











## Research Article

# Enrichment of Argan [*Argania spinosa* (L.) Skeels] Oil with Saffron (*Crocus sativus* L.) Stigma Powder and Induced Changes in Oil Quality Attributes

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Argan oil (AO) is a globally reputed vegetable oil thanks to its nutritional and cosmetic value. AO oxidation induces changes that result in a low quality. In this research, we aimed at fortifying AO using saffron stigmas powder (SSP) and assessing its quality. Dried and grounded saffron stigmas were added to AO at three different concentrations (0.2, 0.5, and 1%). All enriched AOs were stored for six consecutive months under two conditions, namely, light and darkness. Quality indices including free fatty acids (FFA), peroxide value (PV), and specific extinction coefficients K232 and K270 as well as profiling of fatty acids, sterols, and tocopherols were monitored on a monthly basis. Our outcomes reveal that the addition of SSP improved AO oxidation parameters by significantly decreasing ( $p < 0.05$ ) FFA, PV, K232, and K270 of enriched AO (EAO) as compared to unenriched AO (UAO) especially when AO samples are stored in darkness. Such decrease of oxidation parameters was SSP dose-dependent as a general trend. However, AO enrichment did not induce significant changes ( $p < 0.05$ ) in fatty acid and sterol profiles. Furthermore, the quality parameter values of EAO and UAO did not exceed the limits set by the Moroccan standard throughout the storage period. Sensory analysis results indicate that EAO showed a low rancidity but high scores of saffron smell, flavor, and color as compared to UAO especially with SSP 1%. In conclusion, AO enrichment using SSP could be used to improve AO oxidative stability and its nutritional value.

## 1. Introduction

Edible argan oil (AO) is a cold-pressed oil. It is obtained by pressing the roasted kernels of the fruit of the argan tree [*Argania spinosa* (L.) Skeels] [1]. AO is one of the most precious commodities extracted from drupes (fruits) of argan tree [2]. It has been widely evidenced that the AO is endowed with bioactive properties having a positive impact on the health, including the chemoprotective effect on cancer [3], prevention of obesity and cardiovascular diseases [4], prevention of hypothyroidism, and antidiabetic activity [5]. AO also boosts the

immune system [6]. Besides, AO like all vegetable oils is threatened mainly by the phenomenon of oxidation due to its high percentage of unsaturated fatty acids. Oxidation is a spontaneous and unavoidable process, with direct negative effects on the commercial value of AO as well as AO based-products such as cosmetics [7]. Therefore, hindering or delaying lipid oxidation is of crucial importance. For this reason, the oil industry resorts to the incorporation of antioxidants that protect the quality and lengthen the shelf life of vegetable oils and food-based vegetable oil products [8–11]. The most commonly used synthetic antioxidants are the following:

butylhydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and propyl gallate (GP) [12, 13]. Nevertheless, safety problems associated with the use of such synthetic antioxidants and the possibility of their involvement in toxic effects in the health make the use of these compounds questionable and even restricting their use. In addition, the growing consumer demand for natural foods has encouraged the development of alternatives based on the use of natural molecules to delay the oxidation of edible oils [14]. Medicinal and aromatic plants (MAPs) are a major source of natural antioxidants [15]. In fact, previous studies have proven the powerful antioxidant properties of MAPs extracts and essential oils [16–18]. Saffron (*Crocus sativus* L.) is a triploid monocotyledonous herbaceous plant that produces the world's most precious spice [19]. Its origin is the Middle East, while other authors suggest Central Asia or the islands of south-west Greece [20]. From this center of origin, it would have spread to India, China, and Middle East countries. The Arabs spread saffron throughout the Mediterranean basin countries [21] such as Morocco, where it was most likely introduced by the 9th century [22]. Thanks to its therapeutic [20, 23–28], cosmetic [29–33], and culinary properties [19, 34, 35].

As far as we know, no detailed information on the use of saffron to enhance the oxidative stability of edible vegetable oils including AO. Hence, the originality of this paper, which had as goals (i) to assess the potentiality of saffron stigma powder (SSP) as a natural additive to protect AO against oxidation and (ii) to monitor AO quality attributes (routinely measured quality indices and fatty acid and sterol profile) after AO enrichment.

## 2. Material and Methods

**2.1. Plant Material and Experimental Design.** Argan fruits were harvested in August 2020 in Taroudant province (Morocco, 30°28'13" North, 8°52'37" West) and prepared following the methodology used by the local women's cooperative. AO was extracted using an endless press (SMIR Technotour, Agadir, Morocco). *C. sativus* used in this study was purchased from the economic interest grouping Targanine, Morocco, and stored at 4°C until further use.

Predried saffron stigmas were well cleaned (to remove dirt and impurities), grounded, and sieved to a particle size of 125 µm. The obtained powder (SSP) was then used to enrich AO using maceration. The enrichment was carried out using three concentrations of SSP (0.2, 0.5, and 1%). The mixtures (AO and SSP) were maintained in agitation during 48 h and then the samples were kept under two storage conditions (in the darkness and in the light) for a period of six months.

Basic quality indices including free fatty acids (FFA), peroxide value (PV), and UV extinction coefficients (K232 and K270) were measured only at the end of every two months over the experiment period. Other analyses like fatty acid and sterol composition were determined at the beginning and at the end of the storage period.

### 2.2. Measurement of AO Quality Attributes

**2.2.1. Basic Quality Parameters.** FFA, PV, K232, and K270 of all oil samples were determined following the International Standard Organization methods ISO 660:2020, ISO 3960:2017, and ISO 3656:2011, respectively [36–38] with slight modifications.

To determine FFA, 5 g of oil sample was introduced into a 250 mL conical flask; then, 100 mL of an alcoholic solution (ethanol/ether 1:1) was added. After that, a few drops of 1% ethanolic phenolphthalein were added as a color indicator. The obtained mixture was then titrated with a solution of potassium hydroxide (0.1 N). FFA was expressed as g/100 g of oleic acid (oleic acid percent).

For PV; to 3 g of oil sample, 50 mL of the acetic acid/isooctane mixture and 1 mL of freshly prepared saturated potassium iodide solution were added and left to react for 60 s. Then, 100 mL of water was added, and the obtained mixture was shaken vigorously and titrated using sodium thiosulphate solution (0.01 N) and 1 mL of starch solution (1 g/100 mL). PV is expressed as mEq O<sub>2</sub>/kg of oil. Concerning K232 and K270, 0.25 g of oil sample was dissolved in cyclohexane, and the specific absorbance was read at 232 nm and 270 nm using a SCILOGEX SP-UV1100 spectrometer as detailed by Oubannin et al. [39].

**2.2.2. Fatty Acid Composition.** Fatty acids were determined as their respective fatty acid methyl esters (FAME) by transmethylation according to the International Standards Organization (ISO) method [40]. Fatty acids were esterified into fatty acid methyl esters with methanolic sodium hydroxide (MeOH/NaOH, 2 N). Fatty acid composition was performed by gas chromatography (Agilent-6890) coupled to a flame ionization detector (GC-FID). Capillary column consists in CP-Wax 52CB (30 m × 250 µm i.d., 0.25 µm film thickness) was involved. Helium was used as a carrier gas with a flow rate of 1 mL/min. Temperatures of the oven, injector, and detector were 185, 200, and 230°C, respectively. Injection volume was 1 µL in a split mode (split ratio was 1:50). Results were expressed as the relative percentage of the area of each fatty acid peak.

**2.2.3. Phytosterol and Tocopherol Composition.** Phytosterol composition was determined according to ISO 12228-1:2014 [41] following trimethylsilylation of crude sterol fractions, by using a Varian 3800 instrument equipped with a VF-1 ms (30 m, 0.25 mm i.d, and 0.25 µm film thickness), and carrier gas was helium (flow rate 1.6 mL/min). The column temperature was isothermal at 270°C, while the injector and detector temperatures were 300°C. Identification was done according to retention time. Results were expressed as a relative percentage of each phytosterol peak area (g/100 g).

Contents of the total and individual tocopherols were performed as described in ISO 9936:2016 [42] method. The sample was prepared by dissolving oils in hexane and injected into a normal-phase HPLC using a mixture of hexane 99.5% and isopropanol 0.5% as an isocratic eluting system with 1.0 mL/min as a rate of flow. The injection volume

was 10  $\mu\text{L}$ . Detection was performed via a fluorescence detector ( $\lambda_{\text{ex}} = 280 \text{ nm}$  and  $\lambda_{\text{em}} = 340 \text{ nm}$ ), and quantification is obtained by means of external standardization with a mixture of single tocopherol forms preparing different calibration levels.

**2.2.4. Rancimat's Test.** Rancimat's test was used to assess AO oxidative stability by conductimetry of an aqueous solution. In fact, volatile compounds were collected after heating oil samples at 120°C. Polar compounds (formed by oil oxidation) concentration increased conductivity over time. During the induction period, the conductivity of the water increased but very slightly. Thereafter, conductivity increased sharply indicating a massive oxidation and formation of a number of volatile polar compounds soluble in water. Oil stability is given as an induction period [43, 44].

**2.2.5. Sensory Analysis.** Nine panelists were involved in this study including professors and Ph.D. students from Biotechnology, Analytical Sciences, and Quality Control Team (Polydisciplinary Faculty of Taroudant, Ibn Zohr University, Morocco). Such panelists are all argan oil regular consumers and nonsmokers, and the range age was 20-50. They performed a sensory evaluation of the all argan oil samples. Oil rancidity, saffron color, flavor, and smell intensity were rated according to a 4-point scale, 1 being the least intense and 4 being the strongest. Average sensory scores were calculated for the unenriched along with enriched argan oil's control attributes. Samples were presented into amber glasses with a number code and at tasting room temperature ( $22 \pm 2 \text{ }^\circ\text{C}$ ) [45].

**2.3. Statistical Analyses.** The results are presented as mean values  $\pm$  SD of 3 replicates ( $n = 3$ ). Significance level was set at  $p = 0.05$ . Mean values separation was carried out by the Duncan test at a probability level of 5% using R i389 4.1.2 software, and graphs were drawn by OriginPro 2022 software.

### 3. Results and Discussion

#### 3.1. Initial Parameters

**3.1.1. Quality Parameters.** AOs enriched with SSP were subjected to physicochemical characterization at the beginning of the experiment. Basic quality indices (FFA, PV, K232, and K270) and oxidative stability as well as fatty acid, sterol, and tocopherol composition were measured. Satisfactorily, all samples of fresh AO presented the physicochemical properties required to access the extra virgin grade according to the Moroccan norm SNIMA [46]. FFA expressed as a percentage of oleic acid can be used to classify AO and to assess its state of deterioration [47]. According to the Moroccan official AO norm, all the AO samples had the label of the extra-virgin oils since they did not exceed 0.33 g/100 g (Table 1). In addition, the enrichment with SSP at different concentrations decreased FFA. In fact, the initial FFA of unenriched AO (UAO) was much higher than that of enriched AO (EAO) 0.2%, EAO

0.5%, and EAO 1% and already below the 0.8 limit (Table 1). PV was used to estimate oil oxidation degree and must not exceed 15 mEq  $\text{O}_2/\text{kg}$  for extra virgin AO [46]. PV of the analyzed AOs (Table 1) was between  $2.17 \pm 0.25$  and  $2.59 \pm 0.50$  mEq  $\text{O}_2/\text{kg}$  being lower than the maximum values specified by the official regulation of AO [48].

Measurements of specific extinction coefficients can be used to estimate the presence of primary (K232) or secondary (K270) oxidation products [49]. The specific extinction K232 ( $1.33 \pm 0.02$ - $1.40 \pm 0.02$ ) and K270 ( $0.21 \pm 0.01$ - $0.24 \pm 0.01$ ) showed low values for all oils. The results of our study show that the enrichment of AO did not induce significant variations in terms of FFA, PV, K232, and K270 values. In addition, no significant variations in initial fatty acid and sterol composition were observed immediately after enrichment (Table 1). Indeed, fatty acid and sterol composition of UAO and EAO at different concentrations were similar, and their content was found to be in the range of previously published values for AO [46, 50-52]. Given their dualistic function, tocopherols are crucial compounds of AO. Due to their vitamin E activity, they provide significant nutritional benefits; on the other side, they act as effective antioxidants and, therefore, enhance oil stability [53]. In all samples,  $\gamma$ -tocopherol represented 81-92% of the total tocopherols, while  $\alpha$ -,  $\beta$ -, and  $\delta$ - constituted the minority tocopherols with low percentages (Table 1) [52]. Moreover, AO tocopherol richness is not only essential for its preservation but also for its pharmacological activity [3]. After enrichment using SSP,  $\alpha$ - and  $\gamma$ -tocopherol showed an increasing trend,  $\alpha$ -tocopherol content increased from 37.98 (UAO) to 39.85 mg/kg for EAO with 1% SSP. In contrast,  $\delta$ -tocopherol levels of AO were not affected by the addition of SSP. The  $\alpha$ -tocopherol increase could be attributed to SSP antioxidants such as tocopherols including  $\alpha$ -tocopherol (vitamin E) [54]. Generally, tocopherol content increased after the enrichment of oils with medicinal and aromatic plant (MAP) extracts [55].

**3.1.2. Rancimat's Test.** To get an initial overview of the AO oxidative stability, induction period (IP, h) was measured by Rancimat's test. The oxidative stability was expressed as IP measured at 383°K [56]. Figure 1 shows the results of the induction period of AOs enriched with different concentrations of SSP. The initial stability of all the studied oils indicated no significant variations (Figure 1S, supplementary material). In fact, UAO presented an induction time of  $12.65 \pm 0.20$  h. Oils enriched with SSP presented an induction of  $12.48 \pm 0.20$ ,  $12.20 \pm 0.50$ , and  $12.56 \pm 0.15$  for EAO 0.2%, EAO 0.5%, and EAO 1%, respectively. Regardless of SSP concentration, both UAO and EAO presented the same induction time of 12.5 h. This means that the enrichment with SSP had no significant effect ( $p < 0.05$ ) on the initial oxidative stability of AO confirming the quality indices outcomes. The lack of enrichment effect on the initial stability of EAO could be ascribed to the degradation of SSP antioxidants induced by high temperature.

TABLE 1: Mean values of initial physicochemical parameters, fatty acids, sterols, and tocopherols composition of unenriched argan oil (UAO) and enriched argan oil with 0.2% (EAO 0.2%), with 0.5% (EAO 0.5%), and with 1% of saffron stigma powder (EAO 1%).

	UAO	EAO (0.2%)	EAO (0.5%)	EAO (1%)
Free fatty acids (g/100 g)	0.33 ± 0.07 <sup>a</sup>	0.22 ± 0.07 <sup>b</sup>	0.22 ± 0.07 <sup>b</sup>	0.23 ± 0.07 <sup>b</sup>
Peroxide value (mEq O <sub>2</sub> /kg)	2.30 ± 0.50 <sup>a</sup>	2.59 ± 0.50 <sup>a</sup>	2.58 ± 0.32 <sup>a</sup>	2.17 ± 0.25 <sup>b</sup>
K232	1.37 ± 0.02 <sup>a</sup>	1.33 ± 0.02 <sup>a</sup>	1.36 ± 0.02 <sup>a</sup>	1.4 ± 0.02 <sup>a</sup>
K270	0.22 ± 0.07 <sup>a</sup>	0.22 ± 0.07 <sup>a</sup>	0.24 ± 0.01 <sup>a</sup>	0.21 ± 0.01 <sup>a</sup>
Fatty acids (g/100 g)				
SFA*	19.7 ± 0.03 <sup>b</sup>	21.31 ± 0.03 <sup>a</sup>	21.8 ± 0.03 <sup>a</sup>	20.93 ± 0.03 <sup>a</sup>
MUFA**	47.8 ± 0.0 <sup>b</sup>	48.6 ± 0.03 <sup>a</sup>	48.15 ± 0.03 <sup>a</sup>	48.76 ± 0.03 <sup>a</sup>
PUFA***	32.3 ± 0.0 <sup>a</sup>	30.09 ± 0.02 <sup>b</sup>	30.05 ± 0.02 <sup>b</sup>	30.31 ± 0.02 <sup>b</sup>
Phytosterols (g/100 g)				
Campesterol	0.30 ± 0.05 <sup>a</sup>	0.26 ± 0.05 <sup>ab</sup>	0.28 ± 0.05 <sup>a</sup>	0.24 ± 0.05 <sup>b</sup>
Spinasterol	41.71 ± 0.05 <sup>b</sup>	41.06 ± 0.05 <sup>b</sup>	42.05 ± 0.05 <sup>a</sup>	42.39 ± 0.05 <sup>a</sup>
Schottenol	48.85 ± 0.05 <sup>c</sup>	51.40 ± 0.05 <sup>a</sup>	48.72 ± 0.05 <sup>c</sup>	49.80 ± 0.08 <sup>b</sup>
Tocopherols (mg/kg)				
α-T	37.98 ± 0.47 <sup>b</sup>	38.66 ± 0.19 <sup>ab</sup>	39.24 ± 0.07 <sup>a</sup>	39.85 ± 0.37 <sup>a</sup>
γ-T	385.21 ± 0.20 <sup>c</sup>	386.76 ± 0.69 <sup>bc</sup>	387.15 ± 1.49 <sup>ab</sup>	386.20 ± 2.61 <sup>bc</sup>
δ-T	24.00 ± 0.07 <sup>c</sup>	24.78 ± 0.24 <sup>a</sup>	24.31 ± 0.33 <sup>bc</sup>	24.50 ± 0.22 <sup>ab</sup>
Total tocopherols (mg/kg)	447.05 ± 0.6 <sup>c</sup>	449.71 ± 1.12 <sup>b</sup>	449.64 ± 1.75 <sup>b</sup>	452.41 ± 3.19 <sup>a</sup>

SFA\*: saturated fatty acids; MUFA\*\*: monounsaturated fatty acids; PUFA\*\*\*: polyunsaturated fatty acids. Within each line, values followed by the same letter are not significantly different at  $p < 0.05$ .

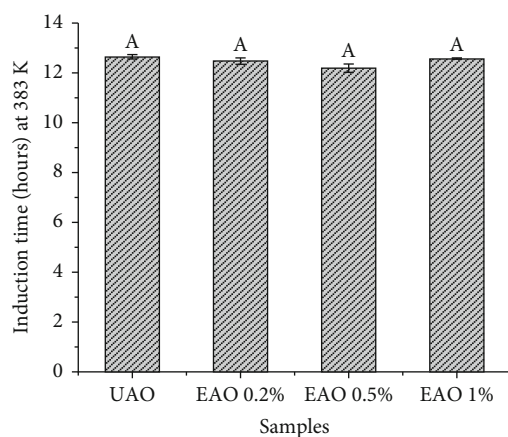


FIGURE 1: Oxidative stability (induction period) of unenriched argan oil (UAO) and enriched argan oils (EAOs). Data are mean values ( $n = 3$ ). Different letters indicate significant differences at  $p < 0.05$ .

### 3.2. Evolution of Oxidation Parameters

**3.2.1. FFA Variations.** The FFA of UAO stored under light increased slightly (about FFA 0.1% per month) during the first four months of storage to reach 0.55 g/100 g (Figure 2). Following four months of storage, FFA increased quickly, suggesting an accelerated deterioration of triacylglycerol. After six months at 25°C in light, FFA of UAO reached the 0.8 limit, losing its extra virgin label [48]. When UAO was stored in darkness, FFA value increased continuously but less rapidly than UAO in the case of storage in light. This induces triacylglyceride oxidation in argan oil. Similar trends were

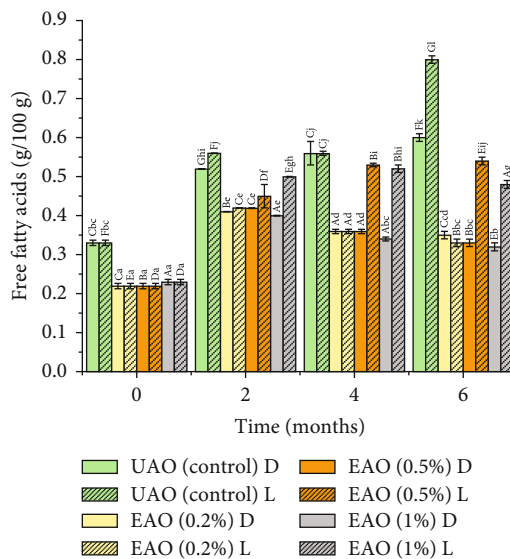


FIGURE 2: Free fatty acids of unenriched argan oil (UAO) and enriched argan oils with 0.2% (EAO 0.2%), 0.5% (EAO 0.5%), and 1% of saffron (EAO 1%) in darkness (D) and light (L). Data are mean values ( $n = 3$ ). Different letters indicate significant differences at  $p < 0.05$ .

observed by other authors for AO [46], olive oil [55], and other vegetable oils [57].

Independently of storage conditions (darkness or light), FFA of AOs increased significantly ( $p < 0.05$ ) over the six-month storage period. The oils enriched with SSP showed the lowest FFA. Indeed, after six months of storage, FFA reached 0.35 g/100 g in darkness and 0.54 g/100 g under light



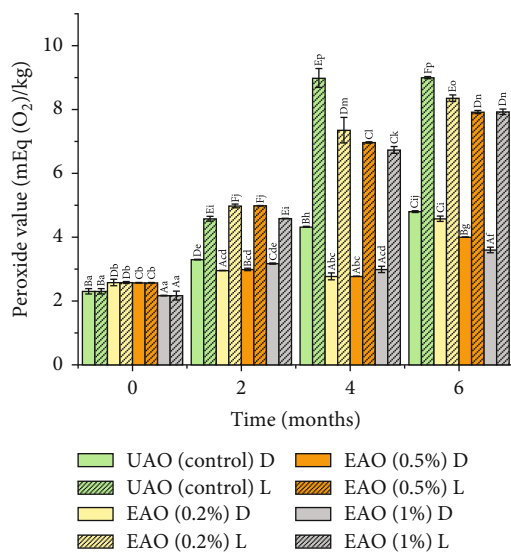


FIGURE 3: Peroxide value of unenriched argan oil (UAO) and enriched argan oils with 0.2% (EAO 0.2%), with 0.5% (EAO 0.5%), and with 1% of saffron (EAO 1%) in darkness (D) and light (L). Different letters indicate significant differences at  $p < 0.05$ .

for EAO 0.2%, 0.33 g/100 g in darkness and 0.54 g/100 g in light for EAO 0.5%, and 0.48 g/100 g in darkness and 0.32 g/100 g in light for EAO 1%. Regarding the storage conditions, it turned out that light/darkness impacted much more on UAO as compared to EAO. Regardless of storage time and conditions, significant differences were observed in FFA between the control sample and oil samples with different SSP levels. This finding agrees with that of Sanmartin et al. [55]. In contrast, other authors reported no significant difference between enriched and unenriched oils in terms of acidity [58].

**3.2.2. PV Variations.** Peroxide value (PV) is used as an indicator for the primary oxidation products (such as hydroperoxides). The measurement of peroxides can be used as an index of oxidation for the first stages of oil oxidation [59]. In general, the peroxide value of UAO and EAO at different concentrations stored under light or in darkness behaved differently according to our results (Figure 3). The enrichment had a significant effect on PV, since the lowest PVs were recorded by the enriched oils. The initial PV of UAO was 2.3 mEq O<sub>2</sub>/kg (Table 1). This value rapidly increased to reach 9 mEq O<sub>2</sub>/kg after six months of being stored at room temperature and exposed to sunlight (under light conditions). When protected from sunlight, a moderate but significant increase was also observed (4.8 mEq O<sub>2</sub>/kg). This clearly illustrates the effect of light on the formation of hydroperoxides [60]. Regardless of storage conditions, all the other enriched oils presented a considerable increase in terms of PV but less intense than the UAO. Indeed, at room temperature and exposed to sunlight, PV of EAO increased for SSP 0.2% (2.59 to 8.36), SSP 0.5% (2.58-7.93), and SSP 1% (2.17-7.93 mEq O<sub>2</sub>/kg) at the end of the storage period. After 6 months of storage, the change of PV was within an acceptable range for all samples during the entire storage period [60]. Such difference in PV between EAO and UAO

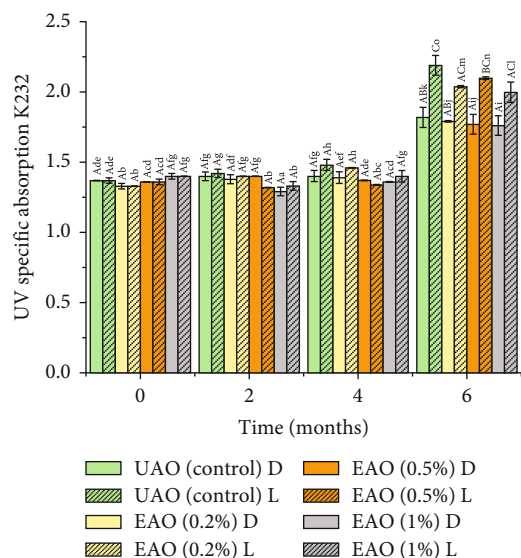


FIGURE 4: UV absorption (K232) of unenriched argan oil (UAO) and enriched argan oils with 0.2% (EAO 0.2%), with 0.5% (EAO 0.5%), and with 1% of saffron (EAO 1%) in darkness (D) and light (L). Data are mean values ( $n = 3$ ). Different letters indicate significant differences at  $p < 0.05$ .

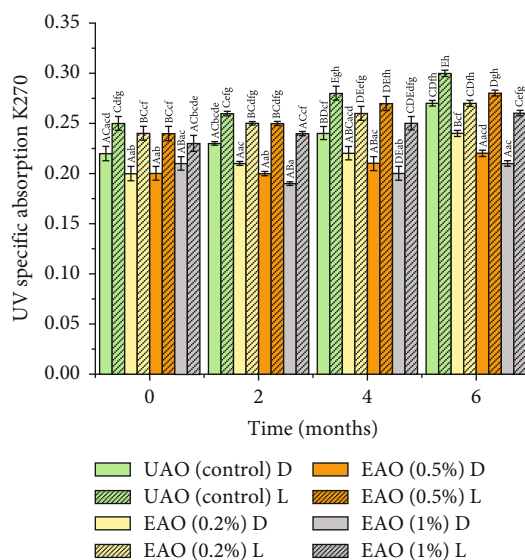


FIGURE 5: UV absorption (K270) of unenriched argan oil (UAO) and enriched argan oil with 0.2% (EAO 0.2%), with 0.5% (EAO 0.5%), and with 1% of saffron stigma powder (EAO 1%) in darkness (D) and light (L). Different letters indicate significant differences at  $p < 0.05$ .

at the end of the storage period could be assigned to crocin, a carotene compound, which is a bioactive constituent of *C. sativus* endowed with significant radical scavenging activity and thus antioxidant activity [25]. Similarly, saffron extracts have shown markedly higher levels of lipid peroxidation products and antioxidant enzymes and lower plasma corticosterone concentrations in rats [61]. Our results are in agreement with other studies involving extracts from MAPs to enrich vegetable oils [62].

TABLE 2: Fatty acids composition (%) of unenriched argan oil (UAO) and enriched argan oils with 0.2% (EAO 0.2%), 0.5% (EAO 0.5%), and 1% of saffron stigma powder (EAO 1%).

Fatty acids	UAO		EAO (0.2%)		EAO (0.5%)		EAO (1%)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
C 16: 0	13.5 <sup>a</sup> ± 0.1	14.42 <sup>b</sup> ± 0.1	14.10 <sup>b</sup> ± 0.1	14.60 <sup>b</sup> ± 0.1	14.72 <sup>b</sup> ± 0.1	14.99 <sup>b</sup> ± 0.1	14.29 <sup>b</sup> ± 0.01	13.96 <sup>a</sup> ± 0.01
C 16: 1	0.10 <sup>a</sup> ± 0.10	0.11 <sup>a</sup> ± 0.01	0.09 <sup>a</sup> ± 0.01	0.10 <sup>a</sup> ± 0.01	0.10 <sup>a</sup> ± 0.01	0.11 <sup>a</sup> ± 0.01	0.10 <sup>a</sup> ± 0.01	0.10 <sup>a</sup> ± 0.01
C 18: 0	6.0 <sup>a</sup> ± 0.01	6.60 <sup>a</sup> ± 0.01	6.84 <sup>a</sup> ± 0.01	6.74 <sup>a</sup> ± 0.01	6.74 <sup>a</sup> ± 0.01	6.75 <sup>a</sup> ± 0.01	6.28 <sup>a</sup> ± 0.01	6.02 <sup>a</sup> ± 0.01
C 18: 1	47.5 <sup>a</sup> ± 0.01	47.82 <sup>a</sup> ± 0.01	48.20 <sup>b</sup> ± 0.01	47.97 <sup>a</sup> ± 0.01	47.77 <sup>a</sup> ± 0.01	47.64 <sup>a</sup> ± 0.01	48.34 <sup>b</sup> ± 0.01	48.62 <sup>b</sup> ± 0.01
C 18: 2	32.2 <sup>a</sup> ± 0.01	30.32 <sup>b</sup> ± 0.01	30.05 <sup>b</sup> ± 0.01	29.83 <sup>c</sup> ± 0.01	29.99 <sup>c</sup> ± 0.01	29.84 <sup>c</sup> ± 0.01	30.25 <sup>b</sup> ± 0.01	30.58 <sup>b</sup> ± 0.01
C 18: 3	0.10 <sup>a</sup> ± 0.01	0.06 <sup>b</sup> ± 0.01	0.04 <sup>c</sup> ± 0.01	0.06 <sup>b</sup> ± 0.01	0.06 <sup>b</sup> ± 0.01	0.05 <sup>c</sup> ± 0.01	0.06 <sup>b</sup> ± 0.01	0.05 <sup>c</sup> ± 0.01
C 20: 0	0.20 <sup>a</sup> ± 0.01	0.39 <sup>b</sup> ± 0.01	0.37 <sup>bc</sup> ± 0.01	0.39 <sup>b</sup> ± 0.01	0.34 <sup>d</sup> ± 0.01	0.38 <sup>bc</sup> ± 0.01	0.36 <sup>bc</sup> ± 0.01	0.36 <sup>bc</sup> ± 0.01
C 20: 1	0.20 <sup>a</sup> ± 0.01	0.30 <sup>b</sup> ± 0.01	0.31 <sup>b</sup> ± 0.01	0.31 <sup>b</sup> ± 0.01	0.28 <sup>c</sup> ± 0.01	0.25 <sup>d</sup> ± 0.01	0.32 <sup>b</sup> ± 0.01	0.32 <sup>b</sup> ± 0.01
SFA*	19.7 <sup>a</sup> ± 0.03	21.41 <sup>b</sup> ± 0.03	21.31 <sup>b</sup> ± 0.03	21.73 <sup>b</sup> ± 0.03	21.8 <sup>b</sup> ± 0.03	22.12 <sup>c</sup> ± 0.03	20.93 <sup>bd</sup> ± 0.03	20.34 <sup>bd</sup> ± 0.03
MUFA**	47.8 <sup>a</sup> ± 0.03	48.23 <sup>b</sup> ± 0.03	48.6 <sup>b</sup> ± 0.03	48.38 <sup>b</sup> ± 0.03	48.15 <sup>b</sup> ± 0.03	48.00 <sup>b</sup> ± 0.03	48.76 <sup>b</sup> ± 0.03	49.04 <sup>bc</sup> ± 0.03
PUFA***	32.3 <sup>a</sup> ± 0.02	30.38 <sup>b</sup> ± 0.02	30.09 <sup>b</sup> ± 0.02	29.89 <sup>b</sup> ± 0.02	30.05 <sup>b</sup> ± 0.02	29.89 <sup>b</sup> ± 0.02	30.31 <sup>bc</sup> ± 0.02	30.63 <sup>bc</sup> ± 0.02

SFA\*: saturated fatty acids; MUFA\*\*: monounsaturated fatty acids; PUFA\*\*\*: polyunsaturated fatty acids. Results are expressed as mean values ± SD ( $n = 3$ ). In each line, values followed by the same letter are not significantly different at  $p < 0.05$ .

**3.2.3. K232 Variations.** Besides the PV, the specific extinction coefficient K232, measured at  $\lambda = 232$  nm, is also a very important indicator of the degree of degradation of the oil in the initial stage [11]. As shown in Figure 4, K232 values confirmed the effect of AO enrichment with SSP on oxidative stability. The highest values of K232 were found in the last month of storage in the UAOs for both storage conditions (1.82 in darkness and 2.19 in light), whereas oils enriched with SSP and stored in darkness showed, after the six months of accelerated aging, the lowest K232 values: 1.79, 1.77, and 1.76 for the three concentrations of SSP 0.2, 0.5, and 1%, respectively. As discussed in Pokorny et al., the antioxidant effect of a given molecule is related to its implication in radical chain reaction formulation [63]. The inhibitory action of an antioxidant compound could be explained by its ability to block the radical chain process. Moreover, crocin is an isomeric molecule that can take part in the initiation of chain reactions during the oxidation of triglycerides, which can subsequently explain the effect of their adjournment on the oxidation stability [62].

**3.2.4. K270 Variations.** Peroxides previously evaluated by PV and K232 evolve over time and give rise to the formation of various products such as unsaturated ketones and diketones, which absorb in the UV zone around 270 nm [64]. As can be observed in Figure 5, K270 of UAOs and EAOs did not overshoot the maximum limit reported by the International Standard Organization ( $\leq 0.35$ ) [48]. In parallel to the previous quality indices, the addition of SSP has also contributed to extend the shelf-life of the oil, as the EAO, after six months of accelerated aging in the light recorded a K270 of 0.3, while that of EAO at 0.2% was only 0.26. These differences may be due to the radical-scavenging properties of crocin and picrocrocin of *C. sativus* as reviewed by Xing et al. [65].

**3.3. Changes in Fatty Acid Composition.** The major constituents of vegetable oils are fatty acids. Characteristics, stability, and nutritive value of a given vegetable oil depend strongly upon the fatty acid composition [66, 67]. In this regard, AO is known to be rich in unsaturated fatty acids, and the nutritional values of unsaturated fatty acids are widely described. As shown in (Figure 2S, supplementary material), oleic acid (43–49 g/100 g) and linoleic acid (29–37 g/100 g) are the major unsaturated fatty acids present in argan oil [50, 68]. The fatty acid composition of UAO and EAOs is reported in Table 2. The enrichment of AO with SSP did not induce any significant modification of the relative percentages of fatty acid distribution of AO in agreement with previous studies on other oilseeds [69, 70]. Likewise, no statistically significant differences were observed among the percentages of polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), and saturated fatty acids (SFA). For all oils, initial and final fatty acid contents were within published values for AO [48, 52, 56].

**3.4. Changes in Phytosterol Composition.** Phytosterols were the second class of compounds investigated since AO is rich in phytosterols. Indeed, its two major phytosterols are schottenol (44–49%) and spinasterol (34–44%) [50]; stigmasta-8-22-dien-3 $\beta$ -ol (3.2–5.7%) and  $\Delta 7$ -avenasterol (4.0–7.0%) were in small amounts, and the campesterol was found at a very low level (Table 3). However, this parameter is interesting for AO authenticity assessment [46]. In addition, the phytosterols are very interesting molecules with several biological properties, and their determination is of major interest owing to their activity and their beneficial impact on health. Therefore, phytosterol composition was evaluated in this study. As observed for fatty acids, no significant variations in sterol composition were

TABLE 3: Phytosterols composition of unenriched argan oil (UAO) and enriched argan oil with 0.2% (EAO 0.2%), with 0.5% (EAO 0.5%), and with 1% of saffron stigma powder (EAO 1%).

Phytosterols	UAO		EAO 0.2%		EAO 0.5%		EAO 1%	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Campesterol	0.30 <sup>a</sup> ± 0.05	0.26 <sup>b</sup> ± 0.05	0.28 <sup>ab</sup> ± 0.05	0.24 <sup>ab</sup> ± 0.05	0.26 <sup>a</sup> ± 0.05	0.28 <sup>ab</sup> ± 0.05	0.25 <sup>ab</sup> ± 0.05	0.25 <sup>ab</sup> ± 0.05
Spinasterol	41.71 <sup>a</sup> ± 0.05	41.06 <sup>a</sup> ± 0.05	42.05 <sup>a</sup> ± 0.05	42.39 <sup>a</sup> ± 0.05	41.28 <sup>a</sup> ± 0.05	42.52 <sup>a</sup> ± 0.05	39.99 <sup>a</sup> ± 0.05	41.06 <sup>a</sup> ± 0.05
Schottenol	48.85 <sup>a</sup> ± 0.05	51.40 <sup>b</sup> ± 0.05	48.72 <sup>a</sup> ± 0.05	49.80 <sup>ab</sup> ± 0.08	48.02 <sup>ab</sup> ± 0.05	49.23 <sup>ab</sup> ± 0.05	50.80 <sup>ab</sup> ± 0.05	51.84 <sup>ab</sup> ± 0.05
Δ7-Avenasterol	4.01 <sup>a</sup> ± 0.05	3.94 <sup>a</sup> ± 0.05	4.49 <sup>a</sup> ± 0.05	4.02 <sup>a</sup> ± 0.05	4.48 <sup>a</sup> ± 0.05	3.95 <sup>a</sup> ± 0.05	4.23 <sup>a</sup> ± 0.05	3.85 <sup>a</sup> ± 0.05

Results are expressed as mean values ± SD. In each line, values followed by the same letter are not significantly different at  $p < 0.05$ .

TABLE 4: Sensorial evaluation of unenriched argan oil (UAO) and enriched argan oil with 0.2% (EAO 0.2%), 0.5% (EAO 0.5%), and 1% of saffron stigma powder (EAO 1%).

	UAO	EAO (0.2%)	EAO (0.5%)	EAO (1%)
Rancidity	4 ± 0 <sup>a</sup>	3 ± 0.5 <sup>b</sup>	1 ± 0 <sup>c</sup>	0 ± 0 <sup>d</sup>
Saffron color	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	1 ± 0 <sup>b</sup>
Saffron flavor	0 ± 0 <sup>a</sup>	1 ± 0 <sup>b</sup>	2 ± 0.5 <sup>c</sup>	4 ± 0 <sup>d</sup>
Saffron smell	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	1 ± 0 <sup>b</sup>	3 ± 0 <sup>c</sup>

Results are expressed as mean values ± SD. In each line, values followed by the same letter are not significantly different at  $p < 0.05$ .

identified over the 6 months storage period. These results suggest that the sterol fraction does not actively participate in the conservation of AO [71]. These results are consistent with those reported for other vegetable oils such as cactus oil [47].

**3.5. Sensorial Analysis.** The acceptability studies of preparations based on argan oil and aromatic plants are very relevant for the implementation of these products on the commercial market. This suitability can be assessed by several parameters such as color, taste, and overall acceptability. Preliminary judgment of sensory quality based on the color of the sample showed that the incorporation of SSP did not affect the color of the argan oil, and a slight discrepancy was noticed in the 1% enriched oil, which can be considered a good result for the acceptability of the product by consumers. However, odor and flavor were impacted by the enrichment. This suggests that argan oil color was not sufficient to discriminate the EAOs. A better categorization of the EAOs was established on the basis of taste and odor, which represent the key sensory attributes of EAOs added with SSP (Table 4). Thus, the typical rancid taste of oxidized oil was detected mainly in the control (UAO) and slightly in the 0.2% enriched oil. These sensory tests have proven the added value of the addition of SSP as an antioxidant. This is shown in the reduction of the rancid smell in the enriched oils. Argan oil added with SPP at 1% and at a lesser magnitude SSP at 0.5% seemed to affect significantly ( $p < 0.05$ ) EAO in terms of flavor, smell, and color. Other enrichment studies have solicited the sensory difference created by the addition of MAPs to vegetable oils, such as the study of Ayadi et al. where the panelists preferred the color and the flavor of the aromatized olive oil [72].

## 4. Conclusions

It can be concluded that *C. sativus* stigma powder can be used as an effective food stabilizer of argan oil as an alternative to synthetic antioxidants, against oxidation reactions and therefore a longer shelf-life. Saffron stigma powder showed varying degrees of antioxidant activity in argan oil in a dose-dependent manner. The results of the different stability indices were in agreement with each other, recommending the use of 1% of saffron stigma powder and strongly suggesting the storage of the oils in a dark place. Crocin-rich saffron can be studied as an effective bioactive pharmaceutical ingredient or as a stabilizer for other foods, dietetics, and cosmetic products.

## Data Availability

The datasets used during the current study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare no conflict of interest.

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## Supplementary Materials

Figure 1S: Rancimat's curve results for unenriched argan oil (UAO) and enriched argan oils (EAOs). Figure 2S: chromatograms of relative fatty acids composition before (a) and after enrichment (b). (*Supplementary Materials*)

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