



Leveraging waste for natural antioxidant production: the effect of direct ultrasound fortification of sunflower oil with saffron by-products to enhance oxidative stability

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Abstract

This investigation evaluates the effect of saffron by-products on the oxidative stability of sunflower oil under accelerated storage conditions. Sunflower oil was directly supplemented, using ultrasound treatment with petals, stamens, and leaves at 0.2%, 0.4%, and 0.6% (wt/wt) for each antioxidant. The treatments underwent an accelerated heating process at 60 °C for 12 weeks. Samples were taken bi-weekly to assess free fatty acids formation, primary oxidation products (peroxide value and K_{232} value), secondary oxidation products (anisidine and K_{270} value), total oxidation value, and pigments content. The Rancimat test, fatty acid profile, iodine value, and oxidisability value were evaluated. The results indicated that the saffron by-product extracts contained high levels of total phenolic compounds (87.23 and 39.23 mg of GAE g⁻¹ in petals and stamens, respectively) and total flavonoid content (109.78 and 33.11 mg / QE g⁻¹ for petals and stamens, respectively), exhibiting significant antioxidant activity as evidenced by ferric reducing antioxidant powder and free radical neutralisation potency assays. The incorporation of saffron by-products has improved the stability of the SO, with the greatest effectiveness observed when combined with the stamens, followed by the petals and leaves. This study shows that the direct ultrasonic enrichment green method offers a rapid approach without recourse to chemicals solvents.

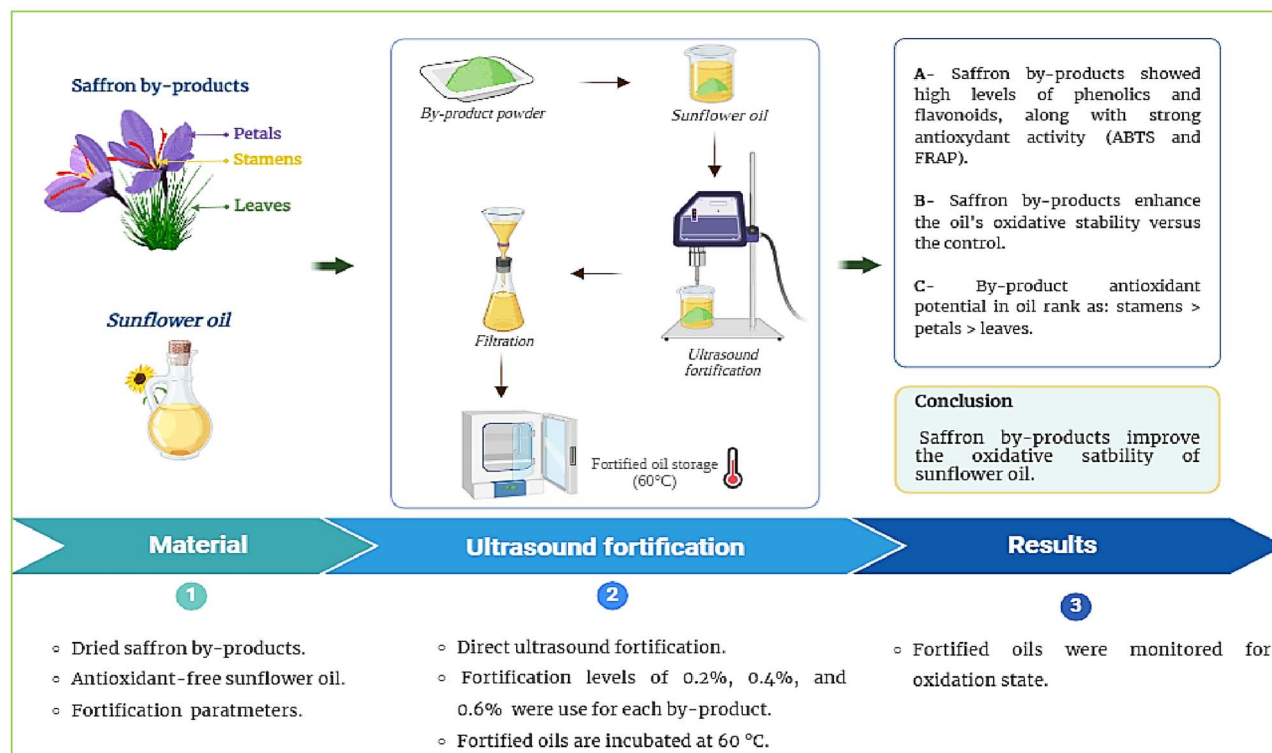
Keywords: accelerated storage, oxidative stability, saffron by-products, ultrasound, vegetable oil

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Graphical abstract



Introduction

The shelf life and quality characteristics of edible oils play a crucial role in the food sector. Researchers, dieticians and consumers all emphasise the dietary value of vegetable oils (Ayyildiz et al., 2015). Sunflower oil (SO) is highly regarded for both industrial and human consumption, as it contains 85% unsaturated fatty acids (UFAs), particularly essential linoleic acid, making it an excellent choice for high-quality vegetable oil (Metzner Ungureanu et al., 2020). Along with their health benefits, UFAs are recognised for their important contribution in cardiovascular risk prevention and their ability to moderate low-density lipoprotein cholesterol levels in plasma (Cherif & Slama, 2022). However, they are particularly sensitive to oxidation, a complex process involving both successive and parallel reactions influenced by various factors. When exposed to external conditions, such as oxygen, heat, and/or light, the double bonds of detached UFAs easily become oxidised, ultimately leading to diminished oxidative stability (Lu et al., 2022).

The stability of SO is influenced not only by its fatty acid profile but also by the primary and secondary oxidation products. Factors such as the presence of antioxidants, metals, and contaminants can either exacerbate or mitigate the oxidative process (Lužaić et al., 2022). Moreover, during the refining process (degumming, neutralisation, decolorisation, and deodorisation), refined oils experience a loss of phenolic compounds, tocopherols, and carotenoids, making them more susceptible to oxidative degradation (Asbbane et al., 2024; Gharby, 2022). The oxidation of oils can lead to sensory changes, such as undesirable odours and flavours, a notable decrease in nutritional value, and the formation of chemical compounds that may pose health risks to consumers (Metzner Ungureanu et al., 2020).

Lipid oxidation is a widespread issue that significantly reduces shelf life (Gharby et al., 2015). This latter is defined as “the

durability or period of time allowed for the stored product to remain efficient, valuable or fit for consumer use” (Syed, 2016). A key strategy for extending shelf life involves adding antioxidants to the oil to inhibit oxidative reactions in the lipids (Lu et al., 2022). However, concerns have been raised about the toxicity and carcinogenicity of synthetic additives, leading to a growing focus on natural antioxidants as a safer alternative for enhancing the oxidative stability of vegetable oils (Metzner Ungureanu et al., 2020).

Saffron (*Crocus sativus* L.), recognised as the world's most expensive spice, is a sterile triploid plant from the *Iridaceae* family (Najafi et al., 2022). This plant is primarily cultivated for its stigmas, which constitute only 7.4% of the flowers, leaving the rest as by-products (Bellachioma et al., 2022). According to Moratalla-López et al. (2019), producing 1 kg of dried saffron stigmas generates approximately 63 kg of by-products, including petals, stamens, and leaves. Generally, these by-products are either discarded as waste or used as animal feed, with limited utilisation in herbal teas or food components (Fazeli Nasab, 2019). However, they contain bioactive metabolites with demonstrated antioxidant activity. Azghandi Fardaghi et al. (2021) highlighted the antioxidant potential of various saffron flower parts, including stigmas, stamens, styles, and petals, as well as Jadouali et al. (2017) found high antioxidant activity in extracts from the stamens, petals, and styles of Moroccan saffron. Saffron petals, in particular, are rich in natural compounds with antioxidant properties (Bellachioma et al., 2022). The beneficial effects of these by-products stem from a diverse range of valuable active compounds, such as flavonoids (kaempferol, anthocyanins, and quercetin) and other components like picrocrocin, crocin, crocetin, and safranal (Moratalla-López et al., 2019). Despite this, considering the high quantities (~90%) of by-products such as waste, their potential health and food applications remain largely

untapped (Lahmass et al., 2018). Therefore, creating innovative food-related applications for these by-products could enhance sustainable cultivation practices.

Based on our knowledge, recent investigations have been conducted on saffron stigmas (Ahmed et al., 2024; Najafi et al., 2022; Nid Ahmed et al., 2024a, 2024b) and pollen (Salamat et al., 2024) as fortifying agents to extend the shelf life of table oils, indicating a need for further studies on saffron by-products for enhancing oil protection against oxidation. Therefore, this study aimed to assess the effectiveness of directly incorporating saffron by-products into SO as natural antioxidants to inhibit oxidation. Furthermore, the antioxidant activity of these by-products in SO was evaluated during accelerated shelf-life testing at an elevated temperature of 60 °C and compared to a control group without antioxidant addition.

Materials and methods

The refined SO sample, which contains no added antioxidants, was purchased from a local supermarket in Taroudant. Saffron by-products, including petals, stamens, and leaves, were supplied directly by a saffron-growing family in the Taliouine region (Tamaloute).

Potassium hydroxide (KOH), potassium iodide, sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$), iso-octane (2,2,4-trimethylpentane), nitric acid, glacial acetic acid (CH_3COOH), para-anisidine reagent ($\text{C}_7\text{H}_9\text{ON}$), methanol (CH_3OH), and ethanol ($\text{C}_2\text{H}_6\text{O}$) were sourced from Merck (Sigma-Aldrich) for the analysis. All reagents and solvents used were of analytical quality.

Antioxidant properties and proximate analysis of saffron by-products

Antioxidant properties

Preparation of saffron by-products extracts

To characterise the by-products involved, 1 g of each powdered by-product was extracted in a 10 ml mixture of methanol–water (80%, vol/vol), which was agitated for 24 hr at ambient temperature. The resulting solution was then filtered through filter paper, and recovered filtrate was preserved for further analysis at 4 °C.

Total phenolic content (TPC)

An assay of the reduction capacity of a sample by-products extract by Folin-Ciocalteu was used for the determination of total polyphenols (Ait Bouzid et al., 2022). For this assay, 0.25 ml of each sample extract mixed with 1.25 ml of Folin-Ciocalteu phenolic reagent (diluted 1:10), followed by alkalisation with 2 ml sodium carbonate Na_2CO_3 (7.5%). The solution was thoroughly mixed using a vortex and incubated at 45 °C for 30 min. A blank was prepared using the same procedure, substituting the extract with distilled water. Absorbance was recorded at 765 nm against the blank solution. A calibration curve was established using gallic acid standards, with known concentrations ranging from 5 to 160 $\mu\text{g ml}^{-1}$. The results were expressed as milligrammes of gallic acid equivalent per gram of dry matter (mg of GAE g^{-1} DM).

Total flavonoids content (TFC)

The total flavonoid content (TFC) of the various by-products extracts was assessed using the aluminium complexation assay, following the colorimetric method described by Ait Bouzid et al. (2022). To achieve this, 1 ml of the extract was mixed with 4 ml of distilled water and 0.3 ml of 5% (wt/vol) aqueous NaNO_2 solution in a 10 ml volumetric flask. The mixture was topped up after 5 min with 0.3 ml of 10% AlCl_3 solution and 1 ml NaOH (2 N), the final

volume was adjusted to 10 ml with distilled water. A blank was prepared using the same procedure, substituting the extract with distilled water. The mixture was incubated at room temperature for 30 min, after which the absorbance was measured at 415 nm using a spectrophotometer (SCIOGEX SP-UV1100 UV/Vis, USA). A calibration curve was established with quercetin standards at concentrations ranging from 10 to 160 $\mu\text{g ml}^{-1}$. The results were expressed as milligrammes of quercetin equivalent per gram of dry matter (mg of QE g^{-1} DM).

Free radical neutralisation potency assay (ABTS)

The “ABTS” assay for each by-products extract was conducted using the methods referred to by Gagour et al. (2022). To begin with, aqueous solutions of ABTS reagent at 2 mM and potassium persulphate $\text{K}_2\text{S}_2\text{O}_8$ at 70 nM were combined to prepare the cationic radical $\text{ABTS}^{\bullet+}$. This mixture was allowed 16 hr to react at ambient temperature in darkness and then was diluted with $\text{C}_2\text{H}_6\text{O}$ until the absorbance was 0.70 ± 0.02 units at 734 nm. Next, 200 μl of each extract was combined with 2 ml of prepared $\text{ABTS}^{\bullet+}$ radical solution, and the obtained solution was kept for 10 min at room temperature in the dark. The absorbance values were measured at a wavelength of 734 nm using the spectrophotometer (SCIOGEX SP-UV1100 UV/Vis, USA) with $\text{C}_2\text{H}_6\text{O}$ as a blank. A calibration curve was established with trolox standards at concentrations ranging from 10 to 160 $\mu\text{g ml}^{-1}$. The results were expressed as milligrammes of trolox equivalent per gram of dry matter (mg of TE g^{-1} DM).

Ferric reducing antioxidant powder assay (FRAP)

The reducing power of methanolic extracts from by-products was assessed using the method described by Işıl Berker et al. (2010). Each 1 ml of methanolic extract was mixed with 2.5 ml of 0.2 N sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide solution ($\text{K}_3\text{Fe}(\text{CN})_6$). The mixture was then incubated in a water bath at 50 °C for 20 min. After cooling, 2.5 ml of 10% trichloroacetic acid was added to the incubated mixture. Portions of 2.5 ml were taken from this mixture and combined with 2.5 ml of water and 0.5 ml of 0.1% ferric chloride (FeCl_3) solution. A blank was prepared by substituting the extract with distilled water. The absorbance of the resulting solution was measured at 700 nm after 30 min, using the solution blank as a reference. A calibration curve was established with trolox standards at concentrations ranging from 10 to 250 $\mu\text{g ml}^{-1}$. The results were expressed as milligrammes of trolox equivalent per gram of dry matter (mg of TE g^{-1} DM).

Proximate analysis

Ash content determination

One gram of the by-product was weighed in incineration crucibles and placed in a muffle furnace at 500 °C until it stabilised at a constant weight (ISO 3632-2, 2010). The ash content was determined using the following equation 1:

$$\text{Ash} = ((W_{\text{Ash}} - W_c) / W_s) \times 100 \quad (1)$$

where: W_{Ash} is weight of crucible with ash (g). W_c is weight of empty crucible (g). W_s is weight of sample (g).

Protein content determination

The protein content of the various spice by-products was evaluated based on the Dumas method, which involves multiplying the total nitrogen content by a factor equivalent to 6.25 (equation 2),

according to the method specified by Gagour et al. (2022).

$$\% \text{ protein} = \% \text{ nitrogen} \times \text{protein factor (6.25)} \quad (2)$$

Mineral profiling determination

To quantify the levels of elements in the by-products for fortification, a method described by Ibourki et al. (2022) was adopted. This involved calcining 1 g of each dried and ground by-product at 500 °C in a muffle furnace for 2 hr to ensure complete calcination. The calcinate sample was then digested with 4 ml of nitric acid and diluted in a 25 ml volumetric flask to the nearest mark with deionised water. The resulting liquid solution was analysed using a Perkin Elmer (Waltham, Optima 8000, USA) inductively coupled plasma optical emission spectrometer (ICP-OES).

Moisture content determination

The moisture content of saffron by-products was gravimetrically measured according to the method outlined in ISO 3632-2 (2010). This involved drying 2.5 g of the powdered by-product sample at 103 ± 2 °C for 16 hr in an oven. The determination of moisture content was based by weighing the sample before drying (W_0) and after drying (W_1) equation 3:

$$MC = ((W_0 - W_1) / W_0) \times 100 \quad (3)$$

Oxidative stability of SO enriched with saffron by-products

Sonication and maceration procedures

The flavoured SO were prepared by initially drying the petals, stamens and leaves in an oven at 103 °C for 2 hr. Subsequently, the dried materials were finely ground using a mortar and pestle. The resulting powders from each by-product were directly mixed with refined oil at three different concentrations: 0.2%, 0.4%, and 0.6% (wt/wt). The mixtures underwent ultrasonication with a probe immersed in the suspension, using an amplitude of 75% for a duration of 15 min. The temperature of the suspensions was maintained at 25 °C through a water reservoir regulated by an ultrasonic temperature sensor. The ultrasound device used was a Biobased UCD-650 model manufactured in China (Figure 1). After the enrichment process, all samples at each concentration were filtered and transferred to well-sealed glass bottles, then stored in an oven at 60 °C. A control sample was also prepared using the same oil without antioxidants. Physicochemical indices such as free fatty acids, peroxide value, anisidine value, TOTOX value, and K232 and K270 coefficients were measured biweekly during storage, while Rancimat, fatty acid composition, iodine value, and COX were determined at the start and end of the experiment. The final oils were coded as follows: SO-C for control SO (without antioxidants); SO-P0.2, SO-P0.4, and SO-P0.6 for SO supplemented with 0.2%, 0.4%, and 0.6% of petals, respectively; SO-S0.2, SO-S0.4, and SO-S0.6 for SO supplemented with 0.2%, 0.4%, and 0.6% stamens, respectively; SO-L0.2, SO-L0.4, and SO-L0.6 for SO supplemented with 0.2%, 0.4%, and 0.6% leaves, respectively.

Free fatty acids (FFA)

The ISO 660 (2020) standard method was used to assess the FFA content of the samples. 10 g of oil sample was introduced into a 250 ml beaker, then 50 ml of absolute C_2H_6O was added followed by three drops of ethanolic phenolphthalein (1%, wt/vol) as a colour indicator. The resulting mixture was titrated with solution of KOH (0.1 N). Free fatty acid was expressed as g 100 g⁻¹ of oleic acid.

Peroxide value (PV)

In accordance with ISO method ISO 3960 (2017), 5 g sample was mixed with 50 ml isooctane/acetic acid (2:3, vol/vol). Saturated potassium iodide (0.5 ml) was added to the mixture, which was then mixed manually without aeration for exactly 60 s (use a timer accurate to ±1 s). Following this, 100 ml demineralized water was added, and the mixture was titrated with $Na_2S_2O_3 \cdot 5H_2O$ solution (0.01 N) in the presence of a 1% starch indicator until a white colour appeared. The PV (mEq O_2 Kg⁻¹) was calculated based on the volume of $Na_2S_2O_3 \cdot 5H_2O$ needed to titrate the solution.

Anisidine value (p-AnV)

Analysis of the p-AnV of the samples was carried out in accordance with ISO 6885 (2016). In a 25 ml volumetric flask, 2.0 g of the sample was dissolved in isooctane to prepare solution A. Solutions B and C were subsequently prepared from solution A. For solution B, 5 ml of solution A was combined with 1 ml of CH_3COOH in a test tube, shaken thoroughly, and stored in the dark at (23 ± 3) °C for 8 min. To prepare solution C, 5 ml of solution A was mixed with 1 ml of anisidine reagent (0.25% in CH_3COOH , wt/vol) in a test tube, shaken well, and stored in the dark at (23 ± 3) °C for 8 min. A blank test was carried out in the same manner as for the preparation of solution C, replacing solution A with isooctane. Absorbances of solutions B, C, and the test blank were measured at 350 nm, with the absorption zero of the spectrometer adjusted using isooctane. The p-AnV value was calculated from the absorbance readings by applying equation 4:

$$p - AnV = [(100 \times Q \times V) / m] \times [1.2 \times (A_0 - A_1 - A_2)] \quad (4)$$

where, V is the volume in which the test sample is dissolved ($V = 25$ ml); Q is the sample content of the measured solution based on which the anisidine value is expressed (g ml⁻¹) ($Q = 0.01$ g ml⁻¹); m is the mass of the test sample (g); A_0 , A_1 , and A_2 are the absorbance values of solutions B, C, and test blank, respectively. 1.2 is the correction factor for diluting the test solution with 1 ml of reagent or CH_3COOH .

Total oxidation (TOTOX) value

The TOTOX value reflects total oxidation, which involves both primary as well as secondary oxidation products. It was estimated from PV/p-AnV employing following equation 5 (Nid Ahmed et al., 2024):

$$TOTOX \text{ value} = 2(PV) + p - AnV \quad (5)$$

where, PV is the peroxide value and p-AnV is the anisidine value.

Specific UV extinction K_{232} and K_{270}

The method described by ISO 3656 (2011) was applied to estimate the specific UV extinction, K_{232} and K_{270} . These coefficients were expressed as the absorbance of a 1% (wt/vol) oil solution in cyclohexane. Practically, 0.5 g of oil was dissolved in 50 ml of cyclohexane, and the absorbance was measured in a 10 mm cuvette using a UV-visible spectrophotometer (SCILOGEX SP-UV1100 UV/Vis, USA).

Rancimat accelerated oxidation test

The Metrohm Rancimat (892 Professional Rancimat, Switzerland) was utilised to carry out the Rancimat accelerated oxidation test, determining the induction time of samples according to the standard method ISO 6886 (2016). A total of 3 g of oil was oxygenated at 110 °C with an air flow rate of 20 L hr⁻¹. The highly

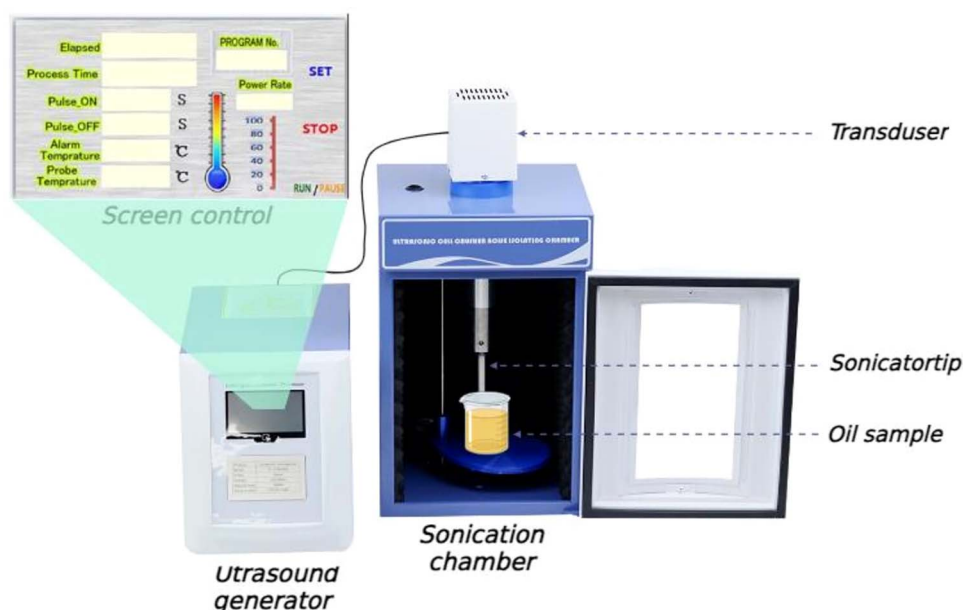


Figure 1. Schematic illustration of the ultrasound-assisted enrichment technique.

volatile secondary oxidation products formed by the oxidation reaction (mainly formic acid and acetic acid) were absorbed by a 60 ml distilled water measurement solution. The release of these volatile acids resulted in an increase in the conductivity of the sample. The instrument software automatically recorded the sample's resistance to oxidation, referred to as the "induction time," and expressed it in hr.

Fatty acid profile (FAs) analysis by GC-FID

The analysis of FAs was performed after their derivatisation into fatty acid methyl esters, utilising a gas chromatograph (GC Agilent Technologies, USA) in accordance with the standard method outlined in the document *ISO 12966-2* (2017). For fatty acid methyl ester derivatisation, 0.1 g of each sample was dissolved in 2 ml of isooctane, followed by the addition of 0.1 ml of 2 N methanolic KOH. The mixture was vortexed for 1 min and allowed to settle for 2 min. Subsequently, the organic phase was washed with 2 ml of saturated NaCl solution. This phase was then dried and filtered using approximately 1 g of anhydrous sodium sulphate. A 1 µl aliquot of the filtrate was injected into the GC equipped with a flame ionisation detector (FID) and a DB 23 AG-TRANS capillary column (60 m length, 320 µm internal diameter, and 0.25 µm film thickness). Helium served as the carrier gas, and the injector and detector temperatures were maintained at 260 °C. The total analysis time was 30 min. Each fatty acid was identified by referring to its retention time. The GC software automatically calculated the values for quantifying the identified fatty acids based on the area of each fatty acid peak relative to the total area of all fatty acid peaks, expressed as a percentage of the total fatty acids.

Iodine value (IV) and oxidability value (COX)

Based on the FAs of the oils examined, the iodine value (Oubannin et al., 2022) and oxidisability (COX) value (Ait Bouzid et al., 2024) were calculated respectively by means of formulas 6 and 7:

$$IV = (1.001 \times \% C_{16:1}) + (0.899 \times \% C_{18:1}) + (1.814 \times \% C_{18:2}) + (2.737 \times \% C_{18:3}) \quad (6)$$

$$COX \text{ value} = [(\% C_{16:1} + \% C_{18:1}) + 10.3 \times (\% C_{18:2}) + 21.6 \times (\% C_{18:3})] / 100 \quad (7)$$

Chlorophyll and carotenoid contents

Carotenoid and chlorophyll content were obtained spectrophotometrically (SCILOGEX SP-UV1100 UV/Vis, USA) according to the method defined by Gharby et al. (2018). Oil samples (7.5 g) were diluted in 25 ml of cyclohexane. The chlorophyll content was measured at an absorbance of 670 nm (equation 8), while the carotenoid content was measured at 470 nm (equation 9). Results were expressed in mg kg⁻¹.

$$\text{Chlorophylls content} = (A_{670} \times 10^6) / (613 \times 100 \times L) \quad (8)$$

$$\text{Carotenoids content} = (A_{470} \times 10^6) / (2000 \times 100 \times L) \quad (9)$$

where A corresponds to the absorbance at the relevant wavelength (668 and 480 nm), L corresponds to the cell thickness of the spectrophotometer (1 cm).

Statistical analysis

All physicochemical analysis experiments in the current study were conducted in triplicate ($n = 3$), and the data are presented as means accompanied by SDs calculated with software Excel version 2019. A comparison of variance was performed using a one-way ANOVA, f and a Tukey test was applied at a significance level of $p < .05$. The ANOVA analysis was conducted using R statistical software version 4.0.5. Principal component analysis and heatmaps with hierarchical cluster analysis and were performed with the OriginPro software (version 9.1, OriginLab Inc.)

Results and discussion

Antioxidant properties of saffron by-products

The extracts of the studied by-products varied significantly in terms of total simple phenolic content (TPC) and TFC ($p < .05$),

Table 1. Proximate analysis and antioxidant properties of saffron by-product.

Analysis name	Measurement unit	Petals	Stamens	Leaves
Ash content	g 100 g ⁻¹	5.90 ± 0.12 ^a	13.87 ± 0.19 ^b	6.54 ± 0.16 ^c
Protein content	g 100 g ⁻¹	11.16 ± 0.17 ^a	16.62 ± 0.22 ^b	20.88 ± 0.32 ^c
Moisture content	g 100 g ⁻¹	8.98 ± 0.09 ^a	7.64 ± 0.11 ^b	6.37 ± 0.04 ^c
Calcium (Ca) (317.933 nm)	mg kg ⁻¹	18.24 ± 0.34 ^a	21.22 ± 0.29 ^b	89.53 ± 0.46 ^c
Sodium (Na) (589.592 nm)	mg kg ⁻¹	6.66 ± 0.08 ^a	5.19 ± 0.18 ^b	7.76 ± 0.33 ^c
Potassium (K) (766.490 nm)	mg kg ⁻¹	172.84 ± 1.0 ^a	610.60 ± 4.67 ^b	167.88 ± 1.72 ^a
Magnesium (Mg) (285.213 nm)	mg kg ⁻¹	14.14 ± 0.18 ^a	34.93 ± 0.95 ^b	31.14 ± 0.88 ^c
Iron (Fe) (239.562 nm)	mg kg ⁻¹	3.89 ± 0.22 ^a	2.89 ± 0.24 ^b	1.65 ± 0.30 ^c
Manganese (Mn) (257.610 nm)	mg kg ⁻¹	0.26 ± 0.06 ^a	0.61 ± 0.09 ^b	0.31 ± 0.10 ^a
Copper (Cu) (324.752 nm)	mg kg ⁻¹	0.06 ± 0.00 ^a	0.15 ± 0.08 ^a	0.06 ± 0.00 ^a
Zinc (Zn) (213.857 nm)	mg kg ⁻¹	0.34 ± 0.06 ^a	0.57 ± 0.08 ^b	0.30 ± 0.02 ^a
Phosphorus (P) (213.617 nm)	mg kg ⁻¹	30.13 ± 0.55 ^a	63.06 ± 1.12 ^b	30.19 ± 0.18 ^a
TPC	mg of GAE g ⁻¹ DM	87.23 ± 0.61 ^a	39.23 ± 0.20 ^b	28.94 ± 1.01 ^c
TFC	mg of QE g ⁻¹ DM	109.78 ± 3.93 ^a	33.11 ± 2.36 ^b	20.89 ± 0.79 ^c
ABTS test	mg of TE g ⁻¹ DM	26.32 ± 0.82 ^a	26.00 ± 0.09 ^a	24.85 ± 0.63 ^a
FRAP test	mg of TE g ⁻¹ DM	76.13 ± 1.95 ^a	41.13 ± 0.44 ^b	40.06 ± 1.77 ^b

Note. Means followed by the same letter in a row indicate no significant differences ($p > .05$) according to Tukey's test ($n = 3$).

with petals exhibiting the highest levels, followed by stamens (Table 1). The leaves extract recorded low TPC (28.94 ± 1.01 mg of GAE g⁻¹ DM) and TFC (20.89 ± 0.79 mg of QE g⁻¹ DM) values. Similarly, Serrano-Díaz et al. (2012) reported that flower petals had high TPC (95.3 mg KE g⁻¹ DW) and TFC (63.9 mg KE g⁻¹ DW), compared with 56.7 and 20.2 mg KE g⁻¹ DW for stamens, respectively. Additionally, Jadouali et al. (2018) recorded TPC and TFC values of 81.69 mg GAE g⁻¹ and 50.64 mg CE g⁻¹ for Moroccan saffron leaves. Azghandi Fardaghi et al. (2021) observed TPC values of 1.96% and 1.06% for saffron petals and stamens, respectively, with corresponding TFC values of 20.18% and 7.80% . Caser et al. (2020) demonstrated that TPC in saffron petals extracted with methanol at various concentrations ranged from 374.8 to 677.7 mg GAE per 100 g. Phenolic constituents contribute greatly to antioxidant activity, as the presence of these compounds, which exhibit reducing, hydrogen-donating, and anti-free radical properties, is directly linked to the plant's richness in phenolics (Phuyal et al., 2020).

The antioxidant potential of the by-product extracts was assessed using ABTS and FRAP tests. The petals showed the higher level of ABTS (26.32 ± 0.82 mg of TE g⁻¹ DM) and FRAP trapping activity (76.13 ± 1.95 mg of TE g⁻¹ DM). While ABTS activity was comparable across all by-products ($p > .05$), FRAP capacity showed no significant differences ($p > .05$) between stamens and leaves. Caser et al. (2020) reported that the FRAP activity for petal extract (using methanol 80%) was 148.6 mmol Fe²⁺ Kg⁻¹, while the ABTS value was 6.79 μmol TE g⁻¹. The superior antioxidant activity of petals observed in our study aligns with findings from Mir et al. (2018), which indicated that the antioxidant potential against ABTS radicals for saffron by-products from Kashmir was ranked as follows: petals > leaves > stamens. The antioxidant efficacy of these by-products can be attributed to their higher content of phenolic compounds and flavonoids, which exhibit biological

activity. A corresponding relationship was noted between TPC and TFC content and the ABTS and FRAP antioxidant activity.

Proximate analysis of saffron by-products

Ash content reflects the total mineral content of the food product (Bilge et al., 2016). The results revealed a different ($p < .05$) ash content in the saffron by-products analysed (Table 1). Ash content was found in the following order: stamens (13.87 ± 0.19 g 100 g⁻¹) > leaves (6.54 ± 0.16 g 100 g⁻¹) > petals (5.90 ± 0.12 g 100 g⁻¹). These results are generally consistent with those from previous studies, which reported ash contents of 7.30 , 13.4 , and 5.63 g 100 g⁻¹ for petals, stamens, and leaves, respectively (Jadouali et al., 2021). The total protein content of the leaves was found to be 20.88 ± 0.32 g 100 g⁻¹, which was significantly higher ($p < .05$) than that of petals (11.16 ± 0.17 g 100 g⁻¹) and stamens (16.62 ± 0.22 g 100 g⁻¹). These results are higher than those reported by Jadouali et al. (2018), who found protein contents of 6.35 g 100 g⁻¹ and 7.24 g 100 g⁻¹ in saffron petals and leaves, respectively. Another study, reported contained a higher percentage of protein (6.35 g 100 g⁻¹) than stamens (5.97%) (Jadouali et al., 2019). The moisture content was measured 8.98 ± 0.09 , 7.64 ± 0.11 , and 6.37 ± 0.04 g 100 g⁻¹ for petals, stamens, and leaves, respectively. In contrast, Jadouali et al. (2018, 2021) reported much higher moisture content for saffron by-products, with values for petals of 92 g 100 g⁻¹ and for leaves of 66 and 76.48 g 100 g⁻¹.

Saffron by-products contain varying quantities of mineral elements, as shown in Table 1. Major elements such as K, P, Ca and Mg were present in abundance, while microelements, such as Mn, Zn, and Cu, were the least abundant. Significant differences ($p < .05$) were observed among the by-products for several mineral elements. The stamens recorded the highest levels of K, Mg, and

P, while the leaves exhibited the highest levels of Ca and Na, and the petals contained the highest levels of Fe. No significant differences ($p > .05$) were found in the levels of K, Mn, Cu, Zn, and P between the petals and leaves. In comparison, Jadouali et al. (2021) conducted a study on saffron by-products from the same region, noting marked differences in Na, Ca, K, N, Fe, and Zn levels, with particularly high levels of Fe (985.8 mg kg^{-1}) and Na (55.4 mg kg^{-1}) in the leaves, as well as elevated Fe levels in the petals. Serrano-Díaz et al. (2013) reported that saffron spice by-products contain high amounts of Ca, K, Mg, P, and Fe. Moroccan saffron is recognised as a rich source of mineral elements, as evidenced by recent studies conducted by Ibourki et al. (2022, 2024). However, the presence of specific elements in excessive quantities, such as iron and copper, can compromise the stability of enriched oil, as they are known to accelerate oxidation (Fathollahy et al., 2021; González-Torres et al., 2023).

Effects of saffron by-products enrichment on oil quality during accelerated ageing conditions Free fatty acids (FFA)

Oils undergo hydrolysis when exposed to moisture and heat, resulting in the breakdown of triacylglycerol structures into FFA, which negatively impacts their oxidative stability (Gharby et al., 2017). More specifically, the accumulation of FFA leads to the development of common rancid aromas (Syed, 2016). Figure 2A shows the evolution of FFAs throughout the oil's storage life after the addition of by-products. Significant increases ($p < .05$) in FFA levels were observed in all samples during storage, with initial FFA values in the enriched samples being slightly higher than those in the control. In the first week (W0), the control oil showed the lowest FFA values, recorded at $0.03 \pm 0.01 \text{ g } 100 \text{ g}^{-1}$. The unheated enriched oils (heating time = 0 hr) showed an identical FFA value of $0.04 \pm 0.01\%$. This minimal increase in FFA, compared to the control, can be attributed to the slight oxidation induced by the sonication process during enrichment. Similarly, Moustakime et al. (2021) a slight increase in free acidity in virgin olive oil enriched with *Pimpinella anisum* through ultrasound-assisted maceration compared to the control. From week 6 onward, a significant increase ($p < .05$) in FFA values was observed in the control oil, showing higher values than enriched oils. A notable increase in FFAs in fortified samples was recorded after the fourth week of storage. With the exception of SO-P0.2, no relevant differences ($p < .05$) were found in FFAs between weeks W10 and W12 for each fortification concentration in each by-product. At the end of storage, for the enriched oils, SO-S0.4 and SO-S0.6 had the lowest FFA levels ($0.16 \pm 0.02 \text{ g } 100 \text{ g}^{-1}$), while SO-L0.6 exhibited the highest value ($0.23 \pm 0.03 \text{ g } 100 \text{ g}^{-1}$), followed closely by SO-P0.2 ($0.22 \pm 0.03 \text{ g } 100 \text{ g}^{-1}$). Therefore, the highest FFA values among enriched oils were recorded for samples containing leaves, particularly SO-L0.6. Overall, the increased concentration of by-products (petals and stamens) was associated with a greater inhibition of glycerides degradation, leading to reduced FFAs production compared to the control. Furthermore, SO supplemented with 0.6% stamens and petals considerably inhibited FFAs production. Our results are in harmony with those of (Najafi et al., 2022; Ahmed et al., 2024; Nid Ahmed et al., 2024), who investigated the stability of soybean or SOs under accelerated shelf-life conditions at 60°C , noting a reduction in FFAs with the addition of saffron stigmas. Furthermore, Taghinia et al. (2019) revealed that ultrasound assisted extraction of *Ferula persica* extract enhanced antioxidant effects, particularly at higher sonication amplitudes, which increased the liberation rate of phenolic compounds and resulted in a bioactive extract with improved antioxidant activity.

Peroxide value (PV)

The hydroperoxide product of primary oxidation was assessed by measuring the PV, which provides valuable insights into the primary oxidation products in oils (Abd-Allah et al., 2018). As illustrated in Figure 2B, the PV of SO, both with and without the addition of by-products, displayed a variable increase over the storage period at 60°C . The SO-C sample (without antioxidants) recorded an initial PV of $1.28 \pm 0.25 \text{ mEq O}_2 \text{ kg}^{-1}$, which significantly increased ($p < .05$) during storage, peaking at $51.38 \pm 3.69 \text{ mEq O}_2 \text{ kg}^{-1}$ at week 8. This was followed by a decrease to $48.08 \pm 3.67 \text{ mEq O}_2 \text{ kg}^{-1}$ at week 10, ultimately reaching $45.73 \pm 2.77 \text{ mEq O}_2 \text{ kg}^{-1}$ by the end of the storage period. This indicates that the SO-C sample had a higher hydroperoxide content compared to those containing by-products. The reduction in PV for SO-C could be attributed to the conversion of hydroperoxides into secondary oxidation products (Ahmadi-Dastgerdi et al., 2019). At elevated temperatures, hydroperoxides can decompose rapidly into volatile and non-volatile components such as dimers, trimers, and alcohols (Lužaić et al., 2022). A similar finding was obtained by Delfanian et al. (2016), who observed a decline in the PV of soybean oil samples after reaching the peak. However, the addition of by-products led to lower PV values compared to the SO-C. The initial PV of the enriched samples varied from 0.97 ± 0.18 (SO-S0.4) to $1.83 \pm 0.22 \text{ mEq O}_2 \text{ kg}^{-1}$ (SO-L0.6), with values progressively increasing ($p < .05$) over the storage duration.

By the second week, PV values surpassed the limits set by the Codex Alimentarius (2009) for refined oils ($10 \text{ mEq O}_2 \text{ kg}^{-1}$), possibly due to oxidation occurring during the fortification process, except for those containing stamens. The observed increase in PV can be linked to the formation of hydroperoxides; stamen supplementation appeared particularly effective at reducing PV, especially at the 0.6%, indicating a concentration dependence effect, where values ranged from 1.05 ± 0.13 to $36.55 \pm 1.94 \text{ mEq O}_2 \text{ kg}^{-1}$ throughout storage. Petal-enriched oils also exhibited a similar trend, whereas those supplemented with leaves had the highest PV levels, ranging from 1.17 ± 0.3 to $47.18 \pm 2.87 \text{ mEq O}_2 \text{ kg}^{-1}$ for SO-L0.2, from 1.47 ± 0.36 to $45.50 \pm 1.98 \text{ mEq O}_2 \text{ kg}^{-1}$ for SO-L0.4, and from 1.83 ± 0.22 to $50.30 \pm 2.14 \text{ mEq O}_2 \text{ kg}^{-1}$ for SO-L0.6. As a result, stamen-enriched oils had the lowest PV, while leaves-enriched oils had the highest PV. The ability of stamen and petal by-products to inhibit the primary oxidation of SO was generally linked to their concentrations. The PV of the enriched samples were ranked as follows: PV (stamens) < PV (petals) < PV (leaves). This finding aligns with research indicating that stamens are highly effective in scavenging peroxy radicals, while petal and styles have moderate activity (Serrano-Díaz et al., 2012). The fact that enriched oils have lower PV values than the control may be linked to the ability of these by-products to mitigate oxidation, as they contain phenolic compounds that function as antioxidants, capable of neutralising reactive free radicals (Kozłowska & Gruczyńska, 2018). Moreover, previous studies have demonstrated that fortified oils with certain extracts (pomegranate and orange peel extracts) can maintain lower PV levels compared to controls during high-temperature storage (65°C) (Abd-Allah et al., 2018). It is well-established that the PV of oils can increase significantly when exposed to elevated temperatures, light, and oxygen (Konuskan et al., 2019).

Anisidine value (p-AnV)

The quantification of secondary oxidation products in oil, comprising volatile and non-volatile carbonyl compounds, is

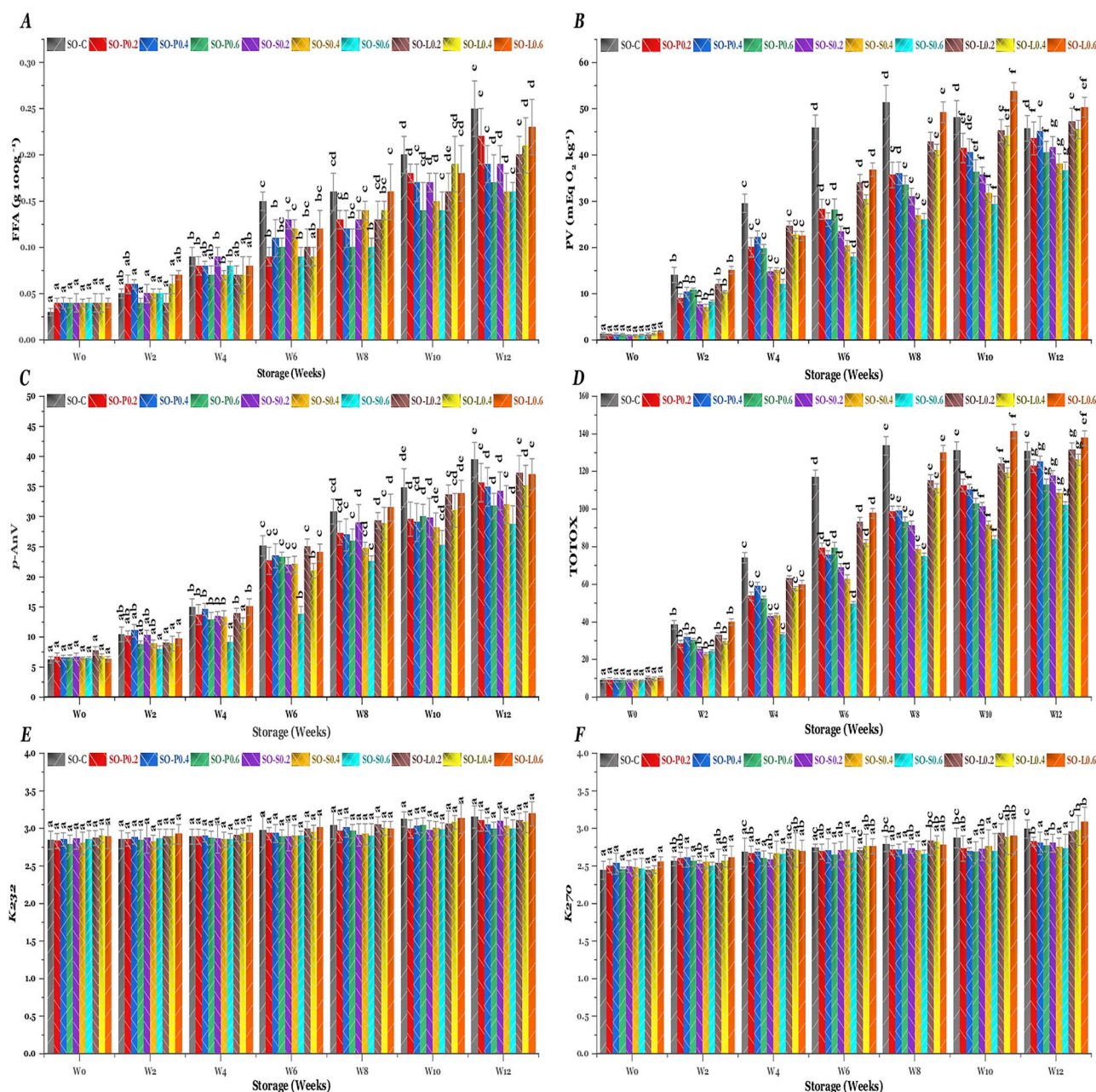


Figure 2. By-products effect on sunflower oil quality indices at 60 °C for 12 weeks. Free fatty acids (A), peroxide value (B), anisidine value (C), oxidation total (D), and specific UV extinction K₂₃₂ (E) and K₂₇₀ (F). Identical letters indicate non-significant difference ($p > .05$) according to Tukey's test ($n = 3$) for each individual treatment throughout the different weeks of storage.

performed using the p -AnV. An increase in p -AnV indicates the presence of secondary oxidation products, with values below 10 signifying high-quality oil (Drinić et al., 2020). As shown in Figure 2C, p -AnV increases significantly ($p < .05$) with extended storage time. However, oils incorporating by-products recorded a smaller increase in p -AnV than control oils. By the end of the storage period, the highest p -AnV values were recorded across all samples. Specifically, the p -AnV of the control oil (SO-C) rose from 6.19 ± 0.55 to 39.40 ± 2.90 after 12 weeks, whereas the low concentration (0.2%) for each by-product increased from 6.62 ± 0.71 to 35.62 ± 3.20 for SO-P0.2, 6.69 ± 0.60 to 34.25 ± 3.15 for SO-S0.2, and 7.69 ± 0.70 to 37.20 ± 2.91 for SO-L0.2. In the high concentration (0.6%) samples, the p -AnV values showed a more modest rise: petals ranged from 6.57 ± 0.42 to

31.77 ± 2.10 , stamens from 6.45 ± 0.22 to 28.67 ± 3.11 , and leaves from 6.27 ± 0.44 to 36.99 ± 2.60 , all of which were lower than the control. Samples SO-L0.2 and SO-L0.6 showed higher p -AnV after the sixth week of storage than the sample containing 0.2%, 0.3%, and 0.6% petals and stamens. Samples SO-P0.2 and SO-P0.4 showed higher p -AnV values than SO-S0.4 by week six, although they remained lower overall than the samples with leaves and the control. Throughout the storage period, SO-S0.6 exhibited very low p -AnV values from weeks 2 to 12, indicating its strong capacity to mitigate secondary oxidation. Overall, saffron by-products effectively inhibited the secondary oxidation of SO, with petals and stamens showing a more pronounced effect correlating with their concentrations. This is consistent with previous research, including findings by Nid Ahmed et al. (2024)

involving the addition of saffron stigmas to SO under accelerated conditions highlighted a reduction in *p*-AnV compared with the control. Similarly, other studies also reported that various natural antioxidants significantly reduced secondary oxidation in oils, reinforcing the conclusions of this study (Pereira et al., 2020; Ling et al. (2015). However, Salamat et al. (2024) reported that saffron pollen at concentrations of 75, 150, and 225 mg kg⁻¹ slightly increased the *p*-AnV in SO after 45 days at 30 °C.

Total oxidation (TOTOX) value

Lipid oxidation can be monitored by measuring primary and secondary oxidation products simultaneously over time, by referring to the TOTOX value as a measure of the total oxidation state of oil. As illustrated in Figure 2D, the TOTOX values of pure and treated oil samples increase with the accelerated oxidation time. The control oil recorded significant increases ($p < .05$) in TOTOX values, rising from 8.75 ± 1.01 at week 0 to 133.56 ± 4.94 by week 8, before stabilising around 130 at weeks 10 and 12. The decrease in TOTOX values during the final two weeks corresponds to the reduction in PV. This indicates that the control oil is particularly susceptible to oxidation, likely due to its high UFAs content and the absence of added antioxidants. However, oils treated with petals and stamens, demonstrated lower TOTOX values throughout the storage period. The inhibitory effect on SO oxidation was found to be concentration dependent. Sunflower containing 0.6% stamen showed the lowest TOTOX values and attained 101.77 ± 2.00 at the end of the storage period. Also, a low concentration of stamen (0.2%) demonstrated a more pronounced inhibitory effect on oxidation compared to both petals and leaves. Although petals showed high antioxidant activities (TPC, TFC, and FRAP test), it is important to recognise that the antioxidant efficacy of antioxidant compounds is influenced by their chemical structure and polarity, rather than solely by their concentration (Baiano et al., 2009). Furthermore, saffron stamens have been reported to contain a high content of flavonol glucosides, such as kaempferol-3-O-sophoroside, quercetin-3-O-sophoroside, and kaempferol-3-O-glucoside, all of which are thought to play a role in antioxidant activity (Lachguer et al., 2024). For SO-L0.6, TOTOX values increased from 9.93 ± 0.99 at week 0 to 141.23 ± 3.76 by week 10, followed by a slight decrease to 137.59 ± 3.96 at week 12. The SO-L0.2 and SO-L0.4 recorded TOTOX values ranging from 10.03 ± 1.00 to 131.56 ± 3.62 and from 9.75 ± 0.87 to 126.09 ± 3.01 , respectively, during storage. Notably, SO-L0.4 exhibited superior antioxidant protection compared to both SO-L0.2 and SO-L0.6. The ranking of antioxidant efficacy was as follows: SO-S0.6 > SO-S0.4 > SO-S0.2 > SO-P0.6 > SO-P0.4 > SO-P0.2 > SO-L0.4 > SO-L0.2 > SO-L0.6 > SO-C. Recent studies, such as those reported by Salamat et al. (2024), highlight the strong antioxidant potential of saffron pollen when added to SO stored at 30 °C for 45 days. Similarly, Zhao et al. (2019) reported that *Moringa oleifera* leaves extract had an antioxidant effect on soybean oil stored under thermal oxidation. Our results suggest an exploitation of saffron by-products, in particular stamens and petals, as an important supply of bioactive components that effectively inhibit both primary and secondary oxidation in SO. Additionally, Zeka et al. (2015) found that saffron petals contain a variety of valuable bioactive compounds (flavonoids, polyphenols, kaempferol, and crocetin glycosides) that offer potential antioxidant activity. Cerdá-Bernad et al. (2022) also indicated that saffron floral by-products provide are rich in antioxidant compounds, making them promising candidates for sustainable and innovative food ingredient development.

Specific UV extinction K_{232} and K_{270}

The oxidation state of oils can be assessed by measuring their absorbance at specific wavelengths of 232 and 270 nm. Absorbance at 232 nm (K_{232}) indicates the presence of primary oxidation products, specifically conjugated dienes, while absorbance at 270 nm (K_{270}) reflects secondary oxidation products, such as conjugated trienes (Turan & Koroğlu, 2020). Figure 2E and F display the K_{232} and K_{270} values for oils supplemented with petals, stamens, and leaves at 60 °C.

During the storage period, all oils exhibited a slight increase in K_{232} , with no significant differences ($p > .05$) observed between enriched and non-enriched samples. The initial K_{232} for control oils was 2.84 ± 0.12 , rising to 3.15 ± 0.15 by week 12. Saffron by-products improved the oil's resistance to the formation of conjugated dienes. Among the enriched oils, SO-L0.4 had the highest initial K_{232} at 2.90 ± 0.09 , followed closely by SO-L0.6 at 2.89 ± 0.16 . At the end of storage, SO-S0.6 and SO-P0.6 recorded the lowest K_{232} values at 2.99 ± 0.12 and 2.99 ± 0.09 , respectively, both lower than the control (SO-C). By week 12, SO-L0.6's K_{232} was only slightly above that of SO-C at 3.19 ± 0.16 . It has been stated that conjugated diene generation is strongly linked to the oxidation of UFAs (Taghinia et al., 2019).

Similarly, K_{270} exhibited significant fluctuations throughout the oxidation process in all samples, with notable differences ($p < .05$) recorded over various weeks of storage, except for the SO-P0.4, SO-P0.6, SO-S0.4, and SO-S0.6 samples. The control recorded the lowest initial K_{270} value of 2.44 ± 0.12 , increasing to 2.99 ± 0.12 at the end of the oxidation period. In contrast, samples containing petals and stamens showed a gradual increase in K_{270} from week 4 until the end of storage, while those with leaves exhibited slightly higher K_{270} values than the control over several weeks, peaking at 3.08 ± 0.20 for the 0.6% concentration in the final week. Antioxidant activity against trienes increases with increasing concentration in petals and stamens. Among the tested by-products, petals and stamens emerged as the most effective in inhibiting the formation of conjugated trienes in SO, thereby enhancing its oxidation stability.

The observed slight increase in K_{232} and K_{270} reflect the formation of primary and secondary oxidation products in the oil, respectively. The present results are consistent with those of Bijla et al. (2024), who found that both SO enriched with used coffee grounds as a natural antioxidant and unenriched oils experienced increased K_{232} and K_{270} values during 120 days of storage at 60 °C. Similarly, Azabou et al. (2020) revealed adding a tomato by-product extract (500 mg kg⁻¹) to olive oil resulted in lower levels of conjugated dienes and trienes compared to the control. Furthermore, it has been evident that *Pterospartum tridentatum* extract offers a protective capacity to prevent an increase in K_{232} and K_{270} values in SO, thus protecting it from oxidation (Neves et al., 2020). Overall, the results suggest that the application of stamens at a concentration of 0.6% leads to the lowest levels of conjugated oxidation products (dienes and trienes), consistent with the findings for PV and *p*-AnV.

Rancimat accelerated oxidation test

To assess the oxidation stability of the oils examined, the Rancimat accelerated oxidation test was conducted at 110 °C. This method involves subjecting the oil to elevated temperatures and controlled oxygen levels to facilitate oxidation, allowing for a rapid estimation of the induction time based on changes in the conductivity of oxidation products. Induction time serves as an effective measure of antioxidant potential in relation to oxidation

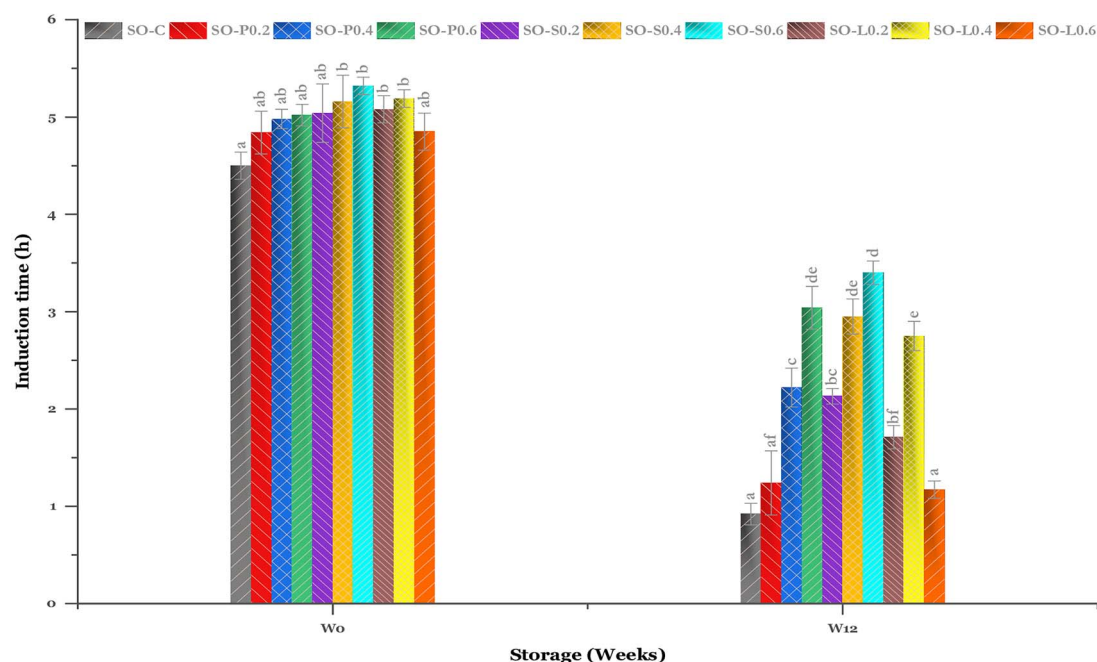


Figure 3. By-products effect on sunflower oil induction time at 60 °C for 12 weeks. Identical letters indicate nonsignificant difference ($p > .05$) according to Tukey's test ($n = 3$) for each individual treatment throughout the different weeks of storage.

stability. The Rancimat test is known for its ease of application and high reproducibility (Yang & Chiang, 2017). Initial and final induction time results for both enriched and control SO are summarised in Figure 3. At baseline, the control oil exhibited a lower induction time of 4.50 ± 0.14 hr, while enriched oils demonstrated significantly longer induction times. A consistent correlation was observed between induction time and the concentration of petals and stamens used for enrichment. The SO enriched with stamens, particularly SO-S0.6, showed the highest resistance to oxidation, achieving an induction time of 5.32 ± 0.09 hr. The induction times of enriched SO, ranked from lowest to highest at day zero, were as follows: SO-P0.2 < SO-L0.6 < SO-P0.4 < SO-P0.6 < SO-S0.2 < SO-L0.2 < SO-S0.4 < SO-L0.4 < SO-S0.6. After 12 weeks of storage at 60 °C, all samples exhibited a significant reduction in induction time ($p < .05$). The control sample, SO-C, displayed the most considerable decrease, with a final induction time of 0.92 ± 0.11 hr, indicating its heightened susceptibility to oxidation. However, oils containing antioxidants maintained higher induction times, with SO-P0.6 recording 3.04 ± 0.22 hr and SO-S0.6 at 3.40 ± 0.12 hr. The samples SO-S0.6, SO-S0.4, and SO-P0.6 experienced minimal changes in induction time post-storage, with values of 3.40 ± 0.12 hr, 2.95 ± 0.18 hr, and 3.04 ± 0.22 hr, respectively, suggesting enhanced oxidative stability. Final induction times for enriched SO after 12 weeks were ranked from lowest to highest as follows: SO-L0.6 < SO-P0.2 < SO-L0.2 < SO-S0.2 < SO-P0.4 < SO-L0.4 < SO-S0.4 < SO-P0.6 < SO-S0.6. All in all, the addition of by-products significantly improved the oxidative stability of SO. The obtained results are in harmony with the study carried out by Najafi et al. (2022), who revealed that the induction time for SO treated with saffron stigma extract ($1,000 \text{ mg kg}^{-1}$) via ultrasonic-assisted extraction was significantly greater than that of the control. Similarly, Assami et al. (2016) demonstrated that *Carum carvi* L. seeds used as natural antioxidants in olive oil yielded higher induction times when treated with ultrasound compared to conventional methods and untreated samples.

Fatty acid profile (FAs)

The potential applications of a vegetable oil and its quality are largely influenced by its FAs (Lužaić et al., 2022). This profile is a crucial factor affecting the oxidation stability of oils. Table 2 demonstrate the effects ($p < .05$) of 12-week storage at 60 °C on the FAs of the investigated oil samples. The SO analysed have a typical FAs, as reported by Anastasi et al. (2010) and Giuffrè et al. (2017). A total of nine fatty acids were identified, encompassing varying proportions of saturated (SFAs), monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs), with a total of $53.30 \pm 0.18\%$, prominently featuring linoleic acid ($C_{18:2}$) at $53.02 \pm 0.09\%$ and oleic acid ($C_{18:1}$) at $35.17 \pm 0.16\%$. However, elevated levels of PUFAs contribute to rapid deterioration of the oils (Symoniuk et al., 2019). The proportion of SFAs was relatively low, identified at 11.29 ± 0.17 g per 100 g, with palmitic acid ($C_{16:0}$) comprising the largest share at $6.85 \pm 0.08\%$, followed by stearic acid ($C_{18:0}$) at $4.07 \pm 0.09\%$. This understanding of SFAs content is noteworthy from a nutritional perspective, particularly concerning its implications for cardiovascular health (Symoniuk et al., 2017). The findings regarding the proportions of various FAs are consistent with the ranges specified in the Codex Alimentarius (2009).

The SO examined has a lower MUFAs content than the PUFAs content, a finding consistent with the results reported by Kozłowska and Gruczyńska (2018). The highest levels of MUFAs were detected in SO-S0.2 ($36.05 \pm 0.22\%$), while SO-S0.6 contained the highest levels of PUFAs ($54.70 \pm 0.13\%$). Over the 12-week storage period, the percentages of MUFA, PUFA, and SFA varied. MUFAs generally increased by the end of the storage period, except for SO-C, which experienced a maximum reduction of 1.07%, and SO-P0.6, which recorded a decrease of 0.14%. However, PUFAs experienced a maximum decrease of 3.58% in SO-S0.2 and 3.19% in SO-P0.2, with the least reduction observed at 0.66% in SO-S0.6 and 0.68% in SO-P0.6. This decline in PUFAs can be attributed primarily to linoleic and linolenic acids, which were most affected during the storage period. Specifically, linoleic acid decreased more significantly in SO-S0.2 (from 54.34 ± 0.15

Table 2. Fatty acid profile, iodine value, and COX value of control and enriched sunflower oils at 60 °C for 12 weeks.

Control and petals-enriched sunflower oils					
SO-C		SO-P0.2		SO-P0.4	
Initial	Final	Initial	Final	Initial	Final
C14:0	0.08 ± 0.01ab	0.07 ± 0.00bc	0.10 ± 0.01a	0.07 ± 0.01bc	0.06 ± 0.01b
C16:0	6.85 ± 0.08bcd	6.56 ± 0.12hi	7.09 ± 0.10bf	6.69 ± 0.12dhi	7.21 ± 0.07ef
C16:1	0.12 ± 0.01a	0.11 ± 0.00a	0.12 ± 0.02a	0.10 ± 0.01a	0.10 ± 0.01a
C18:0	4.07 ± 0.09bcd	3.91 ± 0.07def	4.03 ± 0.08ce	3.76 ± 0.12e	4.18 ± 0.14cf
C18:1	35.17 ± 0.16a	34.67 ± 0.08bc	35.69 ± 0.10f	34.95 ± 0.06ac	35.62 ± 0.09f
C18:2	53.02 ± 0.09b	54.11 ± 0.20ef	52.40 ± 0.11ag	53.87 ± 0.10cdf	52.34 ± 0.08g
C18:3	0.28 ± 0.02bcd	0.31 ± 0.03cd	0.28 ± 0.02bcd	0.27 ± 0.01bcd	0.25 ± 0.02cd
C20:0	0.29 ± 0.02c	0.18 ± 0.01ab	0.20 ± 0.03b	0.14 ± 0.02ad	0.16 ± 0.01bd
C20:1	0.13 ± 0.01ab	0.13 ± 0.00ab	0.08 ± 0.01c	0.16 ± 0.02a	0.09 ± 0.01c
Σ SFA	11.29 ± 0.17bcd	12.84 ± 0.10a	10.72 ± 0.12fg	10.67 ± 0.13gh	11.62 ± 0.16ci
Σ MUFA	35.42 ± 0.14ac	35.04 ± 0.22ab	34.86 ± 0.10bd	35.21 ± 0.11ad	35.81 ± 0.21ce
Σ PUFA	53.30 ± 0.18bc	52.12 ± 0.14a	54.42 ± 0.09fg	54.14 ± 0.12egj	52.59 ± 0.10j
IV	128.68 ± 0.49bcde	126.05 ± 0.88a	130.28 ± 1.06de	128.03 ± 0.60acdf	127.75 ± 0.47ab
COX value	5.87 ± 0.50a	5.73 ± 0.25a	5.99 ± 0.22a	5.96 ± 0.34a	5.80 ± 0.27a
Stamens-enriched sunflower oils					
SO-S0.2		SO-S0.4		SO-S0.6	
Initial	Final	Initial	Final	Initial	Final
C14:0	0.08 ± 0.00ab	0.08 ± 0.01ab	0.08 ± 0.01ab	0.09 ± 0.00ac	0.06 ± 0.00b
C16:0	6.71 ± 0.08dhi	6.82 ± 0.08bci	6.70 ± 0.09dhi	6.52 ± 0.09gh	6.69 ± 0.10dhi
C16:1	0.13 ± 0.02a	0.11 ± 0.00a	0.14 ± 0.02a	0.13 ± 0.01a	0.10 ± 0.01a
C18:0	3.81 ± 0.08be	4.13 ± 0.08cd	4.14 ± 0.10cd	3.88 ± 0.13def	3.87 ± 0.10de
C18:1	34.40 ± 0.17b	35.80 ± 0.10f	34.65 ± 0.12bc	34.43 ± 0.06b	34.73 ± 0.10bc
C18:2	54.34 ± 0.15e	52.46 ± 0.09g	53.71 ± 0.12cf	54.40 ± 0.20e	54.09 ± 0.10ef
C18:3	0.28 ± 0.01bcd	0.20 ± 0.01ef	0.30 ± 0.01cd	0.30 ± 0.01cd	0.25 ± 0.01df
C20:0	0.16 ± 0.01bd	0.26 ± 0.01c	0.20 ± 0.02b	0.16 ± 0.02bd	0.11 ± 0.01d
C20:1	0.08 ± 0.00c	0.14 ± 0.02a	0.08 ± 0.01c	0.08 ± 0.01c	0.10 ± 0.01bc
Σ SFA	10.75 ± 0.09fj	11.29 ± 0.13bcd	11.12 ± 0.09bde	10.64 ± 0.08g	10.73 ± 0.10fg
Σ MUFA	34.61 ± 0.21b	36.05 ± 0.22e	34.84 ± 0.29bd	34.64 ± 0.14b	34.93 ± 0.10ab
Σ PUFA	54.62 ± 0.13f	52.66 ± 0.13j	54.01 ± 0.11dg	54.70 ± 0.13f	54.34 ± 0.19fgi
IV	130.39 ± 0.58ef	128.00 ± 1.12acd	129.54 ± 0.61bcde	130.59 ± 0.45e	130.13 ± 0.40ce
COX value	6.00 ± 0.21a	5.81 ± 0.20a	5.94 ± 0.16a	6.01 ± 0.12a	5.97 ± 0.20a
Leaves-enriched sunflower oils					
SO-L0.2		SO-L0.4		SO-L0.6	
Initial	Final	Initial	Final	Initial	Final
C14:0	0.08 ± 0.01ab	0.08 ± 0.01ab	0.10 ± 0.00a	0.09 ± 0.00bc	0.08 ± 0.00ac
C16:0	6.52 ± 0.08gh	7.20 ± 0.10ef	6.86 ± 0.06bcd	6.80 ± 0.11gij	7.46 ± 0.11ae
C16:1	0.13 ± 0.01a	0.10 ± 0.01a	0.13 ± 0.00a	0.13 ± 0.01a	0.14 ± 0.02a
C18:0	3.92 ± 0.13def	4.05 ± 0.07ce	3.92 ± 0.10def	3.88 ± 0.09def	4.28 ± 0.13c
C18:1	34.55 ± 0.16bc	34.67 ± 0.12bc	34.77 ± 0.19ab	34.46 ± 0.22be	34.66 ± 0.19bc
C18:2	54.20 ± 0.22de	53.46 ± 0.18c	53.73 ± 0.09ef	54.09 ± 0.17ef	53.00 ± 0.14b
C18:3	0.31 ± 0.04cd	0.20 ± 0.02ef	0.28 ± 0.04bcd	0.28 ± 0.01bcd	0.14 ± 0.03ae
C20:0	0.18 ± 0.01ab	0.14 ± 0.01ad	0.14 ± 0.02ad	0.17 ± 0.02ab	0.16 ± 0.02bd
C20:1	0.10 ± 0.01bc	0.10 ± 0.00bc	0.08 ± 0.01c	0.10 ± 0.01bc	0.09 ± 0.01c
Σ SFA	10.69 ± 0.09fg	11.47 ± 0.12ce	11.03 ± 0.10dfh	10.94 ± 0.10bfg	11.99 ± 0.08i
Σ MUFA	34.78 ± 0.18bd	34.87 ± 0.12bd	34.98 ± 0.10ab	34.69 ± 0.10bd	34.89 ± 0.15ab
Σ PUFA	54.51 ± 0.22ef	53.66 ± 0.24bd	54.01 ± 0.16dg	54.37 ± 0.10fgi	53.14 ± 0.12ch
IV	130.36 ± 1.06de	128.79 ± 0.66bcde	129.62 ± 0.59bcde	130.00 ± 0.86bcde	127.82 ± 0.71ac
COX value	6.00 ± 0.33a	5.90 ± 0.14a	5.94 ± 0.24a	5.98 ± 0.20a	5.84 ± 0.19a

Note. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; IV = iodine value; COX value = oxidizability value. Means followed by the same letter in a row indicate no significant differences ($p > .05$) according to Tukey's test ($n = 3$).

to $52.46 \pm 0.09\%$), while linolenic acid saw a greater reduction in SO-C (from 0.28 ± 0.02 to $0.12 \pm 0.01\%$). The SO-L0.4 exhibited the lowest reduction in PUFAs (1.18%), followed by SO-L0.2 (1.55%) among the leaf-supplemented samples. The inhibitory effects on PUFAs oxidation ranged from mild to strong, depending on the concentration of the by-product added, effectively preventing the oxidation of linoleic and linolenic acids. In terms of SFA, an increase was observed across all samples, with the highest increases noted in SO-C (1.54%) and SO-L0.6 (1.05%). This rise in SFAs is linked to the reduction of PUFAs and variations in MUFA levels, which is consistent with the previous study of (Cherif & Slama, 2022).

It appeared that the control sample was more affected by oxidation, resulting in a decrease in both PUFAs and MUFAs. Additionally, the incorporation of leaves at 0.2% and 0.4% in the oil reduced the degradation of PUFAs compared to the control oil samples. The most favourable outcomes were achieved with the addition of 0.4% and 0.6% stamens, as well as 0.6% petals. Consequently, incorporating stamens and petals at higher concentrations, along with leaves at lower concentrations, enhances the protection of fatty acids in SO during periods of accelerated oxidation. Supporting these findings, Pereira et al. (2020) confirmed that by-product extract of star fruit (*Averrhoa carambola* L.) effectively safeguarded PUFAs in sesame oil against oxidative deterioration during accelerated storage at 60 °C for 14 days.

Iodine value (IV)

Iodine value (IV) corresponds to a structural indicator that reflects the amount of double bonds contained in the oil sample (Ayyildiz et al., 2015). The influence of saffron by-products on the IV of SO was examined, with the results reported in Table 2 ($p < .05$). The IV values observed in the oils ranged from 126.05 ± 0.88 to 130.59 ± 0.45 g I₂ 100 g⁻¹, indicating a relatively high level of unsaturation. However, elevated IV suggest a likelihood of oxidation occurring in the UFAs molecule (Oliveira et al., 2017). At the end of the heating period, IV values decreased, with the control sample showing the greatest reduction of 2.04% (from 128.68 ± 0.49 to 126.05 ± 0.88 g I₂ 100 g⁻¹). The incorporation of antioxidants into SO noticeably inhibited the rate of IV reduction. Among the fortified oils, SO-S0.6, SO-S0.4, SP-S0.6, and SL-S0.4 demonstrated the smallest declines in IV, with reductions of 0.35, 0.53, 0.61, and 0.70%, respectively. However, SO-S0.2 demonstrated the greatest decrease in IV, correlating directly with the maximum reduction in PUFAs observed in that sample. Similar IV reduction rates were noted in SO-P0.2 (1.73%) and SO-P0.4 (1.72%). Heat treatment resulted in a decrease in IV in the samples studied, likely associated with the respective decreases in PUFA content and increases in SFA content. In other terms, the decreases in IV correspond to a reduction in the number of double bonds due to oxidation. The IV values obtained in this study align with the FAs results, indicating that saffron by-products effectively mitigated the reduction of UFAs during accelerated heating of SO, showcasing their potent antioxidant activity. These results are in accordance with those published by Asadi and Farahmandfar (2020) and Nid Ahmed et al. (2024). Pereira et al. (2020) concluded that sesame oil containing star fruit by-product extract at 1000 mg kg⁻¹ showed a lower reduction in unsaturation levels compared to the control.

Oxidability (COX) value

The stability of SO can be evaluated by calculating the COX value, which is derived from the ratio of UFAs present in the

oil. This value provides a good indicator for the degree of susceptibility to oxidation. The COX values were statistically similar ($p > .05$) before and after storage, as shown in Tables 2–4. Initial COX value decreased slightly during storage for all SO analysed, particularly for SO-C, which declined by 2.39% (from 5.87 ± 0.50 to 5.73 ± 0.25). The smallest variation was detected for SO-S0.6, SO-S0.4, and SO-P0.6, with values changing from 6.01 ± 0.12 to 5.97 ± 0.20 , 5.98 ± 0.17 to 5.94 ± 0.16 , and 5.97 ± 0.40 to 5.93 ± 0.14 , respectively; all of these samples recorded an identical reduction of 0.67%. As Nid Ahmed et al. (2024) examined the effect of adding various synthetic antioxidants on the oxidative stability of SO and noted a slight decrease in COX values during storage, with a considerable reduction in the control SO compared to the fortified oil.

Chlorophylls and carotenoids contents

Figure 4A illustrates the effects of by-products on oil chlorophyll content over weeks of storage. Initial measurements revealed variable chlorophyll levels among the samples, with the lowest recorded value of 0.09 ± 0.01 mg kg⁻¹ for SO-C. Supplementing SO with saffron by-products slightly increased the chlorophyll content, especially with the addition of 0.6% leaves, which achieved the highest initial value of 0.21 ± 0.03 mg kg⁻¹. Both the control and enriched samples experienced a significant decline in chlorophylls content due to storage. The SO-C and SO-S0.2 had the lowest chlorophyll content, 0.02 ± 0.01 and 0.03 ± 0.01 mg kg⁻¹, respectively, at the end of the storage period. However, SO-L0.6 exhibited the highest chlorophyll content at 0.12 ± 0.01 mg kg⁻¹, followed by SO-L0.4 at 0.08 ± 0.01 mg kg⁻¹. The high chlorophyll content in the 0.6% leaves-enriched oil may explain its increased sensitivity to oxidation compared to other enriched oils, as chlorophyll can be a pro-oxidant and accelerate the oxidation process (Symoniuk et al., 2019). Furthermore, a limited quantity of chlorophyll is required in the oil to prevent the pro-oxidant reaction of chlorophyll pigments, thereby protecting the oils from deterioration (Elouafy et al., 2022). While chlorophyll can act as an antioxidant when stored at low temperatures in the dark, it may have pro-oxidant effects when exposed to light (Redondo-Cuevas et al., 2018).

The carotenoid content throughout storage is shown in Figure 4B. Results showed that enrichment with petals and stamens increased carotenoid content compared with the control. Initially, SO-S0.6 revealed the highest carotenoid content (0.43 ± 0.05 mg kg⁻¹), followed by SO-S0.4 (0.29 ± 0.03 mg kg⁻¹) and SO-P0.6 (0.22 ± 0.01 mg kg⁻¹). It was noted that the carotenoid levels in oils enriched with petals and stamens were dependent on the enrichment concentration. Carotenoids are associated with several health benefits, including a reduced risk of degenerative eye diseases and cardiovascular disease (Elouafy et al., 2022). A significant decrease ($p < .05$) in carotenoid content was observed across all samples after 12 weeks of storage. Completion of the storage period, SO-C and SO-P0.2 recorded the lowest carotenoid levels of 0.04 ± 0.01 and 0.07 ± 0.01 mg kg⁻¹, respectively. However, SO-S0.6, SO-S0.4, and SO-P0.6 presented high levels at 0.26 ± 0.02 , 0.16 ± 0.02 , and 0.12 ± 0.01 mg kg⁻¹, respectively. The SO-C and SO-P0.2 recorded the lowest carotenoid levels of 0.04 ± 0.01 and 0.07 ± 0.01 mg kg⁻¹, respectively. In general, the supplementation by stamens (at 0.2%, 0.4%, and 0.6%) and petals (at 0.4% and 0.6%) resulted in high carotenoid content throughout the storage period, while the addition of leaves yielded lower levels. This finding may explain the high stability of SO containing by-products, as carotenoids not only contribute colour but may also inhibit oxidation (Symoniuk et al., 2019). Our results are consistent with

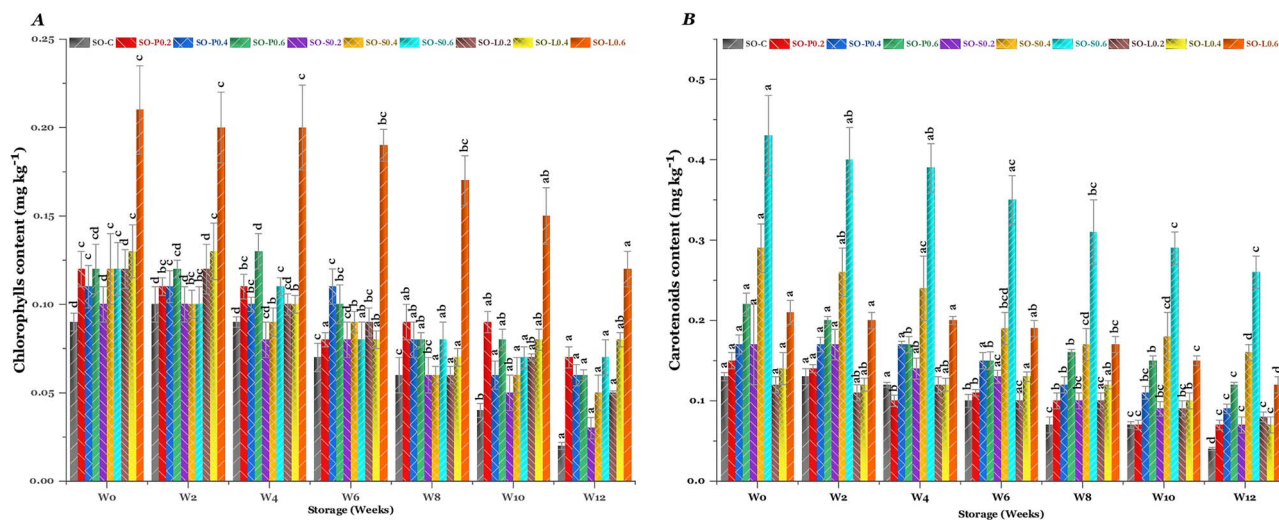


Figure 4. By-products effect on sunflower oil pigment content at 60 °C for 12 weeks. Chlorophyll content (A) and carotenoid content (B). Identical letters indicate non-significant difference ($p > .05$) according to Tukey's test ($n = 3$) for each individual treatment throughout the different weeks of storage.

those recently obtained by Bezzezi et al. (2023) and Nid Ahmed et al. (2024), which also reported decreases in chlorophyll and carotenoid levels over the storage period.

Conclusion

This study revealed that saffron by-product extracts were sources of polyphenols and flavonoids, exhibiting antioxidant properties as evidenced by their scavenging activity (ABTS test) and reducing power (FRAP test). As a result, saffron by-products displayed a significant antioxidant effect that mitigated the peroxidation of SO during accelerated storage at 60 °C. Moreover, the fortified oil proved to be more stable than the control oil. The inhibitory effects of stamens and petals were found to be concentration-dependent, and they were more effective than leaves in delaying the oxidation of SO. Among the tested by-products, stamens, particularly at a concentration of 0.6%, is effective in inhibiting the oxidation process, thereby enhancing the oil's shelf life. These findings underscore the advantages of utilising saffron by-products as natural antioxidants, particularly through ultrasound-assisted direct enrichment methods that can expedite the maceration process, reduce processing time, and eliminate the need for solvents. Such advancements will promote the utilisation of these by-products and contribute to the development of SO with exceptional oxidative stability.

Data availability

The data are contained within the article.

Author contributions

Moussa Nid Ahmed (Writing—original draft, Methodology, Investigation), Abderrahim Asbbane (Writing—review & editing), Laila Bijla (Investigation, Conceptualisation), Otmane Hallouch (Investigation, Formal analysis), Mohamed Ibourki (Data curation, Validation), Brik Guenaze (Supervision, Validation), Khalid Majourhat (Methodology, Validation, Formal analysis), Angelo Maria Giuffrè (Software, Resources, Writing—review & editing, Visualisation,

Project administration), and Said Gharby (Supervision, Resources, Funding acquisition, Writing—review & editing)

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Conflicts of interest

None declared.

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