ASIAN JOURNAL OF AGRICULTURE
Volume 8, Number 1, June 2024
Pages: 10-17

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g080102

Comprehensive characterization of Gerboui olive oil: Quality, composition and aromatic profile

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Manuscript received: 29 October 2023. Revision accepted: 7 December 2023.

Abstract. *Manai-Djebali H, Nait Mohamed S, Madrigal-Martínez M, Martínez-Cañas MA, Sánchez-Casas J, Ben Youssef N. 2023. Comprehensive characterization of Gerboui olive oil: Quality, composition and aromatic profile. Asian J Agric 8: 10-17.* This study presents an in-depth analysis of Gerboui olive oil, a relatively lesser-known Tunisian variety. The investigation encompassed a thorough evaluation of key quality parameters, including acidity, peroxide value, ultraviolet absorbance, and fatty acid composition. The findings establish that Gerboui olive oil not only meets the stringent criteria set by the International Olive Oil Council for extra virgin olive oil but also exhibits remarkable qualities. It demonstrated a robust antioxidant capacity, with a DPPH (2,2-diphenyl-1-picrylhydrazyl) radical inhibition percentage of 94.93%. Furthermore, it boasts a substantial total phenolic content of 564.81 mg CA (caffeic acid)/kg and commendable oxidative stability, lasting 30.44 hours. The oil displayed exceptional resistance to oxidation, showcasing its potential as a premium product with potential health benefits. Additionally, the research delved into the fatty acid composition, highlighting the prevalence of unsaturated fatty acids, particularly oleic acid. The triacylglycerol profile revealed the presence of primary and secondary triacylglycerols, with notable triacylglycerols (TAGs) like Dilinoleoyl oleoyl glycerol (LLO) and Oleoyl linoleoyl oleoyl glycerol (OLO) found in substantial proportions at 5.04% and 17.84%, respectively. Trioleoyl glycerol (OOO) emerged as the most abundant TAG, constituting 30.07% of the TAG content, contributing to the oil's distinct characteristics. Furthermore, the analysis of volatile compounds unveiled a complex aroma profile, with (E)-2-hexenal (19.47%) and n-dodecane (15.66%) as the primary contributors. This comprehensive characterization enhances our understanding of Gerboui olive oil and underscores its potential as a unique and valuable olive oil variety.

Keywords: Antioxidant capacity, fatty acid composition, Gerboui olive oil, triacylglycerol profile, volatile compounds

Abbreviations: EVOO: Extra Virgin Olive Oil, FAs: Fatty Acids, TAGs: Triacylglycerols, LLL: Trilinolein, OOO: Triolein, PPP: Tripalmitin, SSS: Tristearin, LnLnLn: Trilinolenin, PoPoPo: Tripalmitolein, IOOC: International Olive Oil Council, DPPH: 2,2-diphenyl-1-picrylhydrazyl, CA: Caffeic Acid, OS: Oxidative Stability, SFA: Saturated Fatty Acids, UFA: Unsaturated Fatty Acids, MUFA: Monounsaturated Fatty Acids, PUFA: Polyunsaturated Fatty Acids, LLnLn: Dilinolenoyl linoleoyl glycerol, LLLn: Dilinoleoyl linolenoyl glycerol, OLLn: Oleoyl linoleoyl linolenoyl glycerol, LLL: Trilinoleoyl glycerol, PLLn: Palmitoyl linoleoyl linolenoyl glycerol, LLO: Dilinoleoyl oleoyl glycerol, OLnO: Dioleoyl linolenoyl glycerol, PLL: Dipalmitoyl linoleoyl glycerol, OLO: Dioleoyl linoleoyl glycerol, PLO+SLL: Palmitoyl linoleoyl oleoyl glycerol and Dilinoleoyl stearoyl glycerol, PPL: Dipalmitoyl linoleoyl glycerol, OOO: Trioleoyl glycerol, POO: Dioleoyl palmitoyl glycerol, PPO: Dipalmitoyl oleoyl glycerol, PPP: Tripalmitoyl glycerol, SOO: Dioleoyl stearoyl glycerol, SLS+POS: Distearoyl linoleoyl glycerol and Palmitoyl oleoyl stearoyl glycerol

INTRODUCTION

Renowned and savored worldwide for its role as a wholesome source of dietary fat, Extra Virgin Olive Oil (EVOO), renowned and savored worldwide for its role as a wholesome source of dietary fat, stands out as a culinary gem with profound global significance. Derived from freshly ripened olives, it holds a distinguished position as one of the cornerstone agricultural products in the Mediterranean region, a cradle of olive cultivation (Muzammil et al. 2021). The chemical composition of EVOO, this liquid gold of the culinary world, is intricately intertwined with a multitude of factors. These include the specific olive cultivar, the meticulous agronomic practices employed during cultivation, and the geographical locale in which these olive trees thrive (Hmida et al. 2022). Within the context of the Mediterranean diet, EVOO takes center stage as a dietary cornerstone celebrated not only for its exceptional taste but also for its well-documented health-promoting qualities (Jimenez-Lopez et al. 2020). Beyond its role on the plate, olive oil has found itself inextricably woven into the culinary heritage of many cultures, serving as an indispensable ingredient in a diverse array of traditional dishes. This dual role, both as a dietary staple and an essential culinary component, positions it as a vital commodity in the ever-evolving food industry (Fernández-Lobato et al. 2022). Moreover, the production and consumption of EVOO contribute significantly to the economic landscape of several countries, most notably Tunisia (Fernández-Uclés et al. 2020). Tunisia, renowned for its production of high-quality EVOO, has crafted a distinct reputation for its oil, characterized by a unique

flavor profile and chemical composition. Additionally, the trend of producing monovarietal EVOOs, extracted from a single olive cultivar, is steadily gaining momentum in response to evolving culinary preferences and growing consumer demand for unique flavors (Hlima et al. 2017). Examining the composition of minor Tunisian olive oil holds considerable significance for a variety of compelling reasons. Firstly, it plays a pivotal role in the characterization and differentiation of diverse olive oil varieties, as underscored by Chtourou et al. (2021). This distinction is invaluable for both consumers and producers, serving as a safeguard to ensure the genuineness and excellence of the oil they encounter. By accurately identifying the unique chemical profiles of different monovarietal oils, consumers can confidently make informed choices while producers can maintain the integrity of their products. Secondly, the analysis of minor Tunisian olive oil composition contributes to the realm of nutrition studies, as highlighted by Manai-Djebali et al. (2012). The widely acknowledged health advantages of olive oil make it a subject of intense interest in the field of nutrition. By discerning the specific compositions of distinct olive oil varieties, researchers can delve deeper into the nutritional properties and potential health benefits associated with each, enhancing our understanding of how olive oil can positively impact human health. Furthermore, the knowledge surrounding the composition of minor Tunisian olive oil offers significant advantages in the realm of olive breeding projects, as indicated by Monasterio et al. (2013). For instance, understanding the precise sterol fraction of different olive varieties equips breeders with the tools to identify ideal parent trees for the cultivation of new olive cultivars. This selective breeding process can yield offspring with improved characteristics, both in terms of oil quality and other desirable traits, thereby advancing the olive industry and enriching the range of olive oil products available.

The aim of this research is to analyze and describe the chemical composition of Gerboui olive oils from Testour, located in the northern region of Tunisia. Through the analysis of olive oils from this specific variety, the study places its focus on critical chemical parameters, encompassing aspects such as free acidity, peroxide values, overall phenolic content, levels of chlorophylls and carotenoids, as well as tocopherols. Additionally, this investigation assesses the antioxidant potential of these olive oils, emphasizing the varying capabilities of both polar and lipid fractions in neutralizing DPPH radicals, while also scrutinizing the fatty acid and triacylglycerol profiles. The outcomes of this study offer valuable insights into the quality and nutritional properties of Gerboui olive oil, making significant contributions to the fields of scientific research and the olive oil industry.

MATERIALS AND METHODS

Olive oil and chemicals

The Gerboui olive oil was harvested from Testour, Béja, Tunisia (36° 32' 54.228" N, 9° 25' 29.781" E) (Figure 1).

Reagents and standards for spectrophotometric and titration analyses were sourced from Fluka (Buchs, Switzerland), Sigma-Aldrich (Saint Louis, MO, USA), and Merck (Darmstadt, Germany). Triacylglycerol standards, including trilinolein (LLL), triolein (OOO), tripalmitin (PPP), tristearin (SSS), trilinolenin (LnLnLn), and tripalmitolein (PoPoPo), each with a purity exceeding 98%, were obtained from Sigma-Aldrich (Saint Louis, MO, USA). A Fatty Acid Methyl Ester (F.A.M.E.) mix (CRM18918) was provided by Supelco (Bellefonte, PA, USA). α, β, and γ-tocopherol were acquired from Fluka (Buchs, Switzerland). All solvents used for chromatographic techniques adhered to high-performance liquid chromatography (HPLC) standards. Specifically, n-Hexane, diethyl ether, acetone, and acetonitrile were supplied by Panreac (Barcelona, Spain).

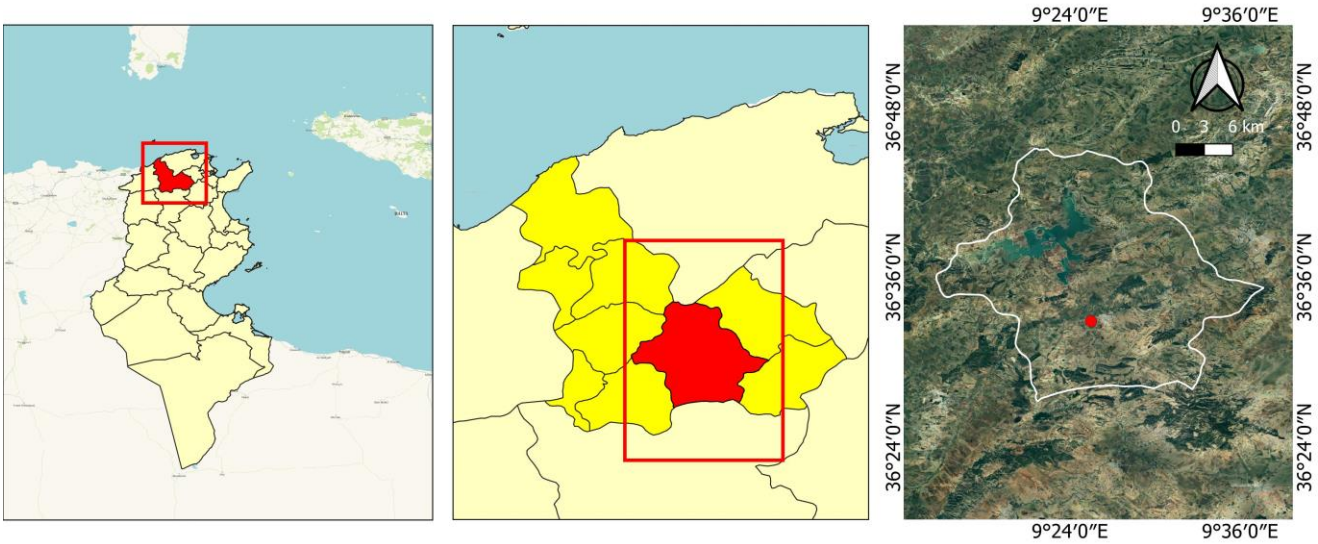

Figure 1. The Gerboui olive harvesting region: Testour, Béja, Tunisia

Sampling of Gerboui olive oil

For this study, we utilized three batches of extra virgin olive oils, each totaling 1000 mL, all derived from the Gerboui olive variety. These olive oils were sourced from a carefully selected farm located in Testour, within the Béja region of Tunisia. The Gerboui olives were harvested when they had reached their optimal ripeness stage, and the farm operators meticulously adhered to proper grove management and oil processing procedures. The extraction of the olive oils was carried out using a state-of-the-art continuous technological plant equipped with a three-phase centrifugal system. The freshly extracted oils were then stored in containers at a temperature of 4°C, shielded from light, until they were ready for analysis.

Determination of basic indicators of Gerboui olive oil quality

In this study, The free acidity was determined by dissolving 2 g of olive oil in a neutralized solvent mix, titrating it with a 0.1 M ethanolic solution of potassium hydroxide in the presence of phenolphthalein, and calculating the free acidity as a percentage of oleic acid by weight (IOOC 2017b). The peroxide value was determined by dissolving 1 g of olive oil in a mixture of chloroform, glacial acetic acid, and potassium iodide, followed by titration with a solution of sodium thiosulfate, expressed in meq O_2/kg of oil (IOOC 2017c). Specific extinction coefficients (K232 and K270) were determined by preparing a 1% oil solution in cyclohexane and measuring absorbances at 232 and 270 nm, and changes in specific extinction coefficients (ΔK) were calculated based on absorbance at wavelengths 266 and 274 nm (IOOC 2019).

Head space sampling

According to Flamini (2007), a 2g sample of olive oil was placed in a sealed 10 mL vial and allowed to equilibrate for 30 minutes. Subsequently, a solid-phase microextraction fiber (PDMS, 100µm, Supelco, Bellefonte, PA, USA) was exposed to the headspace for 50 minutes. The fiber was then retracted into a needle and transferred for analysis using a gas chromatography-mass spectrometry (GC-MS) system.

Volatile compound analysis by gaz chromatography

Gas chromatography analysis was performed using a Varian CP 3800 gas chromatograph equipped with a DB-5 Capillary column and a Varian Saturn 2000 ion trap mass detector. The injector was maintained at 250°C, the transfer line at 220°C, and the oven temperature gradually increased from 60 to 240°C at a rate of 3°C per minute. Helium served as the carrier gas at a flow rate of 1 mL/min. A splitless injection method was employed. The chemical constituents were identified by comparing retention times with authentic reference samples and using computer-assisted matching against the NIST 98 commercial library.

Oxidative stability of oils

According to Manaï et al. (2007), the assessment of oxidative stability in oil was conducted using a Rancimat 743 Metrohm apparatus (Herisau, Switzerland). This method is centered on the determination of the induction time, which signifies the duration required for the oil to undergo a specified level of oxidation, as indicated by a noticeable increase in the oil's electrical conductivity. The procedure involved heating the oils to 110°C and exposing them to a continuous flow of air. The electrical conductivity of the oil was systematically monitored over time, and the induction time was established as the moment when the conductivity exhibited a 2 mS/cm increase.

Antioxidant activity

The assessment of the oil samples' capacity to scavenge the DPPH radical was conducted in accordance with the procedure outlined by Kalantzakis et al. (2006). This involved the addition of 250 µL of an oil solution in ethyl acetate (10% weight/volume) to 1 mL of a freshly prepared DPPH solution (10 mM in ethyl acetate) within a 2 mL test tube. The mixture was vigorously shaken for 10 seconds using a Vortex apparatus and left in the dark for 30 minutes until it reached a stable state. Subsequently, the absorbance of the mixture was measured at 515 nm using a Cary 60 UV–vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) and compared to a blank solution devoid of the radical.

Evaluation of total phenolic content of the extra virgin olive oils

The total phenolic content of the olive oil was assessed using colorimetric analysis and the Folin-Ciocalteu reagent, following the protocols described by Psomiadou and Tsimidou (2002). This analysis focused on the polar oil fraction (2.5 g of each oil in 5 mL of n-hexane and then extracting it with a mixture of 5 mL of methanol and water (60:40, *v/v*)). To perform the assessment, a 20 µL aliquot of the appropriately diluted fraction was combined with 50 µL of distilled water and 20 µL of the Folin-Ciocalteu reagent in a tube. After thorough mixing and a 3-minute resting period, 125 µL of a 7% Na_2CO_3 solution was introduced, and the mixture was adjusted with an additional 100 µL of distilled water. The tube was then allowed to stand at room temperature in the dark for 90 minutes, following which the absorbance was measured at 760 nm. The results were expressed as the equivalent of caffeic acid (mg CA/kg of oil), and three replicates were performed for each sample.

Determination of chlorophyll and carotenoid contents

The determination of carotenoid and chlorophyll contents in the Extra Virgin Olive Oils (EVOOs) was carried out using a spectrophotometric method, following the methodology of Minguez-Mosquera et al. (1991). This involved measuring the absorbance at a wavelength of 470 nm for carotenoids and 670 nm for chlorophylls, respectively. Specific extinction coefficients were applied, with values of 613 L/(g·cm) for pheophytin A as the primary chlorophyll in EVOO and 2000 L/(g·cm) for lutein as the primary carotenoid. The pigment contents were calculated by dividing the absorbance at the specified wavelengths by the reference compound's extinction coefficients, the spectrophotometer cell thickness (1 cm), and a factor of 100. The results were expressed in

milligrams of pheophytin A for chlorophylls and lutein for carotenoids per kilogram of oil. Three replicates were conducted for each sample.

Determination of tocopherol composition

The assessment of tocopherol composition was conducted in accordance with the method established by Manai-Djebali et al. (2012). This analytical procedure involved initially dissolving the oil sample in n-hexane. Subsequently, the solution was subjected to analysis using an Agilent 1200 High-Performance Liquid Chromatography (HPLC) system, featuring a silica gel Lichrosorb Si-60 column with a particle size of 5 µm. The column had dimensions of 25 cm in length and 4 mm in inner diameter and was provided by Agilent Technologies, based in Santa Clara, CA, USA. Elution was achieved by employing a solvent mixture of n-hexane and 2-propanol (99:1, *v*/*v*), while maintaining the flow rate was set at 1 mL/min. Detection was performed utilizing a fluorescence detector, with excitation and emission wavelengths configured at 290 and 330 nm, respectively. Individual tocopherols were quantified and expressed in milligrams per kilogram (mg/kg) of the oil. The identification process relied on comparing retention times for α-, β-, and γ-tocopherols. Quantitative analysis utilized a calibration curve for these three tocopherols, resulting in coefficients of determination (R^2) of 0.948, 0.975, and 0.996 for α-, β-, and γ-tocopherols, respectively. Each sample was subjected to three replicate analyses.

Determination of fatty acid composition of the EVOOs

The determination of the fatty acid composition of the olive oils involved the preparation of fatty acids methyl esters (FAs). This was achieved by vigorously shaking the oils in n-hexane along with 0.2 mL of a 2 M methanolic potassium hydroxide solution, following the procedure outlined by (IOOC 2017a). The determination of FAs was carried out using an Agilent 6890N gas chromatograph (Agilent Technologies, Wilmington, DE, USA) equipped with a Flame Ion Detector (FID). It featured an HP-1 (polydimethylsiloxane) fused-silica capillary column with a length (L) of 50 m, an Inner Diameter (I.D.) of 0.2 mm, and a film thickness of 0.33 mm. Helium was employed as the carrier gas, flowing at a constant rate of 1 mL/min. The oven temperature was programmed to rise from 60 to 250°C at a rate of 2°C/min, followed by an isothermal hold for 20 minutes. The FID temperature was set at 250°C. The identification of fatty acids was carried out by comparing the specific retention time of each compound with those from a Fatty Acid Methyl Ester standard (F.A.M.E. Mix, CRM18918-Supelco, Bellefonte, PA, USA). Three replicates were conducted for each sample, and the results were expressed in terms of area percentages.

Determination of triacylglycerol composition

The analysis of triacylglycerols (TAGs) was conducted by initially dissolving 0.12 g of olive oil in 0.5 mL of n-hexane. Subsequently, the triacylglycerol fraction was purified using Solid-Phase Extraction (SPE) with a Silica column and a mixture of n-hexane and diethyl ether (87:13, *v*/*v*). After purification, the triacylglycerols were reconstituted in 2 mL of acetone and subjected to analysis using an Agilent 1200 HPLC Series system (Agilent Technology, Palo Alto, CA, USA). The HPLC system was equipped with a refractometric detector and utilized a LiChrospher RP-18 column with a particle size of 5 µm, featuring dimensions of 25 cm in length and 4.6 mm in Inner Diameter (I.D). The elution solvent consisted of a mixture of acetone and acetonitrile (50:50, *v*/*v*), with a flow rate set at 1.2 mL/min. Standards for triacylglycerols, including LLL, OOO, PPP, SSS, LnLnLn, and PoPoPo, were used (Sigma-Aldrich, St Louis, MO, USA). To identify the TAGs, retention times were compared with reference chromatograms obtained from soybean oil, a 30:70 (m/m) mixture of soybean oil and olive oil, and pure olive oil, following the methodology outlined in (IOOC 2017d). It was assumed that the total area of peaks representing different TAGs summed up to 100%, and the proportional distribution of each TAG was subsequently calculated.

Data analysis

The data analysis was performed using JMP 14 software (© SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Phytochemical characteristics of Gerboui olive oil

Table 1 provides a comprehensive overview of the phytochemical characteristics of the analyzed olive oil sample, providing critical insights into its quality and composition. Notably, the oil exhibits low free acidity (0.58±0.07%), indicating a high-quality product. The peroxide value (11.5 meq O_2/kg) suggests that the oil is relatively stable concerning oxidation. Furthermore, the K232 (2.37) and K270 (0.201) values further indicate the oil's freshness and potential for degradation. A low ΔK (0.005) signifies stable oil composition. Remarkably, the oil demonstrates a strong antioxidant capability, as evidenced by a significant 94.93% inhibition of DPPH radicals. Furthermore, it presents a significant total phenolic content, measuring at 564.81 mg of caffeic acid equivalents per kilogram of oil (mg CA/kg). Lastly, the oil demonstrates commendable oxidative stability, with a substantial duration of 30.44 hours.

Table 1. Phytochemical characteristics of Gerboui extra virgin olive oils from Testour Region in Tunisia

Phytochemical Characterisation	Values
Free acidity (% oleic acid (w/w))	0.58±0.07
Peroxide value (meq O2/kg)	11.5±1.34
K232	2.37±0.01
K270	0.201±0.002
ΔK	0.005±0.0004
DPPH• Inhibition (%)	94.93±0.46
Total phenolic content (mg CA/kg)	564.81±39.44
Oxidative stability (h)	30.44±0.14

Note: Data are expressed as average of a triplicates ± standard deviation

Fatty acid composition in Gerboui extra virgin olive oil

Table 2 provides a comprehensive overview of the fatty acid composition in Gerboui extra virgin olive oil originating from the Testour Region in Tunisia. This breakdown is crucial for gaining insights into the nutritional and sensory attributes of the olive oil. Notably, the oil is predominantly composed of high presence of oleic acid (C18:1), constituting a substantial 66.88% of the total fatty acids. This high presence of oleic acid is associated with the oil's excellent sensory qualities and potential health benefits. Furthermore, linoleic acid (C18:2), an essential polyunsaturated fatty acid, contributes 14.91% to the composition, enhancing the oil's nutritional value. The balance between saturated and unsaturated fatty acids is reflected in the sum of saturated (∑SFA) and unsaturated (∑UFA) fatty acids, comprising 16.7% and 83.3%, respectively. The ratio of oleic acid (C18:1) to linoleic acid (C18:2) (C18:1/C18:2) is 4.49, providing insights into the oil's fatty acid profile.

Phytochemical composition of the Gerboui extra virgin olive oil from the Testour Region in Tunisia

The data presented in Table 3 offers valuable insights into the phytochemical composition of the Gerboui extra virgin olive oil from the Testour Region in Tunisia. Notably, Table 3 shows a significant presence of α-tocopherol, with a content of 350 mg/kg, which are renowned for their potent antioxidant properties. This high α-tocopherol content enhances the oil's stability and potential health benefits. Furthermore, the presence of β-tocopherols and γ-tocopherols, at 7.42 mg/kg and 13.36 mg/kg, respectively, contributes to the oil's overall antioxidant capacity. The total tocopherol content of 370.86 mg/kg represents the combined antioxidant potential of these compounds. In addition to tocopherols, the oil contains chlorophylls (6.05 mg/kg) and carotenoids (2.71 mg/kg), which not only influence the oil's color but also add to its antioxidant profile.

Triacylglycerol (TAG) compositions in Gerboui olive oil from the Testour Region in Tunisia

The Table 4 presents a comprehensive overview of the triacylglycerol (TAG) compositions in Gerboui olive oil from the Testour Region in Tunisia. TAGs are a vital component of olive oil, influencing both its physical properties and potential health benefits. The table highlights the diversity of TAGs within this oil, with varying concentrations of different TAG species. Notable TAGs include Dilinoleoyl oleoyl glycerol (LLO) and Dioleoyl linoleoyl glycerol (OLO), which are present in substantial proportions at 5.04% and 17.84%, respectively. Trioleoyl glycerol (OOO) is the most abundant TAG, accounting for 30.07% of the TAG content. On the other hand, several TAGs, such as Dilinolenoyl linoleoyl glycerol (LLnLn) and Dilinoleoyl linolenoyl glycerol (LLLn), are present in comparatively minor amounts.

Table 2. Fatty acids composition (% total fatty acids) of the Gerboui extra virgin olive oils from Testour Region in Tunisia

Fatty Acids	% Total Fatty Acids
Palmitic acid (C16:0)	12.53±0.02
Palmitoleic acid (C16:1)	0.39±0.01
Stearic acid (C18:0)	3.65±0.08
Oleic acid (C18:1)	66.88±0.06
Linoleic acid (C18:2)	14.91±0.02
Alpha-linolenic acid (C18:3)	0.78±0.01
Arachidic acid (C20:0)	0.52±0.01
Gadoleic acid (C20:1)	0.35±0.01
Sum of Saturated Fatty Acids (∑SFA)	16.7±0.09
Sum of Unsaturated Fatty Acids (∑UFA)	83.3±0.09
Sum of Monounsaturated Fatty Acids (∑MUFA)	67.61±0.06
Sum of Polyunsaturated Fatty Acids (∑PUFA)	15.69±0.03
C18:1/C18:2	4.49±0.01

Note: Data are expressed as average of a triplicates ± standard deviation

Table 3. Phytochemical composition of the Gerboui extra virgin olive oil from the Testour Region in Tunisia

Phytochemicals	Contents (mg/kg)
α-Tocopherols	350.08± 1.52
β-Tocopherols	7.42±0.21
γ-Tocopherols	13.36±0.41
Total Tocopherols	370.86±2.15
Chlorophylls	6.05±0.23
Carotenoids	2.71±0.10

Note: Data are expressed as average of a triplicates ± standard deviation

Table 4. The triacylglycerol composition (% total triacylglycerols) of the Gerboui extra virgin olive oil from the Testour Region in Tunisia

Triacylglycerols	% total Triacylglycerols
Dilinolenoyl linoleoyl glycerol (LLnLn)	0.10±00
Dilinoleoyl linolenoyl glycerol (LLLn)	0.08±00
Oleoyl linoleoyl linolenoyl glycerol (OLLn)	0.37±00
Trilinoleoyl glycerol (LLL)	0.40±0.01
Palmitoyl linoleoyl linolenoyl glycerol (PLLn)	0.13±00
Dilinoleoyl oleoyl glycerol (LLO)	5.04±0.01
Dioleoyl linolenoyl glycerol (OLnO)	2.58±0.02
Dilinoleoyl palmitoyl glycerol (PLL)	0.85±00
Dioleoyl linoleoyl glycerol (OLO)	17.84±0.05
Palmitoyl linoleoyl oleoyl glycerol and Dilinoleoyl stearoyl glycerol (PLO+SLL)	10.09±0.02
Dipalmitoyl linoleoyl glycerol (PPL)	1.41±0.01
Trioleoyl glycerol (OOO)	30.07±0.09
Dioleoyl palmitoyl glycerol (POO)	21.46±0.04
Dipalmitoyl oleoyl glycerol (PPO)	3.84±00
Tripalmitoyl glycerol (PPP)	0.41±0.01
Dioleoyl stearoyl glycerol (SOO)	4.22±0.13
Distearoyl linoleoyl glycerol and palmitoyl oleoyl stearoyl glycerol (SLS+POS)	1.11±0.14

Note: Data are expressed as average of a triplicates ± standard deviation

Table 5. Volatile compounds of of the Gerboui extra virgin olive oil from the Testour Region in Tunisia

Volatile Compounds	Area %
(E)-2-penten-1-ol	0.52±0.03
(Z)-3-hexenal	6.24±0.31
(E)-2-hexenal	19.47±0.97
(Z)-3-hexen-1-ol	4.85±0.24
Heptanal	0.23±0.01
1-hexanol	3.42±0.17
(E.E)-2.4-Hexadienal	1.33±0.07
3-ethyl-1,5-octadiene*	1.79±0.09
3-ethyl-1,5-octadiene*	1.96±0.1
(Z)-2-Heptenal	0.61±0.03
3,7-decadiene	6.73±0.34
1-hexyl acetate	4.53±0.23
Limonene	1.33±0.07
(E)-β-ocimene	3.11±0.16
4,8-dimethyl-1,3,7-nonatriene	1.52±0.08
n-dodecane	15.66±0.78
Nonanal	0.66±0.03
(E)-2-dodecene	3.26±0.16
(E)-2-decenal	1.67±0.08
α-copaene	2.15±0.11
n-tetradecane	5.95±0.3
(E,E)-α-farnesene	4.03±0.2
Monoterpene hydrocarbons	3.11±0.16
Sesquiterpene hydrocarbons	6.18±0.31
Non-terpene derivatives	81.73±4.09
Total identified	91.02±4.55

Note: Data are expressed as average of a triplicates ± standard deviation, *: isomers

Volatile compounds of the Gerboui extra virgin olive oil

The volatile compound composition, as detailed in Table 5, reveals the intricate aroma profile of Gerboui extra virgin olive oil originating from the Testour Region in Tunisia. Among the most prominent constituents is (E)-2-hexenal, which contributes a significant 19.47% to the overall aroma. Another substantial component is n-dodecane, constituting 15.66%. Notable aromatic contributors also include 3,7-decadiene (6.73%), (Z)-3-hexenal (6.24%), and 1-hexyl acetate (4.53%). These compounds are known for their contributions to the fruity, green, and slightly spicy notes that are often associated with high-quality olive oils. Moreover, the presence of monoterpene hydrocarbons (3.11%) and sesquiterpene hydrocarbons (6.18%) adds complexity and depth to the aroma, while the non-terpene derivatives (81.73%) play a crucial role in providing a rich, well-rounded bouquet to the oil.

Discussion

Characterizing minor olive varieties is essential for several reasons. Firstly, it allows for the recovery and preservation of neglected germplasm, which may have unique genetic traits and contribute to the overall genetic diversity of olive species (Debbabi et al. 2020). Secondly, the characterization of minor compounds in olive oil, such as phenolic and triterpenic compounds, tocopherols, sterols, and free fatty acids, can help in identifying varietal markers and distinguishing the origin of olive oil samples (Olmo-García et al. 2019). This information is valuable for quality control, authentication, and traceability in the olive oil industry. Additionally, the characterization of minor components can contribute to the valorization of typical olive oil productions and aid in optimizing processing techniques (Amiri-Nowdijeh et al. 2018). Finally, the study of minor olive varieties can uncover their nutraceutical potential and identify varieties that may be superior in terms of their oil composition and active substances (Ilyasoglu et al. 2010).

The assessment of Gerboui olive oil, a lesser-known Tunisian variety, was conducted with respect to several critical quality markers, and the findings demonstrated that it complies with the criteria established for this category of olive oils. All the measured parameters conformed to the defined standards for "extra virgin olive oil" as outlined by the International Olive Oil Council (IOOC 2021). These standards include an acidity level of 0.8% or less, a peroxide value not exceeding 20 meq O_2/kg, K270 below 0.22, K232 under 2.5, and ΔK less than or equal to 0.01. Notably, lower values of these parameters typically signify that the olives used were fresh and in good condition, harvested at the ideal stage of ripeness, and promptly processed without prolonged storage. Our study's outcomes are in line with prior investigations on Gerboui olive oil in Tunisia (Hannachi et al. 2006), reinforcing the robustness of our results and contributing valuable insights to the existing knowledge base regarding Gerboui olive oil in Tunisia.

Impressively, the oil demonstrates a robust antioxidant capacity, with a DPPH radical inhibition percentage of 94.93%. Additionally, it boasts a substantial total phenolic content (564.81 mg CA/kg), which contributes to both its quality and potential health benefits. These results are consistent with several prior studies on Tunisian olive oil varieties (Manai-Djebali et al. 2017). Our findings align with the research conducted by Manai-Djebali et al. (2012), which focused on minor Tunisian olive varieties. In their study, they observed a similar range of chlorophyll content, varying from 6.22 to 1.15 mg/kg, and carotenoid content ranging from 3.82 to 1.07 mg/kg in these olive oils. Oxidative stability serves as a crucial parameter in the assessment of the quality of oils and fats. It provides a reliable estimate of their vulnerability to oxidative degradation, which is the primary cause of their deterioration (Carrasco-Pancorbo et al. 2005). Gerboui olive oil exhibits commendable oxidative stability (30.44 hours), a key factor for shelf life and resistance to spoilage. These findings collectively highlight the exceptional quality and promising attributes of the olive oil under examination, making it a noteworthy choice for culinary and health-conscious consumers. After an in-depth literature review, it is worth noting that this is the first report describing the characteristics of Gerboui olive oil.

As proposed by multiple authors, the tocopherol fraction in virgin olive oils is primarily composed of alpha-tocopherols, which exhibit both vitamin and antioxidant properties. Table 3 demonstrates a significant presence of alpha-tocopherol, with a content of 350 mg/kg. These

findings align with previous research, emphasizing that tocopherol content exhibits substantial variability depending on the olive oil variety, especially in the case of minor Tunisian olive oils (Manai-Djebali et al. 2012).

Chlorophylls and carotenoids are the primary pigments found in vegetable oils (Yao et al. 2022). In olive oils, lutein and pheophytin are the predominant carotenoids and chlorophylls, respectively. It's important to note that both chlorophylls and carotenoids play roles in autoxidation and photooxidation processes (Borello and Domenici 2019). Our findings are consistent with the research conducted by Manai-Djebali et al. (2012), which focused on minor Tunisian olive varieties. In that study, they observed a similar range of chlorophyll content, varying from 6.22 to 1.15 mg/kg, and carotenoid content ranging from 3.82 to 1.07 mg/kg in these olive oils. This alignment between our results and their findings underscores the stability and reliability of the measurements and highlights the characteristic pigment profiles of minor Tunisian olive varieties, including Gerboui.

Fatty acids play a crucial role in human nutrition and exert diverse effects on health. They can be categorized into Saturated Fatty Acids (SFAs) and Unsaturated Fatty Acids (UFAs), which encompass Monounsaturated Fatty Acids (MUFAs) and polyunsaturated fatty acids (PUFAs). Existing research has demonstrated that SFAs tend to have adverse health implications, including an elevated cardiovascular disease risk. In contrast, UFAs have been associated with positive health outcomes, such as a reduced risk of cardiovascular disease, as highlighted by Islam et al. (2019). Additionally, the ratio of MUFAs to PUFAs is often regarded as an indicator of the overall fatty acid composition quality, as indicated by Chen and Liu (2020). Our findings align with prior investigations conducted by Ben-Temime et al. (2006), Youssef et al. (2010), Youssef et al. (2012), and Yahia et al. (2012).

Triacylglycerols (TAGs) are the predominant components found in olive oil (Torres-Cobos et al. 2023). These compounds comprise a glycerol molecule bonded to three fatty acid chains and play a pivotal role in determining various physical and chemical characteristics of the oil, including viscosity, stability, and oxidative characteristics, as highlighted in the study by (Sánchez and Harwood 2002). The analysis presented in Table 4 reveals a distinctive triacylglycerol profile in the olive oil under examination. Among these, three primary triacylglycerols, namely OOO (comprising three oleic acid chains), POO (with palmitic and oleic acid chains), and OLO (containing oleic and linoleic acid chains), dominate the composition. In addition to these primary triacylglycerols, the oils exhibit seven secondary ones, including PLO+SLL, LLO, SOO, PPO, OLnO, nPPL, and SLS+POS. It's noteworthy that these triacylglycerols are fundamental in shaping the oil's characteristics and properties. Furthermore, traces (less than 1%) of OLLn, PLLn, PLL, LLL, LLnLn, and PPP are also discernible across all the samples, contributing to the overall complexity of the oils' triacylglycerol composition. Our results are consistent with previous studies conducted by Manai-Djebali et al. (2012).

The intricate aroma profile of Gerboui extra virgin olive oil from the Testour Region in Tunisia, as revealed by the volatile compound composition in Table 5, showcases a diverse array of aromatic constituents. At the forefront of this aromatic symphony is (E)-2-hexenal, making a substantial contribution of 19.47% to the overall aroma. This compound is known for its characteristic fruity and green notes, adding a fresh and vibrant quality to the oil. Another noteworthy participant in the aroma is n-dodecane, which accounts for 15.66% of the composition, contributing a distinctive element to the oil's overall fragrance (Wang et al. 2023). In addition to these key players, several other compounds significantly influence the aromatic profile. Notable among them is 3,7-decadiene (6.73%), recognized for its role in enhancing the green and slightly spicy notes often associated with premium-quality olive oils. (Z)-3-hexenal (6.24%) is another essential component contributing to the oil's aroma, with a characteristic green and grassy scent (Manai-Djebali et al. 2023). The presence of 1-hexyl acetate (4.53%) introduces complexity to the aroma, imparting fruity and sweet undertones. Moreover, the inclusion of monoterpene hydrocarbons (3.11%) and sesquiterpene hydrocarbons (6.18%) adds depth and richness to the aromatic profile. These compounds can introduce floral, herbal, or woody notes, further enhancing the sensory experience. The majority of the aroma (81.73%) is attributed to non-terpene derivatives, which play a crucial role in providing a well-rounded and harmonious bouquet to the oil. This diverse group of compounds contributes to the overall complexity and balance of the oil's aroma, making it an appealing and sensory-rich experience for consumers (Manai-Djebali et al. 2023).

In conclusion, our study on Gerboui olive oil, a lesser-known Tunisian olive variety, demonstrates that this oil meets the stringent standards for "extra virgin olive oil" of the IOOC. It exhibits an exceptional antioxidant capacity with a DPPH radical inhibition percentage and a substantial total phenolic content. Furthermore, it boasts commendable oxidative stability. The fatty acid composition aligns with previous research on Tunisian olive oils, indicating its favorable nutritional profile. The distinctive triacylglycerol profile contributes to its unique properties, while the complex volatile compounds in the aroma enhance its sensory appeal. Our study adds valuable insights to the understanding of Gerboui olive oil and its potential as a high-quality olive oil option with promising health benefits and a unique sensory experience.

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