were harvested with a manual inverted umbrella (MIU) and branch

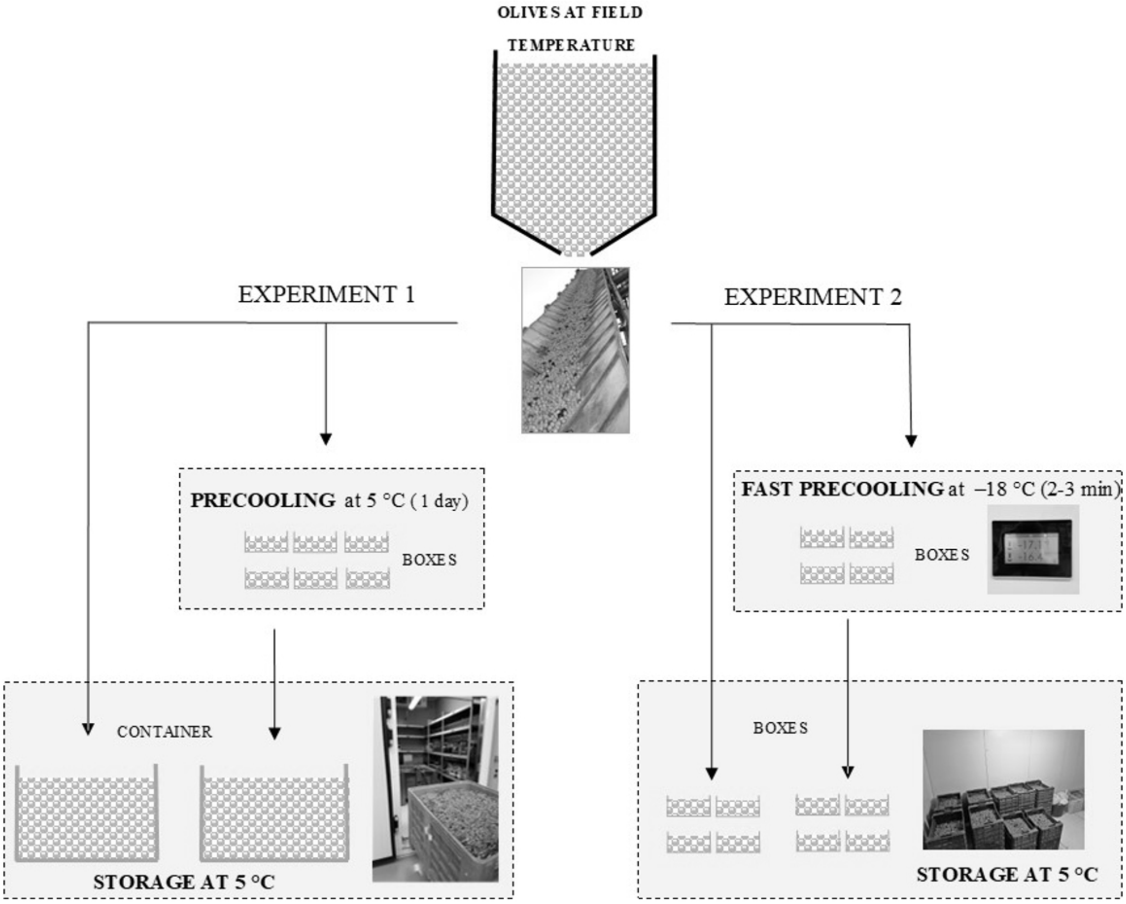

FIGURE 1. Schematic representation of experiment 1 and 2 with their different treatments.

shakers in the 'Del Cetino' plantation in Bollullos del Condado (Huelva, Spain) during the second week of October, 2018. The fruit was healthy and presented optimal ripening characteristics (color index between 1.5 and 2.0). The fruit was collected in perforated plastic boxes (30 x 50 x 40 cm), able to contain a maximum of 20 kg. 50 boxes were recollected, and on the same day transported to the experimental mill of the Instituto de la Grasa (CSIC) in Sevilla in approximately one hour. Upon arrival, the olives were mechanically cleaned of leaves and small twigs but not washed at the outdoor facilities of the experimental mill. The batch of cleaned but dry olives provided the necessary amount of fruit for the two experiments. Minor bruises were produced during the cleaning treatment and passage onto various conveyor belts. The cooling installations were situated within the Research Center and comprised two cooling refrigeration rooms set at 5 °C (± 1 °C), and one freezing room at -18 °C (± 1 °C).

2.2. Trials

2.2.1. Experiment 1

The goal of the experiment was to evaluate the effects of a pre-cooling treatment on the internal temperature of olive containers and the quality of the subsequently extracted oil over 14 days (Figure 1).

Two vented bulk bin containers (100 x 120 x 80 cm; volume of 670 L) were used, each with a capacity of 380 kg olives. One container was filled with fruit at field temperature (FT) immediately after the cleaning procedure. The second container was filled the next day after the olives underwent a preliminary treatment (PC). This treatment consisted of bringing the temperature of the olives to 5 °C by storing the fruit in half-filled boxes (± 10 kg) in a cooling room up to the next day. After controlling that the temperature of the fruit attained 5 °C, the container was filled with the precooled olives.

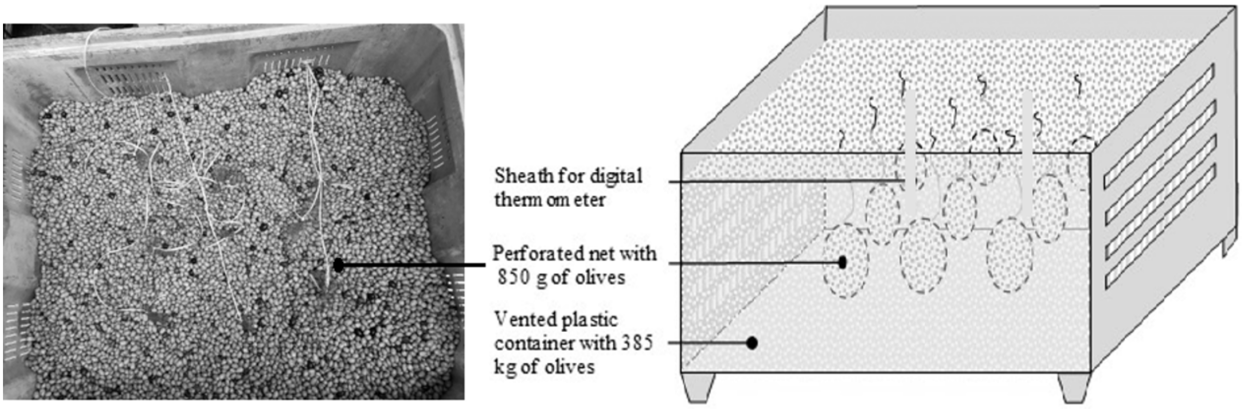

FIGURE 2. Schematic representation of experiments 1 and 2 with their different treatments.

To facilitate the withdrawal of similar samples of olives at a future moment in time, nine plastic nets, each filled with 850 g of olives, were buried within the fruit in each container, evenly distributed, halfway up the height and at the same distance from each other (3 x 3 units) (Figure 2). Every net had a long drawstring attached to facilitate its location and pulling out when needed. To enable the measurement of the internal temperature in each of the containers, two hollow tubes were used as a sheath and situated along the middle ax, at 1/3 of each of the smaller sides.

The cooling room at 5 °C (± 1 °C) was accommodated to store the two containers side by side. One container filled with olive fruit with a field temperature of 18 °C was placed inside once the nets and sheaths were in place. The next day, a second container, filled with pre-cooled fruit at 5 °C was brought inside (Figure 1).

The temperature was measured with a digital thermometer (precision of 0.1 °C) with a wired probe that was glided into the sheaths. The measurement took place every 24 hours, twice, for 14 days.

On day 0, three samples of 750 g of olives were taken from the FT container and the boxes in the pre-cooling treatment, both before entering the cooling room. On days 4, 8, and 14, three nets of olives were pulled out of each container and used to extract oil. Olive oil was physically extracted following the Abencor method as described below, nitrogenized, and stored at -18 °C until analysis.

2.2.2. Experiment 2

The goal of the experiment was to evaluate the effect of a fast-cooling procedure before the cool storage of olives on the quality of the extracted oil (Figure 1).

Two ventilated plastic boxes, each filled with 10 kg olives were placed in a cooling room at 5 °C (+/- 1°C) to attain the same temperature at a slow rate (SR). Four boxes, each filled with 5 kg olives were placed in a freezer room at -18 °C for the time necessary for the fruit to attain a temperature of 5 °C. This cooling process at a fast rate (FR) was continuously monitored with an IR thermometer. The desired temperature was attained after 3 minutes and then the 4 boxes were brought immediately to the cooling room at 5 °C, where the content of 2 boxes was brought together into one.

On days 0, 4, 8, and 14, three olive samples of 750 g were taken from both the SR and the FR-boxes. 100 olives from each sample were visually examined for the presence of ‘chilling injuries’. Virgin olive oil from each of the samples was extracted following the Abencor method, nitrogenized, and stored at -18 °C until analysis.

2.3. Extraction method and sample conservation

The oil was extracted following the procedures for extraction with an Abencor installation (Martinez *et al.*, 1975). Individual samples of 750 g olives were crushed in a hammer mill. 600 g of the resulted paste was weighted in a stainless-steel casserole pot and malaxated in the thermoblender for 30 min at 30 °C. Centrifugation was performed at 1372 G for 1 min. The liquid obtained was placed in a graduated 500-mL test tube for separating the aqueous phase from the lipids. The olive oil was taken using a Pasteur pipette, filtered with filter paper, and placed in an amber glass bottle of 125 ml, filled with nitrogen, and frozen at -18 °C until further examination.

2.4. Physicochemical analysis

The physicochemical analysis consisted of the measurement of the following parameters: Titratable acidity (Free Fatty Acid, FFA), peroxide index (PI) value, and the extinction coefficients at 232 and 270 nm (K232 and K270). The analysis followed the guidelines of the official analytical methods (EEC, 1991). The selected parameters are a crucial part of the internationally established quality standards for olive oil and essential for evaluating the quality of the extracted oils (Conte *et al.*, 2020).

2.5. Statistical analysis

A statistical data analysis of temperature data and physicochemical parameters for both experiments was performed using PASW Statistics 18.0 (SPSS). The effects of 2 treatments at each storage time (ST) were determined by one-way ANOVA.

2.6. Implementation strategy

It was agreed and planned that the outcome of the experiments would be used to adjust the actual working and storage procedures at the farm as far as the results would lead to expect an amelioration of the logistics without jeopardizing the quality of fruit.

3. RESULTS

3.1. Effect of pre-cooling (experiment 1)

The evolution of the internal temperature of both containers was characterized by the profile shown in Figure 3. In the container with the olive fruit at a field temperature (FT), the mean internal temperature descended towards 10 °C during the first week, after which a more stabilized period set in with temperatures that oscillated between 8.8 and 9.5 °C up to the end of the measurement. Within the first week, a more pronounced cooling was observed during the first two days in which the temperature descended from 18 °C to 13°C. The evolution of the temperature in the container with pre-cooled fruit (PC) followed an inverse trajectory. On day 1, the measurements confirmed that the desired internal temperature of 5 °C was present once the container was filled with the fruit. From day 2, a slow but uninterrupted temperature rise set in, amounting to 7.6 °C on day 14. As this rise was almost linear, no flattening the curve tendencies were observable between day 2 and day 14.

In both treatments (FT and PC), none of the examined parameters attained a level that would lead to losing the quality level ‘Extra Virgen Olive Oil (EVOO)’ (Table 1). For the measured acidity, indicating straightforwardly the degree of oil deterioration, the values stayed far below the established maximum level of 0.8% FFA (expressed as oleic acid). Neverthe-

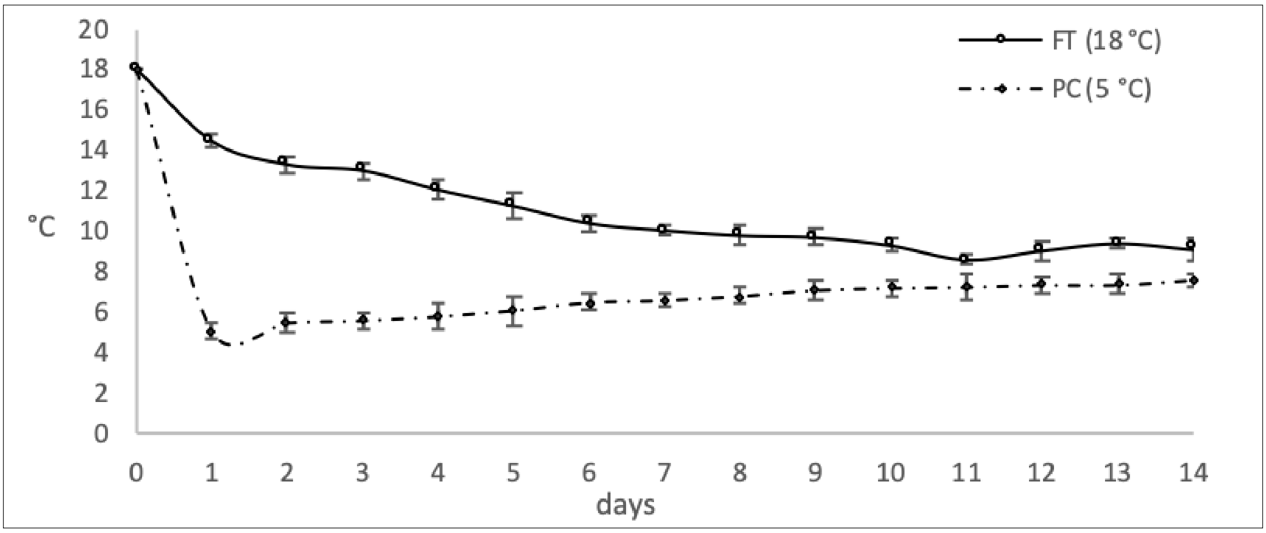

Figure 3. Evolution of the temperature over 14 days within two perforated containers, each filled with 375 kg olives, and placed in the cooling room at 5 °C. FT refers to the fruit with an initial temperature of 18 °C (field temperature); PC refers to fruit brought to 5 °C in small perforated boxes before their transfer to the container (pre-cooled). Each value is the mean of two measurements. Vertical bars express Standard Deviation.

Table 1. Physicochemical parameters of oil extracted from olives stored up to 14 days in perforated plastic bulk containers after applying a pre-cooling treatment before storage (PC) and at field temperature (FT).

Cooling Method (CM)	Storage Time (ST) (days)	FFA (% oleic acid)	PI (meq O_2/kg oil)	K_{232}	K_{270}
FT	0	0.15 ± 0.00 b‡	6.83 ± 0.85	1.69 ± 0.13	0.17 ± 0.01
	4	0.14 ± 0.00 b	7.26 ± 1.56	1.75 ± 0.24	0.16 ± 0.01
	8	0.15 ± 0.01 b	6.66 ± 0.66	1.71 ± 0.05	0.16 ± 0.01
	14	0.18 ± 0.01 a	9.21 ± 1.30	1.92 ± 0.26	0.16 ± 0.01
PC†	0	0.15 ± 0.00	6.83 ± 0.85	1.69 ± 0.13	0.17 ± 0.01
	4	0.13 ± 0.01	7.92 ± 1.16	1.76 ± 0.15	0.15 ± 0.01
	8	0.15 ± 0.01	7.12 ± 0.89	1.77 ± 0.12	0.15 ± 0.01
	14	0.14 ± 0.00	7.90 ± 1.76	1.73 ± 0.05	0.16 ± 0.01
CM		0.011*	0.926	0.617	0.144
ST		0.006**	0.076	0.520	0.005**
CM × ST		0.011*	0.493	0.571	0.826

† The pre-cooling treatment consisted of keeping 10 kg of olives in boxes at 5 °C overnight to guarantee that the fruit attainted an internal temperature of 5 °C before filling the plastic container with 375 kg. The container without treatment was filled with olives at a field temperature of 18 °C (FT). The storage temperature was 5 °C.

‡ In each variable, the values of different treatments followed by different letters are significantly different according to the Tuckey test ($P < 0.05$). The absence of letters means no significant effect due to treatment according to one-way ANOVA ($P < 0.05$). Each value is the mean ± SD of 3 replicates. Significant levels of the factors CM, ST and CM × ST * $p < 0.05$, ** $p < 0.01$.

less, there was a significant difference between both treatments as well as a significant effect of storage time. The significant effect of the interaction between the cooling method (CM) and the storage time (ST) underlined that the effects of applying a pre-cooling treatment increased with storage time. This effect was more prominent in the container filled with fruit at field temperature (FT), where a significant increase was detected at 14 days of storage compared to the other storage times. The olives that underwent a pre-cooling treatment in small boxes (PC), did not experience a significant increase in the acidity level after 14 days of storage compared to the previous ones. The PI values as well as those obtained for K232 and K270 were not influenced in a significant way, either by the CM or the ST.

3.2. Effect of rapid cooling treatment (experiment 2)

Visual examination of triplicate samples of 100 olives at each of the different storage times did not show damages that could be attributed to ‘chilling injuries’ in any of the treatment tested (data not shown).

Subjecting the olives to a rapid cooling at -18 °C for a short time to obtain the desired temperature of 5 °C did not lead to an overall worsening of the examined physicochemical quality parameters (Table 2). No significant differences were observable in the acidity or the K232 and K270. Only the measured PI showed an effect on the speed of cooling (SC). On the other hand, the effect of the ST was significant in all the variables. At punctual storage times, there was a significant difference between the two obtained values. This was the case on day 0 concerning the K270 and, for the level of FFA, on day 4. These slightly deviated results do not influence the overall appreciation of the results.

Finally, it is worth mentioning that the visible bruises that were caused during the cleaning procedure did not produce any problematic rise in any of the examined parameters.

3.3. Implementation

The obtained results were promising for the integration of a pre-cooling procedure as a valuable intermediate step before storage in bins. The filling level of the boxes used during harvesting was reduced to 2/3 to maximize the evacuation of the field heat within each box during the pre-cooling treatment. The filled boxes were transferred to the

TABLE 2. Physicochemical parameters of oil extracted from olives stored at 5 °C for up to 14 days after being subjected to rapid pre-cooling at -18 °C (FC) compared to room cooling at 5 °C (SC).

Storage time (ST) (days)	Speed of cooling (SC)	FFA (% oleic acid)	PI (meq O_2/kg oil)	K_{232}	K_{270}
0	SC	0.14 ± 0.01	11.97 ± 0.29	1.88 ± 0.20	0.21 ± 0.01 a[†]
	FC	0.13 ± 0.01	9.87 ± 1.49	1.89 ± 0.02	0.19 ± 0.01 b
4	SC	0.14 ± 0.00 a	14.83 ± 0.45	1.69 ± 0.06	0.19 ± 0.01
	FC	0.12 ± 0.00 b	13.05 ± 1.87	1.82 ± 0.07	0.19 ± 0.01
8	SC	0.14 ± 0.01	13.89 ± 1.30	1.76 ± 0.07	0.19 ± 0.00
	FC	0.14 ± 0.01	11.21 ± 1.49	1.82 ± 0.07	0.18 ± 0.00
14	SC	0.15 ± 0.00	9.90 ± 1.82	1.70 ± 0.07	0.19 ± 0.01
	FC	0.15± 0.01	10.20 ± 0.68	1.70 ± 0.12	0.20 ± 0.01
ST		0.032*	0.000***	0.034	0.009**
SC		0.162	0.010**	0.245	0.051
ST × SC		0.271	0.257	0.661	0.011*

[†] In each variable, the values for different treatments followed by different letters are significantly different according to the Tuckey test ($P < 0.05$). The absence of letters means no significant effect due to treatment according to one-way ANOVA ($P < 0.05$). Each value is the mean ± SD of 3 replicates. Significant levels of the factors ST, SC and ST × SC * $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$.

cooling room on a very regular basis and whenever 20 boxes were filled. The boxes were placed on pallets and pilled with sufficient space to optimize the airflow between them. The temperature was set at 5 °C (± 1 °C). The next morning, the cooled olives in these boxes were transferred to the presented bins. The emptied boxes were reused during the following picking day.

This procedure was carried out in the harvesting campaigns of 2018 and 2019. Bins were filled and stored within the cooling room. Several of them were stacked on top of each other to make the best use of space. The temperature of the olives in the containers was monitored with a digital thermometer as well as an IR thermometer. The first filled bins were stored for up to 7 days, as the picking had to be postponed for several days due to bad weather. The internal temperature climbed to 7 °C on the day of transport to the mill. Minor problems arose during storage as the capacity of the existing cooling installation was not designed to store such an amount of fruit. The fact that the olives came in in small batches made it possible to remove the field heat over the time of a day and night, although the formation of ice on the slats of the evaporator was inevitable and needed to be removed daily by blowing warm air over it.

The physicochemical characteristics of the obtained oils were analyzed and revealed a high-quality profile for both years (Table 3). The organoleptic quality was not tested by an official sensory panel,

TABLE 3. Physicochemical parameters of the bottled AOVE extracted from olives (c.v. Picual) in the experimental olive mill of the Instituto de la Grasa during the recollection campaigns of 2018 and 2019. The recollected fruit was picked with a Manual Inverted Umbrella and stored at 5 °C with full implementation of the described pre-cooling treatment. Analysis report issued by the Physical-Chemical Laboratory of the Instituto de la Grasa (CSIC).

Harvesting				FFA (% oleic acid)	PI (meq O_2/kg oil)	K232	K270	Delta K
Year	Amount of bins	kg	Max. storage (days)					
2018	10	3.583	7[†]	0.13	6.6	1.65	0.16	< 0.01
2019	13	4.721	5	0.11	5.7	1.75	0.17	< 0.01

[†] The picking was postponed for three days due to bad weather.

but the obtained prizes in various international competitions (New York International Olive Oil Contest 2018: Gold; 2019: Silver; Japanese Olive Oil Contest 2019: Gold; Premio Mezquita de Córdoba 2018: Gold; 2019: Gold) convincingly illustrated their exceptional quality over both years.

4. DISCUSSION

The evolution of the internal temperature showed that instead of a continuous increase in temperature up to even 25 °C, as was reported by García *et al.* (1994) and García and Yousfi (2006), a steady decrease was observed. Some parameters such as the degree of maturity, the degree of damage due to harvesting, and the cooling conditions in which they were kept, come to the fore as possible factors to explain these differences (data not shown). Proper control of the internal temperature was obtained, even without a pre-cooling treatment, and with even better results when the storage of a bulk container started with pre-cooled olives.

Experiment 1 demonstrated that the internal temperature did not rise by more than a few degrees over 2 weeks. As the environment stayed constant at 5 °C, the parameters for the convection stayed the same as well as those for the heat conduction dynamics within the container. It can thus be hypothesized that the excess of energy was generated by the fruit itself. The initial temperature of 5 °C inhibited the respiration of the fruit to a large extent, although it could not impede that over 14 days the heat generated by the respiration exceeded the heat that dissipated from the container by way of transmission or by radiation. In this respect, the results support the assumption of Redding *et al.* (2016) that respiratory heat does have a significant contribution when the (pre)-cooling process is extended.

The vulnerability of olives for chilling injuries is well known and well documented (Kader, 1985). The development of these injuries takes place over an extended storage time and at temperatures below 5 °C. The results of this experiment showed that exposing the olives to a room temperature as low as –18 °C for less than 3 minutes did not cause injuries to the fruit or deterioration to the oil subsequently extracted. The required cooling time of 3 minutes was in line with the observations published by Plasquy *et al.* (2021), who documented the cooling rate of 6 varieties, including the Picual c.v. used in this experiment.

A detailed comparison with the data as published by Garcia *et al.* (1994) was complicated as neither the intactness nor the state of ripening of the fruit was reported. However, the fact that the FT samples did not present excessive acidity levels indicated that additional factors must have exerted a detrimental effect on the fruit and consequently on the quality level of the extracted oil. It must be pointed out that the field temperature of 18 °C is rather low compared the daytime temperatures that can be experienced at the start of the harvesting campaign. Dealing with fruit at temperatures above 25 °C is far from unusual and do caution that more research is needed to document the evolution of the temperature of stored olives in containers.

The respiration rate of fruit increases at higher temperatures and as a consequence so does the respiration heat (Becker *et al.*, 1996). The results of Garcia *et al.* (1994) illustrated that once a given threshold temperature is reached, room cooling, even at 5 °C, becomes insufficient to lower the heat load. This experiment showed that this limit must be above 18 °C. However, further empirical research and modeling are needed to determine at what temperature storage in 400-kg containers becomes critical if not impossible.

Pre-cooling is since long recognized as critical in guaranteeing the quality of fresh fruit and vegetables. Removing the field heat does lead to a drastic reduction in the respiration rate and hence a decline in the deterioration as well (Brosnan and Sun, 2001). The implementation of the experiment illustrated that this technique can be successfully realized at a small scale to optimize cold storage. To what extent the proposed solution can be introduced on a larger scale will depend on the creativity to adapt existing systems to the specific conditions of the olive harvest. The obtained results showed that it is worthwhile to undertake such an endeavor.

5. CONCLUSIONS

The results indicated that olives of the ‘Picual’ variety, stored in containers of 400 kg at an initial temperature of 18 °C, can be kept at 5 °C for 14 days without significant signs of deterioration. Applying a pre-cooling treatment by bringing the fruit temperature to 5 °C before their storage in these containers resulted in a lower internal temperature during the storage time. The slight increase in temperature over time indicated that the respiration of the fruit substantially contributed to the internal heat production, although without provoking substantial deteriorations. Pre-cooling the olives in a fast way did not cause ‘chilling injuries’ nor did it affect the quality parameters of the extracted oil. The pre-cooling treatment offered a workable and affordable solution for the producer and could be successfully implemented as an integral part of the recollection process on the farm. Both experiments raised the expectation that the storage of olives in bin containers be further examined to clarify the vulnerability of the different cultivars and the impact of greater temperature gradients. At the same time, it seems recommendable to explore the possibilities of a fast pre-cooling process in greater depth and at the level of the farm to obtain the desired temperature before olive storage in container bins in a more efficient way.

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REFERENCES

Becker BR, Misra A, Fricke BA. 1996. Bulk refrigeration of fruits and vegetables. Part 1: theoretical considerations of heat and mass transfer. *HVACR Research* **2**, 122–134. https://doi.org/10.1080/10789669.1996.10391338

Brosnan T, Sun DW. 2001. Precooling techniques and applications for horticultural products. A review. *Int. J. Refrig* .**24**, 154-170. https://doi.org/10.1016/S0140-7007(00)00017-7

Canet M, García JM. 1999. Repercusión de la frigoconservación de la aceituna de molino en el proceso de producción de aceite de oliva virgen. *Grasas Aceites* **50**, 181–184.

Clodoveo ML, Delcuratolo D, Gomes T, Colelli G. 2006. Effect of different temperatures and storage atmospheres on Coratina olive oil quality. *Food Chem.* **102**, 571–576. http://doi.org/ 10.1016/j.foodchem.2006.05.035

Conte L, Bendini A, Valli E, Lucci P, Moret L, Manquet A, Lacoste P, Brereton D, García González L, Moreda W, Gallina Toschi T. 2020. Olive oil quality and authenticity: A review of current EU legislation, standards, relevant methods of analyses, their drawbacks and recommendations for the future. *Trends Food Sci. Technol.* **105**, 483–493. https://doi.org/10.3989/gya.1999.v50.i3.653

Dag A, Boim S, Sobotin Y, Zipori I. 2012. Effect of Mechanically Harvested Olive Storage Temperature and Duration on Oil Quality. *Horttechnology* **22**, 528–533. https://doi.org/10.21273/HORTTECH.22.4.5281

EEC. 1991. Commission Regulation No. 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Off. J. Eur. Comm.* **L248**, 1–83.

Faminiani F, Farinelli D, Urbani S, Al Hariri R, Paoletti A, Rosati A. 2020. Harvesting system and fruit storage affect basic quality parameters and phenolic and volatile compounds of oils from intensive and super-intensive olive orchards. *Sci. Hortic.* **263**, 1–14. https://doi.org/10.1016/j.scienta.2019.109045

Ferguson L. 2006. Trends in olive fruit handling previous to its industrial transformation. *Grasas Aceites* **57**, 9–15. https://doi.org/10.3989/gya.2006.v57.i1.17

García P, Brenes M, Romero C, Garrido A. 1995. Respiration and physicochemical changes in harvested olive fruit. *J. Hortic. Sci.* **70**, 925–933

García JM, Gutiérrez F, Barrera MJ, Albi MA. 1996. Storage of Mill Olives on an Industrial Scale. *J. Sci. Food Agric.* **44**, 590–593. https://doi.org/10.1021/jf950479s

García JM, Gutiérrez F, Castellano JM, Perdiguero S, Morilla A, Albi MA. 1994. Storage of Olives destined for oil extraction. *Acta Hortic.* **368**, 673–681. https://doi.org/10.17660/ActaHortic.1994.368.80

García JM, Yousfi K. 2011. Alternativas al aumento de la capacidad de molturación para evitar el deterioro del Aceite de Oliva Virgen. *Mercacei* **68**, 245–251.

García JM, Yousfi K. 2006. The postharvest of mill olives. *Grasas Aceites* **57**, 16–24. htpps://doi.org/10.3989/gya.2006.v57.i1.18

Junta de Andalucía. Consejeria de Agricultura, Pesca y Desarrollo Rural. (2015). *Análisis de las* *plantaciones de Oliver en Andalucía. Año 2015*. Encuestra sobre Superficies y Rendimientos de Cultivos en España (ESYRCE)

Kader A. 1985. An overview of the physiological and biochemical basis of CA effects on fresh horticultural crops, in *Fourth Nat. Contr. Atm. Res. Conf*., North Carolina State University, pp. 1–9. https://hortscans.ces.ncsu.edu/library/all/doc_id/399/

Kalua CM, Bedgood DR, Bishop AG, Prenzler PD. 2008. Changes in Virgen Olive Oil Quality during Low-Temperature Fruit Storage. *J. Agric. Food Chem.* **56**, 2415–2422. https://doi.org/10.1021/jf073027b

Kiritsakis A, Nanos GD, Polymenopoulos Z, Thomai T, Sfakiotakis EM. 1998. Effect of Fruit Storage Conditions on Olive Oil Quality. *J. Am. Oil Chem. Soc.* **75**, 721–724. https://doi.org/10.1007/s11746-998-0212-7

Martinez SJ, Muñoz AE, Alba MJ, RA Lanzón RA. 1975. Informe sobre utilizacion del Analizador de Rendimientos “Abencor”. *Grasas Aceites* **26**, 379–385.

Mercier S, Villeneuve S, Mondor M, Uysal I. 2017. Time-Temperature Management Along the Food Cold Chain: A review of Recent Developments. Compr. *Rev. Food Sci. Food Saf.* **16**, 647–667. http://doi.org/ 10.1111/1541-4337.12269

Morales-Silero A, García JM. 2015. Impact assessment of mechanical harvest on fruit physiology and consequences on oil physicochemical and sensory quality from ‘Manzanilla de Sevilla’ and ‘Manzanilla Cacereña’ super-high-density hedgerows. A preliminary study. *J. Sci. Food Agric.* **95**, 2445–2453. https://doi.org/10.1002/jsfa.6971

Plasquy E, Sola-Guiraldo RR, Florido MC, García JM, Blanco-Roldán G. 2019. Evaluation of a Manual Olive Fruit Recolector for Small Producers. *Res. Agric. Eng.* **65**, 105–111. https://doi.org/10.17221/18/2019-RAE

Plasquy E, García JM, Florido MC, Sola-Guirado RR. 2021. Estimation of the Cooling Rate of Six Olive Cultivars Using Thermal Imaging. *Agriculture* **11**, 164. https://doi.org/10.3390/agriculture11020164

Rallo L, Díez CM, Morales-Silero A, Miho H, Priego-Capote F, Rallo P. 2018. Quality of olives: A focus on agricultural preharvest factors. *Sci. Hortic.* **233**, 491–509. https://doi.org/10.1016/j.scienta.2017.12.034

Redding GP, Yang A, Shim YM, Olatunji J, East A. 2016. A review of the use and design of produce simulators for horticultural forced-air cooling studies. *J. Food En.g* **190**, 80–93. http://doi.org/10.1016/ j.jfoodeng. 2016.06.014

Sola-Guirado RR, Castro-García S, Blanco-Roldán GL, Jiménez-Jiménez F, Castillo-Ruiz FJ, Gil-Ribes JA. 2014. Traditional olive tree response to oil olive harvesting technologies. *Biosys.t Eng.* **118**, 186–193. https://doi.org/10.1016/j.biosystemseng.2013.12.007

Yousfi K, Weiland CM, García JM. 2012. Effect of Harvesting System and Fruit Cold Storage on Virgen Olive Oil Chemical Composition and Quality of Superintensive Cultivated ‘Arbequina’ Olives. *J. Sci. Food Agric.* **60**, 4743–4750. https://doi.org/10.1021/jf300331q

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Chemical composition and thermal properties of Tunisian pecan nut [*Carya illinoinensis* (Wangenh.) K. Koch] oils

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SUMMARY: An investigation on fatty acid, triacylglycerol, tocopherol, and xanthophyll contents and thermal properties of pecan (*Carya illinoinensis*) kernel oils from two cultivars was carried out. The main fatty acids were oleic acid, followed by linoleic and palmitic acids. The predominant triacylglycerols were OOL, OOO, and OLL (where O stands for oleoyl and L for linoleoyl). Pecan kernel oil is a rich source of tocopherols, mainly γ-tocopherol. Two xanthophylls (lutein and zeaxanthin) were investigated, and lutein was found to be the major one. Thermal behavior was studied by differential scanning calorimetry (DSC). Pecan nut oil displayed melting and crystallization transitions at low-temperature zones. The difference between DSC parameter values provides a path for distinguishing among cultivars. These data promote pecan kernel oil as a potential source of bioactive compounds with nutraceutical properties (monounsaturated fatty acids, tocopherols, and xanthophylls) and reveal, for the first time, the thermal properties of *Carya illinoinensis* oil.

KEYWORDS: *Pecan nut; Fatty acids; Tocopherols; Triacylglycerols; Xanthophylls; Thermal properties*

RESUMEN: ***Composición química y propiedades térmicas de aceites de nuez de pecana*** **[Carya illinoinensis (Wangenh.) K. Koch]** ***tunecina.*** Se llevó a cabo una investigación sobre el contenido de ácidos grasos, triacilgliceroles, tocoferoles, xantofilas y propiedades térmicas de aceites de nuez de pecana (*Carya illinoinensis*) de dos cultivares. Los principales ácidos grasos fueron el ácido oleico seguido de los ácidos linoleico y palmítico. Los triacilgliceroles predominantes fueron OOL, OOO y OLL (donde O, oleoilo y L; linoleoilo). El aceite de nuez de pecana es una fuente rica en tocoferoles, principalmente γ-tocoferol. Se investigaron dos xantofilas (luteína y zeaxantina), la luteína fue la mayoritaria. Los comportamientos térmicos se estudiaron mediante calorimetría diferencial de barrido (DSC). El aceite de nuez de pacana mostró transiciones de fusión y cristalización en zonas de baja temperatura. La diferencia entre los valores de los parámetros de DSC proporciona un camino para distinguir entre cultivares. Estos datos presentan al aceite de nuez pecana como una fuente potencial de compuestos bioactivos con propiedades nutracéuticas (ácidos grasos monoinsaturados, tocoferoles y xantofilas) y revelan, por primera vez, las propiedades térmicas del aceite de *Carya illinoinensis*.

PALABRAS CLAVE: *Ácidos grasos; Nuez de pecana; Propiedades térmicas; Tocoferoles; Triacilgliceroles; Xantofilas*

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1. INTRODUCTION

The World Health Organization (WHO) has recommended the dietary consumption of nuts due to their high contents in unsaturated fatty acids which help to prevent cardiovascular diseases, obesity, diabetes, certain cancers, and other chronic diseases (WHO, 2019). Among all nuts, the world production of pecan (*Carya illinoinensis*) oil is promptly increasing. This oil is recognized as a healthy specialty oil. Indeed, pecan kernel oil has been reported to be a good source of fat-soluble bioactive compounds such as unsaturated fatty acids, tocopherols, phytosterols (Miraliakbari and Shahidi, 2008; Bouali *et al.,* 2013), and polyphenols (Kornsteiner *et al.,* 2006; Bouali *et al.,* 2020). Likewise, clinical studies have revealed that regular pecan nut supplementation ameliorates the lipid profiles that are related to cardiovascular risk (Atanosov *et al.,* 2017).

The potential health benefits of pecan kernel oil are often attributed to its high levels of monounsaturated fatty acids (MUFA) (Miraliakbari and Shahidi, 2008; Atanosov *et al.,* 2017), primarily oleic acid (60- 70% of total fatty acids) (Bouali *et al.,* 2013; do Prado *et al.*, 2013).

Pecan kernel oil contains triacylglycerols (96.4 g/100 g of oil) (Miraliakbari and Shahidi, 2008), which are the major components of most vegetable oils included in the human diet. They are considered an important factor in the evaluation of the oxidation of oils (Zeb, 2012). Moreover, triacylglycerols (TAG) are employed in the food industry to assess the authenticity of vegetable oils.

Emphasis has been given to the use of lipid-soluble antioxidants. Indeed, antioxidants such as tocopherols and carotenoids generally enhance oil stability, and consequently, consumers often value oils naturally rich in these compounds. Tocopherols are powerful antioxidants and scavengers of free radicals. They possess potent anti-cancer, anti-inflammatory, antiatherogenic, cardioprotective, and cholesterol lowering properties (Mathur *et al.,* 2015).

Carotenoids are fat-soluble compounds known for their provitamin A activity. Among the carotenoids, we primarily examined two xanthophylls which are lutein and zeaxanthin. They are present in the macula of the retina in the eye. They have protective effects against the evolution of age-related macular degeneration and cataracts (Ma and Lin, 2010). Likewise, many recent epidemiological studies have shown that lutein and zeaxanthin may reduce the risk of cancer and coronary heart disease (Ma and Lin, 2010).

It is important to monitor the thermal behavior of edible vegetable oils and fats in order to evaluate the changes in their comportments when exposed to thermal load disruptions, to verify their authenticity (Nilchian *et al.,* 2020), and to enhance the acquaintance concerning the influence of oil composition on their thermal behavior (Chambre *et al.,* 2019). Differential scanning calorimetry (DSC) is the most commonly used thermo-analytical tool for oils. Numerous studies have focused on the characterization of tree nut oils by DSC such as hazelnut (*Corylus avellana* L.) oil, walnut (*Juglans regia* L.) oil (Che Man and Tan, 2002), Almond (*Prunus dulcis* L.) oil (Beltrán *et al.,* 2011) and pistachio (*Pistacia vera* L.) oil (Ling *et al.,* 2015). So far, no complete research concerning the thermal behavior of pecan nut oil has been performed.

A comparative study of the different pecan cultivars is key for breeding programs and the food industry in the selection of cultivars with the maximum amount of high value-added compounds. In this context, our aim was to evaluate two Tunisian pecan cultivars (Mahan and Moore) through their fatty acids, TAG, tocopherol, and xanthophyll compositions and through their thermal behavior.

2. MATERIALS AND METHODS

2.1. Reagents and standards

Toluene, acetonitrile, methanol, dichloromethane, and hexane were supplied by Carlo Erba (Val de Reuil, France). Petroleum ether was obtained from Fisher Scientific SA (Loughborough, UK). Fatty acid methyl ester (FAME) standards were acquired from Nu-Chek-Prep (Elysian, MN, USA). Tocopherol standards, ammonium acetate, sodium bicarbonate, and 7% boron trifluoride in methanol (BF_3-MeOH) were provided by Sigma–Aldrich (Saint-Quentin Fallavier, France). Lutein and Zeaxanthin standards were generous gifts from Roche (Neuilly-sur-Seine, France).

2.2. Pecan samples

Pecan nuts from Moore and Mahan cultivars were harvested at complete maturity from a restricted zone on the INRGREF experimental farm (National Institute for Research in rural engineering, water and forest), in Mateur, Northern Tunisia (Longitude: 09°48′ E; Latitude: 37°15′ N; Altitude: 5 m above sea level).

Husks were removed And the kernels were detached manually from the shell and dried in an oven at 60 °C. Using a mortar, the dry samples were finely ground for the extraction process.

2.3. Oil extraction

Oil was extracted from dry milled pecan kernels (5 g) using a soxhlet apparatus with light petroleum ether for 6 h at 42 °C. The solvent was removed from the resulting extract by rotary vacuum evaporation at 50 °C. The obtained oil was stored in dark glass bottles at 4 °C until analysis.

2.4. Fatty acid analysis

The fatty acid composition was determined as fatty acid methyl esters (FAME) prepared as described previously by Bouali *et al.* (2013). Twenty milligrams of oil were mixed with 0.7 mL boron trifluoride reagent (BF3/methanol, 7 %) and 0.3 mL toluene under nitrogen and the tube was heated at 95 °C for 2 h and then cooled to room temperature. FAMEs were extracted by adding 5 mL of sodium bicarbonate and 3 x 2 mL hexane. The obtained mixture was centrifuged for 3 min. The solvent was removed under a nitrogen stream. Then, the FAMEs were dissolved in hexane and analyzed on a Hewlett Packard Model 4890 gas-chromatograph (Palo Alto, CA, USA) with a CP-Sil 88 (100 m × 0.25 mm I.D. capillary column, 0.2 µm film, Varian, Les Ulis France). The injector and detector temperatures were 250 and 280 °C, respectively. The initial oven temperature was held at 60 °C for 5 min, increased to 165 °C at 15 °C/min and held for 1 min at this temperature; then increased to 225 °C at 2 °C/min, before being maintained at this temperature for 17 min. The peaks were identified based on their retention times using synthetic and commercial standards. Analyses were performed in triplicate.

2.5. Triacylglycerol analysis

The TAG analysis was performed by gas chromatography (GC) using the same chromatographic conditions as reported by Bootello *et al.* (2016). About 5 mg of pecan kernel oil were dissolved in 1.8 mL of heptane. One µL of this solution was injected into the GC, an Agilent 7890 gas chromatograph (Palo Alto, CA) equipped with a flame ionization detector (FID) and a Quadrex Aluminum-Clad 400-65HT chromatography column (30 m length; 0.25 mm diameter; 0.20 µm film thickness: Bellefonte, PA, USA). Hydrogen was used as the carrier gas at a linear gas rate of 50 $cm \cdot s^{-1}$ and a split ratio of 1:50. The detector and injector temperatures were 370 and 350 °C, respectively; the oven temperature was 335 °C, and a pressure gradient from 100 to 200 kPa was applied. Peak identification was performed by comparing their retention times with those of a standard mixture and known samples. Quantification of the peaks was made by internal normalization of the chromatographic peak area, and the results are expressed in relative percentage.

2.6. Tocopherol and xanthophyll analysis

The tocopherol and xanthophylls analysis was performed by reverse-phase high performance liquid chromatography (RP-HPLC) using the procedure previously described by Bouali *et al.* (2013). Samples (40 mg) were diluted in 1 mL of mixed HPLC mobile phase: acetonitrile/methanol containing 50 mM ammonium acetate/water/dichloromethane (700:150:50:100, V/V/V/V). Then, the mixture was vortexed.

The assays were carried out on a Jasco PU-1580 apparatus plus intelligent pump equipped with an automatic injector system AS300 (Thermo Finnigan, Les Ulis, France) and a Jasco MD-1510 plus multi wavelength detector (JASCO International Co., Ltd., Japan). Chromatographic separation was performed on a VIDAK C18 column (25 mm x 4.6 mm id, 5 µm particle size) connected with a Nucleosil C18 column (25 mm x 4.6 mm id, 5 µm particle size). The mobile phase was a mixture of acetonitrile/ methanol at 50 mM ammonium acetate/ water/ dichloromethane (700:150:50:100, V/V/V/V) at a flow rate of 2 mL/min. Samples of 18 µL were injected, the wavelengths were set at 298 nm for tocopherols and 450 nm for lutein and zeaxanthin. The identification was carried out by comparing the retention times and absorption spectra of unknown peaks to authentic standards and by adding α-, δ-, γ-tocopherol, lutein, and zeaxanthin standards to the sample for co-chromatography. The quantification was based on the external standard method.

2.7. Thermal analysis by DSC

Thermal properties of pecan kernel oils were carried out on a diferential scanning calorimetry (DSC) using a Q2000 V23.5 scanner (TA instruments, New Castle, DE, USA). Dry nitrogen was used as the purge gas at a rate of 20 mL/min. Oil samples (6-8 mg) were weighed directly into aluminium pans. An empty sealed pan was employed as control.

Samples were subjected to the following temperature program: 90 °C isotherm for 5 min, and then cooled to -80 °C at a rate of 10 °C/min, held for 20 min. Oil samples were then heated from -80 °C to 90 °C at a rate of 5 °C/min to acquire the data of the melting thermogram. The crystallization was achieved by heating the oils at 90 °C for 5 min and then decreasing the temperature to -80 °C at a rate of 10 °C/min.

Crystallization and melting parameters: the melting enthalpy ($\triangle H_m$), the temperature of the major peak of melting (P_m), the initial and end temperature of the melting phase (T onset $_m$ and T end $_m$), the crystallization enthalpy ($\triangle H_c$), the temperature of the major peak of crystallization (P_c), the initial temperature of the crystallization phase (T onset $_c$), the range of the transition phase (R: the temperature difference between T onset and T end) were determined.

2.8. Statistical analysis

The results were expressed as mean values and standard deviation and were analyzed using the statistical analysis system XLSTAT (version 2015). The differences between cultivars were analyzed using one-way ANOVA followed by Duncan's test ($P < 0.05$), and principal component analysis (PCA).

3. RESULTS AND DISCUSSION

3.1. Total lipid content

Oil contents, expressed in percentage of dry weight, are reported in Table 1. It can be deduced that the amount of lipids differs among pecan nut cultivars as has been observed previously (Ribeiro *et al.,* 2020). Moore kernels had lipid content significantly higher (72.57 %) than those of Mahan (69.64 %). The kernels investigated in this study exhibited a substantial fount of lipids for food with more than 69% of this macronutrient (Table 1). In the present study, the oil content of both cultivars was significantly higher than that of the Burkett cultivar (68.42% dry weight) as previously studied (Bouali *et al.,* 2013).

Table 1. Oil content (expressed as % dry weight) and fatty acid composition (% of total fatty acids).

	Cultivars	
	Mahan	**Moore**
Total oil content (%)	69.64 ± 0.24^{a}	72.57 ± 1.02^{b}
Fatty acids		
C14:0	0.06±0.017^{a}	0.05±0^{a}
C15:0	0.02±0.015^{b}	0.013±0.005^{a}
C16:0	6.37±0.06^{a}	6.2±0.03^{a}
C16:1n-9	0.03±0^{a}	0.03±0^{a}
C16:1n-7	0.09±0^{b}	0.08±0^{a}
C17:0	0.06±0^{a}	0.06±0.005^{a}
C18:0	2.61±0.005^{a}	2.27±0.005^{a}
C18:1n-9	55.16±0.28^{a}	56.81±0.08^{b}
C18:1n-7	1.05±0.04^{a}	1.12±0.025^{a}
C18:2n-6	32.73±0.24^{a}	31.60±0.06^{a}
C20:0	0.13±0^{b}	0.12±0^{a}
C20:1n-9	0.22±0^{a}	0.26±0.006^{b}
C18:3n-3	1.44±0.035^{b}	0.3±0^{a}
C22:0	0.03±0.005^{a}	0.03±0^{a}
Σ SFA	9.28	8.75
Σ MUFA	56.55	58.30
Σ PUFA	34.17	31.63
Σ UFA	90.72	89.93

Each value is a mean ± standard deviation (SD) of a triplicate analysis performed on different samples. Fatty acids detected: C14:0, myristic acid; C15:0, pentadecylic acid; C16:0, palmitic acid; C16:1n-9, palmitoleic acid; C17:0, margaric acid; C18:0, stearic acid; C18:1n-7, vaccenic acid; C18:1n-9, oleic acid; C18:2n-6, linoleic acid; C18:3n-3, α-linolenic acid; C20:0, arachidic acid, C20:1n-9, eicosanoic acid; C22:0, behenic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids. Means with same letters in the same line were not significantly different according to the multiple range Duncan test at $P < 0.05$.

These values are similar to those published by Miraliakbari and Shahidi, (2008) but higher than those reported by do Prado *et al.* (2013) and Ribeiro *et al.* (2020). Differences in lipid content in pecans could be attributed to cultivar, geographic factors, ripening stage, and variations in extraction methods (Miraliakbari and Shahidi, 2008; Bouali *et al.,* 2013; Ribeiro *et al.*, 2020). Most nuts contain high amounts of fat (almond 60.10%, pistachio nut 22.09%, and walnut 32.95%) (Rabadán *et al.,* 2019).

3.2. Fatty acid composition

Table 1 shows the fatty acid composition of Mahan and Moore cultivars. Fourteen fatty acids were detected. Oleic acid was the predominant one (55.16–56.81% of total fatty acids). Linoleic acid was the second in order of importance (31.60–32.73%). Among the remaining fatty acids only palmitic (6.20–6.37%) and stearic (2.27–2.61%) acids showed considerable amounts. The major fatty acids found in the present study are, in general, comparable to those reported in the literature (do Prado *et al.*, 2013; Ribeiro *et al.*, 2020).

In the current study, the fatty acid composition of both cultivars was significantly different from the Burkett cultivar previously investigated, which showed a particular composition with very high oleic acid (69.04%) and low linoleic acid (20.69%) contents (Bouali *et al.,* 2013). This variation could be attributed to genetic differences.

In pecan kernel oil, saturated fatty acids (SFA) were the minor group (8.75–9.28% of total fatty acids): whereas monounsaturated fatty acids (MUFA) were the highest (56.55 – 58.30%) (Table 1). It is important to mention that the pecan nut oil is exceeded in monounsaturated fatty acids level by olive oil (58.07–71.13%) (Ouni *et al.,* 2011) but followed by other nut oils like pistachio nut (54.28%), cashew (57.54%) and walnut (19.16%) (Al Juhaimi *et al.,* 2018). Polyunsaturated fatty acids (PUFA) were present at high levels (31.63 – 34.17% of total fatty acids).

Overall, the obtained results (MUFA > PUFA > SFA) are similar to those observed in Brazilian pecan nuts (Ribeiro *et al*., 2020) and other geographical origins such as Mexico (Rábago-Panduro *et al.,* 2020).

3.3. Triacylglycerols composition

In a previous study, Miraliakbari and Shahidi (2008) reported that pecan nut oil contains 96.4% of triacylglycerols. Therefore, it was interesting to investigate the TAG compositions among pecan nut cultivars. The TAGs were identified and quantified by GC. Significant differences between the two cultivars were detected. As shown in Table 2, thirteen compounds were identified and quantified: POP, PLP, POS, POO, PLS, POL, PLL, SOO, OOO, SOL, OOL, OLL, LLL (where P, palmitoyl; S, stearoyl; O, oleoyl; and L; linoleoyl). The predominant TAG was OOL (28.05 – 28.54%) in both cultivars, followed by OOO (25.96%) and OLL (15.53%) in the Moore cultivar. In the Mahan cultivar, OLL (20.62%) was the second major TAG and OOO was the third (17.77%). The remaining TAGs had smaller percentages (Table 2). The amount of POS was the lowest in both cultivars. The variation, observed among cultivars, may be attributed to genetic causes, mostly acyltransferase activities during the TAG assembly. Several authors have emphasized the effect of genotype on TAG composition in other nuts (Bouabdallah *et al.,* 2014; Taş and Gökmen, 2015). The TAG composition is considered the most effective parameter for distinguishing cultivars (Bouabdallah *et al.,* 2014), which is confirmed by our findings.

Table 2. Triacylglycerol composition (% of total triacylglycerols)

	Cultivars	
	Mahan	**Moore**
POP	0.49±0.12^a	0.53±0.12^a
PLP	0.44±0.1^a	0.50±0.11^a
POS	0.23±0.04^a	0.28±0.04^a
POO	5.63±0.7^a	7.90±0.95^b
PLS	0.53±0.1^a	0.78±0.1^b
POL	7.80±0.88^a	8.20±0.83^b
PLL	4.31±0.41^b	3.03±0.25^a
SOO	1.82±0.07^a	2.03±0.05^a
OOO	17.77±0.18^a	25.96±0.07^b
SOL	3.36±0.03^b	2.00±0.00^a
OOL	28.05±0.35^a	28.54±0.73^a
OLL	20.62±0 .82^b	15.53±0.82^a
LLL	8.97±0.5^b	4.69±0.84^a
Total	**100**	**100**

Each value is a mean ± standard deviation (SD) of a triplicate analysis performed on different samples. P, palmitoyl; S, stearoyl; O, oleoyl; and L; linoleoyl. Means with same letters in the same line were not significantly different according to the multiple range Duncan test at $P < 0.05$.

Our results are different from those reported by Fernandes *et al.* (2017) for pecan nuts obtained from local grocery stores in Brazil. These same authors reported the predominance of PLP + OOO + PoPP. This difference could be attributed to cultivar and geographical variations, ripeness stages, storage conditions and differences in extraction methods and analytical procedures.

TABLE 3. Tocoperol and xanthophyll contents in pecan nut kernel oils (expressed as mg/kg of oil).

Cultivars	α-tocopherol	δ-tocopherol	γ-tocopherol	Total tocopherols	Lutein	Zeaxanthin	Total xanthophylls
Moore	6.31 ± 0.5^{b}	0.80 ± 0.25^{b}	259.82 ± 10.35^{b}	266.93	0.22 ± 0.01^{a}	ND^{a}	0.22
Mahan	4.64 ± 0.42^{a}	0.50 ± 0.1^{a}	197.30 ± 7.54^{a}	202.44	0.23 ± 0^{a}	ND^{a}	0.23

ND: Not detected. Each value is a mean ± standard deviation (SD) of a duplicate analysis performed on different samples. Means with the same letters in the same column were not significantly different according to the multiple range Duncan test at $P < 0.05$.

For walnut (*Juglans regia*), a member of the same family as the pecan nut (the Juglandaceae family), 19 TAG compounds were detected in this oil, with LLL as the major one (Bouabdallah *et al.,* 2014).

3.4. Tocopherol contents

The amount of tocopherols is a fundamental parameter for assessing the quality of oils. The tocopherol composition is reported in Table 3. γ-Tocopherol was predominant in both analyzed oils, representing about 98% of total tocopherols. The highest amount was observed in the Moore cultivar (259.82 mg/kg of oil); whereas the lowest was detected in the Mahan cultivar (197.30 mg/kg of oil). γ-Tocopherol is considered the most frequent isoform of vitamin E in several nuts (Kornsteiner *et al.,* 2006). α-Tocopherol was the second predominant isoform, accounting for 2.36% and 2.29% of the total tocopherols in Moore and Mahan cultivars, respectively. Several investigations have revealed that γ-tocopherol possesses beneficial activities which are not common to α-tocopherol. γ-Tocopherol has, notably, stronger anti-cancer, anti-inflammatory, antioxidant, cardioprotective properties (Smolarek and Suh, 2011; Mathur *et al.,* 2015), greater detoxification of nitrogen oxides and higher inhibition of cyclooxygenase-2 (COX-2) (Mathur *et al.,* 2015) than α-tocopherol. In addition, γ-tocopherol, but not α-tocopherol, exerts an anti-proliferative effect on lung and prostate cancer cells (Jiang *et al.,* 2004).

Besides γ- and α-tocopherols, δ-tocopherol was also present in pecan kernel oil, with low amounts representing 0.3 and 0.25 % of the total tocopherols in Moore and Mahan cultivars, respectively.

These results indicated that pecan kernel oil provides natural antioxidants, with the Moore cultivar exhibiting the greatest tocopherol content (266.93 mg/kg of oil). In this study, the total tocopherol content of both cultivars was significantly higher than that of the Burkett cultivar previously investigated (Bouali *et al.,* 2013). Similar results have been found by other authors (do Prado *et al.*, 2013) implying that tocopherol content is highly cultivar-dependent.

The total tocopherol contents found in this study were higher than those reported by Kornsteiner *et al.,* (2006) and Fernandes *et al,.* (2017). In contrast, they were lower than those reported by Miraliakbari and Shahidi (2008). It is well-known that tocopherol content is strongly influenced by genetic differences, maturity (Bouali *et al.,* 2013), geographic and climatic conditions (do Prado *et al.*, 2013), as well as differences in extraction methods and analytical procedures (Miraliakbari and Shahidi, 2008).

Miraliakbari and Shahidi (2008) studied the tocopherol composition of oils extracted from several nuts by different solvents and found that the tocopherol content in pecan kernel oil ranges from 453.9 to 491.1 mg/kg oil, exceeded by the total tocopherol content in hazelnut oil. It has been pointed out that pecan kernel oil contains the greatest amount of γ-tocopherol among tree nut oils (Miraliakbari and Shahidi, 2008).

3.5. Xanthophyll contents

Both cultivars were also investigated for their levels of lutein and zeaxanthin. Among xanthophylls, lutein and zeaxanthin prevent the risk of several diseases (Ma and Lin, 2010). Thus, it is beneficial to assess the levels of these compounds in pecan nuts in order to select the cultivar which contains the greatest amount of xanthophylls.

The amounts of lutein and zeaxanthin as well as their sum are reported in Table 3. Concerning total xanthophylls, a similarity was found between the cultivars. In the present study, the total xanthophylls contents in both cultivars (0.22–0.23 mg/kg of oil) was significantly higher than that of the Burkett cultivar (0.14 mg/kg

Table 4. DSC parameters.

	Mahan		Moore	
	Peak 1	**Peak 2**	**Peak 1**	**Peak 2**
Melting Curves:				
$\triangle H_m$ (J/g)	66.2		70.2	
P_m (°C)	-29.2	-21.2	-27.4	-19.4
T onset $_m$ (°C)	-41.7	-26.3	-37.5	-24.7
T end $_m$ (°C)	-26.3	-7.6	-24.7	-4.5
R (°C)	34.1		33.0	
Crystallization Curves:				
$\triangle H_c$ (J/g)	40.1		61.2	
P_c (°C)	-63.3		-58.9	
T onset $_c$ (°C)	-24.6		-24.8	

$\triangle H_m$: melting enthalpy, P_m: temperature of the major peak of melting, T onset $_m$ and T end $_m$: initial and end temperature of the melting phase, $\triangle H_c$: crystallization enthalpy, P_c: temperature of the major peak of crystallization, T onset $_c$: initial temperature of the crystallization phase, R: range of the transition phase (temperature difference between T onset and T end).

of oil) already studied (Bouali *et al.,* 2013). It is important to highlight that *Carya illinoinensis* kernel oil is not a substantial source of dietary xanthophylls.

In pecan kernel oil, lutein was the predominant xanthophyll. Our findings are in disagreement with the previous study of Kornsteiner *et al.,* (2006) who did not detect any lutein. The same authors revealed that pecan nut oil was surpassed in lutein level by pistachio nut oil (15–96 mg/kg of oil) (Kornsteiner *et al.,* 2006). Indeed, pistachio nut oil contains the highest level of lutein among all nut oils; whereas almond, Brazil nut, cashew, hazelnut, macadamia and walnut oils do not contain any lutein (Kornsteiner *et al.,* 2006). Nonetheless, zeaxanthin was not detected in either cultivar of *Carya illinoinensis*.

3.6. Thermal properties

The thermal parameters supplied by the heating and cooling thermograms are given in Table 4. The melting thermograms of both cultivars displayed profiles with two not well-separated endothermic phase transitions. As shown in Table 4, the temperature of the first major peak of melting (P_m) was -29.2 ºC for Mahan cultivar and -27.4 ºC for Moore cultivar with an onset temperature (T onset $_m$) ranging between -41.7 °C (Mahan) and -37.5 °C (Moore) and an end temperature (T end$_m$) varying between -26.3 and -24.7 °C for Mahan and Moore, respectively. The temperature of the second P_m was -21.2 ºC for Mahan and -19.4 ºC for Moore with a T onset $_m$ ranging between -26.3 °C (Mahan) and -24.7 °C (Moore) and a T end $_m$ varying between -7.6 °C (Mahan) and -4.5 °C (Moore). Mahan showed the lowest P_m. The non-significant difference in P_m values is attributed to the fact that the Mahan cultivar presents more unsaturated TAGs and fatty acids, and consequently, this leads to the appearance of a lower melting point (Mansor *et al.,* 2012) than that of the Moore cultivar. As explained in the literature, the first peak detected in both samples at a low-temperature region of the heating curve might be linked to the melting of the lowest stability polymorphic form of TAGs (Rezig *et al.,* 2012). The range of transition phase (R) varied between 33 and 34.1 °C for Moore and Mahan, respectively. The melting enthalpy ($\triangle H_m$) varied significantly among cultivars. The Moore cultivar had the highest value (70.2 J/g). The melting behavior of both cultivars revealed identical patterns but different DSC parameter values which confirm that the melting behavior was affected by cultivar. This difference is in concordance with the variation in TAG composition detected between the two cultivars.

Tan and Che Man (2002) investigated the melting behavior of walnut (*Juglans regia*) oil and reported a broad endothermic peak with smaller ones. These peaks were attributed to the melting of the predominant TAGs detected in this oil, namely LLL, LLLn, and OLL. The same authors also studied the melting behavior of hazelnut oil and found a large peak.

The crystallization profiles showed an exothermic phase transition with one well-defined peak for both cultivars. The temperature of the major peak of crystallization (P_c) was -63.3 for Mahan cultivar and

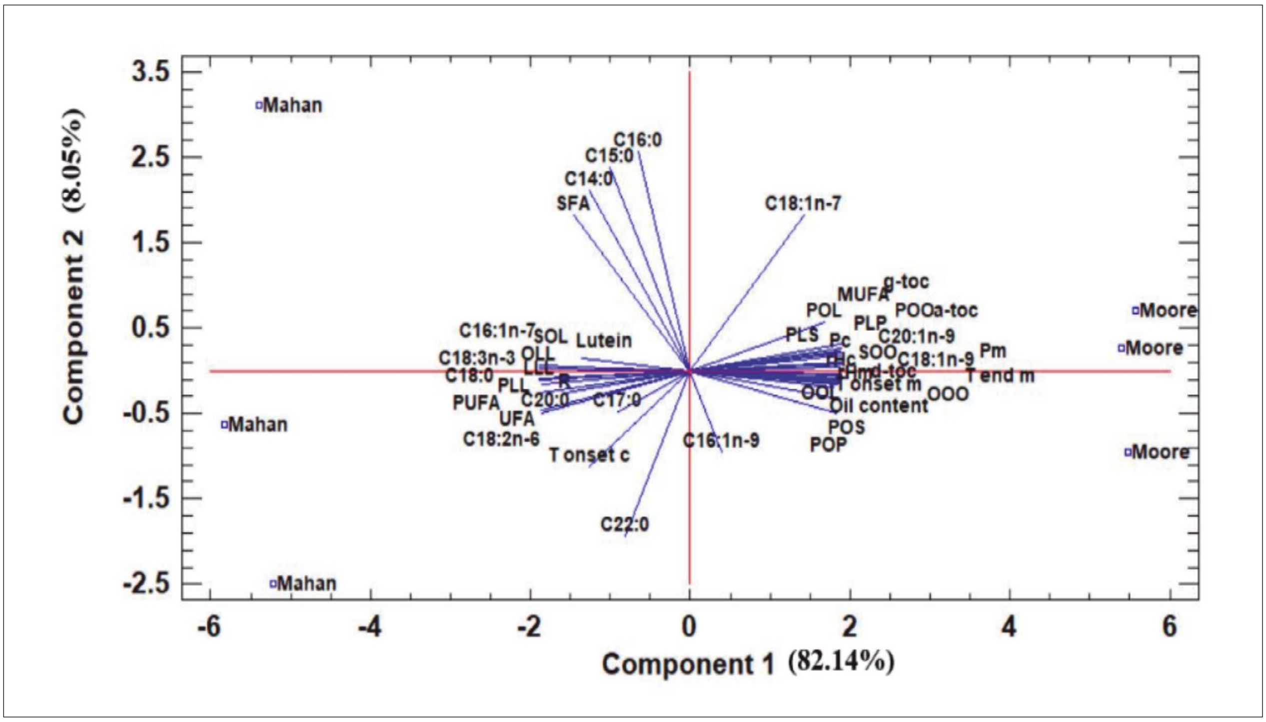

FIGURE 1. Graphical depiction of principal component analysis carried out for Mahan and Moore cultivars.

-58.9 ºC for Moore cultivar (Table 4). As explained previously, here too, the Pc observed for both samples at a low-temperature zone was mainly due to the high contents in OOL, OLL, and OOO, since these unsaturated TAGs are known to crystallize at lower temperatures (Yanty *et al.,* 2013). Both cultivars showed similar values for initial temperature of crystallization (T onset $_c$) (-24.8 – -24.6 ºC). The crystallization enthalpy ($\triangle H_c$) varied significantly among cultivars indicating that the genotype significantly influences the $\triangle H_c$ value.

The crystallization behavior of both cultivars exhibited identical patterns. However, different values for DSC parameters demonstrated that it was linked to cultivar. This significant variability (Table 4) may be explained by the dissimilarity of fatty acid and TAG compositions detected between cultivars.

The crystallization behavior in pecan nut oil differs remarkably from that of walnut oil. Indeed, a wide exothermic peak and two smaller ones were detected in walnut oil with a $\triangle H_c$ value of -57.4 J/g (Che Man and Tan, 2002). The major peak was attributed to the crystallization of the main TAGs found in this oil (Che Man and Tan, 2002).

The differences found in the thermal analysis supply a basic comprehension of the thermodynamic variations linked to the chemical composition. As indicated by the melting and crystallization parameters, the lowering of the transition temperatures is directly related to the high level of unsaturated TAGs present in pecan kernel oil. To our knowledge, no previous studies have investigated the thermal properties of pecan nut oil and hence, it was not possible to compare our findings with prior works.

3.7. Principal component analysis (PCA)

For a deeper investigation of the differences and similarities between the two studied cultivars, a principal component analysis was performed (Figure 1).

According to the PCA results, the first two components explained 90.19% of the total variance with the first one at 82.14% and the second one 8.05% of the total variance.

Moore and Mahan were different, located at the positive and negative sides of component 1. Moore was typified by great levels of oil content, C16:1n-9, C18:1n-9, C18:1n-7, C20:1n-9, MUFA, γ-tocopherol, α-tocopherol, δ-tocopherol, OOO, POO, POL, PLS, POP, POS, SOO, OOL, PLP, and high values for $\triangle H_m$, $\triangle H_c$, P_m, P_c, T end $_m$, T onset $_m$. Mahan was characterized by substantial levels of C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, SFA, C16:1n-7, C18:2n-6, C18:3n-3, PUFA, UFA, SOL, OLL, LLL, PLL, lutein, and high values for T onset $_c$, R.

These findings confirm the great variability in chemical composition and thermal behavior between the two pecan cultivars.

4. CONCLUSIONS

Pecan kernel oil serves as a good source of natural antioxidants and bioactive compounds. The Moore cultivar showed a particular composition with a high level of tocopherols. Mahan was richer in xanthophylls than Moore. Pecan nut oil was found to be rich in TAGs containing oleic and linoleic acids. The pecan nut oil exhibited its main thermal transition at a low-temperature zone. Our DSC data revealed that three parameters, namely $\triangle H_c$ and P_c of the crystallization profile and $\triangle H_m$ of the melting profile are sensitive indicators for distinguishing between cultivars. The genotype appears to affect the chemical composition and thermal properties of pecan kernel oil.

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REFERENCES

Al Juhaimi F, Özcan MM, Ghafoor K, Babiker EE, Hussain S. 2018. Comparison of cold-pressing and soxhlet extraction systems for bioactive compounds, antioxidant properties, polyphenols, fatty acids and tocopherols in eight nut oils. *J. Food Sci. Technol.* **55**, 3163–3173. https://doi:10.1007/s13197-018-3244-5 209-211.

Atanosov AG, Sabharanjak SM, Zengin G, Mollica A, Szostak A, Simirgiotis M, Huminiecki Ł, Horbanczuk OK, Nabavi SM, Mocan A. 2017. Pecan nuts: A review of reported bioactivities and health effects. *Trends Food Sci. Technol.* **71**, 246–257. https://doi.org/10.1016/j.tifs.2017.10.019.

Beltrán SA, Grané TN, Martín CML, Garrigós SMC. 2011. Characterization of almond cultivars by the use of thermal analysis techniques. Application to cultivar authenticity. *J. Am. Oil Chem. Soc.* **88**, 1687–1693. https://doi:10.1007/s11746-011-1847-3.

Bootello MA, Garcés R, Martinez-Force E, Salas JJ. 2016. Effect of the distribution of saturated fatty acids in the melting and cristalization profiles of high-oleic high-stearic oils. *Grasas Aceites*. **67**, 1–7. https://doi.org/10.3989/gya.0441161.

Bouabdallah I, Bouali I, Martinez-Force E, Albouchi A, Pérez-Camino MC, Boukhchina S. 2014. Composition of fatty acids, triacylglycerols and polar compounds of different walnut varieties (*Juglans regia* L.) from Tunisia. *Nat. Prod. Res.* **28**, 1826−1833. https://doi.org/10.1080/14786419.2014.950573.

Bouali I, Trabelsi H, Bou Abdallah I, Albouchi A, Martine L, Gregoire S, Bouzaien G, Gandour M, Boukhchina S, Berdeaux O. 2013. Changes in fatty acid, tocopherol and xanthophyll contents during the development of Tunisian-grown pecan nuts. *J. Am. Oil Chem. Soc.* **90**, 1869–1876. https://doi.org/10.1007/s11746-013-2340-y.

Bouali I, Tsafouros A, Ntanos E, Albouchi A, Boukhchina S, Roussos PA. 2020. Inter-cultivar and temporal variation of phenolic compounds, antioxidant activity and carbohydrate composition of pecan (*Carya illlinoinensis*) kernels grown in Tunisia. *Hortic. Environ. Biotechnol.* **61**, 183–196. https://doi.org/10.1007/s13580-019-00188-8.

Chambre DR, Tociu M, Stanescu MD, Popescu C. 2019. Influence of composition on the thermal behavior of oils extracted from the seeds of some romanian grapes. *J. Sci. Food Agric.* **99**, 6324–6332. https://doi:10.1002/jsfa.9909.

Che Man YB, Tan CP. 2002. Comparative differential scanning calorimetric analysis of vegetable oils: ii. Effects of cooling rate variation. *Phytochem. Anal.* **13**, 142–151. https://doi.org/10.1002/pca.634.

do Prado ACP, Manion BA, Seetharaman K, Deschamps FC, Arellano DB, Block JM. 2013. Relationship between antioxidant properties and chemical composition of the oil and the shell of pecan nuts [*Carya illinoinensis* (Wangenh) C. Koch]. *Ind. Crops Prod.* **45**, 64–73. https://doi:10.1016/j.indcrop.2012.11.042.

Fernandes GD, Gómez-Coca RB, Pérez-Camino MC, Moreda W, Barrera-Arellano D. 2017. Chemical characterization of major and minor compounds of nut oils: almond, hazelnut, and pecan nut. *J. Chem.* **2017**, 1-12. https://doi.org/10.1155/2017/2609549.

Jiang Q, Wong J, Bruce NA. 2004. γ-Tocopherol induces apoptosis in androgen-responsive lncap prostate cancer cells via caspase-dependent and independent mechanisms. *Ann. N Y Acad. Sci*. **1031**, 399–400. https://doi.org/10.1196/annals.1331.056.

Kornsteiner M, Wagner KH, Elmadfa I. 2006. Tocopherols and total phenolics in 10 different nut types. *Food Chem*. **98**, 381–387. https://doi.org/10.1016/j.foodchem.2005.07.033.

Ling B, Yang X, Li R, Wang S. 2015. Physicochemical properties, volatile compounds, and oxidative stability of cold pressed kernel oils from raw and roasted pistachio (*Pistacia vera* L. Var Kerman). *Eur. J. Lipid Sci. Technol*. **118**, 1368–1379. https://doi:10.1002/ejlt.201500336.

Ma L, Lin XM. 2010. Effects of lutein and zeaxanthin on aspects of eye health. *J. Sci. Food Agric*. **90**, 2–12. https://doi.org/10.1002/jsfa.3785.

Mansor TST, Che Man YB, Shuhaimi M. 2012. Employment of differential scanning calorimetry in detecting lard adulteration in virgin coconut oil. *J. Am. Oil Chem. Soc*. **89**, 485–496. https://doi.org/10.1007/s11746-011-1936-3.

Mathur P, Ding Z, Saldeen T, Mehta JL. 2015. Tocopherols in the prevention and treatment of atherosclerosis and related cardiovascular disease. *Clin. Cardiol*. **38**, 570–576.

Miraliakbari H, Shahidi F. 2008. Lipid class compositions, tocopherols and sterols of tree nut oils extracted with different solvents. *J. Food Lipids*. **15**, 81–96. https://doi.org/10.1111/j.1745-4522.2007.00104.x.

Nilchian Z, Ehsani MR, Piravi-Vanak Z, Bakhoda H. 2020. Comparative analysis of butter thermal behavior in combination with bovine tallow. *Food Sci. Technol. (Campinas)*. **40**, 597–604. https://doi.org/10.1590/fst.32019.

Ouni Y, Guido F, Daoud D, Zarrouk M. 2011. Effect of cultivar on minor components in Tunisia olive fruits cultivated in microclimate. *J. Hortic. For*. **3**, 13–20.

Rabadán A, Álvarez-Ortí M, Pardo JE. 2019. A comparison of the effect of genotype and weather conditions on the nutritional composition of most important commercial nuts. *Sci. Hortic*. **244**, 218–224. https://doi:10.1016/j.scienta.2018.09.064.

Rábago-Panduro LM, Martín-Belloso O, Welti-Chanes J, Morales-de la Peña M. 2020. Changes in bioactive compounds content and antioxidant capacity of pecan nuts [*Carya illinoinensis* (Wangenh. K. Koch)] during storage. *Rev. Mex. Ing. Quim*. **19**, 1439–1452. https://doi.org/10.24275/rmiq/Alim1149.

Rezig L, Chouaibi M, Msaada K, Hamdi S. 2012. Chemical composition and profile characterisation of pumpkin (*Cucurbita maxima*) seed oil. *Ind. Crop Prod*. **37**, 82-87. https://doi.org/10.1016/j.indcrop.2011.12.004.

Ribeiro SR, Klein B, Ribeiro QM, dos Santos ID, Gomes Genro AL, Ferreira DF, Hamannb JJ, Barina JS, Cichoskia AJ, Fronzac D, Both V, Wagner R. 2020. Chemical composition and oxidative stability of eleven pecan cultivars produced in Southern Brazil. *Food Res. Int*. **136**, 1–12. https://doi:10.1016/j.foodres.2020.109596.

Smolarek AK, Suh N. 2011. Chemopreventive activity of vitamin E in breast cancer: a focus on γ- and δ-tocopherol. Review. *Nutrients*. **3**, 962-986. https://doi.org/10.3390/nu3110962.

Tan CP, Che Man YB. 2002. Comparative differential scanning calorimetric analysis of vegetable oils: i. effects of heating rate variation. *Phytochem. Anal*. **13**, 129–141. https://doi.org/10.1002/pca.633.

Taş NG, Gökmen V. 2015. Profiling triacylglycerols, fatty acids and tocopherols in hazelnut varieties grown in Turkey. *J. Food Compos. Anal*. **44**, 115–121. https://doi:10.1016/j.jfca.2015.08.010.

Yanty NAM, Marikkar JMN, Che Man YB. 2013. Effect of fractional crystallization on composition and thermal characteristics of avocado (*Persea americana*) butter. *J. Therm. Anal. Calorim*. **111**, 2203–2209. https://doi.org/10.1007/s10973-011-2055-y.

WHO. 2019. World Health Organization. Healthy diet. https://www.who.int/health-topics/healthy-diet#tab=tab_1.

Zeb A. 2012. Triacylglycerols composition, oxidation and oxidation compounds in camellia oil using liquid chromatography–mass spectrometry. *Chem. Phys. Lipids*. **165**, 608–614. https://doi.org/10.1016/j.chemphyslip.2012.03.004.

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Lipid classes and fatty acid composition in two parasitic copepods *Peroderma cylindricum* and *Lernaeocera lusci* and their respective fish hosts *Sardina pilchardus* and *Merluccius merluccius* from the Tunisian waters

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SUMMARY: The present study investigates the detailed lipid classes and their fatty acid (FA) compositions from two parasitic copepods *Lernaeocera lusci* and *Peroderma cylindricum* and their respective fish host species *Merluccius merluccius* and *Sardina pilchardus*. The lipid classes, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), triacylglycerol (TAG), wax ester/cholesterol ester (WE/CE), mono-diacylglycerol (MDG), and free fatty acids (FFA) were separated by thin layer chromatography. The results revealed that TAG and PC were the major lipid classes in parasites; while WE/CE and PS were the most abundant in hosts. As for FA composition, C16:0, C18:0, C18:1n-9, C20:5n-3, and C22:6n-3 were recurrently found to be dominant in all lipid classes of the different organisms studied. However, some differences concerning the abundance and the distribution of several FAs were observed. Overall, the obtained results highlighted that despite the quite strong trophic connection between the parasites and their respective hosts, the parasites could be distinguished by specific lipid profiles.

KEYWORDS: *Copepods; Fatty acid; Hake; Lipid classes; Parasite; Sardine.*

RESUMEN: Clases de lípidos y composición de ácidos grasos en dos copépodos parásitos* Peroderma cylindricum *y* Lernaeocera lusci *y sus respectivos peces hospedadores* Sardina pilchardus *y* Merluccius merluccius *de aguas tunecinas. El presente estudio investiga en detalle las clases de lípidos y sus composiciones de ácidos grasos (AG) de dos copépodos parásitos *Lernaeocera lusci* y *Peroderma cylindricum* y sus respectivas especies de peces hospedadores *Merluccius merluccius* y *Sardina pilchardus*. Las clases de lípidos incluyen fosfatidilcolina (FC), fosfatidiletanolamina (FE), fosfatidilserina (FS), fosfatidilinositol (FI), triacilgliceroles (TAG), ceras/ésteres de colesterol (C/EC), mono-diacilglicerol (MDG) y ácidos grasos libres (AGL), que fueron separados mediante cromatografía en capa fina. Los resultados mostraron que TAG y FC eran las principales clases de lípidos en los parásitos, mientras que C/EC y FS eran las más abundantes en los hospedadores. En cuanto a la composición de AG, se encontró de forma recurrente que C16:0, C18:0, C18:1n-9, C20:5n-3 y C22:6n-3 eran dominantes en todas las clases de lípidos de los diferentes organismos estudiados. Sin embargo, se observaron algunas diferencias en cuanto a la abundancia y distribución de varios AGs. En general, los resultados obtenidos destacaron que a pesar de la fuerte conexión trófica entre los parásitos y sus respectivos hospedadores, los parásitos podían distinguirse por perfiles de lípidos específicos.

PALABRAS CLAVE: *Ácidos grasos; Clases de lípidos; Copépodos; Merluza; Parásito; Sardina.*

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1. INTRODUCTION

Peroderma cylindricum (Heller, 1865) and *Lernaeocera lusci* (Bassett-Smith, 1896) are two common parasitic copepods belonging to the Pennellidae family which infect several marine fish. These parasites have complex and heteroxenous cycles that comprise different larval stages (Brooker *et al*., 2007). While the life cycle of *L. lusci* is well established, that of *P. cylindricum* remains only partially elucidated. The typical host species of *L. lusci* in the Tunisian coastal area are the sole *Solea solea* (intermediate host) and the European hake *Merluccius merluccius* is the definitive host (Kabata, 1979). Once passing through copepodid and chalimus developmental stages, *L. lusci* males and females attain maturity on the gills of their intermediate host. After copulation, the females leave the sole and swim actively to infect the definitive host where they embed deeply into the gill arches and continue to develop (Kabata, 1979). As for *P. cylindricum,* only the adult metamorphosed female embedded into pilchard *Sardina pilchardus* (definitive host) is known to date. It was reported that *P. cylindricum* inserts its holdfast into the pilchard's kidney and spine while its genital segment, producing two egg strings, emerges on the exterior (Becheikh *et al*., 1997). As a result of their attachment and feeding, these two hematophagous parasites *P. cylindricum* and *L. lusci* can affect the survival, physiology and fitness of their hosts (van Damme *et al*., 1994; Hajji *et al*., 1998). Particularly, these parasites were found to be able to inflect substantial alteration in their host's lipids (Hajji *et al*., 2015; Telahigue *et al*., 2017; Telahigue *et al*., 2019). However, little is known about their own lipid profiles.

Lipids and their building block fatty acids are fundamental components for animal health and survival. They are involved in a wide range of biological functions due to their complexity and structural diversity (Tracey *et al*., 2018). Neutral lipids, predominantly constituted by triacylglycerols (TAG) and wax esters (WE), have the primary function of energy storage and are widely related to the physiological status of the organism (Şen Özdemir *et al*., 2019). However, phospholipids and sterols serve as the principal structural constituents of cell membranes (Dufourc, 2008). They may also play significant roles in cell signaling pathways and serve as precursors to bioactive compounds such as steroid hormones (Tocher, 2003). The common phospholipid classes including phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylcholine (PC), and phosphatidylserine (PS), possess significant structural diversity and complexity which mainly depend on the type of fatty acid (length of the fatty acid chain and number of double bonds) which is incorporated (Şen Özdemir *et al*., 2019; Zhou *et al*., 2020).

Owing to their involvement in these diverse cellular and physiological processes, lipids are attractive targets for parasites and are recognized as key players in host-parasite interactions (Vallochi *et al*., 2018). It was reported that various pathogens such as obligate hematophagous and intracellular parasites are able to hijack host's lipids to their own benefit in order to modulate and escape the host response and to complete their own life cycle (O'Neall *et al*., 2020). Although some authors have reported that the fatty acid composition of parasites may largely reflect that of their hosts (Tocher *et al*., 2010), others have highlighted that several parasites may have their own specific fatty acid fingerprints (Taraschewski *et al*., 1995).

The main objective of this study was to illustrate the lipid profiles of two common parasitic copepods from the Mediterranean waters: *L. lusci* and *P. cylindricum*, and to investigate whether these parasites share common patterns with their respective hosts or whether they have their own specific profiles. For this purpose, the lipid class fatty acid (FA) compositions of the two parasitic copepods as well as those of their respective host fish were studied. For more relevance, the specific fixation sites of these parasites (i.e. *M. merluccius* Gills for *L. lusci* and *S. pilchardus* kidney for *P. cylindricum*) were considered in this work. The obtained results will help to better elucidate the trophic connections in the two studied parasite-host systems and to bring new comprehensive knowledges about some biochemical aspects of *L. lusci* and *P. cylindricum*, which remain hitherto poorly understood.

2. MATERIAL AND METHODS

2.1. Sample collection

A total of 243 specimens of *Sardina pilhcardus* (16.5±1.5 cm) and 276 specimens of *Merluccius*

merluccius (18.3±2.2 cm) were purchased from fishermen at the port of Bizerte (Northeast of Tunisia). The samples were directly transported to the laboratory in polystyrene ice-cooled boxes. All specimens were carefully examined for the presence of *P. cylindricum* and *L. lusci*. Specimens from the two parasite species (adult females with egg strings) as well as *M. merluccius* gills and *S. pilhcardus* kidney tissues were collected, weighed, and stored at -20 °C until analysis.

2.2. Total lipid extraction and lipid classes' separation

Total lipids from the whole body (including egg strings) of both *P. cylindricum* and *L. lusci* and their respective hosts' tissues were extracted according to the method of Folch *et al*. (1957) using a mixture of the chloroform-methanol (2:1 V/V) solvent containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant. An analysis of lipid classes was performed by one-dimensional double development high performance thin layer chromatography (HPTLC) as described by Olsen and Henderson (1996). Briefly, aliquots of 500 µl of lipid extracts were spotted onto a TLC plate (20x20 cm, silica gel 60, Merck, Germany), and hexane/diethyl ether/glacial acetic acid (80: 20: 2. V/V) was used as developing solvent system for the neutral lipid classes and methyl acetate/isopropanol/chloroform/methanol/0.25% KCl (25: 25: 25: 10: 9. V/V), for the polar lipid separation. Lipid fractions were visualized under UV light after spraying with 0.1% 2'-7' dichloro-fluorescein in absolute methanol. Eight lipid classes, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), triacylglycerol (TAG), wax ester/cholesterol ester (WE/CE), mono-diacylglycerol (MDG), and free fatty acids (FFA) were identified by comparison with known standards. Each lipid class band was then scraped from the TLC plates and subjected to transmethylation to yield fatty acid methyl esters (FAME) as given by Cecchi *et al*. (1985).

2.3. Fatty acid analysis

FAMEs were analyzed on a HP 6890 gas chromatograph with a split/splitless injector equipped with a flame ionization detector at 275 °C, and a 30 m HP Innowax capillary column with an internal diameter of 250 µm and a film thickness of 0.25 µm. The injector temperature was held at 250 °C. The oven temperature was programmed from 50 to 180 °C at a rate of 40 °C/min, then from 180 to 220 °C at 1.33 °C/min and to stabilize at 220 °C for 7 min. Nitrogen was the carrier gas. Methyl nonadecanoate 19:0 (Sigma) was added as internal standard. The identification of FAMEs was based on the comparison of their retention times with those of a mixture of methyl esters (SUPELCO PUFA-3 and Supelco 37 component FAME). Fatty acid peaks were integrated and analyzed using HP chemstation software.

2.4. Statistical analysis

The R software version 4.0.2. (R Core Team, 2020) was used to carry out statistical analyses. The normality of data distribution and homogeneity of variance were evaluated using the Shapiro-Wilk test and Levene's test, respectively. One-way analysis of variance (ANOVA) followed by the Bonferroni test were performed to check the significant differences between fatty acid amounts of lipid classes from the two studied parasites. The Bonferroni conservative correction method adjusts p values because of the increased risk in a type I error (significance level). Additionally, the hierarchical clustering analysis (HCA) was made by "FactoMineR" R package (Zhao *et al*., 2014) using Ward's method.

3. RESULTS

3.1. Lipid classes and their fatty acid composition from *Lernaeocera lusci* and its host *Merluccius merluccius*

The percentages of each lipid class in relation to total lipid content in the whole body of *L. lusci* and in the gills of *M. merluccius* are presented in Figures 1 and 2, respectively. TAG was the major lipid class in *L. lusci*, constituting ~46% of total lipids followed by PC (with ~26%) and PE (with ~11%). WE/CE was found to represent around 7%, while PI and MDG were found in lesser amounts (~1.5%) (Figure 1). As for the host, it was found that WE/CE was by far the most dominant lipid class (up to 40%) followed by TAG and PS, which constituted around 16% of the total lipids. However, PC and FFA were found to be minor constituents, each representing around 3% of the TL in *M. merluccius* gill tissue (Figure 2).

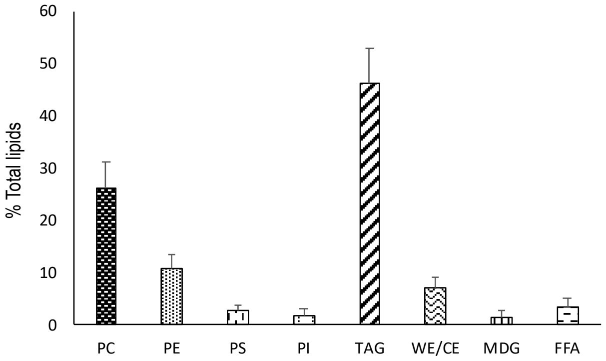

Figure 1. Lipid class composition (% of total lipids) of *Lernaeocera lusci.* The results were expressed as the means with error bar of triplicate analyses (n=3) performed on the pool of 9 samples. PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; WE/CE: wax ester/cholesterol ester; MDG: mono-diacylglycerol; FFA: free fatty acids.

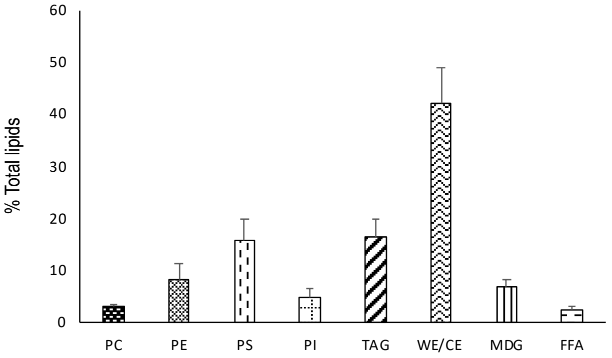

Figure 2. Lipid class composition (% of total lipids) of *Merluccius merluccius* gill tissue. The results were expressed as the means with error bar of triplicate analyses (n=3) performed on the pool of 9 samples. PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; WE/CE: wax ester/cholesterol ester; MDG: mono-diacylglycerol; FFA: free fatty acids.

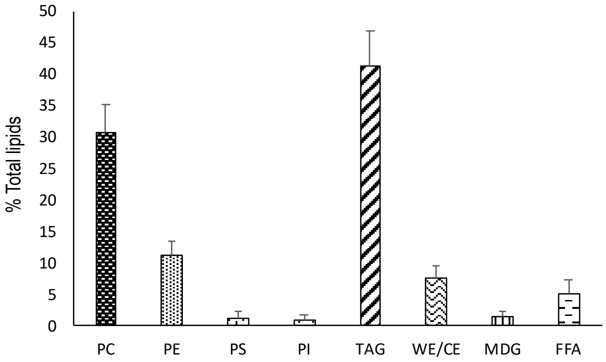

Figure 3. Lipid class composition (% of total lipids) of *Peroderma cylindricum.* The results were expressed as the means with error bar of triplicate analyses (n=3) performed on the pool of 9 samples. PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; WE/CE: wax ester/cholesterol ester; MDG: mono-diacylglycerol; FFA: free fatty acids.

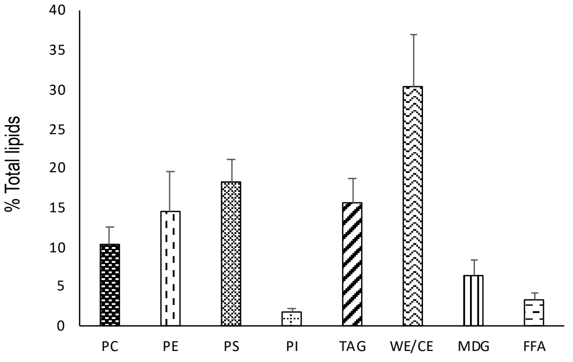

Figure 4. Lipid class composition (% of total lipids) of *Sardina pilchardus* kidney. The results were expressed as the means with error bar of triplicate analyses (n=3) performed on the pool of 9 samples. PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; WE/CE: wax ester/cholesterol ester; MDG: mono-diacylglycerol; FFA: free fatty acids.

The fatty acid composition of the various lipid classes isolated from *L. lusci* and its host *M. merluccius* are respectively given in Tables 1 and 2. Overall, our results revealed that all *L. lusci* lipid classes were dominated by saturated fatty acids (SFA), which accounted for about 50% of the total FA followed by polyunsaturated fatty acids (PUFA), ranging from 28.41 to 39.93% and monounsaturated fatty acids (MUFA) by about 15% (Figure 2). Among the 33 FA species identified*,* palmitic (C16:0), stearic acid (C18:0), oleic acid (C18:1n-9), eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA) were recurrently found to be major in all lipid classes. Among the phospholipid classes, PC had significantly ($p < 0.05$) higher proportions of C14:0 (9.97%) than the other lipid classes. Significantly high amounts of DHA were also recorded in PC and PI fractions. In addition, substantial amounts of C16:0, reaching 30.81 and 32.83% of the total FA were recorded for PI and PS ($p > 0.05$). As for neutral lipids, TAG and FFA appeared to be richer in PUFA, mainly in terms of EPA and DHA when compared to the other lipid classes. The WE/CE fraction was characterized by a higher amount

Table 1. Fatty acid composition of phospholipid and neutral lipid classes from the parasitic copepod *Lernaeocera lusci*. FA: fatty acid; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; WE/CE: wax ester/cholesterol ester; MDG: mono-diacylglycerol; FFA: free fatty acids.

%FA	Polar lipids				Neutral lipids			
	PC	**PE**	**PI**	**PS**	**TAG**	**WE/CE**	**MDG**	**FFA**
C14:0	9.97±1.12^{a}	4.49±0.55^{b}	2.81±0.33^{b}	6.65±0.67^{c}	6.46±0.65^{a}	8.29±0.75^{b}	5.01±0.63^{a}	5.20±0.52^{a}
C15:0	2.09±0.43^{a}	1.41±0.25^{a}	1.51±0.22^{a}	0.09±0.02^{b}	1.49±0.18^{a}	1.80±0.42^{a}	1.79±0.38^{a}	0.42±0.05^{b}
C16:0	27.21±2.24^{a}	27.45±1.88^{a}	30.81±2.30^{a}	32.83±2.01^{a}	21.46±1.95^{a}	19.43±1.64^{a}	27.57±2.08^{b}	22.83±2.16^{ab}
C17:0	0.84±0.11^{a}	2.56±0.44^{b}	2.12±0.25^{bc}	3.76±0.42^{d}	2.47±0.20^{a}	2.33±0.22^{a}	3.20±0.31^{b}	0.03±0.00^{c}
C18:0	10.89±1.02^{a}	9.61±1.02^{a}	11.41±0.88^{a}	10.01±0.95^{a}	13.77±0.15^{a}	14.71±1.68^{a}	8.26±0.62^{b}	16.14±1.15^{a}
C20:0	0.27±0.04^{ab}	0.17±0.03^{b}	0.36±0.05^{ac}	0.18±0.03^{b}	0.52±0.07^{a}	0.17±0.04^{b}	3.39±0.25^{c}	0.13±0.03^{b}
C22:0	0.02±0.00^{a}	0.15±0.04^{b}	0.26±0.04^{c}	0.37±0.05^{d}	0.02±0.01^{a}	0.09±0.03^{a}	1.22±0.18^{b}	0.04±0.02^{a}
C24:0	0.05±0.01^{a}	0.37±0.07^{b}	0.34±0.04^{b}	1.17±0.15^{c}	0.88±0.08^{a}	0.01±0.00^{b}	0.10±0.03^{b}	0.01±0.00^{b}
ΣSFA	**51.34± 6.29^{a}**	**46.21±4.95^{a}**	**49.64±5.55^{a}**	**55.06±6.33^{a}**	**47.08±4.11^{a}**	**46.82±5.15^{a}**	**50.53±4.88^{a}**	**44.81±5.27^{a}**
C14:1	0.31±0.04^{a}	0.51±0.08^{b}	0.23±0.03^{a}	0.38±0.05^{ab}	1.39±0.21^{a}	1.51±0.18^{a}	0.26±0.04^{b}	0.88±0.07^{c}
C15:1	0.37±0.05^{a}	0.83±0.09^{b}	0.42±0.06^{a}	0.77±0.08^{b}	1.40±0.22^{ac}	4.33±0.55^{b}	2.08±0.19^{a}	0.87±0.09^{c}
C16:1n-9	2.38±0.82^{a}	2.64±0.35^{a}	1.02±0.08^{b}	1.81±0.22^{a}	3.57±0.33^{a}	2.42±0.24^{b}	2.65±0.20^{b}	4.37±0.52^{a}
C16:1n-7	0.40±0.05^{a}	1.24±0.21^{b}	0.25±0.03^{a}	3.09±0.31^{c}	0.60±0.04^{a}	1.05±0.15^{a}	2.67±0.25^{b}	1.90±0.21^{c}
C18:1n-9	6.04±0.77^{a}	7.49±0.66 ^{ab}	7.70±0.68^{ab}	8.08±0.75^{b}	5.32±0.63^{a}	6.14±0.66^{a}	5.17±0.54^{a}	6.09±0.58^{a}
C18:1n-7	2.12±0.42^{ab}	2.94±0.33^{a}	2.41±0.32^{ab}	1.52±0.20^{b}	1.40±0.24^{a}	2.43±0.21^{b}	1.13±0.15^{ac}	0.78±0.08^{c}
C20:1	0.11±0.08^{a}	0.31±0.06^{b}	0.00^{a}	0.81±0.07^{c}	0.14±0.03^{a}	0.01±0.00^{b}	0.65±0.06^{c}	0.37±0.05^{d}
C22:1	0.31±0.04^{a}	0.05±0.002^{b}	0.06±0.02^{b}	0.07±0.02^{b}	0.02±0.00^{a}	0.00^{a}	0.17±0.04^{a}	0.01±0.00^{a}
ΣMUFA	**12.04±3.09^{a}**	**16.01±2.25^{a}**	**12.08±1.92^{a}**	**16.53±2.86^{a}**	**13.82±1.04^{a}**	**17.89±2.12^{b}**	**14.79±1.75^{ab}**	**15.26±2.96^{ab}**
C16:2n-4	3.17±0.75^{a}	2.28±0.28^{a}	2.75±0.04^{a}	2.90±0.33^{a}	3.44±0.51^{ab}	2.77±0.28^{b}	4.23±0.51^{ac}	4.86±0.49^{c}
C16:3n-4	0.91±0.08^{a}	0.51±0.08^{b}	0.81±0.07^{a}	0.94±0.07^{a}	1.79±0.20^{ab}	2.31±0.31^{bc}	2.61±0.30^{c}	1.14±0.18^{a}
C16:4	0.01±0.00^{a}	0.55±0.07^{b}	0.43±0.05^{b}	1.50±0.22^{c}	1.62±0.16^{a}	2.10±0.25^{b}	1.09±0.10^{c}	0.21±0.04^{d}
C18:2n-6	3.22±0.35^{a}	4.86±0.49^{b}	1.40±0.21^{c}	1.24±0.25^{c}	1.06±0.15^{a}	0.40±0.08^{b}	1.66±0.22^{c}	1.00±0.11^{a}
C18:3n-6	0.11±0.03^{a}	0.37±0.04^{b}	0.39±0.04^{b}	0.21±0.04^{a}	0.42±0.05^{a}	0.03±0.01^{b}	0.27±0.05^{c}	0.09±0.03^{b}
C18:3n-3	0.61±0.07^{a}	0.45±0.05^{b}	0.39±0.03^{b}	0.33±0.05^{b}	0.56±0.07^{a}	0.14±0.03^{b}	0.41±0.06^{c}	0.72±0.06^{d}
C18:4n-3	0.75±0.08^{a}	0.68±0.00^{a}	0.18±0.03^{b}	1.33±0.23^{c}	2.01±0.24^{ac}	3.11±0.25^{bc}	2.56±0.26^{c}	3.55±0.31^{b}
C20:2n-6	0.58±0.06^{a}	0.47±0.04^{a}	0.54±0.06^{a}	0.48±0.06^{a}	0.82±0.09^{a}	0.20±0.04^{b}	0.47±0.05^{c}	0.39±0.06^{c}
C20:3n-6	0.13±0.04^{a}	0.31±0.05^{b}	0.14±0.04^{a}	0.13±0.04^{a}	0.46±0.05^{a}	0.43±0.07^{a}	0.06±0.01^{b}	1.19±0.20^{c}
C20:4n-6	3.44±0.32^{ab}	2.63±0.32^{b}	3.89±0.44^{a}	4.24±0.43^{a}	1.09±0.11^{a}	1.16±0.21^{a}	3.35±0.31^{b}	3.51±0.34^{b}
C20:3n-3	0.02±0.00^{a}	0.10±0.02^{b}	0.08±0.02^{b}	0.02±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}
C20:4n-3	1.01±0.22^{a}	1.90±0.31^{b}	2.28±0.33^{b}	0.25±0.04^{c}	2.65±0.23^{a}	2.51±0.20^{a}	2.67±0.22^{a}	3.02±0.29^{a}
C20:5n-3	5.96±0.92^{ab}	7.22±0.85^{ab}	7.99±0.85^{a}	5.51±0.45^{b}	8.09±0.66^{ab}	7.42±0.52^{bc}	6.14±0.52^{c}	9.43±0.82^{a}
C22:2n-6	0.27±0.05^{a}	0.71±0.16^{b}	0.57±0.06^{b}	0.55±0.06^{b}	1.24±0.22^{a}	1.53±0.24^{a}	1.33±0.19^{a}	1.47±0.25^{a}
C22:5n-6	0.05±0.02^{a}	0.58±0.07^{a}	0.50±0.05^{a}	0.03±0.00^{a}	0.05±0.02^{a}	0.01±0.00^{a}	0.21±0.04^{a}	0.01±0.00^{a}
C22:5n-3	3.32±0.41^{a}	5.91±0.64^{b}	3.47±0.51^{a}	2.71±0.29^{a}	7.70±0.53^{a}	9.14±0.75^{a}	4.59±0.67^{b}	5.06±0.62^{b}
C22:6n-3	13.06±1.15^{a}	8.25±0.95^{b}	12.45±1.05^{a}	6.01±0.41^{b}	5.08±0.62^{a}	2.02±0.18^{b}	3.01±0.24^{b}	4.30±0.33^{a}
ΣPUFA	**36.62±3.75^{a}**	**37.78±4.08^{a}**	**38.28±3.44^{a}**	**28.41±2.35^{b}**	**39.10±4.52^{a}**	**35.28±3.27^{a}**	**34.68±3.08^{a}**	**39.93±4.04^{a}**

All values are averages of triplicate analyses (n=3) performed on the pool of 9 samples. Values labelled with different letters in the same line by considering polar and neutral lipids separately, are significantly different (by the Bonferroni test at the 5% probability level). SFA (saturated fatty acids that have no double bonds), MUFA (monounsaturated fatty acids that contain one double bond), PUFA (polyunsaturated fatty acids that contain two or more double bonds).

of C15:1 (4.33%, $p < 0.05$) and C22:5n-3 (9.14%), while MDG showed significantly elevated amounts of SFA, mainly C16:0, with 27.57%.

A quite different pattern was observed for the host, where the PUFA group was found to be dominant mainly in the phospholipid classes (with around 50%), followed by SFA (varying from 28.86% in PI to 37.73% in PE) and MUFA (ranging from 14.07 to 21.20% in PE and PI, respectively). Within saturates, C16:0 was the major FA in all lipid classes, exhibiting significantly highest levels in PS (27.16%) and FFA (29.9%) fractions

TABLE 2. Fatty acid composition of phospholipid and neutral lipid classes from the gills of *Merluccius merluccius*. FA: fatty acid; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; WE/CE: wax ester/cholesterol ester; MDG: mono-diacylglycerol; FFA: free fatty acids.

%FA	Polar lipids				Neutral lipids			
	PC	**PE**	**PI**	**PS**	**TAG**	**WE/CE**	**MDG**	**FFA**
C14:0	0.53 ± 0.08^{ac}	2.95 ± 0.13^{b}	1.24 ± 0.48^{a}	0.22 ± 0.02^{c}	1.87 ± 0.19^{a}	3.77 ± 0.42^{b}	1.66 ± 0.13^{a}	4.83 ± 0.66^{b}
C15:0	0.49 ± 0.07^{ac}	3.56 ± 0.78^{b}	1.42 ± 0.15^{a}	0.27 ± 0.02^{c}	3.74 ± 0.36^{a}	2.00 ± 0.83^{b}	1.56 ± 0.20^{b}	1.71 ± 0.26^{b}
C16:0	21.61 ± 3.67^{ab}	16.16 ± 2.15^{b}	12.66 ± 1.07^{b}	27.16 ± 4.50^{a}	17.06 ± 2.44^{a}	21.11 ± 4.60^{ab}	25.89 ± 3.45^{ab}	29.90 ± 3.60^{b}
C17:0	2.87 ± 0.41^{ac}	7.72 ± 0.37^{b}	3.47 ± 0.76^{c}	1.99 ± 0.14^{a}	3.69 ± 0.36^{a}	1.83 ± 0.85^{b}	1.61 ± 0.12^{b}	0.83 ± 0.09^{b}
C18:0	7.98 ± 2.60^{a}	6.92 ± 3.03^{a}	8.74 ± 3.52^{a}	5.98 ± 2.30^{a}	10.97 ± 1.84^{a}	8.29 ± 2.57^{a}	8.46 ± 1.19^{a}	7.60 ± 0.96^{a}
C20:0	0.20 ± 0.03^{a}	0.41 ± 0.05^{b}	0.51 ± 0.06^{b}	1.07 ± 0.09^{c}	2.47 ± 0.25^{a}	0.15 ± 0.01^{b}	0.78 ± 0.11^{c}	0.15 ± 0.03^{b}
C22:0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.81 ± 0.02^{b}	0.00 ± 0.00^{a}	0.08 ± 0.02^{a}	0.09 ± 0.02^{a}	0.02 ± 0.00^{a}	0.44 ± 0.06^{b}
C24:0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.02 ± 0.00^{b}	0.12 ± 0.01^{c}	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}	0.70 ± 0.05^{b}	0.50 ± 0.04^{c}
ΣSFA	**33.68 ± 3.69^{ab}**	**37.73 ± 4.07^{a}**	**28.86 ± 2.82^{b}**	**36.82 ± 3.07^{a}**	**39.90 ± 3.11^{a}**	**37.26 ± 4.10^{a}**	**40.69 ± 5.37^{a}**	**45.96 ± 4.06^{a}**
C14:1	0.69 ± 0.10^{ac}	1.80 ± 0.22^{b}	0.38 ± 0.11^{c}	0.89 ± 0.06^{a}	2.51 ± 0.25^{a}	4.76 ± 0.66^{b}	4.40 ± 0.24^{b}	0.52 ± 0.06^{c}
C15:1	0.16 ± 0.02^{a}	2.38 ± 0.15^{b}	0.81 ± 0.09^{c}	0.08 ± 0.01^{a}	1.21 ± 0.41^{a}	0.47 ± 0.07^{b}	1.17 ± 0.09^{a}	0.21 ± 0.03^{b}
C16:1n-9	4.34 ± 0.69^{a}	2.65 ± 0.43^{b}	1.54 ± 0.66^{b}	1.37 ± 0.10^{b}	2.99 ± 0.56^{a}	3.89 ± 0.36^{a}	2.90 ± 0.60^{a}	7.18 ± 0.88^{b}
C16:1n-7	9.97 ± 1.28^{a}	0.62 ± 0.11^{b}	1.65 ± 0.90^{b}	0.23 ± 0.02^{b}	1.13 ± 0.10^{a}	5.41 ± 0.13^{b}	2.44 ± 0.56^{c}	4.36 ± 0.62^{b}
C18:1n-9	3.76 ± 0.24^{a}	5.34 ± 1.05^{ab}	12.09 ± 2.37^{c}	8.96 ± 1.55^{bc}	6.92 ± 0.39^{a}	9.11 ± 1.56^{a}	9.01 ± 2.08^{a}	8.19 ± 0.95^{a}
C18:1n-7	0.01 ± 0.00^{a}	0.90 ± 0.01^{b}	1.62 ± 0.22^{c}	3.26 ± 0.23^{d}	2.41 ± 0.26^{a}	0.96 ± 0.33^{b}	0.77 ± 0.17^{b}	1.73 ± 0.15^{c}
C20:1	1.76 ± 0.31^{a}	0.39 ± 0.02^{b}	1.35 ± 0.15^{ac}	1.14 ± 0.05^{c}	2.04 ± 0.22^{a}	0.64 ± 0.08^{b}	0.77 ± 0.06^{b}	0.48 ± 0.05^{b}
C22:1	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.78 ± 0.44^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.07 ± 0.02^{a}	0.41 ± 0.07^{b}	0.49 ± 0.06^{b}
ΣMUFA	**20.70 ± 2.10^{a}**	**14.07 ± 1.53^{b}**	**21.20 ± 2.39^{a}**	**15.93 ± 1.20^{b}**	**19.22 ± 1.77^{a}**	**25.30 ± 2.61^{b}**	**21.88 ± 2.05^{ab}**	**23.15 ± 2.08^{b}**
C16:2n-4	4.93 ± 0.28^{a}	4.13 ± 0.55^{ab}	2.00 ± 0.10^{c}	3.30 ± 0.23^{b}	2.28 ± 0.24^{ab}	2.56 ± 0.41^{a}	1.59 ± 0.12^{b}	0.52 ± 0.07^{c}
C16:3n-4	0.14 ± 0.02^{a}	0.50 ± 0.06^{b}	1.36 ± 0.14^{c}	0.91 ± 0.05^{d}	2.21 ± 0.40^{a}	1.76 ± 0.49^{a}	1.05 ± 0.77^{a}	0.90 ± 0.08^{a}
C16:4	0.11 ± 0.02^{a}	1.90 ± 0.72^{b}	2.40 ± 0.81^{b}	0.03 ± 0.00^{a}	0.67 ± 0.09^{a}	0.42 ± 0.08^{b}	0.18 ± 0.06^{c}	0.12 ± 0.03^{c}
C18:2n-6	0.18 ± 0.03^{a}	0.50 ± 0.09^{a}	4.05 ± 0.99^{b}	0.42 ± 0.03^{a}	10.03 ± 2.80^{a}	10.78 ± 1.36^{a}	2.46 ± 0.04^{b}	2.21 ± 0.25^{b}
C18:3n-6	0.01 ± 0.00^{ab}	0.10 ± 0.00^{b}	0.88 ± 0.07^{c}	0.00 ± 0.00^{a}	0.24 ± 0.01^{a}	0.78 ± 0.09^{b}	0.52 ± 0.04^{c}	0.08 ± 0.02^{d}
C18:3n-3	0.21 ± 0.03^{a}	1.64 ± 0.82^{b}	0.27 ± 0.02^{a}	3.58 ± 0.25^{c}	0.38 ± 0.03^{a}	1.60 ± 0.58^{b}	1.15 ± 0.42^{ab}	0.53 ± 0.05^{a}
C18:4n-3	1.16 ± 0.14^{a}	0.39 ± 0.01^{b}	0.74 ± 0.02^{c}	0.31 ± 0.02^{b}	0.40 ± 0.03^{a}	2.87 ± 0.61^{b}	1.69 ± 0.33^{c}	1.88 ± 0.23^{bc}
C20:2n-6	0.56 ± 0.08^{a}	0.67 ± 0.05^{a}	2.93 ± 0.58^{b}	2.50 ± 0.17^{b}	1.21 ± 0.13^{a}	0.16 ± 0.02^{b}	1.32 ± 0.10^{a}	0.26 ± 0.04^{b}
C20:3n-6	0.31 ± 0.04^{a}	0.79 ± 0.10^{b}	0.97 ± 0.09^{b}	0.59 ± 0.25^{ab}	1.30 ± 0.14^{a}	0.10 ± 0.04^{b}	0.31 ± 0.06^{b}	0.96 ± 0.11^{c}
C20:4n-6	5.57 ± 0.66^{a}	4.48 ± 0.41^{a}	2.21 ± 0.77^{b}	4.01 ± 0.83^{ab}	1.73 ± 0.17^{a}	0.40 ± 0.12^{b}	2.02 ± 0.30^{a}	0.55 ± 0.06^{b}
C20:3n-3	0.20 ± 0.03^{a}	0.22 ± 0.01^{a}	0.53 ± 0.10^{b}	0.27 ± 0.07^{a}	0.06 ± 0.01^{a}	0.07 ± 0.03^{a}	0.24 ± 0.07^{b}	0.14 ± 0.03^{ab}
C20:4n-3	4.53 ± 0.65^{a}	4.40 ± 0.94^{a}	3.23 ± 0.97^{a}	3.04 ± 0.49^{a}	2.39 ± 0.20^{a}	0.17 ± 0.07^{b}	3.34 ± 0.26^{c}	0.04 ± 0.01^{b}
C20:5n-3	6.20 ± 1.03^{ab}	5.32 ± 0.02^{a}	8.22 ± 1.39^{b}	6.89 ± 0.68^{ab}	0.27 ± 0.04^{a}	2.57 ± 0.68^{b}	0.38 ± 0.03^{a}	5.43 ± 0.55^{c}
C22:2n-6	0.81 ± 0.12^{ab}	1.22 ± 0.61^{a}	0.19 ± 0.02^{b}	0.33 ± 0.11^{ab}	0.79 ± 0.09^{a}	0.12 ± 0.05^{b}	1.10 ± 0.30^{a}	0.07 ± 0.02^{b}
C22:5n-6	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.73 ± 0.05^{b}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.06 ± 0.03^{a}	0.89 ± 0.13^{b}	0.56 ± 0.22^{b}
C22:5n-3	2.58 ± 0.78^{a}	1.21 ± 0.24^{b}	1.06 ± 0.05^{b}	0.54 ± 0.13^{b}	0.76 ± 0.06^{a}	0.34 ± 0.09^{b}	1.00 ± 0.17^{a}	2.03 ± 0.04^{c}
C22:6n-3	18.13 ± 2.75^{a}	20.72 ± 0.70^{a}	18.15 ± 2.08^{a}	19.53 ± 2.13^{a}	16.17 ± 3.64^{a}	12.67 ± 1.74^{a}	18.20 ± 1.34^{a}	14.58 ± 1.74^{a}
ΣPUFA	**45.61 ± 4.35^{a}**	**48.20 ± 3.71^{a}**	**49.93 ± 4.19^{a}**	**47.25 ± 4.88^{a}**	**40.88 ± 4.04^{a}**	**37.44 ± 3.09^{a}**	**37.43 ± 3.20^{a}**	**30.89 ± 2.85^{b}**

All values are averages of triplicate analyses (n=3) performed on the pool of 9 samples. Values labelled with different letters in the same line by considering polar and neutral lipids separately, are significantly different (by the Bonferroni test at the 5% probability level). SFA (saturated fatty acids that have no double bonds), MUFA (monounsaturated fatty acids that contain one double bond), PUFA (polyunsaturated fatty acids that contain two or more double bonds).

($p < 0.05$). Along with C16:0, C18:0 was also found to be abundant in all analyzed polar and neutral lipid fractions with a percentage exceeding 5% of total FAs ($p > 0.05$). Within the MUFA group, we particularly noticed significantly high amounts of C16:1n-7 in PC (~10%) and C18:1n-9 in PI, reaching 12.09% of total FAs. The latter FA was also found to be dominant in all neutral lipid classes ($p > 0.05$). With respect to PUFA, DHA was by far the most dominant FA in all lipid classes, varying from 14.58% in FFA to 20.72% in PE. In addition to DHA, EPA was also found at quite important

Table 3. Fatty acid composition of phospholipid and neutral lipid classes from the parasitic copepod Peroderma cylindricum. FA: fatty acid; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; WE/CE: wax ester/cholesterol ester; MDG: mono-diacylglycerol; FFA: free fatty acids.

%FA	Polar lipids				Neutral lipids			
	PC	**PE**	**PI**	**PS**	**TAG**	**WE/CE**	**MDG**	**FFA**
C14:0	2.73 ± 0.31^{a}	2.53 ± 0.22^{ab}	1.67 ± 0.25^{b}	3.19 ± 0.45^{a}	4.07 ± 0.45^{a}	4.65 ± 0.52^{a}	4.17 ± 0.55^{a}	3.70 ± 0.44^{a}
C15:0	1.46 ± 0.18^{ab}	1.65 ± 0.18^{ab}	2.14 ± 0.23^{a}	1.19 ± 0.21^{b}	2.11 ± 0.22^{ac}	1.11 ± 0.20^{b}	1.82 ± 0.39^{a}	2.57 ± 0.37^{c}
C16:0	25.83 ± 2.25^{a}	26.08 ± 2.44^{ab}	33.65 ± 2.95^{b}	32.85 ± 3.05^{ab}	33.04 ± 2.75^{a}	29.58 ± 1.98^{a}	30.07 ± 2.45^{a}	29.75 ± 2.66^{a}
C17:0	2.95 ± 0.30^{a}	2.23 ± 0.25^{b}	1.84 ± 0.22^{b}	2.25 ± 0.22^{ab}	2.48 ± 0.25^{a}	1.01 ± 0.12^{b}	2.21 ± 0.20^{b}	2.13 ± 0.17^{ab}
C18:0	8.96 ± 0.85^{a}	7.54 ± 0.66^{a}	12.19 ± 0.19^{b}	12.47 ± 0.15^{b}	6.32 ± 0.82^{a}	8.79 ± 0.93^{b}	8.74 ± 0.83^{b}	7.35 ± 0.65^{ab}
C22:0	0.03 ± 0.0^{ab}	0.01 ± 0.00^{b}	0.09 ± 0.03^{b}	0.07 ± 0.02^{ac}	0.28 ± 0.04^{a}	0.14 ± 0.03^{b}	0.13 ± 0.03^{b}	0.22 ± 0.04^{ab}
ΣSFA	**41.96 ± 3.82^{a}**	**40.04 ± 3.88^{a}**	**51.58 ± 4.75^{b}**	**52.02 ± 4.58^{b}**	**48.30 ± 4.95^{a}**	**45.28 ± 3.11^{a}**	**47.14 ± 4.79^{a}**	**45.72 ± 5.87^{a}**
C22:1	0.05 ± 0.02^{a}	0.02 ± 0.00^{b}	0.01 ± 0.00^{b}	0.00^{b}	0.11 ± 0.03^{a}	0.03 ± 0.01^{b}	0.01 ± 0.00^{b}	0.04 ± 0.01^{b}
C14:1	0.39 ± 0.04^{a}	0.38 ± 0.05^{a}	0.83 ± 0.07^{b}	0.46 ± 0.05^{a}	0.15 ± 0.04^{a}	1.47 ± 0.18^{b}	0.55 ± 0.05^{c}	1.54 ± 0.19^{b}
C15:1	0.18 ± 0.03^{a}	0.09 ± 0.02^{b}	0.14 ± 0.03^{ab}	0.08 ± 0.04^{b}	0.50 ± 0.05^{ab}	0.39 ± 0.06^{a}	0.48 ± 0.04^{ab}	0.59 ± 0.07^{b}
C16:1n-9	2.49 ± 0.28^{a}	2.16 ± 0.22^{a}	3.55 ± 0.32^{b}	1.26 ± 0.18^{c}	2.11 ± 0.19^{a}	2.82 ± 0.23^{b}	3.66 ± 0.31^{c}	3.50 ± 0.33^{bc}
C16:1n-7	0.30 ± 0.01^{a}	1.67 ± 0.17^{b}	0.59 ± 0.08^{ac}	0.72 ± 0.09^{c}	3.33 ± 0.41^{a}	2.45 ± 0.31^{a}	2.83 ± 0.25^{a}	3.34 ± 0.34^{a}
C18:1n-9	4.93 ± 0.45^{a}	6.95 ± 0.55^{b}	1.63 ± 0.19^{c}	8.73 ± 0.85^{d}	8.95 ± 0.58^{a}	9.86 ± 0.95^{a}	10.96 ± 0.95^{a}	9.53 ± 0.84^{a}
C18:1n-7	1.86 ± 0.21^{a}	2.61 ± 0.21^{b}	1.16 ± 0.15^{c}	1.11 ± 0.23^{c}	1.50 ± 0.11^{a}	1.49 ± 0.17^{a}	1.83 ± 0.22^{a}	0.41 ± 0.05^{b}
C20:1	0.03 ± 0.01^{a}	0.22 ± 0.07^{b}	0.03 ± 0.02^{a}	0.07 ± 0.03^{a}	0.04 ± 0.02^{a}	0.55 ± 0.06^{b}	0.24 ± 0.04^{a}	1.57 ± 0.17^{c}
ΣMUFA	**10.23 ± 1.82^{ab}**	**14.10 ± 2.91^{a}**	**7.94 ± 1.77^{b}**	**12.43 ± 1.58^{a}**	**16.69 ± 2.64^{a}**	**19.06 ± 2.17^{a}**	**20.56 ± 2.55^{a}**	**20.52 ± 3.06^{a}**
C16:2n-4	2.77 ± 0.25^{a}	3.20 ± 0.35^{a}	2.54 ± 0.39^{a}	3.22 ± 0.33^{a}	1.56 ± 0.18^{a}	2.11 ± 0.22^{a}	3.76 ± 0.33^{b}	2.03 ± 0.23^{a}
C16:3n-4	0.69 ± 0.08^{ac}	1.23 ± 0.15^{b}	0.84 ± 0.08^{a}	0.46 ± 0.05^{c}	0.95 ± 0.08^{a}	0.68 ± 0.07^{b}	0.50 ± 0.06^{bc}	0.47 ± 0.06^{c}
C18:2n-6	2.78 ± 0.30^{a}	1.11 ± 0.28^{b}	3.19 ± 0.25^{a}	1.12 ± 0.16^{b}	0.75 ± 0.09^{ab}	1.03 ± 0.13^{a}	0.71 ± 0.08^{b}	0.98 ± 0.11^{ab}
C18:3n-4	0.34 ± 0.07^{a}	0.23 ± 0.06^{ac}	0.07 ± 0.03^{b}	0.10 ± 0.04^{bc}	0.18 ± 0.03^{a}	1.23 ± 0.16^{b}	0.48 ± 0.7^{ab}	0.07 ± 0.02^{a}
C18:3n-3	0.38 ± 0.04^{a}	0.34 ± 0.05^{ab}	0.39 ± 0.05^{a}	0.22 ± 0.04^{b}	0.39 ± 0.05^{a}	0.43 ± 0.05^{a}	0.02 ± 0.00^{b}	0.09 ± 0.02^{b}
C20:2n-6	0.41 ± 0.05^{a}	0.44 ± 0.06^{a}	0.23 ± 0.04^{b}	0.34 ± 0.05^{ab}	0.31 ± 0.04^{a}	0.22 ± 0.04^{a}	0.31 ± 0.04^{a}	0.62 ± 0.03^{b}
C20:4n-6	2.80 ± 0.25^{ab}	1.94 ± 0.21^{a}	3.57 ± 0.66^{b}	3.24 ± 0.44^{b}	2.53 ± 0.21^{a}	2.88 ± 0.25^{a}	2.38 ± 0.25^{a}	1.62 ± 0.21^{b}
C20:3n-3	0.05 ± 0.02^{a}	0.08 ± 0.03^{a}	0.08 ± 0.02^{a}	0.33 ± 0.06^{b}	0.08 ± 0.02^{ab}	0.10 ± 0.03^{b}	0.01 ± 0.00^{a}	0.22 ± 0.04^{c}
C20:4n-3	2.98 ± 0.31^{a}	2.06 ± 0.25^{b}	2.17 ± 0.22^{b}	1.64 ± 0.18^{b}	0.15 ± 0.04^{a}	0.78 ± 0.08^{b}	1.32 ± 0.11^{c}	1.71 ± 0.18^{d}
C20:5n-3	11.05 ± 0.85^{a}	10.83 ± 0.85^{a}	8.86 ± 0.75^{a}	11.24 ± 0.95^{a}	8.72 ± 0.65^{a}	6.95 ± 0.66^{b}	5.93 ± 0.66^{bc}	4.71 ± 0.45^{c}
C22:3n-3	1.48 ± 0.18^{a}	0.55 ± 0.07^{b}	1.24 ± 0.26^{a}	0.07 ± 0.02^{c}	0.42 ± 0.05^{a}	0.37 ± 0.05^{a}	2.21 ± 0.26^{b}	0.34 ± 0.06^{a}
C22:5n-6	0.57 ± 0.08^{a}	0.69 ± 0.08^{a}	0.27 ± 0.04^{b}	0.76 ± 0.07^{a}	0.02 ± 0.00^{a}	0.12 ± 0.03^{a}	0.03 ± 0.00^{a}	0.82 ± 0.07^{b}
C22:5n-3	2.49 ± 0.28^{a}	2.94 ± 0.33^{a}	4.15 ± 0.55^{b}	1.37 ± 0.21^{c}	0.71 ± 0.08^{a}	2.33 ± 0.22^{b}	2.05 ± 0.19^{b}	3.35 ± 0.28^{c}
C22:6n-3	19.02 ± 1.88^{a}	20.22 ± 2.15^{a}	12.88 ± 1.05^{b}	11.44 ± 1.95^{b}	18.24 ± 1.75^{a}	16.43 ± 1.66^{a}	12.59 ± 0.96^{b}	16.73 ± 0.88^{a}
ΣPUFA	**47.81 ± 5.18^{a}**	**45.86 ± 4.13^{a}**	**40.48 ± 4.66^{ab}**	**35.55 ± 3.78^{b}**	**35.01 ± 5.54^{a}**	**35.66 ± 3.96^{a}**	**32.30 ± 3.77^{a}**	**33.76 ± 4.15^{a}**

All values are averages of triplicate analyses (n=3) performed on the pool of 9 samples. Values labelled with different letters in the same line by considering polar and neutral lipids separately, are significantly different (by the Bonferroni test at the 5% probability level). SFA (saturated fatty acids that have no double bonds), MUFA (monounsaturated fatty acids that contain one double bond), PUFA (polyunsaturated fatty acids that contain two or more double bonds).

percentages (up to 5%), mainly in phospholipid and FFA fractions. Another FA, C18:2n-6, was also found at significantly high amounts (around 10% of TFA) in both TAG and WE/CE fractions ($p < 0.05$).

3.2. Lipid classes and their fatty acid composition from *Peroderma cylindiricum* and its host *Sardina pilchardus*

PC and PE were the major lipid classes in *P. cylindricum* polar lipids, representing 30.63%, and 11.21% of TL, respectively. Among neutral lipids, a high proportion of TAG (41.38%) was found while WE/CE and FFA represented 7.75 and 5.20%, respectively. The remaining lipid classes (i.e. PI, PS, and MDG) occurred in smaller proportions (ranging from ~1 to 1.6%) (Figure 3). Regarding the host, we found that the lipid fraction of the *S. pilchardus* kidney was characterized by the dominance of the WE/CE, which accounted for over 30% of TL followed by PS (18.16%), TAG (15.62%), PE (14.42%), and

TABLE 4. Fatty acid composition of phospholipid and neutral lipid classes from the kidney of *Sardina pilchardus*. FA: fatty acid; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; WE/CE: wax ester/cholesterol ester; MDG: mono-diacylglycerol; FFA: free fatty acids.

%FA	**Polar lipids**				**Neutral lipids**			
	PC	**PE**	**PI**	**PS**	**TAG**	**WE/CE**	**MDG**	**FFA**
C14:0	3.67±0.35^{a}	3.75±0.38^{a}	1.16±0.12^{b}	1.06±0.21^{b}	7.92±0.85^{a}	6.07±0.55^{a}	3.80±0.39^{b}	6.14±0.74^{a}
C15:0	0.97±0.09^{a}	1.32±0.12^{b}	1.29±0.15^{ab}	0.97±0.11^{a}	0.77±0.08^{a}	0.58±0.06^{a}	2.82±0.32^{b}	3.82±0.41^{c}
C16:0	23.55±2.55^{ab}	19.53±2.78^{a}	25.18±2.66^{ab}	29.13±3.16^{b}	26.18±2.87^{a}	33.05±3.56^{a}	32.46±3.09^{a}	29.08±2.98^{a}
C17:0	1.16±0.14^{a}	1.13±0.15^{a}	0.62±0.07^{b}	1.63±0.21^{c}	0.30±0.04^{a}	0.00^{b}	0.55±0.06^{c}	0.44±0.05^{c}
C18:0	7.57±0.84^{a}	10.65±1.42^{ab}	11.60±1.68^{b}	9.45±0.95^{ab}	17.14±1.94^{a}	13.27±1.66^{ab}	11.30±1.45^{b}	14.30±1.86^{ab}
C20:0	0.00^{a}	0.01±0.00^{ab}	0.07±0.02^{c}	0.04±0.01^{bc}	0.00^{a}	0.03±0.01^{a}	0.13±0.03^{b}	0.01±0.00^{a}
C22:0	0.02±0.01^{a}	0.05±0.02^{a}	0.13±0.03^{b}	0.00^{a}	0.07±0.02^{a}	0.07±0.03^{a}	0.73±0.08^{b}	0.12±0.20^{a}
ΣSFA	**36.94±3.55^{a}**	**36.44±3.05^{a}**	**40.04±4.08^{a}**	**42.28±3.96^{a}**	**52.38±4.95^{a}**	**53.07±4.15^{a}**	**51.79±5.22^{a}**	**53.91±4.76^{a}**
C14:1	0.35±0.04^{a}	0.25±0.03^{a}	0.63±0.07^{b}	0.64±0.06^{b}	0.82±0.09^{a}	0.68±0.07^{a}	1.80±0.21^{b}	2.00±0.23^{b}
C15:1	0.31±0.03^{a}	0.26±0.04^{a}	0.44±0.05^{b}	0.14±0.03^{c}	0.66±0.07^{a}	0.09±0.02^{b}	0.91±0.08^{c}	1.11±0.12^{c}
C16:1n-9	0.41±0.05^{a}	0.89±0.11^{b}	1.19±0.21^{b}	0.93±0.08^{b}	0.45±0.05^{a}	4.01±0.52^{b}	4.22±0.46^{b}	2.37±0.25^{c}
C16:1n-7	0.35±0.04^{ab}	0.27±0.03^{a}	0.67±0.07^{a}	1.11±0.21^{c}	3.82±0.41^{ab}	4.49±0.53^{a}	3.70±0.44^{ab}	2.98±0.29^{b}
C18:1n-9	7.37±0.75^{a}	8.08±0.68 ^{a}	9.30±0.82^{a}	14.44±1.87^{b}	10.99±1.55^{a}	12.01±1.74^{a}	10.04±1.35^{a}	5.42±0.73^{b}
C18:1n-7	1.22±0.11^{a}	3.84±0.35^{b}	1.51±0.22^{a}	2.56±0.31^{c}	1.86±0.17^{a}	2.43±0.32^{ab}	3.15±0.38^{b}	0.98±0.08^{c}
C20:1	0.11±0.03^{a}	0.72±0.08^{b}	0.16±0.03^{a}	0.25±0.04^{c}	0.46±0.05^{a}	0.45±0.06^{a}	0.81±0.09^{b}	0.93±0.11^{b}
C22:1	0.08±0.02^{a}	0.10±0.00 ^{a}	0.08±0.02^{a}	0.06±0.03^{a}	0.90±0.12^{a}	0.17±0.03^{b}	0.51±0.06^{c}	0.10±0.02^{d}
ΣMUFA	**10.20±1.55^{a}**	**14.41±1.78^{a}**	**13.97±1.65^{a}**	**20.13±2.06^{b}**	**19.95±2.12^{a}**	**24.33±2.85^{ab}**	**25.14±2.55^{b}**	**15.89±1.44^{c}**
C16:2n-4	2.02±0.21^{a}	1.17±0.14^{b}	1.44±0.16^{b}	2.01±0.24^{a}	0.83±0.07^{a}	0.30±0.03^{b}	1.01±0.15^{a}	2.20±0.24^{c}
C16:3n-4	0.50±0.06^{a}	0.74±0.08^{b}	0.46±0.05^{ac}	0.32±0.04^{c}	0.39±0.04^{a}	0.46±0.06^{a}	0.71±0.08^{ab}	1.07±0.23^{b}
C18:2n-6	7.16±0.82^{ac}	4.66±0.53^{b}	5.32±0.46^{ab}	8.01±0.92^{c}	7.02±0.84^{a}	9.30±0.78^{b}	4.44±0.56^{c}	6.75±0.71^{a}
C18:3n-6	0.02±0.01^{a}	0.01±0.00^{a}	0.00^{a}	0.34±0.05^{b}	0.87±0.09^{a}	0.42±0.05^{b}	0.11±0.03^{c}	0.00^{c}
C18:3n-3	0.30±0.04^{a}	1.01±0.12^{b}	0.42±0.06^{ac}	0.61±0.07^{c}	0.67±0.08^{a}	1.52±0.22^{b}	0.23±0.03^{c}	0.21±0.04^{c}
C18:4n-3	0.15±0.03^{a}	0.52±0.05^{b}	0.23±0.03^{a}	0.24±0.04^{a}	0.92±0.08^{a}	0.73±0.08^{b}	0.09±0.02^{c}	0.31±0.04^{d}
C20:2n-6	0.81±0.07^{a}	1.32±0.11^{b}	0.63±0.05^{ac}	0.58±0.06^{c}	4.15±0.42^{a}	0.35±0.04^{b}	0.25±0.03^{b}	0.47±0.05^{b}
C20:3n-6	0.00^{a}	0.01±0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.10±0.02^{b}	0.00^{a}
C20:4n-6	0.11±0.03^{a}	0.27±0.04^{b}	0.14±0.03^{a}	0.27±0.04^{b}	0.00^{a}	0.00^{a}	0.01±0.00^{a}	0.19±0.02^{b}
C20:3n-3	3.63±0.35^{a}	2.28±0.22^{b}	1.09±0.1^{c}	3.03±0.34^{ab}	1.78±0.25^{a}	0.24±0.03^{b}	1.26±0.18^{c}	1.05±0.17^{c}
C20:4n-3	1.72±0.22^{a}	1.66±0.18^{a}	0.45±0.05^{b}	1.13±0.15^{c}	0.11±0.03^{a}	0.31±0.04^{b}	0.02±0.01^{a}	0.32±0.04^{b}
C20:5n-3	11.61±1.76^{ab}	15.91±1.84^{a}	14.41±2.02^{ab}	10.07±1.13^{b}	4.66±0.56^{a}	4.34±0.61^{a}	7.66±0.82^{b}	6.70±0.72^{b}
C22:3n-3	0.89±0.11^{a}	0.22±0.03^{b}	0.90±0.08^{a}	0.09±0.02^{b}	0.07±0.02^{a}	0.12±0.03^{a}	0.12±0.04^{a}	0.34±0.05^{b}
C22:5n-6	0.43±0.04^{a}	0.59±0.06^{ab}	0.63±0.07^{b}	0.83±0.09^{c}	0.13±0.02^{a}	1.34±0.15^{bc}	1.74±0.19^{c}	1.20±0.16^{b}
C22:5n-3	3.12±0.45^{a}	2.14±0.26^{b}	3.25±0.36^{a}	2.67±0.27^{ab}	0.45±0.05^{a}	0.81±0.07^{b}	1.24±0.15^{c}	1.62±0.17^{d}
C22:6n-3	18.39±1.77^{a}	16.64±1.85^{a}	15.62±2.03^{a}	9.05±0.85^{b}	5.62±0.7^{a}	2.35±0.25^{b}	4.08±0.52^{ab}	7.77±0.83^{c}
ΣPUFA	**42.86±3.65^{ab}**	**49.15±5.08^{a}**	**45.99±4.92^{a}**	**37.59±3.77^{b}**	**27.67±2.88^{ab}**	**22.60±2.75^{a}**	**23.07±2.81^{a}**	**30.20±3.07^{b}**

All values are averages of triplicate analyses (n=3) performed on the pool of 9 samples. Values labelled with different letters in the same line by considering polar and neutral lipids separately, are significantly different (by the Bonferroni test at the 5% probability level). SFA (saturated fatty acids that have no double bonds), MUFA (monounsaturated fatty acids that contain one double bond), PUFA (polyunsaturated fatty acids that contain two or more double bonds).

PC (10.26%). The other lipid classes, including MDG, FFA and PI were found to be minor components with proportions ranging from 6.27 to 3.30% and to 1.65% of TL, respectively (Figure 4).

The fatty acid compositions of the different P. cylindricum lipid classes are presented in Table 3. The obtained results showed that almost all lipid classes contained higher proportions of SFA than MUFA and PUFA, except for PC and PE fractions, where PUFA constituted the major group with 47.81 and 45.86%, respectively. Among the total of 28 identified FA, five (i.e. C16:0, C18:0, C18:1n-9, EPA and DHA) appeared to be dominant in all lipid fractions. Within phospholipids, PC and PE showed quite similar compositions and exhibited the highest level of DHA, which constitutes almost 20% of total FAs ($p < 0.05$). C16:0 and C18:0 were mostly found in PI and PS (~33%). As for neutral lipid classes, large proportions of C16:0 (33.04%) and DHA (18.24%) were recorded in the TAG fraction. Furthermore, substantial amounts of DHA were also recorded in WE/CE, FFA, and MDG.

The fatty acid profiles of polar and neutral lipid classes extracted from the host kidney are shown in Table 4. All neutral lipids were dominated by SFA (up to 50%), followed by PUFA and MUFA. C14:0, C16:0 and C18:0 were the major FAs of SFA group, while C18:1n-9 was the most abundant MUFA. In the PUFA group, C18:2n-6, EPA and DHA tended to be dominant, mainly in the FFA fraction. Regarding the polar lipid classes, PUFA percentages were higher than SFA and MUFA except for PS, where SFA dominated (by representing 42.28% of total FA). C16:0 and C18:1n-9 were the most abundant SFA and MUFA, culminating to 29.13 and 14.44% ($p < 0.05$) in PS, respectively. EPA and DHA were the major FA within the PUFA group with significantly higher levels of DHA in PC, PE, and PI. In addition, C18:2n-6 was found in relatively important amounts (up to 5%) in all phospholipid fractions.

3.3. Multivariate analysis

To provide an overview of similarities and discrepancies within each studied host-parasite system, hierarchical clustering analysis (HCA) was applied to the whole FA data set (Figure 5). Figure 5a presents the dendrogram of HCA, giving information about *L. lusci* and its host *M. merluccius.* Two major groups, comprising two clusters each, were separated out in the resulting dendrogram. The first group was constituted by the hosta' TAG and PL classes, while the second group involved the remaining data. Interestingly, we found that among the second group, almost all neutral lipid classes (including FFA, WE/CE, and MDG) of the host clustered with the parasite phospholipid. Similarly, the dendrogram generated from *P. cylindricum* and *S. pilchardus* data revealed two major groups. The first one constituted two clusters related to the host neutral lipid moieties. The second group was composed of two distinct clusters: Cluster I comprised the polar lipids of the host; while cluster II grouped all lipid classes of *P. cylindricum*.

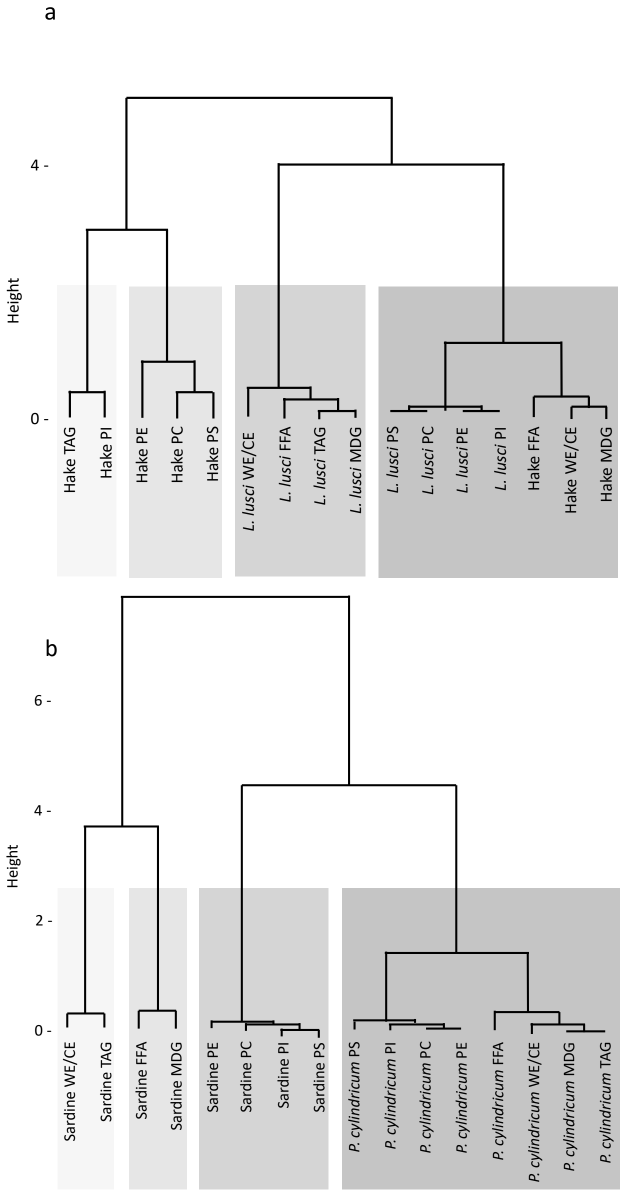

Figure 5. Hierarchical clustering analysis (Euclidien distance, Ward's clustering) of the whole fatty acid data set obtained from the two studied host-parasite systems. (a): Dendrogram obtained for *Lernaeocera lusci* and its host *Merluccius merluccius.* (b): Dendrogram obtained for *Peroderma cylindricum* and its host *Sardina pilchardus.* PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; WE/CE: wax ester/cholesterol ester; MDG: mono-diacylglycerol; FFA: free fatty acids; MDG: mono-diacylglycerol; FFA: free fatty acids.

4. DISCUSSION

It is well established that lipid content and composition in copepods are diverse and vary depending upon several parameters such as species, type of food, latitude, season, developmental stage, and life cycle strategy (van der Meeren *et al*., 2008). Although a substantial amount of literature exists on free-forms, information on the lipid composition of parasitic forms is still limited. Thus, this study was conducted to explore similarities and differences among the lipid fractions and their fatty acid compositions in *L. lusci* and *P. cylindricum,* and their respective hosts.

According to the obtained results, clear differences in the relative percentages of the different lipid classes were noticed between parasites and their respective hosts. Indeed, TAG turned out to be the most predominant neutral lipid class in both *L. lusci* and *P. cylindricum*, while WE/CE prevailed in fish tissues. These results are in accordance with those of Tocher *et al.* (2010) and Kotani (2006), who reported that parasitic and opportunistic feeder copepods store their lipids mainly as TAGs. However, it is worth noting that by feeding regularly on their host's blood, the parasitic copepod females are able to maintain a sufficient lipid content to sustain their own survival and to fuel maturation and egg production as recently reported in *L. lusci* by Hajji *et al.* (2021). Thus, it is thought that TAG is rather stored in lipovitellin as a main source of energy for the next stages (e.g. nauplii and infective copepodids) to ensure development, basic metabolism, swimming activity and infectivity. In this context Tocher *et al.* (2010) demonstrated that the eggs of parasitic copepods contain significantly higher proportions of TAG than adulate females. The same authors reported that, furthermore, due to the short time period between fish host infections, long-term energy deposits such as WEs are not required for parasitic copepods (Tocher *et al*., 2010). In the other hand, the high proportions of WE/CE recorded in the host's tissues (i.e. gills and kidney) could be explained by the essential role played by cholesterol in the physiological regulation of the physical properties of cell membranes (Díaz *et al*., 2016). Differences in the proportion of polar lipid classes were herein observed between the two studied parasites and their respective hosts. Although PC, and to a lesser extent, PE were found to be the major phospholipid classes in parasites, PS appeared among the most dominant phospholipid classes in host tissues. In line with this, Tocher *et al.* (2010) also reported that PC and PE were the major phospholipid classes in both the females and egg strings of parasitic caligid copepods belonging to the genus *Lepeophtheirus.* These key components of biological membranes are known for their functional and structural roles. Moreover, it was reported that PC is the principal lipid component in crustacean lipovitellin and may serve as metabolic energy storage for reproduction and embryonic development (Lee *et al*., 2006).

Regarding the fatty acid composition of the different lipid classes, a general pattern characterized by the dominance of five FAs comprising C16:0, C18:0, C18:1n-9, C20:5n-3 and C22:6n-3 was herein clearly observed in both copepods and fish. Such findings corroborate previous studies which emphasized that the FA profile of the parasite can largely reflect that of its host (Tocher *et al*., 2010; Telahigue *et al*., 2017; Hajji *et al*., 2021). However, some dissimilarities were noticed as for the abundance and the distribution of some fatty acids. For instance, SFA was found to be the major FA group in almost all lipid classes of the two parasites *L. lusci* and *P. cylindricum,* mainly due to the substantial level of palmitic acid (C16:0). Although C16:0 was also found in a considerable amount in the host's tissues, their lipid fractions (mainly polar ones) tended to be more unsaturated and affected by the high DHA and EPA percentages. Remarkably high proportions of saturated fatty acids were also recorded in the phospholipid molecular species of other parasites such as *Isoparorchis hypselobagri* which infect the catfish *Wallago attuand* (Mondal and Dey, 2013) and *Paratenuisentis ambiguous,* an intestinal helminth parasite in eels (Aitzetmüller *et al*., 1994). Next to SFA, relatively high proportions of PUFA were also recorded in all lipid molecular species of the two studied parasitic copepods. Interestingly, it was observed that *L. lusci* neutral lipid classes, mainly TAG and FFA were particularly rich in PUFA (~40%), mostly in terms of long-chain FA such as C20:5n-3, C22:5n-3 and C22:6n-3. This may reflect a direct diversion of these essential FA from the host by the adult female and their probable incorporation in their egg strings. In fact, these fatty acids are known for their key roles in the reproductive success, development, and somatic growth of copepods as reported by Arendt *et al.* (2005). It is worth noting that the high unsaturation level in FFA could also be considered as an indicator of a good conservation of the acyl lipids during the storage and/or extraction process. High proportions of PUFA (up to 40% of the total FAs) with substantial amounts of DHA and EPA were also recorded in *P. cylindricum* phospholipid classes, chiefly in PC and PE. This pattern, reflecting the importance of PUFAs as essential structural components of the cell membrane phospholipids, was also reported by Tocher *et al.* (2010) in the parasitic female lice and its egg strings. According to several authors, these components are involved in various physiological functions such as the modulation of physicochemical properties of the membrane, ion exchange and transport, and cell signaling (Tocher, 2003). Furthermore, it has been reported that some C20-PUFAs such as ARA and EPA, recognized as main precursor molecules for eicosanoids, are thought to be involved in the modulation/suppression of the host's immune responses, which is crucial for parasite survival (Fast *et al.*, 2004; Tocher *et al.*, 2010).

The scarce available literature on the lipid profile of parasitic copepods has pointed out that neutral lipids (principally TAG) are characterized by high levels of MUFA (Lee, 1975; Tocher *et al.*, 2010). This pattern contrasts with that observed in our study where monoenes were found to not exceed 20% of the total FAs in all lipid classes of both *L. lusci* and *P. cylindricum*. This seems to be reflective of the host's lipid profiles, where MUFAs were found to compose approximately 20% of the total FAs across almost all the analyzed lipid classes. According to our results, the MUFA group was mainly comprised of oleic acid (C18:1n-9). This FA, known as a potential metabolic energy source (Tocher, 2003), has been found to occur in high proportions in several free and parasitic forms of marine copepods (Lee, 1975; Tocher *et al.*, 2010; Escribano and Pérez, 2010). Furthermore, this FA is generally considered a trophic marker for omnivorous and carnivorous copepods (Dalsgaard *et al.*, 2003). Although the main source of oleic acid is food intake, its synthesis *de novo* or by conversion of 18:0 was also reported in copepods (Kattner *et al.*, 1994).

To better visualize the trophic connection within each parasite-host system, hierarchical clustering analysis was applied. It was found that *L. lusci* lipid classes clustered with the neutral lipids of its host *M. merluccius,* which suggested that they were closely related. However, the lipid classes of *P. cylindricum* linked more with the phospholipids of its host *S. pilchardus*. These observations could be attributed to several parameters such as the host/parasite species, the fixation site, and the ability of each parasite to modulate its FA composition.

Overall, our data revealed that although the two studied host-parasite systems exhibited differences concerning the abundance of lipid classes as well as the distribution of some of their fatty acids, they appeared to share general similar FA patterns due to the strong trophic connection between them. Yet, to be more conclusive, further investigations (taking apart the eggs from the adult females) are needed to go deeper into the knowledge of their molecular biology and biochemistry in order to better understand host-parasite interactions.

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REFERENCES

Aitzetmüller K, Taraschewski H, Filipponi C, Werner G, Weber N. 1994. Lipids of fish parasites and their hosts: fatty acids of phospholipids of *Paratenuisentis ambiguus* and its host eel (*Anguilla anguilla*). *Comp. Biochem. Physiol.* **109**, 383–389. https://doi.org/10.1016/0305-0491(94)90021-3.

Arendt KE, Jonasdottir SH, Hansen PJ, Gartner S. 2005. Effects of dietary fatty acids on the reproductive success of the calanoid copepod *Temora longicornis*. *Mar. Biol.* **146**, 513–530. https://doi.org/10.1007/s00227-004-1457-9

Becheikh S , Rousset V, Maamouri F, Ben Hassine OK, Raibaut A. 1997. Pathological effects of *Peroderma cylindricum* (Copepoda: Pennellidae) on the kidneys of its pilchard host, *Sardina pilchardus* (Osteichthyes: Clupeidae), from Tunisian coasts. *Dis. Aquat. Org.* **28**, 51–59. https://doi.org/10.3354/dao028051

Brooker AJ, Shinn AP, Bron JE. 2007. A review of the biology of the parasitic copepod *Lernaeocera branchialis* (L. 1767) (Copepoda: Pennellidae). *Adv. Parasitol.* **65**, 297–341. https://doi.org/10.1016/S0065-308X(07)65005-2

Cecchi G, Basini S, Castano C. 1985. Méthanolyse rapide des huiles en solvant. *Rev. Fr .Corps Gras* **4**, 163–164.

Dalsgaard J, John M, Kattner G, Mueller-Navarra D, Hagen W. 2003. Fatty acid trophic markers in the pelagic marine environment. *Adv. Mar. Biol.* **46**, 225–340. https://doi.org/10.1016/S0065-2881(03)46005-7.

Dufourc EJ. 2008. Sterols and membrane dynamics. *J. Chem. Biol.* **1**, 63–77. https://doi.org/10.1007/s12154-008-0010-6

Díaz M, Dópido R, Gómez T, Rodríguez C. 2016. Membrane lipid microenvironment modulates thermodynamic properties of the Na+-K+-ATPase in branchial and intestinal epithelia in euryhaline fish in vivo. *Front. Physiol.* **7**, 589. https://doi: 10.3389/fphys.2016.00589.

Escribano R, Pérez C. 2010. Variability in fatty acids of two marine copepods upon changing food supply in the coastal upwelling zone off Chile: Importance of the picoplankton and nanoplankton fractions. *J. MAR. BIOL. ASSN. UK.* **90**, 301–313. https://doi.org/10.1017/S002531540999083X.

Fast MD, Ross NW, Craft CA, Locke SJ, Mackinnon SL, Johnson SC. 2004. *Lepeophtheirus salmonis*: characterization of prostaglandin E2 in secretory products of the salmon louse by RP-HPLC and mass spectrometry. *Exp. Parasitol.* **107**, 5–13. https://doi.org/10.1016/j.exppara.2004.04.001

Folch J, Lees M, Sloane-Stanley GA. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**, 497–509.

Hajji T, Ben Hassine OK, Farrugio H. 1998. Impact du copépode parasite *Peroderma cylindricum* Heller, 1868 sur la croissance et la fécondité des stocks exploités sur la sardine *Sardina pilchardus* (Walbaum, 1792). *Cah. Options Mediterr.* **35**, 79–86.

Hajji T, Telahigue K, Bennour S, Gharbi M, El Cafsi M. 2015. Impact of *Peroderma cylindricum* (Copepoda: Pennellidae) infection on fatty acid composition and lipid quality of Sardine (*Sardina pilchardus*). *J. Parasitol.* **101**, 682–686. https://doi.org/10.1645/15-777.

Hajji T, Telahigue K, Rabeh I, Ben Ammar R, Mdaini Z, El Cafsi M, Ghali R. 2021. Polar and neutral lipid composition of the copepod *Lernaeocera lusci* and its host *Merluccius merluccius* in relationship with the parasite intensity. *Parasitol. Res.* **120**, 1979-1991. http://doi: 10.1007/s00436-021-07182-z. Epub 2021 May 14. PMID: 33987737.

Kabata Z. 1979. *Parasitic Copepoda of British fishes*. The Ray Society, London.

Kattner G, Graeve M, Hagen W. 1994. Ontogenetic and seasonal changes in lipid and fatty acid/alcohol compositions of the dominant Antarctic copepods *Calanus propinquus*, *Calanoides acutus* and *Rhincalanus gigas*. *Mar. Biol.* **118**, 637–644. https://doi.org/10.1007/BF00347511

Kotani Y. 2006. Lipid content and composition of dominant copepods in the Oyashio waters analyzed by the thin layer chromatography flame ionization detection method. *Plankton Benthos Res.* **1**, 85–90. https://doi.org/10.3800/pbr.1.85

Lee RF. 1975. Lipids of parasitic copepods associated with marine fish. *Comp. Biochem.Physiol.* **52**, 363–364. https://doi.org/10.1016/0305-0491(75)90146-7

Lee RF, Hagen W, Kattner G. 2006. Lipid storage in marine zooplankton. *Mar. Ecol. Prog. Ser.* **307**, 273–306. https://doi.org/10.3354/meps307273

Mondal J, Dey C. 2013. Lipid and fatty acid compositions of a trematode, *Isoparorchis hypselobagri* Billet, 1898 (Digenea: Isoparorchiidae) infecting swim bladder of *Wallago attu* in the district North 24-Parganas of West Bengal. *J. Parasit. Dis.***39**, 67–72. https://doi.org/10.1007/s12639-013-0283-8

O'Neal AJ, Butler LR, Rolandelli A, Gilk SD, Pedra JH. 2020. Lipid hijacking: a unifying theme in vector-borne diseases. *ELife*, **9**, e61675. https://doi.org/10.7554/eLife.61675

Olsen RE, Henderson RJ. 1996. The rapid analysis of neutral and polar marine lipids using double-developed HPTLC and scanning densitometry. *J. Exp. Marine Biol. Ecol.* **129**, 189–197. https://doi.org/10.1016/0022-0981(89)90056-7

R Core Team. 2020. A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Şen Özdemir N, Parrish CC, Parzanini C, Mercier A. 2019. Neutral and polar lipid fatty acids in five families of demersal and pelagic fish from the deep Northwest Atlantic. *ICES J. Mar. Sci.* **76**, 1807–1815. https://doi:10.1093/icesjms/fsz054.

Taraschewski H, Aitzetmüller K, Werner G, Kühs K. 1995. Lipids of fish parasites and their hosts: fatty acid fingerprints of four species of acanthocephalans and of their hosts' intestinal tissues. *Parasitol. Res.* **81,** 522–526. https://doi.org/10.1007/BF00931796

Telahigue K, Hajji T, Gharbi M, Cherif A, El Cafsi M. 2017. The parasitic copepod *Peroderma cylindricum* Heller, 1865 (Copepoda: Pennellidae) and its host *Sardina pilchardus* (Walbaum, 1792): trophic relationships as revealed by fatty acid profiles. *J. Crustacean Biol.* **37**, 453–457. https://doi.org/10.1093/jcbiol/rux054

Telahigue K, Rabeh I, Chetoui I, Bejaoui S, El Cafsi M, Hajji T. 2019. To what extent are hake fat and its oil quality affected by the parasite *Lernaeocera lusci*? *Grasas y Aceites* **70**, e297. https://doi.org/10.3989/gya.0697181

Tocher DR. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. *Rev. Fisheries Sci.* **11**, 107–184. https://doi.org/10.1080/713610925

Tocher JA, Dick JR, Bron JE, Shinn AP, Tocher DR. 2010. Lipid and fatty acid composition of parasitic caligid copepods belonging to the genus *Lepeophtheirus*. *Comp. Biochem. Physiol.* **156**, 107–114. https://doi.org/10.1016/j.cbpb. 2010.02.010

Tracey TJ, Steyn FJ, Wolvetang EJ, Ngo ST. 2018. Neuronal lipid metabolism: Multiple pathways driving functional outcomes in health and disease. *Front. Mol. Neurosci.* **11**, 10. https://doi.org/10.3389/fnmol.2018.00010

Vallochi AL, Teixeira L, Oliveira KDS, Maya-Monteiro CM, Bozza PT. 2018. Lipid Droplet, a Key Player in Host-Parasite Interactions. *Front. Immunol.* **9**, 1022. https://doi.org/10.3389/fimmu.2018.01022

van der Meeren T, Olsen R, Hamre K, Fyhn H. 2008. Biochemical composition of copepods for evaluation of feed quality in production of juvenile marine fish. *Aquaculture* **274**, 375–397. https://doi.org/10.1016/j.aquaculture.2007.11.041

van Damme PA, Ollevier F, Hamerlynck O. 1994. Pathogenicity of *Lernaeocera lusci* and *L. branchialis* in bib and whiting in the North Sea. *Dis. Aquat. Organ.* **19**, 61–65. http://dx.doi.org/10.3354/dao019061

Zhao S, Guo Y, Sheng Q, Shyr Y. 2014. Heatmap3: an improved heatmap package with more powerful and convenient features. *BMC Bioinformatics* **15**, 16.

Zhou L, Yang F, Zhao M, Zhang M, Liu J, Marchioni E. 2020. Determination and comparison of phospholipid profiles in eggs from seven different species using UHPLC-ESI-Triple TOF-MS. *Food Chem.* **339**, 127856. https://doi.org/10.1016/j.foodchem.2020.127856.

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The influence of olive tree fertilization on the phenols in virgin olive oils. A review

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SUMMARY: The total phenols in virgin olive oil are highly dependent on cultivar, but also on ripening stage and other agronomic factors. The focus of most studies on agronomic factors has been irrigation, while fertilization has received less attention. Most of the fertilization works find that nitrogen over-fertilization leads to a decrease in phenol contents in virgin olive oil (VOO) and extra virgin olive oil (EVOO), under rain-fed or irrigation management. Ortho-diphenols also decrease with high doses of nitrogen, with no effect on secoiridoids. Phosphorous has a minor effect on irrigated trees; while the role of potassium is controversial, with a lack of trials with calcium and micro-nutrients. Due to the great impact of the fertilization on the phenol content and quality of VOO, new research is necessary with focus aimed at different cultivars and agronomic factors.

KEYWORDS: *Nitrogen; Nutrients; Ortho-diphenols; Phenols; Secoiridoids*

RESUMEN: ***Influencia de la fertilización del olivo en los fenoles del aceite de oliva virgen. Revisión.*** Los fenoles del aceite de oliva tienen un fuerte componente varietal, estando influenciados por el estado de maduración y otros factores agronómicos. Entre estos últimos, el efecto del riego es el más conocido, mientras que la fertilización está menos estudiada. La mayoría de los trabajos existentes sobre fertilización, muestran que el incremento en el abonado nitrogenado disminuye los niveles de fenoles totales del aceite de oliva virgen o virgen extra, tanto en secano como en regadío. También se han observado niveles inferiores de ortodifenoles en tratamientos con aplicación de nitrógeno, sin detectar diferencias en el contenido de secoiridoides. El fósforo tiene una influencia menor y la del potasio es algo controvertida, quedando por explorar la influencia del calcio y de los micronutrientes. Debido al considerable impacto que tiene la fertilización en los fenoles así como en la calidad del aceite, sería necesario incrementar los estudios de fertilización con diferentes variedades y sistemas productivos.

PALABRAS CLAVE: *Fenoles; Nitrógeno; Nutrientes; Ortodifenoles; Secoiridoides*

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1. INTRODUCTION

Due to its present and future strategic value, olive production systems for the production of olive oil have received a strong push in terms of technological innovation in the last twenty years. A higher number of plants per hectare, mechanized harvesting and the use of fertigation are some of the innovations that involve what is called agricultural intensification. The intensification of olive orchard management implies an increase in irrigation and fertilization, especially nitrogen (N), which can lead to a reduction in virgin olive oil's (VOO[1]) quality and stability, in particular, a decrease in phenol content. There is a negative relationship between irrigation and phenol content, as different authors have demonstrated in studies with linear irrigations, where the total applied irrigation water linearly changed with the effective crop coefficient (Tovar *et al.,* 2001; Berenguer *et al.,* 2006) or deficit irrigations (Gómez-del-Campo, 2013; Rufat *et al.,* 2018), although no differences were found in intermediate irrigation strategies (Sastre *et al.,* 2019), which linearly increased the total amount of applied water. Berenguer *et al.* (2006) found differences among the most contrasting treatments. Phenols are highly influenced by cultivar and ripening stage (Aguilera *et al.,* 2017), and also by other agronomic factors such as harvesting method (Morales-Sillero and García, 2015; Arbonés *et al.,* 2016).

Water availability is important for determining plant response to mineral nutrition (Marschner, 2012) at both the irrigation level and N status, which are highly related to the cultivar. At present, the question stated by Zipori *et al.* (2015) should be answered "Can threshold values being used today be applied indiscriminately to all cultivars, especially in intensive, irrigated orchards?"

The influence of ripeness and irrigation on the phenol content in VOOs phenols has been studied in recent years (Aparicio and Harwood, 2013; Mena *et al*., 2018; Vidal *et al*., 2019), although there are few works related to fertilization (Lanza and Ninfali, 2020; Zipori *et al*., 2020) despite its crucial role in VOO's quality. Fertilization should be carefully monitored to avoid negative impacts on VOO (Rallo *et al*., 2018). This work aims to review existing studies and research related to the influence of olive orchard fertilization on the phenols in VOO.

2. NITROGEN, PHOSPHORUS AND POTASSIUM INFLUENCE ON VOO PHENOLS

Despite the fact that most of the studies show that an increase in N fertilization leads to a decrease in the phenol content of the VOOs in a different way, depending on olive orchard management and cultivars, some results are not so clear (Inglese *et al.,* 2002; Mezghani *et al.,* 2018).

In a long-term trial under rainfed conditions, Fernández-Escobar *et al.* (2002) found that increasing N doses up to 1 kg N·tree^{-1} resulted in a decrease in total phenols in VOO with no increase in yield or vegetative growth, despite higher values in leaf and fruit N content compared to the control. This occurred regardless of the plantation system (Fernandez-Escobar *et al.,* 2006). Moreover, foliar applications of B and N in Koroneiki induced a phenol decrease but a foliar algae solution (derived from fronds and strap-like blades of the seaweed *Ascophyllum nodosum* L. and applied at a concentration of 0.5%) did not (Chouliaras *et al.,* 2009).

A decrease in total phenol content was observed in response to high doses of N and phosphorus (P) while potassium (K) had only a minor effect or no effect at all on irrigated trees (Dag *et al.,* 2009; Lanza and Ninfali, 2020), while Pascual *et al.* (2019) found a significant decrease with K fertilization. Erel *et al.* (2013), who observed a decreased in phenol content as foliar N increased, found the same trend. They also found a weaker relationship between phenol content and fruit N, without finding a correlation between phenol content and fruit P content. In the Barnea cultivar, Dag *et al.* (2018) observed a reduction in total phenol content when high N doses were applied, similar to what Morales-Sillero *et al.* (2007) observed in the Manzanilla de Sevilla cultivar, although significant differences with fruit N content were not found. Phosphorous enhances the accumulation of N in leaves and fruits and thus indirectly increases the nutritional level of N. Total phenol concentration was entirely independent of the leaf or fruit K levels, and therefore the effect of the N on oil properties depended solely on the absolute N content in the leaves or fruits, regardless of the cause. Olive trees can deal with a low soil fertility unless a great deficiency occurs, but an overdose in N, P or K fertilization can lead to a reduction in VOO quality (Tognetti *et al.,* 2008).

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[1] VOO includes Extra Virgin and Virgin Olive Oil categories as are described in the Regulation (EU) No 1308/2013 (EU, 2013).

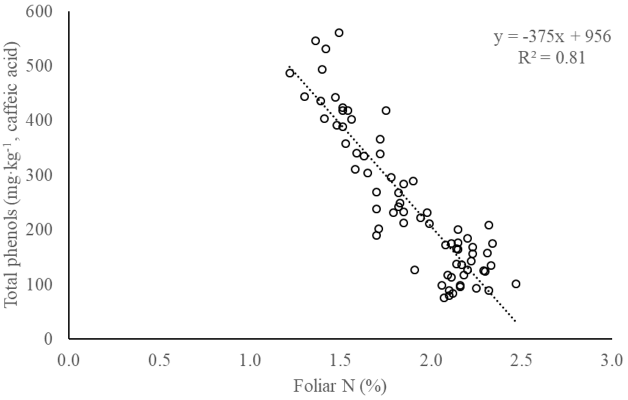

Figure 1. Linear regression between leaf N content and total phenols ($R^2 = 0.806$) in the VOO of cv Arbequina from two super-intensive orchards in Madrid and Lleida (Spain) with different levels of irrigation and fertilization with nitrogen and potassium. N=64.

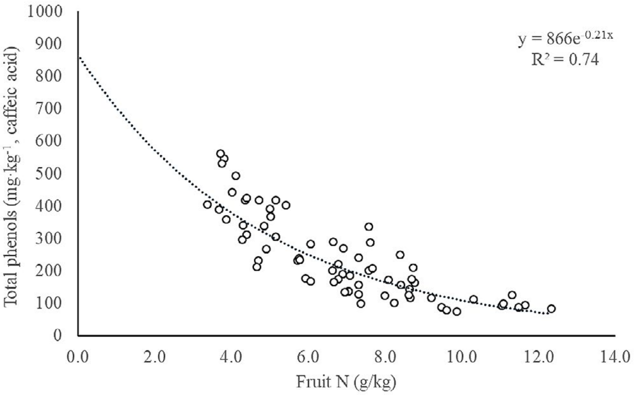

Figure 2. Quadratic regression between fruit N content and total phenols ($R^2 = 0.743$) in the VOO of cv Arbequina from two super-intensive orchards in Madrid and Lleida (Spain) with different levels of irrigation and fertilization with nitrogen and potassium. N=60.

Arbonés *et al.* (2017) also found a negative correlation between total phenols and N foliar content in a trial with N and K fertilization in super-intensive hedgerows of the Arbequina cultivar in Lleida (Spain). Only in ON years (heavy yield) did K application have a positive response, and a N/K interaction was evidenced by the effect of K on increasing leaf N content. Moreover, a high decrease in total phenols with K fertilization was found (Pascual *et al.*, 2019). They also established a critical nutrient range for N leaf in the interval of 1.80–1.94% with no effect on VOO yield, while beyond this threshold, phenol content and oxidative stability were compromised. That statement was confirmed by Arbonés *et al.* (2020), with cv Arbequina in two super-intensive orchards in Madrid and Lleida (Spain) with different levels of irrigation and N-K fertilization, finding a good negative linear correlation (R^2=0.81) between foliar N and total phenol content in VOO (Figure 1). Beyond 1.9% leaf N, the total phenol content decreased to below 200 $mg \cdot kg^{-1}$ (expressed as caffeic acid), a value considered to be the threshold above which phenolic compounds exert their nutraceutical effects as antioxidants (European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies, 2011; Gucci *et al.*, 2012). This fact gives rise to a reduction in VOO quality. The relationship between fruit N (pulp plus stone) and total phenol content is quadratic, and it is highly influenced by crop load (Figure 2). Below 7 $g \cdot kg^{-1}$ fruit N, VOO's phenol content falls below the 200 $mg \cdot kg^{-1}$ (expressed as caffeic acid).

Within the phenol family, increasing foliar and fruit N levels gives rise to a decrease in total oil phenols (around 250 mg per N foliar unit), specially in secoroid compounds, which results in lower VOO quality and stability. This negative impact of N on VOO quality is higher in OFF years (low yield). These results differ from those of Centeno *et al.* (2017) with cv Arbequina, who observed lower ortho-diphenol (o-diphenols) contents in all N-fertilized treatments regardless of doses or products, whilst total secoiridoids were only reduced in treatments with N inhibitors. In Picholine cultivar under irrigation conditions, Tekaya *et al.* (2014) found a significant decrease in total phenols with urea and micronutrient fertilization. The same group did not find a negative effect of N fertilization on total phenols but they observed a great decrease in o-diphenol content (Tekaya *et al.,* 2013).

Nitrogen is one of the nutrients that can modulate the activity of different enzymes related to phenol content and metabolism, like phenylalanine ammonium lyase (PAL) and polyphenol oxidase (PPO). In different plants (e.g. basil, tobacco and olive tree), PAL activity, involved in the synthesis of phenols, can be negatively influenced by N fertilizacion due to a competition between phenols and protein in fruit (Ruiz *et al.,* 1998; Fernández-Escobar, 2011; Jakovljević *et al.*, 2019). Furthermore, PPO activity increases as foliar and fruit N contents increase, with a negative effect on VOO stability due to changes in its fatty acid profile (Pascual *et al.,* 2016).

The synthesis of phenolic compounds is stimulated in response to different plant stresses (Fernández 2014; Rallo *et al.,* 2018). Therefore, in intensive and super-intensive olive orchards, from the point of view of the quality of the VOO, it should be appropiate to exert a certain level of stress, but not too high so as to reduce crop load.

3. OTHER NUTRIENTS THAT INFLUENCE VOO PHENOLS

A decrease in total phenols and o-diphenols has been observed after foliar fertilization with boron (B), magnesium (Mg), manganese (Mn) and sulfur (S), due to the B action (Tekaya *et al.,* 2013). When the content of B in the leaves is low, phenolic biosynthesis increases, but when the level of boron is adequate, the PAL activity is depressed and phenol biosynthesis is limited (Ruiz *et al.,* 1998). Under semiarid conditions, Saadati *et al.* (2013) did not find statistical differences in total phenols for different cultivars after applying boric acid and zinc sulfate. Increases in VOO oil content and phenolic compounds were observed when cv. Beledy olive trees were treated with Zn in the form of amino acid chelate (Bastam *et al.,* 2020). In the Chemlali cultivar, the foliar spraying of amino acids, beet molasses and fish by-products as sources of N, P and K resulted in a decrease in the phenol content, contrary to an application of a formulation based on calcium (Ca), which led to a significant increase in phenolic compound contents (Dabbaghi *et al.,* 2019).

Foliar fertilization with selenium (Se) applied to the Leccino cultivar increased the total Se, alleviated damage caused by drought stress conditions and increased total antioxidant compounds, mainly phenol contents (D'Amato *et al.,* 2018). Silicon (Si), like selenium, is a non-essential element in plant nutrition despite the fact that it plays an important role against drought and other abiotic stresses (Marschner 2012). Different studies have found that Si forms a physical barrier and induces the production of phenolic compounds at sites of infection (Fernandez-Escobar, 2019). Silicon applying can be done through drip irrigation or the most effective foliar spraying, which can be promising for olive orchards, mainly under rainfed conditions.

4. CONCLUSIONS

Nitrogen (N) over-fertilization leads to a total phenol content decrease, regardless of the olive growing system or cultivar type; phosphorus fertilization seems to have a minor influence, and in the case of potassium (K), the effect is controversial.

Olive yield decreases beyond a threshold of foliar N, for this reason it is essential to establish the proper reference levels, which do not impair olive yield whilst maintaining a sufficient phenol content to maintain good VOO quality and stability. This threshold should be different for each cultivar and olive-growing system.

Phosphorus fertilization seems to have an indirect influence on phenol content, although results on the effect of K differ among authors. There is a lack of field trials with these macro-nutrients near or below the deficiency threshold to clarify the role of K regarding phenol content. New trials with potassium-based formulations could be of great interest, in order to increase total phenols in cultivars with low to medium phenol content.

Fertilization with micronutrients and other non-essential microelements have been scarcely studied. These elements must be considered for future studies, mainly boron and selenium elements, which could produce an increase in total phenols, or silicon, which could protect fruit skin, thus avoiding the loss of phenols during mechanical harvesting.

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REFERENCES

Aguilera M, Uceda M, Beltran G. 2017. La calidad del aceite de oliva, in Barranco D, Fernandez-Escobar R, Rallo L (Eds.) *El cultivo del olivo*, 7th ed, Ediciones Muni-Prensa, Madrid, pp. 839-868.

Aparicio R, Harwood J. 2013. *Handbook of Olive Oil: Analysis and Properties*. Springer, Boston, MA: Springer US. https://doi.org/10.1007/978-1-4614-7777-8

Arbonés A, Pascual M, Villar JM, Romero A, Rufat J. 2016. Respuesta cualitativa al riego y a la recolección mecanizada en olivo superintensivo (cv. Arbequina). *Actas del I Congreso Ibérico de Olivicultura / V Jornadas Nacionales del Grupo de Olivicultura de la Sociedad Española de Ciencias Horticolas (SECH) / VII Simpósio Nacion-*

al de Olivicultura da Associação Portuguesa de Horticultura (APH), April 2016, pp. 137.

Arbonés A, Pascual M, Villar JM, Rufat J. 2017. Riego, abonado nitrogenado y calidad de aceite. *Revista de Fruticultura* **56**,120-129. Ed.Tècnica Quatrebcn.

Arbonés A, Sastre B, Perez MÁ, de Lorenzo C, Pascual M, Benito A, Villar JM, Rufat J. 2020. Influence of irrigation and fertilization on the sterol and triterpene dialcohol compositions of virgin olive oil. *Grasas Aceites* **71**, 376. https://doi.org/10.3989/gya.0795191.

Bastam N, Baninasab B, Mobli M, Goli SAH. 2020. Effects of foliar applications of zinc in the forms of free mineral or amino acid complexed on qualitative characteristics of olive oil. *J. Am. Oil Chem. Soc.* **98**, 173-184. https://doi.org/10.1002/aocs.12443.

Berenguer MJ, Vossen PM, Grattan SR, Connell JH, Polito VS. 2006. Tree irrigation levels for optimum chemical and sensory properties of olive oil. *HortScience* **41**, 427-432. https://doi.org/10.21273/HORTSCI.41.2.427.

Centeno A, Garcia JM, Gomez-del-Campo M. 2017. Effects of nitrogen fertilization and nitrification inhibitor product on vegetative growth, production and oil quality in ‘Arbequina’ hedgerow and ‘picual’ vase-trained orchards. *Grasas Aceites* **68**, e215. https://doi.org/10.3989/gya.0441171.

Chouliaras V, Tasioula M, Chatzissavvidis C, Therios I, Tsabolatidou E. 2009. The effects of a seaweed extract in addition to nitrogen and boron fertilization on productivity, fruit maturation, leaf nutritional status and oil quality of the olive (*Olea europaea* L.) cultivar koroneiki. *J. Sci. Food Agric.* **89**, 984-988. https://doi.org/10.1002/jsfa.3543.

Dabbaghi O, Tekaya M, Flamini G, Zouari I, El-Gharbi S, M’barki N, Laabidi F, Cheheb H, Attia F, Mezghani MA, Hammami M, Mechri B. 2019. Modification of phenolic compounds and volatile profiles of chemlali variety olive oil in response to foliar biofertilization. *J. Am. Oil Chem. Soc.* **96**, 585-593. https://doi.org/10.1002/aocs.12201.

Dag A, Erel R, Kerem Z, Ben-Gal A, Stern N, Bustan A, Zipori I, Yermiyahu U. 2018. Effect of nitrogen availability on olive oil quality. *VIII International Olive Symposium* **1199**, 465-469. https://doi.org/10.17660/ActaHortic.2018.1199.74.

Dag A, Ben-David E, Kerem Z, Ben-Gal A, Erel R, Basheer L, Yermiyahu U. 2009. Olive oil composition as a function of nitrogen, phosphorus and potassium plant nutrition. *J. Sci. Food Agric.* **89**, 1871-1878. https://doi.org/10.1002/jsfa.3664.

D’Amato R, De Feudis M, Hasuoka PE, Regni L, Pacheco PH, Onofri A, Businelli D, Proietti P. 2018. The selenium supplementation influences olive tree production and oil stability against oxidation and can alleviate the water deficiency effects. *Front. Plant Sci.* **9**, 1191. https://doi.org/10.3389/fpls.2018.01191.

Erel R, Kerem Z, Ben-Gatt A, Dag A, Schwartz A, Zipori I, Basheer L, Yermiyahu U. 2013. Olive (*Olea europaea* L.) tree nitrogen status is a key factor for olive oil quality. *J. Agric. Food Chem.* **61**, 11261-11272. https://doi.org/10.1021/jf4031585.

EU. 2013. Regulation (EU) No 1308/2013 of the European Parliament and of the Council of 17 December 2013 establishing a common organisation of the markets in agricultural products and repealing Council Regulations (EEC) No 922/72, (EEC) No 234/79, (EC) No 1037/2001 and (EC) No 1234/2007. *OJEU* **L347**, 20 December 2013, pp. 671-854.

European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies. 2011. Scientific opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865). *EFSA J.* **9**, 2033.

Fernández JE. 2014. Understanding olive adaptation to abiotic stresses as a tool to increase crop performance. Environ. Exp. Bot. 103, 158-179. https://doi.org/10.1016/j.envexpbot.2013.12.003.

Fernández-Escobar R. 2011. Use and abuse of nitrogen in olive fertilization. *International Symposium on Olive Irrigation and Oil Quality* **888**, 249-257. https://doi.org/10.17660/ActaHortic.2011.888.28.

Fernández-Escobar R. 2019. Olive nutritional status and tolerance to biotic and abiotic stresses. *Frontiers Plant Sci.* **10**, 1151. https://doi.org/10.3389/fpls.2019.01151.

Fernández-Escobar R, Beltran G, Sánchez-Zamora M, García-Novelo J, Aguilera M, Uceda M. 2006. Olive oil quality decreases with nitrogen over-fertilization. *HortScience* **41**, 215-219. https://doi.org/10.21273/HORTSCI.41.1.215.

Fernández-Escobar R, Sánchez-Zamora MA, Uceda M, Beltran G. 2002. The effect of nitrogen overfertilization on olive tree growth and oil quality. *Acta Horticulturae* **586**, 429-432. https://doi.org/10.17660/ActaHortic.2002.586.88.

Gómez-del-Campo M. 2013. Summer deficit irrigation in a hedgerow olive orchard cv. Arbequina: Relationship between soil and tree water status, and growth and yield components. *Spanish J. Agric. Res.* **11**, 547-557. https://doi.org/10.5424/sjar/2013112-3360.

Gucci R, Caruso G, Canale A, Loni A, Raspi A, Urbani S, Taticchi A, Esposto S, Servili M. 2012. Qualitative Changes of Olive Oils Obtained from Fruits Damaged by *Bactrocera oleae* (Rossi). *HortScience* **47**, 301-306. http://doi.org/10.21273/HORTSCI.47.2.301.

Inglese P, Gullo G, Pace LS. 2002. Fruit growth and olive oil quality in relation to foliar nutrition and time of application. *Acta Hortic.* **586**, 507-509. https://doi.org/10.17660/ActaHortic.2002.586.105.

Jakovljević D, Topuzović M, Stanković M. 2019. Nutrient limitation as a tool for the induction of secondary metabolites with antioxidant activity in basil cultivars. *Ind. Crops Prod.* **138**, 111462. https://doi.org/10.1016/j.indcrop.2019.06.025.

Lanza B, Ninfali P. 2020. Antioxidants in extra virgin olive oil and table olives: Connections between agriculture and processing for health choices. *Antioxidants* **9**, 41. https://doi.org/10.3390/antiox9010041.

Marschner H. 2012. *Marschner's Mineral Nutrition of Higher Plants.* 3rd ed, Academic Press.

Mena C, Gonzalez AZ, Olivero-David R, Perez-Jimenez, MA. 2018. Characterization of 'Castellana' Virgin Olive Oils with Regard to Olive Ripening. *Horttechnol.* **28**,48-57. https://doi.org/10.21273/HORTTECH03845-17.

Mezghani MA, Ayadi M, Attia F, Zouari I, Labidi F, Attia L. 2018. Effects of irrigation and fertigation on tree growth, yield and oil quality of olive cultivars in south tunisia. *Acta Hortic.* **1199**, 255-260. https://doi.org/10.17660/ActaHortic.2018.1199.39.

Morales-Sillero A, García JM. 2015. Impact assessment of mechanical harvest on fruit physiology and consequences on oil physicochemical and sensory quality from 'Manzanilla de Sevilla' and 'Manzanilla Cacereña' super-high-density hedgerows. A preliminary study. *J. Sci. Food Agric.* **95**, 2445-2453. https://doi.org/10.1002/jsfa.6971.

Morales-Sillero A, Jimenez R, Fernandez JE, Troncoso A, Beltran G. 2007. Influence of fertigation in 'manzanilla de sevilla' olive oil quality. *HortScience* **42**, 1157-1162. https://doi.org/10.21273/HORTSCI.42.5.1157.

Pascual M, Villar JM, Arbonés A, Rufat J. 2019. Nitrogen nutrition diagnosis for olive trees grown in super-intensive cropping systems. *J. Plant Nutr.* **42**, 1803-1817. https://doi.org/10.1080/01904167.2019.1628983.

Pascual M, Villar JM, Arbonés A, Rufat J. 2016. Fertilización nitrogenada, actividad enzimática y calidad de producto en melocotonero y olivo. *Acta Hortic.* **75**, ed. SECH, 16-18 October 2016, pp. 54.

Rallo L, Diez CM, Morales-Sillero A, Miho H, Priego-Capote F, Rallo P. 2018. Quality of olives: A focus on agricultural preharvest factors. *Scientia Hortic.* **233**, 491-509. https://doi.org/10.1016/j.scienta.2017.12.034.

Rufat J, Romero-Aroca AJ, Arbonés A, Villar JM, Hermoso JF, Pascual M. 2018. Mechanical harvesting and irrigation strategy responses on 'Arbequina' olive oil quality. *Horttechnology* **28**, 607-614. https://doi.org/10.21273/HORTTECH04016-18.

Ruiz JM, Bretones G, Baghour M, Ragala L, Belakbir A, Romero L. 1998. Relationship between boron and phenolic metabolism in tobacco leaves. *Phytochem.* **48**, 269-272. https://doi.org/10.1016/S0031-9422(97)01132-1.

Saadati S, Moallemi N, Mortazavi S, Seyyednejad S. 2013. Effects of zinc and boron foliar application on soluble carbohydrate and oil contents of three olive cultivars during fruit ripening. *Sci. Hortic.* **164**, 30-34. https://doi.org/10.1016/j.scienta.2013.08.033.

Sastre B, Arbones A, Benito A, Rufat J, de Lorenzo C, Pascual M, Villar JM, Perez MA. 2019. Efecto del riego deficitario en un superintensivo de Arbequina sobre la calidad del aceite de oliva virgen, *Expoliva*, May 2019.

Tekaya M, Mechri B, Cheheb H, Attia F, Chraief I, Ayachi M, Boujneh D, Hammami M. 2014. Changes in the profiles of mineral elements, phenols, tocopherols and soluble carbohydrates of olive fruit following foliar nutrient fertilization. *LWT-Food Science and Technology* **59**, 1047-1053. https://doi.org/10.1016/j.lwt.2014.06.027.

Tekaya M, Mechri B, Bchir A, Attia F, Cheheb H, Daassa M, Hammami A. 2013. Effect of nutrient-based fertilisers of olive trees on olive oil quality. *J. Sci. Food Agric.* **93**, 2045-2052. https://doi.org/10.1002/jsfa.6015.

Tognetti R, Morales-Sillero A, D'Andria R, Fernandez JE, Lavini A, Sebastiani L, Troncoso A. 2008. Deficit irrigation and fertigation practices in olive growing: Convergences and divergences in two case studies. *Plant Biosystems* **142**, 138-148. https://doi.org/10.1080/11263500701872879.

Tovar MJ, Motilva MJ, Luna M, Girona J, Romero MP. 2001. Analytical characteristics of virgin olive oil from young trees (Arbequina cultivar) growing under linear irrigation strategies. *J. Am. Oil Chem. Soc.* **78**, 843-849. https://doi.org/10.1007/s11746-001-0353-5.

Vidal AM, Alcala S, de Torres A, Moya M, Espinola F. 2019. Characterization of Olive Oils from Superintensive Crops with Different Ripening Degree, Irrigation Management, and Cultivar: (Arbequina, Koroneiki, and Arbosana). *Eur. J. Lipid Sci. Technol.* **121,** 1800360. http://doi.org/10.1002/ejlt.201800360.

Zipori I, Erel R, Yermiyahu U, Ben-Gal A, Dag A. 2020. Sustainable management of olive orchard nutrition: A review. *Agriculture-Basel* **10**, 11. https://doi.org/10.3390/agriculture10010011.

Zipori I, Yermiyahu U, Erel R, Presnov E, Faingold I, Ben-Gal A, Dag A. 2015. The influence of irrigation level on olive tree nutritional status. *Irrig. Sci.* **33**, 277-287. https://doi.org/10.1007/s00271-015-0465-5.

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Application of coconut fiber and shell in the bleaching of soybean oil

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SUMMARY: The bleaching process is an important stage in the edible oil refining operation, and is carried out by using acid-activated bleaching earths. The aim of this study was to evaluate the efficiency of coconut fiber ash, shell ash, acid-activated fiber ash and acid-activated shell ash as compared to the commercial bleaching earth in the bleaching of neutralized soybean oil. Bleaching materials were added to neutralized oil at the concentration of 1% (w/v) with agitation under vacuum at 110 °C for 30 minutes. The values for red and yellow colors, carotenoids, chlorophylls, peroxide value, *p*-anisidine value, free fatty acid contents, copper and iron levels of the bleached samples were determined. The results indicated that all coconut-based adsorbents have been significantly more effective than commercial bleaching earth in reducing color and the greatest reductions in carotenoid (84.25%) and chlorophyll (82.30%) contents were obtained by using acid-activated fiber ash. The peroxide value for all treatments decreased. The amounts of iron and copper as peroxide compounds decreased considerably (44.59% and 23.53%) by using acid-activated fiber ash and acid-activated shell ash, respectively. Therefore, coconut fiber and shell as agricultural wastes which have been ignored in the past might be employed as effective agents to bleach crude oils, particularly soybean oil, in refining operations.

KEYWORDS: *Adsorbents; Agricultural waste; Bleaching; Coconut; Soybean oil.*

RESUMEN: Aplicación de fibra y cáscara de coco para decolorar aceite de soja. El proceso de decoloración es una etapa importante en la operación de refinación de aceites comestibles que se lleva a cabo utilizando tierras decolorantes activadas con ácido. El objetivo de este estudio fue evaluar la eficiencia de las cenizas de fibra de coco, cenizas de cáscara, cenizas de fibra activada por ácido y cenizas de cáscara activada con ácido, en comparación con la tierra decolorante comercial, en la decoloración de aceite de soja neutralizado. Los materiales de decoloración se añadieron al aceite neutralizado a una concentración del 1 % (p/v) con agitación a vacío a 110 °C durante 30 minutos. Se determinaron los valores de colores rojo y amarillo, carotenoides, clorofilas, índice de peróxido, índice de *p*-anisidina, contenido de ácidos grasos libres, niveles de cobre y hierro de las muestras decoloradas. Los resultados indicaron que todos los adsorbentes a base de coco han sido significativamente más efectivos que la tierra decolorante comercial para reducir el color y la mayor reducción en los contenidos de carotenoide (84,25 %) y clorofila (82,30 %) se obtuvo mediante el uso de ceniza de fibra activada con ácido. El índice de peróxido en todos los tratamientos disminuyó. Las cantidades de hierro y cobre como compuestos peroxidantes disminuyeron considerablemente (44,59% y 23,53%) al usar ceniza de fibra activada por ácido y ceniza de cáscara activada por ácido, respectivamente. Por lo tanto, la fibra y la cáscara de coco como desechos agrícolas que se han ignorado en el pasado podrían emplearse como agentes efectivos para decolorar los aceites crudos, particularmente el aceite de soja en las operaciones de refinación.

PALABRAS CLAVE: *Aceite de soja; Adsorbentes; Coco; Decoloración; Residuos agrícolas.*

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1. INTRODUCTION

The coconut fruit contains three layers: the exocarp, mesocarp, and endocarp. Both the exocarp (the glossy outer skin, usually yellow-green to yellow-brown in color) and the mesocarp (middle fibrous coat, called coir) make up the "husk" of the coconut, while the endocarp makes up the hard coconut "shell" (Emojevwe, 2013). Therefore, the coconut plant has some useful waste materials such as fronds, husk and shell, which have many traditional and commercial uses.

Coir (the fiber from the husk of the coconut) is used in ropes, mats, doormats, brushes, and sacks, brooms, as caulking for boats, and as stuffing fiber for mattresses. It is also used in horticulture for potting compost (Pauline, 2000).

Different studies have been carried out for the application of these lignocellulosic agricultural wastes in polymeric composites (Ikumapayi *et al*., 2020), the textile industry (Martins and Sanches, 2019), and the oil and gas industry as adsorbents for industrial wastewater (Johari *et al*., 2014). Yuan *et al*. (2021) synthesized an efficient adsorbent by simple doping of Mg–Al composite oxide on coconut shell fiber to remove of phosphorus from domestic sewage. Taksitta *et al*. (2020) modified and used coconut husk as adsorbents for metal ion removal from wastewater.

One of the applications of adsorbents in the food industry concerns the bleaching process in oil refineries. Bleaching is one of the refining stages of oil and fat industries which removes not only pigments but also other minor impurities such as residual soaps, metal, and oxidation products to provide an acceptable product to the consumer with the highest stability. Acid-activated bleaching earth containing silicates combined with metal oxides such as aluminum, magnesium, calcium, iron, sodium, and potassium activated by sulfuric or hydrochloric acids is employed in the conventional bleaching operation (Erten, 2004).

Some researchers have proposed the use of nut shells as adsorbents. Shruthi and Parithra (2018) studied the efficiency of groundnut shells for the removal of led and copper from wastewater. The ability of groundnut shell to remove toxic metals from industrial wastewater was achieved by Aarti Sowmya *et al*., 2018. Almasi *et al*. (2016) stated that the walnut shell has suitable physical properties for the production of activated carbon. Forozan Sepehr *et al*. (2020) used walnut shell ash for the bleaching of soybean oil and reported a reduction in color, carotenoid and chlorophyll contents during this process. Coconut shell ash has been reported to produce the highest activated carbon among the agro-waste materials that can be used as adsorbents (Ikumapayi *et al*., 2020).

Usman *et al*. (2012) indicated that there is a poor correlation between the chemical composition of clays and their adsorptive power. This means that poor adsorbents may have identical composition to active types. Therefore, the scientific basis for evaluating the adsorbent's functionality involves actual testing in the laboratory. According to the literature, the application of coconut fiber and shell is not receiving much application in the food industry. Therefore, the aim of this research is to study the application of these agro wastes as bio adsorbenta in the bleaching of neutralized soybean oil.

2. MATERIALS AND METHODS

2.1. Materials

Coconut was supplied from a local market in Tehran, Iran. Neutralized soybean oil was obtained from the Behshahr Vegetable Oil Company (Tehran, Iran). Commercial activated bleaching earth, Bentonite Type KF, with pH of 3.3, moisture content of 11% and density of 0.88 g/cm^3 was purchased from Kanysaz Jam Industries Ltd., (Tehran, Iran). All the chemicals used in the experiments were pure and of analytical grade, purchased from Merck Chemical Co. (Germany) and Sigma Aldrich Co. (USA).

2.2. Preparation of adsorbents

Coconut fibers and shell were separated from the fruits and dried at 103 ± 2 °C, then ground in a mill (Triplex, France), and passed through a 200-mesh sieve separately. The coconut fiber and coconut shell powders were carbonized in air atmosphere and then heated in a muffle furnace at 800 °C for 5h to prepare the ash. The ash samples were cooled in a desiccator at room temperature, passed through a 200-mesh sieve and stored in amber glass bottles (Forozan Sepehr *et al*., 2020).

In order to acid activate the ash samples, they were mixed with 12N HCl in the ratio of 1:3 (w/v) separately. The suspension was agitated at 750 rpm at 80 ± 2 ºC for 2 h and then diluted with distilled water up to pH of 3.5. The slurry was filtered and dried at 103 ± 2 °C. Activated ashes were ground and passed through a 200-mesh sieve (Forozan Sepehr *et al.*, 2020).

The specific surface area of adsorbents was measured according to Kashani Motlagh *et al.* (2011) by the N_2-BET method with a Micromeritics Gemini 2360 instrument.

2.3. Soybean oil bleaching

100 ml of neutralized soybean oil and 1% (W/V) adsorbent (commercial acid-activated bleaching earth, coconut fiber ash, acid-activated coconut fiber ash, coconut shell ash, acid-activated coconut shell ash separately) were placed into a special three-neck flask equipped with a vacuum pump, thermometer and magnetic stirrer. Vacuum at 9 mmHg was applied and the temperature was increased to 110 °C and stirring was continued at medium speed to mix the oil and adsorbent for 30 minutes. Heating was discontinued after this period but vacuum and agitation were continued for a further period of 15 min. The temperature of the bleached oil was reduced to 50 °C. Bleached oils were filtered under vacuum by using Watman No. 41 filter paper to remove the adsorbent particles and obtain a clear oil. The treated oils were stored for further analysis in sealed vessels at 4 ºC after flushing with nitrogen (Abdi *et al.*, 2021).

2.4. Physical and chemical analyses

A series of physical and chemical tests were carried out on the neutralized and bleached oils according to the British Standard and AOCS Official Methods.

The carotenoid contents of the neutralized and bleached oil samples were determined by a UV-VIS spectrophotometer at 450 nm (PG Instruments T60U UV/VIS Spectrophotometer) according to BS 684, UK (BS, 1993).

The chlorophyll contents of the neutralized and bleached oil samples were determined according to the AOCS method (*Cc* 13d-55) by a UV-VIS spectrophotometer at 630, 670 and 710 nm (AOCS, 2017a).

A color measurement was carried out according to the AOCS standard method, Cc13e-92, by Lovibond Tintometer apparatus in 5.25-inch cell (AOCS, 2009).

The free fatty acid contents of the neutralized and bleached oil samples were determined by dissolving the oil in a diethyl ether-ethanol (1:1) solution followed by titration with a standard solution of 0.01 N potassium hydroxide in the presence of phenolphthalein according to the AOCS method, Cd 3d-63 (AOCS, 2017b).

The peroxide value determination of the neutralized and bleached oil samples was carried out according to the AOCS method, Cd 8–53 by dissolving the oil in an acetic acid-chloroform (3:2) solution, followed by titration with a 0.01 N sodium thiosulfate solution in the presence of potassium iodide and starch indicator (AOCS, 2003).

The determination of the *p*-anisidine value (*p*AV) of the neutralized and bleached oil samples was carried out following the ISO 6885:2006 method (ISO, 2006). A solution of the oil in iso-octane was reacted with p-anisidine in glacial acetic acid to form yellowish reaction products. Then absorbance was measured by a UV-VIS spectrophotometer at 350 nm (PG Instruments T60U UV/VIS Spectrophotometer).

The amounts of copper and iron in the neutralized and bleached oil samples were determined by Atomic Absorption Spectrometer according to the AOCS standard method, Ca 15–75. Oils dissolved in methyl isobutyl ketone were analyzed for metals by direct aspiration (AOCS, 2017c).

2.5. Statistical analysis

All the experiments and/or measurements were carried out in triplicate. The data were statistically analyzed using the Statistical Analysis System software package on replicated test data. Analyses of variance were performed by ANOVA. Significant differences among the means were determined using the Duncan multiple range test.

3. RESULTS AND DISCUSSION

Although carotenoids as the main pigments in most vegetable oils have antioxidant properties, they might act as pro-oxidant under elevated temperatures during oil processing (Zeb and Murkovic, 2011; Zeb and Murkovic, 2013). Therefore, the removal of these compounds from the oil might be desirable for their subsequent applications.

Table 1. Carotenoid and chlorophyll contents in soybean oils bleached with 1% of different adsorbents *

Treatments with	Carotenoids (mg β-Carotene/kg oil)	Chlorophyll (mg Pheophytin a/kg oil)
No treatment (Neutralized oil)	24.96 ± 0.05 ^{a}	2.43 ± 0.12 ^{a}
Commercial Bleaching Earth	6.50 ± 0.09 ^{e}	0.41 ± 0.01 ^{e}
Fiber Ash	11.56 ± 0.36 ^{b}	1.22 ± 0.00 ^{b}
Acid-Activated Fiber Ash	3.93 ± 0.08 ^{f}	0.43 ± 0.05 ^{de}
Shell Ash	10.85 ± 0.28 ^{c}	0.67 ± 0.05 ^{c}
Acid-Activated Shell Ash	7.08 ± 0.07 ^{d}	0.46 ± 0.02 ^{d}

* The values are expressed as means ± standard deviation, n=3. Different letters in each column indicate significant differences ($P < 0.05$) using Duncan's multiple range test.

Table 2. Lovibond color in soybean oils bleached with 1% of different adsorbents*

Treatments with	Red units	Yellow units
No treatment (Neutralized oil)	3.2 ± 0.20 ^{a}	60.0 ± 0.12 ^{a}
Commercial Bleaching Earth	2.4 ± 0.10 ^{e}	10.0 ± 0.20 ^{b}
Fiber Ash	2.5 ± 0.07 ^{de}	8.0 ± 0.12 ^{cd}
Acid Activated Fiber Ash	2.9 ± 0.12 ^{bc}	8.5 ± 0.07 ^{c}
Shell Ash	3.0 ± 0.07 ^{ab}	7.0 ± 0.05 ^{d}
Acid Activated Shell Ash	2.7 ± 0.05 ^{cd}	5.0 ± 0.05 ^{e}

*The values are expressed as means ± standard deviation, n=3. Different letters in each column indicate significant differences ($P < 0.05$) using Duncan's multiple range test.

Table 1 indicates the amounts of carotenoids in soybean oils bleached with different adsorbents. Although the amounts of carotenoids were significantly decreased in all bleached treatments, the highest reduction was obtained by using acid-activated treatments. The percentages of carotenoid reduction in treatments obtained from acid-activated fiber ash and acid-activated shell ash were 84.25 and 71.63%, respectively. Therefore, fiber ash had a better efficiency in reducing carotenoid pigments compared to shell ash. The removal of carotenoids is due to the reaction between these pigments and the active sites of the adsorbents (Jummao *et al.*, 2008). The textural characteristics and surface chemistry of adsorbents which can be modified by acid activation play important roles in their functionality (Hussin *et al.*, 2011). Hambly *et al.* (2021) stated that acid activation attacks and partly dissolves the layered mineral structure, leaving a partially amorphous material with a higher surface area and porosity. The specific surface area of coconut fiber and shell ashes were obtained at 78 and 92 m^2/g, respectively, which were increased by acid activation and reached up to 156 and 143 m^2/g for acid-activated fiber and shell ashes, respectively. Therefore, structural modification of adsorbents is an effective factor in their better efficiency.

One objective of oil bleaching is to remove the green pigments, chlorophylls, due to their decomposition into pheophytins by heat treatment (Zheng *et al.*, 2017) and acting as photooxidation sensitizers which enhance the oxidation reaction (Diosady, 2005), which results an opaque and dark oil with undesirable flavors.

The Removal of chlorophyll as green pigments present in crude soybean oil is considered more difficult than carotenoid pigments (Subramanian, 2001; El-Hamidi and Zaher, 2016) and according to the results shown in Table 1, the amount of chlorophyll in all the bleached oil samples was decreased significantly ($p < 0.05$). Most of the chlorophyll reduction (82.31 %) was obtained by using acid-activated fiber ash and there were no significant differences with commercial bleaching earth. Breaking the tetrapyrrole ring in the chlorophyll structure might be one of the reasons for decreasing chlorophyll content during oil bleaching (Jung *et al.* 1989). Due to the application of acid-activated bleaching agents and materials, the Mg in the center of chlorophyll might be removed and might affect the color intensity.

Bleaching efficiency might be monitored by measuring the reduction in color bodies according to the Lovibond method. The greatest reductions in red

Table 3. Amounts of peroxide value, *ρ*-anisidine value, free fatty acids, iron and copper in soybean oils bleached with 1% of different adsorbents*

Treatments with	Peroxide value (meq/kg oil)	*ρ*-Anisidine value	Free fatty acids (g/100g)	Iron (mg/kg)	Copper (mg/kg)
No treatment (Neutralized oil)	4.55 ± 0.25^{a}	2.79 ± 0.13^{c}	0.44 ± 0.04^{d}	0.74 ± 0.12^{c}	0.17 ± 0.01^{ab}
Commercial Bleaching Earth	2.74 ± 0.35^{c}	2.92 ± 0.04^{ab}	0.56 ± 0.12^{c}	0.63 ± 0.05^{d}	0.15 ± 0.00^{c}
Fiber Ash	1.40 ± 0.05^{d}	3.04 ± 0.02^{a}	0.22 ± 0.01^{f}	0.92 ± 0.11^{a}	0.18 ± 0.05^{a}
Acid-Activated Fiber Ash	2.60 ± 0.12^{c}	2.98 ± 0.21^{ab}	0.67 ± 0.00^{b}	0.41 ± 0.07^{f}	0.16 ± 0.05^{bc}
Shell Ash	1.26 ± 0.09^{d}	2.90 ± 0.03^{ab}	0.33 ± 0.11^{e}	0.86 ± 0.05^{b}	0.18 ± 0.01^{a}
Acid-Activated Shell Ash	3.40 ± 0.07^{b}	2.97 ± 0.17^{ab}	0.78 ± 0.00^{a}	0.48 ± 0.00^{e}	0.13 ± 0.00^{d}

* The values are expressed as means ± standard deviation, n=3. Different letters in each column indicate significant differences ($P < 0.05$) using Duncan's multiple range test.

(21.87 %) and yellow (91.67 %) Lovibond units were w achieved using fiber ash and acid-activated shell ash, respectively (Table 2). All coconut-based adsorbents were significantly more effective than commercial bleaching earth in reducing the yellow color ($p < 0.05$).

Reduction in oil color might be attributed to the absorption and catalytic properties of the bleaching earths. Pigments present in oil, solution or colloidal particles, are adsorbed onto the bleaching earth surface by a weak Van der Waals intermolecular force. Carotene and other pigments might also be affected by the catalytic properties of acid-activated earths. The structural changes in the pigments or the breakdown of their molecular bonds, resulting in the lightening of the oil color, might have occurred due to the presence of hydrogen ions (Mustapha *et al*., 2013).

The free fatty acid content in neutralized oil which was originally 0.44 g/100g was considerably reduced (50% and 25%) during bleaching with fiber ash and shell ash and reached 0.22 and 0.33 g/100g, respectively. Bleaching with acid-activated adsorbents increased the acid value so that the free fatty acid contents of oils bleached with acid-activated fiber ash and acid-activated shell ash reached 0.67 and 0.78 g/100g, respectively. The application of commercial acid-activated earths produced similar results. Different studies have indicated that oil bleaching with acidic adsorbents slightly increases the acid value of oils, which is due to triglyceride hydrolysis and subsequent production of free fatty acids (Boki *et al*., 1992; Usman *et al*., 2012). Considering the effective role of the acidic adsorbents in providing the main purposes of bleaching, slight increases in free fatty acid contents during oil bleaching is not noticeable because these components could be removed simply from the oil during the deodorization stage.

Silva *et al.* (2014) indicated that acid-activated bleaching earths might act as an oxidizer. Therefore, peroxide and *p*-anisidine values were determined as primary and secondary oxidation products, respectively. Table 3 presents the peroxide and *p*-anisidine values for neutralized and bleached oils after treatment by different adsorbents. The peroxide value of neutralized soybean oil was determined as 4.55 meq O_2/kg oil. Bleaching by different adsorbents decreased the peroxide value by 25.27 – 72.31%. The lowest peroxide value (1.26 meq O_2/kg oil) was obtained for oil bleached with shell ash, which indicates better function compared to commercial bleaching earth. According to the peroxide value results, a slight increase in secondary oxidation products occurred due to decomposition of primary oxidation products. There are significant differences between the *p*-anisidine value of the neutralized and bleached oils with different adsorbents ($p < 0.05$).

Usman *et al.* (2012) indicated that peroxides are decomposed into aldehydes and ketones due to further oxidation which might be catalyzed by the acidic nature of adsorbents. These decomposition products are also adsorbed to the bleaching agent (Subramanian *et al.,* 2001; Mirrezaie Roodaki *et al.,* 2016).

According to the results, the amount of iron was increased in bleached oils treated with fiber ash and shell ash, which might be due to the nature of the adsorbents used which contain different metals. Although iron content was significantly decreased in all samples treated with acidic adsorbents, the greatest reduction (44.59 %) was obtained in the oil bleached with acid-activated fiber ash, which might be due to the dealumination of the structure, resulting in organic acid activation.

Usman *et al*. (2012) stated that a number of metal ions in the octahedral layer, such as aluminum, magnesium, and iron cations and impurities such as calcite are also removed by leaching with a mineral acid at elevated temperatures.

The results for changes in the amount of copper in the treated samples are similar to the results obtained for the iron content so that copper content was decreased in the oil treated with acidic adsorbents. However, these changes are not statistically significant.

According to the results, these adsorbents might be recommended for post-bleaching processes carried out after oil hydrogenation with the aim of removing nickel from hydrogenated oil.

4. CONCLUSIONS

Coconut fiber and shell are agricultural wastes and their application in the food industry has received limited attention. Therefore, the efficiency of these compounds for the bleaching of edible oil has been investigated in this research. The results showed that these adsorbents were quite effective in achieving the main goals of the oil bleaching process, reducing oil color and carotenoid and chlorophyll contents. In this respect, acidic treatments with fiber and shell have shown to be more effective, which might be due to the structural modification of adsorbents by acid activation.

Regarding the effect of these compounds on the oil quality factors, the peroxide value and the amounts of iron and copper as prooxidant compounds decreased in all treatments. Although the content in free fatty acids increased in adsorbents with acidic nature, they could be removed from the oil during the deodorization process. Due to the competitiveness of acid-activated coconut fiber and shell with commercial bleaching earth, the use of these low-cost biosorbents can be considered for oil bleaching. Also, based on the effectiveness of the acid-activated fibers compared to acid-activated shells, acid-activated coconut fibers are recommended as adsorbent in the oil industry.

REFERENCES

Abdi E, Gharachorloo M, Ghavami M. 2021. Investigation of using egg shell powder for bleaching of soybean oil. *LWT-Food Sci. Technol*. **140**, 110859. https://doi.org/10.1016/j.lwt.2021.110859

Almasi A, Mousavi SA, Hesari A, Janjani H. 2016. Walnut shell as a natural adsorbent for the removal of Reactive Red 2 form aqueous solution. *Intl. Res. J. Appl. Basic. Sci.* **10**, 551–556. http://webcache.googleusercontent.com/search?q=cache:7R5aUEJ1YxsJ:research.kums.ac.ir/attachmentsjson/download.action%3FmasterCode%3D20295+&cd=1&hl=en&ct=clnk&gl=ir

AOCS. 2003. Declared surplus peroxide value-acetic acid-chloroform method.

AOCS. 2009. Official Method Cc 13e-92. Color Fats Lovibond.

AOCS. 2017a. Official Method Cc 13d-55. Chlorophyll pigments in Refined and Bleached Oils.

AOCS. 2017b. Official Method C 3d-63. Acid value fats and oils.

AOCS. 2017c. Official Method Cd 15-75. Analyses for chromium, copper, iron, and nickel in vegetable oils by atomic absorption spectrophotometry.

Aarti Sowmya T, Gayavajitha E, Kanimozhi R, Subalakshmi R. 2018. Removal of toxic metals from industrial wastewater using Groundnut shell. *Int. J. Pure Appl. Math*. **119**, 629–634. https://www.researchgate.net/publication/326253312_Removal_of_toxic_metals_from_industrial_waste_water_using_groundnut_shell

Boki K, Kubo M, Wada T, Tamura T. 1992. Bleaching of alkali-refined vegetable oils with clay minerals. *J. Am. Oil Chem. Soc.* **69**, 232–236. https://doi.org/10.1007/BF02635892

BS 684. 1993. Fats and fatty oils- Determination of carotene in vegetable oils.

Diosady LL. 2005. Chlorophyll Removal from Edible oils. *Int. J. Appl. Sci. Eng*. **3**, 81–88. https://doi.org/10.6703/IJASE.2005.3(2).81

El-Hamidi M, Zaher FA. 2016. Comparison Between Some Common Clays as Adsorbents of Carotenoids, Chlorophyll and Phenolic Compounds from Vegetable Oils. *Am. J. Food Technol.* **11**, 92-99. http://doi.org/10.3923/ajft.2016.92.99

Emojevwe V. 2013. Cocos nucifera (Coconut) Fruit: A review of its medical properties. *Adv. Agr. Sci. Eng. Res.* **3**, 718–723. https://doi.org/10.13140/RG.2.2.21506.53446

Erten Y. 2004. Use of domestic minerals for vegetable oil blanching. Master ASTER of Science Food Engineering. İzmir Institute of Technology izmir, Turkey.

Forozan Sepehr Z, Gharachorloo M, Yousefi S. 2020. Investigation of soybean oil bleaching by using walnut shell. *J. Nuts*, **11**, 169–177. https://doi.org/10.22034/jon.2020.1889666.1076

Hambly AJ, van Duijneveldt JS, Gates PJ. 2021. Identification of β-carotene oxidation products produced by bleaching clay using UPLC-ESI-MS/MS. *Food Chem.* **353**, 129455. https://doi.org/10.1016/j.foodchem.2021.129455

Hussin F, Aroua MK, Wan Daud WMA. 2011. Textural characteristics, surface chemistry and activation of bleaching earth: A review. *Chem. Eng. J.* **170**, 90–106. https://doi.org/10.1016/j.cej.2011.03.065

ISO 6885. 2006. Animal and vegetable fats and oils – determination of anisidine value, ISO method 6885:2006, geneva, Switzerland.

Ikumapayi OM, Akinlabi ET, Majumdar JD, Akinlabi SA. 2020. Applications of coconut shell ash/particles in modern manufacturing: a case study of friction stir processing. *Mod. Manuf. Process.* 69–95. https://doi.org/10.1016/B978-0-12-819496-6.00004-X

Johari K, Saman N, Song ST, Heng JYY, Mat H. 2014. Study of Hg(II) removal from aqueous solution using lignocellulosic coconut fiber biosorbents: Equilibrium andkinetic evaluation. *Chem. Eng. Commun*. **201**, 1198–1220. https://doi.org/10.1080/00986445.2013.806311

Jung MY, Yoon SH, Min DB. 1989. Effects of processing steps on the contents of minor compounds and oxidation of soybean oil. *J. Am. Oil Chem. Soc.* **66**, 118–120. https://doi.org/10.1007/BF02661798

Junmao T, Zhansheng W, Xifang S, Xiaolin X, Chun L. 2008. Adsorption Kinetics of β- carotene and Chlorophyll an Acid- activation Bentonite in Model oil. *Chin. J. Chem. Eng*. **16** (2), 270–276.

Kashani Motlagh MM, Youzbashi AA, Amiri Rigi Z. 2011. Effect of acid activation on structural and bleaching properties of a bentonite. *Iran. J. Mater. Sci. Eng*. **8**, 50–56. http://ijmse.iust.ac.ir/files/site1/user_files_4qu804/ijmse-A-10-3-159-b328326.pdf

Martins AP, Sanches RA. 2019. Assessment of coconut fibers for textile applications. *Revista Matéria.* **24**. https://doi.org/10.1590/S1517-707620190003.0743

Mirrezaie Roodaki MS, Sahari MA, Ghiassi Tarzi B, Barzegar M, Gharachorloo M. 2016. Effect of refining and thermal processes on olive oil properties. *J. Agric. Sci. Technol.* **18**, 629–641. http://jast.modares.ac.ir/article-23-1727-en.html

Mustapha SI, Mohammed AA, Zakari AY, Mohammed HA. 2013. Performance evaluation of local clays from northern Nigeria for the refining of palm oil. *J. Chem. Eng. Mater. Sci.* **4**, 58–66. https://doi.org/10.5897/JCEMS2013.0152

Pauline DP. 2000. Dictionary of plants used in Cambodia. Imprimerie Olympic. pp. 165–166.

Shruthi KM, Pavithra MP. 2018. A study on utilization of groundnut shell as biosorbent for heavy metals removal. *Int. J. Eng. Tech*. **4,** 411–415. https://www.researchgate.net/publication/342260653

Silva SM, Sampaio KA, Ceriani R, Verhé R, Stevens C, Greyt WD, Meirelles AJA. 2014. Effect of type of bleaching earth on thefinal color of refined palm oil. *LWT - Food Sci. Technol.* **59**, 1258–1264. https://doi.org/10.1016/j.lwt.2014.05.028

Subramanian R, Nabetani H, Nakajima M, Ichikawa S, Kimura T, Maekawa T. 2001. Rejection of Carotenoids in Oil Systems by a Nonporous Polymeric Composite Membrane. *J. Am. Oil Chem. Soc.* **78**, 803–807. https://doi.org/10.1007/s11746-001-0346-4

Taksitta K, Sujarit P, Ratanawimarnwong N, Donpudsa S, Songsrirote K. 2020. Development of tannin-immobilized cellulose fiber extracted from coconut husk and the application as a biosorbent to remove heavy metal ions. *Environ. Nanotechnol. Monit. Manag*. **14**, 100389. https://doi.org/10.1016/j.enmm.2020.100389

Usman MA, Ekwueme VI, Alaje TO, Mohammed AO. 2012. Characterization, acid activation, and bleaching performance of Ibeshe clay, Lagos, Nigeria. *ISRN Ceramics*, 1–5. https://doi.org/10.5402/2012/658508

Yuan J, Zhu Y, Wang J, Gan L, He M, Zhang T, Li P, Qiu F. 2021. Preparation and application of Mg–Al composite oxide/coconut shell carbon fiber for effective removal of phosphorus from domestic sewage. *Food Bioprod. Process.* **126**, 293–304. https://doi.org/10.1016/j.fbp.2021.01.004

Zeb A, Murkovic M. 2011. Carotenoids and triacylglycerols interactions during thermal oxidation of refined olive oil. *Food Chem*. **127**, 1584–1593. https://doi.org/10.1016/j.foodchem.2011.02.022

Zeb A, Murkovic M. 2013. Determination of thermal oxidation and oxidation products of β-carotene in corn oil triacylglycerols. *Food Res. Int.* **50,** 534–544. https://doi.org/10.1016/j.foodres.2011.02.039

Zheng R, Gao H, Ren Z, Cen D, Chen Z. 2017. Preparation of activated bentonite and its adsorption behaviour on oil-soluble green pigment. *Physicochem. Probl. Miner. Process*. **53**, 829−845. http://doi.org/10.5277/ppmp170213

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Influence of different irrigation regimes and planting times on the quality and quantity of calyx, seed oil content and water use efficiency of roselle (*Hibiscus sabdariffa* L.)

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ABSTRACT: This study was carried out to examine the physiological traits, quality of calyx extract and seed oil content of roselle (Hibiscus sabdariffa L.) as affected by irrigation regimes and planting dates. The growth period from seed sowing to calyx harvesting was shortened as planting time was delayed. Stem diameter and plant height were decreased by drought stress or late planting, but calyx yield, total phenolic content, total anthocyanin content, vitamin C, and calyx water use efficiency increased under mild drought condition. In addition, antioxidant activity and calyx water use efficiency were significantly increased by late planting. It was suggested that an increase in calyx harvest index in delay in planting would be due to better photosynthesis activity and higher assimilate use efficiency because of the increase in sink capacity. Seed oil content decreased considerably due to drought stress and delay in planting date. These findings suggest that mild drought stress improves the quality and quantity of calyx and water use efficiency.

Keywords**:** *Antioxidant activity; Harvest index; Phenolic compounds; Sowing date*

RESUMEN: ***Influencia de diferentes regímenes de riego y tiempo de siembra en la calidad y cantidad del cáliz, el contenido de aceite de las semillas y la eficiencia del uso del agua de la roselle (*****Hibiscus sabdariffa** ***L.).*** Este estudio se llevó a cabo para examinar los rasgos fisiológicos, la calidad del extracto de cáliz y el contenido de aceite de semillas de roselle (*Hibiscus sabdariffa* L.) afectado por regímenes de riego y fecha de siembra. El período de crecimiento desde la siembra de la semilla hasta la cosecha del cáliz se acortó debido a que se retrasó el tiempo de siembra. El diámetro del tallo y la altura de la planta disminuyeron por el estrés por sequía o la siembra tardía, pero el rendimiento del cáliz, el contenido fenólico total, el contenido total de antocianinas, la vitamina C y la eficiencia del uso del agua del cáliz aumentaron en condiciones de sequía leve. Además, la actividad antioxidante y la eficiencia del uso del agua del cáliz aumentaron significativamente en la siembra tardía. Se sugirió que el aumento del índice de cosecha del cáliz en la demora en la siembra se debería a una mejor actividad de fotosíntesis y una mayor eficiencia en el uso de asimilación debido al aumento de la capacidad de hundimiento. El contenido de aceite de las semillas disminuyó considerablemente debido al estrés por sequía y al retraso en la fecha de siembra. Estos hallazgos sugieren que el estrés por sequía leve mejora la calidad y cantidad del cáliz y la eficiencia del uso del agua.

PALABRAS CLAVE: *Actividad antioxidante; Compuestos fenólicos; Fecha de siembra; Índice de cosecha*

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1. INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) is an annual medicinal and edible plant belonging to the Malvaceae family and cultivated in South-East Asia as well as other arid and semi-arid regions (Gendy *et al.,* 2012) with a temperature of about 25 to 35 °C. The different useful parts of this plant, including stem fiber, leaves, seeds and flowers have been used as vegetables, oil and drinks. Roselle is generally planted for its red calyx which contains vitamin C, anthocyanin and other phenolic compounds (Da-Costa-Rocha *et al.,* 2014). Many environmental agronomic factors influence the growth, yield and quality of roselle plants (Xu *et al.,* 2019).

Drought stress is the most important obstacle in achieving a high crop yield throughout the world. Furthermore, saving irrigation water and improving crop yields are two related and important global issues. Proper planting date and practices of irrigation management are some effective techniques for maximizing the use of limited water resources (Keshavarz Mirzamohammadi *et al.,* 2021). It has reported that increasing intervals between irrigation and drought condition resulted in a significant decrease in yield and yield components but increase in active constituents of calyx such as vitamin C and total phenols in the roselle plant (Hewidy *et al.,* 2018). However, another researcher reported an increase in flowering and dry yield (Begum *et al.,* 2015). In addition, Seghatoleslami *et al.* (2013) determined that drought stress had no significant effect on the height of the roselle plant, number of branches or calyx yield. In another study, mild drought stress following an increase in irrigation intervals led to an increment in anthocyanin due to carbohydrate accumulation which reshapes into secondary metabolites (El-Boraie *et al.,* 2009). Fallahi *et al.* (2017a) found that drought conditions in light soil did not affect stem diameter or the plant height of roselle but instead calyx yield and number of flowers increased under mild drought stress. Khalil and Yousef (2014) defined that drought stress led to a reduction in biological yield, calyx yield and quality of roselle.

Maximum production potential due to agronomic practices such as time of sowing have been related to high yield. An appropriate sowing date promotes plant growth and development, resulting in higher biological yield and better economic use of the land. The optimum sowing date ensures that the susceptible growth stage of the plant does not coincide with harmful environmental conditions. The roselle is short day plant with a critical photoperiod of 12-12.5 hours for flowering. Long days at the wrong developmental stage lead to losses in yield (Ghayour *et al.,* 2020). Morphological traits and yield components like stem diameter, plant hight and branch numbers are influenced by planting due to a longer growth period from the first planting date (Asadpour *et al.,* 2020). The results of Seghatoleslami *et al.* (2013) for roselle (*Hibiscus sabdariffa* L.) highlighted a significant correlation between plant dry matter and sowing date and the wrong sowing date brought about loss on economic yield by affecting yield components. Asadpour *et al.* (2020) showed that a delayed sowing date decreased the grain yield of maize (*Zea mays* L.). In a study on the effect of five sowing dates for rice, Basyouni Abou-Khalifa (2010) concluded that the greatest grain yield was achieved with the sowing dates of May 10 and April 30 (stage of milky and maturity, respectively). It was reported that a delay in sowing date from mid-May to mid-July led to a 60% flower yield loss and 58% reduction in the yield of calyx (Ghayour *et al.,* 2020). Parsa Motlagh *et al.* (2018) stated that a delay in sowing from 30th March to 22nd May resulted in lower plant height, harvest index and calyx yield per unit. Also, Alizade Moradi *et al.* (2018) showed that a delay in sowing time led to losses in flower yield and biological yield but increased the water use efficiency of the calyx and flower.

A study on the effect of irrigation and planting date on the quality of the calyx and water use efficiency is still rare. Changing irrigation schedule, water deficit technique and planting date have been widely used in to improve the harvest index and water use efficiency of plants. Given the importance of determining desirable irrigation regimes and sowing dates for the cultivation of a newly-introduced plant to this region, the present study aimed to examine the influence of different irrigation regimes and panting dates on yield quantity and quality and water use efficiency of roselle (*Hibiscus sabdariffa* L.), and to determine the exact quantity of irrigation water to be applied on roselle plant grown.

2. MATERIALS AND METHODS

2.1. Site study

This study was carried out at the farm station of Azad Islamic University, Karaj, Iran to study the effect of water deficit stress and sowing date on the quantitative and qualitative yield of Roselle (*Hibiscus sabdariffa* L.) during the two consecutive growing seasons of 2017 and 2018. This experiment included nine treatments which were included interactions among three irrigation regimes (well-watered, mild and drought stress) and three planting dates (4th June, 18th June and 2nd July). The climate in this province is semi-dry with an annual rain fall of about 250 mm which mostly occurs in autumn and winter between November and April. Average minimum and maximum temperatures and precipitation in both years of the present study are shown in Figure 1. The soil type of the field was sandy-loam and low in organic matter (0.28%) with total N (0.031%) as measured by the Kjeldahl method (Beljkas *et al.,* 2010), available P (35 mg kg^{-1}), using the Olsen procedure (Olsen and Sommers, (1982) and available K (225 mg kg^{-1}) after extraction with ammonium acetate, with pH and EC of 6.96 and 0.82 dS m^{-1}, respectively.

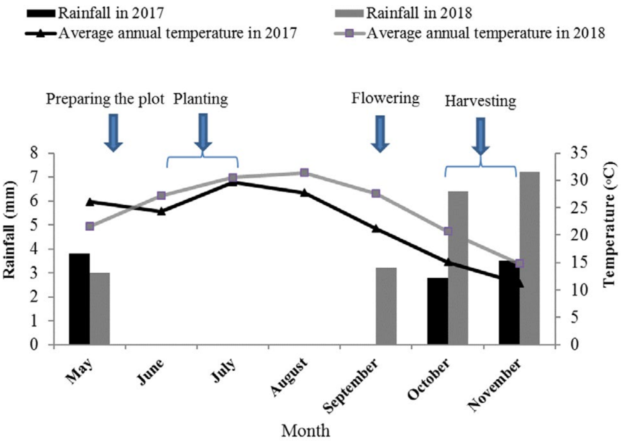

FIGURE 1. The average temperature and the total rainfall in the months of the growing season in 2017 and 2018.

2.2. Experiment design

The nine treatments were employed in split plot with three replicates in a randomized complete block design (RCBD). Irrigation regimes were distributed among the main plots and included three irrigation levels (30, 45 and 60% depletion of available water, labelled control, mild and drought stress, respectively) (Keshavarz Mirzamohammadi *et al.,* 2021). Planting date treatments were arranged in subplots. Roselle (*Hibiscus sabdariffa* L.) seeds were directly sown in prepared plots (divided into four rows at 60 cm apart and 5 cm between the plants on each row) on 4th June, 18th June and 2nd July in both years. After germination, the seedlings were thinned to reach optimum density. The seedlings were irrigated when needed until they completely developed. Soil moisture content in each plot was monitored daily using a time domain Reflectometry (TDR). Irrigation water needed prior to irrigation (V_w in m^3) was estimated based on the soil water content (θ_i) (by TDR) and effective rooting depth (*D*, 0.3m here) according to the following equation:

$$V_w\text{: } (\theta_{F.C} - \theta_i) \times D \times A$$

Where, $\theta_{F.C}$ is the volume of soil moisture at field capacity and *A* is the main plot area (m^2). Data on volumetric water content were collected daily prior to setting the experiment calibrates before seed sowing and during the growing season to calculate the time of irrigation. The amount of water irrigation applied was according to the soil deficit to bring it back to field capacity. All plots were fertilized uniformly, during soil preparation in the spring of each year.

2.3. Data collection

The leaves and calyxes were harvested within four weeks after flowering. The fresh calyxes of each plot were washed with water, air-dried at room temperature, and weighed. The seeds were removed manually, air dried at room temperature and the whole calyces were used for the analysis of chemical properties. The extraction of dried calyxes with water at a ratio of 1:10 was conducted in a water bath (50 °C, 30 min) (Chumsri *et al.,* 2008). The total phenolic and anthocyanin contents of calyx extract were assessed according to the Folin-Ciocalteu reagent method (Temraz and El-Tantawy, 2008) and Krizek *et al.* (1993), respectively. Vitamin C was measured spectrophotometrically (Hitachi, U-1800, Tokyo, Japan) by measuring Fe^{2+} complexes with 2, 2-dipyridyl (Sarker and Oba, 2018) and reading the

Table 1. Analysis of variance (mean squares) of physiological traits, yield, water use efficiency and harvest index of roselle (*Hibiscus sabdariffa* L.) in irrigation and sowing date treatments

Source of variation	d.f	Plant height	Stem diameter	Biological yield	Calyx yield	Total phenolic content	Total anthocyanin content	Vitamin C	Antioxidant activity	Seed oil content	Calyx water use efficiency	Calyx harvest index
Replication	2	818.92^{ns}	0.02 ^{ns}	0.0034 ^{ns}	0.05 *	0.01 ^{ns}	15.59 ^{ns}	37.3 ^{ns}	5.25 ^{ns}	1.072 ns	0.10 *	2.80 *
Irrigation	2	4637.37**	1.52 **	34.42 **	0.28 *	1.53 *	206.92 **	36580 **	1294.4 **	81.35 **	0.247 **	2.41 *
Main error	4	144.81	0.054	0.039	0.0043	0.17	4.92	299.9	2.48	1.75	0.010	299.3
Sowing date	2	831.59 **	0.071 ^{ns}	2.58 **	0.13 *	0.022 ^{ns}	1.03 ^{ns}	1393 ^{ns}	140.48 *	3.40 *	0.24 *	1393.7 ^{ns}
Irrigation × sowing date	4	51.81 ^{ns}	0.068 ^{ns}	0.19 ^{ns}	0.023 ^{ns}	0.012 ^{ns}	18.37 ^{ns}	412.27 ^{ns}	49.45 ^{ns}	0.75 ^{ns}	0.040 ^{ns}	412.2 ^{ns}
Error	12	107.57	0.049	0.13	0.051	23.9	10.03	377.27	23.96	0.85	0.043	0.72
CV%		6.51	9.048	2.71	9.6	9.6	9.46	5.78	6.43	6.86	10.51	8.56

^{ns} – not significant, *: significant at $P \leq 0.05$ and **: significant at $P \leq 0.01$

absorbance of the sample solution at 525 nm. Antioxidant activity was measured according to Sarker and Oba (2018) by means of the diphenyl-picrylhydrazyl (DPPH) radical degradation method.

Harvesting was done at the physiological maturity stage on the first of October until the first of November (in both years) by harvesting the four middle rows. The traits examined included plant height, stem diameter, biological yield and calyx yield. Biological yield was determined after oven drying at 75 °C for 72 hours to constant weight. Then the dried biological part of plant was weighed by a digital 0.01-precision scale. Stem diameters and plant height were measured manually from the ground level with a caliper and a barcoded height stick (1 mm resolution), respectively. Therefore, they were considered to be precise and accurate. For this purpose, five individual plants from each plot were measured, and the averaged stem diameter and plant height were used as the plot-level. Seed oil content was measured by Inframatic 8620 Percor, England. Calyx water use efficiency (WUE_{calyx}) and calyx harvest index (HI_{calyx}) were calculated by dividing the calyx yields by the volume of applied water and biological yield, respectively.

2.4. Statistical analysis

Analyses of variance (ANOVA) were performed using the SAS software (SAS Institute Inc. ver. 9.2). Two years' data were analyzed by combined years because the Bartlett test was not significant for all traits measured. Differences among mean values for the roselle plant responses to the level of irrigation regimes and planting date were analyzed with the least significant difference (LSD) test at a significance of $\alpha \leq 0.05$. Principal Component Analysis (PCA) based on biplot (SAS 9.1) and coefficient of correlation were applied to consider the visualization of similarities or differences and interrelationships by acute and obtuse angles among all parameters. Clustering analysis (S-PLUS ver. 6.1 software, Insightful Corporation, USA) aimed at classifying objects based on the minimum variance linking method and similarity of input data for the existing parameters using Ward's hierarchical approach and Euclidean distance to organize data into groups.

3. RESULTS AND DISCUSSION

The results showed that the different irrigation regimes differed among the studied traits (Table 1). Sowing date treatments differed significantly in terms of plant height, biological yield, calyx yield, antioxidant activity, seed oil content and calyx water use efficiency. However, the two-way interactions of irrigation regimes × sowing date treatments was not significant in any traits (Table 1). The results of the cluster analysis showed that all the treatments were divided into three separate groups, so that the interaction of severe drought + 4th June, severe drought + 18th June, and severe drought + 2nd July (T_7, T_8 and T_9, respectively) treatments were placed into one group and well-watered + 4th June, well-watered

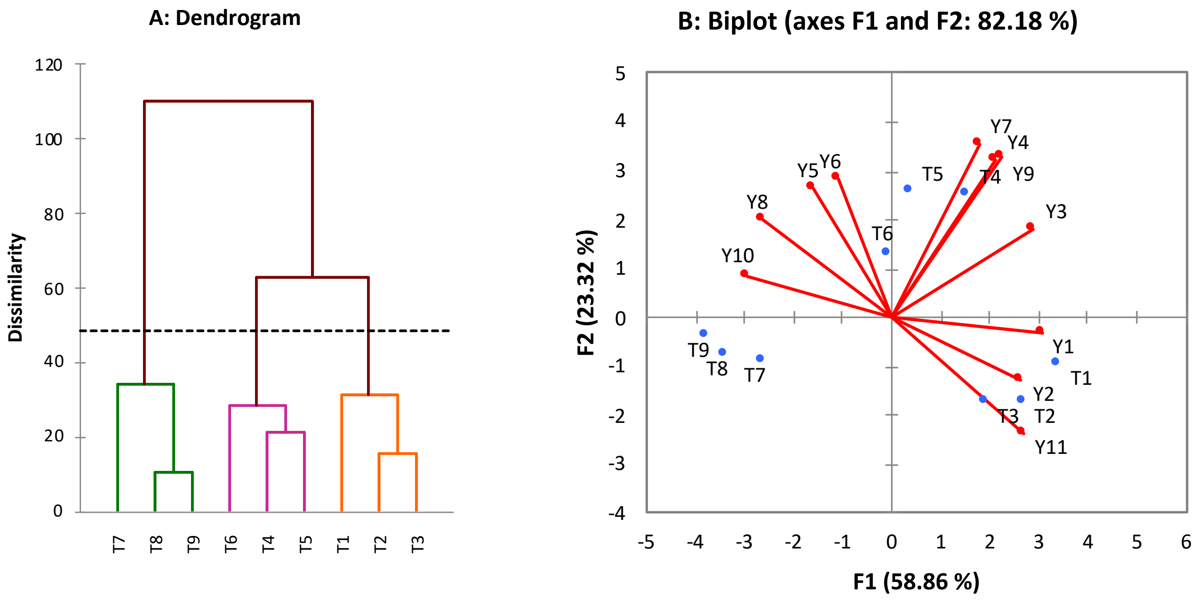

Figure 2. The results of dendrogram based on cluster analysis (A) and (B) biplot of first and second components based on principal component analysis. T1: well-watered+4th June, T2: well-watered+18th June, T3: well-watered+2nd July, T4: mild drought+4th June, T5: mild drought+18th June, T6: mild drought+2nd July, T7: severe drought+4th June, T8: severe drought+18th June, T9: severe drought+2nd July. Y1: plant height, Y2: stem diameter, Y3: biological yield, Y4: calyx yield, Y5: calyx water use efficiency, Y6: calyx harvest index, Y7: total phenolic content, Y8: total anthocyanins content, Y9: vitamin c, Y10: antioxidant activity, Y11: seed oil content.

+ 18th June and well-watered + 2nd July (T_1, T_2 and T_3, respectively) treatments were placed in the other group. In addition, based on the results of the cluster analysis, it was found that mild drought + 4th June, mild drought + 18th June and mild drought + 2nd July (T_4, T_5 and T_6) treatments were more closely related to all studied traits and were placed in another group (Figure 2). The results of the principal component analysis showed that the first and second components presented the highest relative variance with 58 and 23%, respectively, and accounted for a total of 82% of the total variance. Based on the biplot obtained from the first and second components, it was observed that the TPC, calyx yield, vitamin c and biological yield had the highest correlation with T5 and T4 treatments. Also, T1, T2 and T3 treatments showed a strong relationship with plant height, stem diameter and seed oil content (Figure 2).

3.1. Plant height and stem diameter

The data related to plant height (Tables 2 and 3), the well-watered irrigation (181.2 cm) and first sowing date (4th June) (168.7 cm) showed maximum value for plant height. The well-watered plants measured significantly higher stem diameters (2.85 cm) than the mild and severe drought stress by 14.03 and 28.7%, respectively. Our results are in agreement with those found by Karami *et al.* (2016) for soybean cultivars [*Glycine max* (L.) Merr.], which indicated that drought stress reduced plant growth, plant height and stem diameter due to disturbance in photosynthesis and low carbohydrate production. In addition, prolonged growth periods allowed the crops to use growth resources like light, water and nutrients which finally increased the growth of the crops. Similar findings with respect to drought stress and sowing date have been reported by Apeyuan *et al.* (2017) and Rah Khosravani *et al.* (2017) for roselle (*Hibiscus sabdariffa* L.) and maize (*Zea mays* L.) hybrids, respectively.

3.2. Biological yield

A decrease of 14.69 ton.ha^{-1} to 11.40 ton.ha^{-1} in biological yield of roselle was recorded due to decreasing the irrigation supply from control to severe drought stress. However, there were no significant difference between well-watered and mild drought stress treatments (Table 2). This may be related as stress conditions reduce the biological yield production because of limitations in the water and essential fertilizer uptake by the plants (Seghatoleslami *et al.,* 2013). Reductions in total dry matter of rice (*Oryza*

Table 2. Morphological and physiological traits of roselle (*Hibiscus sabdariffa* L.) from three different irrigation treatments.

Irrigation	**Plant height**	**Stem diameter**	**Biological yield**	**Calyx yield**	**Total phenolic content**	**Total anthocyanin content**	**Vitamin C**	**Antioxidant activity**	**Seed oil content**	**Calyx water use efficiency**	**Calyx harvest index**
	(cm)	(cm)	(ton.ha^{-1})	(ton.ha^{-1})	(mg.100 g^{-1} DW)	(mg.100 g^{-1} FW)	(mg.100 g^{-1} FW)	(%)	(%)	(kg.kg^{-1})	(%)
Well-watered	181.2±16. a	2.85±0.18 a	14.69±0.48 a	1.37±0.16 b	2.33±0.22 ab	28.11±3.14 b	342.1±23 b	63.71±5 c	16.88±1.2 a	1.79±0.17 b	9.32±0.84 b
Mild drought stress	160.6±16 b	2.45±0.25 b	14.88±0.69 a	1.52±0.23 a	2.77±0.25 a	35.00±3.24 a	395.7±19 a	77.04±7 b	12.21±1.1 b	2.11±0.24 a	10.19±1.25 a
Severe drought stress	135.8±11 c	2.03±0.25 c	11.40±0.50 b	1.16±0.09 c	1.94±0.25 b	37.33±3.24 a	268.7±19 c	87.64±3 a	11.27±0.7 b	2.02±0.29 a	10.24±0.72 a

DW – dry weight; FW – fresh weight. Means donated by similar letter(s) indicate no significantly ($p \leq 5\%$) by Least Significant Difference test. Values represent the mean of three replicates ± SD.

Table 3. Morphological and physiological traits of roselle (*Hibiscus sabdariffa* L.) from three different sowing date treatments

Sowing date	**Plant height**	**Biological yield**	**Calyx yield**	**Antioxidant activity**	**Seed oil content**	**Calyx water use efficiency**
	(cm)	(ton.ha^{-1})	(ton.ha^{-1})	(%)	(%)	(Kg.kg^{-1})
4th June	168.7±24.8 a	14.24±1.7 a	1.48±0.23 a	71.57±12.7 b	14.14±3 a	1.81±0.17 b
18th June	159.4±24.1 ab	13.55±1.8 b	1.33±0.24 b	78.62±12.1 a	13.27±2.5 ab	1.97±0.30 ab
2nd July	149.5±20.6 b	13.18±1.5 b	1.24±0.10 b	78.20±8.8 a	12.95±2.6 b	2.14±0.23 a

Means followed by similar letters indicate no significance ($p \leq 5\%$) by Least Significant Difference test. Values represent the mean of three replicates ± SD.

sativa L.) under drought conditions seemed to be attributed to the reduction in water availability, which reduced cell division, lowered LAI and plant height and finally resulted in lower dry matter and biological yield (Aghdasi *et al.,* 2018a). The authors stated that an increased allocation of photosynthates to the plant root relative to the shoots was another reason for the reduction in biological yield. Regarding sowing dates, a significant maximum biological yield (14.24 ton.ha^{-1}) was achieved when the plants were sown on 4th June with the minimum biological yield (13.18 ton.ha^{-1}) in the case of the crop sown on 2nd July. However, there were no significant difference between 18th June and 2nd July. These results are in accordance with those of Keshavarz and Khodabin (2019) and Aghdasi *et al.* (2018b). Higher biological yield with early sowing was mainly due to a greater number of effective branches per m^2, more plant height and greater stem diameter (some data not show). Another reason for this result is probably due to the fact that the first sowing dates resulted in longer growing periods, and therefore, greater total rainfall received during the growing periods. An adequate irrigation supply would eventually yield the higher growth rate. These results are in line with Rah Khosravani *et al.* (2017). There was a significant positive correlation between calyx yield, total phenolic content, vitamin C and seed oil yield with biological yield. However, biological yield had a negative correlation with total anthocyanin content and antioxidant activity (Table 4). In a similar study on roselle, Fallahi *et al.* (2017b) concluded that biological yield had a positive effect on the number of capsules per plant and plant height which may be attributed to genetic effects rather than environmental ones and these traits may be good selections for improving seed and calyx yield in roselle.

3.3. Calyx yield

An increase from 1.37 ton.ha^{-1} to 1.52 ton.ha^{-1} in calyx yield was recorded for roselle with an increase

Table 4. Coefficient of correlation matrix based on all studied traits

	1	2	3	4	5	6	7	8	9	10	11
1- Plant height	1										
2-Stem diameter	0.59 **	1									
3-Biological yield	0.73 **	0.63 **	1								
4-Calyx yield	0.35 ns	0.16 ns	0.75 **	1							
5-Total phenolic content	0.35 ns	0.36 ns	0.64 **	0.42 *	1						
6-Total anthocyanin content	-0.76 **	-0.57 **	-0.50 **	-0.082 ns	-0.14 ns	1					
7-Vitamin C	0.54 **	0.48 *	0.87 **	0.68 **	0.76 **	-0.24 ns	1				
8-Antioxidant activity	-0.79 **	-0.59 **	-0.77 **	-0.51 **	-0.25 ns	0.74 **	-0.43 *	1			
9-Seed oil content	0.71 **	0.63 **	0.56 **	0.27 ns	-0.02 ns	-0.75 **	0.24 ns	-0.84 **	1		
10-Calyx water use efficiency	-0.58 **	-0.28 ns	-0.16 ns	0.24 ns	0.081 ns	0.52 **	-0.03 ns	0.34 ns	-0.48 **	1	
11-Calyx harvest index	-0.35 ns	-0.50 **	-0.068 ns	0.59 **	-0.11 ns	0.49 **	-0.03 ns	0.14 ns	-0.26 ns	0.58 **	1

ns – not significant, *: significant at $P \leq 0.05$ and **: significant at $P \leq 0.01$

drought severity from well-watered to mild drought stress but severe drought stress significantly decreased calyx yield to 1.16 ton.ha^{-1} (Table 2). It was demonstrated that well-watered irrigation is needed to guarantee high crop yield but in the case of medicinal plants a mild drought stress could be beneficial to the quality and quantity of these plants (keshavarz *et al.,* 2018). Increases in roselle calyx may be attributed to the mild water stress imposed. This was in agreement with the reports of Fallahi *et al.* (2017a), who claimed that the calyx yield of roselle increased under mild drought stress compared to full irrigation. El-Dissoky *et al.* (2020) studied the effect of irrigation frequency on the calyx yield of the roselle plant and found that mild drought stress improved the calyx production but severe drought condition reduced the calyx yield. Stress conditions have been shown to decrease the number of flowers and calyx (depending on the time of the stress severity), because the flowering phase involves several processes that are vulnerable to stress conditions (Jasim *et al.,* 2020). From Table 3, it is clear that first sowing dates (4th June) registered maximum calyx yield (1.48 ton.ha^{-1}) while the lowest calyx yield (1.24 ton.ha^{-1}) was recorded for the third sowing date (2nd July). Given the favorable growth conditions such as temperature and the sun light on 4th June, plants produced more assimilates and yielded greater dry matter. Due to a shortened vegetative phase, flowering occurred when summer temperatures were high and the flowers aborted. Previous research has reported that optimal planting time leads to better developed plants with higher LAI than those sown later (Rah Khosravani *et al.,* 2017). Accordingly, flowering abortion would be expected to increase during drought stress as it decreases the flux of photosynthate supply from source leaves to the vegetative tissues. Also, drought stress may change the concentration of ABA in the plants, and thereby induce flower abortion in drought-stressed crops (Salih, 2019).

3.4. Total phenolic content (TPC)

The highest TPC (2.77 mg.100 g^{-1} DW) was recorded for plants treated with mild drought stress, while the lowest TPC (1.94 mg.100 g^{-1} DW) was recorded for severe drought stress treatment. Although, mild drought stress increased TPC, there was no significant difference between well-watered and mild drought stress treatments (Table 2). However, a greater increase in drought severity, to 55% FC, lowered the TPC of roselle. TPC, as a group of non-enzymatic antioxidant compounds with carbon skeleton, by osmotic regulation of plant cells serves to respond to drought conditions, stabilize proteins, prevent lipid peroxidation, and act as an osmolyte in the permeability of cell membranes (Keshavarz Mirzamohammadi *et al.,* 2021). Under severe drought conditions, TPC decreases, which can be because of an increase in the production of oxygen radicals, peroxidation of chlorophyll pigments, degradation of genes related to the chlorophyll biosynthetic path, reduction in the photosynthetic level and increasing the allocation of carbon to the root.

3.5. Total anthocyanin content (TAC)

The effect of irrigation treatment was significant for the TAC of roselle (Table 1). On average, TAC increased by 19.68 and 24.69% compared to the well-watered treatment when the plants were treated with mild and severe drought stress, respectively (Table 2). However, mild and severe stress were statistically at the same level. As documented in many papers (An *et al*., 2020; Cirillo *et al*., 2021), the total anthocyanin content of roselle was increased by drought stress to reduce excess light availability and provide useful protection to leaves without significantly reducing photosynthesis efficiency. This shows a mechanism to modulate light absorption and reduce photo-oxidative injury. It has been reported that anthocyanin has the potential to reduce photo-oxidative damage in leaves, both by scavenging reactive oxygen species and protecting chloroplasts from excess high-energy quanta (He *et al.,* 2020). The influence of drought stress on TAC is in agreement with the findings of Fallahi *et al*. (2017a) and Hinojosa-Gómez *et al*. (2020) on roselle, which stated that the improvement in the plants' qualitative traits under simultaneous drought stress, such as anthocyanins, has been related to improved secondary metabolism.

3.6. Vitamin C

Vitamin C content was affected significantly by irrigation levels (Table 1) and the amount of these compounds under mild drought stress (395.7 mg 100 g^{-1}) was higher than well-watered irrigation (13% on average). In contrast, severe drought stress sharply decreased the vitamin C content by 21 and 32% compared to the well-watered and mild stress, respectively. Changes in vitamin C under drought stress have been shown to depend on stress severity, drought duration and cultivar (Dolatabadian *et al*., 2010). Mataa *et al*. (2020) showed that the concentrations of vitamin C, TPC, and TAC showed similar trends and were reduced in severe drought stress. Therefore, it is possible that in this study, the concentration of other antioxidants, such as superoxide dismutase and catalase (not measured in this study), can be increased in roselle under drought stress.

3.7. Antioxidant activity

In this investigation, antioxidant activity (DPPH) was significantly increased with the increase in the severity of drought stress in the order: control < mild < severe drought stress (Table 2). In general, with delay in planting date, the percentage of antioxidant activity in the plant increased and 4th June exhibited the lowest (71.57%) antioxidant activity for septal extract, whilst the highest antioxidant activities were observed on 18th June and 2nd July (78.62 and 78.20, respectively) (Table 3). Free radicals are generated under stress conditions and the antioxidant capacity of plants includes enzymatic and non-enzymatic compounds, free radical species are oxidized by releasing electrons to free radicals (Keshavarz, 2020). A significant decrease was observed for total anthocyanin content ($y = -0.76^{**}$) and antioxidant activity ($y = -0.79^{**}$) with the increase in plant height (Table 4). This is due to the difference in red (R): far red (FR) ratio perceived by the plants grown at different irrigation regimes, planting time and neighbor detection (Khattak *et al.,* 2016). The plants develop a strategy to grow taller in order to capture more photosynthetic radiation at the expense of physiological changes (Smith, 1982).

3.8. Seed oil content

On average, the seed oil content (% of seed dry matter) of the plants under mild and severe drought treatment was significantly low (12.21 and 11.27%, respectively) compared to 16.88% in the seeds of the well-watered plants (Table 2). Delay in planting time significantly decreased the seed oil content in fruits compared to on-time sowing dates, where the highest values for seed oil content were recorded on 4th June (14.14%) with an average of 6.15 and 8.41% higher than 18th June and 2nd July (Table 3). As previously explained, water stress conditions affected plant growth and development, leading to an effect on seed oil content. The optimal water supply throughout the growing period could enhance oil content in seed oil plants, while drought stress reduces it (Soheili-Movahhed *et al.*, 2019). A reduction in seed oil content might be attributed to the polyunsaturated fatty acid oxidation by free radicals under drought conditions (Keshavarz, 2020). The reduction in seed oil content when drought stress occurred during the growing season is supported by various reports (Keshavarz *et al.,* 2020). The quantity of seed oil content is mainly determined by water balance, soil fertilizers, sun light and other environmental factors throughout the growth period. The high temperature and consequently changes in water balance in the seed filling stage could likely be the reason for lower oil content on 2nd July compared to 4th June. Asadpour *et al.* (2020) and Yazdanpanah *et al.* (2018) reported that high seed oil content in roselle (*Hibiscus sabdariffa* L.) was related to lower temperature and water availability at the seed filling phase.

3.9. Calyx water use efficiency (WUE_{calyx}) and calyx harvest index (HI_{calyx})

The highest water use efficiency for calyx (2.02 $kg.kg^{-1}$) was obtained with severe drought treatment and the lowest (1.79 $kg.kg^{-1}$) was with the well-watered treatment (Table 2). The results showed that water use efficiency increased when the sowing date was delayed to after June 4th. Based on our results (Table 3) the highest use efficiency was observed on 2nd July and it was higher by 15.42 and 7.94% than the first and second planting dates, respectively. However, there were no significant difference between the first and second planting dates. Based on harvested calyx yield and the amount of irrigation, severe drought stress was determined to be the best irrigation regime in terms of the WUE_{calyx}. As previously mentioned, the biological yield of roselle was decreased by drought stress. In our study, since the average amount of irrigation water was reduced among the drought stress or delayed planting treatments, higher calyx yield compared to biological yield led to the greater WUE_{calyx}.

A limited supply of carbohydrates for dry matter production leads to a reduction in biological yield. Previous research has reported that higher values for HI_{calyx} in roselle (*Hibiscus sabdariffa* L.) under drought conditions might be due to higher air temperature and heat stress, which could negatively affect biological yield and plant growth, resulting in earlier flowering due to shortened vegetative stages and increased economical yield/biological yield ratio (Javadzadeh, 2018). In fact, drought conditions reduced biological yield more than calyx yield. As shown in Table 2, calyx yield is higher under mild drought conditions. It was thought that the increase in calyx under mild drought conditions resulted from the increase in assimilate partitioning to flower due to a shortened vegetative phase. On the hypothesis that short distance translocation of is limited to first/second node (Liu *et al.,* 2020), the improvement in HI_{calyx} means the increase in sink demand to source. So, we can conclude that the increase in calyx yields improved HI_{calyx} by increasing sink demand, therefore, the use efficiency of assimilates was increased under drought conditions.

CONCLUSIONS

Plant height and stem diameter decreased with decreasing water availability; while TAC, antioxidant activity, WUE_{calyx} and HI_{calyx} increased with decreasing water availability. However, in term of biological yield and calyx yield, there was no significant difference between well-watered and mild drought stress. Sowing date, plant height, biological yield, calyx yield and oil seed content decreased with delayed planting while antioxidant activity and WUE_{calyx} increased. In brief, the planting date on 2nd July that improved WUE_{calyx} (probably due to reducing irrigation supply) was a good strategy to increase calyx production and WUE. These results suggest that the delay in on-time planting date from 4th June to early July (2nd July) is possible to improve calyx WUE in arid and semi-arid regions like Karaj.

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REFERENCES

Aghdasi S, Modares Sanavy SAM, Aghaalikhani M, Keshavarz H. 2018a. Effect of foliar application of iron and manganese on yield and yield components of mungbean under water deficit stress. *Water Soil Sci*. **28** (3), 13-25.

Aghdasi S, Modares Sanavy SAM, Aghaalikhani M, Keshavarz H. 2018b. Impact of water deficit stress and foliar application of iron and manganese on some morphological and physiological of mungbean (*Vigna radiata* L.). *J. Plant. Proc. Func*. **7** (26), 101-116. http://jispp.iut.ac.ir/article-1-670-en.html.

Alizade Moradi M, Ramroudi M, Asgharipour MR. 2018. Response of roselle (*Hibiscus sabdariffa* L.) as a medicinal plant to planting date and micronutrient spraying in alley cropping. *Applied Res. Field Crop*. **31** (2), 119-134.

An JP, Zhang XW, Bi SQ, You CX, Wang XF, Hao YJ. 2020. The ERF transcription factor MdERF38 promotes drought stress-induced anthocyanin biosynthesis in apple. *Plant J.* **110** (3), 573-589. https://doi.org/10.1111/tpj.14555.

Apeyuan K, Nwankiti A, Oluma O, Ekefan E. 2017. Effect of different sowing dates on disease initiation and development of roselle (*Hibiscus sabdariffa* L.) leaf spot disease caused by *Coniella musaiensis* Var. hibisci in Makurdi, Central Nigeria. *J. Geosci. Environ. Protec.* **5**, 94-101. https://doi.org/10.4236/gep.2017.511007.

Asadpour S, Madani H, Nour Mohammadi Gh, Majidi Heravan I, Heidari Sharif Abad H. 2020. Improving maize yield with advancing planting time and nano-silicon foliar spray alone or combined with zinc. *Silicon.* Online Availabe. https://doi.org/10.1007/s12633-20-00815-5.

Basyouni Abou-Khalifa AA. 2010. Response of some rice varieties to irrigation withholding under different sowing dates. *Agri. Bio. J. North America.* **1** (1), 56-64.

Begum H, Medagam TR, Rao NH, Neelam S, Pandravada SR, Natarajan S. 2015. Genetic diversity and variability in landraces for key agroeconomic traits in vegetable roselle (*Hibiscus Sabdariffa* Var. Sabdariffa L.). *Jordan J. Biologic. Sci.* **8** (2), 113-125. https://doi.org/10.12816/0027557

Beljkas B, Matic J, Milovanovic I, Jovanov P, Misan A, Saric L. 2010. Rapid method for determination of protein content in cereals and oilseeds: validation, measurement uncertainty and comparison with the Kjeldahl method. *Accredit Qual. Assur*. **15**, 555-561.

Chumsri P, Airichote A, Itharat A. 2008. Songklanakarin. *J. Sci. Tech*. **30**, 133-139.

Cirillo, V., D'Amelia, V., Esposito, M., Amitrano, C., Carillo, P., Carputo, D., Maggio, A. 2021. Anthocyanins are key regulators of drought stress tolerance in tobacco. Biology. **10**, 139. https://doi.org/10.3390/biology10020139

Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M. 2014. Hibiscus sabdariffa L. A phytochemical and pharmacological review. *Food Chem*. **165**, 424-443. https://doi.org/10.1016/j.foodchem.2014.05.002

Dolatabadian A, Modarres Sanavy SAM, Asilan KS. 2010. Effect of ascorbic acid foliar application on yield, yield component and several morphological traits of grain corn under water deficit stress conditions. *Not Sci Biol*. **2** (3), 45-50. https://doi.org/10.15835/nsb234717

El-Boraie FM, Gaber AM, Abdel-Rahman G. 2009. Optimizing irrigation schedule to maximize water use efficiency of *Hibiscus sabdariffa* under Shalatien conditions. *World J. Agri. Sci*, **5** (4), 504-514.

El-Dissoky R, Attia AM, Awad AM. 2020. Managing roselle plant (*Hibiscus sabdariffa* L.) requirements of rertilizers and irrigation grown under upper Egypt conditions. *J. Soil Sci. Agric. Enginee*. **11** (12), 693-700. https://doi.org/10.21608/jssae.2020.159758

Fallahi HR, Ghorbany M, Aghhavani-Shajari M, Samadzadeh AR, Asadian AH. 2017a. Qualitative response of roselle to planting methods, humic acid application, mycorrhizal inoculation and irrigation management. *J. Crop Improvement*. **31** (2), 192-208. http://dx.doi.org/10.1080/15427528.2016.1269378

Fallahi HR, Ramezani SHR, Ghorbani M, Aghhavani-Shajari M. 2017b. Path and factor analysis of roselle (*Hibiscus sabdariffa* L.) performance. *J. Appl. Res. Med. Aromatic Plants* **6**, 119-125. https://doi.org/10.1016/j.jarmap.2017.04.001

Gendy ASH, Said-Al Ahl HAH, Abeer Mahmoud A. (2012). Growth, productivity and chemical constituents of roselle (*Hibiscus sabdariffa* L.) plants as influenced by cattle manure and biofertilizers treatments. *Australian J. Basic. Appl. Sci*. **6** (5), 1-12.

Ghayour M, Taherian M, Baghban S, Khavari S. 2020. Effect of Early Planting Dates and Different Treatments of Seed Priming on Germination and Seedling Establishment of Roselle (Hibiscus sabdariffa). *Iranian J. Seed Res*. **6** (2), 95-109. https://doi.org/10.29252/yujs.6.2.95

He YF, Xie YF, Li X, Yang J. 2020. Drought tolerance of transgenic rice overexpressing maize C4-PEPC gene related to increased anthocyanin synthesis regulated by sucrose and calcium. *Biologia Plantarum*. **64**, 136-149. https://doi.org/10.32615/bp.2020.031

Hewidy M, Sultan E, Elsayed M. 2018. Water schedule of roselle (*Hibiscus sabdariffa* L.) under organic fertilization. *Egypt J. Hortic*. **45** (1), 53-64.

Hinojosa-Gómez J, San Martín-Hernández C, Heredia JB, León-Félix J, Osuna-Enciso T, Muy-Rangel MD. 2020. Anthocyanin Induction by Drought Stress in the Calyx of Roselle Cultivars. *Molecules* **25**, 1555; https://doi.org/10.3390/molecules25071555.

Jasim AA, Jabr AK, Rawdhan SA, Abdullatif ZA. 2020. Effect of subsurface irrigation and drip irrigation on growth and yield of roselle under different irrigation periods. *Indian J. Ecology* **47** (12), 98-100.

Javadzadeh S. 2018. The effect of irrigation intervals and different ratios of manure and chemical fertilizer on yield and yield components of roselle in Iranshahr climate conditions. *Agroecol. J.* **13** (4), 71-86. https://doi.org/10.22034/aej.2018.539479.

Karami S, Modarres-Sanavy SAM, Ghanehpoor S, Keshavarz H 2016. Effect of foliar zinc application on yield, physiological traits and seed vigor of two soybean cultivars under water deficit. *Not. Sci. Biol.* **8** (2), 181-191. https://doi.org/10.15835/nsb.8.2.9793.

Keshavarz H, Khodabin G. 2019. The role of uniconazole in improving physiological and biochemical attributes of bean (*Phaseolus vulgaris* L.) subjected to drought stress. J Crop Sci Biotech. **22** (2), 161-168. https://doi.org/10.1007/s12892-019-0050-0

Keshavarz H. 2020. Study of water deficit conditions and beneficial microbes on the oil quality and agronomic traits of canola (*Brassica napus* L.). *Grasas Aceites* **71** (3), e373. https://doi.org/10.3989/gya.0572191

Keshavarz H. Modarres-Sanavy SAM, Mahdipour Afra M. 2018. Organic and chemical fertilizer affected yield and essential oil of two mint species. *J. Essential. Oil. Bear. Plant*. **21** (6), 1674-1681. https://doi.org/10.1080/0972060X.2018.1497545

Keshavarz Mirzamohammadi H, Modarres-Sanavy SAM, Sefidkon F, Mokhtassi-Bidgoli A, Mirjalili MH. 2021. Irrigation and fertilizer treatments affecting rosmarinic acid accumulation, total phenolic content, antioxidant potential and correlation between them in peppermint (*Mentha piperita* L.). *Irrigation Sci* Online Available: https://doi.org/10.1007/s00271-021-00729-z

Khalil S, Yousef RMM. 2014. Study the effect of irrigation water regime and fertilizers on growth, yield and some fruit quality of *Hibiscus sabdariffa* L.. *Intern. J. Adv. Res*. **2** (5), 738-750.

Khattak AM, Sajid M, Sarwar HZ, Rab A, Ahmad M, Khan MA. 2016. Effect of Sowing Time and Plant Density on the Growth and Production of Roselle (*Hibiscus sabdariffa*). *Int. J. Agric. Biol*. **18**, 1219-1224. https://doi.org/10.17957/IJAB/15.0215.

Krizek DT, Kramer GF, Upadhyaya A, Mirecki RM. 1993. UV- B response of cucumber seedling grown under metal halid and high-pressure sodium/deluxe lamps. *Physiol. Plant*. **88**, 350-358. https://doi.org/10.1111/j.1399-3054.1993.tb05509.x

Liu B, Qu D, Liu J. 2020. Light enrichment, flowering asynchrony and reproduction success in two field-grown soybeans in Northern China. *Legume Res*. **43 (2)**, 241-246.

Mataa M, Siziya IN, Shindano J, Moonga HB, Simon JE. 2020. Variation in leaf macro-nutrient and anti-nutrient contents associated with maturity in selected roselle (*Hibiscus sabdariffa*) genotypes. *J Medicinally Active Plant*. **9** (3), 133-144. https://doi.org/10.7275/wkzz-2070

Olsen SR., Sommers LE. 1982. Phosphorus. In: Methods of soil analysis. Part 2. Chemical and microbiological properties, Page AL, Miller RH, Keeney DR (Eds). American Society of Agronomy, Soil Science Society of America, Madison, Wisconsin, pp. 404.

Parsa Motlagh B, Rezvani Moghaddam P, Azami Sardooei Z. 2018. The effect of sowing date and intra-row space on leaf area index, dry matter accumulation and physiological characteristics of roselle (*Hibiscus sabdariffa* L.) as medicinal plant. J. Plant. Proc. Fun. **7** (26), 171-183.

Rah Khosravani AT, Mansouriear C, Modarres-Sanavy SAM, Asilan KS, Keshavarz H. 2017. Effects of sowing date on physiological characteristics, yield and yield components for different maize (*Zea mays* L.) hybrids. *Not. Sci. Bio.* **9** (1), 143-147. https://doi.org/10.15835/nsb919913.

Salih RF. 2019. Effect of sowing dates and varieties of cotton (*Gossypium hirsutum* L.) on growth and yield parameters. *Agric. Environ. Rese.* **31** (3), 64-70. https://doi.org/10.21271/zjpas.31.3.9

Sarker U, Oba S. 2018. Response of nutrients, minerals, antioxidant leaf pigments, vitamins, polyphenol, flavonoid and antioxidant activity in selected vegetable amaranth under four soil water content. *Food Chem.* **252**, 72-83. https://doi.org/10.1016/j.foodchem.2018.01.097

Seghatoleslami MJ, Mousavi SG, Barzgaran T. 2013. Effect of irrigation and planting date on morphphysiological traits and yield of roselle (*Hibiscus sabdariffa*). *J. Animal. Plant Sci.* **23** (1), 256-260.

Smith H. 2000. Phytochromes and light signal perception by plants – an emerging synthesis. *Nature.* **407**, 585–591.

Soheili-Movahhed S, Khomari S, Sheikhzadeh P, Alizadeh B. 2019. Improvement in seed quantity and quality of spring safflower through foliar application of boron and zinc under end season drought stress. *J. Plant Nutrition.* **42**(8): 942-953. https://doi.org/10.1080/01904167.2019.1584214

Temraz A, El-Tantawy WH. 2008. Characterization of antioxidant activity of extract from Artemisia vulgaris. *Pak J Pharm Sci.* **21** (4), 321-326.

Xu HJ, Wang XP, Zhao CY, Zhang XX. 2019. Responses of ecosystem water use efficiency to meteorological drought under different biomes and drought magnitudes in northern China. 278. *Agri. Forest Meteo.* **278**, 107660. https://doi.org/10.1016/j.agrformet.2019.107660

Yazdanpanah M, Rezvani Moghaddam P, Asadi G, Shahriari A. 2018. The impact of different levels of irrigation, planting date and fertilizer management on yield and yield components of roselle (*Hibiscus sabdariffa* L.) in Minab. *Iranian J. Field Crop. Res.* **16** (4), 707-721. https://doi.org/10.22067/gsc.v16i4.57794

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Antioxidant properties of two novel lipophilic gallic acid derivatives

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SUMMARY: The effectiveness of two lipophilic derivatives of the natural phenol, gallic acid (GA), synthesized using methyl gallate as starting material was investigated. The antioxidant activities of these novel phenolics compared to GA, *tert*-butylhydroquinone (TBHQ) and butylated hydroxytoluene (BHT) were evaluated in bulk oil, emulsion and the DPPH systems. The results showed that the new compounds effectively delayed lipid oxidation much better than GA and other antioxidants under Rancimat (100–140 °C) and emulsion tests. In the bulk oil system at 65 °C, they still behaved better than GA, but TBHQ had the highest activity. Thus, replacing the electron-withdrawing carboxylic group on GA by covalently linking sterically hindered phenols to its phenyl ring increased its lipophilicity and also resulted in synergistic effects which improved overall antioxidant activity through stabilization of the phenoxy radical. These new antioxidant variants satisfy industrial demands for bioactive ingredients with strong antioxidant potentials under different food processing conditions.

KEYWORDS: *Antioxidant activity; Free radical scavenging; Gallic acid derivatives; Lipophilicity; Oil-in-water emulsion; Rancimat test*

RESUMEN: ***Propiedades antioxidantes de dos nuevos derivados lipofílicos del ácido gálico.*** Se aporta información sobre de la eficacia de dos derivados lipofílicos de fenoles naturales derivados del ácido gálico (GA) y sintetizados utilizando galato de metilo como material de partida. Las actividades antioxidantes de estos nuevos compuestos fenólicos en comparación con el GA, terc-butilhidroquinona (TBHQ) y butil hidroxitolueno (BHT) se evaluaron en aceites, sistemas emulsionados y mediante DPPH. Los resultados mostraron que los nuevos compuestos retrasaron efectivamente la oxidación de lípidos mucho más fuerte que el GA y otros antioxidantes mediante Rancimat (100–140 °C) y pruebas de emulsión. En el aceite a 65 °C, se comportaron mejor que el GA, pero el TBHQ tuvo la actividad más alta. Por lo tanto, reemplazar el grupo carboxílico en GA al unir covalentemente fenoles impedidos estéricamente a su anillo de fenilo ayudó a aumentar su lipofilia y también dio como resultado efectos sinérgicos que mejoraron la actividad antioxidante general a través de la estabilización del radical fenoxi. Estas nuevas variantes de antioxidantes satisfacen la demanda industrial de ingredientes bioactivos con un fuerte potencial antioxidante en diferentes condiciones de procesamiento de alimentos.

PALABRAS CLAVE: *Actividad antioxidante; Derivados del ácido gálico; Eliminación de radicales libres; Emulsión de aceite en agua; Lipofilia; Rancimat*

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1. INTRODUCTION

Lipid oxidation generally deteriorates vegetable oils and animal fats by producing a wide range of reactive radicals that adversely affect their quality, making them unpalatable and even unfit for human consumption. Several factors affect the rate and/or occurrence of lipid oxidation, such as the composition and degree of unsaturation of fatty acids, the presence of pro-/antioxidants, and processing and storage conditions. Among the different methods employed to prevent lipid oxidation in food, the addition of natural and synthetic antioxidants remains the most effective strategy (Olajide *et al.,* 2018).

Phenolic antioxidants have become an important group of food additives due to their unique ability to conveniently preserve the sensory and nutritional qualities of food. Synthetic antioxidants such as *tert*-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate (PG) are widely used in the food industry because of their ready availability and good antioxidant capacity. Nevertheless, due to the sensitivity of consumers to the their presence in foods (Alavi Rafiee *et al.,* 2018) and weak potency during food preparation involving high temperatures (Olajide *et al.,* 2020), continuous efforts are being made to replace them with bioactive, non-toxic, high molecular weight, thermally stable and generally more potent semi-synthetic and/or natural alternatives (Torres de Pinedo *et al.,* 2007).

Gallic acid (3,4,5-trihydroxybenzoic acid, GA) is a prevalent bioactive phenolic compound found in many edible and medicinal plants (Farhoosh and Nyström, 2018). It is of significant interests to the food and pharmaceutical industries, although hydrophilic groups such as hydroxyl and carboxyl groups generally limit its application under lipophilic conditions (Crauste *et al.,* 2016). Consequently, the need to improve the functionality of GA while retaining its bioactivity has led scientists to synthesize and investigate some gallic acid derivatives including its alkyl esters (Asnaashari *et al.,* 2014; Farhoosh and Nyström, 2018; Mansouri *et al.,* 2020) and bis-gallate analogues with varying length of aliphatic chains (AL Zahrani *et al.,* 2020; Dodo *et al.,* 2008). Interestingly, studies on the *in-vitro* antioxidant investigation of GA derivatives and hybrid molecules in lipid-based foods, especially under high temperature processing conditions are still lacking. To our knowlwdge, there is no report of any derivative or hybrid ever tested in bulk oils at these temperatures (i.e. ≥ 140 °C) except for propyl gallate (PG) which is easily degraded by high heat and thus provides poor carry-through properties (i.e. retardation of rancidity) in foods that are baked or fried.

Lipophenols, as opposed to free polyphenols, contain both polar groups and lipophilic alkyl components in one molecule, and are usually synthesized by joining acids with phenols *via* ester bonds (Zhong and Shahidi, 2012). Similarly, structural modification involving the introduction of *tert*-butyl moiety to the phenyl ring of phenolics has been reported, which is more efficient than long-chain alkanes in producing compounds with better lipid solubility and antioxidant activity at high temperatures (Huang *et al.,* 2014; Silva *et al.,* 2000). Moreover, it has been proven that synergistic effects may result from a medley of phenolic antioxidants linked by a covalent bond in one molecule, which can be greater than individual antioxidants (Kancheva *et al.,* 2010; Teixeira *et al.,* 2013). Additionally, covalent bonds in the form of aliphatic bridge between poly-gallates may increase the Log P value (hydrophobicity), resulting in improved bioactivity (Dodo *et al.,* 2008).

Therefore, taking into account the bioactivity of gallic acid and practical application of novel semi-synthetic GA variants with improved functionalities such as stronger antioxidant efficiency, thermal stability and lipid solubility, two novel gallic acid derivatives, 5-(3,5-di-tert-butyl-4-hydroxybenzyl)benzene-1,2,3-triol (**5a**) and 5-(3(4)-(tert-butyl)-2,5-dihydroxybenzyl)benzene-1,2,3-triol (**5b**), were synthesized and their antioxidant activities were evaluated by Rancimat, DPPH, Schaal oven, and emulsion tests.

2. MATERIALS AND METHODS

2.1. Materials

All solvents and chemicals used in this study were of analytical grade and mainly purchased from Shanghai Macklin Biochemical Co., and Sinopharm Chemical Reagent Co. Ltd (Shanghai, China).

NMR spectra were recorded on a Bruker Avance 600 MHz spectrometer (USA) and data are presented as chemical shift δ (ppm). Mass spectra were obtained using Thermo Scientific Q Exactive plus LTQ Orbitrap XL (Thermo Fisher Scientific, USA). TLC was carried out on 0.25-mm pre-coated silica gel plates visualized under UV light at 254 nm. Induction periods (IP) were meausred on Rancimat 743 (Metrohm, Herisau, Switzerland) and UV spectra on a UV-2450 spectrophotometer (Shimadzu Corp, Kyoto, Japan).

2.2. General synthesis of compounds

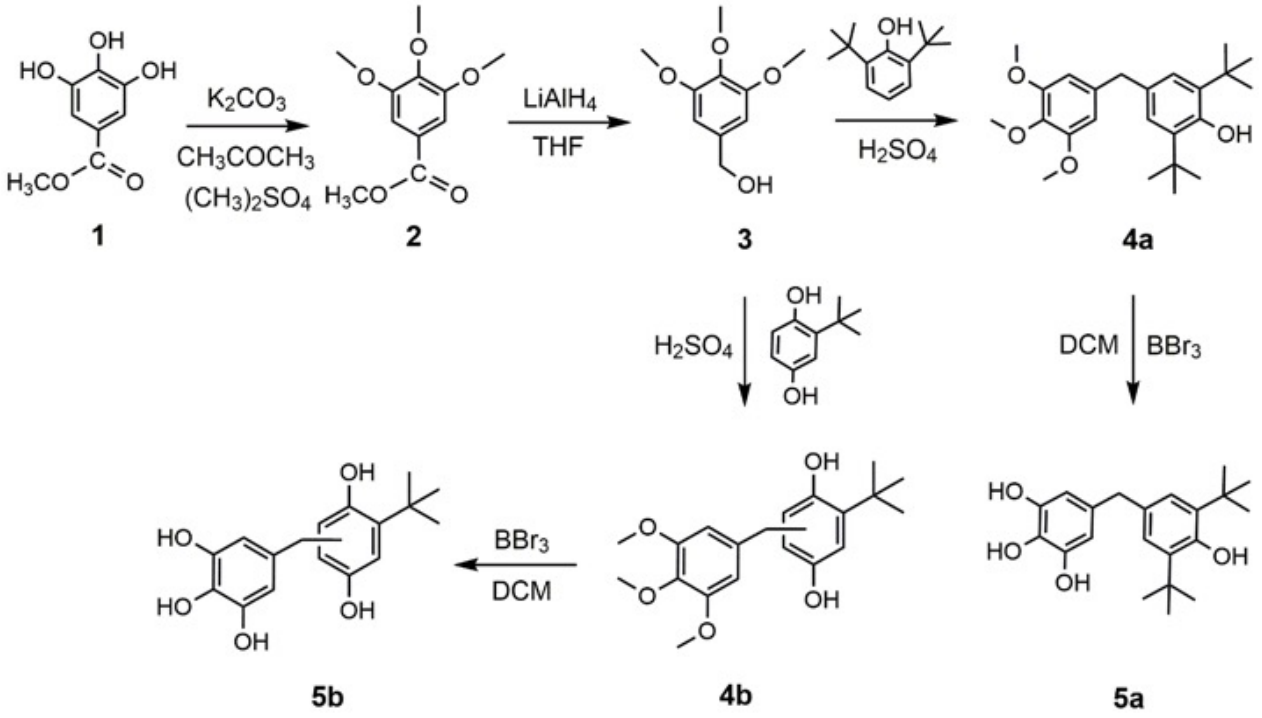
The new compounds were synthesized *via* a four-step reaction starting from methyl gallate (Scheme 1).

Methyl gallate trimethyl ether (**2**): Dimethyl sulfate (7.6 ml, 80 mmol) was slowly and uniformly added to a mixture of MG (7.4 g, 40 mmol) and K_2CO_3 (11.1 g, 80 mmol) in acetone (50 ml), and heated to reflux for 12 h. After completion, the reaction mixture was concentrated under reduced pressure and the desired compound **2** (97% yield) was isolated as a white solid by flash column chromatography using PE/EA (10:1, v/v) as eluent. Analytical data were as reported by Hirose *et al.,* (2014).

3,4,5-trimethoxybenzylalcohol (**3**): **2** (6 g, 30 mmol) was added to a stirred solution of $LiAlH_4$ (2.28 g, 60 mmol) in anhydrous THF (180 ml) at 0 °C under nitrogen conditions. Then, 0.5 M HCl solution (150 ml) was added to the resulting mixture at 0 °C after stirring at room temperature for 7 h. The acidic mixture was washed with EA (50 ml × 3), and concentrated under low pressure and subjected to flash column chromatography using PE/EA (2:1, v/v) as eluent to afford **3** (93% yield) as a colorless solid. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 6.57 (s, 2H), 4.59 (s, 2H), 3.83 (s, 6H), 3.82 (s, 3H). ^{13}C NMR (150 MHz, Chloroform-*d*) δ 153.22, 137.06, 136.88, 103.73, 65.21, 60.82, 56.02.

2,6-di-tert-butyl-4-(3,4,5-trimethoxybenzyl)phenol (**4a**): 2.5 mmol of **3** (0.5 g) were added to a mixture of 2,6-DTBP (0.78 g, 3.8 mmol) and H_2SO_4 (0.6 ml) in ethanol under nitrogen conditions. After 24 h at reflux temperature, the mixture was washed with brine (30 ml) followed by EA (30 ml), dried over $MgSO_4$, filtered and evaporated in a vacuum, yielding **4a** (69%) as a white solid after purifying by column chromatography (PE/ EA: 30:1, v/v). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.00 (s, 2H), 6.43 (s, 2H), 3.85 (s, 2H), 3.82 (s, 3H), 3.81 (s, 6H), 1.42 (s, 18H). ^{13}C NMR (150 MHz, Chloroform-*d*) δ 153.10, 137.37, 135.88, 125.31, 105.89, 60.89, 56.06, 41.97, 34.33, 30.34.

5-(3,5-di-tert-butyl-4-hydroxybenzyl)benzene-1,2,3-triol (**5a**): BBr_3 (1 mol/L in DCM, 15 ml) was added dropwise to a stirred solution of compound **4a** (0.9 g) in DCM (10 ml) at 0 °C. The reaction mixture after stirring for 16 h at room temperature was



Scheme 1. Synthetic route leading to gallic acid (GA) lipophilic derivatives, 5a and 5b.

poured into ice water and extracted with EA. The organic layers were then concentrated and purified by column chromatography (PE/EA: 4:1, v/v) to afford **5a** as a beige colored oil (92 % yield). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 6.96 (s, 2H), 6.32 (s, 2H), 3.72 (s, 2H), 1.41 (s, 18H). ^{13}C NMR (150 MHz, Chloroform-*d*) δ 152.10, 143.88, 135.83, 134.52, 131.52, 129.64, 125.45, 108.48, 41.32, 34.31, 30.34. HRMS (ESI) m/z calcd for $C_{21}H_{29}O_4^+$ $(M+H)^+$ 345.20604, found 345.20612.

2-(tert-butyl)-5(6)-(3,4,5-trimethoxybenzyl)benzene-1,4-diol (**4b**): 2.5 mmol of **3** (1.0 g) was added to a mixture of TBHQ (1.26 g, 3.8 mmol) and sulfuric acid (1.6 ml) in ethanol under nitrogen conditions. After 12 h at reflux temperature, the mixture was washed twice with brine (50 ml) followed by EA (30 ml), dried over $MgSO_4$, filtered and evaporated in vacuum, yielding **4b** (85%) as a white solid after purifying by column chromatography (PE/ EA: 5:1, v/v). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 6.73 (s, 1H), 6.46 (s, 5H), 6.41 (s, 1H), 6.37 (s, 1H), 3.97 (s, 4H), 3.83 (s, 3H), 3.82 (s, 6H), 1.38 (s, 18H). ^{13}C NMR (150 MHz, Chloroform-*d*) δ 153.35, 147.97, 135.44, 124.91, 118.28, 114.81, 105.84, 60.87, 56.10, 36.10, 34.32, 29.53.

5-(3(4)-(tert-butyl)-2,5-dihydroxybenzyl)benzene-1,2,3-triol (**5b**): BBr_3 (1 mol/L in DCM, 15 ml) was added dropwise to a stirred solution of compound **4b** (1.0 g) in DCM (15 ml) at 0 °C. The reaction mixture after stirring for 16 h at room temperature was washed with ice water and extracted with EA. The organic layers were then combined, dried over $MgSO_4$, filtered, concentrated at low pressure and the residue was purified by column chromatography (PE/EA: 2:1, v/v) to afford **5b** as a beige colored oil (95% yield). ^{1}H NMR (600 MHz, Methanol-d_4) δ 6.67 (s, 1H), 6.58 (d, J = 3.0 Hz, 1H), 6.35 (s, 1H), 6.27 (d, J = 2.9 Hz, 1H), 6.20 (s, 4H), 3.69 (s, 2H), 3.62 (s, 2H), 1.37 (s, 9H), 1.33 (s, 9H). ^{13}C NMR (150 MHz, Methanol-d_4) δ 148.41, 147.11, 145.51, 133.88, 132.35, 130.70, 125.84, 117.74, 113.56, 107.93, 34.*34*, 33.95, 29.72. HRMS (ESI) m/z calcd for $C_{17}H_{19}O_5^-$ $(M-H)^-$ 303.12380, found 303.12332.

2.3. Soybean oil purification

The soybean oil (250 g) used in this study was bought from the local market and stripped of endogenous antioxidants and pro-oxidants by column chromatography according to an earlier method described by Asnaashari *et al.,* (2014) with minor modification. Briefly, the glass column (50 x 5 cm I.D.) was packed sequentially with aluminum oxide 60 (100 g), silica gel (120 g; 200 mesh) and activated carbon (10 g) at the top. The absorbents were activated by heat prior to purification. The column tube and collection flasks were covered with aluminum foil, and the oil was passed through the column by suction force without solvent.

2.4. Oil and emulsion samples preparation for oven test

Soybean oil samples (50 g) spiked with 200 ppm antioxidants dissolved in acetone were placed in the oven at 65 ± 0.5 °C after agitation for 2 min at room temperature. The peroxide (PV) and *p*-Anisidine (*p*-AV) values of the samples were determined at 3 day intervals. Similarly, a 20% oil-in-water (o/w) stable emulsion was prepared by vigorously mixing purified soybean oil (20 g) containing 200 ppm antioxidant with Tween 80 (10 g) and a phosphate buffer solution (70 g) followed by sonication in an ice bath. The emulsions were oxidized at 65 ± 0.5 °C and PV was measured daily. The IP of samples was dependent on the time taken to reach a PV of 80 meq O_2/kg oil and a *p*-AV of 10 (Maszewska *et al.,* 2018).

2.5. Evaluation of antioxidant activity

2.5.1. Rancimat test

The antioxidant activity compounds were evaluated under Rancimat based on an earlier method reported by Olajide *et al.,* (2020) with some modification. 3 ± 0.05 g lard samples containing 200 ppm antioxidants were subjected to accelerated oxidation between 100 and 140 °C at an air flow rate of 20 L/h. The results were expressed as IP of antioxidant-spiked samples in hours relative to those without antioxidants. The protection factors (Pf) of antioxidants were also calculated with the formula below:

$$Pf = IP_s/IP_{b,}$$

where IP_s is the induction period of oil with added antioxidant, and IP_b is that of oil without antioxidant.

2.5.2. DPPH assay

The DPPH radical scavenging capacity was carried out according to the procedure described by Jiang *et al.,* (2014) with slight modification. Briefly, 0.5 ml antioxidant in methanol (1.5, 3, 6, 12, 24, and 48 μM) was added to 2.5 ml DPPH methanolic solution (0.1 mM), vigorously mixed, left to react in a dark chamber for 30 min, and the decreasing absorbance of DPPH was read at 517 nm against a blank on a spectrophotometer. Methanol served as blank and 2.5 ml solution of DPPH plus 0.5 ml methanol were used as the control. EC_{50}, the effective concentration required to obtain 50% antioxidant capacity of compounds was calculated from the linear regression of plots between the scavenging activity (%) of antioxidants and their concentrations. DPPH radical scavenging activity was calculated with the formula below:

$$\textit{Scavenging activity}\ (\%) = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

2.5.3. Peroxide value (PV), p*-Anisidine value (*p*-AV) and total oxidation value (Totox)*

The peroxide and p-anisidine contents in oil samples were measured according to the AOCS Official Methods Cd 8b-90 (AOCS, 2011) and 18-90 (AOCS, 1995) with some modification.

Finally, Totox was calculated with the following equation:

$$\text{Totox} = 2\text{PV} + p\text{-AV}$$

2.6. Statistical analysis

All the tests in this study were performed in triplicate and data are expressed as mean ± standard deviation (SD). The level of significance between means was examined by analysis of variance (ANOVA) with Duncan's multiple range test ($P < 0.05$) on OriginPro and IBM SPSS.

3. RESULTS AND DISCUSSION

3.1. Preparation and characterization of compounds

The new lipophilic GA derivatives were obtained starting from MG following a four-step reaction (Scheme 1). Briefly, the three hydroxyl groups on MG were methylated and the resulting trimethyl ether was reduced using $LiAlH_4$ to trimethoxybenzyl alcohol. Subsequently, the protecting methyl groups were cleaved (Jiang *et al.,* 2014) to yield **5a** and **5b** in good yields, respectively, following alkylation of the trimethoxybenzyl alcohol with 2,6-DTBP and TBHQ.

The identity of the new compounds was confirmed by NMR and HRMS-ESI. Compound **5b** is composed of two structurally similar isomers that were only distinguishable by NMR but chromatographically inseparable on the TLC plate. This means that they displayed the same retention factor (Rf). The retention factors of GA, **5a** and **5b** on TLC were 0.06, 0.62, and 0.44, respectively (PE/ EA: 1:1, v/v) and their theoretical partition coefficient (Log P_{theor}) values on ChemBioDraw Ultra Software were calculated as 0.42, 5.98 and 3.88. Rf correlated with Log P_{theor} values, showing the following order: GA < **5b** < **5a**. This indicates that GA had the highest polarity of all the evaluated antioxidants in this study, with a similar Log P as that reported by Farhoosh *et al.,* (2016). **5a** and **5b** were both less hydrophilic due to the presence of dense carbon skeletons existing in the form of bulky hydrophobic *tert*-butyl substituents and methylene bridges attached to their phenyl rings. Their UV spectra were quite similar, showing absorptions at 273 and 264 nm, respectively.

In the ^{1}H NMR spectrum of **5a**, two singlets around 6.96 and 6.32 ppm were assigned to the aromatic protons, while proton signals at 3.72 and 1.41 ppm were observed for the methylene bridge and the tert-butyl moieties linked to the aromatic ring. The ^{13}C NMR spectrum exhibited 11 carbon signals including eight aromatic carbon signals between 152.10 and 108.48ppm, a methylene group carbon signal at 41.32 ppm, and *tert*-butyl group car-bon signals at 34.31 and 30.34 ppm. The HRMS (ESI) spectrum of **5a** exhibited a protonated specie at m/z 345.20612, which was assigned to $C_{21}H_{29}O_4^+$ $(M+H)^+$. As expected, the ^{1}H NMR spectrum of **5b** revealed it as an isomeric mixture (i.e. 80% 5-(4-(tert-butyl)-2,5-dihydroxybenzyl)benzene-1,2,3-triol (5bi) and 20% 5-(3-(tert-butyl)-2,5-dihydroxybenzyl)benzene-1,2,3-triol (5bii). Two singlets around 6.67 and 6.35 ppm (for 5bi), two doublets around 6.58 and 6.27 ppm (for 5bii) and a sharp singlet at 6.20 (for both) were assigned to the aromatic protons, while proton signals at 3.69 and 3.62 ppm were observed for the methylene groups and *tert*-butyl group proton signals linked to the aromatic ring of 5bii and 5bi, which were observed at 1.37 and 1.33ppm, respectively. The ^{13}C NMR spectrum of **5b** exhibited 13 carbon signals including 10 aromatic carbon signals between 148.41 and 107.93 ppm, a methylene group carbon signal at 34.34 ppm, and tert-butyl group

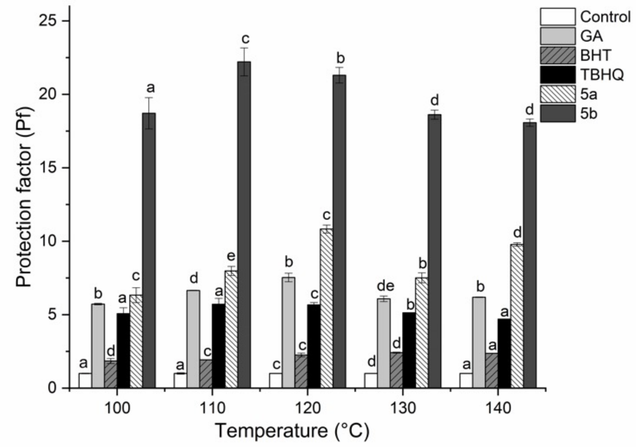


FIGURE 1. Pf values of oil samples containing 0.02% (w/w) antioxidants at different temperatures. Values are expressed as mean ± SD (n=3). Different letters are significantly different according to Duncan's multiple range test (P < 0.05).

carbon signals at 33.95 and 29.72 ppm. The HRMS (ESI) spectrum of **5b** exhibited a molecular specie at m/z 303.12332, which was assigned to $C_{17}H_{19}O_5^-$ $(M\text{-}H)^-$. The spectra data completely characterized **5a** and **5b**, neither of which has been previously described in any literature.

3.2. Evaluation of antioxidant activity of compounds

3.2.1. Rancimat test

The structure-antioxidant activity of the new antioxidants, **5a** and **5b** was evaluated in comparison with GA, BHT and TBHQ under temperatures of up to 140 °C at 0.02% (w/w) under air saturation conditions. The results are expressed as the IP of samples compared to the oxidative stability of lard without antioxidants (Table 1), and the Pf of the antioxidants (Figure 1). Pf values greater than 3 indicate strong antioxidant activity and those lower than 1 indicate pro-oxidant activity (Weng and Huang, 2014). In this experiment, the concentration was set at 200 ppm according to the safety limit established for frequently used and emerging commercial antioxidants in oils (Saad *et al.,* 2007).

The IPs of GA, BHT and TBHQ were less than **5a** and **5b** at the different temperatures. i.e., the novel lipophilic GA derivatives exhibited much stronger oxidative stability than GA and the common commercial synthetic antioxidants, TBHQ and BHT. Compound **5b,** however, showed the highest IP, which was 146 h higher than **5a** and 159, 209, and 170 h more than GA, BHT and TBHQ, respectively. For all the antioxidants evaluated at 0.02% (w/w) (Figure 1), a Pf greater than 1 was observed, which affirms a protective capacity against accelerated oxidation in the lipid matrix. Nevertheless, **5b** similarly exhibited the highest Pf (110 °C, Pf = 22.21; 140 °C, Pf = 18.07), followed by **5a** (110 °C, Pf = 7.98; 140 °C, Pf = 9.78), GA (110 °C, Pf = 6.65; 140 °C, Pf = 6.19), TBHQ (110 °C, Pf = 5.71; 140 °C, Pf = 4.70) and finally, BHT (110 °C, Pf = 1.93; 140 °C, Pf = 2.37). Briefly, the antioxidant activity of the evaluated antioxidants under Rancimat conditions decreased as follows: **5b** >> **5a** > GA > TBHQ > BHT > Control.

Previous studies have shown GA and its alkyl esters to exert better antioxidant activities than free polyphenols at moderate temperatures (Farhoosh *et al.,* 2016) and even TBHQ (Jung and Choi, 2016)

TABLE 1. Induction periods (IPs) of oil samples containing 0.02% (w/w) antioxidants at different temperatures under Rancimat accelerated test.

	IP (h)				
Sample	**100 °C**	**110 °C**	**120 °C**	**130 °C**	**140 °C**
Control	6.65 ± 0.04^{a}	3.00 ± 0.11^{a}	1.20 ± 0.01^{c}	0.53 ± 0.00^{d}	0.27 ± 0.00^{a}
GA	38.02 ± 5.26^{b}	19.96 ± 0.04^{d}	9.04 ± 0.57^{b}	3.22 ± 0.38^{de}	1.67 ± 0.03^{b}
BHT	12.31 ± 0.33^{d}	5.78 ± 0.02^{c}	2.72 ± 0.26^{c}	1.28 ± 0.07^{d}	0.62 ± 0.02^{a}
TBHQ	33.74 ± 1.61^{a}	17.13 ± 0.93^{a}	6.80 ± 0.30^{c}	2.72 ± 0.03^{b}	1.27 ± 0.01^{a}
5a	42.11 ± 3.02^{c}	23.94 ± 0.62^{e}	13.0 ± 0.54^{c}	3.98 ± 0.26^{b}	2.64 ± 0.24^{d}
5b	124.45 ± 6.64^{a}	66.64 ± 1.90^{c}	25.56 ± 1.06^{b}	9.87 ± 0.61^{d}	4.88 ± 0.49^{d}

Results are expressed as mean ± SD (n=3). Means in the same row with different letters are significantly different according to Duncan's multiple range test ($P < 0.05$). Control: lard; GA: gallic acid; BHT: butylated hydroxytoluene; TBHQ: *tert*-butylhydroquinone; 5a and 5b: novel gallic acid derivatives.

in lipid matrices. Nonetheless, the much stronger antioxidant effects were exhibited by **5a** and **5b** compared to GA, The TBHQ and BHT in this study may be attributed to their higher molecular weight, which contributed to less partial volatilization/decomposition at high temperatures, especially when air is blown and a large amount of steam is formed (Huang *et al.,* 2014; Olajide *et al.,* 2018; Olajide *et al.,* 2020). In addition, various authors have proven that a synergistic effect may result from the combination of two or more phenolic antioxidants linked by a covalent bond in a single molecule, which is greater than individual antioxidants (Kancheva *et al.,* 2010; Teixeira *et al.,* 2013). Herein, the Log P value (hydrophobicity) of the resulting compounds increased due to the presence of a methylene bridge and bulky *tert*-butyl moiety (an excellent electron-donating group), thus leading to substantial activity of the antioxidants in oil. The electron-withdrawing effect of the carboxylic group on GA generates unstable phenoxy radicals (Torres de Pinedo *et al.,* 2007), however, replacing this with electron-donating molecules (Scheme 1) resulted in a well-stabilized phenoxy radical. Moreover, the excellent antioxidant capacity of the new compounds, especially that of **5b**, can be attributed to the strong steric synergy exhibited separately in the form of hydrogen bonding between hydroxyl groups in the pyrogallol (Farhoosh *et al.,* 2016) and sterically hindered hydroquinone units (Huang *et al.,* 2014) of the molecule, a phenomenal effect which allowed the less stable free radical of **5b** to easily convert to a more stable form intramolecularly. Furthermore, the pyrogallol component may easily synergistically regenerate the sterically-hindered hydroquinone one by donating hydrogen to it radical forms (Guo *et al.,* 2017).

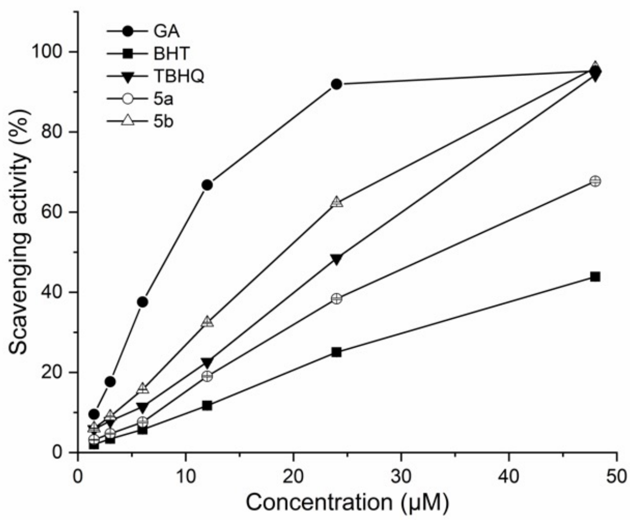


FIGURE 2. Radical scavenging activity of different antioxidants towards DPPH. Values are expressed as mean ± SD (n=3). Statistical significance at $p \leq 0.05$ according to Duncan's multiple range test.

3.2.2. DPPH assay

This method is frequently used to evaluate the potency of antioxidants as radical scavengers in that it is easily reproducible, rapid and sensitive. The scavenging activities of the antioxidants studied are shown in Figure 2. All the tested compounds showed a steady increase in scavenging activities between 1.5 and 48 μM, except GA, which exhibited a rapid one. At 12 μM, the scavenging abilities of GA, BHT, TBHQ, **5a** and **5b** were 67, 12, 23, 19 and 32%, respectively. Similarly, EC_{50}, the effective concentration needed to reduce the initial DPPH concentration by 50% is provided in Table 2. GA exhibited the highest EC_{50} value (19.09 μM) while BHT expectedly showed the least (54.84 μM). The antioxidant activity of the antioxidants in the DPPH system decreased as follows: GA > **5b** > TBHQ > **5a** > BHT.

This finding is in agreement with previous studies (Alavi Rafiee *et al.,* 2018; Asnaashari *et al.,* 2014; Farhoosh *et al.,* 2016; Mansouri *et al.,* 2020), where GA was found to be the most potent anti-radical agent compared to a subset of other phenolic antioxidants. The ability of phenolics to scavenge radicals is dependent on the number of electron-donating hydroxyl groups in the phenyl ring, which increase phenoxy

TABLE 2. The EC_{50} values of the lipophilic gallic acid derivatives (5a and 5b), GA, BHT and TBHQ.

Compound	**EC_{50} (μM)**	**Log P_{theor}**
GA	19.09 ± 0.04^{b}	0.42
BHT	54.84 ± 0.03^{c}	5.54
TBHQ	25.46 ± 0.05^{a}	2.96
5a	33.66 ± 0.04^{cd}	5.98
5b	23.60 ± 0.07^{b}	3.88

EC_{50}, 50% effective concentration. Values are expressed as mean ± SD (n=3). Means within a column with the same lowercase letters are not significantly different according to Duncan's multiple range test ($P < 0.05$).

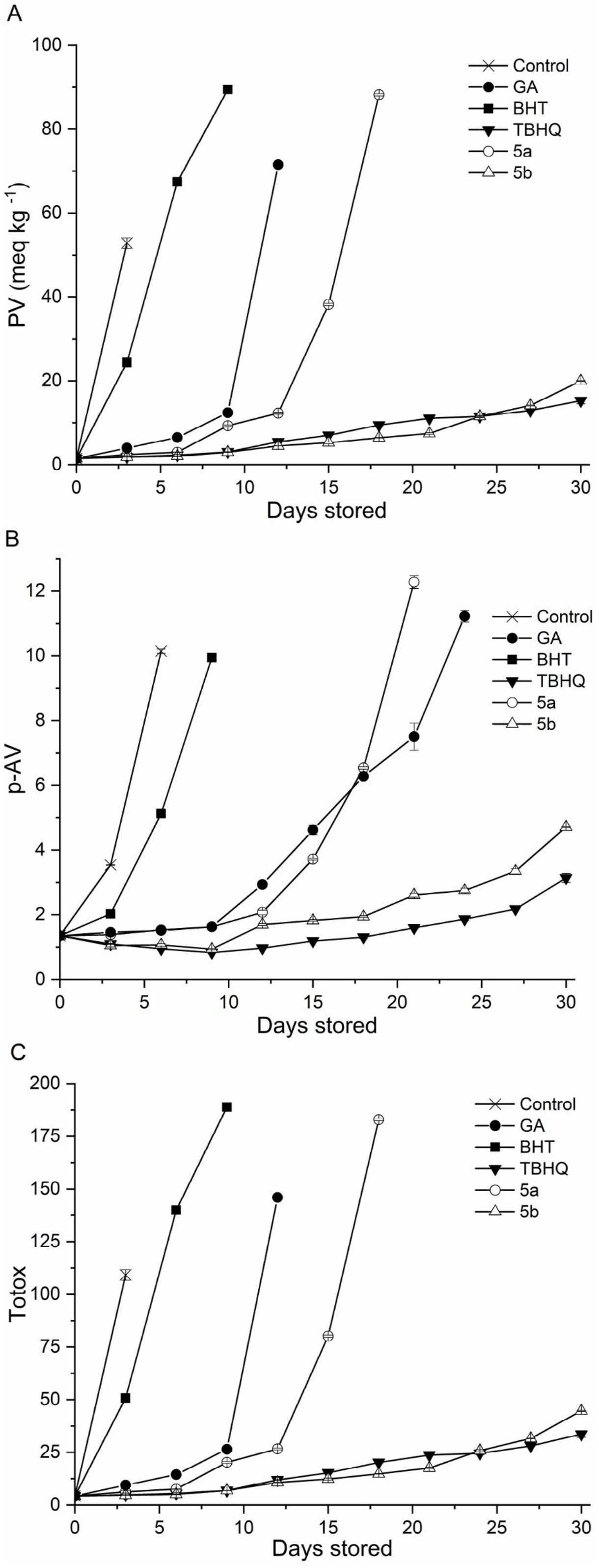


FIGURE 3. Changes in peroxide value (A), p-anisidine value (B) and Totox (C) during oxidation of soybean oil spiked with 0.02% (w/w) antioxidants at 65 °C. Values are expressed as mean ± SD (n=3). Statistical significance at p ≤ 0.05 according to Duncan's multiple range test.

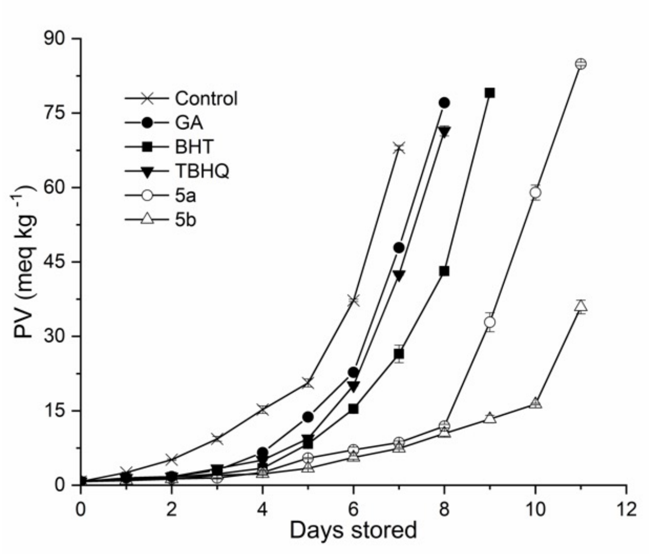


FIGURE 4. Changes in peroxide value during oxidation of soybean oil-in-water emulsion spiked with 0.02% (w/w) antioxidants at 65 °C. Values are expressed as mean ± SD (n=3). Statistical significance at p ≤ 0.05 according to Duncan's multiple range test.

radical stability. As a result, GA, with a carboxyl and three hydroxyl substituents, was more active than **5b,** TBHQ, **5a** and BHT, as more hydrogen can be donated from the phenolic hydroxyls to stabilize the free radicals (de Pinedo *et al.,* 2007). Moreover, despite the electron-withdrawing effect of the carboxyl group (–COOH), it can readily dissociate in polar media to carboxylate anion (–COO–), an electron-donating moiety which supports the formation of more stable phenoxyl radicals (Mansouri *et al.,* 2020). The pattern of interaction between phenol and the DPPH radical occurs in the form of phenolic hydrogen abstraction and the transfer of a second hydrogen and/or formation of dimers (reactive or non-reactive) from the phenoxyl radical (Guitard *et al.,* 2016). TBHQ, with a hydroquinone structure, can release two hydrogens to DPPH. However, the presence of *tert*-butyl creates a steric hindrance effect on the *ortho*-OH substituent and thus lowers the abstraction of hydrogen by the DPPH (Weng and Huang, 2014).

This effect was apparent in the scavenging effect of **5b**, but nevertheless, the additional presence of a pyrogallol unit clearly increased its overall scavenging ability, thereby surpassing that of TBHQ. Strongly sterically hindered monophenols like BHT can only transfer one hydrogen per molecule and are unable to form reactive dimers (Guitard *et al.,* 2016), leading to low DPPH scavenging activity compared to GA and other antioxidants studied here. Nonetheless, 5a displayed a higher scavenging activity (EC_{50} = 33.66 µM) compared to BHT (EC_{50} = 54.84 µM), mainly due to the presence of pyrogallol component in its molecule.

Meanwhile, the radical scavenging activity of antioxidants may also be illustrated by the degree of polarity, which determines their solubility and availability to oxidative compounds such as DPPH (Mansouri *et al.,* 2020). Compounds with higher log P values are more hydrophobic. Therefore, based on theoretical partition coefficient (Log P_{theor}) values (Table 2), GA had higher polarity than other compounds studied herein, leading to better interactions between it and DPPH. Briefly, as seen in this study, molecular configuration, ability to donate hydrogen atoms and subsequently stabilize phenoxyl radicals all influenced the antioxidant activity of the different phenolic antioxidants (Silva *et al.,* 2000).

3.2.3. Peroxide (PV), **p**-*anisidine (*p-*AV) and totox changes in oil*

As shown in Figure 3A, the initial PV of the samples was 1.47 ± 0.06 mEq/Kg oil, depicting that the oil was in good condition (PV < 15 mEq O_2/Kg) (Maszewska *et al.,* 2018). As expected, BHT and control displayed a sharp increase in PV, reaching 80 mEq/kg at 4.6 and 8.5 days of storage, respectively. At the same time, GA and **5a** samples oxidized slowly at the initial stage, and then steeply increased, reaching a maximum on days 12 (71.52 ± 0.85 mEq/kg) and 18 (88.20 ± 0.26 mEq/kg), respectively. However, both TBHQ and **5b** had better oxidative stability by exhibiting a gradual increase in PV throughout the storage period.

A *p*-AV below 10 depicts good quality oil (Maszewska *et al.,* 2018). Results from Figure 3B showed that after 30 days of storage at 65 ± 0.5 °C, most of the samples had a *p*-AV exceeding 10 from an initial value of 1.35 ± 0.03, except for the TBHQ (3.14 ± 0.14) and **5b** (4.71 ± 0.02) groups. The control and BHT samples showed similar result trends as earlier presented in PV with *p*-AV of 10.14 ± 0.07 and 9.94 ± 0.07 on days 6 and 9, respectively. Nevertheless, GA extended the oxidative stability of oil samples in terms of *p*-AV by an additional 12 days compared to what was observed for PV, thereby reaching a maximum value of 11.22 ± 0.18 on the 24th day of storage.

Totox considers both peroxides and aldehydes generated during oxidation and is thus a better indicator of overall oxidative deterioration (i.e. lower Totox value depicts better oil quality and vice versa). The results presented in Figure 3C are similar in trend to those of PV and *p*-AV under the same storage conditions except for GA, which displayed extended oxidative stability by delaying the production of secondary oxidation products for 12 more days. However, this effect showed little significance in terms of total oxidation. Nonetheless, the antioxidant capacity of the compounds evaluated in bulk oil under the Schaal Oven test decreased as follows: TBHQ ≈ **5b** > **5a** > GA > BHT > control.

The dissimilarity in the antioxidant activities of compounds may depend on their chemical structures, which influence their ability to stabilize their own phenoxy radicals. Compounds **5a** and **5b** both exhibited stronger oxidative stability than TBHQ at high temperatures (> 100 °C) under Rancimat, but at moderate thermal temperatures (65 °C) the protective activity of **5a** was lower and that of **5b** was close to TBHQ. This observation is in accordance with previous reports (Olajide *et al.,* 2020; Zhang *et al.,* 2004). The phenolic hydroxyl groups on TBHQ have strong steric synergy and can transfer hydrogen atoms to active peroxyl radicals in lipid matrices to interrupt the oxidative process. Similar to a previous study (Farhoosh *et al.,* 2016), despite the lower polarity and DPPH scavenging activities of **5a** and **5b** (Figure 2; Table 2), they were more effective antioxidants than GA in bulk oil. This is probably due to their fundamental carry-through properties—i.e., the ability to resist decomposition by heat and/or loss via volatilization. Moreover, in the lipid media, hydrogen atoms from sterically hindered moieties linked to **5a** and **5b** may interact intramolecularly with the meta-hydroxyl substituents of their pyrogallol component, subsequently leading to the stabilization of phenoxy radicals and thus increased antioxidant activities (Asnaashari *et al.,* 2014).

3.2.4. Peroxide value (PV) changes in oil-in-water (O/W) emulsion

The activity of antioxidants evaluated in emulsion following accelerated oxidation in the oven until PV equaled 70 mEq/kg contrasted that observed for bulk oil, and the relative decreasing order was: **5b** > **5a** > BHT > TBHQ > GA > control (Figure 4). Similarly, this finding was different from the trend observed in the DPPH assay which suggests antioxidant performance depends greatly on the method of analysis and/or system used (Farhoosh *et al.,* 2016). The effectiveness of antioxidants in the emulsion system were generally reduced compared to those in the soybean bulk oil, indicating lower antioxidant concentration in the hydrophobic center of oil droplets, thus leading to a faster oxidation rate. Gallic acid, with the highest scavenging activity under DPPH assay, was the weakest in the emulsion system. This conforms with previous studies, where the antioxidant activities of GA (Asnaashari *et al.,* 2014) and rosmarinate alkyl esters (Panya *et al.,* 2010; Schwarz *et al.,* 2000) in emulsion increased as their polarity decreased. These lower polar compounds up to their dodecyl derivatives were more effective antioxidants in that they predominantly concentrated themselves at the oil–water interface where oxidation occurs. However, GA and rosmarinic acid with the highest polarity incorporated a larger amount of their molecules to the aqueous phase than the interface.

Comparably, TBHQ, with the strongest antioxidant activity in bulk oil (Figure 3), exhibited weak antioxidant activity compared to the more lipophilic BHT, **5a** and **5b**. Antioxidants partition on the basis of polarity, which is determined by their molecular structural properties (i.e. hydrophobic compounds display higher log P values and vice versa) (Asnaashari *et al.,* 2014). Zhang *et al.,* (2004) also reported weak antioxidant activity for TBHQ, which was due to its low hydrophobicity leading to more migration towards the aqueous phase in the emulsion than the oil-water interface (oxidation site).

According to Panya *et al.,* (2010), butyl rosmarinate, an alkyl ester of rosmarinic acid, with similar molecular structure and Log P_{theor} value to **5b**, predominantly concentrated at the interface and thus exerted stronger antioxidant capacity than the more polar rosmarinic acid and least polar eicosyl rosmarinate (20 carbon atoms). As a result, the combined hydrophobic nature of the methylene bridge and the *tert*-butyl substituent in **5b** must have shifted the molecule to the oil-water interface, leading to its greater oxidative stability compared to other antioxidants studied herein. Meanwhile, BHT, with the highest hydrophobicity among all the phenolics studied, may have partitioned more into the oil phase away from the interface, thus exhibiting reduced effectiveness compared to **5a** and **5b**. This was in agreement with the results obtained by Li *et al.,* (2006) for BHT in soybean oil-in-water emulsions. Hence, contrary to the performance in the DPPH assay, the lipophilic derivatives—**5a** and **5b**, mostly exhibited stronger antioxidant activities than their individual parent molecules in emulsion and bulk oil systems, including the high temperature (100 – 140 °C) Rancimat experiment.

This can be attributed to the fact that alkylation of the pyrogallol unit with sterically hindered hydroquinone or 2,6-DTBP moieties in **5b** and **5a** molecules, respectively, decreased the electron-withdrawing effect of –COOH linked to the phenyl ring of GA, resulting in a phenoxy radical with better stabilization (Farhoosh and Nyström, 2018). Indeed, the effectiveness of phenolic antioxidants in oil indicates that their stabilizing capacity is considerably related to the properties of functional groups present, steric synergy and intramolecular hydrogen bonding. Meanwhile, in the emulsion system, it may depend on variables such as polarity and solubility, emulsifier used, concentration and type of antioxidants, radical-scavenging properties and the complex effects at the oil–water interfaces. Moreover, the polar paradox theory, which denotes that more polar antioxidants exhibit lower capacity in more polar media (Alavi Rafiee *et al.,* 2018), is not applicable in many cases.

4. CONCLUSIONS

The present study showed that the novel lipophilic derivatives of gallic acid, **5a** and **5b**, demonstrated excellent oxidative stability in emulsion and bulk oil systems at high and moderate temperatures. Their efficiency in the alcoholic medium of the polar DPPH system compared to GA slightly decreased, indicating that the polar paradox theory may not always be applicable. Overall, the better antioxidant effectiveness of the new antioxidants compared to other phenolics studied herein justifies the initial premise—i.e., a synergistic effect leading to stronger antioxidant activity may result from a medley of phenolic antioxidants covalently bonded together in one molecule. Thus, these new lipophilic antioxidants may be utilized industrially as functional ingredients with strong antioxidant potential in different food processing conditions following a further study on the proper characterization of their safe consumption.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this work.

REFERENCES

AL Zahrani NA, El-Shishtawy RM, Asiri, AM. 2020. Recent developments of gallic acid derivatives and their hybrids in medicinal chemistry: A review. *Eur. J. Med. Chem.* **204**, 112609. https://doi.org/10.1016/j.ejmech.2020.112609

Alavi Rafiee S, Farhoosh R, Sharif A. 2018. Antioxidant activity of gallic acid as affected by an extra carboxyl group than pyrogallol in various oxidative environments. *Eur. J. Lipid Sci. Technol.* **120**, 1–8. https://doi.org/10.1002/ejlt.201800319

AOCS. 1995. *Official Methods and Recommended practices of the American Oil Chemists' Society* (4th ed.). Champaign, IL (USA): AOCS Press.

AOCS. 2011. *Official methods and recommended practices of the American Oil Chemists' Society*. AOCS Method Cd8b-90. Champaign, IL. (USA): AOCS Press.

Asnaashari M, Farhoosh R, Sharif A. 2014. Antioxidant activity of gallic acid and methyl gallate in triacylglycerols of Kilka fish oil and its oil-in-water emulsion. *Food Chem.* **159**, 439–444. https://doi.org/10.1016/j.foodchem.2014.03.038

Crauste C, Rosell M, Durand T, Vercauteren J. 2016. Omega-3 polyunsaturated lipophenols, how and why? *Biochimie* **120**, 62–74. https://doi.org/10.1016/j.biochi.2015.07.018

Dodo K, Minato T, Noguchi-Yachide T, Suganuma M, Hashimoto Y. 2008. Antiproliferative and apoptosis-inducing activities of alkyl gallate and gallamide derivatives related to (-)-epigallocatechin gallate. *Bioorg. Med. Chem.* **16**, 7975–7982. https://doi.org/10.1016/j.bmc.2008.07.063

Farhoosh R, Johnny S, Asnaashari M, Molaahmadibahraseman N, Sharif A. 2016. Structure-antioxidant activity relationships of o-hydroxyl, o-methoxy, and alkyl ester derivatives of p-hydroxybenzoic acid. *Food Chem.* **194**, 128–134. https://doi.org/10.1016/j.foodchem.2015.08.003

Farhoosh R, Nyström L. 2018. Antioxidant potency of gallic acid, methyl gallate and their combinations in sunflower oil triacylglycerols at high temperature. *Food Chem.* **244**, 29–35. https://doi.org/10.1016/j.foodchem.2017.10.025

Guitard R, Paul JF, Nardello-Rataj V, Aubry JM. 2016. Myricetin, rosmarinic and carnosic acids as superior natural antioxidant alternatives to α-tocopherol for the preservation of omega-3 oils. *Food Chem.* **213**, 284–295. https://doi.org/10.1016/j.foodchem.2016.06.038

Guo Y, Guo Y, Xie Y, Cheng Y, Qian H, Yao W. 2017. Regeneration of tert-butylhydroquinone by tea polyphenols. *Food Res. Int.* **95**, 1–8. https://doi.org/10.1016/j.foodres.2017.02.009

Hirose T, Takai H, Watabe M, Minamikawa H, Tachikawa T, Kodama K, Yasutake M. 2014. Effect of alkoxy terminal chain length on mesomorphism of 1,6-disubstituted pyrene-based hexacatenar liquid crystals: Columnar phase control. *Tetrahedron* **70**, 5100–5108. https://doi.org/10.1016/j.tet.2014.05.111

Huang Y, Jiang Z, Liao X, Hou J, Weng X. 2014. Antioxidant activities of two novel synthetic methylbenzenediol derivatives. *Czech J. Food Sci.* **32**, 348–353. https://doi.org/10.17221/283/2013-cjfs

Jiang ZW, Weng XC, Huang Y, Hou JP, Liao XY. 2014. Synthesis and antioxidant activity of two novel tetraphenolic compounds derived from toluhydroquinone and tertiary butylhydroquinone. *Grasas Aceites* **65** (2), e016. https://doi.org/10.3989/gya.086513

Jung MY, Choi DS. 2016. Protective effect of gallic acid on the thermal oxidation of corn and soybean oils during high temperature heating. *Food Sci. Biotechnol.* **25**, 1577–1582. https://doi.org/10.1007/s10068-016-0243-z

Kancheva VD, Boranova PV, Nechev JT, Manolov II. 2010. Structure-activity relationships of new 4-hydroxy bis-coumarins as radical scavengers and chain-breaking antioxidants. *Biochimie* **92**, 1138–1146. https://doi.org/10.1016/j.biochi.2010.02.033

Li J, Wang T, Wu H, Ho C-T, Weng X. 2006. 1,1-Di-(2 ',5 '-dihydroxy-4 '-tert-butylphenyl)ethane: A novel antioxidant. *J. Food Lipids* **13**, 331–340.

Mansouri H, Farhoosh R, Rezaie M. 2020. Interfacial performance of gallic acid and methyl gallate accompanied by lecithin in inhibiting bulk phase oil peroxidation. *Food Chem.* **328**, 127128. https://doi.org/10.1016/j.foodchem.2020.127128

Maszewska M, Florowska A, Dłuzewska E, Wroniak M, Marciniak-Lukasiak K, Zbikowska A.

2018. Oxidative stability of selected edible oils. *Molecules* **23**, 15–17. https://doi.org/10.3390/molecules23071746

Olajide TM, Pasdar H, Weng XC. 2018. A novel antioxidant: 6,6’-(butane-1,1-diyl)bis(4-methylbenzene-1,2-diol). *Grasas y Aceites* **69**, 1–7. https://doi.org/10.3989/gya.0344181

Olajide TM, Liu T, Liu H, Weng X. 2020. Antioxidant properties of two novel lipophilic derivatives of hydroxytyrosol. *Food Chem.* **315**, 126197. https://doi.org/10.1016/j.foodchem.2020.126197

Panya A, Laguerre M, Lecomte J, Villeneuve P, Weiss J, McClements JD, Decker EA. 2010. Effects of chitosan and rosmarinate esters on the physical and oxidative stability of liposomes. *J. Agric. Food Chem.* **58***,* 5679–5684. https://doi.org/10.1021/jf100133b

Saad B, Sing YY, Nawi MA, Hashim NH, Mohamed Ali AS, Saleh MI, Sulaiman SF, Talib KM, Ahmad K. 2007. Determination of synthetic phenolic antioxidants in food items using reversed-phase HPLC. *Food Chem.* **105**, 389–394. https://doi.org/10.1016/j.foodchem.2006.12.025

Schwarz K, Huang S, German JB, Tiersch B. 2000. A*ctivities of antioxidants are affected by colloidal properties of oil-in-water and water-in-oil emulsions and bulk oils*. *J. Agric. Food Chem*. **48***,* 4874–4882. https://doi.org/10.1021/jf991289a

Silva FAM, Borges F, Guimarães C, Lima JLFC, Matos C, Reis S. 2000. Phenolic acids and derivatives: Studies on the relationship among structure, radical scavenging activity, and physicochemical parameters. *J. Agric. Food Chem*. **48**, 2122–2126. https://doi.org/10.1021/jf9913110

Teixeira J, Silva T, Benfeito S, Gaspar A, Garrido EM, Garrido J, Borges F. 2013. Exploring nature profits: Development of novel and potent lipophilic antioxidants based on galloyl-cinnamic hybrids. *Eur. J. Med. Chem.* **62**, 289–296. https://doi.org/10.1016/j.ejmech.2012.12.049

Torres de Pinedo A, Peñalver P, Morales JC. 2007. Synthesis and evaluation of new phenolic-based antioxidants: Structure-activity relationship. *Food Chem.* **103**, 55–61. https://doi.org/10.1016/j.foodchem.2006.07.026

Weng XC, Huang Y. 2014. Relationship structure-antioxidant activity of hindered phenolic compounds. *Grasas Aceites* **65,** e051. https://doi.org/10.3989/gya.0225141

Zhang CX, Wu H, Weng XC. 2004. Two novel synthetic antioxidants for deep frying oils. *Food Chem.* **84**, 219–222. https://doi.org/10.1016/S0308-8146(03)00205-X

Zhong Y, Shahidi F. 2012. Lipophilised epigallocatechin gallate (EGCG) derivatives and their antioxidant potential in food and biological systems. *Food Chem.* **131**, 22–30. https://doi.org/10.1016/j.foodchem.2011.07.089

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Removal of DBP from evening primrose oil with activated clay modified by chitosan and CTAB

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SUMMARY: The pollution of phthalic acid esters (PAEs) in edible oils is a serious problem. In the current study, we attempt to remove dibutyl phthalate ester (DBP) from evening primrose oil (EPO) with modified activated clay. The activated clay, commonly used for de-coloration in the oil refining process, was modified by chitosan and hexadecyl trimethyl ammonium bromide (CTAB). The modifications were characterized by SEM, XRD, and FT-IR. We further tested the DBP adsorption capacity of CTAB/chitosan-clay and found that the removal rate was 27.56% which was 3.24 times higher than with pristine activated clay. In addition, the CTAB/chitosan-clay composite treatment had no significant effect on the quality of evening primrose oil. In summary, the CTAB/chitosan-clay composite has a stronger DBP adsorption capacity and can be used as a new adsorbent for removing DBP during the de-coloration process of evening primrose oil.

KEYWORDS: *Activated clay; Evening primrose oil; Dibutyl phthalate ester.*

RESUMEN: ***Eliminación de DBP en aceite de onagra mediante arcilla activada modificada por chitosán y CTAB.*** La contaminación por ésteres de ácido ftálico (PAEs) en los aceites comestibles es un problema grave. En el presente estudio, intentamos eliminar el éster de ftalato de dibutilo (DBP) del aceite de onagra (EPO) con arcilla activada modificada. La arcilla activada, comúnmente utilizada en la decoloración en el proceso de refinación de los aceites, fue modificada con chitosán y bromuro de hexadecil trimetil amonio (CTAB). Las modificaciones se caracterizaron mediante SEM, XRD y FT-IR. Además, probamos la capacidad de adsorción de DBP de CTAB / chitosán-arcilla y descubrimos que la tasa de eliminación era del 27,56%, que era 3,24 veces mayor que la arcilla activada pura. Además, el tratamiento compuesto de CTAB/chitosán-arcilla no tuvo un efecto significativo sobre la calidad del aceite de onagra. En resumen, el compuesto CTAB/chitosán-arcilla tiene una capacidad de adsorción de DBP más fuerte y se puede utilizar como un nuevo adsorbente para eliminar DBP durante el proceso de decoloración del aceite de onagra.

PALABRAS CLAVE: *Aceite de onagra; Arcilla activada; Éster de ftalato de dibutilo.*

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1. INTRODUCTION

Phthalates, or phthalate esters, are esters of phthalic acid. They are mainly used as plasticizers in industry to produce hundreds of products including packaging materials, pharmaceuticals, blood bags and tubing, and personal care products (Kamrin, 2009; Net *et al.,* 2015; Giuliani *et al.,* 2020). PAEs are used primarily to soften polyvinyl chloride. PAEs are connected to polyvinyl chloride through intermolecular forces such as van der Waals forces or hydrogen bonds, which means PAEs can be released from the polymer and cause pollution to food, water, air, and soil (Gao and Wen, 2016). Studies have shown that PAEs have acute and chronic toxic effects on both aquatic organisms and terrestrial organisms (including humans) (Martino-Andrade and Chahoud, 2010; Benjamin *et al.,* 2017). Thus, many governments have issued relevant standards to limit PAE contents in products. The U.S. Environmental Protection Agency has included 6 types of PAEs in the list of priority pollutants, and the European Union, Japan and China have their own standards to limit the content of PAEs in products (Zhang *et al.,* 2015).

Phthalates are characterized as highly soluble in fat, so food products containing a higher fat content are more likely to contain higher levels of phthalate (Liu *et al.,* 2020). Researchers have already evaluated the pollution level of PAEs in edible oils in various countries and regions (Kong *et al.,* 2022; Xiang *et al*., 2019; Zhao *et al*., 2014). Wei *et al.* (2020) collected 1016 samples of edible oil blends, soybean oil, peanut oil and rapeseed oil from all over China. They found 13.48% of the samples contained DBP, while 7.78% of the samples contained DEHP. Similar work was also done by Bi *et al.* (2013). They tested the content of PAEs in 21 edible vegetable oil samples which were collected from the US market. About 90.5% of the samples contained DBP and all the samples contained DEHP. Moreover, the highest concentration in DBP was 95.8 μg/kg, while the highest concentration in DEHP was 6166 μg/kg. The high concentration in bis(2-ethylhexyl) phthalate (DEHP) in oil is higher than the maximum residual levels which set by the Chinese Government (1.5 mg/kg), (Pereira *et al.,* 2019). Thus, DBP and DEHP in oil should be removed.

However, the current research on the removal of PAEs in oil is relatively simple (Kotowska *et al.,* 2020; Pang *et al.,* 2021). Adsorption, steam distillation and molecular distillation are the three most widely investigated methods. Among them, molecular distillation has the highest removal efficiency of PAEs, generally up to 90% or more (Chen *et al.,* 2019; Xiong *et al.,* 2013; Gelmez *et al.,* 2017), but this method is difficult to be widely used due to its high cost and difficulties in industrialization. Adsorption and steam distillation are widely used in the refining process of de-coloration and deodorization processes in edible oil. Some researchers have reported that steam distillation has a certain ability to remove PAEs. Chen *et al.* (2019) successfully removed 82% of DBP and 66% of DEHP in sea buckthorn fruit oil with two consecutive steam distillation treatments. In our work, we applied steam distillation to the refining process of evening primrose oil. We found significant differences in different batches. That is, in a certain batch, the PAE can be removed successfully. However, in another batch nearly no PAEs could be removed. We cannot solve this problem at present. Hence, we referred to the adsorption method. The adsorbents (activated carbon, activated clay, attapulgite and diatomite) commonly used in the oil refining process have very little adsorption capacity for PAEs. Therefore, a novel type of adsorbent, which is low in cost and easy to operate is needed for removing PAEs from evening primrose oil.

Activated clay is the most commonly used adsorbent in the de-coloration process of edible oil (Zhang *et al*., 2021). There are some studies that have shown that after modification with CTAB and chitosan, the adsorption capacity of activated clay can be greatly increased. Cao *et al.* (2014) used CTAB to organically modify Na-bentonite. The results of XRD and BET showed that the pore size and interlayer spacing of bentonite became larger after modification, and the removal rate of phenol in water by modified clay could reach 81.36%. Rahardjo *et al.* (2011) prepared CTAB modified bentonite. XRD and FT-IR results showed that CTAB was successfully inserted between the layers so that the distance between the bentonite layers became larger, that the surface structure changed to a certain extent, and that the affinity for hydrophobic substances became stronger. Compared to unmodified bentonite, modified bentonite exhibited stronger adsorption capacity for organic matter and heavy metal ions. Guo *et al*. (2012) prepared a chitosan/CTAB combined modified activated clay. After modification, the adsorption efficiency of the clay on the weakly-acidic scarlet dye could reach up to 85%. Therefore, in the current work, we prepared a CTAB/chitosan-clay composite. Then we studied the surface characteristics of the adsorbent and the adsorption capacity of DBP in evening primrose oil (EPO), and investigated the effect of CTAB/chitosan-clay composite treatment on the quality of EPO. At the same time, the adsorption mechanism of the CTAB/chitosan-clay composite to adsorb DBP in EPO was determined.

2. MATERIALS AND METHODS

2.1. Materials

Evening primrose oil (EPO) was collected from the Baili Biotech Company in Changchun, Jilin, China. Activated Clay was purchased from a Chinese market (Decolorization rate ≥ 98%, Activity ≥ 100 mmol/kg, free acid content ≤ 0.5%, moisture content ≤ 12.0%). CTAB (purity ≥ 98%) and chitosan (Viscosity = 50-100 mpa, 95% degree of deacetylation) were purchased from Roche. Standard dibutyl phthalate ester was purchased from Aladdin, China. The chemical reagents involved in GC-MS detection were of chromatographic purity (purchased from CNW, Germany). All other chemical reagents (including solvents) were of analytical grade and purchased from local suppliers. Water was purified by the Milli-Q water purification system (Millipore, Bedford, USA).

2.2. Synthesis of CTAB/chitosan-clay composite

The CTAB/chitosan-clay composite was prepared in the ratio chitosan: CTAB: clay = 0.2:0.3:1. The chitosan (2 g) was dissolved in a 300 mL 3% acetic acid solution, and stirred at 60 °C in a temperature-controlled water bath until the chitosan was completely dissolved. Then the chitosan solution was mixed with 10 g activated clay and 3 g CTAB. The mixture was again subjected to moderate stirring in a temperature-controlled water bath at 60 °C for 6 h. The mixtures were centrifuged and washed with deionized water. The wet mixture was dried at 80 °C for 48 h. After the formation of CTAB/chitosan-clay, the composite was sieved to obtain a powder.

2.3. Characterization techniques of CTAB/chitosan-clay composite

The preparation of CTAB/chitosan-clay composite was verified by a series of characterization methods. The surface morphologies of the pristine activated clay and CTAB/chitosan-clay composite were determined by scanning electron microscope (SEM, ZEISS Gemini SEM500), with 2-3 kV working voltage and 10-100 K magnification, by spraying gold-plated palladium alloy onto the sample surface before testing. CTAB/chitosan-clay composite and pristine activated clay were recorded on a powder X-ray diffractometer (XRD, Smartlab 9 kW, operating at 40 kV). The CuKa radiation source with a wavelength of 1.54 Å was used and the data was collected for a wide-angle region ranging between 5 and 80° on a 2θ scale with a scan rate of 5°/min. The surface area of the composite was measured by BET measurements, using a Micromeritics ASAP 2020 instrument at liquid nitrogen temperature (77 K) via nitrogen gas adsorption. The Fourier-transformed infrared (FT-IR) spectra of the CTAB/chitosan-clay composite and chitosan and pristine activated clay were recorded on an IR Prestige-21 FTIR in KBr pellets. The detailed work is shown in a former paper.

2.4. Methodology for adsorption of DBP in EPO

Adsorption experiments were carried out in batch mode in a temperature-controlled water bath with constant stirring under vacuum. The removal rate of DBP in oil was determined and the adsorption efficiency was calculated as:

$$\text{Adsorption efficiency (\%)} = \frac{C_o - C_e}{C_o} * 100\%$$

Where c_o and c_e (mg/kg) are the initial and equilibrium concentrations of DBP in the reaction medium. Adsorption kinetics were obtained by treating 2 g of CTAB/chitosan-clay composite with 20 g EPO of 10 mg/kg DBP of initial concentration in the time range 0-540 min under optimized adsorption conditions. The adsorption isotherms were obtained by treating 2 g of CTAB/chitosan-clay composite with 20 g EPO of DBP of initial concentration in the range of 1-25 mg/kg under optimized adsorption conditions.

2.5. DBP composition analysis in EPO by GC-MS

Oil sample preparation was performed according to Chinese national standard GB 5009.271-2016. DBP determination was performed using a Shimadzu GC-MS QP-2010 Ultra. A Rxi-5MS column (30 m × 0.25 mm × 0.25 μm) was from Shimadzu Inc. Oven temperature was set initially at 60 °C for 1 min, programmed to increase at 20 °C/min to 220 °C and held for 1min, then increased to 250 °C at 5 °C/min, held for 1min and then increased to 290 °C at 20 °C/min, the temperature was then maintained for 6 min. Helium (99.999% purity) was used as a carrier gas at a constant flow rate of 1.0 mL/min and the injection volume was 1 μL. The injector, transfer and ion source temperatures, were set at 260, 230 and 280 °C, respectively. EI was used as the electron bombardment ion source and the ionization energy was 70 eV. Scan mode (Scan) was used for qualitative and selective ion monitoring and (SIM) was used for the quantitative analysis. The qualifier ion of DBP was 91, 206, 238(m/z), while the quantitative ion was 149 (m/z).

2.6. Analysis of physicochemical properties of evening primrose oil

The peroxide value (POV) was determined according to Chinese national standard GB 5009.227-2016 and the results were expressed as millimole (mmol)/kg of oil. The acid value (AV) was measured according to Chinese national standard GB/T 5009.229-2016 and expressed in mg KOH/g. The iodine value (IV) was evaluated by reference to Chinese national standard GB/T 5532-2008. The *p*-anisidine value (*p*-AV) was measured by Chinese national standard GB/T 24304-2009.

2.7. Analysis of fatty acid composition

The fatty acid composition was determined according to the method proposed by Pan *et al*. (2020) with few modifications. Briefly, 100 mg (accurate to 0.1 mg) EPO were mixed with 2 mL of n-heptane and 2 mL of 2 M KOH-methanol, and shaken vigorously until well mixed. After the mixture was allowed to stand for stratification, the supernatant was taken out, and an appropriate amount of anhydrous Na_2SO_4 was added to remove the water. Finally, after passing the mixture through a 0.22 μm organic filter membrane, the supernatant could be analyzed. Samples were subjected to a Shimadzu GC-MS QP2010Ultra and a Rxi-5MS column (30 m × 0.25 mm × 0.25 μm). The oven temperature was set at an initial temperature of 160 °C, held for 5 min, then increased at 2 °C/min to 220 °C, held for 10 min, programmed to increase at 4 °C/min to 240 °C with a final holding time of 10 min. Helium (99.999% purity) was used as carrier gas in a constant flow of 1.0 mL/min and the injection volume was 1 μL, with an AOC-20i autosampler split ratio of 1:50. The temperatures of injector, transfer and ion source were set at 260, 200 and 250 °C, respectively. The ionization energy was 70 eV and the change in total ion current in the range of m/z 50-500 was recorded. Fatty acids were identified by comparison with data and the NIST Mass Spectrometry Library (National Institute of Standards and Technology, Gaithersburg, MD, USA). The results were recorded across the percentage of the relative peak areas.

2.8. Statistical analysis

The samples were measured in triplicate, and the results were expressed as mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1. Characterization of the CTAB/chitosan-clay composite

The SEM results clearly indicated that the surface morphology of clay changed after modification. As shown in Figure 1(a), the pristine activated clay showed a typical agglomerated flake structure. The surface of unmodified clay was relatively smooth, flat and compact. On the contrary, the CTAB/chitosan-clay composite had a heavier curling degree at the edge, and with a looser interlayer structure and rougher surface. The looser interlayer structure could increase the diffusion of PAEs into the CTAB/chitosan-clay composite (Alshameri *et al.,* 2018). These characters changed together mean that more functional groups might be exposed to the environments and the functional group could interact with PAE more efficiently. Thus, the modified clay could remove the DBP from evening promise oil more efficiently.

The absorption peak positions of pristine activated clay before and after modification were basically the same according to the XRD results (Figure 1(b)). The results indicated that the addition of CTAB and chitosan did not change the basic structure, which is an agglomerated flake structure, of activated clay. The main changes after modification might refer to the looser interlayer structure. According to pervious research, the silicate group in the pristine activated clay may interact with the hydroxyl group of chitosan by hydrogen bonding. Thus, the silicate group played the role of an anchor and held the chitosan (Mohd *et al.*, 2018). The silicate group in the pristine activated clay was considered to remain constant while the long carbon chain of CTAB was directly inserted between the layers of the pristine activated clay. The position of the characteristic diffraction peak of d (001) existing in the clay close to 2θ=5° shifted to the left. From the Bragg equation (2dsinθ=nλ), it could be seen that the layer spacing of pristine activated clay increased after modification. In addition, the material composition analysis of the XRD structure showed that the main components of the adsorbent before and after the modification were basically the same, which were quartz, albite and illite.

Furthermore, the Brunauer-Emmett-Teller (BET) surface area of the pristine activated clay was measured as 137.0916 m^2/g, while the BET surface area of the CTAB/chitosan-clay composite was 18.4283 m^2/g. Furthermore, the pore volume of the pristine activated clay was determined to be 0.1909 cm^3/g, and the CTAB/chitosan-clay composite's pore volume was 0.0843 cm^3/g. The decrease in the surface area and pore volume was attributable to the intercalation of CTAB and chitosan molecules in the interlayers of pristine activated clay which resulted in the pore blockage (Budyak *et al.*, 2016; Zhang *et al.*, 2009). However, CTAB/chitosan-clay composite (15.9528 nm) had a larger pore size than pristine activated clay (6.6768 nm). Pore size influences the behavior of the adsorbate−adsorbent system (Wang *et al.*, 2020). A suitable size of pore may help in the absorptions process.

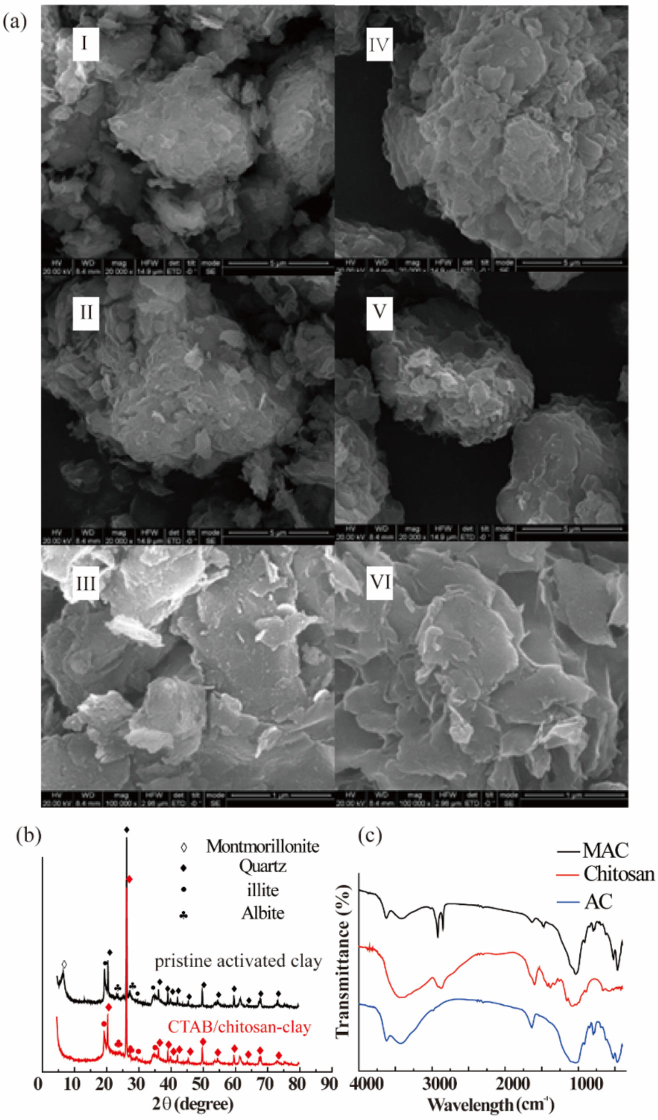

FIGURE 1. Characterization test results of activated clay and CTAB/chitosan-clay composite: (a)SEM(I, II, III for activated clay and IV, V, VI for CTAB/chitosan-clay composite), (b)XRD, (c)FT-IR.

The FT-IR (Figure 1(c)) of chitosan, pristine activated clay and CTAB/chitosan-clay composite revealed the changes in the functional groups in the composite. The peak shape of the infrared spectrum of the activated clay before and after modification did not change significantly, and the characteristic absorption peaks of the activated clay appeared, indicating that the modification did not change the typical agglomerated flake structure of the activated clay, which was consistent with the XRD results. There were four new peaks at 2920 cm^{-1}, 2850 cm^{-1}, 1482 cm^{-1} in CTAB/chitosan-clay composite. The peaks at 2920 cm^{-1} and 2850 cm^{-1} were asymmetric and symmetric stretching vibration peaks of CTAB's and chitosan's -CH-, respectively. The peak at 1482 cm^{-1} was a symmetric bending vibration of -CH-, which was detected at 1443 cm^{-1} in original activated clay (Fu *et al.*, 2017). These results indicated that there were certain interactions between the CTAB, chitosan and activated clay.

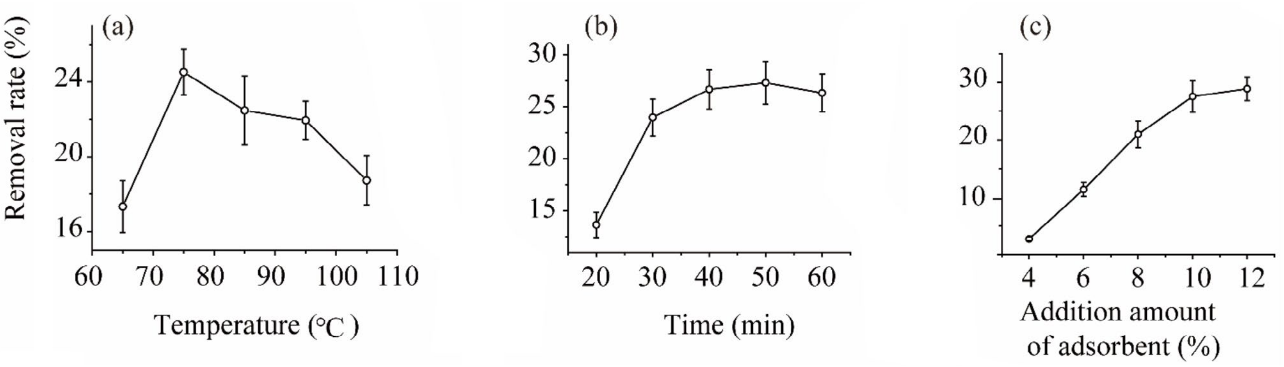

Figure 2. Effect of (a) temperature, (b) time, (c) adsorbent doses (%, Madsorbent/MEPO) on adsorption efficiency of DBP in evening primrose oil, initial concentration of DBP=2 mg/kg, weight of evening primrose oil=20 g. Each value in the figure represents the mean ± SD (n=3).

3.2. The optimization of adsorption conditions

To optimize the parameters for removing DBP from EPO, we investigated the influence of temperature, time and the additional amount of adsorbent on the adsorption. The entire adsorption was carried out under vacuum conditions. The adsorption results are shown in Figure 2.

The influence of temperature is shown in Figure 2(a). The adsorption rate of DBP could be increased from 17.33 to 24.52% when the temperature increased from 65 to 75 °C. However, when the temperature ranged from 75 to 105 °C, the adsorption rate of DBP showed a downward trend. This might be due to the higher temperature accelerating the resolution of DBP on the adsorbent, resulting in a decrease in its adsorption rate. Hence, the optimal temperature was chosen as 75 °C. The influence of time is shown in Figure 2(b), When the adsorption time increased from 20 to 40 min, the adsorption rate of DBP increased rapidly from 14 to 28%. After that, the adsorption rate slowed down and fluctuated at 30%. Hence, considering the efficiency, the optimal time was chosen as 40 min. Similar trends were found in the adsorbent dose. As shown in Figure 2(c), the adsorption rate of DBP increased with the increase in adsorbent dose. However, when the amount of adsorbent added exceeded 10% (w/w), the growth of DBP adsorption efficiency slowed down significantly. This might be because too much adsorbent would reduce the adsorption capacity per unit mass of adsorbent and affect the utilization rate of the adsorbent. Hence, the optimal amount of adsorbent was chosen as 10%. In short, under the optimal conditions described above, the adsorption rate of the adsorbent to DBP was 27.56%, while the removal rate of pristine activated clay was only 8.51% under the same conditions.

3.3. The change in the properties of evening primrose oil

The physical and chemical properties of evening primrose oil treated by activated clay (named as AC-EPO) and CTAB/chitosan-clay composite (named as MAC-EPO) were determined under the same conditions. The physical and chemical indicators of evening primrose oil processed with different clay are shown in Table 1. There were no significant differences in acid value, peroxide value, or *p*-AV, among AC-EPO and MAC-EPO, and iodine value.

The *p*-AV can be used to measure the number of secondary products such as aldehydes, ketones, and

Table 1. Comparison of the quality of evening primrose oil treated by activated clay and CTAB/chitosan-clay composite

	AV (KOH) mg/g	POV mmol/kg	IV $g\cdot 100g^{-1}$	*p*-AV	C16:0 (%)	C18:0 (%)	C18:1 (%)	C18:2 (%)	C18:3n3 (%)	C18:3n6 (%)
AC-EPO	0.38±0.04a	1.38±0.09b	163.22±2.19c	6.22±0.24d	8.6±0.09e	2.89±0.09f	5.77±0.21g	71.27±0.39h	0.30±0.03i	9.73±0.18j
MAC-EPO	0.40±0.02a	1.29±0.07b	161.01±1.48c	7.68±0.14d	8.52±0.16e	2.9±0.07f	6.37±0.32g	70.45±0.46h	0.47±0.02i	9.52±0.21j

Each value in the table represents the mean ± SD(n=3). AV: Acid value, POV: Peroxide value, IV: Iodine value, *p*-AV: *p*-Anisidine value. Letters in the same column indicate the significance of evening primrose oil treated by different adsorbent: the same letters represent no significant difference ($p > 0.05$), and different letters represent significant difference ($p < 0.05$), compared by ANOVA (Tukey-Kramer HSD test).

quinones in the oil. In the oil refining process, the *p*-AV value of the oil in the decolorization process can be the highest. This is because the clay can catalyze the decomposition of hydroperoxides to generate aldehydes and ketones (Kreps *et al.*, 2014). The CTAB/chitosan-clay had a stronger catalytic ability to hydroperoxide in oil, which increased the contents in aldehydes and ketones, and finally made the *p*-AV value of MAC-EPO higher than AC-EPO.

AV is an important indicator of oil quality, and IV is the response to the degree of unsaturation of oils. The AV of AC-EPO and MAC-EPO were both around 0.4, while the IV in MAC-EPO dropped slightly. This might be due to insufficient vacuum during the adsorption process, so some unsaturated fatty acids were oxidized.

3.4. Comparison between the fatty acid compositions of evening primrose oil with different treatments

The main fatty acids contained in EPO are α-linolenic acid, γ-linolenic acid, linoleic acid, oleic acid, stearic acid and palmitic acid, which can reach more than 98% (Zhao *et al.*, 2019). Linoleic acid (C18:2) is the major fatty acid in EPO, which can reach more than 70%. γ-linolenic acid (C18:3n6), which is a characteristic nutrient of evening primrose oil, and its content is required to be above 9%. Table 1 lists the fatty acid contents in AC-EPO and MAC-EPO, respectively. The fatty acid composition of AC-EPO and MAC-EPO was not significantly different. The total amount of 5 fatty acids in AC-EPO was 98.56% and the amount of unsaturated fatty acids (UFA) was 87.07%, while the total amount of 5 fatty acids in MAC-EPO reached 98.23% and the amount of UFA was 86.81%. Therefore, the CTAB/chitosan-clay composite adsorption treatment had almost no effect on the fatty acid composition of EPO.

3.5. Adsorption kinetics

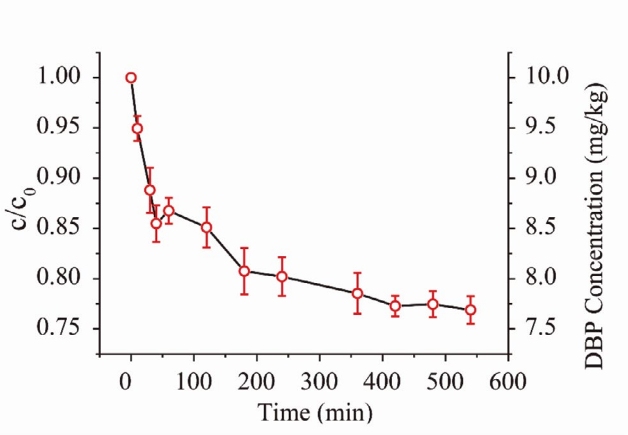

FIGURE 3. Changes in the remaining concentration of DBP in evening primrose oil over time. Adsorption kinetics were obtained by treating 2 g of CTAB/chitosan-clay composite with 20 g evening primrose oil of co=10 mg/kg DBP of initial concentration in the time range 0-540 min under optimized adsorption conditions. c was the concentration of DBP at time t.

The adsorption kinetics test was performed. 10 mg/kg DBP were added to the evening primrose oil and the amount of DBP was recorded over time. The residual concentration of DBP and the interaction time of the CTAB/chitosan-clay composite adsorption treatment was plotted. The results are shown in Figure 3. It can be seen that the adsorption amount of DBP by the CTAB/chitosan-clay composite material increased with the increase in contact time, to reach an equilibrium state at about 420 minutes. We employed two kinetic models, pseudo-first-order and pseudo-second-order kinetic models, to study the adsorption process of DBP on CTAB/chitosan-clay composite. The pseudo-first-order kinetic model is represented by the following formula (Mohd *et al.*, 2018):

$$\log(q_e - q_t) = log q_e - \frac{k_1}{2.303} t$$

$$q_e = \frac{c_o - c_e}{m_{adsorbent}} * m_{EPO}$$

The pseudo-second-order kinetic model is represented as (Mohd *et al.*, 2018):

$$\frac{t}{q_t} = \frac{1}{k^2 q_e^2} + \frac{t}{q_e}$$

Where k_1 and k_2 are the rate constants of the pseudo-first-order and pseudo-second-order adsorption kinetic equations. c_0 and c_e are the initial concentration and equilibrium concentration of DBP, respectively. q_e and q_t are the adsorption capacity of the adsorbent in equilibrium and at time t, respectively. The slope and intercept of the log(q_e-q_t) versus t and t/q_t versus t curves were used to derive k_1 and k_2, respectively.

Figure 4 shows the linear fitting of two kinetic models, and the parameters obtained after fitting are listed in Table 2. The linear regression coefficient value of the pseudo-second-order kinetic model was

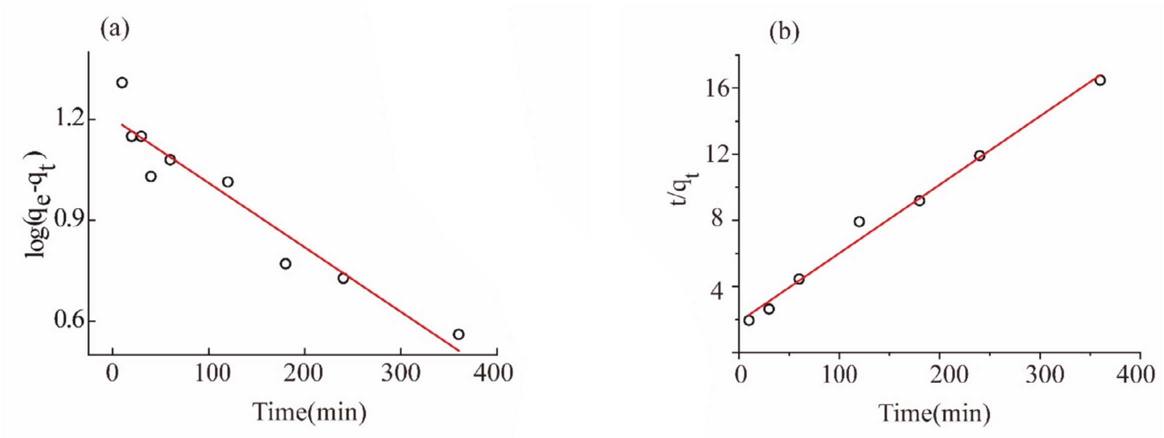

Figure 4. Adsorption kinetics of DBP in the CTAB/chitosan-clay composite fitted with (a) pseudo-first-order model; (b) pseudo-second-order model. Adsorption kinetics were obtained by treating 2 g of CTAB/chitosan-clay composite with 20 g evening primrose oil of co=10 mg/kg DBP of initial concentration in the time range 0-540 min under optimized adsorption conditions.

higher (R^2=0.9902), which could better describe the adsorption of DBP on CTAB/chitosan-clay composite. The pseudo-second-order kinetic model assumes that the rate-limiting step is chemical sorption or chemisorption and predicts the behavior over the whole range of adsorption (Bujdák, 2020). In other words, the adsorption rate is dependent on adsorption capacity and not on the concentration of adsorbate. Thus, the future research should focus on the modification of clay and increase the adsorption capacity of the clay. In addition, the q_e value obtained by the pseudo-second-order kinetic model was closer to the q_e value obtained from the experiment. Furthermore, the pseudo-second-order kinetic model favors the chemisorption concerning valency forces through the sharing or exchanging of electrons between adsorbent and adsorbate. Hence, the results indicate that there might be an electron exchange between the adsorbent and the adsorbate (Ho *et al.*, 2006). The chitosan and CTAB are typical positive molecules, while the dibutyl phthalate esters are negative. The electrostatic interactions between them may contribute during absorptions.

3.6. Adsorption isotherms

The adsorption isotherm curve is the relationship curve of the concentration of the reaction solute molecules in the two phases when the adsorption process reaches equilibrium at a certain temperature. The DBP adsorption on CTAB/chitosan-clay composite was studied with Langmuir and Freundlich adsorption isotherm models. The initial concentrations of the DBP (c_o) in evening primrose oil were taken in the range of 1-25 mg/kg. Determined the equilibrium concentration of DBP (c_e), and the q_e value of the adsorbent. The linear Freundlich adsorption isotherm model is represented by the following formula (Acikyildiz *et al.*, 2015):

$$\log q_e = logK_F + \frac{1}{n} logc_e$$

Among them, K_F and n are Freundlich isothermal constants, which are related to adsorption capacity and strength, respectively. Usually, it is considered that the reaction conditions are conducive to the pro-

Table 2. **Adsorption capacity and adsorption kinetics for DBP sorption by CTAB/chitosan-clay fitted with pseudo first order and pseudo second order models**

Sample	$q_{e,exp}$ (mg/kg)	Pseudo first order kinetic			Pseudo second order kinetic		
		q_{1e} (µg/g)	k_1 (min^{-1})	R^2	q_{2e} (µg/g)	k_2 (min^{-1})	R^2
CTAB/chitosan-clay	25.51	16.08	0.005	0.9143	24.10	0.001	0.9902

k_1 and k_2 are the rate constants of the pseudo-first-order and pseudo-second-order adsorption kinetic equations. q_e and $q_{e\ exp.}$ are the adsorption capacity of the adsorbent in equilibrium in theory and reality, respectively.

Table 3. **Adsorption isotherm parameters for DBP sorption by CTAB/chitosan-clay composite**

Langmuir parameters			Freundlich parameters		
R^2	q_m (μg/g)	K_L	R^2	K_F	n
0.9307	73.53	0.033	0.9886	2.16	1.13

K_F and n are Freundlich isothermal constants and K_L is the constant of the Langmuir equation. q_m is the theoretical maximum adsorption capacity of DBP derived from the Langmuir equation.

gress of adsorption when n>1. Values of K_F, n and R^2 are given in Table 3.

The linear Langmuir adsorption isotherm model is represented as (Acikyildiz *et al*., 2015):

$$\frac{c_e}{q_e} = \frac{1}{q_m K_L} + \frac{c_e}{q_m}$$

The equilibrium parameter, R_L is evaluated as:

$$R_L = \frac{1}{1 + (1 + K_L c_o)}$$

Where q_m (μg/g) is the theoretical maximum adsorption capacity of the adsorbent. K_L is the constant of the Langmuir equation. R_L is the dimensionless constant separation factor. When $0 < R_L < 1$, the adsorption process is preferential adsorption. The K_L, q_m and R^2 values are given in Table 3.

In Figure 5, the correlation coefficients R^2 of Langmuir and Freundlich were both above 0.9, which were 0.9307 and 0.9886, respectively. This indicated that the adsorption process was dominated by monolayer adsorption, which might include physical and chemical adsorption. Similarly, the value of R_L (between 0.35 and 0.5) and the value of n (n > 1) also showed a favorable adsorption process.

4. CONCLUSIONS

In this study, we modified the activated clay modified by chitosan and CTAB and applied this composite for the absorption of DBP in evening primrose oil. We optimized the absorption conditions for this CTAB/chitosan-clay composite. When the amount of CTAB/chitosan-clay composite, temperature and time were 10%, 75 °C, and 40 min, respectively, the maximum adsorption rate of the adsorbent to DBP was 27.56%, which is 3.24 times higher than the adsorption efficiency of pristine activated clay. In addition, we tested the physicochemical properties and fatty acid composition of evening primrose oil treated with activated clay and CTAB/chitosan-clay. The results indicate that the modification did not influence evening primrose oil, which indicated that the CTAB/chitosan-clay could be applied in the industry.

We also investigated the possible mechanism of the absorptions of the CTAB/chitosan-clay composite. According to the SEM and XRD, we found that the specific surface area and pore volume of the CTAB/chitosan-clay composite were reduced, while the pore diameter and interlayer spacing became larger. This phenomenon may be due to the loading of functional groups to the clay. The larg-

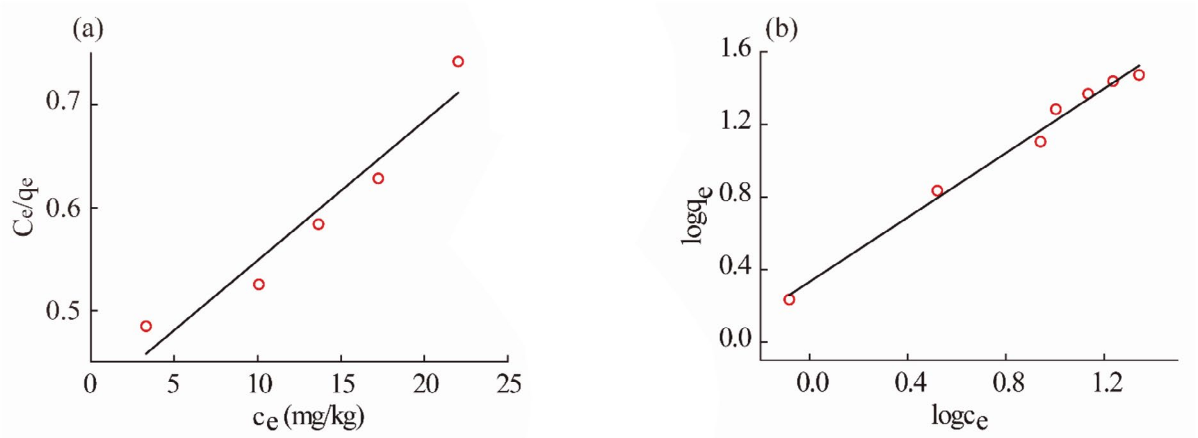

Figure 5. Adsorption isotherms of DBP in CTAB/chitosan-clay composite linearly fitted by: (a) Langmuir adsorption isotherm; (b) Freundlich adsorption isotherm. Adsorption isotherms were obtained by treating 2 g of CTAB/chitosan-clay composite with 20 g evening primrose oil and the initial DBP concentrations ranged between co = 1 and 25 mg/kg, under optimized adsorption conditions.

er pore size and interlayer spacing could help in the diffusion DBP in the CTAB/chitosan-clay composite. We also performed the absorption kinetic of the CTAB/chitosan-clay composite. The kinetic results showed that the pseudo-second-order kinetic model better described the adsorption process of DBP in the CTAB/chitosan-clay composite. The thermodynamic analysis showed that the Freundlich model more accurately described the adsorption process of DBP in the CTAB/chitosan-clay composite, and the value of R_L (from Langmuir model, between 0 and 1) and the value of n (from Freundlich model, $n > 1$) showed a favorable adsorption process. The results of the kinetics and thermodynamic analyses indicated that adsorption was dominated by monolayer adsorption, and electrostatic forces and the hydrogen bonding forces contributed most during the absorption. In conclusion, the CTAB/chitosan clay composite can be a new choice of adsorbent that can be used in the de-coloration process of evening primrose oil.

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REFERENCES

Acikyildiz M, Gurses A, Gunes K, Yalvac D. 2015. A comparative examination of the adsorption mechanism of an anionic textile dye (RBY 3GL) onto the powdered activated carbon (PAC) using various the isotherm models and kinetics equations with linear and non-linear methods. *Surf. Sci.* **354**, 279–284. https://doi.org/10.1016/j.apsusc.2015.07.021

Alshameri A, He H, Zhu J, Xi Y, Zhu R, Ma L, Tao Q. 2018. Adsorption of ammonium by different natural clay minerals: characterization, kinetics and adsorption isotherms. *Appl. Clay Sci.* **159**, 83–93. https://doi.org/10.1016/j.clay.2017.11.007

Benjamin S, Masai E, Kamimura N, Takahashi K, Anderson RC, Faisal PA. 2017. Phthalates impact human health: epidemiological evidences and plausible mechanism of action. *J. Hazard. Mater*. **340**, 360–383. https://doi.org/10.1016/j.jhazmat.2017.06.036

Bujdák J. 2020. Adsorption kinetics models in clay systems. The critical analysis of pseudo-second order mechanism. *Appl. Clay Sci.* **191,** 105630. https://doi.org/10.1016/j.clay.2020.105630

Bi XL, Pan XJ, Yuan SJ. 2013. Plasticizer Contamination in Edible Vegetable Oil in a U.S. Retail Market. *J. Agric. Food Chem.* **61**, 9502-9509. https://doi.org/10.1021/jf402576a

Budyak TM, Yanovska ES, Kichkiruk OY, Sternik D, Tertykh VA. 2016. Natural minerals coated by biopolymer chitosan: synthesis, physicochemical and adsorption properties. *Nanoscale Res. Lett.* **11**, 492. https://doi.org/10.1186/s11671-016-1696-y

Cao C, Meng L, Zhao Y. 2014. Adsorption of phenol from wastewater by organo-bentonite. *Desalin. Water Treat.* **52**, 19-21. https://doi.org/10.1080/19443994.2013.803649

Chen L, Liu Y, Deng J. 2019. Removal of phthalic acid esters from sea buckthorn (Hippophae rhamnoides L.) pulp oil by steam distillation and molecular distillation. *Food Chem.* **294**, 572-577. https://doi.org/10.1016/j.foodchem.2019.05.091

Fu L, Yang H, Tang A, Hu Y. 2017. Engineering a tubular mesoporous silica nanocontainer with well-preserved clay shell from natural halloysite. *Nano Res.* **10** (8), 2782-2799. https://doi.org/10.1007/s12274-017-1482-x

Gao DW, Wen ZD. 2016. Phthalate esters in the environment: A critical review of their occurrence, biodegradation, and removal during wastewater treatment processes. *Sci. Total Environ.* **541**, 986-1001. https://doi.org/10.1016/j.scitotenv.2015.09.148

Gelmez B, Ketenoglu O, Yavuz H. 2017. Removal of di-2-ethylhexyl phthalate (DEHP) and mineral oil from crude hazelnut skin oil using molecular distillation-multiobjective optimization for DEHP and tocopherol. *Eur. J. Lipid Sci. Technol.* **116**, 1600001. https://doi.org/10.1002/ejlt.201600001

Giuliani A, Zuccarini M, Cichelli A, Khan H, Reale M. 2020. Critical review on the presence of phthalates in food and evidence of their biological impact. International Int. *J. Environ. Res. Public Health*, **17** (16), 5655. https://doi.org/10.3390/ijerph17165655

Guo J, Chen S, Liu L. 2012. Adsorption of dye from wastewater using chitosan-CTAB modified bentonites. *J. Colloid Interface Sci.* **382**, 61-66. https://doi.org/10.1016/j.jcis.2012.05.044

Ho YS. 2006. Review of second-order models for adsorption systems. *J. Hazard. Mater.*

136(3), 681-689. https://doi.org/10.1016/j.jhazmat.2005.12.043

Kamrin MA. 2009. Phthalate Risks, Phthalate Regulation, and Public Health: A Review. *J. Toxicol. Env. Health-Pt b-Crit. Rev.* **12**, 157-174. https://doi.org/10.1080/10937400902729226

Kreps F, Vrbikova L, Schmidt S. 2014. Influence of industrial physical refining on tocopherol, chlorophyll and beta-carotene content in sunflower and rapeseed oil. *Eur. J. Lipid Sci. Technol.* **116**, 1572-1582. https://doi.org/10.1002/ejlt.201300460

Kong X, Bai Z, Jin T, Jin D, Pan J, Yu X, Cernava T. 2022. *Arthrobacter* is a universal responder to di-n-butyl phthalate (DBP) contamination in soils from various geographical locations. *J. Hazard. Mater*. **422**, 126914. https://doi.org/10.1016/j.jhazmat.2021.126914

Kotowska U, Kapelewska J, Sawczuk R. 2020. Occurrence, removal, and environmental risk of phthalates in wastewaters, landfill leachates, and groundwater in Poland. *Environ. Pollut.* **267**,115643. https://doi.org/10.1016/j.envpol.2020.115643

Liu JM, Li CY, Zhao N, Wang ZH, Lv SW, Liu JC, Chen LJ, Wang J, Zhang,Y, Wang S. 2020. Migration regularity of phthalates in polyethylene wrap film of food packaging. *J. Food Sci*, **85** (7), 2105-2113. https://doi.org/10.1111/1750-3841.15181

Martino-Andrade AJ, Chahoud I. 2010. Reproductive toxicity of phthalate esters. *Mol. Nutr. Food Res.* **54**, 148-157. https://doi.org/10.1002/mnfr.200800312

Mohd AS, Dutta RK, Sen AK. 2018. Removal of diethyl phthalate via adsorption on mineral rich waste coal modified with chitosan. *J. Mol. Liq.* **261**, 271-282. https://doi.org/10.1016/j.molliq.2018.04.031

Net S, Sempere R, Delmont A, Paluselli A, Ouddane B. 2015. Occurrence, Fate, Behavior and Ecotoxicological State of Phthalates in Different Environmental Matrices. *Environ. Sci. Technol.* **49**, 4019–4035. https://doi.org/10.1021/es505233b

Pan FG, Li YY, Luo XD, Wang XQ, Wang CS, Wen BL, Guan XR, Xu YF, Liu BQ. 2020. Effect of the chemical refining process on composition and oxidative stability of evening primrose oil. *J. Food Process Preserv.* **44**, e14800. https://doi.org/10.1111/jfpp.14800

Pang X, Skillen N, Gunaratne N, Rooney DW, Robertson PK. 2021. Removal of phthalates from aqueous solution by semiconductor photocatalysis: A review. *J. Hazard. Mater*. 402, 123461. https://doi.org/10.1016/j.jhazmat.2020.123461

Pereira J, Selbourne MD, Pocas F. 2019 Determination of phthalates in olive oil from European market. *Food Control* **98**, 54-60. https://doi.org/10.1016/j.foodcont.2018.11.003

Rahardjo AK, Susanto MJJ, Kurniawan A, Indraswati N, Ismadji S. 2011. Modified Ponorogo bentonite for the removal of ampicillin from wastewater. *J. Hazard. Mater.* **190**, 1001-1008. https://doi.org/10.1016/j.jhazmat.2011.04.052

Wang X, Cheng H, Chai P, Bian J, Wang X, Liu Y, Yin X, Pan S, Pan Z. 2020. Pore characterization of different clay minerals and its impact on methane adsorption capacity. *Ene. Fuels* **34** (10), 12204-12214. https://doi.org/10.1021/acs.energyfuels.0c01922

Wei X, Qin G, Jian X, Kailong L, Fengxiang Y, Ting C, Si Q, Can L, Fangbin W. 2020. Cumulative risk assessment of phthalates inedible vegetable oil consumed by Chinese residents *J. Sci. Food Agric.* **100**, 1124–1131. https://onlinelibrary.wiley.com/doi/epdf/10.1002/jsfa.10121

Xiang W, Gong Q, Xu J, Li KL, Yu FX, Chen T, Qin S, Li C, Wang FB. 2019. Cumulative risk assessment of phthalates in edible vegetable oil consumed by Chinese residents. *J. Sci. Food Agric.* **100**, 1124-1131. https://doi.org/10.1002/jsfa.10121

Xiong Y, Zhao ZM, Zhu LP, Chen YT, Ji HB, Yang DP. 2013. Removal of three kinds of phthalates from sweet orange oil by molecular distillation. *Food Sci. Technol.* **53**, 487-491. https://doi.org/10.1016/j.lwt.2013.04.012

Zhang Y, Liang Q, Gao R, Hou H, Tan W, He X, Yu M, Ma L, Xi B, Wang X. 2015. Contamination of Phthalate Esters (PAEs) in Typical Wastewater-Irrigated Agricultural Soils in Hebei, North China. *PLoS ONE* **10** (9), e0137998. https://doi.org/10.1371/journal.pone.0137998

Zhang A, Xiang J, Sun L, Hu S, Li P, Shi S, Fu P, Su S. 2009. Preparation, characterization and application of modified chitosan sorbents for elemental mercury removal. *Ind. Eng. Chem.* **48**, 4980-4989. https://doi.org/10.1021/ie9000629

Zhang T, Wang W, Zhao Y, Bai H, Wen T, Kang S, Song G, Song S, Komarneni S. 2021. Removal of heavy metals and dyes by clay-based adsorbents: From natural clays to 1D and 2D nano-composites. *Chem. Eng. J.* **420**, 127574. https://doi.org/10.1016/j.cej.2020.127574

Zhao BB, Gong HD, Li H, Zhang Y, Deng JW, Chen ZC. 2019. Fatty Acid, Triacylglycerol and unsaponifiable matters profiles and physicochemical properties of chinese evening primrose oil. *J. Oleo Sci.* **68**, 719-728. https://doi.org/0.5650/jos.ess19091

Zhao ZH, He GX, Peng XY, Lu L. 2014. Distribution and sources of phthalate esters in the top soils of Beijing, China. *Environ. Geochem. Health* **36**, 505-515. https://doi.org/10.1007/s10653-013-9577-0

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Influence of grafting on fatty acid profile and some physicochemical properties of watermelon seed and seed oil

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SUMMARY: This study aimed to investigate the effects of grafting on the fatty acid profile and some physicochemical properties of watermelon seed and seed oil. The 'Crimson Tide' cultivar was used as the scion while two wild watermelon (*Citrullus lanatus* var. *citroides* (A1 and A2)), one *Lagenaria siceraria* (A3) and one *Cucurbita maxima* Duchesne x *Cucurbita moschata* Duchesne (A4) were used as rootstocks. The use of rootstock significantly influenced the fatty acid profile and the physical parameters of seeds and seed oils. The highest linoleic acid ratio was found in the seed oil from A1 and A2, the oil from A3 had the highest oleic acid ratio. The results showed that the content and acid value in seed oils were improved, and that total phenolic compounds and antioxidant activity of both seed and oil were decreased by grafting. Wild rootstocks can be used in watermelon cultivation to obtain a watermelon seed which is rich in linoleic acid.

KEYWORDS: *Citron watermelon; Grafting; PCA; Rootstock; Watermelon seed; Watermelon seed oil*

RESUMEN: ***Influencia de un injerto en el perfil de ácidos grasos y algunas propiedades fisicoquímicas de la semilla y el aceite de semillas de sandía.*** El objetivo de este estudio fue investigar los efectos del injerto en el perfil de ácidos grasos y algunas propiedades fisicoquímicas de la semilla y el aceite de semillas de sandía. El cultivar 'Crimson Tide' se utilizó como vástago, mientras que dos sandías silvestres (*Citrullus lanatus* var. *Citroides* (A1 y A2)), una *Lagenaria siceraria* (A3) y una *Cucurbita maxima* Duchesne x *Cucurbita moschata* Duchesne (A4) se utilizaron como portainjertos. El uso de portainjertos influyó significativamente en el perfil de ácidos grasos y los parámetros físicos de semillas y aceites de semillas. La proporción de ácido linoleico más alta se encontró en el aceite de semillas de A1 y A2, el aceite de A3 tuvo la proporción de ácido oleico más alta. Los resultados mostraron que el contenido de aceite y el índice de acidez mejoró y los compuestos fenólicos totales y la actividad antioxidante tanto de la semilla como del aceite se redujeron mediante el injerto. Para obtener un aceite de semillas de sandía rico en ácido linoleico, se pueden utilizar portainjertos silvestres en el cultivo de sandía.

PALABRAS CLAVE: *Aceite de semilla de sandía; Injerto; PCA; Portainjerto; Sandía cidra; Semilla de sandía*

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1. INTRODUCTION

The watermelon (*Citrullus lanatus*), which belongs to the family Cucurbitaceae, is one of the most nutritionally and economically important horticultural crops worldwide (Acar *et al*., 2012; Kombo and Sari, 2019). According to data from the Food and Agriculture Organization (FAO), a total of 100,414,933 tons per year of watermelon was produced in the world (FAOSTAT, 2020). China (60.4%) is the leading producer of watermelon, followed by Turkey (3.9%) and India (2.5%). Most of the fruit's seeds are usually discarded, and some are considered by-products. The seeds, which constitute 1-4% of the total watermelon weight, are highly nutritious and are rich in protein, vitamin B, minerals and oil (Braide *et al*., 2012; Duduyemi *et al*., 2013; Kiin-Kabari and Akusu, 2014; Seidu and Otutu, 2016; Tabiri *et al*., 2016). Watermelon seed oil, whose content value is generally between 19-35% is used as edible oil in the food industry and as an ingredient in the cosmetic industry (Jensen *et al*., 2011; Tabiri *et al*., 2016; Rezig *et al*., 2019). Unsaturated fatty acids (UFAs) are dominant in the fatty acid profile of watermelon seed oil. The saturated (mainly palmitic (16:0) and stearic (18:0) acids), monounsaturated (mainly oleic acid (18:1(ω-9)) and polyunsaturated (mainly linoleic acid (18:2(ω-6)) fatty acid contents in the seed oil are generally 17.90, 16.31 and 65.79%, respectively (de Conto *et al*., 2011; Eke *et al.,* 2021).

The quality of an oil obtained from a vegetable or seed is highly related to its unsaturated fatty acid profile (Nayeri and Yarizade, 2014). The proportions of oleic and linoleic acids in edible oils are generally indicative of their oxidative stability and nutritional properties (Nayeri and Yarizade, 2014). Polyunsaturated fatty acids are the most beneficial for the preservation of human health and for controlling diseases such as cancer, inflammation, rheumatoid arthritis, cardiovascular disorders, and coronary heart diseases. In fact, the higher the degree of unsaturation in ω-3, 6, 9 fatty acids, the more positive are the effects on human health. Another important aspect of linoleic acid is that it is essential and must be consumed because it cannot be synthesized in the human body (Asif, 2015). Grafting plants on coherent rootstocks ensures some advantages like augmenting plant vigor, prolonging harvesting period and post-harvest life, providing water efficiency and tolerance to drought and salt (Yetisir *et al*., 2003; Davis *et al*., 2008; Lee *et al*., 2010; Yetisir and Uygur, 2010; Zhao *et al*., 2011; Kumar *et al*., 2017; Solmaz *et al*., 2018). Several studies stated that grafting alters the content and quality of fruit. That being said, for successful grafting, choosing suitable rootstock is important (Davis *et al*., 2008a; Davis *et al*., 2008b; Turhan *et al*., 2012). Grafting is commonly used in watermelon cultivation. *Cucurbita maxima* × *Cucurbita moschata* hybrids and *Lageneria siceraria* are the most common commercial rootstocks. On the other hand, wild watermelons (*Citrullus lanatus* var. *citroides*) are important gene sources and are used in rootstock and breeding studies.

Although many research studies regarding the effects of grafting on watermelon seed content are available, there has been no report on the analysis in terms of fatty acid profile and some physicochemical properties (except thousand grain weight) of seeds and seed oils from grafted watermelons in the literature. The aim of this study was to determine the effect of grafting on the seed and the seed oil of the Crimson Tide variety, which was grafted on *Cucurbita maxima* × *Cucurbita moschata, Lageneria siceraria* and two wild watermelon rootstocks. In this context, the effects of grafting, namely total phenolic compounds, antioxidant activity and reflectance color values (*L*, a*, b*,* C* and h) of the watermelon seeds and seed oils were evaluated. Also, thousand grain weight and width-length-thickness dimensions of the seeds, and seed oil content, kernel oil content, fatty acid profile, and acid value of the seed oils were investigated.

2. MATERIALS AND METHODS

2.1. Plant material and planting

The research was carried out in Selçuk University, Faculty of Agriculture, Sarıcalar Research and Application Farm, in 2017. The trial zone is located between 38° 05' northern latitudes and 32° 36' east longitudes and its average height from the sea is 1006 m. In the study, two wild rootstocks *Citrullus lanatus* var. *citroides* (A1 and A2), one open-pollinated *Lagenaria siceraria* (A3) and one *Cucurbita maxima* Duchesne x *Cucurbita moschata* Duchesne hybrid ('TZ-148') (A4) were used as rootstocks.

'Crimson Tide' (CT), a commercial watermelon variety widely grown in Turkey, was grafted on these four rootstocks and production materials were obtained. Ungrafted CT (K) was used as control. A total of 5 trial subjects were used in three replications according to the randomized block design. All cultural practices up to the harvest were applied to all applications equally. Watermelon fruits were harvested on time and after harvest, watermelon seeds from each application were hand collected seperately, washed to remove the fruit pulp residue and dried in an environment free from sunlight at room temperature for a week. The seeds were kept in freezer bags at 4 °C until oil extraction and analyses.

2.2. Determination of thousand grain weight and seed size

Seeds (50 samples) were counted and weighed, and the weight was multiplied by 20 to obtain a thousand grain weight (Pradhan, 2010). The width, length and thickness of the seed grains were determined in mm using a caliper.

2.3. Determination of oil content in seeds and kernels

According to AOCS (1980) with some modifications, approximately 2 g of the milled seeds and kernels (shelled seeds) were extracted using 250 ml of petroleum ether in the Soxhlet device for 4 hours. The solvent was removed from the miscella by a rotary vacuum evaporator (RE100-Pro, Scilogex, CT, USA) set at 40 °C, and the oil contents of the samples were calculated.

2.4. Determination of acid numbers in seed oils

The acid number analysis was determined by making some modifications according to TSI (2003). Firstly, 25 ml of ethyl alcohol-diethyl ether mixture (1:1) were added to 2 g oil sample. The mixture was titrated with a 0.1 N ethanolic NaOH solution with 1 ml phenolphthalein indicator. Results were given as mg NaOH/g oil.

2.5. Determination of fatty acid profile of seed oils

The fatty acid methyl esters of seed oils were prepared according to the method described by Williams (1984) with some modifications. The sample was shaken vigorously in isooctane (0.4 g in 4 ml) and combined with 0.2 ml of 2 N methanolic potassium hydroxide. After the duration in the dark for 6 min, a drop of methyl orange and 0.45 ml of 1 N HCl were added, mixed and centrifuged (2000 rpm/5 min). The supernatants were then analyzed by a GC (Agilent, Santa Clara, CA) equipped with an injector, a capillary column (Innowax, 100 m length, 0.25 mm i.d., 0.20 µm of thickness), and a flame ionization detector (FID). Hydrogen was used as carrier gas. The temperature of the detector and injector was set as 250 °C. The initial oven temperature was 180 °C and increased to 220 °C at 30 °C/min.

2.6. Determination of reflectance color values of seeds and seed oils

Color measurements of the seeds and their oils were determined as *L** (lightness-darkness), *a** (redness-greenness), *b** (yellowness-blueness), C* (chroma) and h (hue) values in the CIE color system profile using Minolta colorimeter (CM-5, Minolta, Japan).

2.7. Extraction of polyphenols and antioxidant effective compounds from seeds and seed oils

To extract the polyphenols and antioxidant effective compounds from seeds, 0.1 g of defatted ground seed obtained at the end of the oil extraction was weighed and 40 ml of 80% methanol solution were added. Homogenization was obtained by an ultra-turrax operation for 2 minutes at 200 rpm. Similarly, 1 g of oil sample was mixed with 2 ml methanol for 10 minutes in a shaker. The supernatants of oil-methanol and seed-methanol obtained from the centrifugation at 4100 rpm for 10 minutes was used for total phenolic compounds and antioxidant activity analyses.

2.8. Determination of total phenolic compounds

The total phenolic compounds (TPC) of oils and seeds were obtained using the Folin-Ciocalteu method (Singleton and Rossi, 1965), with slight modifications. The Folin-Ciocalteu reagent of 1.25 ml (0.2 N) and 1 ml of sodium carbonate (75 g/l) solution were added to 250 µl of extract. Before determining the absorbance values of samples by spectrophotometer at 765 nm, they were kept in the dark for 2 hours. Results were expressed as mg gallic acid equivalent (GAE) /kg.

TABLE 1. The thousand grain weight, dimensions, total phenolic compounds and antioxidant activity values of the watermelon seeds

Rootstocks	Thousand grain weight (g)	Width (mm)	Length (mm)	Thickness (mm)	Total phenolic compounds (mg GAE/kg)	Antioxidant activity (mmol TE/kg)
K	44.00 ± 0.20^{b}	5.36 ± 0.10^{b}	8.87 ± 0.17^{a}	1.95 ± 0.03^{a}	2340.87 ± 17.39^{a}	6.80 ± 0.53^{a}
A1	40.70 ± 0.30^{d}	5.07 ± 0.10^{c}	8.55 ± 0.19^{b}	1.70 ± 0.11^{b}	1366.96 ± 121.74^{d}	5.22 ± 0.53^{b}
A2	41.50 ± 0.10^{c}	5.22 ± 0.13^{bc}	8.63 ± 0.08^{b}	1.72 ± 0.05^{b}	1575.65 ± 86.96^{c}	6.54 ± 0.26^{a}
A3	48.70 ± 0.10^{a}	5.77 ± 0.12^{a}	9.05 ± 0.14^{a}	1.91 ± 0.04^{a}	1140.87 ± 110.00^{e}	3.57 ± 0.99^{d}
A4	42.20 ± 0.80^{c}	5.04 ± 0.07^{c}	8.38 ± 0.11^{b}	1.66 ± 0.08^{b}	1801.74 ± 139.13^{b}	4.16 ± 0.53^{c}

The results are expressed as means ± standard deviation of 3 replicates (n = 3). Different letters in the same column indicate significant differences (p < 0.01) among rootstocks according to the one-way ANOVA/ Duncan's multiple range test.

TABLE 2. Color values of watermelon seeds

Rootstocks	***L****	***a****	***b****	C*	h
K	37.73 ± 1.07^{a}	5.25 ± 0.28^{a}	12.92 ± 1.11^{a}	67.77 ± 1.00	13.92 ± 1.15^{a}
A1	31.42 ± 2.46^{bc}	3.39 ± 0.56^{b}	7.65 ± 1.74^{b}	65.90 ± 1.93	8.37 ± 1.81^{b}
A2	31.56 ± 1.32^{bc}	3.17 ± 0.51^{b}	7.38 ± 1.33^{b}	66.72 ± 0.95	8.04 ± 1.42^{b}
A3	29.77 ± 1.52^{c}	3.69 ± 0.13^{b}	7.45 ± 0.87^{b}	63.49 ± 2.15	8.31 ± 0.82^{b}
A4	33.65 ± 1.89^{b}	3.93 ± 0.55^{b}	8.46 ± 1.41^{b}	64.99 ± 0.68	9.33 ± 1.51^{b}

The results are expressed as means ± standard deviation of 3 replicates (n = 3). Different letters in the same column indicate significant differences (p < 0.01) among rootstocks according to the one-way ANOVA/ Duncan's multiple range test.

2.9. Determination of antioxidant activity

The antioxidant activity of watermelon seeds and their oils was determined using the 2, 2-diphenyl-1-picrylhydracyl (DPPH) radical scavenging method, according to an adapted colorimetric procedure (Akbulut *et al*., 2009). After adding 1.95 ml of DPPH reagent to 50 µl extract, the mixture was kept in the dark for 30 minutes. The absorbance values of the samples were measured by spectrophotometer at 515 nm and antioxidant activity values were calculated in mmol Trolox Equivalent (TE)/kg.

2.10. Statistical analysis

Significant differences among samples were determined through analysis of variance (ANOVA) and Duncan's multiple range test using SPSS package program version 22.0. Differences were considered significant at $p < 0.01$. The JMP 13.0 software was used to analyze the important parameters by Principal Component Analysis (PCA). The relation between rootstocks was compared by drawing a loading plot graph using the same software and the relationship between each parameter and another was presented on the score plot graph.

3. RESULTS AND DISCUSSION

3.1. Effect of grafting on watermelon seeds

Some physicochemical properties of the seeds obtained from watermelon fruits are given in Tables 1 and 2. According to Table 1, the rootstock had statistically significant effects on thousand grain weight, width-length-thickness dimensions, total phenolic compounds (TPC) and antioxidant activity (AA) values for the seeds. Thousand grain weight results of seeds ranged from 40.70 g to 48.70 g, and watermelons grafted on the bottle gourd rootstock (A3) with the highest thousand grain weight were statistically separated from other samples according to Duncan test results. While the thousand grain weight of the seeds of watermelons grafted on Citroides 1 (A1), Citroides 2 (A2) and TZ148 (A4) rootstocks was significantly decreased, that of watermelon seeds grafted on the bottle gourd (A3) was significantly increased ($p < 0.01$). Similar to the results of this study, the grafting of Crimson Sweet (CS) watermelon (*Citrullus lanatus*) steel on three different rootstocks (*Cucurbita maxima* x *Cucurbita moschata* 'NUN-9075', *Lagenaria siceraria* 'Argentario', and citron watermelon *Citrullus amarus*

TABLE 3. Oil content in seeds and some physicochemical, and phytochemical properties of watermelon seed oils

Rootstocks	Oil content (%)	Kernel oil content (%)	Acid value (mg NaOH/g)	Total phenolic compound (mg GAE/kg)	Antioxidant activity (mmol TE/kg)
K	24.05±0.19^{d}	45.61±0.11	5.35±0.20^{a}	51.86±0.26	0.52±0.03^{a}
A1	25.90±0.77^{ab}	46.44±0.34	4.33±0.19^{d}	50.69±0.65	0.45±0.01^{bc}
A2	26.34±0.38^{a}	44.19±0.84	4.86±0.06^{b}	53.56±1.96	0.44±0.01^{c}
A3	24.75±0.36^{cd}	44.44±1.52	4.76±0.13^{bc}	51.50±0.68	0.46±0.01^{bc}
A4	25.46±0.35^{bc}	45.19±0.99	4.50±0.15^{cd}	49.25±2.87	0.49±0.01^{ab}

The results are expressed as means ± standard deviation of 3 replicates (n = 3). Different letters in the same column indicate significant differences ($p < 0.01$) among rootstocks according to the one-way ANOVA/ Duncan's multiple range test.

TABLE 4. The fatty acid profile (%) of watermelon seed oils

Rootstocks	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2
K	11.90±0.00^{b}	7.97±0.00^{a}	16.74±0.41^{c}	63.40±0.40^{b}
A1	11.63±0.11^{c}	7.59±0.11^{b}	15.98±0.11^{d}	64.81±0.33^{a}
A2	12.27±0.00^{a}	7.27±0.15^{c}	15.78±0.02^{d}	64.68±0.13^{a}
A3	12.25±0.08^{a}	7.95±0.14^{a}	17.86±0.16^{a}	61.88±0.00^{c}
A4	11.96±0.11^{b}	7.40±0.01^{bc}	17.33±0.07^{b}	63.32±0.04^{b}

The results are expressed as means ± standard deviation of 3 replicates (n = 3). Different letters in the same column indicate significant differences ($p < 0.01$) among rootstocks according to the one-way ANOVA/ Duncan's multiple range test. Standard deviation values below 0.01 were equal to ± 0.00

Schard 'PI296341') had a significant effect on the thousand grain weight of the seeds obtained (Kombo and Sari, 2019).

As seen in Table 1, the width, length and thickness of the watermelon seeds varied from 5.0-5.8 mm, 8.4-9.0 mm and 1.7-1.9 mm, respectively. A decrease in the total amount of phenolic compounds of all watermelon seeds due to grafting was observed. It was determined that the seeds with the highest TPC belonged to the control sample (2340.9 mg GAE/kg) and the lowest to the A3 sample (1140.9 mg GAE/kg). In an analysis of the seeds of *Citrullus lanatus* var. *citroides*, Acar *et al.* (2012) found that the TPC of the seeds were 130 mg GAE/kg, and the reason for the decrease in the TPC of A1 and A2 samples may be due to the low content in the citroides species. Many recent studies (Seidu and Otutu, 2016; Tabiri *et al.*, 2016) have shown that watermelon seeds have much more TPC than those of this study. These differences in TPC may depend on factors such as variety and environmental conditions. The antioxidant activity values of watermelon seeds varied between 3.57 and 6.80 mmol TE/kg. As in the amount of phenolic compounds, a statistically significant decrease (except A2 sample) was observed in the antioxidant activity values of the seeds obtained as a result of grafting. Tabiri *et al.* (2016) reported the antioxidant activity values of the seeds of "Charleston gray", "Black diamond" and "Crimson sweet" watermelon varieties as 82.59, 96.63 and 130.29 mmol TE/kg, respectively.

The decrease in color values of the watermelon seeds was found to be significant for L^*, a^*, b^* and h, but not for C* (Table 2). The L^*, a^*, b^*, C* and h values varied between 29.77-37.73, 3.17-5.25, 7.38-12.92, 63.49-67.77 and 8.04-13.92, respectively. If we expanded the results for watermelon seeds, no significant difference was observed only in the C* values after grafting.

3.2. Effect of grafting on watermelon seed oil

The seed oil content, kernel oil content, acid value, TPC and AA values of the samples are presented in Table 3 and the fatty acid profile in Table 4. It has been determined that the differences that occured in these values except for the kernel oil content and TPC results are statistically significant ($p < 0.01$). The increase observed in the oil efficiency of all the seeds by grafting was found statistically significant. Sample A2 (26.34%) had the highest seed oil content, followed by A1 (25.90%), A4 (25.46%),

Table 5. Color values of watermelon seed oils

Rootstocks	***L****	***a****	***b****	**C***	h
K	87.09±0.01^{e}	-0.47±0.00^{e}	80.37±0.01^{a}	80.37±0.01^{a}	90.34±0.00^{e}
A1	88.08±0.01^{d}	-0.60±0.02^{d}	74.63±0.03^{c}	74.64±0.04^{c}	90.46±0.01^{d}
A2	88.30±0.03^{c}	-0.75±0.01^{c}	77.43±0.01^{b}	77.43±0.01^{b}	90.56±0.01^{c}
A3	88.60±0.03^{b}	-1.08±0.02^{b}	74.60±0.00^{c}	74.61±0.00^{c}	90.83±0.01^{b}
A4	89.08±0.04^{a}	-1.21±0.01^{a}	62.11±0.03^{d}	62.12±0.02^{d}	91.12±0.01^{a}

The results are expressed as means ± standard deviation of 3 replicates (n = 3). Different letters in the same column indicate significant differences (p < 0.01) among rootstocks according to the one-way ANOVA/ Duncan's multiple range test. Standard deviation values below 0.01 were equal to ± 0.00

Table 6. PCA results regarding some seed characteristics of scion nn different rootstocks

Items	PC1	PC2
Eigenvalue	5.91	4.15
Percentage of variance	53.7	37.7
Cumulative variance	53.7	91.4
Eigenvectors		
Thousand grain weight	-0.01574	**0.48529**
Width	0.00410	**0.47570**
Length	0.13154	**0.43907**
Thickness	0.19524	**0.43071**
*L**	**0.39446**	-0.09372
*a**	**0.38036**	0.12672
*b**	**0.40644**	0.04465
C*	**0.30853**	-0.25675
h	**0.40444**	0.05557
Total phenolic compounds	**0.38396**	-0.13156
Antioxidant activity	0.26956	-0.21624

PC1 and PC2 indicate first and second components, respectively.

A3 (24.75%) and lastly K (24.05%). However, there was no similarity between seed oil content and kernel oil content in the samples. Kernel oil content values were listed in descending order as follows: A1, K, A4, A3, A2. The oil contents in the seeds and kernels of *Citrullus lanatus* var. *citroides* were reported by Acar *et al.* (2012) as 26.83 and 52.34%, respectively. In addition, four *Lagenaria siceraria* cultivars were studied by Essien *et al.* (2013) and the content in seed oils, which is close to our results, was obtained in the range of 23.0-29.5%. Tabiri *et al.* (2016) reported that the seed oil contents in "Crimson sweet", "Charleston gray" and "Black diamond" varieties were 26.50, 26.83 and 27.85%, respectively. In one of the studies, the seed oil content values of the "Crimson" cultivar was 19.23% (de Conto *et al.*, 2011), while in another, in the "Mateera" and "Sugar baby" varieties were 25.53% (46.85% kernel oil content) and 21.93% (38.88% kernel oil content), respectively (Wani *et al.*, 2013). Considering all of this evidence, it seems that oil content values can vary based on the watermelon variety.

The acid values for the samples (4.33 to 5.35 mg NaOH/g) were relatively high compared to that reported for watermelon (*Citrullus lanatus* L.) seeds (2.37 mg NaOH/g) (Duduyemi *et al.*, 2013). With the effect of grafting, there was a significant decrease in acid value as given in Table 3 ($p < 0.01$). Among the grafted rootstocks, A2 (4.86 mg NaOH/g) had the highest acid value, while A1 (4.33 mg NaOH/g) had the lowest. Essien *et al.* (2013) determined the acid values for the seed oils from four gourd cultivars as between 2.1-2.4 mg KOH/g. For this value, the decrease in A3 compared to the control sample may be due to the low acidity of the oil from the gourd seeds. Wani *et al.* (2013) obtained oil from "Mateera" and "Sugar baby" cultivars and reported that the acid values in mg KOH/g oil were 4.27 and 6.46, respectively. In another investigation, in which cold press was applied, the acid value of the "Crimson" variety seed oil was found to be 9.5 mg KOH/g oil (Rezig *et al.*, 2019). The TPC of the seed oils from grafted (49.25-53.56 mg GAE/kg) and ungrafted (51.86 mg GAE/kg) watermelons was lower than that of Iranian watermelon seed oils (111 mg GAE/kg), as reported by Hashemi *et al.* (2017). In addition, the TPC of the seed oils from four gourd cultivars was found to be between 80 and 105 mg GAE/kg as reported by Essien *et al.* (2015). The decrease observed in the antioxidant activity of oils by grafting was found statistically significant ($p < 0.01$). The control sample (K) (0.52 mmol TE/kg) had the highest antioxidant

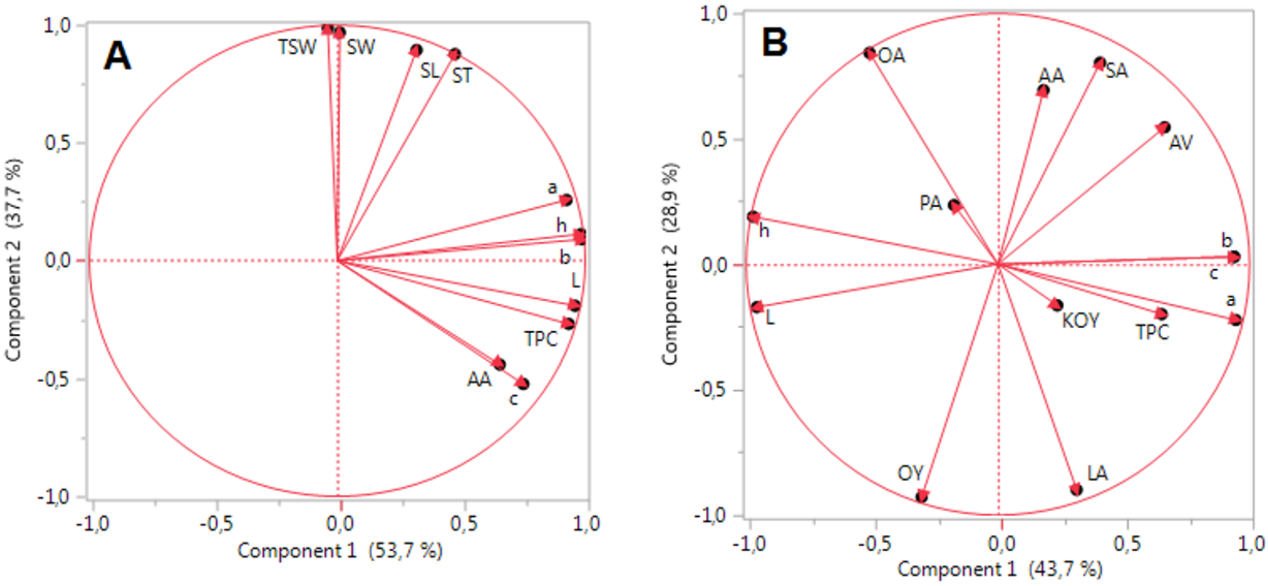

Figure 1. Loading plot based on PC1 and PC2 obtained by principal component analysis using some seed (A) and oil (B) characteristics of scion on different rootstocks

*PC1: First component; PC2: Second component; OY (Oil yield); KOY (Kernel oil yield); TPC (Total phenolic compounds); AA (Antioxidant activity); *L** (Lightness); *a** (redness-greeness); *b** (yellowness-blueness); C* (Chroma); h (Hue); AV (Acid Value); LA (Linoleic Acid); PA (Palmitic Acid); SA (Stearic Acid); OA (Oleic Acid); TSW (thousand grain weight); SW (width); SL (length); ST (thickness)

activity, followed by the A4 (0.49 mmol TE/kg), A3 (0.46 mmol TE/kg), A1 (0.45 mmol TE/kg), A2 (0.44 mmol TE/kg) samples.

The polyunsaturated, monounsaturated and saturated fatty acid profiles of the watermelon seed oils statistically changed with the application of grafting (p<0.01) (Table 4). The fatty acid distribution in the ungrafted control sample (Crimson Tide) was as follows: 63.40% linoleic acid, 16.74% oleic acid, 11.90% palmitic acid and 7.97% stearic acid. When compared to the control sample, an increase in the amount of unsaturated fatty acids (UFAs) in the A1, A2 and A4 samples and of saturated fatty acids (SFAs) in the A3 was observed. The highest linoleic acid content was determined in the seed oils from the citroides rootstock samples as 64.81% (A1) and 64.68% (A2), the lowest oleic acid content was also determined in the same samples as 15.98% and 15.78%, respectively. According to the control sample, the grafting application in citroides samples caused a statistically significant increase in linoleic acid content, with a significant decrease in the oleic acid content. The seed oil from the bottle gourd rootstock sample (A3) had the highest oleic acid content (17.86%), but the lowest linoleic acid (61.88%). No work regarding the effect of grafting on the fatty acid profile of the seed oils from watermelons or the other plants in the Cucurbitaceae family could be found. This situation is also valid for all analyses performed on the seeds and their oils except thousand grain weight. The obtained results for the fatty acid profiles show similarity with the studies performed by Wani *et al.* (2013), Hashemi *et al.* (2017), Rezig *et al.* (2019), and Eke *et al.* (2021). With the effect of grafting, a significant difference was observed in the color values of watermelon seed oils (Table 5). The increase in L^* and h values and the decrease in a^*, b^* and C* values were statistically significant ($p < 0.01$). The values for L^*, a^*, b^*, C* and h in the oils were between 87.09-89.08, (-)0.47-(-)1.21, 62.11-80.37, 62.12-80.37 and 90.34-91.12, respectively.

3.3. Principal component analysis (PCA)

3.3.1. PCA of seeds

PCA is a practical and commonly used method to estimate the agricultural practices on product content and quality (Seymen *et al*., 2019). The seeds of the watermelon grafted with different rootstocks, thousand grain weight, width, length, thickness, color values (L^*, a^*, b^*, C*, h), total phenolic compounds and antioxidant activity results obtained were subjected to PCA (Table 6). As a result of PCA, the study explained a high rate of 91.4%

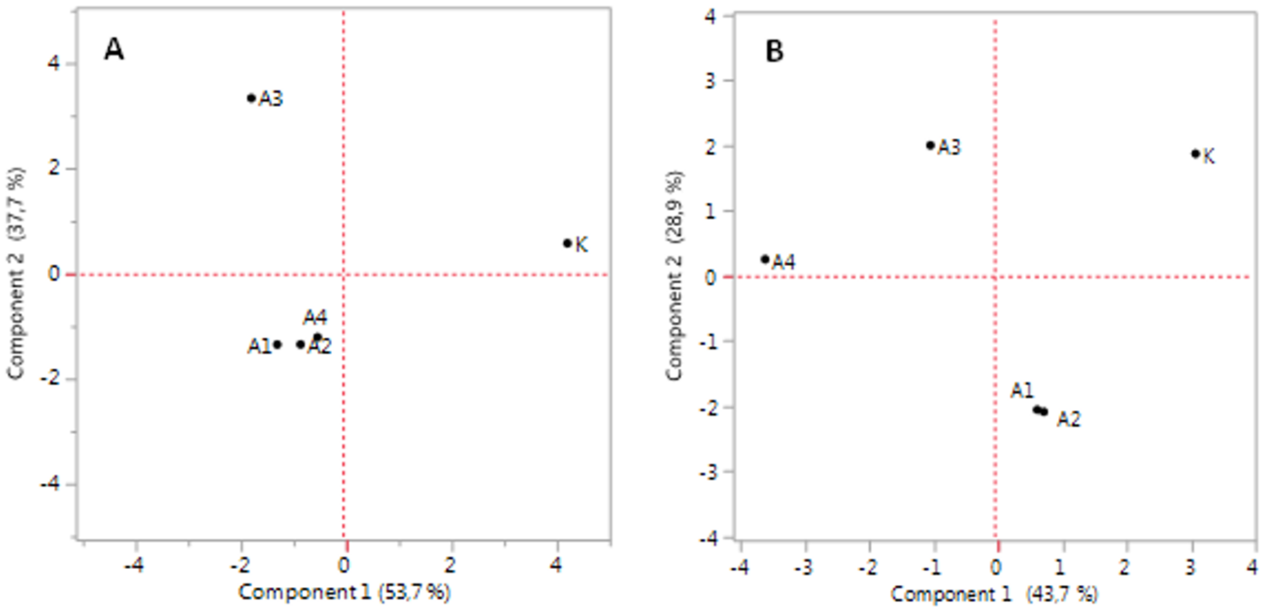

FIGURE 2. Score plot based on PC1 and PC2 obtained from principal component analysis using some seed (A) and oil (B) characteristics of scion on different rootstocks

*PC1: First component; PC2: Second component; K (Control); A1 and A2 (*Citrullus lanatus* var. *citroides*. A3 *(Lagenaria siceraria)*; A4 (*Cucurbita maxima* Duchesne x *Cucurbita moschata* Duchesne hybrid (‘TZ-148’))

in two components. Seymen *et al.* (2019) reported that the first two components should be explained by more than 25% of the study to use PCA. According to the results obtained, 91.4% of two components studied were detected and it was revealed that this analysis will give important results about the usability and the parameters examined. The first component (PC1) explained 53.7% of the study and L^*, a^*, b^*, C*, h and total phenolic compound parameters were the highest declared parameters positively. The second component (PC2) explained 37.7% of the study and thousand grain weight, width, length, and thickness parameters were the highest explained parameters.

A loading chart was created to examine the relationships among thousand grain weight, width, length, thickness color (L^*, a^*, b^*, C*, h), total phenolic compounds and antioxidant activity (Figure 1-A). If the angle between the vectors in the figure is < 90°, there is a positive relationship; if it is > 90°, there is a negative relationship; and if it is equal to 90°, there is no relationship. As seen in the figure, the highest positive relationships were seen among antioxidant activity-C*, h-b^* and thousand grain weight-width. Similarly, Seymen *et al.* (2019) reported positive relationships between thousand grain weight-seed width and thousand grain weight-length in pumpkin seeds. On the other hand, the dimensions of seeds and thousand grain weight parameters showed no relationship with seed color or other chemical contents. In the score plot (Figure 2-A), control (K) was in the positive region of both components, which showed significant results for the parameters in both components. As a result, the control had significant effects on some physicochemical properties of watermelon seeds. On the other hand, wild rootstocks (A1 and A2) and A4 showed similarity in terms of seed characteristics and were located in the same region on the plot.

3.3.2. PCA of seed oil

The seed oil from the watermelon grafted with different rootstocks, oil content, kernel oil content, total phenolic compounds, antioxidant activity, color values (L^*, a^*, b^*, C*, h), acid value, stearic acid, palmitic acid, oleic acid and linoleic acid results obtained were subjected to PCA (Table 7). As a result of PCA, the study explained a high rate of 94.07% in three components.

According to the results obtained, 94.1% of three components studies were detected and it was revealed that this analysis will give important results about the usability and the parameters examined. The first component (PC1) and the second component (PC2) explained 43.7 and 28.9% of the study, respectively. *a*, b*,* and C* parameters in PC1 were the highest declared parameters positively while *L** and h parameters were the highest declared negatively. In PC2 antioxidant activity, oleic acid and stearic acid were the highest parameters declared positively; oil content, and linoleic acid were the highest parameters declared negatively. The third component (PC3) explained 21.5% of the study and total phenolic compounds and palmitic acid parameters were the highest positive parameters and kernel oil content was the highest negative parameter.

A loading chart was created to examine the relationships among oil content, kernel oil content, total phenolic compounds, antioxidant activity, color (*L**, *a**, *b**, C*, h), acid value, stearic acid, palmitic acid, oleic acid and linoleic acid (Figure 1-B). The highest positive relationships were found between *a**- total phenolic compounds, *b**-C*. The highest negative relationships were found between linoleic acid-oleic acid, oil content-antioxidant activity. Similar to the result of this research, Gangadhara and Nadaf (2018) reported strong negative relationships between oleic acid and linoleic acid in two backcross generations of groundnut. Delta-12 fatty acid desaturase 2 (FAD2) enzyme, which is specific to plant seeds, converts oleic acid to linoleic acid by desaturation and leads to high linoleic acid contents in some seed oils (Nayeri and Yarizade, 2014). The negative relationship between oleic and linoleic acids in this study may be explained by conversions among fatty acids. Higher linoleic acid ratios in the oils of A1 and A2 samples can arise from the activities of such enzymes. In the score plot, control (K) was in the positive region of both components (Figure 2-B). As a result, the control had significant effects on the some physicochemical properties of watermelon seed oil. However, A1 and A2 wild rootstocks differed from other rootstocks and the control sample with its high linoleic acid content. When taking into consideration the importance of linoleic acid for human health and linoleic acid ratios of A1 and A2 samples, wild watermelon rootstocks can be an alternative to commercial rootstocks in watermelon grafting in terms of seed oil production.

TABLE 7. PCA results regarding some oil characteristics of scion in different rootstocks

Items	PC1	PC2	PC3
Eigenvalue	6.11	4.04	3.02
Percentage of variance	43.7	28.9	21.5
Cumulative variance	43.7	72.5	94.1
Eigenvectors			
Oil content	-0.12247	**-0.46143**	0.12545
Kernel oil content	0.09545	-0.08155	**-0.54450**
Total phenolic compounds	0.26307	-0.09902	**0.42191**
Antioxidant activity	0.07336	**0.34514**	-0.26465
*L**	**-0.38725**	-0.08516	0.11911
*a**	0.38234	-0.11057	-0.13621
*b**	**0.37950**	0.01483	0.16105
C*	**0.37947**	0.01485	0.16096
h	**-0.39349**	0.09420	0.05256
Acid Value	0.26798	0.27154	0.16867
Palmitic acid	-0.07043	0.11719	**0.54856**
Stearic acid	0.16425	**0.39950**	-0.09502
Oleic acid	-0.20623	**0.41906**	0.01803
Linoleic acid	0.12656	**-0.44726**	-0.11687

PC1, PC2, and PC3 indicate first, second, and third components, respectively.

4. CONCLUSIONS

The present study demonstrated that the application of grafting to watermelon significantly influenced the fatty acid profile and physicochemical properties of seeds and seed oils apart from C* values for the seeds, kernel oil content and total phenolic compounds of seed oils. Although the use of rootstocks decreased the total phenolic compounds and the antioxidant activity of the seeds, it increased the oil efficiency of the seeds and improved the use of them for seed oil. In addition, the use of citroides rootstocks can be recommended in terms of high content in linoleic acid. This study is the first to investigate the detailed characteristics of grafted watermelon seeds. Therefore, more studies are needed on this subject.

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REFERENCES

Acar R, Ozcan MM, Kanbur G, Dursun N. 2012. Some physico-chemical properties of edible and forage watermelon seeds. *Iran J. Chem. Eng.* **31**, 41–47. https://doi.org/10.30492/IJCCE.2012.5919

Akbulut M, Ozcan MM, Coklar H. 2009. Evaluation of antioxidant activity, phenolic, mineral contents and some physicochemical properties of several pine honeys collected from western Anatolia. *Int. J. Food Sci. Nutr.* **60**, 577–589. https://doi.org/10.3109/09637480801892486

AOCS. 1980. *Official Methods and Recommended Practices of The American Oil Chemist's Society.* 4th edn. Champaign, Illinois.

Asif M. 2015. Chemical characteristics and nutritional potentials of unsaturated fatty acids. *Chem. Int.* **1**, 118–133.

Braide W, Odiong IJ, Oranusi S. 2012. Phytochemical and antibacterial properties of the seed of watermelon (*Citrullus lanatus*). *P. J. Microbiol. Res.* **2**, 99–104.

Davis AR, Perkins-Veazie P, Sakata Y, López-Galarza S, Maroto JV, Lee SG,Huh YC, Sun Z, Miguel A, King SR, Cohen R, Lee JM. 2008. Cucurbit grafting. *Crit. Rev. Plant Sci.* **27**, 50–74. https://doi.org/10.1080/07352680802053940

Davis AR, Perkins-Veazie P, Hassell R, Levi A, King SR, Zhang X. 2008. Grafting effects on vegetable quality. *Hortscience* **43**, 1670–1672. https://doi.org/10.21273/HORTSCI.43.6.1670

de Conto LC, Gragnani MAL, Maus D, Ambiel HCI, Chiu MC, Grimaldi R, Gonçalves LAG. 2011. Characterization of crude watermelon seed oil by two different extractions methods. *J. Am. Oil Chem. Soc.* **88**, 1709–1714. https://doi.org/10.1007/s11746-011-1850-8

Duduyemi O, Adebanjo SA, Kehinde O. 2013. Extraction and determination of physico-chemical properties of watermelon seed oil (*Citrullus lanatus* L.) for relevant uses. *Int. J. Sci. Res.* **2**, 66–68.

Eke R, Ejiofor E, Oyedemi S, Onoja S, Omeh N. 2021. Evaluation of nutritional composition of *Citrullus lanatus Linn.* (watermelon) seed and biochemical assessment of the seed oil in rats. *J. Food Biochem.* **45**, e13763. https://doi.org/10.1111/jfbc.13763

Essien EE, Udo II, Umoh SD. 2013. Fatty acids composition and seed oils quality of *Lagenaria siceraria* cultivars grown Northern Nigeria. *Int. J. Nat. Prod. Sci.* **3**, 1–8.

Essien EE, Antia BS, Peter NS. 2015. *Lagenaria siceraria* (Molina) standley. Total polyphenols and antioxidant activity of seed oils of bottle gourd cultivars. *World J. Pharm. Res.* **4**, 274–285.

FAOSTAT. 2020. FAO Statistical Database, http://www.fao.org.

Gangadhara K, Nadaf HL. 2018. Genetic analysis of oleic acid and linoleic acid content in relation to oil quality in groundnut. *Electron J. Plant Breed.* **9** (1), 283–294. https://doi.org/10.5958/0975-928X.2018.00033.9

Hashemi SMB, Khaneghah AM, Koubaa M, Lopez-Cervantes J, Yousefabad SHA, Hosseini SF, Karimia M, Motazediana A, Asadifard S. 2017. Novel edible oil sources: microwave heating and chemical properties. *Food Res. Int.* **92**, 147–153. https://doi.org/10.1016/j.foodres.2016.11.033

Jensen BD, Hamattal MA, Touré AT, Nantoumé AD. 2011. Watermelons in the Sand of Sahara: cultivation and use of indigenous landraces in the Tombouctou Region of Mali. *Ethnobot. Res. Appl.* **9**, 151–162. https://doi.org/10.17348/era.9.0.151-162

Kiin-Kabari DB, Akusu OM. 2014. Effect of processing on the proximate composition, functional properties and storage stability of watermelon (*Citrullus lanatus*) seed flour. *J. Int. J. Biotechnol. Food Sci.* **2**, 143–148.

Kombo MD, Sari N. 2019. Rootstock effects on seed yield and quality in watermelon. *Hortic. Environ. Biotechnol.* **60**, 303–312. https://doi.org/10.1007/s13580-019-00131-x

Kumar P, Rouphael Y, Cardarelli M, Colla, G. 2017. Vegetable grafting as a tool to improve drought resistance and water use efficiency. *Front. Plant Sci.* **8**, 1130. https://doi.org/10.3389/fpls.2017.01130

Lee JM, Kubota C, Tsao SJ, Bie Z, Echevarria PH, Morra L, Oda, M. 2010. Current status of vegetable grafting: diffusion, grafting techniques, automation. *Sci. Hortic.* **127**, 93–105. https://doi.org/10.1016/j.scienta.2010.08.003

Nayeri FD, Yarizade K. 2014. Bioinformatics study of delta-12 fatty acid desaturase 2 (FAD2) gene in oilseeds. *Mol. Biol. Rep.* **41** (8), 5077–5087. https://doi.org/10.1007/s11033-014-3373-5.

Pradhan RC, Meda V, Naik SN, Tabil L. 2010. Physical Properties of Canadian Grown Flaxseed in Relation to Its Processing. *Int. J. Food Propert*. **13** (49), 732–743. https://doi.org/10.1080/10942910902818137

Rezig L, Chouaibi M, Meddeb W, Msaada K, Hamdi S. 2019. Chemical composition and bioactive compounds of Cucurbitaceae Seeds: Potential sources for new trends of plant oils. *Process Saf. Environ. Prot.* **127**, 73–81. https://doi.org/10.1016/j.psep.2019.05.005

Seidu KT, Otutu OL. 2016. Phytochemical composition and radical scavenging activities of watermelon (*Citrullus lanatus*) seed constituents. *Croat J. Food Sci. Technol.* **8**, 83–89. https://doi.org/10.17508/CJFST.2016.8.2.07

Seymen M, Yavuz D, Dursun A, Kurtar ES, Türkmen Ö. 2019.Identification of drought-tolerant pumpkin (*Cucurbita pepo l.*) genotypes associated with certain fruit characteristics, seed yield, and quality. *Agric. Water Manag.* **221**, 150–159. https://doi.org/10.1016/j.agwat.2019.05.009

Singleton VL, Rossi JAJ. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Curr. Contents* **16**, 144–158.

Solmaz I, Sari N, Kombo MD, Şimşek İ, Hussein S, Namli M. 2018. Rootstock capacity in improving production and quality of triploid watermelon seeds. *Turk. J. Agric. For.* **42**, 298–308. https://doi.org/10.3906/tar-1801-59

Tabiri B, Agbenorhevi JK, Wireko-Manu FD, Ompouma FEI. 2016. Watermelon seeds as food: nutrient composition, phytochemicals and antioxidant activity. *Int. J. Food Sci. Nutr.* **5**, 139–144. https://doi.org/10.11648/j.ijnfs.20160502.18

TSI. 2003. TS 342, *Methods of analysis for edible olive oils*. Turkish Standards Institution, Ankara.

Turhan A, Ozmen N, Kuscu H, Serbeci MS, Seniz V. 2012. Influence of rootstocks on yield and fruit characteristics and quality of watermelon. *Hortic. Environ. Biotechnol.* **53**, 336–341. https://doi.org/10.1007/s13580-012-0034-2

Wani AA, Sogi DS, Singh P, Götz A. 2013. Impacts of refining and antioxidants on the physico-chemical characteristics and oxidative stability of watermelon seed oil. *J. Am. Oil Chem. Soc.* **90**, 1423–1430. https://doi.org/10.1007/s11746-013-2277-1

Williams S. 1984. Official methods of analysis of the association official analytical chemists. 14th edn. Arlington VA, USA.

Yetisir H, Sari N, Yucel S. 2003. Rootstock resistance to fusarium wilt and effect on fruit yield and quality of watermelon. *Phytoparasitica* **31,** 163–169. https://doi.org/10.1007/BF02980786

Yetisir H, Uygur V. 2010. Responses of grafted watermelon onto different gourd species to salinity stress. *J. Plant Nutr.* **33**, 315-327. https://doi.org/10.1080/01904160903470372

Zhao X, Guo Y, Huber DJ, Lee J. 2011. Grafting effects on postharvest ripening and quality of 1-methylcyclopropene-treated muskmelon fruit. *Sci. Hortic.* **130**, 581–587. https://doi.org/10.1016/j.scienta.2011.08.010

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Biochemical appraisal of the underutilized *Hura crepitans* seed oil: functional and inflammatory responses in albino rats

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SUMMARY: *Hura crepitans* seed oil (HCSO) remains under-utilized, largely due to the scarcity in data regarding its biochemical properties. To investigate the functional and pro-inflammatory responses to HCSO, twenty-four male rats were grouped into four and received compounded diets containing 5%-HCSO; 10%-HCSO; 15%-HCSO; and 15%-AHO (as control) for eight weeks. The functional responses and the expression of pro-inflammatory cytokines and their receptors were appraised. The organ function biomarkers in rats fed with HCSO-supplemented diets were statistically similar to those of control rats, except for uric acid and creatine levels, which were significantly lower in the HCSO-fed groups, and the urea level, which was elevated in all HCSO-fed groups. Also, HCSO significantly downregulated the expression of pro-inflammatory cytokines (TNF-α, IL-1α, IL-1β, and IL-6) and their receptors (IL-1R and IL-6R), when compared to the control group. Our results highlight the reno- and cardio-protective potentials of HCSO, as well as its anti-inflammatory potentials.

KEYWORDS: *Arachis hypogea; Hura crepitans; Inflammation; Organ function; Seed oil.*

RESUMEN: ***Evaluación bioquímica del aceite de semilla de* Hura crepitans *infrautilizado: respuestas funcionales e inflamatorias en ratas albinas.*** El aceite de semilla de Hura crepitans (ASHC) sigue estando infrautilizado en gran parte debido a la escasez de datos sobre sus resultados bioquímicos. Para investigar las respuestas funcionales y proinflamatorias al ASHC, veinticuatro ratas macho se agruparon en grupos de cuatro y recibieron dietas compuestas que contenían 5%-ASHC; 10%-ASHC; 15%-ASHC y 15%-AC aceite de cacahuete (aceite de cacahuete control), durante ocho semanas. Se evaluaron las respuestas funcionales y la expresión de citocinas proinflamatorias y sus receptores. Los biomarcadores de la función de los órganos en ratas alimentadas con dietas suplementadas con ASHC fueron estadísticamente similares a los de las ratas de control, excepto por los niveles de ácido úrico y creatina, que fueron significativamente más bajos en los grupos alimentados con ASHC, y el nivel de urea, que fue elevado en todos los grupos alimentados con ASHC. Además, ASHC disminuyó significativamente la expresión de citocinas proinflamatorias (TNF-α, IL-1α, IL-1β e IL-6) y sus receptores (IL-1R e IL-6R), en comparación con el grupo de control. Nuestros resultados destacan los potenciales renoprotectores y cardioprotectores del ASHC, así como su potencial antiinflamatorio.

PALABRAS CLAVE: *Aceite de semilla; Arachis hypogea; Función de los órganos; Hura crepitans; Inflamación.*

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1. INTRODUCTION

Seeds, which are valuable sources of fats and oils, comprise an essential component of the human diet (Hao *et al.,* 2020). Seed oils are finding more and more uses in various industries, e.g., as flavors and textures in food industries and as oleo-chemicals for petrochemical industries (Anyasor *et al.,* 2009). Africa, like most other (sub-)tropical continents, has these seeds and nuts in abundance but is yet to wholly utilize them, owing to the scarcity in data concerning their physical, chemical, biochemical, and industrial properties (Abdulkadir *et al.,* 2013). Nevertheless, some seed oils, like those from *Arachis hypogea* (groundnut) and *Glycine max* (soya beans), have received considerable attention and already play important roles in countries such as Nigeria (Esonu *et al.,* 2014). However, most seeds remain underutilized, one of which is *Hura crepitans* (Abdulkadir *et al.,* 2013; Hao *et al.,* 2020).

H. crepitans L. (common name: Sandbox tree) is an evergreen, perennial and dicotyledonous plant of the spurge family (Euphorbiaceae). It has short, dark, thickly parked, and pointed spines on the trunk and branches and is often planted as shade trees in towns and villages in Nigeria (Ezeh *et al.,* 2012). *H. crepitans* seeds embody a very vital source of oil, with diverse potentials. The seeds contain amino acids at levels comparable to the other utilized seeds, with even higher lysine, cysteine methionine, threonine, and histidine (Ezeh *et al.,* 2012; Esonu *et al.,* 2014). Moreover, the bark has been used as a traditional medicine to treat constipation, skin irritations, microbial and fungal diseases in humans and in veterinary practices (Adindu *et al.*, 2015). In Nigeria, however, the seeds are discarded as waste since there is no definite use for the *H. crepitans* seed oil (Adewuyi *et al.*, 2014). Oil from *Arachis hypogea* (AHO), known as groundnut oil or peanut oil, is one of the major vegetable oils, with as much as 6.05 million metric tons of AHO produced globally in 2019/20 (Akhtar *et al.*, 2014; Arya *et al.*, 2016). AHO is rich in essential vitamins and unsaturated fats but contains a low proportion of saturated fats; it also has good antioxidant properties (Arya *et al.*, 2016). It is therefore not surprising that it is one of the most utilized seed oils, whereas that from *H. crepitans* remain under-utilized, despite boasting comparable physical and chemical properties. The physicochemical properties and fatty acid composition of the seed and seed oil of *H. crepitans* have been reported (Oyeleke *et al.*, 2012; Oyekunle and Omode, 2008). Besides, previous studies demonstrated the antimicrobial potentials of essential oil from *H. crepitans* (Abdulkadir *et al.,* 2013; David *et al.,* 2014) but left issues regarding its safety and other biochemical outcomes unexplored.

This study reports, for the first time, the functional and inflammatory responses to *H. crepitans* seed oil, in comparison with that of *A. hypogea* oil (a well-utilized oil), in a bid to fill the dearth of information regarding the biochemical characteristics of this under-utilized seed oil.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Tris salt, n-hexane, diethyl ether, heparin, Tris, EDTA, boric acid, ethidium bromide, agarose, and hydrogen chloride were obtained from British Drug Houses Chemicals Limited, Poole, England. These and all other reagents used were of the purest grade available.

2.2. Plant materials and oil extraction

H. crepitans seeds, of good quality, were collected from Covenant University in Ota, Ogun state. The seeds were handpicked, air-dried, and then preserved. The seeds were identified and authenticated at the Department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta, with the herbarium voucher number - FUNAABH-0082. The oil was extracted from *H. crepitans* seeds using the Soxhlet extraction technique, with analytical grade n-hexane as extraction solvent (Oniya, 2017), while unadulterated *A. hypogea* oil was purchased from the Kurmi market in the city of Kano, Kano State, Nigeria. Both oils were stored at 25 ^{0}C, in glass vials. Yield (%) was calculated as the percentage of the weight of oil divided by the weight of the seeds (Brühl, 1997). The color and smell of the oils were determined by visual observation and sense of smell.

2.3. In-vitro assays

Peroxide value. An oil sample (1 g) was weighed into a 200-ml conical flask, followed by 25 ml of glacial acetic acid:chloroform solvent (2:1 v/v); saturated potassium iodide (1ml) was then added, and the mixture was left in the dark for 1 minute. Next, 30 ml of water were added, and the mixture was titrated with a

Table 1. Composition of *H. crepitans* seed oil (HCSO) and *A. hypogea* oil (AHO) -compounded diet.

Composition	5% HCSO diet (g/100g)	10% HCSO diet (g/100g)	15% HCSO diet (g/100g)	15% AHO diet (g/100g)
Maize	40	40	40	40
Flour binder	10	10	10	10
Soy beans	7	7	7	7
Groundnut cake	10	10	10	10
Fish meal	9	9	9	9
W/Offal	5	5	5	5
Bone	1.4	1.4	1.4	1.4
Premix (Broiler)	2	2	2	2
Lysine	0.1	0.1	0.1	0.1
Salt (NaCl)	0.2	0.2	0.2	0.2
Methionine	0.3	0.3	0.3	0.3
A. hypogea oil	10	5	-	15
H. crepitans seed oil	5	10	15	-

0.02 N thiosulphate solution using 5 M starch as the indicator. A blank determination was similarly carried out. Peroxide value was calculated from the equation:

Peroxide value (mEq/kg) = [100(V1 -V2)]/W

W = weight of sample (g); V1 = volume (ml) of thiosulphate solution in test; V2 = volume (ml) of thiosulphate solution in blank (Brühl, 1997).

Acid value. The acid value of the oil sample was determined by dissolving 0.20 g of oil in 2.5 ml ethanol:diethyl ether solvent (1:1 v/v) and titrating with 0.1 N potassium hydroxide (KOH) while swirling using phenolphthalein as indicator. The calculation is as follows:

Acid Value (mg KOH/g) = [56.1 x N x V]/W

N = Normality of NaOH; V = Volume (ml) of NaOH; W = Weight of sample (Brühl, 1997).

Saponification value. The Sample oil (1 g) was weighed and transferred into an Erlenmeyer flask, after which 4 mls of ethanol and 2 mls of KOH were added. The flask (equipped with a reflux condenser) was heated in a water bath for 30 minutes with occasional shaking. The flask was cooled, a few drops of phenolphthalein were added, and the excess KOH was immediately titrated with 0.5 M hydrochloric acid (HCl). A blank test was also carried out.

Saponification value (mg KOH/g) = [(a - b) × 28.05]/W

a = volume (ml) of 0.5 M HCl consumed in the blank; b = volume (ml) of 0.5 M HCl consumed in the test; W= weight (g) of sample (Brühl, 1997).

2.4. Experimental animals

Twenty-four (24) male albino rats (150-170g) used in this study were obtained from a reputable animal farm in Ota, Ogun State Nigeria, housed in separate cages under ambient conditions in the animal house of our department, and served food and water *ad libitum*. This study received ethical approval from the ethics committee of the Department of Biochemistry, Federal University of Agriculture, Abeokuta (Ref No: FUNAAB/CBS/BCH/PG/14-0054). All conditions of animal experimentation conformed to the guidelines outlined by Percie du Sert *et al.* (2010).

After a two-week acclimation period, the rats were divided randomly into four groups of six animals each. The first group received a compounded diet containing 5% H. crepitans seed oil (HCSO), and the second group received a 10% HCSO-compounded diet, the third group received a 15% HCSO-compounded diet, while the last group (serving as the control) received a 15% AHO-compounded diet. The 15% AHO group was used as the control since this group received the standard seed oil (AHO) to which HCSO was being compared. The diets, compounded as shown in table 1, were given daily for eight (8) weeks with fresh water *ad libitum*.

2.5. Sample collection

After eight weeks, the animals were sacrificed after an overnight fast under diethyl ether anaesthesia. Blood samples, collected via cardiac puncture, were centrifuged immediately for 10 minutes at 4000 rpm to obtain the plasma. Small portions of organs (liver, kidney, and heart) were stored in RNase-free water (-80 ^{0}C) for gene expression profiling.

2.6. Biochemical analysis

Liver function tests [direct and total bilirubin levels, and the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT)], kidney function tests (levels of uric acid, creatinine, and urea), and heart function tests (lactate dehydrogenase (LDH) and creatine kinase activities) were determined in the plasma, using diagnostic kits obtained from Randox Laboratories Limited (Crumlin, United Kingdom).

2.7. Gene expression profiling

The gene expression profiles for pro-inflammatory cytokines and their receptors [tumor necrosis factor-alpha (TNF-α), interleukin-1alpha (IL-1α), interleukin-1beta (IL-1β), IL-1 receptor (IL-1R), interleukin-6 (IL-6), and IL-6 receptor (IL-6R)] were assessed using semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) techniques. RNA was extracted from the organs using an Easy-Spin plus RNA mini extraction Kit (Sigma-Aldrich, Germany). The RT-PCR was carried out using the Transgen *EasyScript*® one-step RT-PCR super mix kit with gene-specific primers (Table 2). The intensity of the amplicon bands on 1% agarose was analyzed using a UV Transilluminator and the image band was quantified with Image J software (Rotimi *et al.,* 2017).

2.8. Statistical analysis

Values are expressed as mean ± standard error of mean (SEM). The levels of homogeneity among the groups were tested using one-way analysis of variance and Tukey's test, with ($p < 0.05$) consid-

TABLE 2. Gene-specific primers

Genes	Sequence (5`-3`)	Template	TM (ºC)
TNF-α	Forward: ATCCGAGATGTGGAACTGGC Reverse: AAATGGCAAATCGGCTGACG	NM_012675.3	55.0
IL-6	Forward: CACTTCACAAGTCGGAGGCT Reverse: AGCACACTAGGTTTGCCGAG	NM_012589.2	55.0
IL-6R	Forward: GTCAACGACACTGGGCACTA Reverse: TTCAGCGGTCCCAAGGGATA	NM_017020.3	50
IL-1R	Forward: CTATACTTGCCGCACGTCCT Reverse: AAGCACAGAACACGGCTGTA	NM_013123.3	54.4
IL-1β	Forward: GGGCCTCAAGGGGAAGAATC Reverse: ATGTCCCGACCATTGCTGTT	NM_031512.2	56.2
IL-1 α	Forward: CCATCCAACCCAGATCAGCA Reverse: TCTCCTCCCGATGAGTAGGC	NM_017019.1	55.7
β-Actin	Forward: GTCAGGTCATCACTATCGGCAAT Reverse: AGAGGTCTTTACGGATGTCAACGT	NM_031144.3	54.4

TABLE 3. Some physicochemical properties of *A. hypogea* oil (AHO) and *H. crepitans* seed oil (HCSO)

Sample	Saponification value (mg KOH/g)	Peroxide value (meqO$_2$/kg)	Acid value (mg KOH/g)	Color	Odor
AHO	185.95 ± 1.67	15.70 ± 1.44	1.29 ± 0.19	Light brown	Agreeable
HCSO	167.93 ± 3.75	10.50 ± 1.16	0.64 ± 0.02	Golden yellow	Agreeable
P value	0.011	< 0.0001	0.028	-	-

Values are mean ± standard error means of three replicates, with the level of significance determined by student's T-test.

ered significant. For the *in-vitro* assays (two sample groups), the post-hoc test used was student's T-test. All analyses were done using GraphPad Prism (version 8.0).

3. RESULTS

3.1. In-vitro and physiochemical results

The saponification value, peroxide, and acid value of AHO were significantly ($p < 0.05$) higher than that of HCO by 10, 33, and 50%, respectively. HCSO had a golden-yellow color compared to light brown for AHO. At room temperature (25 °C), the two oils were in the liquid state, and both had agreeable odors; while the observed percentage yield for HCSO and AHO were 35.28 and 32.00%, respectively (Table 3).

3.2. Effect of HCSO on liver function biomarkers compared to AHO

There was no significant difference ($p < 0.05$) in the assessed markers for liver function (i.e., levels of direct and total bilirubin, as well as activities of AST, ALT, and GGT) across the groups (Figure 1A-E).

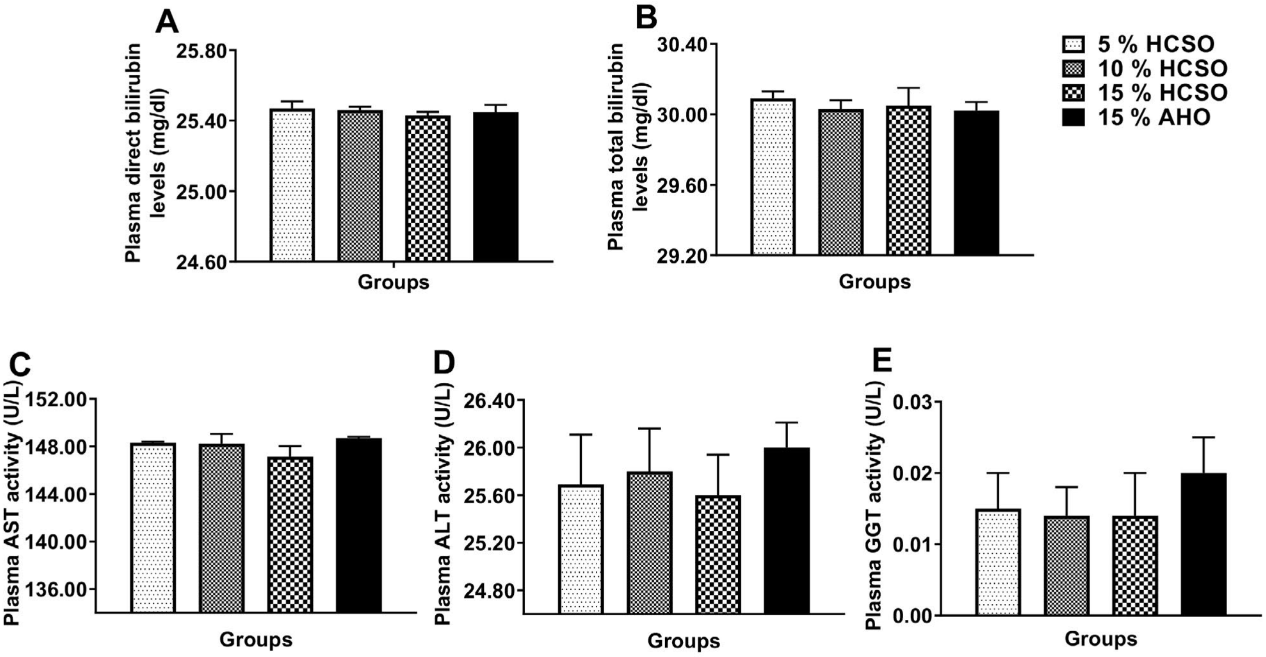

FIGURE 1: **Effect of *H. crepitans* seed oil (HCSO) and *A. hypogea* oil (AHO) on liver function markers in the plasma of experimental rats.** The oils were compounded into the animal diets, with varying percentage compositions (5 – 15%). AST = Aspartate transaminase; ALT = Alanine transaminase; GGT = Gamma-glutamyl transferase. Bars are mean ± standard error of mean (n=6). Bars bearing different letters are significantly different ($p < 0.05$; one-way ANOVA and Tukey test were used to analyze the results).

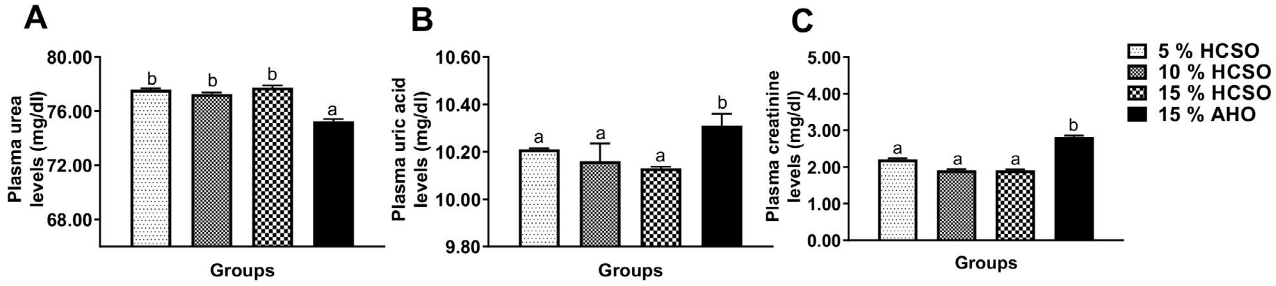

FIGURE 2: **Effect of *H. crepitans* seed oil (HCSO) and *A. hypogea* oil (AHO) on kidney function markers in the plasma of experimental rats.** The oils were compounded into the animal diets, with varying percentage compositions (5 – 15%). Bars are mean ± standard error of mean (n=6). Bars bearing different letters are significantly different ($p < 0.05$; one-way ANOVA and Tukey test were used to analyze the results).

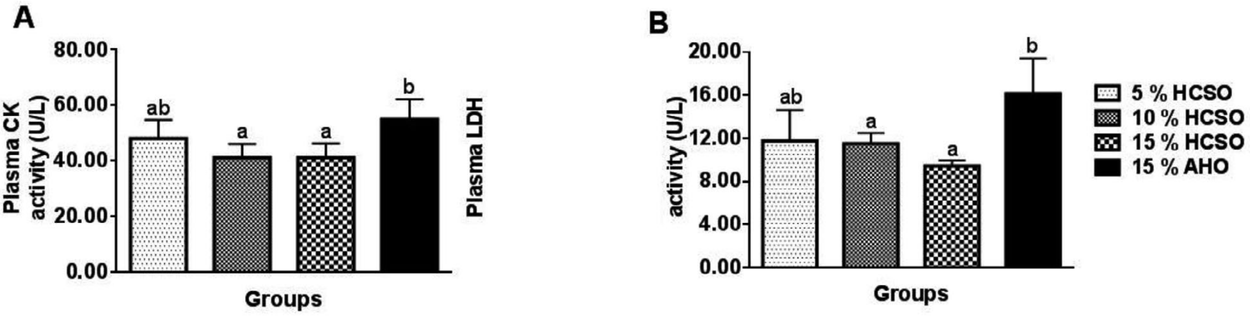

FIGURE 3: **Effect of *H. crepitans* seed oil (HCSO) and *A. hypogea* oil (AHO) on cardiac function markers in the plasma of experimental rats.** The oils were compounded into the animal diets, with varying percentage compositions (5 – 15%). CK = Creatine kinase; LDH = Lactate dehydrogenase. Bars are mean ± standard error of mean (n=6). Bars bearing different letters are significantly different ($p < 0.05$; one-way ANOVA and Tukey test were used to analyze the results).

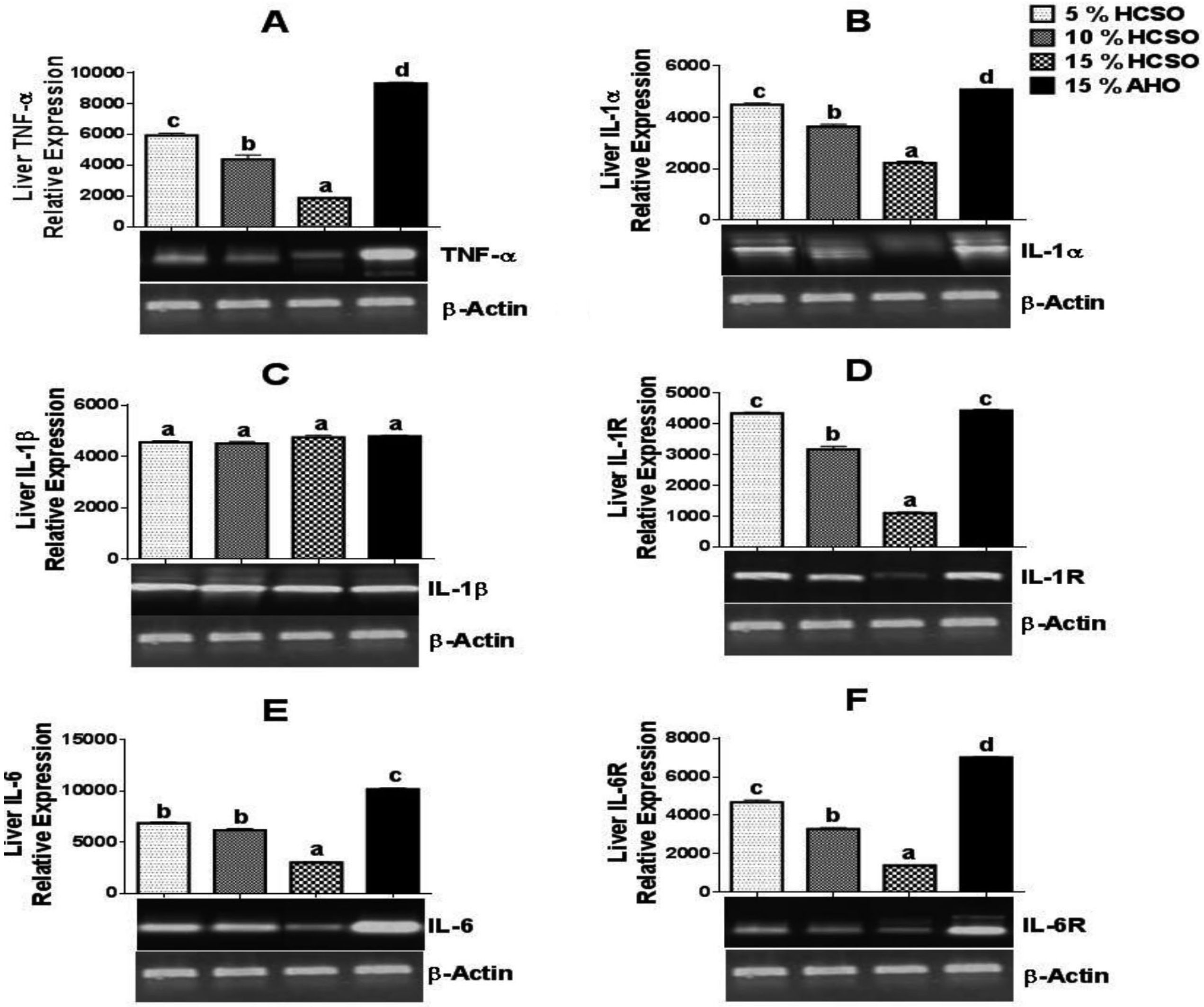

FIGURE 4: **Effect of *H. crepitans* seed oil (HCSO) and *A. hypogea* oil (AHO) on the relative gene expression of pro-inflammatory cytokines and their receptors in the liver of experimental rats.** The oils were compounded into the animal diets, with varying percentage compositions (5 – 15%). TNF-α = Tumour necrosis factor alpha; IL-1α = Interleukin 1 alpha; IL-1β = Interleukin 1 beta; IL-1R = Interleukin 1 receptor; IL-6 = Interleukin 6; IL-6R = Interleukin 6 receptor. Bars are mean ± standard error of mean (n=3). Bars bearing different letters are significantly different ($p < 0.05$; one-way ANOVA and Tukey test were used to analyze the results).

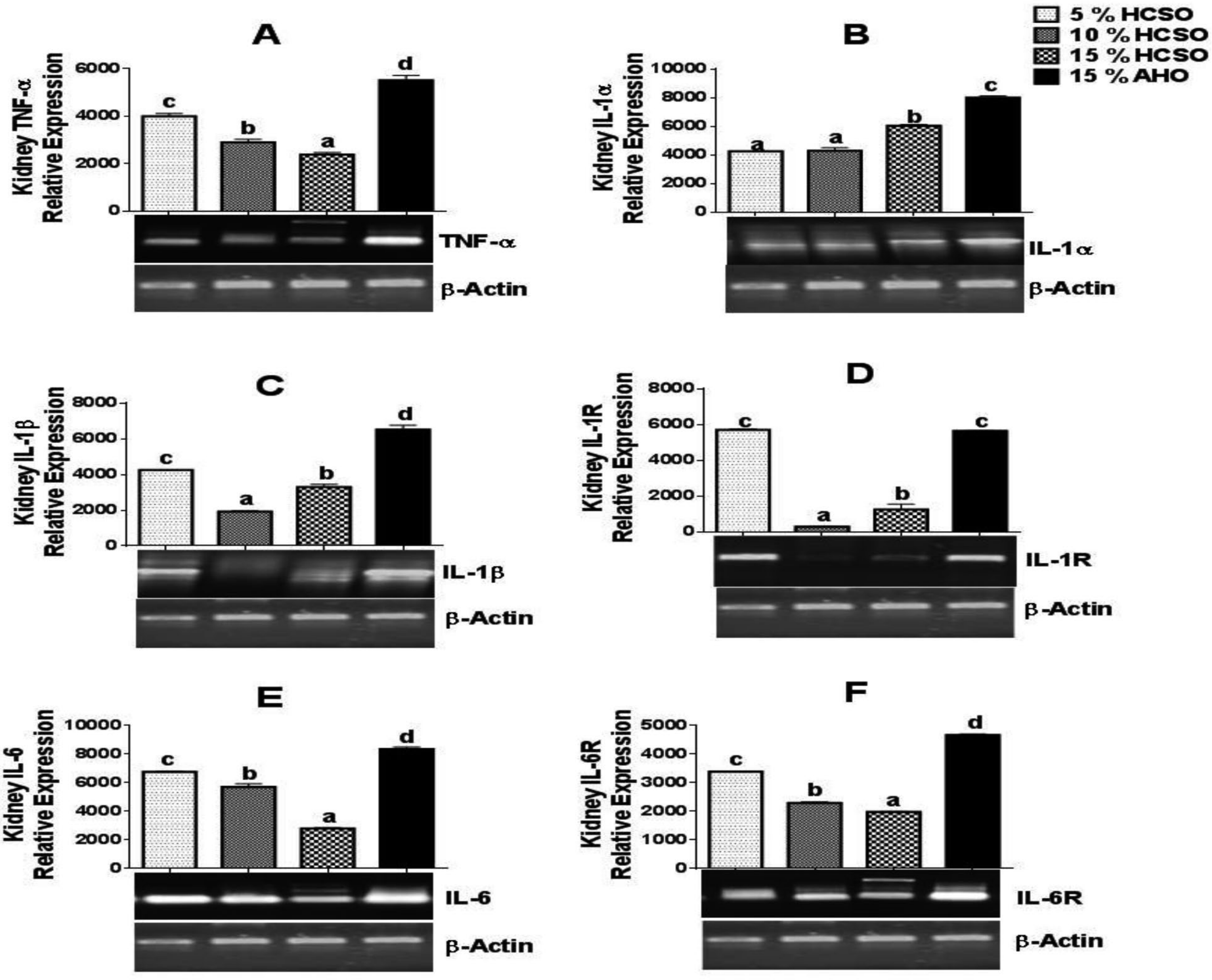

Figure 5: **Effect of *H. crepitans* seed oil (HCSO) and *A. hypogea* oil (AHO) on relative gene expression of pro-inflammatory cytokines and their receptors in the kidney of experimental rats.** The oils were compounded into the animal diets, with varying percentage compositions (5 – 15%). TNF-α = Tumour necrosis factor alpha; IL-1α = Interleukin 1 alpha; IL-1β = Interleukin 1 beta; IL-1R = Interleukin 1 receptor; IL-6 = Interleukin 6; IL-6R = Interleukin 6 receptor. Bars are mean ± standard error of mean (n=3). Bars bearing different letters are significantly different ($p < 0.05$; one-way ANOVA and Tukey test were used to analyze the results).

3.3. Effect of HCSO on kidney function biomarkers compared to AHO

Plasma urea levels were significantly higher ($p < 0.05$) in all HCSO groups compared to the 15%-AHO; while both uric acid and creatinine levels were significantly ($p < 0.05$) decreased in the plasma of the HCSO groups compared to the 15%-AHO group (Figure 2).

3.4. Effect of HCSO on cardiac function biomarkers compared to AHO

The activities of CK and LDH were significantly ($p < 0.05$) lower in 10% and 15%-HCSO groups compared to the 15%-AHO; while in the 5%-HCSO group, CK and LDH enzyme activities were not significantly ($p > 0.05$) different from that of the 15%-AHO (Figure 3).

3.5. Effect of HCSO on relative gene expression of pro-inflammatory cytokines and their receptors in the liver of albino rats, compared to AHO

The gene expressions of TNF-α, IL-1α, IL-1R, and IL-6R, compared to β-actin, followed a similar pattern in the liver (Figure 4A, 4B, 4C, and 4F). Compared to the 15%-AHO group, the relative expressions of these genes were significantly ($p < 0.05$) decreased, with the least expression of these

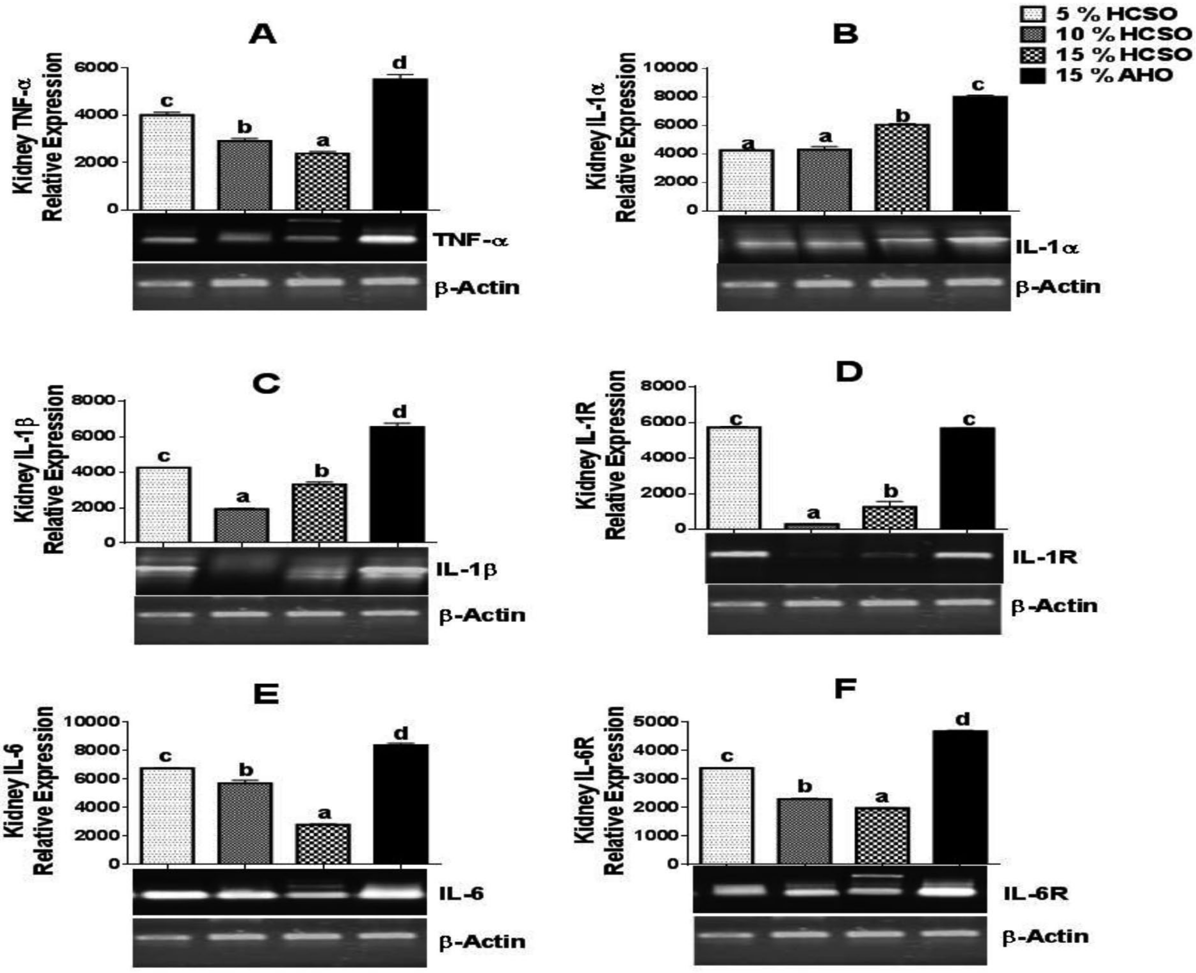

Figure 6: **Effect of *H. crepitans* seed oil (HCSO) and *A. hypogea* oil (AHO) on the relative gene expression of pro-inflammatory cytokines and their receptors in the heart of experimental rats.** The oils were compounded into the animal diets, with varying percentage compositions (5 – 15%). TNF-α = Tumour necrosis factor alpha; IL-1α = Interleukin 1 alpha; IL-1β = Interleukin 1 beta; IL-1R = Interleukin 1 receptor; IL-6 = Interleukin 6; IL-6R = Interleukin 6 receptor. Bars are mean ± standard error of mean (n=3). Bars bearing different letters are significantly different ($p < 0.05$; one-way ANOVA and Tukey test were used to analyze the results).

pro-inflammatory mediators observed in the 15%-HCSO group, followed by the 10%-HCSO group, and then the 5%-HCSO group (i.e., in a dose-dependent manner). The HCSO groups had downregulated expression of IL-6 compared to the 15%-AHO group, with the 15%-HCSO group having the lowest expression; while the 5% and 10%-HCSO groups had statistically similar 1L-6 expression levels (Figure 4E). No significant difference ($p < 0.05$) was observed in the expression of IL-1β (relative to β-actin) across all experimental groups of animals (Figure 4C).

3.6. Effect of HCSO on relative gene expression of pro-inflammatory cytokines and their receptors in the kidney of albino rats, compared to AHO

In a similar manner, the relative expression of gene coding for the pro-inflammatory cytokines and their receptors were significantly ($p < 0.05$) lower in the kidneys of the HCSO groups compared to the 15%-AHO group, except for the IL-1 receptor, where the 5%-HCSO showed no significant ($p > 0.05$) difference from the 15%-AHO group (Figure 5). Notably, the decreased relative expressions of TNF-α, IL-6, and IL-6R followed a dose-dependent trend.

3.7. Effect of HCSO on the relative gene expression of pro-inflammatory cytokines and their receptors in the heart of albino rats, compared to AHO

Similarly, in the heart, the relative expression of the pro-inflammatory cytokines and their receptors were significantly ($p < 0.05$) downregulated, particularly by the 10 and 15%-HCSO diet, when compared to the 15% AHO group (Figure 6).

4. DISCUSSION

The unprecedented surge in the world's population, along with the resultant increased consumption of food and fuel, has made the search for alternative food and fuel sources a priority in the area of study. Seed oils now occupy influential positions in human diets and as alternative biofuels, owing mostly to their tolerability, availability, inexpensiveness, and a plethora of applications (Anyasor *et al.*, 2009; Lei *et al.*, 2018; Pachuau *et al.*, 2019). Despite the intensifying enthusiasm to elucidate the diverse health relevance and bioactivities of these seed oils, many are still under-studied, and one of such is *H. crepitans* (Abdulkadir *et al.,* 2013), partly due to lack of information regarding its biochemical properties. This study thus investigates the functional and pro-inflammatory responses to *H. crepitans* seed oil (HCSO), in comparison with *A. hypogea* oil (AHO), in a bid to fill that dearth of information.

Saponification value correlates inversely with average molecular weight (or, by extension, chain-length) of fatty acids in oil, implying that the lower the saponification value the higher the average molecular weight (or longer the fatty-acid chain) and vice-versa (Gunstone, 2009). Peroxide value is used to monitor the oxidative deterioration (rancidity) of oils. Thus, a high peroxide value may indicate increased oxidation and formation of hydroperoxides (Gordon, 2004). The acid number is a measure of the number of carboxylic acid groups, i.e., the acidity of the oil sample, and a high acid value indicates oil with a reduced quality (Kardash and Tur'yan, 2005). *In-vitro* evaluations revealed similar physical properties (color, odor, and yield) between HCSO and AHO. However, the saponification, peroxide, and acid values were lower in the HCSO, indicating that HCSO may be of even better quality than AHO. Moreover, the values of these chemical properties obtained for HCSO compared favorably with previous values obtained for more utilized seed oils like those of soy bean, sunflower, olive, linseed, etc. (Gunstone, 2009), which is suggestive of the quality of HCSO.

We also examined the effects of HCSO on the function of some vital organs. The liver, the largest internal organ, performs various metabolic functions that are essential for the continuity of life. Due to its strategic position and diverse roles, it is particularly susceptible to diseases (Owojuyigbe *et al.*, 2020). Liver disease, currently among global health issues (Byass, 2014), can be detected by carrying out liver function tests in the blood, such as levels of bilirubin and albumin, as well as the activities of some liver enzymes (Cheesbrough, 2006; Owojuyigbe *et al.*, 2020). Bilirubin is formed from the breakdown of heme. This water-insoluble bilirubin (unconjugated or indirect bilirubin) is transported to the liver, via the blood, where it is conjugated with glucuronic acid (by glucuronosyltransferase) to form water-soluble bilirubin glucuronides (conjugated or direct bilirubin), which are then excreted via biliary excretion. Total bilirubin refers to both direct and indirect bilirubin (Cheesbrough, 2006). Liver enzymes (such as AST, ALT and GGT) are generally useful and rather sensitive markers of liver disease. Localized within the hepatocytes, these enzymes are released into circulation, following the compromise of the cell membrane (resulting from liver injury or disease) (Niemelä and Alatalo, 2010). Increased levels or activities in these biomarkers typically characterize liver damage or disease (Owojuyigbe *et al.*, 2020). Indeed, the hepatoprotective properties of many treatments are assessed based on the reductions in the blood levels of these liver biomarkers. In this study, the activities and levels of these biomarkers in rats fed with HCSO-supplemented diets were not significantly different from that in rats fed with 15% AHO, indicating that HCSO did not damage the liver or impair its function. These findings are corroborated by Igwenyi *et al.* (2017), who reported decreased liver biomarkers (AST, ALT, total and direct bilirubin) following treatment of diabetic rats with *H. crepitans* seed extract (HCSE). They attributed these effects to its rich phytochemical constituents, such as alkaloids, carotenoids, flavonoids, etc., previously characterized by Adindu *et al.* (2015).

The kidney, another major organ, primarily excretes wastes from the blood and are involved in other regulatory processes. However, because they are metabolically active and receive a quarter of cardiac output (despite weighing below 1% of total body weight); while also filtering out water from the filtrate (and may thus concentrate and accumulate toxic substances), the kidneys are particularly vulnerable to injury (Gheshlaghi, 2012; Shaterzadeh-Yazdi *et al.*, 2018). Clinically relevant biomarkers of renal damage include urea, uric acid, and creatinine. Creatinine is produced by the muscle during unaltered catabolism excreted by the kidney, and may thus serve as an important criterion for kidney function. Urea, a waste product from dietary protein, is also filtered into the urine by the kidneys (Burns and Wortmann, 2011). Uric acid, a product of purine metabolism, is a normal component of urine produced in conditions where there is cellular destruction and thus, degradation of the nuclear material and release of purine bases. These purines are, via a series of reactions, converted into either hypoxanthine or xanthine, which are then broken down by xanthine oxidase into uric acid. The excessive build-up of uric acid results in gout (Kanwal *et al.,* 2018). However, these biomarkers (creatinine, urea, and uric acid) are liberally filtered by the glomerulus and efficiently excreted via the urine with negligible metabolism by the kidney. Thus, increased levels in the blood, in most cases, may evince the onset of kidney failure (Burns and Wortmann, 2011). While this current study did not carry out urinalysis, in light of the observed significantly ($p < 0.05$) reduced plasma levels of uric acid and creatinine in all HCSO groups compared to the AHO group, we hypothesize that HCSO promotes renal clearance of these excretory products, suggestive of its reno-beneficial potentials. Interestingly, the levels of urea were significantly ($p < 0.05$) higher in rats fed with the HCSO-supplemented diet compared to those fed the AHO supplemented diet. We attribute this elevation in urea level to the high amino acid content of the *H. crepitans* seed oil (Esonu *et al.*, 2014), especially glutamate (about 14.41 g/100 g protein), as per the reports of Fowomola and Akindahunsi (2007). Thus, the increased urea level may be a consequence of increased amino acid catabolism, which obligates increased urea production (Higgins, 2016).

The heart, a muscular organ, pumps blood that carries nutrients and oxygen to other parts of the body and metabolic waste away from these parts through the blood vessels of the circulatory system (Gaze, 2012). Clinically relevant cardiac tests include CK and LDH activities in the plasma, which are quantifiable markers of the health/disease condition of the heart. CK couples the phosphorylation of creatine to phosphocreatine (PCr) with the dephosphorylation of ATP to ADP. PCr serves as an *in-situ* energy store for the swift regeneration of ATP. Creatine kinase (CK) is primarily cytosolic and is examined as a damage biomarker of CK-rich tissues, like the heart (Moghadam-Kia *et al.,* 2016). LDH, another cytoplasmic enzyme, converts pyruvate to lactate during anaerobic respiration and is extensively expressed in metabolically active tissues like the heart (Hu *et al.*, 2015). Following cardiac injury/damage, these cytosolic enzymes are released into the blood. Thus, elevated activities correlate positively with various heart diseases (Hu *et al.*, 2015; Moghadam-Kia *et al.,* 2016). Our results showed that the CK and LDH activities in rats fed the HCSO-supplemented diet were not significantly different from those obtained from rats fed with AHO. From these results, the HCSO did not provoke any significant damage to the organs (kidney, liver, or heart), at least in comparison with AHO.

Gene analyses revealed a dose-dependent inhibition of the relative expression of genes coding for pro-inflammatory cytokines (TNF-α, IL-1α, IL-1β, and IL-6) and receptors (IL-1R and IL-6R) in the HCSO-fed groups compared to the 15%-AHO group. To the best of our knowledge, this current study is the first to provide data regarding the *in-vivo* anti-inflammatory effects of *H. crepitans* seed oil following oral supplementation. The previously available study on the anti-inflammatory effect of *H. crepitans* focused on its hexane and ethyl acetate extracts and used topical application on rat paws (Avoseh *et al.*, 2018). Although inflammation is a defence mechanism in response to noxious stimuli, e.g., infectious agents, irritants, damaged tissues, etc., if left uncontrolled, it quickly becomes damaging, and this is a contributing factor to the pathogenesis of a plethora of chronic inflammatory diseases (Chen *et al.*, 2018). During the inflammatory response, immune cells are typically activated and they, in turn, secrete cytokines that initiate inflammatory pathways. These pro-inflammatory cytokines (e.g., TNF-α, IL-1β, and IL-6) facilitate inflammation via interaction with the receptors of TNF (TNFR), IL-1 (IL-1R), IL-6 (IL-6R), as well as the Toll-like receptors (TLRs). Following activation, the receptor initiates intracellular signalling cascades, such as the nuclear factor kappa-B (NF-κB), mitogen-activated protein kinase (MAPK), activator of transcription (STAT), and Janus kinase (JAK)-signal transducer cascades

(Zhang *et al.*, 2019). These cascades play major roles in the development of many leading causes of death, like cancer, cardiovascular diseases, chronic obstructive pulmonary disease, diabetes, etc. (WHO, 2020).

The suppression of inflammatory mediators by HCSO, in a dose-dependent manner, clearly evinces its anti-inflammatory properties. It remains to be seen if these properties will prove beneficial in different models of disease conditions. Previous studies have characterized different parts of *H. crepitans* and enumerated various inherent phytochemical constituents, such as the nitrogen-containing alkaloids, aromatic ring-containing phenolics, and isoprene-containing terpenoids (Oyekunle and Omode, 2008; Oyeleke *et al.*, 2012; Adindu *et al.*, 2015). These phytochemicals have been reported to possess antioxidant and anti-inflammatory properties (Chen *et al.*, 2018; Zhu *et al.*, 2018) and may account for the observed effects of HCSO on organ function and inflammatory markers, and even contribute to the lower saponification, peroxide, and acid values obtained for HCSO.

CONCLUSIONS

Our results demonstrate that HCSO did not significantly affect the function of major organs, nor did it provoke adverse inflammatory responses. Instead, it suppressed the expression of pro-inflammatory mediators. Having characterized some of the biochemical effects of HCSO, further research may investigate its potential beneficial effects on various disease states, particularly those involving inflammation.

REFERENCES

Abdulkadir MN, Amoo IA and Adesina AO. 2013. Chemical composition of *Hura crepitans* seeds and antimicrobial activities of its oil. *Int. J. Sci. Res.* **2** (3), 440–445. https://www.ijsr.net/search_index_results_paperid.php?id=IJSRON2013625.

Adewuyi A, Awolade PO and Oderinde RA. 2014. *Hura crepitans* seed oil: an alternative feedstock for biodiesel production. *J. Fuels,* **2014**, 464590. https://doi.org/10.1155/2014/464590.

Adindu EA, Elekwa I and Ogwo JI. 2015. Phytochemical comparative screening of aqueous extracts of the leaves, stem barks, and roots of *Hura crepitans* (L) using GC–FID. *Nat. Sc.* **13** (12), 112–119. http://www.dx.doi.org/10.7537/marsnsj131215.15.

Akhtar S, Khalid N, Ahmed I, Shahzad A and Suleria HAR. 2014. Physicochemical characteristics, functional properties, and nutritional benefits of peanut oil: a review. *Crit. Rev. Food Sci. Nutr.* **54** (12), 1562–1575. https://doi.org/10.1080/10408398.2011.644353.

Anyasor GN, Ogunwenmo KO, Oyelana OA, Ajayi D and Dangana J. 2009. Chemical analyses of groundnut (*Arachis hypogaea*) oil. *Pak. J. Nutr.* **8** (3), 269–272. https://doi.org/10.3923/pjn.2009.269.272.

Arya SS, Salve AR and Chauhan S. 2016. Peanuts as functional food: a review. *J. Food Sci. Tech.* **53** (1), 31–41. https://doi.org/10.1007/s13197-015-2007-9.

Avoseh ON, Ogunbajo LO, Ogunwande IA, Ogundajo AL and Lawal OA. 2018. Anti-inflammatory activity of hexane and ethyl acetate extracts of *Hura crepitans* L. *Eur J Med Plants* **24,** 1–6. https://doi.org/10.9734/EJMP/2018/41439.

Brühl, L. 1997. Official methods and recommended practices of the American oil chemist's society, physical and chemical characteristics of oils, fats and waxes, section I. Ed. *Eur. J. Lipid Sci. Technol.* **99** (5), 197–197. https://doi.org/10.1002/lipi.19970990510

Burns CM, Wortmann RL. 2011. Gout therapeutics: new drugs for an old disease. *Lancet* **377** (9760), 165–177. https://doi.org/10.1016/S0140-6736(10)60665-4.

Byass P. 2014. The global burden of liver disease: a challenge for methods and for public health. *BMC Med.* **12** (1), 1–3. https://doi.org/10.1186/s12916-014-0159-5.

Cheesbrough M. 2006. *District Laboratory Practice in Tropical Countries, Part 1.* Cambridge University Press, Cambridge, UK. https://doi.org/10.1017/CBO9780511543470.

Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. 2018. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* **9** (6), 7204–7218. https://dx.doi.org/10.18632%2Foncotarget.23208.

David OM, Ojo OO, Olumekun VO and Famurewa O. 2014. Antimicrobial activities of essential oils from *Hura crepitans* (L.), *Monodora* *myristica* (Gaertn Dunal) and *Xylopia aethiopica* (Dunal A. Rich) seeds. *Brit. J. Appl. Sci. Tech.* **4** (23), 3332–3341. https://doi.org/10.9734/BJAST/2014/5088.

Esonu BO, Ozeudu E, Emenalom OO, Nnaji C and Onyeikegbulem IK. 2014. Nutritional value of sandbox (*Hura crepitans*) seed meal for broiler finisher birds. *J. Nat. Sci. Res.* **4** (23), 95–99. http://www.iiste.org/Journals/index.php/JNSR/article/view/18523/18794

Ezeh IE, Umoren SA, Essien, EE and Udoh AP. 2012. Studies on the utilization of *Hura crepitans* L. seed oil in the preparation of alkyd resins. *Ind. Crops Prod.* **36** (1), 94–99. https://doi.org/10.1016/j.indcrop.2011.08.013.

Fowomola MA, Akindahunsi AA. 2007. Nutritional quality of sandbox tree (*Hura crepitans* Linn.). *J. Med. Food* **10** (1), 159–164. https://doi.org/10.1089/jmf.2005.062.

Gaze DC. 2012. *The Cardiovascular System: Physiology, Diagnostics and Clinical Implications*. BoD–Books on Demand, Norderstedt, Germany. https://doi.org/10.5772/2266.

Gheshlaghi F. 2012. Toxic renal injury at a glance. *J. Renal Inj. Prev.* **1** (1), 15–16. https://doi.org/10.12861/jrip.2012.07.

Gordon MH. 2004. Factors affecting lipid oxidation, in Steele R (Ed.) *Understanding and Measuring the Shelf-Life of Food*, Woodhead Publishing, Cambridge, pp.128–141. https://doi.org/10.1533/9781855739024.1.128.

Gunstone F. 2009. *Oils and Fats in the Food Industry*, vol. 6. John Wiley & Sons. Hoboken, New Jersey. https://doi.org/10.1002/9781444302424.

Hao W, Zhu H, Chen J, Kwek E, He Z, Liu J, Ma N, Ma KY, Chen ZY. 2020. Wild melon seed oil reduces plasma cholesterol and modulates gut microbiota in hypercholesterolemic hamsters. *J. Agric. Food. Chem.* **68** (7), 2071–2081. https://pubs.acs.org/doi/abs/10.1021/acs.jafc.9b07302

Higgins C. 2016. Urea and the clinical value of measuring blood urea concentration. *Acutecaretesting Org.* 1–6. https://acutecaretesting.org/en/articles/urea-and-the-clinical-value-of-measuring-blood-urea-concentration.

Hu EC, He JG, Liu ZH, Ni XH, Zheng YG, Gu Q, Zhao ZH, Xiong CM. 2015. High levels of serum lactate dehydrogenase correlate with the severity and mortality of idiopathic pulmonary arterial hypertension. *Exp. Ther. Med.* **9** (6), 2109–2113. https://doi.org/10.3892/etm.2015.2376.

Igwenyi IO, Agu EA, Awoke JN, Edwin N, Famurewa AC, Obasi NA, Obasi DC. 2017. Antidiabetic and hepatoprotective effect of *Hura crepitans* seed extract in alloxan-induced diabetic albino rats. *Int. J. Biol. Pharm. Allied Sci.* **6** (9), 1771–1780. https://ijbpas.com/pdf/2017/September/1504287339MS%20IJBPAS%202017%204257.pdf.

Kardash E and Tur'yan YI. 2005. Acid value determination in vegetable oils by indirect titration in aqueous-alcohol media. *Croatica Chem. Acta.* **78** (1), 99–103 https://hrcak.srce.hr/2797.

Kanwal A, Abida L, Sana A, Ahlam S. 2018. A systematic review on the prevalence, pathophysiology, diagnosis, management and treatment of gout (2007-2018). *GSC Biol. Pharm. Sci.* **5** (1), 50–55. https://doi.org/10.30574/gscbps.2018.5.1.0077.

Lei L, Chen J, Liu Y, Wang L, Zhao G and Chen ZY. 2018. Dietary wheat bran oil is equally as effective as rice bran oil in reducing plasma cholesterol. *J. Agric. Food. Chem.* **66,** 2765−2774. https://pubs.acs.org/doi/abs/10.1021/acs.jafc.7b06093.

Moghadam-Kia S, Oddis CV and Aggarwal R. 2016. Approach to asymptomatic creatine kinase elevation. *Clev. Clin. J. Med.* **83** (1), 37–42. https://dx.doi.org/10.3949%2Fccjm.83a.14120.

Percie du Sert N, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P. 2020. Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. *PLoS Biol.* **18** (7), e3000411. https://doi.org/10.1371/journal.pbio.3000411.

Niemelä O and Alatalo P. 2010. Biomarkers of alcohol consumption and related liver disease. *Scand. J. Clin. Lab. Inv* **70** (5), 305–312. https://doi.org/10.3109/00365513.2010.486442.

Oniya OO, Oyelade JO, Ogunkunle O, Idowu DO. 2017. Optimization of solvent extraction of oil from sandbox kernels (*Hura crepitans* L.) by a response surface method. *Energy and Policy Res.* **4** (1), 36–43. https://doi.org/10.1080/23815639.2017.1324332.

Owojuyigbe OS, Firempong CK, Larbie C, Komlaga G and Emikpe BO. 2020. Hepatoprotective potential of *Hura crepitans* L.: a review of ethnomedical, phytochemical and pharmacological studies. *J. Complement. Altern. Med. Res.* **9** (2), 1–10. https://doi.org/10.9734/jocamr/2020/v9i230136.

Oyekunle JAO and Omode AA. 2008. Chemical composition and fatty acid profile of the lipid fractions of selected Nigerian indigenous oilseeds. *Int. J. Food Prop.* **11** (2), 273–281. https://doi.org/10.1080/10942910701302598.

Pachuau L, Devi CM, Goswami A, Sahu S, Dutta RS. 2019. Seed oils as a source of natural bio-active compounds, in: Akhtar M, Swamy M, Sinniah U (Eds.) *Natural Bio-active Compounds*, Springer, Singapore, pp. 209–235. https://doi.org/10.1007/978-981-13-7154-7_8

Rotimi OA, Rotimi SO, Duru CU, Ebebeinwe OJ, Abiodun AO, Oyeniyi BO, Faduyile FA. 2017. Acute aflatoxin B1–Induced hepatotoxicity alters gene expression and disrupts lipid and lipoprotein metabolism in rats. *Toxicol. Rep.* **4,** 408–414. https://doi.org/10.1016/j.toxrep.2017.07.006.

Shaterzadeh-Yazdi H, Noorbakhsh MF, Samarghandian S and Farkhondeh T. 2018. An overview on renoprotective effects of thymoquinone. *Kidney Dis.* **4** (2), 74–82. https://doi.org/10.1159/000486829.

Oyeleke GO, Olayiwola OA, Latona DF. 2012. Chemical Examination Of Sandbox (Hura Crepitans) Seed: Proximate, Elemental And Fatty Acid Profile. *Magnesium* **112**, 0–1. https://doi.org/10.9790/5736-0121013

WHO. World Health Organization. 2020. *The Top 10 Causes of Death*. Available: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death.

Zhang L, Virgous C, Si H. 2019. Synergistic anti-inflammatory effects and mechanisms of combined phytochemicals. *J. Nutr. Biochem.* **69**, 19–30. https://doi.org/10.1016/j.jnutbio.2019.03.009.

Zhu F, Du B and Xu B. 2018. Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: A review. *Crit. Rev. Food Sci. Nutr.* **58** (8), 1260–1270. https://doi.org/10.1080/10408398.2016.1251390.

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A research on the chemical, mineral and fatty acid compositions of two almond cultivars grown as organic and conventional in southeastern Turkey

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SUMMARY: Organic farming is a human and environment friendly production system that is based on soil fertility and food safety without using chemical fertilizers and pesticides in production, aiming to re-establish the deteriorated ecological balance as a result of harmful production practices. Organic products attract the interest of consumers as they are strongly perceived as healthier products compared to conventional food. This study aimed to determine the differences in chemical, mineral, and fatty acid characteristics between conventionally and organically cultivated Ferragnes and Ferraduel almond cultivars. When conventional and organic almonds were evaluated in terms of fatty acids, proximate compositions, and minerals, crude oil and Mg were statistically insignificant, while Cu was significant ($P < 0.05$) and all others were quite significant ($P < 0.001$). Total sugar was higher in organic samples compared to conventional samples in both cultivars. The crude oil and linoleic acid (12.93% for Ferragnes and 14.99% for Ferraduel) were higher in conventional samples but oleic acid (78.9% for Ferragnes and 81.08% for Ferraduel) was higher in organic samples. In addition, organic samples contained higher Mg and Fe but lower P, K, Ca, Na, Zn, Mn and Cu when compared with conventional samples. The results indicate that conventionally cultivated almonds present higher mineral content and lower fatty acid value compared to organically cultivated almonds.

KEYWORDS: *Conventional; Fatty acid; Minerals; Organic; Proximate; Prunus dulcis.*

RESUMEN*: Investigación sobre la composición química, minerales y ácidos grasos de dos cultivares de almendra cultivados como orgánico y convencional en el sudeste de Turquía*. La agricultura orgánica es un sistema de producción amigable con el ser humano y el medio ambiente, basado en la fertilidad del suelo y la seguridad alimentaria. Este sistema suele prescindir del uso de fertilizantes químicos y pesticidas en la producción, con el objetivo de prefijar el equilibrio ecológico previamente destruido como resultado natural de prácticas de producción incorrectas. Los productos orgánicos están atrayendo cada vez más el interés de los consumidores ya que se perciben como productos más saludables en comparación con los alimentos convencionales. Este estudio tenía como objetivo determinar las diferencias en las características químicas, minerales y ácidos grasos existentes entre los cultivares de almendra de Ferragnes y de Ferraduel, cultivados tanto convencionalmente como orgánicamente. Cuando se evaluaron las almendras convencionales y las orgánicas en términos de ácidos grasos, composiciones proximal y minerales, el aceite crudo y el Mg fueron estadísticamente insignificantes, mientras que los valores de Cu fue significativo ($P<0.05$) y todos los demás fueron bastante significativos ($P<0.001$). El azúcar total fue mayor en muestras orgánicas en comparación con muestras convencionales en ambos cultivares. El aceite crudo y el ácido linoleico (12.93% para Ferragnes y 14.99% para Ferraduel) fue mayor en muestras convencionales, pero el ácido oleico (78.9% para Ferragnes y 81.08% para Ferraduel) fue mayor en muestras orgánicas. Además, las muestras orgánicas contenían mayor Mg y Fe pero menor P, K, Ca, Na, Zn, Mn y Cu cuando se compararon con las muestras convencionales. Los resultados indican que las almendras convencionales presentan mayor contenido mineral y menor valor de ácidos grasos en comparación con las cultivadas orgánicamente.

PALABRAS CLAVE: *Ácido graso; Composición proximal; Convencional; Minerales; Orgánico; Prunus dulcis.*

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1. INTRODUCTION

The cultivated almond is classified into the subgenus Amygdalus from the genus Prunus of the family Rosaceae (Kester and Gradziel, 1998). The *Prunus amygdalus* L. (almond) is an important nut crop and cultivated and produced for various purposes compared to other commercial nuts in the world (Simsek *et al*., 2018). Turkey is one of the leading countries in almond production. The agricultural data shows that Turkey (150.000 tons) is the forth largest almond producer after USA (1.936.840 tons), Spain (340.420 tons) and Iran (177.015 tons) (FAO, 2020; Gülsoy and Şimşek, 2020).

The almond is one of the fruit species whose production and consumption is increasing around world because it contains valuable nutrients that have many benefits on human health (Gülsoy and Balta, 2014). The almond is a source of important compounds to maintain a healthy metabolic function because of its high levels of antioxidants, dietary fiber, vitamins (mainly E, A, B1, and B2) minerals, fat, protein, essential fatty acids, and polyphenols (Welna *et al*., 2008; Barreca *et al*., 2020). Recent studies have documented a wide range of health benefits including possible protection from cancer (Davis and Iwahashi, 2001), obesity (Foster *et al*., 2012), diabetes (Lovejoy *et al*., 2002), high cholesterol (Fulgoni *et al*., 2002) and coronary heart disease (Jenkins *et al*., 2002). It is not only beneficial for muscle and joint pain, rheumatism, and skin diseases, but it also reduces headache, sore throat, urinary infections, and kidney disorders (Mushtaq *et al*., 2015).

As organic foods are perceived by consumers as healthier, more beneficial for a sustainable environment, more nutritious compared to conventionally produced foods and higher in quality, (Magnusson *et al*., 2001; Macllwain, 2004; Gomiero, 2018), the organic food industry is one of the fastest growing food and agriculture industries worldwide (Ballester-Costa *et al*., 2013). With the increasing number of consumers who care about healthy lifesyles and the environment, the tendency towards organically produced foods is increasing (Gülnur *et al*., 2016). However, whether there is a significant difference in terms of nutritional value between organic foods and foods grown according to conventional methods is a subject of ongoing discussion among consumers (Akan and Yılmaz, 2015). For this reason, in recent years, many studies have been conducted about the nutritional value of organically produced foods compared to those produced conventionally. These studies have revealed that organic foods have high dry matter content and amino acid quality (Zorb *et al*., 2006), high polyunsaturated fatty acids (Dangour *et al*., 2009), low nitrate and mycotoxin (Pussemier *et al*., 2006), high content in minerals (Lairon, 2010), a high amount of vitamin C, anthocyanin and antioxidant capacities (Woese *et al*., 1997), rich in carotenoids (Hoefkens *et al*., 2010) and phenolic compounds (Wang *et al*., 2008).

There are various studies in the literature comparing the quality characteristics of organic and conventional almonds. In these studies, Sánchez-Bel *et al*. (2008) compared the effect of organic and inorganic fertilizer applications on the chemical quality properties of the Guara almond cultivar. Venkatasubramanian (2011) compared some nutritional quality properties of organic and conventional market foods, including almonds. Vrastil (2018) compared some mineral contents of convetionally and organically grown almonds purchased from local markets. Karaat (2019) compared the pomological parameters, chemical properties and volatile aroma compounds of two organic and conventionally grown almond cultivars. However, there are limited number of studies, especially for detalied fatty acid compounds and macro-micro element contents in organic and conventionally grown almonds.

According to previous research, cultivation and retail market studies are two common methods used to examine the differences between organic and conventional products. In both methods, the advantages and disadvantages are highlighted. This research was performed on the food compositions of almonds picked directly from organic and conventional orchards and not by purchasing products from markets.

Environmental circumstances, harvest and post-harvest influences and accessible information on the products are some of the characteristics that distinguish farm and cultivation studies from retail market studies. For this reason, the notion that retail studies are representative of the expanding system on a large scale has been accepted (Magkos *et al*., 2006). Considering all of these reasons, this research was carried out to assess and to compare some chemical properties, mineral contents, and fatty acid compositions of Ferragnes and Ferraduel almond cultivars grown by organic and conventional methods in the Dicle region (Diyarbakır) of Turkey.

2. MATERIALS AND METHODS

2.1. Plant materials and sampling

The study was conducted in the Dicle district of the Diyarbakir province of Southeast Turkey in 2020. The plant material of the study was composed of 6-7-year old Ferragnes and Ferraduel cultivars grafted on seedling rootstocks. The conventional almond orchard is situated between 38˚21'04"N and 40˚04'31"E coordinates, while the organic orchard is situated between 38˚20'51"N and 40˚03'55"E. The distance between both orchards is about 1000 meters. Ecological conditions and cultural practices such as drip irrigation, tillage, pruning and planting distance (5x6 meters) of the two almond orchards were similar.

In this study, 5 trees of each cultivar were selected and 3 repetitive readings on each tree were done. The study was designed according to the randomized complete block design. In the study, 5 trees for conventional growing, 5 trees for organic growing from each of the Ferragnes and Ferraduel cultivars were chosen to make a total of 20 trees.

2.2. Almond kernel analyses

2.2.1. Proximate analyses

Immediately after harvesting, the outer green sheels of the almonds were removed, and the almond fruits were dried for 20 days at room temperature. Then, fruit samples were ground, and their moisture content was determined. For moisture analysis, 3 g samples were weighed in nickel dry matter containers (tared), and oven dried at 105 ºC when their weight was constant. The results were calculated according to the methods given by the Turkish Standard Institute (AOAC, 1990; Simsek *et al*., 2018). Total ash content was determined by the combustion of 1 g of kernel almond into the muffle oven at 560 °C according to Gönül *et al*. (1988). For crude oil analysis, 5 g samples were homogenized and subjected to extraction for 6 h with 60-80 ml of hexane (boiling range 30-60 °C) in a Soxhlet apparatus. After 6 h of extraction, the samples were evaporated under vacuum, and then weighed and their oil yield was determined (James, 1995; Simsek *et al*., 2018). The crude protein ingredient was calculated by multiplying the percentage of nitrogen (N%) with a constant factor of 6.26 (AOAC, 1990; Simsek *et al*., 2018). Total sugar content was determined by the Anthrone method (Kaplankıran, 1984; Simsek *et al*., 2018).

2.2.2. Mineral composition

Analyses were performed by Atomic Absorption Spectrometry (Perkin-Elmer 703) for magnesium (Mg), calcium (Ca), iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn), Flame Emission Spectrometry for potassium (K) and sodium (Na). Phosphorus (P) were determined by the colorimetric method (Saura Calixto and Canellās, 1982; Simsek *et al*., 2018; Simsek, 2021).

2.2.3. Fatty acid composition

For the the fatty acid methyl ester (FAME) extraction, 0.1 g of exemplary fat was melted in 2 ml of heptane, and 0.2 ml of a 2M methanolic KOH solution. The solution was shaken strongly for 30 seconds and was left to stand until the supernatant liquid became clear. Then, the heptane solution was injected into the gas chromatograph (GC). FAME analysis was performed on a 60 m capillary column (ID = 0.25 mm) covered with an Agilent 6890 series gas chromatography, adorned with a flame ionization detector, and 0.25 µm and 50% cyanopropyl methylpolysiloxane (J & W Scientific, Folsom, CA, the US) (Simsek, 2021). Helium gas was used as carrier gas at a flow rate of 30 ml/min and 1:50 ratio, and the temperature of the injector and the detector were set at 260 and 280 °C, respectively. The oven temperature was scheduled in accordance with a retention time of 1 minute at 120 °C, raised to 170 °C at 6.5 °C/min, and finally to 215 °C at 2.15 °C/min. Fatty acid methyl esters were defined by using standard FAMEs (Supelco – 47885 - U) and calculated in accordance with their percentage values (Dieffenbacher and Pocklington, 1992; Batun *et al*., 2017). In addition to individual fatty acid data, saturated fatty acids (SFA), unsaturated fatty acids (UFA) and UFA/SFA ratios were calculated.

2.3. Statistical analysis

All measurements were carried out in triplicate and analyses of variance (one-way Anova) of the results were performed using SPSS-22 software. The means obtained from each set were compared using the Duncan's multiple range test at 0.05 confidence level.

3. RESULTS AND DISCUSSION

Significant differences ($P < 0.001$) for the evaluated proximate parameters were detected except for crude oil which was not significantly different between cultivars or growing systems (Table 1).

In the Ferraduel cultivar, there was a statistical difference in crude protein, moisture, and ash contents between conventional and organic growing, but no difference was found for crude oil or total sugar contents. However, while moisture, ash and total sugar were significantly different in Ferragnes, crude fat and protein were not.

The proximate chemical content results are presented in Table 1. The crude oil and crude protein contents of conventional Ferragnes samples were higher than the organic samples (48.32 and 48.01%, 24.34 and 23.97%, respectively). In the Ferraduel cultivar, crude oil was higher in the conventional samples than in the organic ones, (47.85 and 47.21% ,respecitvely) while crude protein was higher in the organic samples (24.08 and 26.44%, respectively). The highest moisture content was found in organic Ferragnes (2.90%), while the lowest was found in organic Ferraduel (2.57%). The lowest and highest ash values were found in Ferraduel. The highest total sugar content was found in the organic Ferragnes samples (5.20%) (Table 1).

There are very few studies investigating the chemical differences between organic and conventionally grown almonds. Among these studies, Venkatasubramanian (2011) compared the chemical properties of some organic and conventionally grown nuts and reported higher protein and carbohydrate, lower fat, moisture, and energy in organic production compared to conventional. These results were in parallel with the findings of the present study related to the protein in the Ferraduel cultivar, while incompatible results were obtained related to Ferragnes, and similar results were obtained with both cultivars for crude oil. Gulsoy and Balta (2014) investigated the total oil and protein compositions of conventionally grown Ferragnes and Texas cultivars in Aydın, Turkey and found 22.8% protein and 54.% crude oil for Ferragnes, and 33.1% protein, 49.8% crude oil for Texas. Rabadán *et al.* (2017) studied the crude oil contents of ten almond cultivars in Spain and reported crude oil contents as 54.14% for Ferragnes and 53.96% for Ferraduel. Karaat (2019) reported higher crude fat and lower crude protein, ash, and moisture in traditional compared to organic production of Ferragnes and Ferrudal cultivars grown in Adıyaman.

The differences in chemical composition may be caused by numerous factors such as cultivar, genetic factors, environmental conditions, irrigation, soil, tree age, growing technique, post-harvest processing, packaging, and storage conditions (Mele *et al.*, 2018).

The statistical analysis results for the conventional versus organic almond mineral content comparisons are given in Table 2. Results from the data analyses indicate that there were statistically significant differences at $P < 0.001$ when comparing phosphorus, potassium, calcium, sodium, zinc, manganese iron and cupper between conventionally and organically grown almonds. However, magnesium was found to be insignificant. The micro and macro element contents in the almond kernels are presented in Table 2.

The highest amounts of macro and micro minerals were found in the Ferraduel cultivar except

TABLE 1. Sugar and proximate chemical composition of almond cultivars from conventional and organic orchards

	Ferragnes		**Ferraduel**		**Level of significance p**
	Conventional	**Organic**	**Conventional**	**Organic**	
Crude oil (%)	[+]48.32±1.42	48.01±2.40	47.85±1.78	47.21± 1.46	NS
Crude protein (%)	24.34±0.88 b	23.97±2.02 b	24.08±1.64 b	26.44±1.35 a*	0.000**
Moisture (%)	2.63±0.40 b	2.90±0.26 a	2.96± 0.39 a	2.57±0.34 b	0.000
Ash (%)	3.41± 0.26 a	3.25± 0.13 b	3.51±0.25 a	3.21±0.18 b	0.002
Total sugar (%)	3.22±0.21 c	5.20±0.34 a	3.78±0.97 b	3.84±0.43 b	0.000

* Means followed by the same letter in the each row are insignificant at the 95% probability level according to Duncan's multiple range test
NS,**: non-significant, significant at $P \leq 0.001$, respectively. The results are expressed as mean value ± standard deviation
[+]: The study was carried out with three replicates and each replicate corresponded to five plants. Therefore, the values are based on fifteen plants.

TABLE 2. Macro and micro element contents in almond cultivars from conventional and organic orchards (mg/100 g).

	Ferragnes		**Ferraduel**		**Level of significance p**
	Conventional	**Organic**	**Conventional**	**Organic**	
P	544.28±73.36 b	538.31±73.51 b	687.35±77.68 a	503.35±67.27 b*	0.000***
K	752.12±70.16 b	794.67±149.22b	916.49±93.67 a	653.43±116.01 c	0.000
Ca	343.30±110.69 b	247.33±27.18 c	445.42±94.37 a	294.79±52.18 c	0.000
Mg	222.40±19.73	229.12±17.77	236.38±34.79	242.59±14.96	NS
Na	2.15±0.42 a	0.87±0.12 c	1.15±0.20 b	1.08±0.18 b	0.000
Zn	4.66±0.55 b	5.77±0.81 a	6.18±0.64 a	6.02±0.31 a	0.000
Mn	1.84±0.34 a	1.58±0.21b	1.91±0.35 a	1.84±0.15 a	0.000
Fe	5.89±0.50 c	6.73±0.73 b	7.00±0.79 b	8.01±0.31 a	0.000
Cu	1.72±0.51 b	1.96±0.20 a	1.99± 0.24 a	1.72± 0.19 b	0.010**

* Means followed by the same letter in the each row are insignificant at the 95% probability level according to Duncan's multiple range test
NS,**: non-significant, significant at P ≤ 0.001, respectively. The results are expressed as mean value ± standard deviation
+: The study was carried out with three replicates and each replicate corresponded to five plants. Therefore, the values are based on fifteen plants.

sodium (Na). The highest contents in phosphorus (P), potassium (K), calcium (Ca) and zinc (Zn), manganese (Mn) and copper (Cu) were found in the conventional samples of Ferraduel 687.35 mg/100g, 916.49 mg/100g, 445.42 mg/100g, 6.18 mg/100g,1.91 mg/100g and 1.99 mg/100g, respectively. The highest magnesium (Mg) and iron (Fe) contents were found in organically grown Ferraduel with 242.59 mg/100g and 8.01 mg/100g, respectively (Table 2). The results indicated higher mineral contents for the conventional samples of the Ferraduel cultivar compared to the organic samples. Phosphorus (P), calcium (Ca), sodium (Na) and manganese (Mn) contents were found to be higher in conventional Ferragnes cultivar than in the organic samples, while potassium (K), magnesium (Mg), zinc (Zn), iron (Fe) and copper (Cu) contents were higher in the organic samples.

Generally, the Mg and Fe contents in organic almonds were found to be higher than those of conventional almonds and P, K Ca, Na, Zn, Mn, Cu contents were observed to be lower than in conventional almonds.

In a comparative review conducted by Worthington (2001), higher levels of iron and magnesium in organic vegetables than in conventional ones were found consistent with the findings of the present. Fertilization significantly affects the mineral content of plants. Conventional potassium fertilizers dissolve more easily in soil water, while organically grown soils retain moderate amounts of potassium and magnesium in the root area of the plant. Therefore, organic products would be expected to contain more magnesium and phosphorus than conventional products (Crinnion, 2010).

Conventional almond samples contained more sodium compared to the organic ones. Since easily soluble fertilizers are used in conventional agriculture, soil solution ions are easily absorbed by the roots. Most mineral fertilizers contain highly soluble sodium and are quickly taken up by plants (Gastol and Domagala- Świątkiewicz, 2012).

Venkatasubramanian (2011) found that organic almonds have higher Fe, Ca, and K contents, and lower Na content compared to conventional almonds. The findings of the present study for Ferragnes were in line with Venkatasubramanian's (2011) findings for Fe, K, and Na, but not for Ca. The findings for Ferraduel were compatible with Fe and Na, but incompatible with Ca and K.

Vrastil (2018) compared some mineral (Ca, Fe, Mg, K, Zn) contents in traditionally and organically grown almonds purchased from local markets in three states of the USA and he found a small effect for Fe, a medium-size effect for Ca and K, and no effect for Mg or Zn between conventional and organic production statistically. Yada *et al.* (2011) stated that the nutrient composition of almonds may be changed depending on genotype and cultivar dependents, and may also be influenced by environmental factors, such as growing region, cultivation methods, and climatic conditions. On the other hand, differences in the mineral contents

TABLE 3. Fatty acid compositions (percent) of almonds from organic and conventional orchards

	Ferragnes		**Ferraduel**		**Level of significance p**
	Conventional	**Organic**	**Conventional**	**Organic**	
Palmitic Acid (C16:0)	6.02±0.26 d	6.69±0.18 b	6.26±0.31 c	7.61±0.09 a*	0.000**
Stearic Acid (C18:0)	2.04±0.06 d	2.26±0.06 c	2.39±0.18 b	2.77±0.06 a	0.000
Heptadecanoic Acid	0.09±0.01	0.09±0.00	0.09±0.01	0.14±0.18	NS
Arachidic Acid (C20:0)	0.09±0.01 b	0.09±0.01 b	0.10±0.01 a	0.08±0.01 b	0.000
Tricosanoic Acid (C23:0)	0.07±0.04a	0.04±0.02 b	0.05±0.03 b	0.07±0.03 a	0.004
Palmitoleic Acid (C16:1)	0.64±0.03 d	0.77±0.03 b	0.74±0.03 c	0.85±0.03 a	0.000
Oleic Acid (C18:1)	79.01±0.35 c	79.53±0.63 b	76.36± 0.70 d	81.08±0.49 a	0.000
Linoleic Acid (C18:2)	12.93±0.40 b	11.51±0.67 c	14.99±0.70 a	8.54±0.48 d	0.000
Linolenic acid (C18:3)	0.05±0.01	0.05±0.01	0.04±0.01	0.04±0.01	NS
SFA	8.06±0.26 d	8.96±0.21 b	8.65±0.39 c	10.38±0.12 a	0.000
UFA	89.62±0.26 a	91.04±0.21 c	91.35±0.39 b	89.62±0.12 d	0.000
UFA/SFA	11.42±0.38 a	10.17±0.26 c	10.58±0.53 b	8.64±0.11 d	0.000

* Means followed by the same letter in each row are insignificant at the 95% probability level according to Duncan's multiple range test

NS,**: non-significant, significant at P ≤ 0.001, respectively. The results are expressed as mean value ± standard deviation

+: The study was carried out with three replicates and each replicate corresponded to five plants. Therefore, the values are based on fifteen plants.

SFA: saturated fatty acids, UFA: unsaturated fatty acids, UFA/SFA: saturated fatty acids/ unsaturated fatty acids

of almonds may be a result of specific practices, such as tillage or fertilization. Since mineral content depends on soil composition, differences may be less dependent on conventional or organic status, and more dependent on fertilization methods.

The analysis of variance for the fatty acids investigated in the present study and the multiple comparison test results are given in Table 3. There were significant differences ($P < 0.001$) for the evaluated fatty acids between cultivars and growing systems, except for heptadecanoic and linolenic acid. The fatty acid contents in the conventionally and organically grown almond samples are reported in Table 3. The prevalent fatty acids found in the almond cultivars were oleic acid, linoleic acid, palmitic acid, and stearic acid. Other fatty acids were found in trace amounts.

Oleic acid contents were found higher in organically grown Ferragnes and Ferraduel, while linoleic acid contents were lower in both cultivars. The highest oleic acid content was found in organic growing, which was 79.53% for Ferragnes and 81.08% for Ferraduel (Table 3). Conventional Ferraduel samples were found to possess the highest linoleic acid content (14.99%) followed by conventional Ferragnes samples (12.93%). On the other hand, palmitic, palmitoleic and stearic acid were determined at the highest level in organic production for both cultivars. Their values were higher in organic Ferraduel samples (7.61, 0.85, and 2.77% respectively). In addition, unsatured fatty acids (UFA) and unsatured fatty acid/ satured fatty acid ratios (UFA/SFA) were found to be higher, and satured fatty acids (SFA) were found to be lower in both cultivars of organic almonds compared to conventional growing (Table 3). The results showed that organic almonds contain a higher amount of fatty acids compared to conventional ones.

In previous works, Samman *et al.* (2008), reported no consistent difference between the overall fatty acid composition of commercially available certified organic and conventionally produced edible oils. (coconut, canola, sesame and sunflower). Soares *et al.* (2013), reported that conventional and organic cultivation methods had little effect on the fatty acid composition of cashew nuts. Rabadan *et al.* (2017) reported that oleic, linoleic, and palmitic acid for Ferragnes and Ferraduel cultivars were 71.81, 17.62, 6.49 and 67.52, 21.47, and 6.64% respectively. Karaat (2019) compared the fatty acid composition of almond kernels grown organically and conventionally and reported that the most significant differences were observed in oleic and linoleic acid contents. The highest oleic acid content (82.4% in both cultivars) was recorded in organic fruits when compared to conventional samples (78.9% for Ferragnes and 75.8% for Ferraduel). Linoleic acid was found at the lowest ratio in organic samples for both cultivars. The results of this study were similar to Karaat's work (2019), although slightly higher. Simsek (2021) reported that oleic, linoleic, and palmitic acid contents ranged from 69.21 to 71.88%, from 18.76 to 22.02% and 5.62 to 7.35% in the almond genotypes from the Adiyaman province, respectively. In the present study, the oleic acid contents in conventional almond samples were somewhat different from those reported in previous works. (Rabadan *et al*., 2017; Simsek, 2021) but the linoleic acid content was lower. These differences and similarities in composition may be due to a diversified ecological condition of the soil, climate, location, genetics, and routine agronomic practices. Amaral *et al*. (2006) reported that genetic factors, as well as environmental factors, such as year of production and growing location, strongly influence the fatty acid composition. The difference between the fatty acid contents found in the present study compared to that found by other authors may be due to the methodologies used for extraction and quantification, growing conditions, and climate, time of harvest, variety, ripening, and processing methods.

4. CONCLUSIONS

In recent years, in parallel with the determination of the close relationship between nutrition and health and the increase in awareness in society, there has been a demand for healthier food among consumers. In order to meet this need, production systems that do not use chemical pesticides and fertilizers in both cultivation and processing stages, known as organic or ecological agriculture, have been developed in contrast to conventional production.

The purpose of this study was to explore the differences in biochemical contents between conventionally and organically produced almonds from two different cultivars. The results indicate that the growing system affects the biochemical contents of the Ferragnes and Ferraduel cultivars. Significant differences were found between the biochemical compositions of organically and conventionally grown samples of both cultivars. According to the results of the analysis, conventional almonds were found to be richer in terms of mineral content; whereas fatty acids were detected at higher levels in organic almonds.

Differences in the biochemical contents of the almond kernels may be due to various agricultural processes, such as location, soil composition, irrigation and fertilization, as well as many environmental factors. Although many studies have compared various organically and conventionally grown fruits and vegetables, the number of studies on almonds is limited.

Moreover, with the increasing demand for organic food products, including almonds, the acreage dedicated to organically grown almonds is likely to increase. In this context, it is thought that the findings from this study will make several contributions to the current literature.

REFERENCES

Akan S, Yanmaz R. 2015. Organik gıdaların besin kalitesi ve insan sağlığına etkileri yönünden değerlendirilmesi. *Doğu Karadeniz II. Organik Tarım Kongresi*, 6–9.

Amaral JS, Cunha SC, Santos A, Alves MR, Seabra RM, Oliveira BP. 2006. Influence of cultivar and environmental conditions on the triacylglycerol profile of hazelnut (*Corylus avellana* L.). *J. Agric. Food Chem.* **54** (2), 449–456. https://doi.org/10.1021/jf052133f

AOAC. 1990. *Official Methods of Analysis*, 14th edn. Washington, DC: Association of Official Analytical Chemists.

Ballester-Costa C, Sendra E, Fernández-López J, Pérez-Álvarez JA, Viuda-Martos M. 2013. Chemical composition and in vitro antibacterial properties of essential oils of four Thymus species from organic growth. *Indus. Crops Prod.* **50,** 304–311. https://doi.org/10.1016/j.indcrop.2013.07.052

Barreca D, Nabavi SM, Sureda A, Rasekhian M, Raciti R, Silva AS, Mandalari G. 2020. Almonds (*Prunus dulcis* Mill. DA Webb): a source of nutrients and health-promoting compounds. *Nutrients*, **12** (3), 672. https://doi.org/10.3390/nu12030672

Batun P, Bakkalbaşı E, Kazankaya A, Cavidoğlu İ. 2017. Fatty acid profiles and mineral contents of walnuts from different provinces of Van lake. *Gıda*, **42** (2) 155–162. https://dergipark.org.tr/tr/download/article-file/272305

Crinnion WJ. 2010. Organic foods contain higher levels of certain nutrients, lower levels of pesticides, and may provide health benefits for the consumer. *Altern. Med. Rev.* 15 (1) 4–12.

Dangour A, Dodhia SK, Hayter A, Allen E, Lock K, Uauy R. 2009. Nutritional quality of organic foods: a systematic review. *Am. J. Clinical Nutrit.* **90** (3), 680–685 https://doi.org/10.3945/ajcn.2009.28041

Davis PA, Iwahashi CK. 2001.Whole almonds and almond fractions reduce aberrant crypt foci in a rat model of colon carcinogenesis. *Cancer Lett.* **165** (1), 27–33. https://doi.org/10.1016/S0304-3835(01)00425-6

Dieffenbacher A, Pocklington WD. 1992. IUPAC *Standart Methods for The Analysis of Oils, Fats and Derivatives*, 6th Edition (Fifth Edition Method II.D.19) pgs. 96–102, Pergamon Press Oxford.

FAO, 2020. Food and Agriculture Organisation. http://www.fao.org/faostat/en/#home

Foster GD, Shantz KL, Vander Veur SS, Oliver TL, Lent MR, Virus A, Szapary P, Rader DJ, Zemel BS, Gilden-Tsai A. 2012. A randomized trial of the effects of an almond-enriched, hypocaloric diet in the treatment of obesity. *Am. J. Clinical Nutrit.* **96**, 249–254. https://doi.org/10.3945/ajcn.112.037895

Fulgoni VL, Abbey M, Davis P, Jenkins D, Lovejoy J, Most M, Sabate J, Spiller G. 2002. Almonds lower blood cholesterol and LDL-cholesterol but not HDL-cholesterol in human subjects: results of a meta-analysis. *FASEB J.* **16,** A981–A982.

Gąstoł M, Domagała-Świątkiewicz I. 2012. Comparative study on mineral content of organic and conventional apple, pear and black currant juices. *Acta Sci. Pol.*, *Hortorum Cultus*, **11** (3), 3–14. https://www.researchgate.net/publication/258238191

Gomiero T. 2018. Food quality assessment in organic vs. conventional agricultural produce: findings and issues. *Appl. Soil Ecol.* **123**, 714–728. https://doi.org/10.1016/j.apsoil.2017.10.014

Gönül M, Altug T, Boyacıoglu D, Noka Ü, 1988. Gıda Analizleri. Ege Üniversitesi Mühendislik Fakültesi Çogaltma Yayın **64**, 179s., İzmir.

Gülnur ETİ, Anıl NK, Kılıç B. 2016. Tüketicilerin organik gıda satın alma tercihlerini etkileyen faktörler. *Kırklareli Üniversitesi İktisadi ve İdari Bilimler Fakültesi Dergisi*, **5** (2), 93–108. https://dergipark.org.tr/tr/pub/klujfeas/issue/26889/283270

Gülsoy E, Balta F, 2014. Determination of protein, oil and fatty acid contents of some selected almond (*Prunus amygdalus* Batch) Genotypes from Karacasu and Bozdoğan Yenipazar of Aydın Province. *J. Instit. Sci. Technol.* **4** (1), 9–14.

Gülsoy E, Şimşek M, 2020. *Almond Growing*, Dora Publishing. 116 syf. Bursa

Hoefkens C, Sioen I, Baert K, Meulenaer BD, Henauw SD, Vandekinderen I, Devlieghere F, Opsomer A, Verbeke W, Camp JV. 2010. Consuming organic versus conventional vegetables: The effect on nutrient and contaminant intakes. *Food Chem. Toxicol.* **48**, 3058–3066. https://doi.org/10.1016/j.fct.2010.07.044

James CS, 1995. *Analytical Chemistry of Foods*. Publisher Blackie Academic and Professional, London, pp. 176.

Jenkins DJ, Kendall CW, Marchie A, Parker TL, Connelly PW, Qian W, Spiller GA. 2002. Dose response of almonds on coronary heart disease risk factors: blood lipids, oxidized low-density lipoproteins, lipoprotein(a), homocysteine, and pulmonary nitric oxide: a randomized, controlled, crossover trial. *Circulation,* **106** (11), 1327–1332.

Kaplankiran M. 1984. Research on the relationship between natural hormone, carbohydrate and plant nutrient levels and growth of some citrus rootstocks. – PhD Thesis, *C. U. Science Institute*, Adana, Turkey.

Karaat FE. 2019. Organic vs conventional almond: market quality, fatty acid composition and volatile aroma compounds. *Appl. Ecol. Environment. Res.* **17** (4), 7783–7793. http://dx.doi.org/10.15666/aeer/1704_77837793

Kester DE, Gradziel TM. 1998. The University of California almond breeding programme: I. historical aspects. *Nucis,* **7**, 8-10.

Lairon D. 2010. Nutritional quality and safety of organic food. A review. *Agron. Sustaın. Dev.* **30** (1), 33–41. https://link.springer.com/content/pdf/10.1051/agro/2009019.pdf

Lovejoy JC, Most MM, Lefevre M, Greenway FL, Rood JC. 2002. Effect of diets enriched in almonds on insulin action and serum lipids in adults with normal glucose tolerance or type 2 diabetes. *Am. J. Clin. Nutrit.* **76**, 1000–1006. https://doi.org/10.1093/ajcn/76.5.1000

Macllwain C. 2004. Organic: is it the future of farming?. *Nature*, **428**, 792–793. https://pubmed.ncbi.nlm.nih.gov/15103346/

Magkos F, Arvaniti F, Zampelas A. 2006. Organic food: buying more safety or just peace of mind? A critical review of the literature. *Critical Rev. Food Sci. Nutrit.* **46**, 23–56. https://doi.org/10.1080/10408690490911846

Magnusson M K, Avrola A, Hursti Koivisto UK, Aberg L, Sjoden PO. 2001. Attitudes towards organic foods among Swedish consumers. *British Food J.* **103**, 209–226.

Mele MA, Islam MZ, Kang HM, Giuffrè AM. 2018. Pre-and post-harvest factors and their impact on oil composition and quality of olive fruit. *Emirates J. Food Agric.* **30** (7), 592–603. https://doi.org/10.9755/ejfa.2018.v30.i7.1742

Mushtaq A, Khaliq M, Saeed A, Azeem MW, Ghania JB. 2015. Almond (*Prunus amygdalus* L.): A review on health benefits, nutritional value and therapeutic applications. *Int. J. Chem. Biochem. Sci.* **8**, 103–106. https://www.researchgate.net/profile/Jihene-Ben-Ghnia/publication/342153576

Pussemier L, Larondelle Y, Van Peteghem C, Huyghebaert A. 2006. Chemical safety of conventionally and organically produced foodstuffs: a tentative comparison under Belgian conditions, *Food Control* **17**, 14–21. https://www.favv-afsca.be/scientificcommittee/publications/articles/_documents/organic_final.pdf

Rabadán A, Álvarez-Ortí M, Gómez R, Pardo-Giménez A, Pardo JE. 2017. Suitability of Spanish almond cultivars for the industrial production of almond oil and defatted flour. *Scientia Horticult.* **225**, 539–546. https://www.cabdirect.org/cabdirect/abstract/20173322066

Samman S, Chow JW, Foster MJ, Ahmad ZI, Phuyal JL, Petocz P. 2008. Fatty acid composition of edible oils derived from certified organic and conventional agricultural methods. – *Food Chem.* **109** (3), 670–674. https://doi.org/10.1016/j.foodchem.2007.12.067

Sánchez-Bel P, Egea I, Martínez-Madrid MC, Flores B, Romojaro F. 2008. Influence of irrigation and organic/inorganic fertilization on chemical quality of almond (Prunus amygdalus cv. Guara). *J. Agric. Food Chem.* **56** (21), 10056–10062. https://doi.org/10.1021/jf8012212

Saura Calixto F, Canellās J. 1982. Mineral composition of almond varieties (*Prunus amygdalus* L.). *Z. Lebensm. Unters. Forsch.* **174** (2), 129–131.

Simsek M. 2021. Evaluation of some physical properties and fatty acid compositions of native almondgenotypes grown in Adiyaman province of Turkey. *Fresenius Environmental Bulletin*, **30** (06A), 6349–6359.

Simsek M, Gulsoy E, Yavic A, Arikan B, Yildirim Y, Olmez N, Erdogmus B, Boguc F. 2018. Fatty acid, mineral and proximate compositions of various genotypes and commercial cultivars of sweet almond from the same ecologıcal condıtıons. *Appl. Ecol. Environment. Res.* **16** (3), 2957–2971. http://www.aloki.hu/pdf/1603_29572971.pdf

Soares DJ, Vasconcelos PHMD, Camelo ALM, Longhinotti E, Sousa PHMD, Figueiredo R WD. 2013. Prevalent fatty acids in cashew nuts obtained from conventional and organic cultivation in different stages of processing. *Food Sci. Technol.* **33**, 265–270.

Venkatasubramanian C. 2011. Nutritional quality and acceptability of organic and conventional foods. – *Indian J. Sci. Technol.* **4** (3), 361–365. https://doi.org/10.17485/ijst/2011/v4i3.41

Vrastil JA. 2018. A Comparison of Mineral Content in Conventionally and Organically Grown Almonds. *California State University*, Long Beach.

Wang SY, Chen CT, Sciarappa W, Wang CY, Camp MJ. 2008. Fruit quality, antioxidant capacity, and flavonoid content of organically and conventionally grown blueberries, *J. Agric. Food Chem.* **56**, 5788–5794. https://doi. org/10.1021/jf703775r

Welna M, Klimpel M, Zyrnicki W. 2008. Investigation of major and trace elements and their distributions between lipid and non-lipid fractions in Brazil nuts by inductively coupled plasma atomic optical spectrometry. *Food Chem.* **111,** 1012–1015. https://doi. org/10.1016/j.foodchem.2008.04.067

Woese K, Lange D, Boess C, Bogl KW. 1997. A comparison of organically and conventionally grown foods-results of a review of the relevant literature. *J. Sci. Food Agric.* **74** (3), 281–293.

Worthington V. 2001. Nutritional quality of organic versus conventional fruits, vegetables, and grains. *J. Alternat. Complement. Med.* **7**, 161–173. https://doi.org/10.1089/107555301750164244

Yada S, Lapsley K, Huang G. 2011. A review of composition studies of cultivated almonds: Macronutrients and micronutrients. *J. Food Composit. Anal.* **24** (4-5), 469–480. https://doi.org/10.1016/j.jfca.2011.01.007

Zorb C, Langenkamper G, Betsche T, Niehaus K, Barsch A. 2006. Metabolite profiling of wheat grains (*Triticum aestivum* L.) from organic and conventional agriculture. *J. Agric. Food Chem.* **54** (21), 8301–8306. https:// doi.org/ 10.1021/ jf0615451

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