GRASAS Y ACEITES 74 (3)
July-September 2023, e516
ISSN-L: 0017-3495
https://doi.org/10.3989/gya.0984221

Stabilization of organic extra virgin olive oil using maceration and ultrasound-assisted extraction of natural antioxidants from *Artemisia absinthium* leaves

A. Bezzezi[a,✉], M. Boulares[a], M. Arfaoui[a], O. Ben Moussa[a] and M. Hassouna[a]

[a]University of Carthage, Technological Innovation and Food Security "LR 22-A6R 01", 58 Alain Savary Street, El Khadhra City, 1003, Tunis, Tunisia

[✉]Corresponding author: bezzezi.asma@gmail.com

Submitted: 11 September 2022; Accepted: 12 December 2022; Published online: 10 October 2023

SUMMARY: Olive oil is the most popular product derived from *Olea europaea* L. due to its organoleptic characteristics and its beneficial effects on human health. Nowadays, there is a growing interest in olive oil enrichment with medicinal plants. In this regard, *Artemisia absinthium* L. is an edible source of antioxidants. The aim of the present study was to improve the oxidative stability and the nutritional value of Organic Tunisian *Chetoui* Olive oil by its enrichment with the phenolic compounds in *Artemisia absinthium*. The enrichment carried out by maceration and ultrasound-assisted extraction did not affect the organic criteria of olive oil. The comparative study, performed during one year of storage, showed that ultrasound-assisted extraction led to the lowest final peroxide and extinction coefficient values. At the end of storage, this method increased oleic acid content and biophenol contents. Furthermore, the anti-radical activities of enriched samples were higher than the control.

KEYWORDS: Artemisia absinthium *leaves; Enrichment; Maceration; Organic extra virgin olive oil; Ultrasound-assisted extraction.*

RESUMEN: ***Estabilización de aceite de oliva virgen extra ecológico con antioxidantes naturales de hojas de* Artemisia absinthium *obtenidos por maceración y extracción asistida por ultrasonido***. El aceite de oliva es el producto derivado de *Olea europaea* L. muy popular debido a sus características organolépticas y sus efectos beneficiosos para la salud humana. En la actualidad existe un interés creciente por enriquecer el aceite de oliva con plantas medicinales. En este sentido, *Artemisia absinthium* L. es una fuente comestible de antioxidantes. El objetivo del presente estudio fue la mejora de la estabilidad oxidativa y el valor nutricional del aceite de oliva Chetoui tunecino orgánico mediante su enriquecimiento con compuestos fenólicos de *Artemisia absinthium*. El enriquecimiento realizado por maceración y extracción asistida por ultrasonidos no afectó a los criterios ecológicos del aceite de oliva. El estudio comparativo, realizado durante un año de almacenamiento, mostró que la extracción asistida por ultrasonidos condujo a los valores finales de peróxido y coeficientes de extinción más bajos. Al final del almacenamiento, este método aumentó el contenido de ácido oleico y de biofenoles. Además, las actividades antirradicales de las muestras enriquecidas fueron mayores que las del control.

PALABRAS CLAVE: *Aceite de oliva virgen extra ecológico; Enriquecimiento; Extracción asistida por ultrasonido; Hojas de* Artemisia absinthium; *Maceración.*

Citation/Cómo citar este artículo: Bezzezi A, Boulares M, Arfaoui M, Ben Moussa O, Hassouna M. 2023. Stabilization of organic extra virgin olive oil using maceration and ultrasound-assisted extraction of natural antioxidants from *Artemisia absinthium* leaves. *Grasas Aceites* **74** (3), e516. https://doi.org/10.3989/gya.0984221

1. INTRODUCTION

In recent years, the interest in natural extracts from plants and by-products which are rich in bioactive compounds has increased in the agronomic, pharmaceutical, and cosmetic industries. In fact, there is a growing demand to produce novel functional foods with significant health benefits. The use of plant extracts as natural preservatives for various vegetable oils has been extensively researched (Allane and Benamara, 2019). In this respect, wormwood is a promising medicinal plant due to its richness in a large variety of secondary metabolites such as saponins, tannins, alkaloids, sterols, triterpenoids, phenolic, flavonoids and glycosides, exhibits biological activities (Dahal *et al.,* 2021). In fact, the powerful antioxidant, antimicrobial, anti-inflammatory and anti-diabetic properties of wormwood are due to its high content in lipophilic antioxidant molecules (Allane and Benamara, 2019). Nowadays, literature reports that *Artemisia* extracts and Covid-Organics exhibit some anti-viral activity against SARS-CoV-2 in vitro (Nie *et al.,* 2021). Furthermore, *Artemisia absinthium* leaves are used in the food industry as the main ingredient in the famous absinthe drink, in many herbal tea blends (Judžentiene, 2016) and for the improvement in the oxidative stability of butter. In addition, the essential oils and phenolic extracts of *Artemisia* areal parts presented strong antimicrobial and antioxidant capacities (Dahal *et al.,* 2021). These biological activities have sparked the interest in extracting the antioxidant compounds of these plants to take advantages of their properties and incorporate them into food products. Indeed, new extraction techniques were developed including ultrasound-assisted extraction. This method is an inexpensive, simple and efficient alternative to conventional extraction techniques such as heating, boiling, and maceration, which present disadvantages such as the loss of phenolic compounds due to oxidation, ionization and hydrolysis during processing with long extractions times (Chemat *et al.,* 2017). Ultrasound-assisted extraction contributes to environmental preservation by reducing the use of water and solvents and leads to high yield extraction in a short time. It maintains the good quality of extracts and improves the oxidative stability and organoleptic properties of newly-developed products (Chemat *et al.,* 2017).

In Tunisia, olive oil with its exceptional nutritional quality is a strategic branch in the national economy and is the number one exported Tunisian agricultural product. Thanks to its richness in antioxidants, olive oil is partially protected against oxidation but remains sensitive to photo-oxidation which causes the degradation of its organoleptic and nutritional quality during storage (Sanmartin *et al.,* 2018). In this context, several works studied the enrichment of olive oil using the maceration of vegetable matrices (oregano, Rosemary, Laurel, Basil, olive leaves) (Sousa *et al.,* 2015; Boulares *et al.,* 2022), and their essential oils (Boulares *et al.,* 2022) in order to delay oxidation or to improve their nutritional and organoleptic qualities. As far as we knew, no studies have been performed on the enrichment of olive oil with the natural extracts of *Artemisia absinthium*.

In this study, the oxidative stability of Organic Tunisian *Chetoui* Olive oil (OTCOO) was determined following its enrichment with natural antioxidants from *Artemisia absinthium* L., which were extracted by maceration or ultrasound-assisted extraction. The quality parameters, antioxidant activity and sensorial characteristics of enriched olive oil were assessed over one year of storage.

2. MATERIALS AND METHODS

The *Artemisia absinthium* L. plant originating from north Tunisia (Bizerte) was collected during January 2021. This plant was identified by a specialist in botany and certified specimens (VS1-QS2020/01) were deposited at the Herbarium run. The leaves were dried at room temperature for three weeks.

2.1. Chemical characterization

The moisture, ash, protein and fat of *Artemisia absinthium* leaf powder were determined according to the AOAC (2000) procedures.

2.2. Preparation of raw materials

In the current study, the "*Chetoui*" variety was used. All samples were purchased during the 2019/2020 crop season from the Aljazzira-Morneg oil mill in Tunis. *Artemisia absinthium* leaves were collected from the region of Bizerte in the North of Tunisia. They were washed, dried at room temperature and stored until use. The enrichment of OTCOO

with the natural antioxidants from *Artemisia* leaves was carried out using two methods, maceration and ultrasound-assisted extraction. For this reason, the sample of organic extra virgin olive oil was subdivided into three batches. The first batch served as the untreated control. In the second batch, the natural antioxidants were extracted using the maceration method by incorporating the *Artemisia absinthium* leaves into the OTCOO at a concentration of 2% (w/v) (Arfaoui *et al.,* 2021). The remained batch was enriched by natural polyphenols using ultrasound-assisted extraction. For this reason, twenty grams of dried and crushed *Artemisia absinthium* leaves were added to 1 liter of OTCOO (2%). The extraction was performed for 45 min using an ultrasonicator PEX3 (R.E.U.S., Contes, France) under optimal conditions of 60 W and 16 °C (Achat *et al.,* 2012). Finally, the control and enriched OTCOO were stored in black glass bottles in the dark at room temperature for 12 months. The sampling was done each three months during storage with three replicates.

2.3. Pesticides contents measurement

The OTCOO was analyzed to detect the presence of pesticides using the high-performance liquid chromatography (shimadzu RP-HPLC-PDA system), at the beginning and the end of storage. The described analytical procedure was validated according to the Arfaoui *et al.* (2021) protocol for analytical techniques for pesticide residue analysis in food and feed. This procedure fulfils the European Decision 2002/657/EC requirements to verify if the flavoring affects the organic criterion of the studied oil.

2.4. Fatty acids composition

In the beginning of storage, the total fatty acid compositions of all the studied olive oil samples were determined according to the international standard ISO 5509 (2000). The fatty acid composition was determined by preparing the methyl esters, and analyzing them by gas chromatography (GC) (HP. Hewlett. Packard model 6890) according to the ISO 5509 (2000).

The separation and determination of the fatty acid methyl esters were carried out by GC using a Hewlett HP chromatograph. Hewlett. Packard model 6890 equipped with a capillary column of 30 m length and 0.52 mm internal diameter with a film thickness of 1µm. This technique for separating the components of a mixture is based on the difference in affinity of the substances to be analyzed with respect to a common mobile phase and a stationary phase. In GC, the mobile phase is called carrier gas (nitrogen), which had a flow rate of 13 ml/min. The split-splitless injector (division 1/10) was heated to 240 °C. At the exit of the column, which was held at an isothermal temperature of 230 °C throughout the analyses, the compounds were detected by a FID (Flame Ionization Detector) heated to 260 °C. Elution was carried out in ascending order of molecular weight and number of unsaturation.

2.5. Quality parameters measurements

The fatty acidity (FA), peroxide value (PV) and extinction coefficients (K_{232}, K_{270}) were carried out every three months of storage, according to Boulares *et al.* (2022). The chlorophyll and carotenoid levels were determined according to the method described by Boulares *et al.* (2022). A total of 7.5 g of oil sample were placed in a 25 ml volumetric flask, filled to the mark with cyclohexane. The absorbance of the fat solution obtained was measured against that of the solvent at 670 nm for chlorophylls and 470 nm for carotenoids, using a spectrophotometer Jenway Model 6315. The pigment content was calculated using the following formula:

Chlorophylls (mg/kg) = (A670 x 10^6) / (E1x 100 x d)

Carotenoïds (mg/kg) = (A470 x 10^6) + (E2 x 100 x d)

With: d: optical path = 1 cm; A 670: absorbance at 670 nm; A 470: absorbance at 470 nm; E1: coefficient linked to the spectrophotometer = 613; E2: coefficient linked to the spectrophotometer = 2000.

2.6. Biophenol content

The determination of biophenol content was made in accordance with IOC (2009). The minor polar compounds were directly extracted from olive oil samples with a methanolic solution. Then, high performance liquid chromatography (Agilent 1260 Infinity HPLC chromatograph), using an UV developer at 280 nm was carried out. The biophenol contents were expressed in mg / kg of tyrosol. Syringic acid was used as internal standard.

2.7. Antioxidant activity measurements

The anti-radical activity of OTCOO samples was determined using the DPPH (2,2-diphenyl-1-picryl-

hydrazyl) assay. It was expressed as a percentage inhibition of the DPPH. 1g of the sample was mixed with 10 ml of ethanol/water (4V/1V). The mixture was left to stand for 1.5 hours in the dark. Finally, this mixture was filtered. 0.1 ml aliquot of the extract was added to 1000 µL of a DPPH solution (0.2 mM) and 2.9 mL of ethanol. After vigorous shaking of the mixture, it was kept in the dark for 30 min. The absorbance was measured at 517 nm using a UV/visible spectrophotometer, referring to a control without extract (Antoniewska *et al.,* 2018). The calculation of antioxidant activity was determined according to the following formula:

% Inhibition = 100 x [(control OD - extract OD) / (control OD)]

Inhibition rate in % OD control: absorbance at 517 nm of the control, OD extract: absorbance at 517 nm of the extract solution.

2.8. Sensory evaluation

The sensorial analysis of all olive oils samples was performed at the beginning and the end of the storage period. A descriptive sensory evaluation was carried out by eight experts from the Tunisian National Oil Office. Olive oil samples were served at room temperature in tasting glasses. The expert panelists noted on a profile sheet the intensity of each negative (fusty/muddy, musty, winey, metallic, rancid, wet wood) and positive (fruity, bitter, pungent) descriptor IOC (2018).

2.9. Statistical analysis

Statistical analysis of all data was performed using the ANOVA test according to Duncan's multiple range in SPSS 23.0 (SPSS IBM 2017). All the tests were processed in triplicate.

3. RESULTS AND DISCUSSION

3.1. Chemical characterization

In order to valorize the wormwood, improve its value and its potential application in food industries to produce functional foods, the biochemical properties of its leaf powder were determined. The moisture content in the aerial parts of *Artemisia absinthium* was 4.18 ± 0.04%. The ash content representing the total amount of minerals was 3.34 ± 0.03%. This finding was in accordance with the findings of Iqbal *et al.* (2012), who observed 7% ash content in *Artemisia annua*. The average protein and carbohydrate contents were 11.97 ± 0.03% and 6.93 ± 0.06%, respectively. In this study, a higher fat content (8.8 ± 0.3%) was obtained in wormwood when compared to that (6.07%) reported in the study of Iqbal *et al.* (2012) on *Artemisia annua*. This could be due to variations in climate, soil and species (Riahi *et al.,* 2015). Thus, the promising nutritional value of wormwood leaves which contributes to their health promoting efficiency was shown.

3.2. Organic criteria and initial composition

The obtained results on the pesticide level in olive oil samples, at the beginning and the end of the storage period, showed that their enrichment with *Artemisia* leaves maintained the organic criteria of the olive oil. In fact, the results proved that the phthalate levels remained below the quantification limit recommended by the norm throughout storage and for the three analyzed olive oils (data not shown). In addition, the identification and quantification of 11 analyzed pesticides showed that they remained absent throughout the storage period (data not shown), which confirms the organic criteria of the analyzed olives.

Moreover, the initial values for moisture (0.066%) and impurities (0.0062%) of the analyzed samples demonstrated the high quality of extracted olive oils. These values were lower than those required by the IOC (2019), which are 0.2 and 0.1%, respectively. Furthermore, the initial obtained peroxide value (PV), free fatty acid (FFA) content and extinction coefficients (K_{232}, K_{270}) were 7.38 meq O_2/Kg; 0.23%; 1.87 and 0.17, respectively. These findings were in agreement with the recommended standards IOC (2019), which confirmed that the studied OTCOO had good quality and was under the nomination "extra virgin".

3.3. Effect of enrichment using *Artemisia absinthium* on the quality of OTCOO

3.3.1. Effect on the fatty acid composition

The results on the variations in fatty acid compositions in all samples are shown in table 1. In fact, the control showed the predominance of oleic acid (C18:1) and a richness in linoleic and palmitic acids with respective values of 64.02 ± 0.28%;

Table 1: Initial fatty acids composition (%) of control and enriched organic extra-virgin olive oils

	OC	OM	OU	Limit (IOC, 2019)
Palmitic acid $C_{16:0}$	10.78 ±0.38^{a}	10.78 ±0.12^{a}	11.38 ±0.22^{b}	7.20-20.00%
Palmitoleic acid $C_{16:1}$	0.48 ±0.04^{a}	0.44 ±0.02^{a}	0.49 ±0.04^{a}	0.30-3.50%
Margaric acid $C_{17:0}$	0.05 ±0.00^{a}	0.05 ±0.01^{a}	0.05 ±0.00^{a}	≤0.40
Ginkgolic $C_{17:1}$	0.05 ±0.01^{a}	0.05 ±0.00^{a}	0.06 ±0.01^{a}	≤0.60
Stearic acid $C_{18:0}$	3.06 ±0.0^{a}	3 ±0.01^{a}	3.11 ±0.00^{b}	0.5-5.00%
Oleic acid $C_{18:1}$	64.02 ±0.28^{b}	63.46 ±0.52^{a}	65.38 ±0.23^{c}	55.00-83.00%
Linoleic acid $C_{18:2}$	20.65 ±0.53^{b}	20.37 ±0.47^{b}	19.15 ±0.18^{a}	2.50-21.00%
Linolenic acid $C_{18:3}$	0.71 ±0.02^{a}	0.7 ±0.00^{a}	0.71 ±0.03^{a}	≤1.00
Arachidic acid $C_{20:0}$	0.43 ±0.02^{a}	0.45 ±0.00^{a}	0.48 ±0.01^{b}	≤ 0.60%
Gadoleic acid $C_{20:1}$	0.36 ±0.06^{a}	0.39 ±0.01^{b}	0.38 ±0.04^{ab}	≤0.50

OC: control olive oil; **OM:** enriched olive oil using maceration; **OU:** enriched olive oil using ultrasound-assisted extraction.The values are expressed as means ± standard deviation, n=3. Different letters in each column indicate significant differences ($P < 0.05$) using Duncan's multiple range test.

20.65 ± 0.53% and 10.78 ± 0.38%. These findings are higher than those reported by Boudiche *et al.* (2003) on Spanish, Greek and Italian olive oils. The enrichment of olive oil using the maceration of olive leaves resulted in no significant difference ($p > 0.05$) in linoleic acid content compared to the control sample, although the oleic acid content was significantly ($p< 0.05$) lower than that of the control sample. This result was attributed to the greater richness of olive leaves from the variety "*Chetoui*" in oleic acid than in linoleic acid as described before. The richness in C18:1, known as the major monounsaturated fatty acid in olive oil is associated with beneficial effects on prevention of cancer, inflammatory and autoimmune diseases (Boulares *et al.,* 2022).

On the other hand, olive oil enriched with natural antioxidants obtained using ultrasound extraction had a significantly high amount ($p < 0.05$) of oleic acid 65.38 ± 0.23% and low amount of linoleic acid (19.15 ± 0.18%) compared to the control. Similar results were found by Ammar *et al.* (2017) where the oleic acid content increased significantly with the addition of *O. ficus-indica* flowers in olive oil. In addition, the results obtained by Sousa *et al.* (2015) revealed that the amounts of mono-unsatured fatty acids increased significantly with the addition of bay leaf and oregano to olive oil. These findings are in perfect agreement with those highlighted by Jaber *et al.* (2012), who showed that the enrichment of a refined olive oil with a chlorophyll extract of olive leaves caused an increase in the content of oleic acid and a decrease in that of linoleic acid.

3.3.2. Effect on free acidity

Initially, no significant differences ($p < 0.05$) were detected between the free acidity values of the control and enriched samples. A significant increase in initial free acidity was noted for all olive oil samples during storage. Then, acidity increased especially in macerated olive oil to reach the values of 0.61 ± 0.01%; 0.53 ± 0.03% and 0.72 ± 0.01%, respectively, for the control (OC), olive oil enriched by ultrasonic extracts (OU) and using maceration (OM). These findings can be explained by the enzymatic activity caused by lipolytic reactions in olive oil (Sousa *et al.,* 2015). In general, adding medicinal and aromatic plants or their extracts to olive oil leads to a slight increase in acidity, which has been shown by several authors. In fact, the addition of the phenol extract of olive leaves during the mixing step resulted in an increase in acidity (Arfaoui *et al.,* 2021). Similar results were found by Arfaoui *et al.* (2021), who reported that adding olive leaves or their ultrasonic extracts significantly increased acidity values compared to the control.

3.3.3. Effect on peroxide value

In this study, the peroxide value (PV) increased significantly ($p > 0.05$) during the storage period for all the analyzed samples (Table 2). At the beginning of storage, no significant differences ($p < 0.05$) were observed between any of the studied olive samples. Besides, after 6 months of storage, only the PV of the OU sample remained below the recommended limit. Furthermore, the enriched oils were more stable than the control sample during storage especially the olive

Table 2: Evaluation of quality parameters, pigment, biophenol contents and inhibition percentage (IP) of control and enriched organic extra-virgin olive oil during 12 months of storage

Analyses	Storage time (Months)	OC	OM	OU
Acidity (%)	M_0	0.23 ± 0.01^{aA}	0.25 ± 0.02^{aA}	0.25 ± 0.01^{aA}
	M_3	0.30 ± 0.01^{aB}	0.32 ± 0.04^{aBC}	0.32 ± 0.01^{aBC}
	M_6	0.42 ± 0.01^{aC}	0.35 ± 0.01^{aC}	0.36 ± 0.01^{aC}
	M_9	0.54 ± 0.04^{bD}	0.49 ± 0.02^{bD}	0.42 ± 0.02^{aCD}
	M_{12}	0.61 ± 0.01^{bE}	0.72 ± 0.01^{cE}	0.53 ± 0.03^{aD}
PV (meq O_2/Kg)	M_0	$7.38 \pm 0,45^{aA}$	6.85 ± 0.29^{aA}	7.6 ± 0.33^{aA}
	M_3	10.77 ± 0.48^{bB}	10.47 ± 0.21^{bB}	9.09 ± 0.58^{aB}
	M_6	13.38 ± 0.52^{bC}	13.12 ± 0.52^{bC}	12.23 ± 0.39^{aC}
	M_9	16.84 ± 0.52^{cD}	16.17 ± 0.49^{bD}	15.65 ± 0.55^{aD}
	M_{12}	21.23 ± 0.15^{cE}	18.64 ± 0.22^{bE}	17.07 ± 0.42^{aE}
K_{232}	M_0	1.87 ± 0.04^{aA}	1.8 ± 0.03^{aA}	1.86 ± 0.03^{aA}
	M_3	2.05 ± 0.04^{bB}	2.02 ± 0.04^{aB}	2.01 ± 0.02^{aB}
	M_6	2.33 ± 0.02^{bC}	2.27 ± 0.05^{aC}	2.28 ± 0.05^{aC}
	M_9	2.47 ± 0.11^{bC}	2.37 ± 0.11^{bC}	2.32 ± 0.05^{aC}
	M_{12}	2.87 ± 0.02^{bD}	2.53 ± 0.02^{bD}	$2.42 \pm 0,05^{aD}$
K_{270}	M_0	0.17 ± 0.01^{aA}	0.17 ± 0.02^{aA}	0.17 ± 0.02^{aA}
	M_3	0.19 ± 0.01^{aAB}	0.18 ± 0.01^{aAB}	0.18 ± 0.01^{aAB}
	M_6	0.22 ± 0.01^{bB}	0.19 ± 0.01^{aB}	0.19 ± 0.01^{aB}
	M_9	0.22 ± 0.02^{aB}	0.20 ± 0.03^{aB}	0.19 ± 0.02^{aB}
	M_{12}	0.23 ± 0.03^{bB}	0.21 ± 0.02^{abB}	0.20 ± 0.02^{aB}
Chlorophylls	M_0	4.51 ± 0.05^{bE}	4.61 ± 0.04^{aE}	4.78 ± 0.03^{cE}
	M_3	4.07 ± 0.03^{aD}	3.98 ± 0.04^{bD}	4.32 ± 0.07^{cD}
	M_6	3.13 ± 0.06^{aC}	3.41 ± 0.03^{bC}	3.86 ± 0.08^{cC}
	M_9	2.89 ± 0.05^{aB}	3.26 ± 0.04^{aB}	3.67 ± 0.03^{aB}
	M_{12}	2.42 ± 0.06^{aA}	2.97 ± 0.03^{aA}	3.48 ± 0.04^{aA}
Carotenoids	M_0	1.52 ± 0.04^{aD}	1.56 ± 0.03^{aC}	1.69 ± 0.06^{bE}
	M_3	1.14 ± 0.04^{aC}	1.38 ± 0.05^{bB}	1.42 ± 0.07^{cD}
	M_6	0.54 ± 0.04^{aB}	1.06 ± 0.04^{bA}	1.21 ± 0.02^{cC}
	M_9	0.21 ± 0.01^{aA}	0.96 ± 0.04^{bA}	1.05 ± 0.05^{cB}
	M_{12}	n.d	0.52 ± 0.01^{aA}	0.94 ± 0.02^{bA}
Biophenols	M_0	231 ± 0.05^{aC}	269 ± 0.02^{bD}	338 ± 0.01^{cD}
	M_3	128 ± 0.06^{aB}	162 ± 0.03^{bC}	233 ± 0.05^{cC}
	M_6	61 ± 0.05^{aA}	132 ± 0.05^{bB}	197 ± 0.05^{cB}
	M_9	n.d	64 ± 0.05^{aA}	102 ± 0.05^{bA}
	M_{12}	n.d	0. n.d	n.d
IP (%)	M_0	37.82 ± 0.14^{aE}	44.74 ± 0.12^{bE}	49.39 ± 0.18^{cE}
	M_3	25.9 ± 0.11^{aD}	38.73 ± 0.12^{bD}	42.73 ± 0.12^{cD}
	M_6	13.16 ± 0.16^{aC}	29.89 ± 0.12^{bC}	35.72 ± 0.12^{cC}
	M_9	9.17 ± 0.09^{aB}	22.82 ± 0.12^{bB}	26.39 ± 0.12^{cB}
	M_{12}	1.32 ± 0.03^{aA}	8.09 ± 0.01^{bA}	16.48 ± 0.04^{cA}

OC: control olive oil; **OM:** enriched olive oil using maceration; **OU:** enriched olive oil using ultrasound-assisted extraction. The values are expressed as means ± standard deviation, n=3 using Duncan's multiple range test. Mean values with different lowercase letters indicate significant differences ($p < 0.05$) between samples. Mean values with different uppercase letters indicate significant differences ($p < 0.05$) during storage period.

oil sample enriched with ultrasonic extracts. In fact, enriched samples had the lowest PV values of 18.64 ± 0.22 and 17.07 ± 0.42 for OM and OU, respectively, at the end of the storage period. The obtained results showed that enrichment using maceration or ultrasonic phenolic extracts from *Artemisia absinthium* leaves reduced the formation of lipid hypdroperoxides and consequently reduced the oxidation of the oil compared to the control. This result was attributed to the antioxidant activity of the alkaloid polyphenols, flavonoids, saponin, tannin, glycosides, phenols, and anthroquinononones present in this plant (Dahal *et al.,* 2021).

3.3.4. Effect on specific extinction coefficients

With regards to the specific extinction coefficients K_{232} and k_{270}, which give an indication of primary

and secondary oxidation, a significant ($p > 0.05$) increase in these parameters was observed during the 12 months of storage for all analyzed samples. The increase rate of the enriched samples was slightly slower that of the control sample. The obtained results proved that enrichment using ultrasound-assisted extraction is more efficient in preventing oxidation than the maceration method. These findings were in agreement with those of Sousa *et al.* (2015), who showed that enriched olive oils are more stable against primary oxidation. Indeed, from the third month of storage, the specific extinction coefficient K_{232} of the control sample became significantly higher ($p > 0.05$) than those of enriched samples.

Based on all findings related to quality parameters, it was concluded that olive oil enriched with the ultrasonic extracts of *Artemisia absinthium* remained the only sample classified as "extra virgin olive oil" after 12 months of storage according to the IOC (2019). Therefore, this treatment was the most efficient for oxidative stability due to the richness of *Artemisia* in natural antioxidants (Dahal *et al.,* 2021).

3.3.5. Effect on pigment contents

As shown in Table 2, chlorophyll contents decreased in all samples to reach values of 2.42 ± 0.06 ppm, 3.48 ± 0.04 ppm and 2.97 ± 0.03 ppm, respectively, for the control, OU and OM at the end of storage. In addition, at the end of storage, the carotenoids were not detected in the control sample. In fact, the obtained chlorophyll contents showed a significant decrease ($p < 0.05$) during storage. These findings were in accordance with those of Jaber *et al.* (2012), who reported a decrease in pigment contents throughout the storage period. However, carotenoid contents were 0.94 ± 0.02 ppm and 0.52 ± 0.01 ppm, respectively in OU and OM samples. It was observed that the chlorophyll and carotenoid contents in enriched olive oil with ultrasonic extracts were significantly ($p < 0.05$) higher than those of the macerated sample. These findings can be explained by the mechanical effect caused by the implosion of micro-bubbles of ultrasonic extraction, which cause rapid breakdown of the tissues, allowing the release of compounds into the solvent (Toma *et al.,* 2001). This richness in pigments can contribute to a promising antioxidant activity compared to the other samples. These findings are in line with those of Malheiro *et al.* (2013), who showed that OTCOO enriched by the addition of olive leaves during the extraction process presented much higher lutein and β-carotene contents than the control.

3.3.6. Effect on biophenol content

The term «biophenol» is used to designate the bioactive phenols in order to replace the more common chemically vague term "polyphenol" (Arfaoui *et al.,* 2021).

The registered initial biophenol contents in the analyzed olive oil samples showed that the highest value (338 ± 0.01 ppm) was noted for OU followed by OM (269 ± 0.02 ppm) and then the control (231 ± 0.05 ppm). This result can be explained by the richness of *Artemisia absinthium* in polyphenols (Riahi *et al.,* 2015). During one year of storage, a significant decrease ($p < 0.05$) was registered in terms of biophenol contents for all the analyzed OTCOO samples. These findings were in agreement with those of previous researchers, who showed that total biophenol content decreased in olive oil even after its enrichment with chlorophyll pigment (Jaber *et al.,* 2012) or natural antioxidants extracted from plants using various methods. Furthermore, from the ninth month, the results showed that the phenolic compounds were absent from the control sample and that their content was significantly higher in the OU compared to the macerated sample.

The olive oil enriched with natural antioxidant extracted from *Artemisia absinthium* leaves can be considered a functional food. In fact, its consumption could reduce the risk of chronic diseases such as cardiovascular diseases, diabetes and hypertension due to its high polyphenol content (Boulares *et al.,* 2022).

3.3.7. Effect on variation in antioxidant activity

The obtained results on all analyzed samples showed a significant decrease ($p < 0.05$) in the antioxidant activity over the 12 months of storage. The initial inhibition percentage (IP %) decrease to reach values of 1.32 ± 0.03, 16.48 ± 0.04 and 8.09 ± 0.01%, respectively, for the control, OU and OM at the end of storage. These findings can be assigned to the auto-oxidation of natural antioxidants such as pigments, sterols and polyphenols, which lead to

their degradation and a decrease in anti-radical activity (Antoniewska *et al.,* 2018). Furthermore, the antioxidant activities of enriched olive oil samples were higher ($p < 0.05$) than that of the control after 12 months of storage. The highest radical scavenging activity of the enriched samples with ultrasonic extracts throughout the storage period was attributed to the mechanism of applied ultrasound-assisted extraction. In fact, cavitation, mechanical forces and thermal impact lead to the disruption of cells walls, and the reduction in particle size, which enhance mass transfer across cell membranes, resulting in higher contents of phenolic compounds and pigments (Şahin *et al.,* 2017). These results are in agreement with those of Boulares *et al.* (2022), who showed that olive oils stored in black glass boxes for 90 days and incorporated with various essential oils are characterized by a better antioxidant activity compared to control samples.

3.3.8. Effect on sensorial properties

The sensory analysis was carried out at the beginning and at the end of the storage period all the studied samples (Table 3). At the beginning of storage, all the OTCOO samples were devoid of defects. Regarding the positive attributes (fruity, bitter, pungent), a significant difference ($p < 0.05$) was observed among all samples. In fact, the sample enriched with ultrasonic extracts had a stronger fruity smell (4), bitter smell (3.5) and pungent taste (3.5), which are the most important criteria for the Tunisian consumers. These findings were in agreement with the results found by Şahin *et al.* (2017), who proved that sensorial attributes were enhanced in terms of fruity taste and green color with the addition of olive leaves. In this context, wormwood was known to contain bitter substances such as absinthin (Judžentiene, 2016). Therefore, these molecules may have caused the bitter taste in the enriched olive oils, especially the OU sample. Moreover, at the end of the storage period, the fusty defect was detected in the control, which also had the lowest fruity intensity (0.8). In addition, the macerated OTCOO was musty (0.8) and winey (2), with an unacceptable taste and odor. However, the extraction of natural antioxidants using ultrasound-assisted extraction in olive oil had no negative effect on the product, which remained devoid of defects until the end of storage. These findings showed that the enrichment of olive oil using ultrasonic extracts improved the sensory characteristics of this product and inhibited the musty and fusty defects in olive oil. This finding confirmed that the ultrasound-assisted extraction method is a good alternative to conventional methods.

4. CONCLUSIONS

The present data proved that the enrichment of OTCOO with *Artemisia absinthium* leaf extracts was a preventive tool which gave better oxidative stability to the olive oil by reducing its PV, free acidity and the primary metabolite of oxidation compared to the control. Moreover, the enrichment of OTCOO with natural antioxidants using ultrasound-assisted extraction was shown to be the most efficient technique compared to conventional methods such as maceration by significantly improving the oxidative

Table 3: Variation in sensory properties of control and enriched organic extra-virgin olive oils at the beginning and end of the storage period

		Musty	Winey	Wet wood	Metallic taste	Rancid taste	Fruity taste	Pungent taste	Bitter taste
OC	M_0	n.d	n.d	n.d	n.d	n.d	3.00 ± 0.30^{aB}	3.10 ± 0.20^{aB}	3.0 ± 0.20^{aB}
	M_{12}	1.00 ± 0.10^{bA}	1.50 ± 0.13^{cA}	n.d	n.d	2.02 ± 0.25^{dA}	0.81 ± 0.10^{aA}	0.80 ± 0.11^{aA}	1.5 ± 0.13^{cA}
OM	M_0	n.d	n.d	n.d	n.d	n.d	3.25 ± 0.40^{aB}	3.04 ± 0.20^{aB}	3.2 ± 0.15^{aB}
	M_{12}	0.40 ± 0.13^{aA}	2.02 ± 0.11^{aA}	n.d	n.d	1.50 ± 0.13^{bA}	1.51 ± 0.12^{bA}	1.50 ± 0.21^{bA}	2.2 ± 0.10^{cA}
OU	M_0	n.d	n.d	n.d	n.d	n.d	4.01 ± 0.50^{bB}	3.50 ± 0.00^{aB}	3.5 ± 0.10^{aB}
	M_{12}	n.d	0.50 ± 0.10^{aA}	n.d	n.d	0.51 ± 0.10^{aA}	2.50 ± 0.10^{dA}	2.10 ± 0.10^{bA}	2.2 ± 0.10^{cA}

OC: control olive oil; **OM:** enriched olive oil using maceration; **OU:** enriched olive oil using ultrasound-assisted extraction. The values are expressed as means ± standard deviation, n=3 using Duncan's multiple range test. Mean values with different lowercase letters indicate significant differences ($p < 0.05$) between samples. Mean values with different uppercase letters indicate significant differences ($p < 0.05$) during storage period.

stability of olive oil. This treatment also showed a number of particular advantages, such as increasing oleic acid content, pigments and biophenols and improving antioxidant activity compared to control and macerated oil. Furthermore, the sensorial analysis showed an improvement in taste and odor of the olive oil enriched with ultrasonic extracts. These findings encourage the application of *Artemisia absinthium* leaves as an alternative source of natural antioxidants, especially using ultrasound-assisted extraction to accelerate mass transfer with shorter processing times. All of these advantages will contribute to decreasing operating costs and environmental issues and lead to the production of olive oil with good nutritional value.

Conflict of interest:

All the authors declare that there is no conflict of interest in this work.

Acknowledgements

This study was sponsored by the «Tunisian Ministry of Higher Education and Scientific Research» and the «Tunisian Institution for Agricultural Research and Higher Education ». This work was performed in the Research Laboratory: "Technological Innovation and Food Safety LR22-AGR 01-ESIAT", in the Higher Institute of Food Industries of Tunisia.

REFERENCES

Achat S, Tomao V, Madani K, Chibane M, Elmaataoui M, Dangles O, Chemat F. 2012. Direct enrichment of olive oil in oleuropein by ultrasound-assisted maceration at laboratory and pilot plant scale. *Ultrasonics Sonochem.* **19**, 777–786. https://doi.org/10.1016/j.ultsonch.2011.12.006.

Allane T, Benamara S. 2019. Determination of reducing power of 56 Algerian plant products using olive (Olea europaea) oil as extraction solvent. *Chem. Eng. Communic.* **206**, 12–21. https://doi.org/10.1080/00986445.2018.1469016.

Ammar I, BenAmira A, Khemakem I, Attia H, Ennouri M. 2017. Effect of Opuntia ficus-indica flowers maceration on quality and on heat stability of olive oil. *J. Food Sci. Technol*. **54**, 1502–1510. https://doi.org/10.1007/s13197-017-2581-0.

Antoniewska A, Rutkowska J, Pineda MM, Adamska A. 2018. Antioxidative, nutritional and sensory properties of muffins with buckwheat flakes and amaranth flour blend partially substituting for wheat flour. *LWT* **89**, 217–223. https://doi.org/10.1016/j.lwt.2017.10.039.

AOAC. 2000. *Official methods and Recommended Practices of the American Oil Chemists' Society* (5th ed.). USA, AOCS Press, Champaign, Illinois

Arfaoui M, Boulares M, Bezzezi A, Ayachi S, Ghrab M, Jouin NE, Hassouna M, Boudiche S. 2021. Effect of the enrichment with natural antioxidants obtained by maceration or ultrasound-assisted extraction from olive leaves on organic extra virgin olive oil. *Riv. Ital. Sostanze Grasse* **99**, 121-129

Boudiche S, Bornaz S, Kachouri F. 2003. La competitivite du secteur de l ' huile d ' olive en Tunisie: prix, qualite et avantage concurrentiel national, 6–14.

Boulares M, Bezzezi A, Arfaoui M, Boulares A, Ghrab M, Ben-Moussa O, Hassouna M, Boudiche S. 2022a. Improvement of Tunisian 'Chemlali' extra virgin olive oil stability with rosemary and laurel herbs and essential oils. *Riv. Ital. Sostanze Grasse* **99** (2), 131–140.

Chemat F, Rombaut N, Sicaire AG, Meullemiestre A, Fabiano-Tixier AS, Abert-Vian M. 2017. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrason. Sonochem.* **34**, 540–560. https://doi.org/10.1016/j.ultsonch.2016.06.035.

Dahal P, Bista G, Dahal P. 2021. Phytochemical screening, antioxidant, antibacterial, and antidandruff activities of leaf extract of Artemisia indica. *J. Rep. Pharmac. Sci*. **10**, 231–239. https://doi.org/10.4103/jrptps.JRPTPS_110_20.

IOC. 2009. International Olive Council. Determination of biophenols in olive oils by HPLC. COI/T.20, **29**, 1–8.

IOC. 2018. International Olive Council. Olive oil sensory analysis method: organoleptic evaluation of virgin olive oil, T.20/Doc, 270–278. https://doi.org/10.18188/sap.v16i3.15154.

IOC. 2019. International Olive Council. *Trade standard applying to olive oils and olive pomace oils*, **17**.

Iqbal S, Younas U, Chan K W, Zia-Ul-Haq M, Ismail M. 2012. Chemical composition of artemisia annua L. leaves and antioxidant potential of extracts as a function of extraction solvents. *Molecules*,

17, 6020–6032. https://doi.org/10.3390/molecules17056020.

ISO 5509. 2000. Animal and vegetable fats and oils, ISO method 5509:2000, geneva, Switzerland

Jaber H, Ayadi M, Makni J, Rigane G, Sayadi S, Bouaziz M. 2012. Stabilization of refined olive oil by enrichment with chlorophyll pigments extracted from Chemlali olive leaves. *Eur. J. Lipid Sci. Technol.* **114**, 1274–1283. https://doi.org/10.1002/ejlt.201100176.

Judžentiene A. 2016. Wormwood (Artemisia absinthium L.) oils. *Essential Oils in Food Preservation, Flavor and Safety*, Ed: Victor R. Preedy. pg. 849–856. https://doi.org/10.1016/B978-0-12-416641-7.00097-3

Malheiro R, Casal S, Teixeira H, Bento A, Pereira J A. 2013. Effect of Olive Leaves Addition during the Extraction Process of Overmature Fruits on Olive Oil Quality. *Food Bioproc. Technol.* **6**, 509–521. https://doi.org/10.1007/s11947-011-0719-z.

Nie C, Trimpert J, Moon S, Haag R, Gilmore K, Kaufer B B, Seeberger P H. 2021. In vitro efficacy of Artemisia extracts against SARS-CoV-2. *Virology J.* **18**, 1–7. https://doi.org/10.1186/s12985-021-01651-8.

Riahi L, Ghazghazi H, Ayari B, Aouadhi C, Klay I, Chograni H, Cherif A, Zoghlami N. 2015. Effect of environmental conditions on chemical polymorphism and biological activities among Artemisia absinthium L. essential oil provenances grown in Tunisia. *Indus. Crops Prod.* **66**, 96–102. https://doi.org/10.1016/j.indcrop.2014.12.036.

Şahin S, Sayım E, Bilgin M. 2017. Effect of olive leaf extract rich in oleuropein on the quality of virgin olive oil. *J. Food Sci. Technol.* **54**, 1721–1728. https://doi.org/10.1007/s13197-017-2607-7.

Sanmartin C, Venturi F, Sgherri C, Nari A, Macaluso M, Flamini G, Quartacci M F, Taglieri I, Andrich G, Zinnai A. 2018. The effects of packaging and storage temperature on the shelf-life of extra virgin olive oil. *Heliyon* **4**, e00888. https://doi.org/10.1016/j.heliyon.2018.e00888.

Sousa A, Casal S, Malheiro R, Lamas H, Bento A, Pereira J A. 2015. Aromatized olive oils: Influence of flavouring in quality, composition, stability, antioxidants, and antiradical potential. *LWT* **60**, 22–28. https://doi.org/10.1016/j.lwt.2014.08.026.

Toma M, Vinatoru M, Paniwnyk L, Mason T J. 2001. Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. *Ultrasonics Sonochem.* **8**, 137–142. https://doi.org/10.1016/S1350-4177(00)00033-X.