Future Foods 10 (2024) 100430

Contents lists available at ScienceDirect

Future Foods

journal homepage: www.elsevier.com/locate/fufo

Synergistic potential of co-millinged edible argan oil (*Argania spinosa* L.) and oregano leaves (*Origanum vulgare* L.) in food applications

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ARTICLE INFO

Keywords:
Co-processing
Enrichment
Medicinal and aromatic plants
Oxidative stability
Phenolics

ABSTRACT

By including oregano (*Origanum vulgare* L.) leaves (OL) during the extraction process, the nutraceutical benefits of argan oil (AO) can be significantly increased. Thanks to the employed technique (enrichment through extraction), without resorting to organic solvents. The current work aimed at developing of a green enrichment of argan oil with 2 and 5 % of OL. The purpose of this research was to evaluate the effectiveness of adding OL and its impact on AO oxidative stability under accelerated oxidation for four months. AO physicochemical characterization consisted in measuring quality indices, total phenolic contents, antioxidant activity, pigments, and sensory analysis throughout the storage period. In general, AO samples containing OL exhibited increased phenolic, chlorophylls, and carotenoid contents and lower peroxide and *p*-anisidine values. AO shelf-life was extended thanks to OL, which slowed down the process of lipid complete oxidation. From a sensory standpoint, a rancid taste was found in the unenriched AO, while the taste and color of oregano were more intense in the AOs supplemented with OL at 5 %. This innovative approach boost AO value in various industries and exemplifies the endless possibilities that emerge when we explore the synergy between different natural elements.

1. Introduction

Consuming natural foods in the future is very important safeguard human health and the well-being of the environment. Natural foods, devoid of synthetic additives and chemical treatments, have a higher nutritional value, which helps to improve general health. By adopting natural foods, priority is given to sustainable farming practices, thereby reducing the ecological footprint associated with food production. Natural foods also promote biodiversity by preserving the genetic diversity of plant and animal species, which is essential for the resilience of ecosystems. Adopting natural foods is part of a holistic approach to well-being, promoting not only individual health, but also the health of our planet for generations to come (Román et al., 2017). Argan oil (AO) is composed of 99 % of glycerides, including 95 % of triacylglycerols, while the remaining 4 % are monoacylglycerols, diacylglycerols, phospholipids, and other compounds (Mechqoq et al., 2021). These glycerides are constituted of fatty acids, which are represented by 80 % of unsaturated fatty acids of which linoleic and oleic acids constitute about 29–37 g/100 g and 43–49 g/100 g, respectively (Gharby and Charrouf, 2022).

The Moroccan AO sector is expected to reach more than 507 million dollars in revenues by 2027, producing thousands of tons of oil at an annual growth rate of 10.8 % (Zeghlouli et al., 2022). Many scientific research studies dedicated to the development of argan oil for more than 30 years have revealed several valuable nutritional characteristics of this product in human health (Charrouf and Guillaume, 2018).

Among the therapeutic effects, AO is known for its anti-inflammatory, antidiabetic, immune system enhancing, cytoprotective and anticarcinogenic properties (El Abbassi et al., 2014). Furthermore, medicinal and aromatic plants (MAPs) are a significant source of nutrients and natural medicines. Moreover, some MAPs are developed into industrial crops that are grown all over the world for their therapeutic and dietary benefits (Ait Bouzid et al., 2023). In particular, oregano (*Origanum vulgare* L.) an herbaceous Mediterranean plant of the Lamiaceae family (Soltani et al., 2021). The long-standing application of *Origanum vulgare* in conventional medicine spurs increased interest in

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https://doi.org/10.1016/j.fufo.2024.100430
Received 4 March 2024; Received in revised form 20 June 2024; Accepted 8 August 2024
Available online 18 September 2024
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developing novel pharmacological formulations in numerous fields, thanks to its main bioactive constituents; the phenols thymol and carvacrol as well as the phenolic acids rosmarinic acid and origanoside, which have demonstrated to be potent antioxidant, anti-inflammatory, and cardioprotective agents (Morshedloo et al., 2018; Oniga et al., 2018). Thus, the purpose of this research is to replace synthetic anti-oxidants, used to treat the phenomenon of oxidation of vegetable oils, due to their unfavorable effects on health, with oregano in the enrichment of argan oil (using a green approach, obviously) (Fadda et al., 2022). Enriching AO with oregano is a way to combine the beneficial properties of both matrixes, creating a unique product that may offer supplementary health benefits and culinary uses. Also, the addition of oregano to argan oil can potentially have a number of positive effects on the economy, including product diversification, marketability, and value-added product creation.

As far as we know, there are no studies on the enrichment during the extraction of AO with oregano (*Origanum vulgare* L.) in the literature. This method is quicker and simpler to use than maceration. Additionally, this aromatization method is eco-friendly procedure where no uses of organic solvents.

2. Material and methods

2.1. Samples

Both *Argania spinosa* L. kernels and *Origanum vulgare* L. leaves were employed as a plant material. The argan fruit was harvested in the Tighanimine area of Agadir, Morocco (35° 12′ 40″ north, 6° 18′ 10″ west). Next, using the technique adopted by the women's cooperative (Tighanimine cooperative), argan kernels were obtained. The cleaned oregano leaves (OL) are from the Targanine cooperative in Agadir, Morocco.

2.2. Enrichment procedure

This enrichment method is also called; co-processing, co-extraction or enrichment during extraction method (Gharby et al., 2022). To produce AO enriched with oregano using a mechanical extractor where no heat or chemicals, start by ensuring the argan kernels and oregano leaves are both clean and dry. Measure the oregano leaves at 2 % (or 5 %) of the total weight of the argan kernels. Carefully mix the oregano leaves with the argan kernels to ensure even distribution. Feed this mixture into the mechanical extractor. The mechanical extractor will press the kernels and extract the oil, which will be infused with the aroma and beneficial compounds of the oregano leaves. After extraction-enrichment, filter the using a suitable filtration device.

2.3. Accelerated ageing study

In an oven de type Binder (BINDER GmbH, Bergstr 14, D-78532 Tuttlingen, Germany), enriched (EAOs) and unenriched oils (UAO) were kept in 30 mL dark glass bottles for an accelerated ageing of four months (120 days). Once a month, quality indicators were measured. Free fatty acids (FFA), peroxide value (PV), *p*-anisidine value (*p*-AV), Totox index (Totox), and chlorophyll and carotenoid pigments are some of the parameters that were measured, while fatty acids profiling was evaluated at the commencement (Day 0) and the end (120 days) of the four-month storage period.

2.4. Oil yield (OY)

After crushing the argan kernels in the presence of OL using a blender, the extraction of the oil was accomplished. A 20 g quantity of ground mixture (Argan kernels-OL) was extracted for 6 h utilizing a Soxhlet instrument and the extraction solvent n-hexane (200 mL). The solvent was evaporated under reduced pressure in a rotavapor. The oil was purged with nitrogen and stored at 4 °C until further analysis and extraction yield (oil) was calculated gravimetrically (Bijla et al., 2022) based on the Eq. (1):

$$\textbf{Oil Yield } (\textbf{OY},\ \%) = \frac{\textbf{Weight of extraction oil}}{\textbf{Weight of total oil}} \times\ 100 \quad (1)$$

2.5. Total phenolic compounds (Simple phenolic compounds (SPC) and total flavonoid content (TFC)) and antioxidant activities (AA)

2.5.1. Preparation of oils polar extracts

Basically, Hexane and methanol:water (60:40, v/v) in the ratio of 5.0 mL each were added to a centrifuge tube containing 2.5 g of AO. After vortexing this mixture for 2 min, the tubes were centrifuged at 5000 rpm for five minutes. The polar phase (at the bottom) was removed before being subjected to analyses of its total phenolic content and its antioxidant capacity (Ballus et al., 2015b).

2.5.2. Simple phenolic compounds (SPC)

Simple phenolic content (SPC) was forecast using the Folin-Ciocalteu technique. In this procedure, 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10 in ultrapure water) was combined with 0.5 mL of polar extract from oil samples, and the mixture was left in the dark for 5 min. Following the addition of 2 mL of 7.5 % sodium carbonate, the tubes underwent a 2-hour incubation period in the dark. The absorbance was then measured at 760 nm. A quantitative analysis of a gallic acid analytical curve produced data that were represented as mg of gallic acid equivalents per kg of oil (mg GAE g oil) (Ballus et al., 2015).

2.5.3. Total flavonoid content (TFC)

The colorimetric method was used to calculate the samples' total flavonoid content (TFC). 1 mL of the oil polar solvent and 0.3 mL of NaNO2 (0.5 M) were combined in a 10 mL test flask and let to stand for 5 min at room temperature. The mixture was then included after that to 0.3 mL of 10 % AlCl3. (The mixture was poured into 1 mL of NaOH (2 M) and filled to the mark with distilled water after 6 min. Using a SCILOGEX (SP-UV1100, USA) spectrophotometer, the samples' absorbance was determined at 415 nm. TFC was expressed as g of oil equivalent to quercetin (mg QE/g oil) (Park et al., 2019).

2.5.4. Antioxidant activities (AA)

2.5.4.1. Radical scavenging activity by DPPH• assay. This test consists in putting the DPPH• (2, 2-diphenyl picryl hydrazine) radical (purple color), in the presence of molecules called "antioxidants" in order to measure their capacity to neutralize this radical. The neutralized form (yellow color) does not absorb anymore, which is translated by a decrease of the absorbance at this wavelength. In this test, the antioxidants reduce the purple DPPH• to a yellow compound, DPPH•, their color saturation is inversely related to their capacity antioxidants present in the medium (Aljaiyash et al., 2018). The absorbance values were measured at 517 nm in spectrophotometry (SCILOGEX SP- UV1100, USA) and translated into a percentage of antioxidant activity (AA, %) according to the Eq. (2):

$$\text{DPPH} \bullet\ (\%) = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times\ 100 \quad (2)$$

Where, $\textbf{Abs}_{\textbf{blank}}$ is the is the solvent's absorbance, $\textbf{Abs}_{\textbf{control}}$ is the DPPH• solution's absorbance diluted to 3.5 mL without extract, and Abs sample is the sample's absorbance at a specified concentration.

2.5.4.2. Ferric-reducing antioxidant power (FRAP). The total antioxidant potential of oil samples was also determined using a ferric reduction capacity test (FRAP) as a measure of "antioxidant power" of (Chatterjee et al., 2013; Pérez-Jiménez et al., 2008) with some modifications.

The test sample (the extracted polar portion) or the blank (solvent) are combined with distilled water and the FRAP reagent, which is composed of TPTZ (2, 4, 6-tripyridyl-s-triazine), $FeCl_3$, and acetate buffer. After 30 min, maximum absorbance values at 595 nm are measured at 37 °C using a SCILOGEX SP-UV1100 spectrometer. For calibration, solutions with known trolox concentrations are employed. Results were expressed as mmol Trolox equivalents (TE) per 100 g of oil (Marfil et al., 2011).

2.6. Quality indexes

The quality indices were assessed following the analytical methods described by the International Standard Organization (ISO 660, 2020; ISO 3960, 2017; ISO 6885, 2006).

Free fatty acids (FFA) were given as oleic acid as a percentage by mass of the oil. FFA, was evaluated by the titration of a mixture of oil sample (10 g) and ethanol (80 mL) with a potassium hydroxide at 0.1 N. Peroxide value (PV) was expressed as milliequivalents of active oxygen per a kilogram of oil (mEq O_2 kg^{-1})). It was measured on a sample of five grams of AO, which was dissolved into 60 mL of isooctane–acetic acid (3:2, v/v). The mixture was mixed manually, without air intake, for exactly 60 sec, with 0.5 mL of saturated solution of potassium iodide. Iodine released by the peroxides was then titrated using a standardized solution of sodium thiosulphate solution ($Na_2S_2O_3$) at 0.01 N using starch as an indicator.

The evaluation of secondary oxidation products in oil is known as *p*-AV. 2 g of oil was dissolved into 25 mL of isooctane and the absorbance was measured at 350 nm with isooctane as a blank. 5 mL of the solution was taken and mixed with 1 mL of *p*-AV reagent (0.25 % (w/v) glacial acetic acid). After 10 min of reaction, the absorbance was measured again at 350 nm, with the isooctane/anisidine reagent mixture (5:1, v/v) as a blank. Absorbance was measured using a dual beam UV–Vis spectrophotometer (SCILOGEX (SP-UV1100, USA) (Tavakoli et al., 2019). The *p*-AV is determined according to the formula (3):

$$p-\mathbf{AV} = \mathbf{25}(\mathbf{1.2As}-\mathbf{Ab})/\mathbf{W} \quad (3)$$

Where, *p*-AV: *p*-Anisidine value. A_s: absorbance of oil solution after reaction with *p*-anisidine. A_b: absorbance of the unreacted test solution. 25 is the volume of isooctane used to dissolve the oil and 1.2 is a correction factor for dilution of sample solution with 1 mL of *p*-anisidine solution. W is the weight of sample. The total oxidation (Totox) value can be calculated as the sum of twice the peroxide value and the anisidine value. using the formula (4):

$$\mathbf{Totox} = \mathbf{2PV} + p - \mathbf{AV} \quad (4)$$

Where, *p*-AV stands for *p*-anisidine value and PV for peroxide value (Tavakoli et al., 2019).

2.7. Pigments content

For the measurement of chlorophyll and carotenoid pigments, Each sample weighed precisely 7.5 g, and then a final volume of 25 mL of cyclohexane was added to it, then the value was determined using the absorbance (A) at 670 and 470 nm, respectively (Gagour et al., 2022) as indicated in (4) and (5):

$$\mathbf{Chlorophylls}\left(\frac{\mathbf{mg}}{\mathbf{100g}}\right) = \frac{\boldsymbol{A}670\times 10^6}{613\times 100\times \boldsymbol{L}} \quad (4a)$$

$$\mathbf{Carotenoids}\left(\frac{\mathbf{mg}}{\mathbf{100g}}\right) = \frac{\boldsymbol{A}470\times 10^6}{2000\times 100\times \boldsymbol{L}} \quad (5)$$

Where A is the absorbance measured and L is the thickness of the spectrophotometer cell (10 mm).

2.8. Fatty acids composition

The fatty acids were processed into the respective fatty acid methyl esters (FAME) by transmethylation according to the International Standards Organization (ISO) method (ISO 12966-2, 2017). The methodology used hydrolyzing esterified fatty acids to fatty acid methyl esters with methanolic sodium hydroxide (NaOH / MeOH, 2 M). Gas chromatography (Agilent-6890, USA) coupled to a flame ionization detector (GC-FID) was used to determine the composition of fatty acids. CP-Wax 52CB capillary column (30 m 250 µm i.d., 0.25 µm film thickness) was employed. 1 mL/min of helium was employed as a carrier gas in this experiment. The oven, injector, and detector had respective temperatures of 200, 200, and 230 °C. The samples were injected in a split mode at a volume of 1 µL with a split ratio of 1:50. The proportion of each fatty acid's surface area peak was used to express the results.

2.9. Sensory evaluation

Sensory evaluation was done based on the protocol of Benkhoud et al. (2022). A panel of 10 trained evaluators, including an expert professor from the team of biotechnology, analytical sciences and quality control, all regular consumers of argan oil, aged 20 to 50 years and nonsmokers, conducted the sensory evaluation of three samples of argan oil not enriched and enriched with 2 and 5 % of OL. The samples were distributed in amber glasses bearing a numerical code and at the temperature of the tasting room (22 ± 2 °C). The parameters evaluated are: The rancidity of the oil often created by the long storage time, the intensity of color, flavor and odor created by the addition of oregano on a scale of 4 points, 0 being the absent, 1 the least intense and 4 the strongest (Benkhoud et al., 2022).

2.10. Statistical analysis

For each sample, analyses were typically performed three times (n = 3). Standard deviations and mean values were calculated (Mean ± SD). The one-way analysis of variance (ANOVA) followed by Duncan test at $p < 0.05$ test was run using R i389 4.1.2 software and the graphs were created using OriginPro 2023b software.

3. Results and discussion

3.1. Oil yield (OY)

Argania spinosa kernels are well recognized for their high oil content, which may exceed 50 % (Harhar et al., 2010; Oubannin et al., 2022). Table 1 illustrates the oil yield of the examined samples. The UAO presented an oil yield of 51.56 ± 1.81 **%**, the EAO with 2 % of oregano recorded the highest oil yield 58.44 ± 0.77 **%**, while, the EAO (5 %) achieved only 50.55 ± 0.02 **%** which is slightly lower than the oil yield of UAO. This rise in EAO oil yield with 2 % of oregano can be explained by the oregano oil content that was also extracted and added to argan kernels. On the other hand, this drop in the EAO with 5 % of oregano can be attributed to the absorption/adsorption process (Squeo et al., 2020). Similar results have been reported by other studies such as the one of

Table 1
SPC, TFC, DPPH•, FRAP and oil yield of enriched argan oil with 2 % EAO (2 %) and EAO (5 %) of oregano and unenriched argan oil (UAO). *At $p < 0.05$, values in each line that are separated by the same letter are not statistically different.*

Parameters	UAO	EAO (2 %)	EAO (5 %)
SPC (mg GAE/kg)	$70.05^{a} \pm 9.1$	$169.5^{b} \pm 10.6$	$269.9^{c} \pm 18.1$
TFC (mg QE/g oil)	$0.07^{a} \pm 0.02$	$0.14^{b} \pm 0.06$	$0.19^{c} \pm 0.03$
DPPH• (%)	$0.07^{a} \pm 0.01$	$0.16^{b} \pm 0.08$	$0.27^{c} \pm 0.03$
FRAP (mmol Trolox/kg)	$0.94^{a} \pm 0.06$	$1.49^{ab} \pm 0.09$	$1.99^{bc} \pm 0.10$
Oil yield (%)	$51.56^{a} \pm 1.81$	$58.44^{b} \pm 0.77$	$50.55^{a} \pm 0.02$

Peres et al. (2021) where the authors demonstrated that adding *Thymus mastichina* to oil had a considerable negative impact on oil yield. This was explained by the adsorption of the oil to the lignocellulosic material of the thyme during the mixing operation, hindering the mechanical extraction of the oil (Peres et al., 2021).

3.2. Total phenolic compounds (Simple phenolic compounds (SPC) and Total Flavonoid Content (TFC)) and Antioxidant Activities (AA)

Bioactive substances like phenols and flavonoids are a broad category of secondary metabolites that are widely distributed in the kingdom of seeds, phenolic compounds are of great interest due to their antioxidant and anti-inflammatory properties (Sharaf Eldin et al., 2018). As clearly shown in Table 1, differences between unenriched and enriched oils were statistically significant, the simple phenolic content (SPC) rises gradually with increasing OL concentration by recording the following values; 70.05 ± 9.11, 169.50 ± 10.60 and 269.90 ± 18.10 mg GAE/kg for the UAO, EAO (2 %) and EAO (5 %) respectively. The SPC of argan oil found in this study is much higher than that found by Marfil et al. (2011) (0.697 mg GAE)/kg oil) (Marfil et al., 2011). A similar trend to that of SPC is reported when evaluating the total flavonoids content (TFC) of the oils since UAO did not exceed 0.07 ± 0.02 while EAO (5 %) reached 0.19 ± 0.03 mg QE/g. The phenols content of argan oil is generally considered to be low (Charrouf and Guillaume, 2007). So, this improvement is obviously linked to the addition of oregano. Moreover, when assessing the SPC of oregano extracts, Ilioara et al. (2018) found a value of 94.69 mg GAE/g DM, and Ait Bouzid et al. (2023) presented an amount of 93.41 mg QE/g DM in the TFC (Ait Bouzid et al., 2023). Flavonoids and phenolic acids are among the most abundant and well-studied phytochemical compounds in oregano species, indeed this is what confers to this plant its potential health benefits, namely potential antioxidant, anti-inflammatory and cancer-fighting benefits (Gutiérrez-Grijalva et al., 2018).

Using both methods (DPPH• and FRAP) to evaluate antioxidant activity, it was revealed that the enrichment of argan oil with oregano is capable of increasing antioxidant capacity. The antioxidant capacity values for the UAO using DPPH• method was 0.08 ± 0.01 %. While, for the EAOs values from 0.16 ± 0.08 to 0.27 ± 0.03 % were found for EAO (2 %) and EAO (5 %) respectively.

Also, with the ferric reducing ability of plasma (FRAP) method the peak antioxidant capacity is recorded by EAO 5 % 1.99 ± 0.10 mmol Trolox/kg. It is interesting to note that enrichment has greatly enhanced the antioxidant activity of argan oil, since oxidation is the main challenge for the oil industry. This could be attributed to its high phenolic and flavonoid content present in large quantities in oregano, as mentioned above and also to the chlorophyll present in OL (Damechki et al., 2001). In fact, the AA findings were also expected since the antioxidant activity of *Origanum vulgare* is prominently demonstrated by its quantified levels, which measure approximately 34.77 ± 0.26 mg AAE/g DM through the DPPH• assay and 1012.56 ± 16.77 (μmol TE/g DM) via the FRAP assay (Ait Bouzid et al., 2023). Besides, enriched edible argan oils with oregano, boasting high total phenolic compound, present numerous advantages for foods. These compounds offer potent antioxidant properties, combating free radicals and potentially reducing

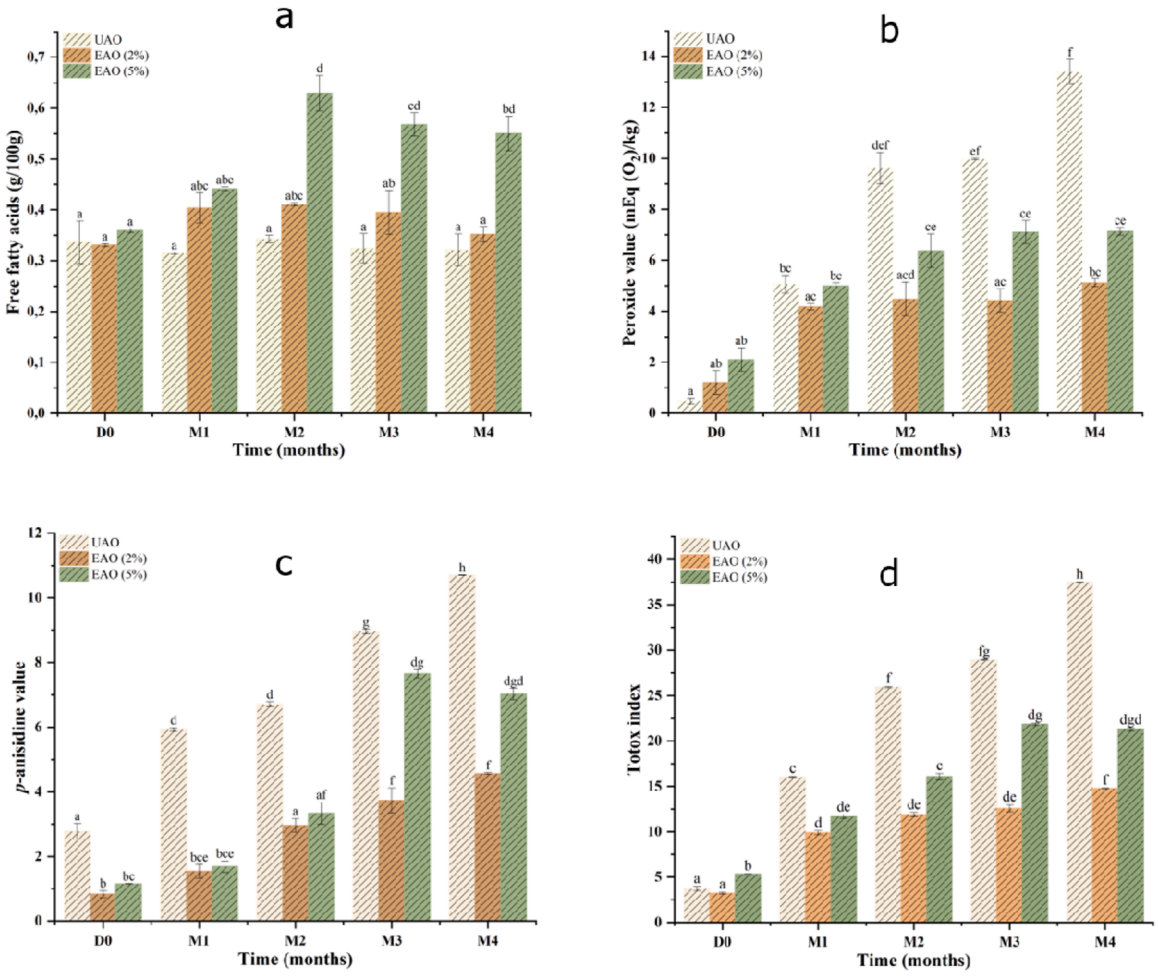

Fig. 1. Quality indexes: (a) FFA, (b) PV, (c) *p*-AV and (d) Totox of enriched argan oil with 2 % EAO (2 %) and EAO (5 %) of oregano and unenriched argan oil (UAO). *Bars with the same letter are not significantly different at* $p < 0.05$.

the risk of oxidative stress-related diseases while extending the shelf life of food products through inhibition of lipid oxidation.

3.3. Quality indexes

Free fatty acids (FFA)

Free acidity is used as a sign of lipid degradation since vegetable oils are susceptible to hydrolysis and oxidation (Gagour et al., 2022). Fig. 1, (a), shows the FFA concentration in control (unenriched argan oil) and in argan oils enriched with OL at two concentrations (2 and 5 %). On the first day of enrichment, the FFA values of the control and enriched oils were clearly similar. The difference began to appear after the first month of storage, when the enriched argan samples showed higher FFA values than the control. As the four months of storage progressed, all the oils (UAO and EAOs) revealed an increase in acidity, which is obviously due to the increase in hydrolysis of triacylglycerols and decomposition of hydroperoxides (Javidipour et al., 2017). However this rise was more intense in the enriched oils, especially in EAO 5 %, this might be read as a rise in concentration or acidity of the plant juice because the increase in acidity is proportional to the increase in concentration, moreover, a high level of FFA it is not an enough sign of lipid degradation because it could be caused by either process (Mihaylova et al., 2020). A similar variation in the FFA was reported using the same enrichment method (Oubannin et al., 2023). Elsewhere, Ayadi et al. (2009) stated that adding the leaves of a number of aromatic plants (lemon, thyme, rosemary, and sage) in olive oil caused a slight elevation in FFAs (Ayadi et al., 2009). Whereas, Clodoveo et al., 2016 reported no significant differences between olive oils enriched with thyme and oregano and unenriched olive oils (Clodoveo et al., 2016).

3.3.1. Peroxide value (PV)

The peroxide value is an unstable balance between two different processes: an oxidation step which ends in the production of peroxides and a decomposition step that results in the elimination of peroxide by producing secondary oxidation products (Oubannin et al., 2022). All the considered oils showed similar trends (Fig. 1,(b)), with an increase in PV of the control eight times higher than the EAO (2 %), the UAO reached a maximum value of 13.39 + 0.48 mEq(O_2)/kg after four months of storage, whereas the enriched oils did not exceed 7.13 + 0.15 mEq (O_2)/kg even after the period storage. The protective effect of OL is proven by the reduce of PV in enriched samples during storage. In oils enriched with OL the best results were achieved with the highest concentration employed, showing a concentration-dependent effect. Antioxidant compounds that contain the added oregano, that prevent the formation of hydroperoxides could be a possible explanation for this behavior. Similar results to the addition of *Origanum vulgare* L. to olive oils have been reported by Gambacorta et al. (2007) (Gambacorta et al., 2007).

3.3.2. p-anisidine value (p-AV)

Low peroxide results are insufficient as a quality indicator because in the following step of oxidation, the hydroperoxide level drops (Gagour et al., 2022). To accurately determine the true oxidation condition of oils, it is crucial to combine the peroxide index with another assessment of the secondary oxidation products, the para-anisidine index, which measures a nonvolatile aldehyde's presence produced during the breakdown of hydroperoxides, is used to achieve this (Gharby et al., 2021). The *p*-AV of UAO (control), and EAOs with 2 and 5 % of oregano were analyzed (Fig. 1 (c)). These results of *p*-AVs obtained 4 months at 60 °C demonstrated that the formation of secondary oxidation products increases significantly ($p < 0.05$) during incubation. The less stable primary oxidation products at high temperature, which later disintegrate to generate secondary oxidation products, most notably ketones and aldehydes, can be the cause of the increase in *p*-AV in all oils with increasing heating duration. The values of *p*-AV were at day 0; 2.77 ± 0.24, 0.83 ± 0.10 and 1.15 ± 0.08 for the UAO, EAO (2 %) and EAO (5 %) respectively. Then, the p-AV of the control increased from an initial value of 2.77 ± 0.24 due to accelerated aging to a maximum level of 10.7 ± 0.89. The oil samples, which included two different concentrations of oregano (2 and 5 %), had fewer secondary products than the control (which did not surpass 8 at the end of storage). However, enrichment with 2 % of OL was more effective than 5 % in preventing the development of secondary products. These results are in agreement with those reported by Gambacorta et al. 2006 where they proved the effective effect of adding oregano in delaying the production of oxidation secondary products (Gambacorta et al., 2007).

3.3.3. Total oxidation (Totox)

The total oxidation value, often abbreviated as Totox, is a parameter used to measure the level of oxidation in oils. It is commonly applied in the context of assessing the quality and freshness of edible oils (Wai et al., 2009). Control samples (without OL) showed higher Totox values than flavored oils (with OL) (Fig. 1,(d)). After 4 months of accelerated ageing, UAO Totox index values are almost double compared to those of argan oil enriched with 5 % of OL (EAO 5 %), and 2.5 times higher than those of EAO 2 %, bringing values of 37.49 ± 0.97, 21.29 ± 0.48 and 14.78 ± 0.33 for UAO, EAO 5 % and EAO 2 % respectively. Consequently, the enrichment of AO with oregano during the extraction process appears to be beneficial in enhancing the antioxidant properties of argan oil, the antioxidant activity of oregano and its increased phenol content, both of which have been previously reported (table x), may justify this advantageous impact. Similar variation of Totox index have been reported after the evaluation of the oxidative stability of oregano (*Origanum vulgare* L.) enriched chia seed oil (Kiani Aliabadi et al., 2023). The low oxidation levels observed in enriched edible oils represent a promising advancement for food formulations. This phenomenon effectively hampers lipid oxidation, thereby preserving the freshness and overall quality of food items. Consequently, it prolongs the shelf life of these products while minimizing the reliance on synthetic preservatives. Furthermore, the retention of essential fatty acids and nutrients in these oils ensures the nutritional robustness of the food, thereby fostering general health and well-being. The heightened resistance to oxidation also endows enriched oils with aromatic and medicinal plants superior sensory attributes, characterized by flavors and aromas.

3.4. Chlorophyll and carotenoid contents

Thanks to their high antioxidant activity, the presence of pigments (chlorophylls and carotenoids) influences the oxidative stability of oils and prevents the auto-oxidation, as it can also be used to determine the quality of oil (Lapčí-ková et al., 2018). As it is clearly shown in Fig. 2,(a, b), Chlorophyll and carotenoid levels reduced in all unenriched and enriched oil samples under accelerated ageing oxidation, demonstrating that this pigment deteriorates during storage at high temperatures. As it can be seen in Fig. 2,(b), the addition of oregano during the extraction of argan oil had significantly enhanced the amount of chlorophylls. Once enriched, the UAO went from 0.125 ± 0.056 to 3.467 ± 0.010 and 9.179 ± 0.713 mg/kg for the EAOs with 2 and 5 % of oregano respectively. A comparable attitude of chlorophylls' levels has been found by Damechki et al. (2001) while enriching olive oil with oregano (Damechki et al., 2001).

Carotenoids are most likely to be involved in the scavenging of reactive oxygen species, singlet molecular oxygen, and peroxyl radicals among the numerous defensive mechanisms (Stahl and Sies, 2003). The carotenoid rate of all argan oil samples declined during the four months' storage period (Fig. 2,(a)). Also, significant variations in carotenoid content were noted between the UAO and the EAOs, notably; in the first month the UAO carotenoids' content was 0.814 ± 0.142 mg/kg while the EAO (5 %) reached a value of 3.695 ± 0.061 mg/kg, even after the accelerated aging, the enriched oil maintained the highest levels of carotenoids; 0.068 ± 0.003 mg/kg and 0.722 ± 0.010 mg/kg for the UAO

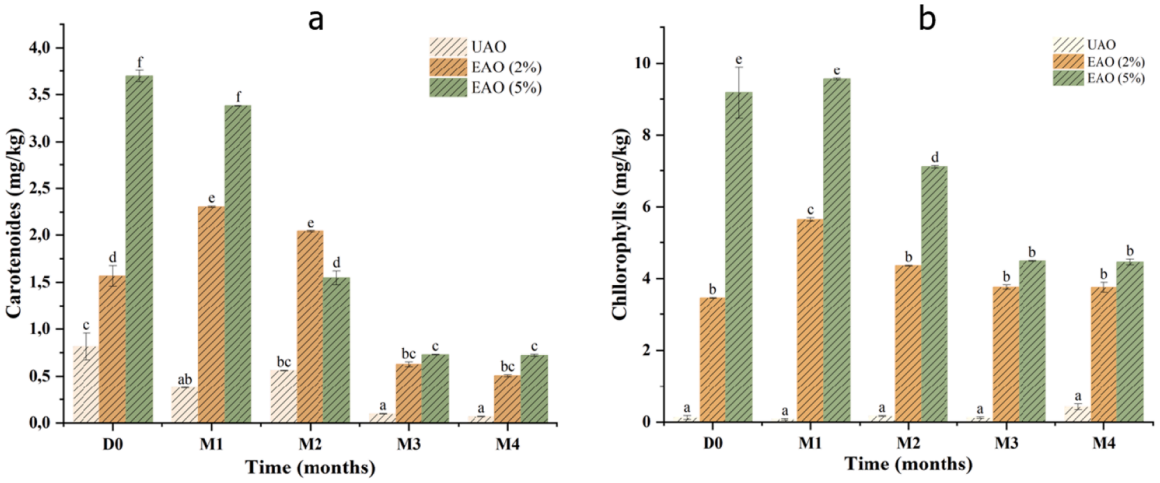

Fig. 2. Pigments: (a) carotenoids and (b) chlorophylls of enriched argan oil with 2 % EAO (2 %) and EAO (5 %) of oregano and unenriched argan oil (UAO). *Bars with the same letter are not significantly different at $p < 0.05$.*

and EAO (5 %) respectively. Which may be inferred from the pigment content of oregano (Asensio et al., 2011).

3.5. Fatty acids profiling

Vegetable oils are characterized mainly by the fatty acid levels that they contain; these fatty acid composition reflects their stability, and it is also an essential indicator of their nutritional value (Dhibi et al., 2014; Gagour et al., 2022) Since fatty acids are thought to constitute the major components (up 98 to 99 %) of vegetable oils, it is crucial to evaluate their composition (Gharby, 2022). The variation in fatty acid levels for each oil before and after the accelerated ageing is seen in Table 2. First off, it is quite noteworthy to see that the quantities of fatty acids did not go above the Moroccan standard's limitations even after four months' storage (Table 2) (SNIMA, 2003). When comparing the control (UAO) with enriched oils (EAOs), we find no significant change ($p < 0.05$) in the percentage of SFA, although there was a significant decrease in the proportion of predominant fatty acids in argan oil; the oleic acid (C18:1) for UAO, and in the linoleic acid (C18:2) for both UAO and EAO (2 %). The percentage of linoleic acid C18:2 was reduced significantly from 35.52 to 31.19, when nothing has changed for the EAO (5 %) after 120 days for the UAO and the EAO 5 % respectively. In short, enriching argan oil with OL during extraction can minimize the oxidation rate of unsaturated fatty acids, which can be credited to the phenolic compounds that are responsible for the prevention of unsaturated fatty acid oxidation. In the meantime, polyphenols have already been proven to have three different pathways via which they can influence lipid oxidation: as potent metal ion chelators, carbonyl scavengers, or free radical scavengers (Papuc et al., 2017). The change in fatty acid composition after enrichment varies from research to another, with some finding no significant difference such as Liu et al., 2021, who assessed the stability during storage of different oils enriched with carotenoids (Liu et al., 2021), and others that observed a noticeable modification as in Kiani Aliabadi et al., 2023 and Dhibi et al., 2014 studies (Dhibi et al., 2014; Kiani Aliabadi et al., 2023).

3.6. Sensory evaluation

Market product acceptability heavily depends on sensory analysis, because it enables us to assess the consumer's general preference (Issaoui et al., 2016). Fig. 3 present the results of the evaluated sensory parameters of the tree samples scale in summary form. Rancidity, and the color, odor and flavor of oregano are the five sensory parameters evaluated.

It was made clear that only the unenriched oil (UAO) and the oil enriched with 2 % OL (EAO 2 %) displayed a modest degree of rancidity in the final condition, with a score of 4/4 and 1/4 for these two oils, respectively, according to the sensory analysis of the rancidity aroma of the oils. In the AO enhanced with 2 % of TL, the flavor of OL was noticeable, and it was particularly noticeable in the EAO with 5 %. It was also noted that the aroma of the oils in the final form was less, which may have contributed to the oregano's ability to partially cover the nutty aroma of the AO. This might be a result of the volatile chemicals that give a perfume its scent degrading (Venskutonis, 1997). As anticipated, the inclusion of OR created a Flavor Enhancement, the enriched argan oil with oregano oil showed a strong, aromatic, and aromatized flavor. It imparts a pleasant and distinctive taste, making it a versatile ingredient for cooking and seasoning various dishes. Moreover, as functional food ingredients, the enriched AO have versatile applications across various food products, promising both culinary delight and potential health-promoting effects in the diet of the future.

Table 2
Fatty acids profiling of enriched argan oil with 2 % EAO (2 %) and EAO (5 %) of oregano and unenriched argan oil (UAO). *At $p < 0.05$, values in each line that are separated by the same letter are not statistically different.*

Fatty acids	UAO		EAO (2 %)		EAO (5 %)		Moroccan Institute of Standardization norm
	0 day	120 days	0 day	120 days	0 day	120 days	
C16: 0	$14.39^{a} \pm 1.10$	$13.53^{a} \pm 1.20$	$14.01^{a} \pm 1.20$	$14.48^{a} \pm 2.10$	$14.15^{a} \pm 0.10$	$14.18^{a} \pm 1.98$	**11,5 – 15,0**
C18: 0	$4.81^{ab} \pm 0.10$	$5.20^{a} \pm 0.10$	$5.16^{a} \pm 0.10$	$5.66^{a} \pm 0.10$	$4.73^{ab} \pm 0.10$	$5.15^{a} \pm 0.10$	**4,3 – 7,2**
C18: 1	$47.03^{a} \pm 2.10$	$45.09^{b} \pm 1.10$	$44.20^{b} \pm 1.10$	$44.20^{b} \pm 1.10$	$45.69^{b} \pm 2.10$	$46.27^{abc} \pm 1.10$	**43,0 – 49,1**
C18: 2	$35.52^{a} \pm 1.11$	$31.19^{bc} \pm 1.17$	$35.61^{a} \pm 2.10$	$33.62^{ac} \pm 1.12$	$34.41^{d} \pm 2.10$	$34.38^{ad} \pm 1.10$	**29,3 – 36,0**
ΣSFA*	19.20	18.73	19.17	20.14	18.88	19.33	-
ΣUSFA**	82.55	76.28	79.81	77.82	80.38	80.65	-

SFA*:Saturated fatty acids; USFA**:Unsaturated fatty acids.

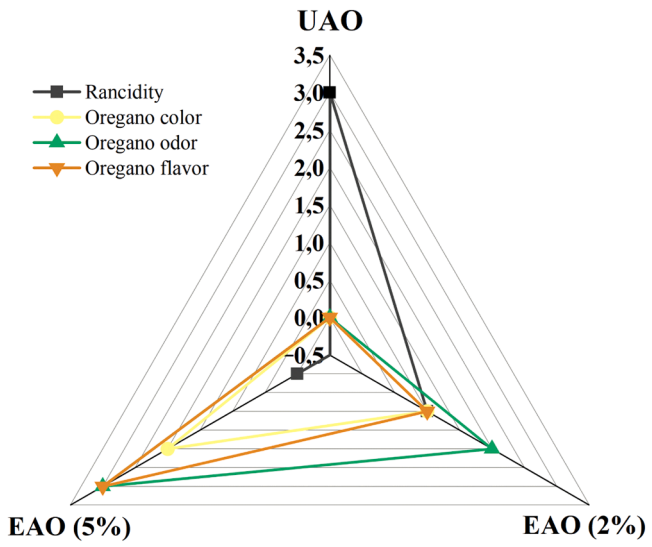

Fig. 3. Radar chart of sensory attributes of unenriched argan oil (UAO) and enriched argan oils with 2 % (EAO 2 %) and 5 % (EAO 5 %) of oregano leaves.

4. Conclusion

the integration of oregano into the argan oil extraction process represents a promising innovation in the production of natural oils. Thanks to the positive results demonstrated by oregano in enhancing oxidation stability and the potential benefits of argan oil, coupled with the advantages of the green method employed, its scalability makes it an invaluable tool for industries such as pharmaceuticals, nutraceuticals, cosmetics, and food manufacturing. This synergistic combination not only enhances the quality and potential benefits of argan oil but also exemplifies the power of harnessing nature's diversity to create unique and valuable products. As a result, such offerings cater to consumer preferences for top-tier, nutritious, and palatable food choices.

Ethical Statement

1) This material is the authors' own original work, which has not been previously published elsewhere.
2) The paper is not currently being considered for publication elsewhere.
3) The paper reflects the authors' own research and analysis in a truthful and complete manner.
4) The paper properly credits the meaningful contributions of co-authors and co-researchers.
5) The results are appropriately placed in the context of prior and existing research.
6) All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.
7) All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.
8) Animals were not involved in this study.

The violation of the Ethical Statement rules may result in severe consequences.

CRediT authorship contribution statement

Samira Oubannin: Writing – original draft, Software, Methodology, Conceptualization. **Abderrahim Asbbane:** Formal analysis, Data curation. **Otmane Hallouch:** Resources, Formal analysis. **Angelo Maria Giuffrè:** Writing – review & editing, Visualization, Software, Resources. **El Hassan Sakar:** Resources, Formal analysis. **Said Gharby:** Writing – review & editing, Validation, Supervision, Project administration.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgements

This work was performed in the frame of two projects: Project 1: Citizen Research Projects and financially supported by Cowater, AND-ZOA and REFARM. Project 2: Valorization of Medicinal and Aromatic Plants (3rd edition, VPMA3 2021/09), funded by the National Agency of Medicinal and Aromatic Plants (Taounate, Morocco) and National Center for Scientific and Technical Research (CNRST, Morocco).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2024.100430.

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