Effect of thermal pre-treatment methods on the chemical composition of grape seed oil: a comparative study

Nour Ksibi [1,2]
Edyta Symoniuk [3]
Aleksander Siger [4]
Anna Grygier [5]
Katarzyna Ratusz [3]
Majdi Hammami [2]
Iness Bettaieb Rebey [2]
Wissem Aidi Wannes [2] ✉
Malgorzata Wroniak [3]
Moufida Saidani Tounsi [2]

[1] Faculty of Sciences of Tunis
University of Tunis El Manar, Tunisia

[2] Laboratory of Aromatic
and Medicinal Plants (LPAM)
Center of Biotechnology of Borj
Cedria (CBBC), Tunisia

[3] Faculty of Food Sciences
Department of Food Technology
Warsaw University of Life Sciences (SGGW)
Nowoursynowska Street 166,
Warsaw, Poland

[4] Faculty of FoodSciences
Department of Food Biochemistry and Analysis
Poznań University of Life
Sciences, Poznań, Poland

[5] Institute of Food Technology of Plant Origin
Faculty of Food Science and Nutrition
Poznań University of Life Sciences
Wojska Polskiego 31
Poznan, Poland

✉ CORRESPONDING AUTHOR
Wissem AIDI WANNES
Laboratoire des Plantes Aromatiques et Médicinales
Centre de Biotechnologie
du Technopole de Borj Cedria
B.P. 901, 2050 Hammam-Lif, Tunisie.
Tel: +216 71 430 855
fax: +216 79 412 638
email: aidiwissem@yahoo.fr

Received: January 10, 2025
Accepted: October 2, 2025

Although grape seed waste is increasingly valorized through oil extraction and biomass use, it still remains underused in several Mediterranean regions, such as Tunisia, where inadequate management may still pose environmental challenges. Valorization of grape seed waste through oil extraction offers a sustainable solution, turning by-products into valuable resources. In this study, the influence of thermal pre-treatment of grape seeds by microwave radiation for 4 min was investigated in comparison with roasting pretreatment at 120°C for 1h before oil extraction. Thermal treatment improved the grape seed oil (GSO) yield, which amounted to 13.01% after the roasting pretreatment and 11.72% after the microwave pretreatment as compared to the control (10.35%). GC analysis of GSO showed that the fatty acid composition was modified after thermal pretreatment and the predominant fatty acids were palmitic acid, oleic acid, and linoleic acid. The main tocochromanols were γ-tocotrienol (9.46 mg/100 g oil) and α-tocopherol (4.62 mg/100 g oil). β-sitosterol was the major sterol identified in GSO and reached 1.16 mg/g after grape seed treatment with microwaves. The oxidative stability of extracted oils was investigated at different temperatures (90, 100, 110, and 120°C). Results revealed that oxidative stability prolongation from 18.19h up to 20.94h was observed at 90°C after the microwave pretreatment of grape seeds in comparison with non-treated grape seeds. The findings of this research indicated that microwave pretreatment can emerge as a promising strategy to enhance the nutraceutical content of oil extracted from grape seeds.

Keywords: grape seed oil, polyunsaturated fatty acids, oxidative stability, Rancimat test,

1. INTRODUCTION

Grape products involve the creation of large quantities of by-products, which mostly consist of organic waste, water waste, emission of greenhouse gases, and inorganic residues [1]. The researchers decided to evaluate the composition and bioactive molecules of these agro-industrial by-products [2].
Grape seeds can yield up to 22% oil, varying based on the grape variety, the maturation degree of the seeds, the environmental conditions during ripening, and the method of extraction [3]. This oil, characterized by its high content of unsaturated fatty acids, particularly linoleic and oleic acids, along with vitamin E and sterols [4], is gaining popularity in the market. Vitamin E, or tocochromanols, is classified into two main classes, tocopherols and tocotrienols, comprising eight naturally occurring isoforms [5]. Sterols play a vital role in reinforcing cell membranes and regulating biological processes [6]. Collectively, these bioactive compounds exhibit various health benefits, including lowering blood cholesterol [7], reducing cardiovascular risk [8], and exerting anti-inflammatory activity. It is worth noting that excessive intake of linoleic acid, especially in diets with low ω-3 intake, may promote inflammatory responses through the

synthesis of pro-inflammatory eicosanoids. Therefore, the health effects of linoleic acid depend on the overall dietary ω-6/ω-3 balance [9]. Numerous studies have shown that grape seed oil (GSO) has health-promoting properties, particularly highlighted in *in vitro* studies, demonstrating antioxidant, anti-inflammatory, cardiovascular protective, antimicrobial, and anti-proliferative effects against human colon cancer. These effects may involve interactions with cellular and molecular pathways [10,11]. This research indicated that GSO consumption reduced inflammation and insulin resistance in overweight and/or obese women, likely due to its tocotrienols and phenolic compounds. Additionally, grape seed extracts were found to significantly lower cholesterol levels [12]. Its application as an edible oil has also been recommended due to its appealing sensory qualities. Grape seed oil has been reported to exhibit pleasant sensory characteristics, mainly attributed to its volatile compound profile. Volatile compounds (alcohols, esters, aldehydes, terpenes, ketones …) such as ethylacetate, hexanal, trans-2-hexanal, trans-2-heptenal, linalool and 3methylbutanol have been identified as major contributors to the fruity, slightly nutty, and fresh aroma of the oil, depending on grape variety and extraction method [13,14]. Tocopherols, unsaturated fatty acids, phytosterols and phenolic compounds (e.g., quercetin, procyanidins, and carotenoids) contribute to its nutritional and oxidative stability [15].
Recently, pretreatment methods for seeds have gained considerable interest in various sectors such as food, nutraceuticals, pharmaceuticals, and cosmetics, due to the enhanced quality of the resulting products [16]. Several pretreatment technologies have been employed, including enzymatic digestion, ultrasonication, microwaving, and roasting. Microwave treatment alters the seed structure, leading to improved oil extraction. Microscopic analyses of microwave-treated rapeseed (*Brassica napus* L.) have shown structural changes in the lipoprotein membranes surrounding lipid bodies, facilitating oil movement through the cell membranes and enhancing extraction efficiency [17]. Given the growing importance of environmental sustainability in agro-industrial practices, further research on integrating grape seed oil extraction and bioactive compound recovery with sustainable methods is needed to address the challenges of waste management and resource use in the Euro-Med region [18].
This study aimed to investigate the impact of grape seed pretreatment on oil quality, focusing on the fatty acid profile, phytosterol, tocopherol, and tocotrienol levels, while also assessing the oil's oxidative stability at temperatures ranging from 90°C to 120°C. Additionally, the kinetic parameters of the oils were examined post-Rancimat testing. To our knowledge, this is the first study to apply and compare these pretreatment methods for grape seeds cultivated in Tunisia.

2. EXPERIMENTAL

2.1. SAMPLE

Grape (*Vitis vinifera* L.var. Carignan) seeds were provided from a wine company in Tunisia and separated by hand picking from foreign materials before pretreatments and oil extraction.

2.2. MICROWAVE PRETREATMENT

The microwave pretreatment was performed in a microwave oven (Model: Panasonic, 800 W, 2450 Mhz). 300 g of whole seeds were placed in a Pyrex petri dish for 4 min with stirring every 1 min. A seed sample with no pretreatment was used as a control.

2.3. ROASTING PRETREATMENT

300 g of whole grape seeds were spread out on a stainless steel plate tightly covered with aluminum foil and heated for 1 h at 120°C in the oven with ventilation (SLW 115 SIMPLE, pol-eko-aparatura, 2500W) with stirring every 15 min. Seeds were cooled to a temperature of 25°C prior to oil extraction.

2.4. OIL EXTRACTION AND PHYTOCHEMICAL ANALYSIS

Oil extraction was performed following the method of Fathi-Achachlouei et al., [19]. The pretreated grape seeds were ground into powder with a steel grinder and 100 g of powder was mixed with 300 ml of hexane in an Erlenmeyer flask on a shaker for 1 h at room temperature. The obtained mixture was filtered and hexane was evaporated using a rotary evaporator (R210, Buchi, Germany) at 55-60°C. Additionally, the oils after evaporation were purged with nitrogen.
Fatty acid methyl esters (FAMES) were used to identify fatty acids of the extracted oils [20] (Harbeoui et al., 2018). 0.1 g of grape seed oil obtained under the different pretreatment conditions was mixed with 2 mL of hexane and 1 mL of methanolic potassium hydroxide (KOH) (0.5M). After 1 min of mixing, 1 mL of the upper phase (hexane phase) was transferred to 2 mL capacity vials. The oil composition analysis was performed by gas chromatography using a Thermo Scientific Trace 1300 gas chromatograph equipped with a flame ionization detector (FID), with a split injector (120:1) operating at a temperature of 285°C with a capillary column (SGE BPX70 60 m × 0.25 mm × 0.22 μm) and the programming conditions were as follows: initial temperature of the oven was 100°C for 4 min, raised to 240°C at a rate of 3°C/min and ending at 240°C for 10 min. The carrier gas was helium used at a flow rate of 40 mL/min. The fatty acids were compared to their retention times with those of the authentic standard mixture of 37 FAME methyl esters from Restek. Esterification and injection (0.8 μL) were performed in triplicate.

To determine tocochromanols, the method of Siger et al., [21] was used. 200 mg of oil was dissolved in 10 ml of n-hexane. Separation was then performed using a Waters HPLC system (Waters, Milford, MA, USA) coupled with a fluorescence detector (FLD) detector (Waters 474), a PDA detector (Waters 2998), and a LiChrosorb Si 60 column (250 × 4.6 mm, 5 μm, Merck Millipore, Darmstadt, Germany). The mobile phase was a mixture of n-hexane with 1,4-dioxane (96:4 v/v) at a flow rate of 1.0 mL/·min. The quantification of tocochromanols was conducted using data from the FLD with excitation/emission wavelengths of 295/330 nm, respectively. The determination was carried out in duplicate for each sample.
Phytosterols were analyzed according to the method defined by Raczyk et al. [22]. Briefly, oil samples (50 mg) with the internal standard (5α-cholestane, 50μL) were saponified with 1M KOH in methanol, at room temperature, for 18 h. The unsaponifiable fraction was extracted three times with hexane/methyl tetr-butyl ether (1:1, v/v) and after the solvent was evaporated under nitrogen. Sterols were silylated with sylon BTZ and piridyne (Sigma-Aldrich, St. Louis, MO, USA) and analyzed in an Agilent 7820A GC (Agilent Technologies, Wilmington, DE, USA) equipped with a DB-35MS (Agilent Technologies) capillary column (30 m, 0.20 mm, and 0.33 μm). Analysis parameters were as follows: the oven temperature was initially 100°C, held 5 min, and increased to 250°C at 25°C/min., and to 290°C at 3°C/min; injector and flame ionization detector (FID) temperature was 250°C; carrier gas, helium at 1mL/min. Phytosterols were identified by comparison of their retention times (relative to 5α-cholestane) with commercially available standards. The determination was carried out in duplicate for each oil.

2.5. RANCIMAT TEST

A Rancimat test was used to determine the oxidative stability of oils and fats under accelerated conditions based on the induction of oxidation of a sample by exposure to high temperatures and continuous air-flow. This causes oxidation of the oil, releasing volatile compounds. The time can be measured by taking a sample and allowing it to completely oxidize and become rancid [20]. Oxidative stability of the oils was determined using a Rancimat 743 Metrohm apparatus (Herisau, Switzerland). The Rancimat test was conducted using samples of 3 g oil that were oxidized at four different temperatures: 90°C, 100°C, 110°C and 120°C, at a constant air flow of 20 L/h. The volatile products formed from the oxidation reaction were soluble in 0.06 L of deionized water. The kinetic analysis was determined following the method of Farhoosh and Seyedeh-zohreh [23].

3. RESULTS AND DISCUSSION

3.1. GRAPE SEED OIL YIELD

Oil yield is a crucial metric for evaluating oilseeds. As illustrated in Figure 1, thermal pretreatments significantly influenced the oil yield. Specifically, the yield of grape seed oil (GSO) increased to 13.01% following microwave treatment and 11.72% after roasting treatment, compared to a control yield of 10.35%. Habeoui et al. [20] noted that oil content in Tunisian grape seeds varied between 6.31% and 12.70%, depending on the variety and environmental conditions. Romanian GSO yield varied between 11.67% and 12.52% [24] while Vietnamese GSO yield was 16.60% [3].
Several factors also impact oil content, including temperature, pressure, extraction time, solvent type, and the extraction method used [25]. Oomah et al. [26] observed that 5 min of microwave treatment increased GSO yield to 15.3%, while a 24-min treatment yielded 15.4%, both exceeding the control yield of 14.6%. Similarly, Lee et al. [27] reported a progressive increase in GSO yield, achieving a 6% increase with 10 minutes of microwave heating and a 5% increase with 5 min of heat treatment compared to the control. These results suggested that dry heat may enhance oil release from the seeds.

3.2. FATTY ACID ANALYSIS

Table I highlights the primary fatty acids present in grape seed oil (GSO), including linoleic acid, oleic acid, palmitic acid, and stearic acid. GSO was characterized by a high proportion of unsaturated fatty acids (UFA) and a low proportion of saturated fatty acids. In untreated GSO, monounsaturated fatty

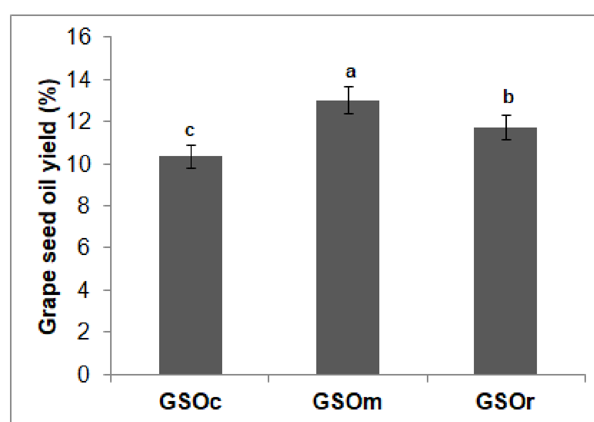

Figure 1 - Grape seed oil yields before and after pretreatments.
GSOc: grape seed oil control; GSOm: grape seed oil after microwave pretreatment; GSOr: grape seed oil after roasting pretreatment.
The small letters (a–c) indicated significant differences ($p < 0.05$) between GSOc, GSOm and GSOr, respectively.

acids (MUFA) comprised 20.78%, while polyunsaturated fatty acids (PUFA) reached 66.86%. The findings of the present study aligned with previous studies on the fatty acid composition of untreated GSO, known to be rich in linoleic acid, containing 61-75%, [3,19,23,15]. Linoleic acid is widely recognized for its beneficial effects on cardiovascular health particularly through its ability to reduce cholesterol levels [28]. However, grape seed oil contained minimal linolenic acid (<1%), which differentiated it from other oils like Nigella seed oil, containing higher linolenic acid levels that can negatively affect oil stability, leading to shorter shelf life due to its susceptibility to oxidation [29]. Fats can be influenced by the variety of the plant, the cultivation environment, and the extraction methods [15].

Chromatography analysis revealed changes in the fatty acid profile following microwave and roasting pretreatments (Table I). After these treatments, the proportions of saturated fatty acids (SFA) slightly increased, while significant alterations were observed in MUFA and PUFA levels – 28.06% and 27.62% for MUFA, and 59.36% and 60.20% for PUFA, respectively, compared to untreated GSO. The reduction in PUFA proportions was likely linked to the heating temperature and the thermal effects of microwave treatment.

Table I - Fatty acid composition of extracted grape seed oils before and after pretreatments

Fatty acid (%)	Grape seed oil		
	GSOc	GSOm	GSOr
C14:0	-	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}
C16:0	6.78 ± 0.21^{a}	5.33 ± 0.12^{b}	5.68 ± 0.15^{b}
C16:1	0.14 ± 0.01^{b}	0.25 ± 0.05^{a}	0.23 ± 0.04^{a}
C18:0	4.57 ± 0.12^{b}	5.44 ± 0.36^{a}	4.96 ± 0.29^{b}
C18:1	20.24 ± 0.56^{b}	27.43 ± 0.85^{a}	27.09 ± 0.98^{a}
C18:2	66.25 ± 1.26^{a}	58.85 ± 1.56^{b}	59.52 ± 1.87^{b}
C18:3	0.34 ± 0.05^{a}	0.25 ± 0.05^{b}	0.24 ± 0.03^{b}
C20:0	0.41 ± 0.06^{c}	0.58 ± 0.07^{a}	0.49 ± 0.06^{b}
C20:1	0.40 ± 0.09^{a}	0.26 ± 0.02^{b}	0.18 ± 0.01^{c}
C22:0	-	0.57 ± 0.08^{a}	0.54 ± 0.09^{a}
C22:1	-	0.12 ± 0.01^{a}	0.12 ± 0.02^{a}
C22:5	0.27 ± 0.02^{b}	0.26 ± 0.01^{b}	0.44 ± 0.02^{a}
C23:0	-	0.12 ± 0.01^{a}	0.11 ± 0.01^{a}
C24:0	-	0.46±0.03^{a}	0.37±0.02^{b}
ΣSFA	11.76 ± 0.33^{c}	12.54 ± 0.11^{a}	12.19 ± 0.72^{b}
ΣMUFA	20.78 ± 0.99^{c}	28.06 ± 0.52^{a}	27.62 ± 0.31^{b}
ΣPUFA	66.86 ± 1.03^{a}	59.36 ± 0.93^{c}	60.20 ± 1.12^{b}

Values are expressed as % of fatty acids (mean ± SD, n = 3). Minor fatty acids (< 0.05%) are not listed; Means within the same row followed by different letters (a,b,c) were significantly different at p< 0.05 using Turkey test.

GSOc: grape seed oil control, GSOm: grape seed oil after microwave pretreatment, GSOr: grape seed oil after roasting pretreatment

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids

It is important to note that while the profile indicated a decrease in specific acids, an increase in proportion does not necessarily equate to an increase in total content. Therefore, the rise in the proportion of SFA and MUFA mainly resulted from the decline in polyunsaturated acid content.

Theoretically, heat treatment should reduce oil viscosity and facilitate fatty acid release. However, studies by Lee et al. [26] and Pardo et al. [30] found that GSO fatty acid composition remained unchanged after various heat treatments. Similarly, Suri et al. [31] reported a slight increase (1.19%) in SFA content in linseed oil from microwave-treated seeds, alongside minor decreases in MUFA (0.31%) and PUFA (0.87%). Ahmed et al. [32] reported a notable reduction in the levels of polyunsaturated fatty acids (PUFAs), specifically linolenic acid, in sesame oil when roasted at 220°C for 30 min. However, the concentrations of saturated fatty acids (SFAs) and other unsaturated fatty acids remained relatively unchanged.

The stability of the fatty acid composition can likely be explained by the high processing temperatures, which surpassed the melting points of saturated and unsaturated oils. The reduction in specific unsaturated fatty acids may be due to the increase in saturated fatty acids and the breakdown of unsaturated fatty acids during prolonged heating [33].

3.3. TOCOCHROMANOL ANALYSIS

Table II presents significant differences in tocochromanol content between treated and untreated Grape Seed Oil (GSO) samples. The main tocochromanols identified were γ-tocotrienol (γ-T3), α-tocotrienol (α-T3), and α-tocopherol (α-T), with concentrations ranging from 6.55 to 9.46 mg/100 g, 5.28 to 8.10 mg/100 g, and 2.08 to 4.62 mg/100 g, respectively. These findings align with Irandoost et al. [10], who reported that GSO has a notably high concentration of tocotrienols, particularly α- and γ-tocotrienol, compared to other vegetable oils.

HPLC analysis revealed that total tocochromanol content in GSO was significantly influenced by the type of pretreatment applied. After roasting, the total tocol content increased to 18.10 mg/100 g, while microwave pretreatment resulted in an even higher total of 24.82 mg/100 g, compared to the control GSO (15.59 mg/100 g). Notably, microwave pretreatment led to a 59.40% increase in total tocochromanols. Additionally, the content of PC-8 rose from 0.24 mg/100 g in the control to 0.47 mg/100 g in the oil extracted after microwave treatment.

Both roasting and microwave pretreatments

significantly enhanced ($p < 0.05$) the levels of tocopherols and tocotrienols. These results suggested that thermal pretreatment ruptures cellular structures, facilitating the release of tocochromanols and subsequently increasing their concentrations in the extracted oil. This study is the first to report the contents of tocochromanol and PC-8 in oils extracted from treated grape seeds using microwave and roasting pretreatments.

Harbeoui et al. [19] examined total tocopherol content in nine Tunisian GSO varieties, finding values between 1.389 and 18.135 mg/100 g oil. Dimić et al. [11] investigated the effect of extraction techniques on tocopherol content in GSO, reporting a maximum of 7.96 mg/100 g oil via microwave-assisted extraction. These findings suggested that GSO was a valuable source of natural liposoluble antioxidants, offering potential health benefits [11].

3.4. STEROL ANALYSIS

Table III details the sterol content in oils from treated and untreated grape seeds. The primary sterols identified include campesterol, stigmasterol, β-sitosterol, sitostanol, Δ5-avenasterol, Δ7-avenasterol, and 24-methylenecycloartanol. Total phytosterol contents were measured at 1.40 mg/g for untreated GSO, and 1.81 mg/g and 1.61 mg/g for GSO subjected to microwave and roasting pretreatments, respectively. Notably, β-sitosterol was the predominant phytosterol, reaching 1.16 mg/g after microwave treatment, indicating that this pretreatment enhances phytosterol levels.

Table II - Tocochromanol and Plastochromanol-8 contents of extracted grape seed oils before and after pretreatments

Compound (mg/100g oil)	Grape seed oil		
	GSOc	GSOm	GSOr
α-Tocopherol	2.08 ± 0.05[c]	4.62 ± 0.05[a]	2.50 ± 0.07[b]
β-Tocopherol	0.05 ± 0.01[b]	0.21 ± 0.01[a]	0.04 ± 0.01[b]
γ-Tocopherol	0.95 ± 0.02[c]	1.47 ± 0.01[a]	1.09 ± 0.04[b]
δ-Tocopherol	0.05 ± 0.01[b]	0.10 ± 0.01[a]	0.06 ± 0.01[b]
α-Tocotrienol	5.28 ± 0.04[c]	8.10 ± 0.01[a]	6.14 ± 0.1[b]
β-Tocotrienol	0.15 ± 0.01[b]	0.25 ± 0.01[a]	0.23 ± 0.04[a]
γ-Tocotrienol	6.55 ± 0.08[c]	9.46 ±0,04[a]	7.55±0,08[b]
δ-Tocotrienol	0.48 ± 0.01[b]	0.61 ± 0.06[a]	0.49 ± 0.03[b]
Total	15.59 ± 0.09[c]	24.82 ± 0.12[a]	18.10 ± 0.20[b]
Plastochromanol-8	0.24 ± 0.01[c]	0.47± 0.02[a]	0.31 ± 0.01[b]

All values are means of two repetitions with standard deviation
Means within the same row followed by different letters (a,b,c) were significantly different at $p< 0.05$
GSOc: grape seed oil control, GSOm: grape seed oil after microwave pretreatment, GSOr: grape seed oil after roasting pretreatment

Table III - Phytosterols content of extracted grape seed oils before and after pretreatments

Sterols (mg/g)	Grape seed oil		
	GSOc	GSOm	GSOr
Campesterol	0.13 ± 0.02[c]	0.17 ± 0.01[a]	0.16 ± 0.01[ab]
Stigmasterol	0.17 ± 0.01[c]	0.20 ± 0.01[a]	0.18± 0.02[b]
β-Sitosterol	0.91 ± 0.06[c]	1.16 ± 0.03[a]	1.05 ± 0.03[b]
Sitostanol	0.05 ± 0.01[bc]	0.08 ± 0.02[a]	0.06 ± 0.01[b]
Δ5-Avenasterol	0.04 ± 0.01[bc]	0.06 ± 0.01[a]	0.05 ± 0.01[b]
Δ7-Avenasterol [mg/g]	0.08 ± 0.01[c]	0.11 ± 0.00[a]	0.10 ± 0.05[b]
24-Methylenecycloartanol [mg/g]	0.02 ± 0.01[b]	0.03 ± 0.00[a]	0.02± 0.01[b]
Total sterols	1.40 ± 0.08[c]	1.81 ± 0.01[a]	1.61 ± 0.04[b]

All values are means of two repetitions with standard deviation
Means within the same row followed by different letters (a,b,c) were significantly different at $p< 0.05$
GSOc: grape seed oil control, GSOm: grape seed oil after microwave pretreatment, GSOr: grape seed oil after roasting pretreatment

Pardo et al. [30] reported that β-sitosterol constituted about 70% of the sterol profile in Spanish GSO, though its concentration was slightly lower (67%) in grape seeds dried at room temperature. They also identified significant amounts of stigmasterol, campesterol, clerosterol, and brassicasterol in ambient temperature-dried grape seed oil. In linseeds, β-sitosterol was the most prevalent phytosterol, followed by campesterol and stigmasterol. Furthermore, roasted linseed oil exhibited higher overall phytosterol content than raw seeds. The highest levels of β-sitosterol (1540.89 mg/kg), cycloartenol (1330.57 mg/kg), campesterol (748.17 mg/kg), and stigmasterol (288.17 mg/kg) were recorded after roasting at 160°C for 8 min.

Other sources, such as *Plukenetia huayllabambana* seed oil, predominantly contained β-sitosterol (60.1 mg/100 g), campesterol (24.8 mg/100 g), and stigmasterol. In walnut oil, β-sitosterol represented over 60% of total phytosterols. The highest levels of β-sitosterol, campesterol, and stigmasterol in walnut oil were observed when the seeds were roasted at temperatures of 140°C, 160°C, and 180°C for 10 min, yielding better results than those obtained with 5 or 15 min of roasting [34].

These findings underscored the impact of thermal pretreatments on enhancing phytosterol content, particularly through microwave treatment, making grape seed oil a valuable source of these beneficial compounds. By optimizing the use of agro-industrial by-products like grape seeds, this approach not only enhanced the nutritional value of the oil but also supported environmental sustainability in the region.

3.5. OXIDATIVE STABILITY BY RANCIMAT TEST

The Rancimat test effectively simulates the oxidation process of oils, allowing researchers to obtain results in hours that would typically take months or years. The findings indicated that the induction time for GSO decreased as the temperature increased, ranging from 20.97 h at 90°C to 2.32 h at 120°C. According to the Van't Hoff rule, the rate of a reaction typically decreases by two to three times for every 10°C reduction in temperature.

Figure 2 illustrates that the induction time for GSO at 90°C increased after both roasting and microwave pretreatments, rising from 18.09 h to 20.15 h and 20.97 h, respectively. At 120°C, the induction time recorded after both pretreatments was 2.51 h, consistent with findings by Maszewska et al. [35], who reported an induction time of 2.4 h at the same temperature.

Ramos et al. [36] studied the oxidative stability of GSO extracted via cold pressing, followed by filtration and refining. They found higher induction times for GSO at 100°C (15.96 h), 110°C (7.54 h), and 120°C (3.68 h) compared to the current study. The assessment of GSO stability using the Rancimat method remains limited, highlighting the need for further research in this area to understand the oxidative stability of GSO under various conditions.

3.6. KINETIC ANALYSIS OF THE RANCIMAT TEST

The kinetic parameters for GSO oxidation are given in Table IV. The activation energy (Ea) for the tested GSO ranged from 77.49 kJ/mol (untreated) to 79.87 kJ/mol (microwave-treated). These values were in line with those reported by Symoniuk et al. [37] for linseed oils, which ranged from 74.03 to 77.76 kJ/mol. However, higher Ea values were reported by Ramos et al. [36] for grape seed, sunflower seed, and sesame oils, with values of 89.83, 86.58, and 90.37 kJ/mol, respectively. The GSO samples

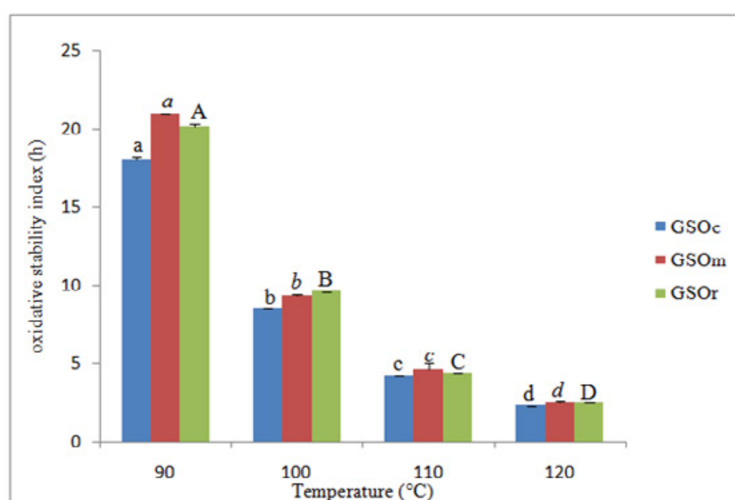

Figure 2 - Oxidative stability of extracted grape seed oils before and after pretreatments
GSOc: grape seed oil control; GSOm: grape seed oil after microwave pretreatment; GSOr: grape seed oil after roasting pretreatment
The small letters (a–d), small italic letters (*a-d*) and capital letters (A-D) indicate significant differences (p< 0.05) between GSOc, GSOm and GSOr, respectively.

Table IV - Calculated values of kinetic parameters of grape seed oil based on Rancimat test

Kinetic parameter	Calculation based on Rancimat		
	GSOc	GSOm	GSOr
Equation 1			
-a	0.030	0.031	0.031
b	3.923	4.061	4.050
R^2	0.998	0.996	0.996
Equation2			
A	4.256	4.387	4.377
-B	10.469	10.772	10.750
R^2	0.999	0.998	0.997
*Ea	77.488	79.872	79.686
Z	2.94×10^{10}	5.91×10^{10}	5.62×10^{10}
k at 120°C	1.489	1.443	1.453
k at 110°C	0.802	0.763	0.810
k at 100°C	0.418	0.390	0.393
k at 90°C	0.210	0.192	0.194
Q10	1.922	1.961	1.961
ΔH	74.344	76.728	76.542
**ΔS	-122.88	-121.87	-117.39

*Ea, ΔH in kJ mol^{-1}
** ΔS in J $molK^{-1}$
Z and k in h^{-1}
(Ea): The activation energy was adapted for an isothermal process:
R: the universal gas constant (8.314 J/mol·K).
The equation of rate constant (*k*) for lipid oxidation of oils:
Q10: the increase in the reaction rate with a 10°C temperature rise
k1 and *k2*: the rate constants at temperatures *T1* and *T2*, respectively.
The equation of the activation enthalpy (*ΔH*) and entropy (*ΔS*):
kB: the Boltzmann constant ($1.3806586 \times 10^{-23}$ J/K)
h: Planck's constant ($6.62607556 \times 10^{-34}$ J·s).
GSOc: grape seed oil control, GSOm: grape seed oil after microwave pretreatment, GSOr: grape seed oil after roasting pretreatment

exhibited relatively low activation energies, which are influenced by their polyunsaturated fatty acid (PUFA) content. According to Adhvaryu et al. [38], a high PUFA content typically decreases Ea, whereas a higher oleic acid content tends to increase it. The substantial presence of PUFAs in GSO implies that less energy is required to initiate oxidation reactions.
The kinetic constant rate (k) of GSO oxidation, shown in Table IV, increased with rising temperature (363.15 K to 393.15 K). High k values indicated rapid lipid oxidation [39]. The oils extracted from grape seeds exhibited the lowest oxidation rates across all temperatures, with k values at 90°C and 120°C being 0.192 h^{-1} and 1.443 h^{-1}, respectively. The order of lipid oxidation rates was GSOc > GSOr > GSOm. Factors affecting the k value included fatty acid composition, triacylglycerol structure, and the presence of catalysts or inhibitors [23].
The Q10 number, which indicated how the oxidation rate changes with a 10°C temperature increase, was 1.92 for untreated GSO and 1.96 for both microwave- and roasting-treated GSO. Higher Q10 values suggest that only small temperature variations significantly impact the lipid oxidation rate. Farhoosh and Seyedeh-zohreh [23] reported Q10 values for various oils (canola, soybean, sunflower, corn, and olive) ranging from 2.08 to 2.18, highlighting that Q10 values can vary by oil type. Table IV also includes thermodynamic parameters for lipid oxidation, specifically enthalpy (ΔH) and entropy (ΔS). According to the activated complex hypothesis, reactants must form a thermodynamically stable complex before undergoing reaction. Lower ΔH and ΔS values are associated with slower oxidation rates [19]. The ΔH values ranged from 74.34 kJ/mol (GSOc) to 76.73 kJ/mol (GSOm), while ΔS values ranged from

Table V - Correlation between bioactive compound content of oils, oxidative stability and kinetic oxidation parameters

Quality parameter	OSI at 90°C	Ea	*k* at 90°C
OSI at 90°C	-	-	-
Ea	0.98 ± 0.13	-	-1.00 ± 0.12
k at 90°C	-0.99 ± 0.09	-0.99 ± 0.01	-
Tocochromanols	0.88 ± 0.05	0.76 ± 0.12	-0.78 ± 0.33
TocopherolsT	0.82 ± 0.11	0.69 ± 0.05	-0.71 ± 0.22
TocotrienolsT3	0.90 ± 0.07	0.80 ± 0.12	-0.82 ± 0.5
Sterols	0.97 ± 0.05	0.90 ± 0.11	-0.92 ± 0.11
SFA	0.98 ± 0.09	0.92 ± 0.09	-0.94 ± 0.33
MUFA	0.97 ± 0.11	1.00 ± 0.33	-1.00 ± 0.53
PUFA	-0.98 ± 0.07	-1.00 ± 0.12	1.00 ± 0.15

OSI: oxidative stability index, Ea: activation energy, *k* at 90°C: rate constant at temperature at 90°C, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.
All values are means of three repetitions with standard deviation.

-122.88 J/mol K (GSOc) to -117.39 J/mol K (GSOr). A positive ΔH indicates an endothermic process for the activated complex's formation, implying that reaction rates increase with temperature. Negative ΔS values suggest the formation of activated complexes, indicating greater oxidative stability [40].
Overall, the oxidative stability and kinetic parameters of GSO varied according to seed pretreatment, primarily due to differences in chemical composition and concentrations of bioactive compounds. The analysis focused on induction time and oxidation rate constant at 90°C due to their variability. Notably, the oxidative stability was highly correlated with sterol content (r = 0.97) and fatty acid composition (Table V). Among tocochromanols, tocotrienols significantly influenced oxidative stability (r = 0.90). The increase in tocotrienol content from treatments positively affected the activation energy of the oxidation reaction (r = 0.80), while decreasing the oxidation rate (r = -0.82). These findings aligned with the growing focus on environmental integration in major world regions, where the optimization of agricultural by-products, such as grape seeds, can significantly enhance the sustainability of oil extraction processes. By improving oxidative stability through targeted pretreatment methods, this approach not only improves the quality of GSO but also promotes energy-efficient practices, reducing waste and supporting regions' sustainable agricultural goals.

4. CONCLUSION

In this study, the microwave and roasting pretreatments were applied to grape seed (*Vitis vinifera* L.) prior to oil extraction, resulting in significant changes to the fatty acid composition and an increase in nutraceutical content in the treated oils. The use of the Rancimat accelerated test demonstrated that microwave pretreatment enhanced the oxidative stability of the oil, effectively extending its shelf life. Kinetic parameter analysis further enabled the prediction of oxidation processes under various conditions. The key novelty of this research is its demonstration that microwave pretreatment not only improved the oxidative stability of GSO, but also amplified its nutraceutical properties, offering potential for better-quality oil production. These findings could have broader implications for the agro-industrial sector in all world regions, where optimizing the use of agriculturalby-products like grape seeds could support both sustainability and the development of high-value oils with enhanced health benefits.

Conflict of interest
On behalf of all authors, the corresponding author states that there is no conflict of interest

REFERENCES

[1] O.E. Constantin, F. Stoica, R.N. Rațu, N. Stănciuc, G.E. Bahrim, G. Râpeanu, Bioactive components, applications, extractions, and health benefits of winery by-products from a circular bioeconomy perspective: A review. Antioxidants (Basel) 13(1), 1-30, (2024).
[2] V. Lavelli, L. Torri, G. Zeppa, L. Fiori, G. Spigno, Recovery of winemaking by-products for innovative food applications. Italian Journal of Food Sciences 28, 542-564, (2016).
[3] N.H. Bui, B.V. Nguyen, T.N.L. Nguyen, T.T.T. Tran, H.C. Mai, Physicochemical properties of seed oil of the cardinal grape (*Vitis vinifera* L.) originated in Vietnam. Food Research 6(5), 161-167, (2022).
[4] N. Rombaut, R. Savoire, B. Thomasset, T. Bélliard, J. Castello, E. Van Hecke, J.L. Lanoisellé, Grape seed oil extraction: Interest of supercritical fluid extraction and gas-assisted mechanical extraction for enhancing polyphenol co-extraction in oil. Comptes Rendus Chimie 17(3), 284-292, (2014).
[5] S. Rizvi, S.T. Raza, F. Ahmed, A. Ahmad, S. Abbas, F. Mahdi, The role of vitamin E in human health and some diseases. Sultan Qaboos University Medical Journal 14(2), e157-e165, (2014).
[6] E.J. Dufourc, Sterols and membrane dynamics. Journal of Chemical Biology 1(1-4): 63-77(2008).
[7] R.T. Ras, J.M. Geleijnse, E.A. Trautwein, LDL-cholesterol-lowering effect of plant sterols and stanols across different dose ranges: A meta-analysis of randomized controlled studies. British Journal of Nutrition 112, 214-219, (2014).
[8] J. Plat, S. Baumgartner, R.P. Mensink, Mechanisms underlying the health benefits of plant sterol and stanol ester consumption. Journal of AOAC International 98(3), 697-700, (2015).
[9] Matthäus, B. (2008). Virgin grape seed oil: Is it really a nutritional highlight? European Journal of Lipid Science and Technology. 110, 645–650. DOI:10.1002/ejlt.200700276.
[10] P. Irandoost, M. Ebrahimi-Mameghani, S. Pirouzpanah, Does grape seed oil improve inflammation and insulin resistance in overweight or obese women? International Journal of Food Sciences and Nutrition 64(6), 706-710, (2013).
[11] I Dimić, N. Teslić, P. Putnik, D. Bursać Kovačević, Z. Zeković, B. Šojić, Ž. Mrkonjić, D. Čolović, D. Montesano, B. Pavlić, Innovative and conventional valorizations of grape seeds from winery by-products as a sustainable source of lipophilic antioxidants. Antioxidants 9, 568 (2020).

[12] S. Ngamukote, K. Mäkynen, T. Thilawech, S. Adisakwattana, Cholesterol-lowering activity of the major polyphenols in grape seed. Molecules 16, 5054-5061, (2011).
[13] Bail, S., Stuebiger, G., Krist, S., Unterweger, H., Buchbauer, G. Characterisation of various grape seed oils by volatile compounds, triacylglycerol composition, total phenols and antioxidant capacity. Food Chemistry. 108 (3), 1122-1132. DOI:10.1016/j.foodchem.2007.11.063, (2008)
[14] Sevindik, O., Kelebek, H., Rombolà, A.D., Selli, S. Grape seed oil volatiles and odour activity values: a comparison with Turkish and Italian cultivars and extraction methods. J Food Sci Technol. 59(5):1968-1981. DOI: 10.1007/s13197-021-05212-3, (2022).
[15] J. Garavaglia, M.M. Markoski A. Oliveira, A. Marcadenti, Grape seed oil compounds: Biological and chemical actions for health. Nutrition and Metabolic Insights 9, 59-64, (2016).
[16] C. Segatto, C. Arruda Souza, C. Reschke Lajus, M.A. Fiori, L.L. Silva, H.G. Riella, C.M. Medeiros Coelho Pretreatment of maize seeds with different magnesium nanoparticles improves the germinating performance and storability. Australian Journal of Crop Science 14, 1473-1478, (2020).
[17] M. Wroniak, A. Rekas, A. Siger, M. Janowicz, Microwave pretreatment effects on the changes in seeds microstructure, chemical composition and oxidative stability of rapeseed oil. LWT-Food Science and Technology 68, 634-641, (2016).
[18] G. Peron, I. Ferrarese, N. Carmo Dos Santos, F. Rizzo, G. Gargari, N. Bertoli, E. Gobbi, A. Perosa, M. Selva, S. Dall'Acqua, Sustainable extraction of bioactive compounds and nutrients from agri-food wastes: Potential reutilization of berry, honey, and chicory byproducts. Applied Sciences 14*(*23), 10785, (2024).
[19] B. Fathi-Achachlouei, S. Azadmard-Damirchi, Y. Zahedi, Y. Zahedi, R. Shaddel, Microwave pretreatment as a promising strategy for increment of nutraceutical content and extraction yield of oil from milk thistle seed. Industrial Crops and Products 128, 527-533, (2019).
[20] H. Harbeoui, I. Bettaieb Rebey, I. Ouerghemmi, W. Aidi Wannes, H. Zemni, N. Zoghlami, N. Akhtar Khan, R. Ksouri, M. Saidani Tounsi, Biochemical characterization and antioxidant activity of grape (*Vitis vinifera* L.) seed oils from nine Tunisian varieties. Journal of Food Biochemistry 42, e12595, (2018).
[21] A. Siger, P. Kachlicki, J. Czubinski, D. Polcyn, K. Dwiecki, M. Nogala-Kałucka, Isolation and purification of plastochromanol-8 for HPLC quantitative determinations. European Journal of Lipid Science and Technology 116, 413-422, (2014).
[22] M. Raczyk, D. Kmiecik, R. Przybylski, M. Rudzinska, Roasting pumpkin seeds and changes in the composition and oxidative stability of cold-pressed oils. Journal of the American Oil Chemists Society 94, 71-711, (2017).
[23] R. Farhoosh, H.Y. Seyedeh-Zohreh, Evolution of oxidative values during kinetic studies on olive oil oxidation in the Rancimat test. Journal of the American Oil Chemists Society 91, 281-293, (2014).
[24] M.A. Gitea, D. Gitea, D. Mirela Tit, S.G. Bungau, M.A. Bogdan, A.F. Radu, F.V. Dulf, M.B. Pasca, Organically cultivated vine varieties-distinctive qualities of the oils obtained from grape seeds. Sustainability 15(14), 11037, (2023).
[25] P. Górnàs, M. Rudzińska Seeds recovered from industry by-products of nine fruit species with a high potential utility as a source of unconventional oil for biodiesel and cosmetic and pharmaceutical sectors. Industrial Crops and Products 83, 329-338, (2016).
[26] B.D. Oomah, J. Liang, D.V. Godfrey, G. Mazza, Microwave heating of grape seed: Effect on oil quality. Journal of Agricultural and Food Chemistry 69, 187-193, (1998).
[27] K.T. Lee, K.S. Park, S.W. Choi, Changes in flavonoid contents of safflower leaf during growth and processing. Preventive Nutrition and Food Science 9, 144-149, (2004).
[28] K.H. Jackson, W.S. Harris, M.A. Belury, P.M. Kris-Etherton, P.C. Calder, Beneficial effects of linoleic acid on cardiometabolic health: an update. Lipids in Health and Disease 23, 1-8, (2024).
[29] G. Mercola, C.R. D'Adamo, Linoleic acid: A narrative review of the effects of increased intake in the standard American diet and associations with chronic disease. Nutrients 15(4), 3129 (2023).
[30] J.E. Pardo, E. Fernández, M. Rubio, A. Alvarruiz, G.L. Alonso, Characterization of grape seed oil from different grape varieties (*Vitis vinifera*). European Journal of Lipid Science and Technology 2, 188-193, (2009).
[31] K. Suri, B. Singh, A. Kaur, M.P. Yadav, N. Singh, Influence of microwave roasting on chemical composition, oxidative stability and fatty acid composition of flaxseed (*Linum usitatissimum* L.) oil. Food Chemistry 326, 126974, (2020).
[32] I. Ahmed, N. Uslu, M. Özcan, F. Al-Juhaimi, K. Ghafoor, E.E. Babiker, M.A. Osman, A.S.A. Alqah, Effect of conventional oven roasting treatment on the physicochemical quality attributes of sesame seeds obtained from different locations. Food Chemistry 338, 128109, (2021).
[33] Z. Cai, K. Li, W.J. Lee, M.T.J. Reaney, N. Zhang, Y. Wang, Effect of microwave pretreatment of sunflower kernels on the aroma-active compo-

sition, sensory quality, lipid oxidation, tocopherols, heterocyclic amines, and polycyclic aromatic hydrocarbons of sunflower oil. Fundamental Research 1, 767-784, (2021).

[34] P. Gao, Y. Cao, R. Liu, Q. Jin, X. Wang, Comparison of solvents for extraction of walnut oils: Lipid yield, lipid compositions, minor-component content, and antioxidant capacity. European Journal of Lipid Science and Technology 121, 1800292, (2019).

[35] M. Maszewska, A. Florowska, E. Dłużewska, M. Wroniak, K. Marciniak-Lukasiak, A. Żbikowska, Oxidative stability of selected edible oils. Molecules 23, 1746, (2018).

[36] T.C.P.M. Ramos, A.R. Fiorucci, C.A.L. Cardoso, M.S. da Silva, Kinetics of lipid oxidation in ternary mixtures of grape, sesame and sunflower oils by Rancimat method. Ciência e Natura 42, e53, (2020).

[37] E. Symoniuk, K. Ratusz, K. Krygier, Comparison of the oxidative stability of linseed (*Linum usitatissimum* L.) oil by pressure differential scanning calorimetry and Rancimat measurements. J Food Sci Technol 53(11), 3986-3995, (2016).

[38] A. Adhvaryu, S.Z. Erhan, Z.S. Liu, J.M. Perez, Oxidation kinetic studies of oils derived from unmodified and genetically modified vegetables using pressurized differential scanning calorimetry and nuclear magnetic resonance spectroscopy. Thermochemica Acta 364, 87-97, (2000).

[39] E.N. Frankel, Lipid oxidation. Elsevier, Amsterdam (2014).

[40] R. Upadhyay, H.N. Mishra, Multivariate optimization of a synergistic blend of oleoresin sage (*Salvia officinalis* L.) and ascorbyl palmitate to stabilize sunflower oil. Journal of Food Science and Technology 53, 1919-1928, (2016).