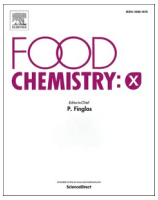
Food Chemistry: X 24 (2024) 101875

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Assessment of the impact of microwave roasting on nutrient content, lipid profile, and oxidative stability of pomegranate seed oil

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ARTICLE INFO

Keywords:
Pomegranate seeds
Oil
Punicic acid
Oxidadtion
Antioxidant
Food processing
γ-Tocopherol
β-Sitosterol
Isomerization

ABSTRACT

The pomegranate, *Punica granatum* L. (*Punicaceae*), stands as one of the most widely employed oils in the cosmetic industry. However, due to its higher content of conjugated linoleic acid, its susceptibility to oxidation is a major challenge, with the most prominent being punicic acid. This study aimed to evaluate the effects of traditional roasting in a microwave on the lipid content, nutritional value, and oxidative stability of Moroccan pomegranate seed oil. The findings indicated a rise in the amount of oil after 15 min of roasting at 650 W, the amount of oil rose from 27.03 to 30.10 (g/100 g). However, the protein content, UV absorbance values, iodine, and saponification values were not significantly affected by a longer roasting time. The peroxide value increases with roasting (1.00 to 5.00 M.eq. O_2/kg oil). The roasting process under 350 W did not significantly alter the fatty acid composition. The total tocopherol content exhibits a decrease with increasing roasting time and power, ranging from 333.36 mg/100 g for unroasted seeds to 316.84 mg/100 g for seeds roasted under the conditions of 650 W for 15 min. The roasting process has proven to be critical for the immediate and long-term preservation of the nutritional and physico-chemical properties of pomegranate seed oil.

1. Introduction

Pomegranate (*Punica granatum*), belonging to the Punicaceae family, is one of the oldest edible fruits, extensively used in traditional medicine (Schubert et al., 1999). Its delicious fruits are consumed worldwide and widely cultivated in India and the Mediterranean region. They are also grown sporadically in the USA, Japan, Russia, and China (Fadavi et al., 2006). Pomegranate fruits are eaten raw or used to make fresh juice from their edible sections (Fadavi et al., 2006). There are significant amounts of seeds in these fruits, ranging from 40 to 100 g per kilogram of fruit weight (Fadavi et al., 2006). Unfortunately, these seeds are often considered waste byproducts of fruit processing (Hajib et al., 2021). The seeds of pomegranates are an abundant source of lipids, making up around 12 % to 20 % of the total seed weight, with variations among different cultivars (Hajib et al., 2021; Kýralan et al., 2009).

Conjugated linolenic acids (CLnAs), commonly known as trienoic acids, are abundant in pomegranate seed oil (PSO) (Lansky & Newman, 2007; Vroegrijk et al., 2011). It has been determined that there are four unique geometric isomers of conjugated linolenic acid, specifically punicic acid (C18:3 cis-9, trans-11, cis-13), catalpic acid (C18:3 trans-9, trans-11, cis-13), α-eleostearic acid (C18:3 (cis-9, trans-11, trans-13), and *β*-eleostearic acid (C18:3 trans-9, trans-11, trans-13) (Aruna et al.,

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https://doi.org/10.1016/j.fochx.2024.101875
Received 11 August 2024; Received in revised form 30 September 2024; Accepted 3 October 2024
Available online 5 October 2024

2016; Hajib et al., 2021; Melo et al., 2014). Among these, punicic acid, the primary CLnA found in pomegranate seed oil, exhibits a diverse range of biological properties (Melo et al., 2014). These properties include its potential as an antidiabetic agent, anti-inflammatory effects, hypolipidemic qualities, and its ability to demonstrate anticarcinogenic activity against various types of cancer (Arao, Wang, et al., 2004; Arao, Yotsumoto, et al., 2004; Boussetta et al., 2009; Koba et al., 2007; Tsuzuki et al., 2004).

Roasting is a pre-treatment technique that allows for microstructural, physical, and chemical modifications of a material under the influence of heat, leading to the formation of new aromatic compounds, distinct flavors, and modified physical and chemical properties (Lee et al., 2004). Roasting seeds enables the desired flavors and colors to be achieved while enhancing the oil's palatability. While it plays a role in eliminating undesired microorganisms and deactivating enzymes that contribute to product spoilage during storage, it is also accountable for improving oil yield and generating antioxidant compounds through non-enzymatic reactions (Chandrasekara et al., 2012). Furthermore, previous studies have shown a positive correlation between the duration of roasting and the stability of prickly pear, pistachios, argan, and rapeseeds (Harhar et al., 2011; Nounah et al., 2021; Rabadán et al., 2018; Rękas et al., 2017). However, if not controlled, the roasting process can result in negative changes, such as the creation of undesirable aromas and side effects, such as the production of off-flavors and toxic compounds like acrylamide (Stadler et al., 2002).

Considering the lower resistance of PSO to oxidation, this study aims to explore whether roasting, including a microwave-based method, can enhance the oxidative stability, lipid profile, and nutritional value of Moroccan 'Sefri' pomegranate oil.

2. Materials and methods

2.1. Plant material collection and preparation

In this study, we used dried pomegranate seeds available in the Flora cooperative of the 'Sefri' variety (*Punica granatum*). September 2022 was the date of the fruit collection from Boujad city, Morocco. The seeds have been sorted and washed, and finally dried in the dark at ambient temperature. Subsequently, plastic bags were for storing the seeds in seven samples of 100 g each until their use. Each of these samples was roasted at two power levels in the microwave: 350 W and 650 W for durations of 5, 10, and 15 min.

2.2. Reagents and solvents

Every reagent employed in this investigation was analytical grade, and Professional Labo (Casablanca, Morocco) supplied the chromatographic quality mobile phase for HPLC.

2.3. Seed analysis

The proximate composition was conducted on roasted and unroasted 'Sefri' seed powder. A triplicate of each test was performed, and the averages were reported. According to the ISO 665 standard (2020), the moisture content (**MC**) was determined. Approximately 5 g of samples were dried at 103 ± 2 °C in a ventilated oven until the weight reached a steady amount.

The total nitrogen content of the seed powder was evaluated using the Kjeldahl procedure. Samples of 0.3 g were digested for 5 h with a mixture of sulfuric acid, salicylic acid, and selenium at 300 °C using a digestion block. 5.5 mL of buffer solution (sodium hypochlorite and sodium nitroprusside) were combined with the resulting filtrate. After incubation in the dark at 37 °C for 15 min, absorbance was measured at 650 nm.

The protein content (**PC**) was determined by multiplying the nitrogen content obtained by a conversion factor of 6.25. The oil content (**OC**) of pomegranate seeds was determined following the ISO 659 standard (2009). Approximately 100 g of powder seed were extracted for 8 h using a Soxhlet apparatus; the solvent used was hexane (300 mL). The oils were collected and stored at about −4 °C for further analysis. The extraction yield was calculated gravimetrically.

2.4. Oil analysis

2.4.1. Oil quality index

a. **Free fatty Acid Value**

The determination of free fatty acid value (**FFA**) in PSO was conducted following the ISO 660 standard (2020) method. Titration was carried out with a 0.1 N KOH solution. The results were expressed as grams of punicic acid per 100 g of oil.

b. **Peroxide Value**

The peroxide value (**PV**) was measured following the ISO 3960 standard (2017) method. The titration was carried out with a sodium thiosulfate solution. The results were expressed in milliequivalents of oxygen per kilogram of oil, providing a measure of primary oxidation in the oil.

c. **UV Absorption (K232 and K270)**

The determination of absorbance at 232 nm and 270 nm was conducted in accordance with the ISO 3656 standard (2011). This method allows for assessing the oxidative state of the oil by detecting peroxides and secondary oxidation products, providing valuable information about the oil's stability and quality.

d. **Saponification Index**

The determination of the saponification value (**SV**) of vegetable oil was carried out according to the ISO 3657 standard (2020) method. Titration was carried out with 0.5 N hydrochloric acid (HCl). The result was expressed in milligrams of KOH per gram of oil.

e. **Iodine Value**

The determination of the iodine value (**IV**) was performed following the ISO 3961 (2018) method. Titration was carried out with sodium thiosulfate solution. The results were expressed in grams of halogen fixed per 100 g of fat.

2.4.2. Oil composition

a. **Fatty Acids composition**

The transformation of fatty acids into their methyl ester derivatives (FAMEs) was performed following the ISO method (12966–2 - 2017) and the composition of fatty acids was analyzed using the ISO method (12966–4 - 2015).

b. **Phytosterol Content and Composition**

The determination of individual phytosterols and total phytosterols was conducted following the ISO method (12228–1 - 2014). Quantification was based on the internal standard (α-cholestanol), and the sterol content was expressed in mg/100 g of the oil.

c. **Tocopherol Content and Composition**

The analysis of tocopherol content and composition was conducted

utilizing HPLC in accordance with the ISO protocol (9936–2016).

The identification of tocopherols was based on retention time, the external standards (α-tocopherol) were used for the quantification of tocopherols. The results were expressed as mg/100 g of oil.

2.5. Statistical analysis

The data were presented as means ± standard deviation, and an analysis of variance was conducted using Tukey's test at a 95 % confidence level with IBM SPSS Statistics 21.

With the use of the metan package in RStudio version (1.3.1093), associations between the physicochemical properties of pomegranate seed oils in this study were evaluated using the Pearson correlation coefficient (r).

3. Results and discussion

3.1. Seed analysis

3.1.1. Moisture content

The moisture content is a critical and essential factor that offers valuable insights into the stability of oilseeds, especially during storage. It significantly affects the quality and yield of oil (Nounah et al., 2021). Elevated water content in seeds can accelerate acylglycerol hydrolysis, resulting in the formation of oxidation byproducts, thereby altering the organoleptic characteristics of the oil, as reported by Harhar et al. (2010).

As indicated in the data presented in Table 1, our results are consistent with the suggested moisture content limit for secure seed storage (8 g/100 g), reducing the potential for oxidation (Brooker et al., 1992; Patterson & Henry, 1989). The moisture content of roasted seeds (RS) did not exceed 8 g/100 g, except for the unroasted sample (URS), which exhibited a slightly higher moisture content at 8.50 ± 0.86 g/100 g. It was observed that the humidity levels decreased as the roasting time and power increased. The lowest moisture content level was achieved with the 650 W/15 min treatment, measuring 1.16 ± 0.41 g/100 g. In summary, moisture content plays a pivotal role in the quality and stability of edible oil kernel seeds, and the data in Table 1 illustrates how roasting conditions impact this crucial parameter, with potential implications for oil quality and yield as reported by (Kaseke et al., 2020; Raigar et al., 2017).

3.1.2. Oil content

The oil extraction yield from unroasted pomegranate seeds, obtained using a Soxhlet apparatus, was 27.03 ± 0.74 g/100 g (Table 1). This outcome closely higher than the results of Hajib et al. (2021), who documented a value of 22.63 ± 0.5 g/100 g for 'Sefri' pomegranate seeds from Morocco. In South Africa, Tafadzwa Kaseke et al., observed values ranging from 23 g/100 g to 32 g/100 g, whereas in Morocco, as reported by Loukhmas et al. (2021), the range spanned from 17.59 g/100 g to 24.69 g/100 g. Meanwhile, in India, Gaikwad et al. (2017) determined an oil content of approximately 20.72 g/100 g. In contrast, Jing et al. (2012) reported lower oil content in Chinese pomegranate seeds, ranging from 11.4 g/100 g to 14.9 g/100 g. Additionally, Fernandes et al. (2015) found pomegranate seeds analyzed in Spain to contain 13.7 g/100 g of oil, while (Fadavi et al., 2006) reported an even lower oil content of 6.63 g/100 g for seeds from Iran. It is important to note that this variation in oil content may be attributed to several factors, including the extraction method employed, the specific pomegranate cultivars under study, regional climatic variations, and environmental conditions, as suggested by Taoufik et al. (2015).

Additionally, our findings demonstrated a notable impact of the roasting process on the oil content of pomegranate seeds under certain conditions. The oil content increased as the roasting time and power increased. The highest value was recorded in roasted seeds under 650 W/10 min (33.12 ± 2.32 g/100 g). Roasted seeds under 350 W/15 min also revealed a significant increase in oil content compared to unroasted seeds. This rise in oil content during roasting could be attributed, as suggested by Ji et al. (2018), to the thermal degradation of cell walls and bonding between lipids and the cellular matrix. Consistently, earlier research has suggested that roasting treatments have the potential to enhance seed oil yield (Anjum et al., 2006; Hama, 2017; Ji et al., 2019).

3.1.3. Protein content

Proteins serve as the fundamental building blocks for growth, tissue renewal, and are integral constituents of muscles in all organisms. Their significance in human nutrition cannot be overstated, as highlighted by Nounah et al. (2021). The protein content of unroasted pomegranate seeds was 11.26 ± 0.10 g/100 g (Table 1). This value closely aligns with the findings of Saeed Dadashi et al. (2013), who reported a protein content of 11.3 ± 0.06 g/100 g in 'Malas' variety pomegranate seeds, and Viuda-Martos et al. (2012), who observed a protein content of about 10.94 ± 0.12 g/100 g in the 'Mollar de Elche' variety pomegranate seed. In contrast, de Wanderley et al. (2023) reported a lower protein value of 7.38 ± 0.04 g/100 g in pomegranate seeds from Brazil. These proteins hold promise as nutritious ingredients for the development of health-conscious foods and as a potential food source across various sectors.

When examining the impact of roasting conditions, it becomes evident that the protein content shows a small loss during roasted treatment. Indeed, Coghe et al. (2006) suggested that the roasting process could potentially lead to a reduction in the protein content of seeds. This reduction is believed to arise from Maillard-type non-enzymatic reactions occurring when reducing sugars interact with proteins during roasting. The small loss in protein content observed during our study can be attributed to the specific roasting method employed. According to Coghe et al. (2006), the Maillard-type non-enzymatic reactions are more pronounced under the high temperatures typically seen in traditional roasting methods. These reactions can lead to a greater reduction in protein content. However, in our study, roasting was carried out using microwave technology, which generally results in more controlled and less intense heat distribution compared to traditional methods. This suggests that microwave roasting, due to its gentler thermal impact, may limit the extent of protein degradation compared to conventional roasting techniques.

3.2. Oil quality index

3.2.1. Free fatty acid

The acidity of seed oils is assessed by the quantification of free fatty acids. It has emerged as a simple and effective means for the quantitative

Table 1
Influence of roasting time and power on proximate composition (g/100 g) of pomegranate seeds Moroccan "Sefri".

Proximate composition	URS	RS 350 W/5 min	RS 350 W/10 min	RS 350 W/15 min	RS 650 W/5 min	RS 650 W/10 min	RS 650 W/15 min
MC (g/100 g)	8.50 ± 0.86^{a}	5.83 ± 0.41^{b}	3.33 ± 0.41^{c}	1.66 ± 0.41^{d}	3.10 ± 0.14^{c}	1.85 ± 0.17^{d}	1.16 ± 0.41^{d}
OC (g/100 g)	27.03 ± 0.74^{c}	29.75 ± 0.44^{b}	30.41 ± 0.65^{b}	32.74 ± 0.04^{a}	32.21 ± 1.26^{a}	33.12 ± 2.32^{a}	30.10 ± 1.12^{b}
PC (g/100 g)	11.26 ± 0.10^{a}	11.40 ± 0.89^{a}	10.90 ± 1.15^{a}	11.63 ± 0.16^{a}	11.18 ± 0.18^{a}	10.82 ± 0.41^{a}	10.84 ± 0.95^{a}

URS: unroasted seeds; **RS:** roasted seeds; **MC:** moisture content; **OC:** oil content; **PC:** protein content. Mean values ± SD of determination for triplicate samples. Means followed by similar letters superscript in the same line are not significantly different according to the Tukey's test ($p < 0.05$).

and qualitative evaluation of oils (Rahmani, 2005). Typically, if the oil is freshly extracted from healthy seeds and processed according to good milling practices, it exhibits very low acidity (Chimi, 2001). However, during pretreatment, extraction, or storage, oil can undergo alteration, leading to an increase in acidity due to the release of free fatty acids through the hydrolysis of triglycerides (Pilz & März, 2008).

The results obtained have shown that all oils from the studied seeds (Table 2), both roasted and unroasted, meet the standards set by CODEX ALIMENTARIUS, (2021), as the free fatty acid content of the analyzed samples remains below 4 mg of KOH/g of oil. The results showed that the quality of the oil samples was good during the roasting process. In addition, we observed that roasting had a slight effect on the value of free fatty acids. Specifically, a slight decrease was observed from 0.78 ± 0.23 to 0.44 ± 0.01 and to 0.53 ± 0.06 under the conditions of 350 W/15 min and 650 W/15 min, respectively. The reduction in the free fatty acid index of the oil can be attributed to the inactivation of lipase enzymes due to thermal pretreatment. Comparable results were observed by Mazaheri et al. (2019), who noted a decrease in the acidity index of *Nigella sativa* oil samples during the roasting process compared to the control sample. A similar trend was also observed by Suri et al. (2022), with acidity progressively decreasing as microwave power increased from 350 W to 650 W over 5 to 15 min.

Fukuda (1990) noted that oils obtained from roasted sesame seeds have higher acidity compared to oils from unroasted seeds. Comparable findings were reported by Harhar et al. (2011) for argan seed oil. This increase could be attributed to higher microwave power and the disruption of existing bonds between glycerol and fatty acids, leading to the release of free fatty acids into the oil.

3.2.2. Peroxide value

The peroxide value is used to assess the state of primary oxidation and hydroperoxides in oil during storage or thermal treatment (Gotoh & Wada, 2006). It is one of the most widely used quality indicators for evaluating the quality of oil (Gotoh & Wada, 2006; Zhang et al., 2021). Peroxides are important intermediate products of oxidative reactions, as they decompose under the influence of transition metals and high temperatures to produce free radicals. The peroxide value is also correlated with organoleptic properties and indicates the freshness of the oil, with a recommended limit not exceeding 15 M.Eq. (O_2)/kg (CODEX ALIMENTARIUS, 2021; Gotoh & Wada, 2006; Zhang et al., 2021).

It was observed that the peroxide value of unroasted pomegranate seed oil was 1.00 ± 0.14 M.eq O_2/kg, well below the accepted standards (Table 2). The same applies to roasted pomegranate oil, where the peroxide value remains below the limit recommended by the CODEX ALIMENTARIUS committee. Oil prepared from seeds roasted for 15 min at 650 W exhibits the highest peroxide value of 5.00 ± 0.00 M.eq O_2/kg oil. This indicates the development of primary oxidation products during prolonged roasting.

The peroxide value increases with roasting at both microwave power levels (360 W and 650 W). This increase may be attributed to the attack of free radicals on unsaturated fatty acids and the accumulation of peroxides. Bakhshabadi et al. (2017) found that increasing microwave power leads to oil oxidation. The production of reactive radicals is a result of oxidation occurring when seeds are exposed to microwave radiation. Similar results have been obtained by Uquiche et al. (2008), Kittiphoom and Sutasinee (2015), and Rękas et al. (2015) for hazelnut, almond, mango, and *Brassica napus* oils, respectively. When roasting time and microwave power are higher, primary oxidation products (peroxides) generated can break down and reduce to secondary oxidation products (aldehydes, epoxy-hydroperoxy, epoxy-hydroxy, and ketones) because of their instability. A previous study concluded a similar trend in the peroxide value of sesame oil with increasing temperature and roasting duration (Ji et al., 2019).

3.2.3. UV absorption at 232 and 270 nm

Specific absorptions at 232 and 270 nm are useful for assessing the oxidative deterioration and purity of pomegranate seed oil by detecting peroxides (at $\lambda = 232$ nm) and secondary oxidation products (at $\lambda = 270$ nm) (Gharby et al., 2012). The wavelength at 232 nm is employed to measure the level of conjugated dienes and primary oil oxidation, while the wavelength at 270 nm indicates the level of conjugated trienes and secondary oil oxidation products (El Idrissi et al., 2022). In this study, the specific absorptions of pomegranate seed oil prepared from unroasted seeds were recorded as 2.07 ± 0.01 (K_{232}) and 2.06 ± 0.00 (K_{270}), as shown in Table 2. When pomegranate seeds are roasted at 350 W and 650 W, the obtained oils do not exhibit significant changes in specific absorptions after 5, 10, and 15 min of roasting. However, Nounah et al. (2021) indicate that after 20 min of traditional roasting, primary and secondary oxidation products increase rapidly. They propose that the formation of primary and secondary oxidation products may result in a decrease in antioxidant molecules and promote the development of lower-quality seed oil.

3.2.4. Saponification and iodine values

The saponification index evaluates the amount required to saponify 1 g of fat, reflecting the degree of unsaturation of its fatty acids. The results obtained indicate that all the oils from the studied seeds, whether roasted or unroasted, showed minimal changes and remained stable under roasting conditions (350 W to 650 W for 5, 10, and 15 min) for both the iodine and saponification indices.

However, we observed a significant difference in the saponification index value compared to Maissa Khemakhem et al. (2021) (161.33 ± 1.53 mg KOH/g). The iodine index value reported by Maissa Khemakhem et al. (2021) (153.00 ± 1.00 I_2/100g oil) differs notably from the (248.14 ± 2.40 g I_2/100g oil) value we obtained. An explanation for this discrepancy is the fact that the value reported by Maissa Khemakhem et al. (2021) was calculated based on unidentified fatty acids and did not employ the volumetric analysis method (REF: NF EN ISO 3961 – August 2018) for determination.

3.3. Oil composition

3.3.1. Fatty acids composition

The fatty acid composition of vegetable oils reflects its stability. Pomegranate seeds are rich in oils. Their fatty acid profiles in the

Table 2
Influence of roasting time and power pomegranate seeds oil quality.

Quality index	URS	RS 350 W/5 min	RS 350 W/10 min	RS 350 W/15 min	RS 650 W/5 min	RS 650 W/10 min	RS 650 W/15 min
FFA (mg KOH/g oil)	$0.78 \pm 0.23^{a,b}$	0.83 ± 0.11^{a}	$0.67 \pm 0.23^{a,b,c}$	0.44 ± 0.01^{d}	$0.47 \pm 0.06^{c,d}$	$0.58 \pm 0.06^{b,c,d}$	$0.53 \pm 0.06^{c,d}$
PV (M.eq O_2/kg)	1.00 ± 0.14^{e}	$1.75 \pm 0.01^{c,d,e}$	$2.15 \pm 0.00^{b,c,d}$	3.00 ± 1.00^{b}	$1.38 \pm 0.75^{d,e}$	$2.50 \pm 0.30^{b,c}$	5.00 ± 0.18^{a}
K_{232}	2.07 ± 0.01^{a}	2.04 ± 0.00^{b}	2.01 ± 0.02^{c}	1.98 ± 0.01^{d}	1.97 ± 0.00^{d}	1.96 ± 0.00^{e}	1.95 ± 0.01^{e}
K_{270}	2.06 ± 0.00^{a}	2.03 ± 0.01^{b}	2.00 ± 0.02^{c}	1.98 ± 0.01^{d}	$1.97 \pm 0.00^{d,e}$	$1.95 \pm 0.00^{e,f}$	1.94 ± 0.00^{f}
SV	167.90 ± 0.49^{b}	160.50 ± 0.59^{e}	159.60 ± 0.91^{f}	159.50 ± 0.76^{f}	161.30 ± 0.20^{d}	168.90 ± 0.81^{a}	163.50 ± 0.49^{c}
IV	248.14 ± 2.40^{a}	248.55 ± 2.08^{a}	248.43 ± 1.71^{a}	248.43 ± 1.78^{a}	248.37 ± 2.06^{a}	237.27 ± 2.70^{b}	233.64 ± 2.50^{b}

URS: unroasted seeds; **RS:** roasted seeds; **FFA:** free fatty acids; **PV:** peroxide value; **SV:** saponification value; **IV:** iodine value. Mean values ± SD of determination for triplicate samples. Means followed by similar letters superscript in the same line are not significantly different according to the Tukey's test ($p < 0.05$).

analyzed oil by gas chromatography are shown in Table 3. (See Tables 4 and 5.)

The results in Table 3 show that saturated fatty acids (SFAs) of unroasted seeds represent only 4.64 ± 0.45 g/100 g, with palmitic acid being the most abundant at 2.68 ± 0.28 g/100 g, followed by stearic acid at 1.57 ± 0.14 g/100 g, arachidic acid at 0.35 ± 0.03 g/100 g, and margaric acid at 0.03 ± 0.00 g/100 g. These values are close to those found by Gecgel et al. (2015), which were SFAs at 4.47 g/100 g, palmitic acid at 2.34 g/100 g, stearic acid at 1.57 g/100 g, and arachidic acid at 0.38 g/100 g.

The content of unsaturated fatty acids (UFAs) was 95.36 ± 2.55 g/100 g, with only 4.11 ± 0.24 g/100 g of monounsaturated fatty acids (MUFAs) and 91.25 ± 2.31 g/100 g of polyunsaturated fatty acids (PUFAs). The primary acid was punicic acid (C18:3, c9, t11, c13), representing 78.96 ± 1.12 g/100 g, catalpic acid (C18:3, t9, t11, c13) came after by 4.78 ± 0.54 g/100 g and then linoleic acid with 4.49 ± 0.34 g/100 g. Four linolenic acids (CLnAs) isomers were identified, with punicic acid (C18:3, c9, t11, c13) being the predominant isomer, followed by catalpic acid (C18:3, t9, t11, c13), α-eleostearic acid (C18:3, c9, t11, t13) at 2.60 ± 0.21 g/100 g, and *β*-eleostearic acid (C18:3, t9, t11, t13) at 0.41 g/100 g. These results are in accordance with previously reported results on pomegranate oil (Elfalleh et al., 2011; Fadavi et al., 2006; Hajib et al., 2021; Jing et al., 2012).

The results of fatty acids in pomegranate seeds roasted at 350 W for 5, 10, and 15 min showed almost no variation (Table 3). However, under the conditions of roasting at 650 W, a remarkable variation in fatty acids was observed. Consequently, the level of punicic acid decreased from 78.96 ± 1.12 g/100 g (URS) to 70.89 ± 2.15 g/100 g (650 W/10 min) and 55.37 ± 2.60 g/100 g (650 W/15 min), with an increase in catalpic acid from 4.78 ± 0.54 g/100 g (URS) to 10.91 ± 1.15 g/100 g and 13.83 ± 1.03 g/100 g under 650 W conditions for 10 and 15 min, respectively. A considerable increase in *β*-eleostearic acid from 0.41 ± 0.10 g/100 g to 10.81 ± 0.74 g/100 g was also observed at 650 W for 15 min. This is due to a *cis-trans* isomerization of punicic acid under these roasting conditions into catalpic and *β*-eleostearic acids, which are chemically more stable configurations. Consequently, if an oil undergoes *cis-trans* isomerization due to excessive heat or other transformation processes, the proportion of *trans*-fatty acids can increase (Liu & Lu, 2018). This transformation can lead to a deterioration in the nutritional quality of the oil and have an impact on its stability and health effects (Ascherio & Willett, 1997; Slattery et al., 2001).

Our results were in correlation with those reported by Salamatullah et al. (2021), which indicated that extreme electric roasting conditions (at 200 °C and 220 °C) can lead to an increase in *trans* fatty acids. Similarly, Durmaz and Gökmen (2010) reported that severe heat treatment of lipid-rich foods may induce the isomerization of double bonds, leading to the formation of trans fatty acids. Arab et al. (2022) also reported that the roasting treatment of sesame seeds may result in the production of trans fatty acids. Zyzelewicz et al. (2014) reported also an increase in *trans* fatty acids during the roasting of cocoa beans. In addition, Budryn et al. (2012) observed the formation of small amounts of *trans* fatty acids under extensive roasting conditions of coffee seeds. Furthermore, Bruhl (1996) explained that elevated temperatures can cause various alterations in fatty acids, including the geometric isomerization of double bonds, which leads to the formation of *trans* fatty acids.

3.3.2. *Phytosterols content and composition*

Phytosterols, natural bioactive compounds found in the unsaponifiable part of the oil, are of nutritional interest due to their potential to reduce total blood cholesterol and LDL cholesterol. Furthermore, the presence of phytosterols is important because of their impact on health and their antioxidant activity (El-Beltagi et al., 2022; Gharby et al., 2018).

The total phytosterol content of unroasted pomegranate seed oil was 540.91 ± 9.21 mg/100 g, which is close to values previously published for this oil in the literature. Fernandes et al. (2015) reported a value of 552.7 mg/100 g, Hajib et al. (2021) found a value of 494.61 mg/100 g, and Habibnia et al. (2012) observed a range of 363.6–575.8 mg/100 g in pomegranate seed oil.

The phytosterol composition of pomegranate oil in our study differed from the results reported by Amri et al. (2017), Fernandes et al. (2015), and Habibnia et al. (2012). *β*-Sitosterol was the main sterol, accounting for approximately 448.34 ± 4.89 mg/100 g, which is consistent with the findings of Hajib et al. (2021), who reported a value of approximately 404.59 mg/100 g. Among sterols, *β*-sitosterol has been the subject of numerous studies for its beneficial physiological effects on human health. Furthermore, *β*-sitosterol reduces cholesterol levels, boosts immunity, and has antipyretic, anti-inflammatory, and anticancer effects, particularly in prostate health.

The second major component was campesterol, representing approximately 42.90 mg/100 g followed by Δ5-stigmastenol (16.29 mg/

Table 3
Influence of roasting time and power on fatty acids composition of pomegranate seeds Moroccan "Sefri".

Fatty acids	URS	RS 350 W/5 min	RS 350 W/10 min	RS 350 W/15 min	RS 650 W/5 min	RS 650 W/10 min	RS 650 W/15 min
Myristic acid (C14:0)	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.02 ± 0.00^{a}
Palmitic acid (C16:0)	2.68 ± 0.28^{b}	2.43 ± 0.16^{b}	2.66 ± 0.04^{b}	2.69 ± 0.06^{b}	2.73 ± 0.12^{b}	$2.90 \pm 0.16^{a,b}$	3.32 ± 0.41^{a}
Palmitoleic acid (C16:1)	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.01 ± 0.00^{b}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}
Margaric acid (C17:0)	0.03 ± 0.00^{b}	$0.04 \pm 0.00^{a,b}$	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	$0.04 \pm 0.00^{a,b}$	$0.04 \pm 0.00^{a,b}$	$0.04 \pm 0.00^{a,b}$
Stearic acid (C18:0)	1.57 ± 0.14^{b}	1.54 ± 0.04^{b}	$1.62 \pm 0.06^{a,b}$	1.56 ± 0.02^{b}	$1.60 \pm 0.03^{a,b}$	$1.77 \pm 0.17^{a,b}$	1.86 ± 0.10^{a}
Oleic acid (C18:1)	$3.63 \pm 0.16^{b,c}$	3.59 ± 0.05^{c}	3.59 ± 0.03^{c}	$3.63 \pm 0.08^{b,c}$	$3.64 \pm 0.07^{b,c}$	$3.99 \pm 0.31^{a,b}$	4.14 ± 0.20^{a}
Linoleic acid (C18:2)	4.49 ± 0.34^{b}	4.42 ± 0.04^{b}	4.29 ± 0.11^{b}	4.51 ± 0.14^{b}	4.66 ± 0.35^{b}	4.67 ± 0.04^{b}	5.37 ± 0.41^{a}
Linolenic acid (C18:3)	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
Arachidic acid (C20:0)	0.35 ± 0.03^{b}	0.34 ± 0.00^{b}	0.35 ± 0.00^{b}	0.38 ± 0.04^{b}	0.35 ± 0.00^{b}	0.70 ± 0.06^{a}	0.11 ± 0.14^{c}
Gadoleic acid (C20:1)	0.46 ± 0.08^{b}	0.48 ± 0.01^{b}	0.47 ± 0.00^{b}	0.50 ± 0.04^{b}	0.48 ± 0.00^{b}	0.57 ± 0.08^{b}	0.85 ± 0.09^{a}
Punicic acid (C18:3, c9, t11, c13)	$78.96 \pm 1.12^{a,b}$	79.76 ± 1.81^{a}	$79.05 \pm 2.53^{a,b}$	78.12 ± 2.29^{b}	$79.04 \pm 1.68^{a,b}$	70.89 ± 2.15^{c}	55.37 ± 2.60^{d}
α-eleostearic acid (C18:3, c9, t11, t13)	2.60 ± 0.21^{c}	2.26 ± 0.04^{c}	2.60 ± 0.16^{c}	2.53 ± 0.31^{c}	2.67 ± 0.20^{c}	3.08 ± 0.18^{b}	4.04 ± 0.11^{a}
Catalpic acid (C18:3, t9, t11, c13)	4.78 ± 0.54^{c}	4.59 ± 0.04^{c}	4.74 ± 0.04^{c}	5.45 ± 0.17^{c}	4.07 ± 1.27^{c}	10.91 ± 1.15^{b}	13.83 ± 1.03^{a}
β-eleostearic acid (C18:3, t9, t11, t13)	0.41 ± 0.10^{b}	0.43 ± 0.02^{b}	0.46 ± 0.00^{b}	0.53 ± 0.00^{b}	0.37 ± 0.00^{b}	0.36 ± 0.00^{b}	10.81 ± 0.74^{a}
SFA	$4.64 \pm 0.45^{a,b}$	4.36 ± 0.19^{b}	$4.68 \pm 0.11^{a,b}$	$4.68 \pm 0.09^{a,b}$	$4.73 \pm 0.15^{a,b}$	5.42 ± 0.38^{a}	5.35 ± 0.37^{a}
UFA	95.36 ± 2.55^{a}	95.55 ± 1.61^{a}	95.22 ± 2.49^{a}	95.29 ± 1.89^{a}	94.96 ± 2.33^{a}	94.49 ± 2.76^{a}	94.42 ± 3.56^{a}
CLnA	86.75 ± 1.97^{a}	87.03 ± 1.71^{a}	86.85 ± 2.41^{a}	86.63 ± 2.15^{a}	$86.15 \pm 2.75^{a,b}$	$85.24 \pm 3.11^{a,b}$	84.05 ± 4.26^{b}
MUFA	4.11 ± 0.24^{b}	4.09 ± 0.06^{b}	4.08 ± 0.03^{b}	4.14 ± 0.12^{b}	4.14 ± 0.07^{b}	$4.58 \pm 0.40^{a,b}$	5.01 ± 0.29^{a}
PUFA	$91.25 \pm 2.31^{a,b}$	91.46 ± 1.67^{a}	$91.14 \pm 2.52^{a,b}$	$91.15 \pm 2.01^{a,b}$	$90.82 \pm 2.40^{a,b}$	$89.91 \pm 3.15^{a,b}$	89.42 ± 3.85^{b}

URS: unroasted seeds; **RS:** roasted seeds; **SFA**: saturated fatty acid; **UFA**: unsaturated fatty acid; **CLnA**: conjugated linoleic acid; **MUFA**: monounsaturated fatty acid; **PUFA**: polyunsaturated fatty acid. Mean values ± SD of determination for triplicate samples. Means followed by similar letters superscript in the same line are not significantly different according to the Tukey's test ($p < 0.05$).

Table 4
Influence of roasting time and power on phytosterol content and composition (mg/100 g) of pomegranate seeds Moroccan "Sefri".

Phytosterols composition	URS	RS 350 W/5 min	RS 350 W/10 min	RS 350 W/15 min	RS 650 W/5 min	RS 650 W/10 min	RS 650 W/15 min
Cholesterol	0.64 ± 0.27[a]	0.68 ± 0.33[a]	0.61 ± 0.20[a]	0.66 ± 0.37[a]	0.59 ± 0.35[a]	0.57 ± 0.20[a]	0.59 ± 0.23[a]
Campesterol	42.90 ± 1.17[a]	42.48 ± 0.95[a]	43.78 ± 3.05[a]	45.43 ± 2.10[a]	42.82 ± 2.69[a]	41.02 ± 1.13[a]	41.41 ± 1.17[a]
Stigmasterol	20.94 ± 1.15[a]	21.81 ± 2.81[a]	21.76 ± 3.17[a]	21.25 ± 0.66[a]	22.18 ± 5.01[a]	21.40 ± 3.32[a]	22.59 ± 2.62[a]
β-Sitosterol	448.34 ± 4.89[a]	446.99 ± 6.97[a]	450.26 ± 2.15[a]	447.27 ± 1.26[a]	449.32 ± 6.57[a]	446.54 ± 7.40[a]	452.75 ± 5.77[a]
Δ5-avenastenol	17.91 ± 1.00[a]	18.22 ± 0.57[a]	17.97 ± 1.03[a]	18.79 ± 0.47[a]	18.48 ± 1.23[a]	18.45 ± 0.65[a]	18.60 ± 1.51[a]
Δ7 stigmastenol	5.44 ± 0.52[a]	5.47 ± 0.62[a]	5.75 ± 0.04[a]	5.59 ± 0.25[a]	5.70 ± 0.04[a]	5.77 ± 0.08[a]	5.16 ± 1.02[a]
Δ7-avenasterol	4.76 ± 0.22[a]	4.71 ± 0.26[a]	5.15 ± 0.23[a]	4.67 ± 1.07[a]	4.91 ± 0.25[a]	4.99 ± 0.03[a]	5.34 ± 0.64[a]
Total phytosterols (mg/100 g)	540.91 ± 9.21[a]	540.36 ± 4.46[a]	545.27 ± 2.66[a]	543.64 ± 3.54[a]	543.99 ± 0.54[a]	538.73 ± 3.92[a]	546.42 ± 3.34[a]

URS: unroasted seeds; **RS:** roasted seeds. Mean values ± SD of determination for triplicate samples. Means followed by similar letters superscript in the same line are not significantly different according to the Tukey's test ($p < 0.05$).

Table 5
Influence of roasting time and power on tocopherol content and composition (mg/100 g) of pomegranate seeds Moroccan "Sefri".

Tocopherol composition	URS	RS 350 W/5 min	RS 350 W/10 min	RS 350 W/15 min	RS 650 W/5 min	RS 650 W/10 min	RS 650 W/15 min
α-Tocopherol	6.77 ± 0.06[a]	5.98 ± 0.14[b]	5.70 ± 0.11[c]	5.62 ± 0.14[c]	6.09 ± 0.08[b]	3.73 ± 0.13[d]	3.25 ± 0.04[e]
γ- Tocopherol	320.37 ± 2.26[a]	306.49 ± 1.02[b]	306.27 ± 1.92[b]	271.30 ± 3.94[d]	295.31 ± 3.64[c]	235.01 ± 3.22[e]	222.35 ± 1.11[f]
δ- Tocopherol	6.23 ± 0.69[a]	4.90 ± 0.27[b,c]	4.87 ± 0.11[b,c]	4.32 ± 0.41[c,d]	5.32 ± 0.40[b]	4.81 ± 0.23[b,c]	3.82 ± 0.37[d]
Total Tocopherols (mg/100 g)	333.36 ± 1.63[a]	317.37 ± 1.43[b]	316.84 ± 2.14[b]	281.24 ± 3.67[d]	306.71 ± 4.12[c]	243.54 ± 3.13[e]	229.42 ± 1.52[f]

URS: unroasted seeds; **RS:** roasted seeds. Mean values ± SD of determination for triplicate samples. Means followed by similar letters superscript in the same line are not significantly different according to the Tukey's test ($p < 0.05$).

100 g) and stigmasterol (19.09 mg/100 g). Minor sterols were also detected (Δ5-stigmasterol, Δ7-stigmastenol, Δ7-avenasterol, and cholesterol). The phytosterol content of pomegranate seed oil from Morocco was like that found by Fernandes et al. (2015). We observed no notable variation in total sterol content according to roasting pretreatment. This is consistent with previous findings by Nounah et al. (2021) in roasted prickly pear seeds.

3.3.3. Tocopherol content and composition

Tocopherols are natural lipophilic antioxidants that play a role in preventing prolonged peroxidation, thereby extending the shelf life and stability of seed oil. They are the primary bioactive components of food and offer several health benefits to humans (El Idrissi et al., 2023). They also contribute to various therapeutic and antioxidant properties through their capability of scavenging free radicals (Matthäus et al., 2010).

The total tocopherol content of pomegranate seed oil was 333.36 ± 1.63 mg/100 g, which is comparable to the value reported by Hajib et al. (2021) for Moroccan 'Sefri' pomegranate seed oil, which was 332.44 mg/100 g. Only α-, γ-, and δ-tocopherols were detected in all analyzed oils. γ-tocopherol is the main component, accounting for approximately 320.37 ± 2.26 mg/100 g of oil, followed by α-tocopherol (6.77 ± 0.06 mg/100 g) and δ-tocopherol (6.23 ± 0.69 mg/100 g). The profile of the tocopherol in oil is comparable to the one found by Fernandes et al. (2015), who found that the main tocopherol in pomegranate seed oil is γ-tocopherol, with values ranging from 123.0 to 449.7 mg/100 g. Additionally, Jing et al. (2012) observed that δ-tocopherol was the primary tocopherol in pomegranate seed oil with a value of 141.42–351.32 (mg/100 g). Nevertheless, this result differs from the study of Pande and Akoh (2009), who found that α-tocopherol was the main tocopherol in pomegranate seed oil, with a value of 161.2–173.7 mg/100 g. This variation in tocopherols may be due to genetic differences.

In this research, a notable decrease in total tocopherol content was observed, from 333.36 ± 1.63 mg/100 g for unroasted seeds to 316.84 ± 2.14 mg/100 g for seeds roasted under the conditions of 350 W/15 min. However, the total tocopherol content in the oil decreased to 229.42 ± 1.52 mg/100 g for seeds roasted under the conditions of 650 W/15 min. This reduction in tocopherols, known for their antioxidant activity, could be explained by the fact that tocopherol content is sensitive to oxidation. Yoshida & Takagi (1996) found that the tocopherol levels decreased gradually in oils prepared from microwaved roasted soybeans, and the percentage of losses increased significantly after 12 min. These results demonstrate that temperature and duration of roasting have an influence on the tocopherol levels of oils. According to Anjum et al. (2006).

3.4. Association among proximate composition and oil quality index and composition

The heatmap revealed several noteworthy positive correlations among the variables, providing insights into their relationships (Fig. 1). Firstly, there was a strong correlation between total tocopherols (TT) and iodine value (IV), with a value of 0.91, significant at the $p < 0.01$ level, indicating a strong association between these two variables. Similarly, TT was positively correlated with punicic acid (PA) and moisture content (MC), with coefficients of correlation of 0.85 and 0.80, respectively, both significant at the $p < 0.05$ level. IV also showed a positive correlation with PA (0.93), significant at $p < 0.01$, suggesting that these variables were closely related.

Peroxide value (PV) and catalpic acid (CA) exhibited a significant positive correlation ($r = 0.83$; $p < 0.05$), indicating a strong relationship between these two variables. PV also correlated positively with C18:0, with a coefficient of correlation value $r = 0.77$; $p < 0.05$. CA was positively associated with both C18:1 and C18:0, with correlation values of 0.99 and 0.96, respectively, both significant at $p < 0.001$. Additionally, C18:0 and C18:1 were positively correlated with a coefficient of correlation value $r = 0.97$; $p < 0.001$.

In the context of fatty acids, C16:0 showed a very strong positive correlation with α-eleostearic acid (α-EA) ($r = 0.99$; $p < 0.001$), C18:0 ($r = 0.93$; $p < 0.01$), and C18:1 ($r = 0.92$; $p < 0.01$). α-EA was also positively correlated with C18:0 ($r = 0.95$; $p < 0.001$) and C18:1 ($r = 0.94$; $p < 0.01$), highlighting the close relationships among these fatty acids. Furthermore, C18:1 and C18:0 had a positive correlation of $r = 0.97$, significant at $p < 0.001$.

Additionally, PV was positively correlated with C18:2 ($r = 0.87$; $p < 0.05$), and CA showed a similar positive correlation with C18:2 ($r = 0.85$; $p < 0.05$). C18:0 was positively correlated with C18:2 at $r = 0.83$,

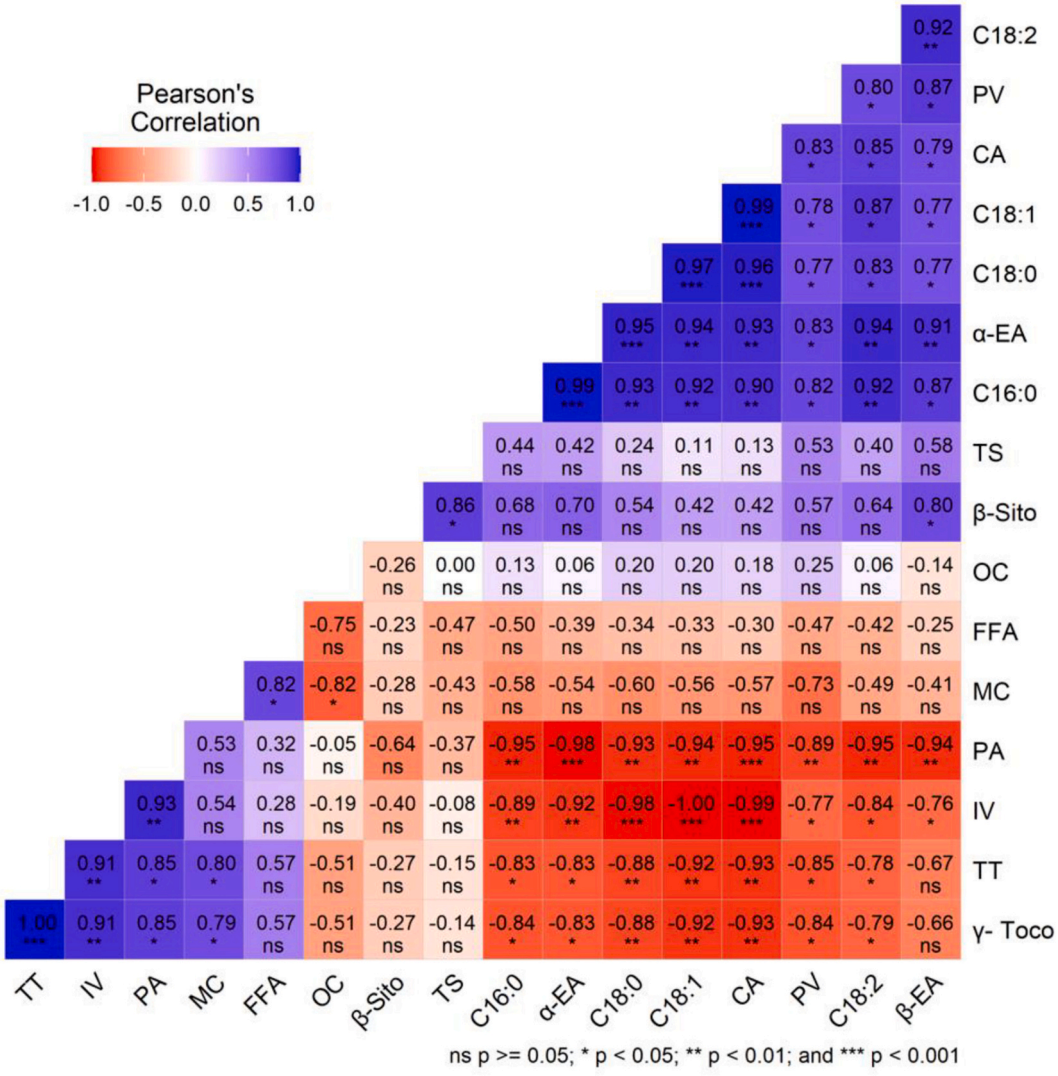

Fig. 1. Pearson's correlation between the variables: **C18:2** (linoleic acid), **PV** (peroxide value), **CA** (catalpic acid), **C18:1** (oleic acid), **C18:0** (stearic acid), **α-EA** (α-eleostearic acid), **β-EA** (β-eleostearic acid), **C16:0** (palmitic acid), **TS** (total sterol), **β-sito** (β-sitosterol), **OC** (oil content), **FFA** (free fatty acid), **MC** (moisture content), **PA** (punicic acid), **IV** (iodine value), **TT** (total tocopherol), **γ-toco** (tocopherol) of the different samples of pomegranate seeds Moroccan "Sefri".

significant at $p < 0.05$, and α-EA showed a positive correlation with C18:2 at $r = 0.94$; $p < 0.01$. Lastly, C16:0 and C18:2 had a strong positive correlation of 0.92, significant at $p < 0.01$. These correlations illustrated the interconnectedness of these variables, with many showing significant positive associations, suggesting potential relationships that could be further explored in related studies.

The heatmap also revealed several negative correlations among the variables, indicating inverse relationships (Fig. 1). C16:0 and γ-Tocopherol (γ-Toco) had a significant negative correlation of $r = -0.84$; $p < 0.05$. α-EA and γ-Toco were also correlated ($r = -0.83$; $p < 0.05$), and C18:0 and γ-Toco were at $r = -0.88$; $p < 0.01$. C18:1 and γ-Toco showed a coefficient of correlation of $r = -0.92$, significant at $p < 0.01$. Additionally, MC and OC had a negative correlation of $r = -0.82$ at the $p < 0.05$ level.

IV had a very strong negative correlation with C18:1 ($r = -1.00$), C18:0 ($r = -0.98$), and CA ($r = -0.99$), all significant at the $p < 0.001$ level, and with α-EA ($r = -0.92$) and C16:0 ($r = -0.89$), both significant at the $p < 0.01$ level. IV also had a negative correlation with PV ($r = -0.77$), C18:2 ($r = -0.84$), and β-EA ($r = -0.76$), all significant at $p < 0.05$. Additionally, TT was negatively correlated with C18:0 ($r = -0.88$), C18:1 ($r = -0.92$), and CA ($r = -0.93$) at $p < 0.01$. TT also had a negative correlation with C16:0 ($r = -0.83$), α-EA ($r = -0.83$), PV ($r = -0.85$), and C18:2 ($r = -0.78$), all significant at $p < 0.05$.

The very strong negative correlation of PA with CA ($r = -0.95$; $p < 0.001$), α-EA ($r = -0.98$; $p < 0.001$), and β-EA ($r = -0.94$; $p < 0.01$) could be explained by the possible isomerization of PA during seed roasting pretreatment. PA also had a negative correlation with C16:0 ($r = -0.95$; $p < 0.01$), C18:0 ($r = -0.93$; $p < 0.01$), C18:1 ($r = -0.94$; $p < 0.01$), PV ($r = -0.89$; $p < 0.01$), and C18:2 ($r = -0.95$; $p < 0.01$). These correlations, particularly the significant ones, highlighted strong inverse relationships that could be crucial for understanding the interactions and behaviors of these variables in various contexts.

4. Conclusions

This study's findings have demonstrated that the roasting duration of pomegranate seeds decreases the free fatty acids (FFA) and water content in the seeds. A significant increase in oil content was also observed with the roasting treatment. Findings from CPG and HPLC analyses, following the roasting of pomegranate seeds at 350 W and 650 W for 5, 10, and 15 min, reveal that pomegranate seed oil is rich in phytosterols, notably *β*-sitosterol, and has a very high tocopherol content. Regarding the fatty acid composition, roasting pomegranate seeds at 650 W led to interesting variations, particularly in conjugated linolenic acid (CLnAs)

composition during 10 and 15 min of roasting. This is due to a transformation or cis-trans isomerization of punicic acid into catalpic and β-eleostearic acids, which are chemically more stable configurations. Based on these findings, roasting is an indispensable treatment to improve the oil content of pomegranate seeds. However, it is recommended to roast the seeds at 650 W for only 5 min to preserve their unique fatty acid composition.

CRediT authorship contribution statement

Mohammed Amakhmakh: Writing – original draft, Resources, Methodology, Data curation. **Ahmed Hajib:** Writing – original draft, Investigation, Data curation. **Walid Belmaghraoui:** Writing – review & editing, Investigation, Data curation. **Hicham Harhar:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **El Asri Mohammed:** Writing – review & editing, Software, Methodology, Data curation. **Waleed Al Abdulmonem:** Writing – review & editing, Investigation, Funding acquisition. **Khang Wen Goh:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Abdelhakim Bouyahya:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Abdeslam Meliani:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The Researchers would like to thank the Deanship of Graduate Studies and Scientific Research at Qassim University for financial support (QU-APC-2024-9/1).

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