



# Blending cold-pressed peanut oil with omega-3 fatty acids from walnut oil: Analytical profiling and prediction of nutritive attributes and oxidative stability

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## ABSTRACT

This study aimed to explore the possibility of enriching cold-pressed Virginia (VIO) and Valencia (VAO) peanut oils with omega-3 fatty acids (FAs) from walnut oil (WO) to produce blended oils with improved nutritional value. The oxidative stability of pure and blended oils was examined under accelerated conditions (60 °C) for 28 days. The FA and tocopherol profiles, as well as nutritional quality indices, were determined. As the proportion of WO increased in the blends, the levels of linoleic and α-linolenic essential FAs increased, while oleic acid content decreased. Furthermore, γ- and δ-tocopherol levels rose, whereas α-tocopherol declined. Among the studied blends, VIO:WO blends, especially at a (70:30) ratio, were nutritionally favorable with a balanced FA profile. During storage, notable changes were observed in tocopherol levels, along with subtle alterations in the FA profile of the blended oils. Hence, the oxidative stability of pure VIO and VAO decreased with WO incorporation.

## 1. Introduction

Vegetable oils, the primary source of fat in our diet, are crucial for providing nourishment and sustaining the human body's regular physiological functions (Jing et al., 2024; Kačániová et al., 2024; Shahid et al., 2024). Their consumption has witnessed a significant surge over the decades (Sharma et al., 2023). However, the intake of a single vegetable oil does not meet the consumers' desires and criteria for an ideal edible oil in terms of its physicochemical characteristics, nutritional attributes, and oxidative stability (Pattnaik & Mishra, 2022). For instance, perilla seed oil, linseed oil, and sea buckthorn seed oil possess a high level of α-linolenic acid (C18:3, ω3 series); yet, their susceptibility

to oxidation poses a constraint on their potential utilization (Dhyani et al., 2022; Romanić et al., 2021). Palm oil has notable oxidative stability, characterized by reduced levels of essential fatty acids (FAs) and a notable abundance of saturated fatty acids (SFA) (Hashempour-Baltork et al., 2016). However, elevated levels of SFA have been linked to the development of diabetes, obesity, and hyperlipemia (Chen & Liu, 2020).

In recent times, there has been a growing focus among nutritionists and consumers on the nutritional composition of vegetable oils (Pattnaik & Mishra, 2022; Fadda et al., 2022). In order to enhance their industrial applications, vegetable oils are frequently subjected to four distinct techniques; namely, hydrogenation, interesterification, fractionation, and blending (Pattnaik & Mishra, 2022). Blending is a simple, cost-

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effective, non-invasive, and generally recognized technique for customizing oils, which is becoming increasingly popular globally (Hashempour-Baltork et al., 2016). Blending of two or more oils with distinct properties not only alters the composition of FAs, but also enhances the concentrations of bioactive lipids and natural antioxidants in the blends, such as tocopherol, sterols, squalene, and polyphenols, resulting in higher quality oils and greater nutritional value at reasonable costs (Sharma et al., 2023; Fadda et al., 2022). For instance, the blends of cold-pressed black cumin seed oil with sunflower oil showed improved physicochemical properties and thermal stability (Kiralan et al., 2017). Blending rapeseed oil with black cumin and rice bran oils improved the polyunsaturated and saturated FAs (PUFA/SFA) and  $\omega 6/\omega 3$  ratios, and increased the contents of tocopherols, tocotrienols, and phytonutrients (Rudzińska et al., 2016). Moreover, blending perilla seed oil with extra virgin olive showed promising potential as an innovative vegetable oil blend, offering enhanced nutritional properties, oxidative stability, and sensory acceptability (Torri et al., 2019).

Peanut (*Arachis hypogaea* L.), an annual legume belonging to the *Leguminosae* family, ranks among the world's largest and most valuable oilseed crops, cultivated across more than 29.5 million hectares globally as of 2024 (USDA, 2024). Peanut oil is abundant in oleic acid (C18:1,  $\omega 9$  series, 35–80 %) and contains a satisfactory concentration of linoleic acid (C18:2,  $\omega 6$  series, 4–43 %), yet it lacks a significant amount of C18:3 (0.0–0.5 %), resulting in an imbalanced FA composition (Idrissi et al., 2022). From a nutritional perspective, excessive consumption of  $\omega 6$  PUFAs leads to a significantly elevated  $\omega 6/\omega 3$  ratio, which can contribute to the development of several diseases, including cardiovascular diseases, diabetes, depression, immunological disorders, and neurological dysfunction (Chen & Liu, 2020). Peanut oil is considered a high-quality cooking and frying oil due to its exceptional resistance to oxidation, which is attributed to its high C18:1 content (Sunil et al., 2015). Peanut oil contains sterols and tocopherols with concentrations ranging from 900 to 4344 mg/kg and 137 to 934 mg/kg, respectively (El Idrissi et al., 2023). These compounds offer several advantages, such as enhancing immune function, reducing blood cholesterol levels, and enhancing the oxidative stability of the oil.

According to the World Health Organisation (WHO), it is recommended to consume a ratio of 1:1.5:1 of saturated- and mono- and polyunsaturated FAs (SFA:MUFA:PUFA) and a ratio of 5–10:1 of  $\omega 6/\omega 3$  in the diet (WHO, 2008). Given that an individual oil cannot meet all these criteria, blending is a very effective and cost-efficient method to achieve these recommendations. Considering consumers' demand for a diversity of healthy oils, walnut oil (WO), a high-value functional oil from seeds of *Juglans regia* L., has been used to blend with peanut oil. WO mostly consists of triglycerides, with C18:2 and C18:3 being the most significant PUFAs, ranging from 50 to 63 % and 11 to 19 %, respectively (Pan et al., 2020). The selection of this particular oil source for the current study was based on its unique balance of  $\omega 6/\omega 3$  FAs, with a ratio of 4:1, which has been demonstrated to reduce the occurrence of cardiovascular risk (Bordón et al., 2019). Additionally, WO is rich in significant minor bioactive components such as tocopherols, phytosterols, and polyphenols (Elouafy et al., 2022). Furthermore, it possesses a nutty flavor that has received a high sensory rating. In this context, Bordón et al. (2019) demonstrated that blending chia oil with other specialty oils such as walnut, almond, virgin, and roasted sesame oils, produced blends with improved oxidative stability, essential FA presence, and sensory characteristics.

To the best of our knowledge, the majority of published studies focus on blends of different seed oils (Sharma et al., 2023; Pattnaik & Mishra, 2022; Hashempour-Baltork et al., 2016), while only a few explore the mixing of peanut oil with other vegetable oils, including palm olein, stearin oils (Ndomou et al., 2023), cottonseed oil (Neeharika et al., 2017), seinat seed oil (Siddeeg & Xia, 2015), alhydwan seed oil (Al-Farga et al., 2020), and rice bran oil (Sunil et al., 2015), with the aim of enhancing their FA profile, frying performance, oxidative stability and sensory attributes.

Herein, the main objective of this study was to produce an  $\omega 3$ -enriched peanut oil blend with enhanced nutritional and health-promoting value. The blending of Virginia and Valencia-peanut type oils, which differ in their FA profile and endogenous antioxidant content, with  $\omega 3$  FA-rich walnut oil was explored. The present study was therefore conducted to evaluate the effect of blending on various aspects, including FA profile, tocopherol composition, nutritional quality indices, and pigment content. An accelerated storage test was carried out at  $60 \pm 2$  °C for 28 days. The evolution of the chemical oxidation parameters and the theoretical flavor scores of the pure and blended oils during accelerated storage were also monitored. In addition, the data were submitted to principal component analysis, hierarchical cluster analysis, and chi-squared automatic interaction detector to identify any discrimination among pure and blended peanut oils.

## 2. Material and methods

### 2.1. Plant material and oil extraction

Two peanut (*Arachis hypogaea* L.) varieties, namely Virginia (ssp. *hypogaea* var. *hypogaea*) and Valencia (ssp. *fastigiata* var. *fastigiata*), were acquired from a market in Kenitra, Morocco, located at coordinates  $34^{\circ} 15' 0''$  N and  $6^{\circ} 35' 0''$  W. Additionally, shelled seeds of the *Juglans regia* L. plant were procured from a local market in Rabat, Morocco, situated at coordinates  $34^{\circ} 15' 0''$  N and  $6^{\circ} 35' 0''$  W.

Extraction of oils from Virginia (VIO), Valencia (VAO), and walnut (WO) was conducted with a Komet screw press (IBG Monforts Oekotec GmbH, Mönchengladbach, Germany) at a temperature of  $50 \pm 5$  °C, according to El Idrissi et al. (2023). The obtained extract (oil + impurities) was then centrifuged (Anke KA-1000, Shanghai, China) at 4000 rpm for 10 min at ambient temperature (22–25 °C). The oils were subsequently placed in amber bottles to protect them from sunlight and stored at 4 °C for a maximum of three days.

### 2.2. Oil blends preparation

The binary blends were prepared by mixing VIO and VAO with WO, designated as VIO:WO and VAO:WO, in the following respective proportions: 95:5, 90:10, 80:20, and 70:30 (v/v). The oils were thoroughly mixed for 15 min using a magnetic stirrer (VELP AREX CerAlTop™, Usmate Velate, MB, Italy) to form uniform blends, according to the methodology described by Pan et al. (2020). To ensure consistency, the pure oils underwent the same stirring process.

### 2.3. Accelerated thermal oxidation of pure and blended oils (Schaal oven test)

Pure oils and oil blends were placed in a series of opaque glass bottles of 30 mL (to avoid the effect of light on oxidative stability). The bottles were completely filled with VIO, VAO, WO, and their blend, with no remaining headspace to minimize exposure to atmospheric oxygen, a major factor in oxidation. After filling, the bottles were securely sealed to maintain the integrity of the contents. The thermal oxidation reaction was accelerated in a forced-air oven (VWR, Sheldon manufacturing, INC. Cornelius, Oregon, USA) set at  $60 \pm 2$  °C in the dark for a period of 28 days (4 weeks), according to AOCS Official method Cg 5–97 (AOCS, 1997). This process mimics long-term aging and was conducted in the dark to prevent light-induced degradation (Lakhlifi El Idrissi et al., 2024). Oil samples were withdrawn weekly and stored at 4 °C for triplicate analysis to monitor oxidative changes. As a control, untreated samples were utilized.

### 2.4. Analytical procedures for fresh and stored oils

#### 2.4.1. Chemical oxidation markers

The evolution of oxidative deterioration of pure and blended oils

during storage was monitored by measuring weekly changes in the following chemical oxidative parameters, according to ISO standard methods:

Acid value (AV) (ISO 660, 2020) was determined using a volumetric titration of oil dissolved in 1:1 (v/v) ethanol/ether with 0.1 N ethanolic potassium hydroxide (KOH) using phenolphthalein as an indicator. AV was expressed as milligrams of KOH per gram of oil.

Peroxide value (PV) (ISO 27107, 2017) was determined by iodine titration of an oil dissolved in a 2:1 (v/v) mixture of isooctane and acetic acid with a 0.01 N sodium thiosulfate solution, using starch aqueous solution as an indicator. PV was represented as milliequivalents of active oxygen per kilogram of oil (meq O<sub>2</sub>/kg).

UV-spectrophotometric indexes (E<sub>232</sub> and E<sub>270</sub>) (ISO 3656, 2011) of a 1 % (w/v) oil solution in cyclohexane were determined in a 10 mm cuvette at excitation wavelengths of 232 and 270 nm using a UV-5800PC spectrophotometer (Shanghai Metash Instruments Co., Ltd., Shanghai, China).

p-anisidine value (p-AnV) (ISO 6885, 2016) was measured spectrophotometrically at 350 nm of an oil solution in isooctane before and after reaction with p-anisidine in glacial acetic acid.

The oxidation states of the oils were analyzed by employing Holm's equation,  $OV = p\text{-AnV} + (2 \times PV)$  Eq. (1), commonly to assess the extent of oxidation in oils (El Idrissi et al., 2023). Additionally, the corresponding flavor scores were also computed using List's equation,  $F = 7.7 - (0.35 \times OV)$  Eq. (2), to predict theoretical flavor scores (Herch et al., 2014).

#### 2.4.2. Determination of pigment content

The carotenoids and chlorophyll levels in fresh and stored oils were assessed following the methodology outlined by Espínola et al. (2021). In brief, 7.5 g of oil was weighed and dissolved in 25 mL cyclohexane. Absorbance values of the resulting mixture were measured using a UV-5800PC spectrophotometer (Shanghai Metash Instruments Co., Ltd., Shanghai, China) at 670 nm for chlorophyll and 470 nm for carotenoids. The specific extinction coefficient values for lutein and pheophytin, a major component of the carotenoid and chlorophylls fractions, were  $\epsilon_0 = 2000$  and  $\epsilon_0 = 613$ , respectively. The pigment contents were calculated utilizing Equations (3) and (4):

$$\text{Chlorophyll (mg/kg)} = \frac{A_{670} \times 10^6}{613 \times 100 \times l} \quad (3)$$

$$\text{Carotenoid (mg/kg)} = \frac{A_{470} \times 10^6}{2000 \times 100 \times l} \quad (4)$$

Where 'A' represents the absorbance and 'l' represents the thickness of the spectrophotometer cell (1 cm). The carotenoids and chlorophylls were quantified in terms of milligrams of  $\alpha$ -pheophytin and lutein per kilogram of oil, respectively.

#### 2.4.3. Fatty acid composition

In accordance with the ISO 12966-2 (2017), FAs of oils were transformed into their methyl ester derivatives (FAMES) by agitating a solution containing 0.1 g of oil and 2 mL of n-hexane with 100  $\mu$ L of 2 N methanolic potassium hydroxide. The methyl ester-containing hexane layer was dehydrated after being separated with 1 g of anhydrous sodium hydrogen sulfate. The FA composition was identified according to the ISO 12966-4 (2015) method. A Chromatec-Crystal 9000 (Chromatec Company, Yoshkar-Ola, Russia) gas chromatography system equipped with a flame ionization detector and a BPX70 capillary column (60 m  $\times$  0.32 mm inner diameter; 0.25  $\mu$ m film thickness, Varian Inc., Middelburg, Netherlands) was used for FAMES analysis. As the carrier gas, helium was utilized at a rate of 1 mL/min. The injector and detector were operated at 250 °C. The oven temperature was initially regulated at 170 °C for 3 min and programmed to increase up to 230 °C at 4 °C/min, and then maintained for 15 min. A split mode injection of 1  $\mu$ L was

performed at a ratio of 1:50. Peak identification was accomplished by comparing retention times with FAMES from oils with known FA profiles analyzed under the specified conditions of this method. Results were reported as weight percentages.

#### 2.4.4. Nutritional quality indices

The nutritional value of FAs present in parent and blended oils was evaluated using the indices provided in Table 1S in Supplementary file 1 (S1). Additionally, the potential of these oils in disease prevention and treatment was investigated. The nutritional indices were computed based on the quantities of FAs, following the formula provided by Szpunar-Krok and Wondolowska-Grabowska (2022) as well as Chen and Liu (2020). A detailed description of each index was provided in the results and discussion section.

#### 2.4.5. Tocopherol composition

Tocopherol determination was carried out according to ISO 9936 (2016) standards. The HPLC analysis was performed using a Shimadzu LC-2050C 3D (Shimadzu, Kyoto, Japan) equipped with a fluorescence spectrophotometer detector (RF-20 A, Shimadzu, Japan) (wavelengths for excitation 290 nm and emission 330 nm) and a LiChrospher Si-60 column (25 cm  $\times$  4.6 mm inner diameter, 5  $\mu$ m film thickness, Merck, Darmstadt, Germany). A 0.45  $\mu$ m PTFE membrane-filtered solution of 2 g oil dissolved in 25 mL n-heptane was injected directly by an autosampler. The mobile phase comprised a mixture of isopropanol and isooctane (1:99, v/v), with a 1.2 mL/min flow rate. Peak integration and quantitative calculation were conducted with LabSolution software and retention time served as the basis for identification. Total and individual amounts of tocopherol were ascertained utilizing external standards of tocopherol isomers. Results were expressed in milligrams of tocopherols per kilogram of oil (mg/kg). Vitamin E activity, representing the vitamin E equivalent of all tocopherol homologs, and the ratio between  $\alpha$ -tocopherol equivalent and polyunsaturated fatty acids ( $\alpha$ -TE/PUFA), were obtained computationally (Romanić et al., 2021).

#### 2.5. Statistical data analysis

The analytical experiments were conducted in triplicate, and the results were reported as the mean  $\pm$  standard deviation. The findings underwent an analysis of variance (ANOVA) and Tukey's test at a 95.0 % confidence level, utilizing IBM SPSS Statistics Version 21 software (SPSS Inc., Chicago, IL, USA). The statistical significance threshold was established at a p-value below 0.05, and the Pearson test was employed to examine the level of correlation between the variables.

The FA profile (MUFA, PUFA, and SFA), nutritional indices (including PUFA/SFA,  $\omega$ 6/ $\omega$ 3, DFA, OFA, HH, AI, TI, and COX), tocopherol isomers content ( $\alpha$ -tocopherol,  $\gamma$ -tocopherol, and  $\delta$ -tocopherol), vitamin E activity,  $\alpha$ -TE/PUFA ratio, and OV were investigated using principal component analysis (PCA) to discern any differences between pure and blended peanut oils. In addition, a hierarchical cluster analysis (HCA) was conducted to investigate the interrelationships among pure and blended peanut oils based on clustering. The Ward cluster's method and the squared Euclidean distance, considered as similarity factors, were utilized to establish the dendrogram. The Chi-squared Automatic Interaction Detector (CHAID) was employed to ascertain whether descriptive characteristics can effectively forecast which oil has a substantial amount of PUFA and a calculated oxidizability value (COX). If this is the case, guidelines will be established to assist in categorizing the oils based on all the assessed data. PCA, HCA, and CHAID analyses were performed using the XLSTAT 2014 software produced by Microsoft® in Redmond, Washington, USA.

### 3. Results and discussion

#### 3.1. Oil blends and their initial chemical quality

In order to determine the effect of blending VIO and VAO with WO, it's crucial to examine the FA and tocopherol profiles. These compounds play a crucial role in indicating nutritional values, particularly in terms of unsaturated FAs. Tocopherols, in particular, act as antioxidants, preventing fat oxidation and the development of rancidity, thereby contributing to enhanced preservation.

##### 3.1.1. Initial chemical characteristics

The initial values for hydrolytic (AV) and oxidative (PV, p-AnV, E<sub>232</sub>, E<sub>270</sub>, and OV) degradation indicators, theoretical flavor scores value as well as chlorophyll and carotenoid content of parent and blended oils are shown in Table 1. All chemical parameter values for pure WO were significantly ( $p < 0.05$ ) higher than those for VIO and VAO. In the VIO:WO and VAO:WO blends, data of AV, PV, E<sub>232</sub>, E<sub>270</sub>, and OV varied proportionally to the amount of peanut oil and WO used in the mixture, in accordance with the published literature (Bordón et al., 2019; Choudhary et al., 2015).

The addition of WO to VIO and VAO resulted in a slight increase in AV, ranging from 0.67 to 0.79 mg KOH/g for VIO:WO blends and from 0.79 to 0.90 mg KOH/g for VAO:WO blends, much lower than the Codex-allowed average of 4.00 mg KOH/g. The initial PV for VIO, VAO, and WO was 0.45, 1.95, and 2.55 meq O<sub>2</sub>/kg, respectively. An increase in PV was observed with increasing WO proportion, remaining within the limits specified by the Codex Alimentarius Commission for cold-pressed oils (15 meq O<sub>2</sub>/kg). These results align with the finding of Neeharika et al. (2017), who reported that groundnut and cottonseed oil blends in proportions ranging from 80:20 to 20:80 exhibited low AV and PV. Therefore, the p-AnV of the blended peanut oils was considerably higher compared to the pure oils, and their values increased with increasing WO concentration. This might be due to the presence of saturated and unsaturated medium- and high-molecular-weight aldehydes, mainly resulting from the oxidation of PUFAs, such as C18:2 and C18:3, which is present in elevated concentration in WO (Bordón et al., 2019). A recent study conducted by Ndomou et al. (2023) showed that the increase in the proportion of stearin and groundnut oil in quaternary blends leads to an increase of p-AnV; whilst an increase in the olein ratio leads to a decrease in this parameter (2.63 to 26.66).

As regards OV, a similar trend of change was observed as for PV and p-AnV, with VAO:WO blends exhibiting higher levels (8.96–16.47).

However, these values remained within the industry-approved standards for edible oil (30), reflecting a higher quality of the oils. Conversely, Ndomou et al. (2023) reported that the increase in the olein and groundnut oils proportion resulted in a significant rise in OV from 11.96 to 32.55 in the quaternary blends. Although the equation was developed for a particular set of experiments, VIO exhibited a quite acceptable flavor score value of 7.31, whereas VAO:WO blends at ratios of (90:10), (80:20), and (70:30) showed low acceptance. The remaining oils demonstrated average flavor score values ranging from 4.35 to 5.68.

Preliminary results on the sensory acceptance of sunflower oil enriched with  $\omega$ 3 FAs from flaxseed oil showed that samples with an 80 % refined sunflower oil and 20 % flaxseed oil blend received higher scores for color ( $4.4 \pm 0.7$ ), average rating ( $4.0 \pm 0.7$ ), and total acceptability ( $3.9 \pm 0.7$ ) compared to pure refined sunflower oil ( $3.3 \pm 0.9$ ,  $3.8 \pm 0.9$ , and  $3.8 \pm 1.0$ , respectively), indicating better sensory acceptability of the blend containing 20 % flaxseed oil compared to refined sunflower oil (Romanić et al., 2021). In addition, blends of cottonseed and groundnut oils were evaluated for their sensory attributes, receiving scores for color, taste, texture, flavor, and overall acceptability ranging from 8.25 to 8.75, 7.63 to 8.88, 7.63 to 8.63, and 7.75 to 8.88, respectively. These scores represent increases of approximately 11.35, 29.07, 25, 30.16, and 25.54 % in each attribute compared to unblended groundnut oil (Neeharika et al., 2017).

In Table 1, it can be observed that the recorded values of E<sub>232</sub> and E<sub>270</sub> for VIO and the corresponding blends were significantly ( $p < 0.05$ ) lower than those for VAO:WO blends. As expected, the latter behaved similarly to PV and p-AnV, due to their correlation. Moreover, WO displayed lower chlorophyll and carotenoid contents of 0.50 and 0.57 mg/kg, respectively, in comparison to VIO (1.06 and 0.84 mg/kg) and VAO (0.81 and 0.66 mg/kg). Nonetheless, as the proportion of WO in the mixtures increased, the pigment contents dropped. Torres et al. (2011) observed a comparable pattern, wherein the incorporation of WO into virgin olive oil at 20, 40, 60, and 80 % resulted in a gradual decrease in chlorophylls (7.31–2.58  $\mu$ g/g oil) and carotenoids content (3.00–1.99  $\mu$ g/g oil).

##### 3.1.2. Fatty acid profile of pure oils and binary blends

Table 2 presents the FA composition of the parent oils and their blends in various proportions. The primary FAs found in VIO and VAO are oleic acid (C18:1; 50.95 and 38.28 %, respectively) and linoleic acid (C18:2; 29.76 and 38.53 %, respectively). Notably, VIO has the highest C18:1 content, whereas VAO exhibits a nearly equal proportion of C18:1 and C18:2. The main SFAs include palmitic acid (C16:0), stearic acid

**Table 1**

Initial chemical oxidation parameters, theoretical flavor scores, and pigment contents of pure Virginia (VIO), Valencia (VAO), and walnut (WO) oils, and their binary blends.

Parameters	Pure oils			VIO:WO blends (v/v)				VAO:WO blends (v/v)			
	VIO	VAO	WO	(95:5)	(90:10)	(80:20)	(70:30)	(95:5)	(90:10)	(80:20)	(70:30)
Acid value (AV, mg/kg)	0.67 ± 0.00 <sup>a</sup>	0.67 ± 0.00 <sup>a</sup>	0.90 ± 0.00 <sup>b</sup>	0.67 ± 0.00 <sup>a</sup>	0.67 ± 0.00 <sup>a</sup>	0.73 ± 0.00 <sup>c</sup>	0.79 ± 0.00 <sup>d</sup>	0.79 ± 0.00 <sup>d</sup>	0.79 ± 0.00 <sup>d</sup>	0.79 ± 0.00 <sup>d</sup>	0.90 ± 0.00 <sup>b</sup>
Peroxide value (PV, meq O <sub>2</sub> /kg)	0.45 ± 0.05 <sup>a</sup>	1.95 ± 0.05 <sup>b</sup>	2.55 ± 0.05 <sup>c</sup>	1.27 ± 0.02 <sup>d</sup>	2.22 ± 0.02 <sup>e</sup>	2.55 ± 0.05 <sup>c</sup>	2.75 ± 0.05 <sup>c</sup>	2.67 ± 0.07 <sup>c</sup>	3.45 ± 0.05 <sup>f</sup>	3.65 ± 0.05 <sup>f</sup>	5.32 ± 0.02 <sup>g</sup>
p-anisidine value (p-AnV)	0.19 ± 0.03 <sup>a</sup>	0.46 ± 0.02 <sup>b</sup>	1.95 ± 0.01 <sup>c</sup>	3.21 ± 0.05 <sup>d</sup>	3.51 ± 0.03 <sup>e</sup>	3.78 ± 0.04 <sup>f</sup>	4.05 ± 0.06 <sup>g</sup>	3.61 ± 0.00 <sup>ef</sup>	3.97 ± 0.04 <sup>fg</sup>	4.37 ± 0.02 <sup>h</sup>	5.83 ± 0.02 <sup>i</sup>
Oxidation value (OV)	1.09 ± 0.13 <sup>a</sup>	4.36 ± 0.08 <sup>b</sup>	7.05 ± 0.11 <sup>c</sup>	5.76 ± 0.00 <sup>d</sup>	7.96 ± 0.01 <sup>e</sup>	8.88 ± 0.14 <sup>f</sup>	9.55 ± 0.04 <sup>g</sup>	8.96 ± 0.14 <sup>f</sup>	10.87 ± 0.14 <sup>h</sup>	11.67 ± 0.12 <sup>i</sup>	16.47 ± 0.01 <sup>j</sup>
Theoretical flavor score	7.31 ± 0.04 <sup>a</sup>	6.17 ± 0.02 <sup>b</sup>	5.23 ± 0.03 <sup>c</sup>	5.68 ± 0.00 <sup>d</sup>	4.91 ± 0.00 <sup>e</sup>	4.59 ± 0.05 <sup>f</sup>	4.35 ± 0.01 <sup>g</sup>	4.56 ± 0.05 <sup>f</sup>	3.89 ± 0.05 <sup>h</sup>	3.61 ± 0.04 <sup>i</sup>	1.93 ± 0.00 <sup>j</sup>
E <sub>232</sub>	0.99 ± 0.01 <sup>a</sup>	1.03 ± 0.00 <sup>ac</sup>	1.22 ± 0.01 <sup>b</sup>	1.03 ± 0.00 <sup>ac</sup>	1.08 ± 0.00 <sup>cd</sup>	1.13 ± 0.02 <sup>d</sup>	1.18 ± 0.00 <sup>b</sup>	1.41 ± 0.01 <sup>e</sup>	1.50 ± 0.01 <sup>f</sup>	1.54 ± 0.00 <sup>fg</sup>	1.58 ± 0.01 <sup>g</sup>
E <sub>270</sub>	0.05 ± 0.00 <sup>a</sup>	0.10 ± 0.01 <sup>b</sup>	0.16 ± 0.00 <sup>cd</sup>	0.04 ± 0.00 <sup>ac</sup>	0.07 ± 0.00 <sup>ac</sup>	0.06 ± 0.00 <sup>ab</sup>	0.07 ± 0.00 <sup>a</sup>	0.14 ± 0.00 <sup>de</sup>	0.15 ± 0.00 <sup>e</sup>	0.12 ± 0.00 <sup>e</sup>	0.19 ± 0.00 <sup>f</sup>
Chlorophylls (mg/kg)	1.06 ± 0.01 <sup>a</sup>	0.81 ± 0.00 <sup>b</sup>	0.50 ± 0.00 <sup>cd</sup>	0.96 ± 0.02 <sup>a</sup>	0.76 ± 0.02 <sup>b</sup>	0.58 ± 0.01 <sup>cd</sup>	0.41 ± 0.02 <sup>df</sup>	0.77 ± 0.03 <sup>b</sup>	0.55 ± 0.03 <sup>ce</sup>	0.45 ± 0.00 <sup>ef</sup>	0.28 ± 0.00 <sup>g</sup>
Carotenoids (mg/kg)	0.84 ± 0.00 <sup>a</sup>	0.66 ± 0.00 <sup>b</sup>	0.57 ± 0.00 <sup>c</sup>	0.76 ± 0.01 <sup>d</sup>	0.47 ± 0.02 <sup>e</sup>	0.31 ± 0.00 <sup>f</sup>	0.20 ± 0.00 <sup>g</sup>	0.54 ± 0.00 <sup>b</sup>	0.37 ± 0.00 <sup>h</sup>	0.28 ± 0.00 <sup>f</sup>	0.19 ± 0.01 <sup>g</sup>

Values (mean ± SD, n = 3) with different superscripts within a row differ significantly ( $p < 0.05$ ) among peanut samples.

Table 2

Fatty acid profile (%) and nutritional quality indices (%) of pure Virginia (VIO), Valencia (VAO), and walnut (WO) oils, and their binary blends.

Fatty acids (%)	Pure oils			VIO:WO blends (v/v)				VAO:WO blends (v/v)			
	VIO	VAO	WO	(95:5)	(90:10)	(80:20)	(70:30)	(95:5)	(90:10)	(80:20)	(70:30)
C16:0	9.00 ± 0.00 <sup>a</sup>	10.81 ± 0.01 <sup>b</sup>	7.02 ± 0.02 <sup>c</sup>	8.92 ± 0.02 <sup>d</sup>	8.85 ± 0.01 <sup>e</sup>	8.57 ± 0.00 <sup>f</sup>	8.41 ± 0.01 <sup>g</sup>	10.64 ± 0.01 <sup>h</sup>	10.48 ± 0.00 <sup>i</sup>	10.01 ± 0.00 <sup>j</sup>	9.67 ± 0.00 <sup>k</sup>
C18:0	2.79 ± 0.01 <sup>a</sup>	3.66 ± 0.01 <sup>b</sup>	2.82 ± 0.00 <sup>ac</sup>	2.84 ± 0.01 <sup>c</sup>	2.84 ± 0.01 <sup>a</sup>	2.87 ± 0.01 <sup>c</sup>	2.86 ± 0.01 <sup>c</sup>	3.61 ± 0.01 <sup>bd</sup>	3.59 ± 0.01 <sup>d</sup>	3.47 ± 0.01 <sup>e</sup>	3.42 ± 0.01 <sup>e</sup>
C18:1	50.95 ± 0.03 <sup>a</sup>	38.28 ± 0.03 <sup>b</sup>	15.08 ± 0.02 <sup>c</sup>	49.35 ± 0.06 <sup>d</sup>	47.51 ± 0.03 <sup>e</sup>	43.71 ± 0.03 <sup>f</sup>	40.21 ± 0.02 <sup>g</sup>	37.13 ± 0.03 <sup>h</sup>	36.02 ± 0.03 <sup>i</sup>	33.48 ± 0.02 <sup>j</sup>	31.47 ± 0.02 <sup>k</sup>
C18:2	29.76 ± 0.03 <sup>a</sup>	38.53 ± 0.03 <sup>b</sup>	61.32 ± 0.04 <sup>c</sup>	31.19 ± 0.03 <sup>d</sup>	33.15 ± 0.04 <sup>e</sup>	36.30 ± 0.03 <sup>f</sup>	39.52 ± 0.03 <sup>g</sup>	39.83 ± 0.02 <sup>h</sup>	41.04 ± 0.04 <sup>i</sup>	43.60 ± 0.05 <sup>j</sup>	45.65 ± 0.03 <sup>k</sup>
C18:3	0.08 ± 0.00 <sup>a</sup>	0.09 ± 0.00 <sup>a</sup>	12.94 ± 0.02 <sup>b</sup>	0.72 ± 0.01 <sup>c</sup>	1.40 ± 0.02 <sup>d</sup>	1.40 ± 0.02 <sup>e</sup>	3.94 ± 0.00 <sup>f</sup>	0.75 ± 0.01 <sup>c</sup>	1.43 ± 0.01 <sup>d</sup>	2.78 ± 0.02 <sup>e</sup>	3.91 ± 0.01 <sup>f</sup>
C20:0	1.47 ± 0.01 <sup>a</sup>	1.71 ± 0.01 <sup>b</sup>	0.19 ± 0.00 <sup>c</sup>	1.39 ± 0.01 <sup>d</sup>	1.31 ± 0.00 <sup>e</sup>	1.16 ± 0.01 <sup>f</sup>	0.99 ± 0.00 <sup>g</sup>	1.61 ± 0.01 <sup>h</sup>	1.49 ± 0.01 <sup>a</sup>	1.33 ± 0.01 <sup>e</sup>	1.18 ± 0.00 <sup>f</sup>
C20:1	1.20 ± 0.00 <sup>a</sup>	1.03 ± 0.00 <sup>b</sup>	0.28 ± 0.00 <sup>c</sup>	1.11 ± 0.00 <sup>d</sup>	1.05 ± 0.00 <sup>b</sup>	0.99 ± 0.00 <sup>e</sup>	0.86 ± 0.00 <sup>f</sup>	0.99 ± 0.00 <sup>e</sup>	0.92 ± 0.00 <sup>g</sup>	0.83 ± 0.00 <sup>i</sup>	0.74 ± 0.00 <sup>j</sup>
C22:0	2.98 ± 0.01 <sup>a</sup>	3.75 ± 0.02 <sup>b</sup>	0.00 ± 0.00 <sup>c</sup>	2.79 ± 0.02 <sup>d</sup>	2.52 ± 0.01 <sup>e</sup>	2.30 ± 0.01 <sup>f</sup>	2.05 ± 0.00 <sup>g</sup>	3.57 ± 0.01 <sup>h</sup>	3.30 ± 0.01 <sup>i</sup>	2.94 ± 0.01 <sup>a</sup>	2.59 ± 0.01 <sup>e</sup>
C24:0	1.39 ± 0.00 <sup>a</sup>	1.59 ± 0.01 <sup>b</sup>	0.00 ± 0.00 <sup>c</sup>	1.31 ± 0.01 <sup>d</sup>	1.01 ± 0.01 <sup>e</sup>	1.01 ± 0.00 <sup>f</sup>	0.79 ± 0.00 <sup>g</sup>	1.53 ± 0.02 <sup>g</sup>	1.36 ± 0.00 <sup>a</sup>	1.23 ± 0.00 <sup>h</sup>	1.04 ± 0.00 <sup>e</sup>
MUFA	52.32 ± 0.04 <sup>a</sup>	39.43 ± 0.03 <sup>b</sup>	15.50 ± 0.01 <sup>c</sup>	50.62 ± 0.06 <sup>d</sup>	48.71 ± 0.03 <sup>e</sup>	44.86 ± 0.04 <sup>f</sup>	41.22 ± 0.02 <sup>g</sup>	38.25 ± 0.03 <sup>h</sup>	37.06 ± 0.04 <sup>i</sup>	34.44 ± 0.02 <sup>j</sup>	32.35 ± 0.03 <sup>k</sup>
PUFA	29.84 ± 0.03 <sup>a</sup>	38.62 ± 0.03 <sup>b</sup>	74.26 ± 0.02 <sup>c</sup>	31.91 ± 0.02 <sup>d</sup>	34.55 ± 0.06 <sup>e</sup>	39.03 ± 0.05 <sup>f</sup>	43.47 ± 0.04 <sup>g</sup>	40.58 ± 0.03 <sup>h</sup>	42.48 ± 0.03 <sup>i</sup>	46.39 ± 0.08 <sup>j</sup>	49.56 ± 0.04 <sup>k</sup>
SFA	17.74 ± 0.01 <sup>a</sup>	21.66 ± 0.03 <sup>b</sup>	10.18 ± 0.03 <sup>c</sup>	17.35 ± 0.00 <sup>d</sup>	16.63 ± 0.01 <sup>e</sup>	16.03 ± 0.04 <sup>f</sup>	15.22 ± 0.03 <sup>g</sup>	21.05 ± 0.01 <sup>h</sup>	20.32 ± 0.04 <sup>i</sup>	19.09 ± 0.03 <sup>j</sup>	18.01 ± 0.01 <sup>k</sup>
<b>Nutritional quality indices (%)</b>											
ω6/ω3	374.92 ± 2.54 <sup>a</sup>	430.61 ± 2.83 <sup>b</sup>	4.74 ± 0.01 <sup>c</sup>	43.47 ± 0.79 <sup>d</sup>	23.68 ± 0.30 <sup>e</sup>	13.30 ± 0.09 <sup>f</sup>	10.02 ± 0.01 <sup>cf</sup>	53.25 ± 0.80 <sup>g</sup>	28.59 ± 0.31 <sup>e</sup>	15.67 ± 0.13 <sup>f</sup>	11.66 ± 0.04 <sup>f</sup>
MUFA/PUFA	1.75 ± 0.00 <sup>a</sup>	1.02 ± 0.00 <sup>b</sup>	0.21 ± 0.00 <sup>c</sup>	1.58 ± 0.00 <sup>d</sup>	1.41 ± 0.00 <sup>e</sup>	1.15 ± 0.00 <sup>f</sup>	0.95 ± 0.00 <sup>g</sup>	0.94 ± 0.00 <sup>h</sup>	0.87 ± 0.00 <sup>i</sup>	0.74 ± 0.00 <sup>j</sup>	0.65 ± 0.00 <sup>k</sup>
UFA/SFA	4.63 ± 0.01 <sup>a</sup>	3.60 ± 0.00 <sup>b</sup>	8.82 ± 0.03 <sup>c</sup>	4.75 ± 0.00 <sup>d</sup>	5.00 ± 0.00 <sup>e</sup>	5.23 ± 0.02 <sup>f</sup>	5.56 ± 0.01 <sup>g</sup>	3.74 ± 0.00 <sup>h</sup>	3.91 ± 0.00 <sup>i</sup>	4.23 ± 0.01 <sup>j</sup>	4.55 ± 0.00 <sup>k</sup>
PUFA/SFA	1.68 ± 0.00 <sup>a</sup>	1.78 ± 0.01 <sup>b</sup>	7.29 ± 0.02 <sup>c</sup>	1.84 ± 0.00 <sup>d</sup>	2.07 ± 0.01 <sup>e</sup>	2.43 ± 0.01 <sup>f</sup>	2.85 ± 0.00 <sup>g</sup>	1.93 ± 0.00 <sup>h</sup>	2.09 ± 0.00 <sup>e</sup>	2.43 ± 0.01 <sup>f</sup>	2.75 ± 0.01 <sup>i</sup>
DFA	84.95 ± 0.08 <sup>a</sup>	81.71 ± 0.01 <sup>b</sup>	92.59 ± 0.03 <sup>c</sup>	85.37 ± 0.04 <sup>d</sup>	86.11 ± 0.02 <sup>e</sup>	86.76 ± 0.08 <sup>f</sup>	87.55 ± 0.05 <sup>g</sup>	82.45 ± 0.01 <sup>h</sup>	83.12 ± 0.01 <sup>i</sup>	84.30 ± 0.04 <sup>j</sup>	85.33 ± 0.02 <sup>d</sup>
OFA	9.03 ± 0.00 <sup>a</sup>	10.85 ± 0.01 <sup>b</sup>	7.08 ± 0.02 <sup>c</sup>	8.95 ± 0.02 <sup>d</sup>	8.88 ± 0.01 <sup>d</sup>	8.60 ± 0.00 <sup>e</sup>	8.44 ± 0.01 <sup>f</sup>	10.67 ± 0.01 <sup>g</sup>	10.51 ± 0.01 <sup>h</sup>	10.04 ± 0.01 <sup>i</sup>	9.70 ± 0.01 <sup>j</sup>
HH	8.94 ± 0.01 <sup>a</sup>	7.09 ± 0.01 <sup>b</sup>	12.62 ± 0.04 <sup>c</sup>	9.08 ± 0.02 <sup>d</sup>	9.23 ± 0.01 <sup>e</sup>	9.61 ± 0.01 <sup>f</sup>	9.91 ± 0.02 <sup>g</sup>	7.28 ± 0.01 <sup>h</sup>	7.46 ± 0.01 <sup>i</sup>	7.95 ± 0.00 <sup>j</sup>	8.35 ± 0.01 <sup>k</sup>
AI	0.11 ± 0.00 <sup>a</sup>	0.14 ± 0.00 <sup>b</sup>	0.08 ± 0.00 <sup>f</sup>	0.11 ± 0.00 <sup>a</sup>	0.11 ± 0.00 <sup>a</sup>	0.10 ± 0.00 <sup>c</sup>	0.10 ± 0.00 <sup>c</sup>	0.14 ± 0.00 <sup>b</sup>	0.13 ± 0.00 <sup>d</sup>	0.12 ± 0.00 <sup>e</sup>	0.12 ± 0.00 <sup>e</sup>
TI	0.29 ± 0.00 <sup>a</sup>	0.37 ± 0.00 <sup>b</sup>	0.13 ± 0.00 <sup>c</sup>	0.27 ± 0.00 <sup>d</sup>	0.26 ± 0.00 <sup>d</sup>	0.23 ± 0.00 <sup>f</sup>	0.22 ± 0.00 <sup>f</sup>	0.35 ± 0.00 <sup>g</sup>	0.32 ± 0.01 <sup>h</sup>	0.28 ± 0.00 <sup>a</sup>	0.26 ± 0.00 <sup>d</sup>
ODR	36.93 ± 0.01 <sup>a</sup>	50.22 ± 0.04 <sup>b</sup>	83.12 ± 0.01 <sup>c</sup>	39.27 ± 0.04 <sup>d</sup>	42.10 ± 0.06 <sup>e</sup>	47.17 ± 0.01 <sup>f</sup>	51.95 ± 0.01 <sup>g</sup>	52.21 ± 0.04 <sup>h</sup>	54.11 ± 0.03 <sup>i</sup>	58.08 ± 0.06 <sup>j</sup>	61.16 ± 0.04 <sup>k</sup>
LDR	0.26 ± 0.01 <sup>a</sup>	0.23 ± 0.00 <sup>a</sup>	17.42 ± 0.03 <sup>b</sup>	2.25 ± 0.04 <sup>c</sup>	4.05 ± 0.05 <sup>d</sup>	6.99 ± 0.01 <sup>e</sup>	9.07 ± 0.01 <sup>f</sup>	1.84 ± 0.02 <sup>g</sup>	3.38 ± 0.04 <sup>h</sup>	6.00 ± 0.05 <sup>i</sup>	7.89 ± 0.01 <sup>k</sup>
COX	3.59 ± 0.01 <sup>a</sup>	4.37 ± 0.00 <sup>b</sup>	9.26 ± 0.00 <sup>c</sup>	3.86 ± 0.00 <sup>d</sup>	4.19 ± 0.01 <sup>e</sup>	4.76 ± 0.01 <sup>f</sup>	5.32 ± 0.01 <sup>g</sup>	4.63 ± 0.01 <sup>h</sup>	4.90 ± 0.00 <sup>i</sup>	5.43 ± 0.01 <sup>j</sup>	5.86 ± 0.01 <sup>k</sup>
S/P	0.14 ± 0.00 <sup>a</sup>	0.19 ± 0.00 <sup>b</sup>	0.11 ± 0.00 <sup>c</sup>	0.14 ± 0.00 <sup>a</sup>	0.14 ± 0.00 <sup>a</sup>	0.14 ± 0.00 <sup>a</sup>	0.13 ± 0.00 <sup>d</sup>	0.18 ± 0.00 <sup>e</sup>	0.18 ± 0.00 <sup>e</sup>	0.17 ± 0.00 <sup>f</sup>	0.16 ± 0.00 <sup>g</sup>
OL/(LA + ALA)	1.71 ± 0.00 <sup>a</sup>	0.99 ± 0.00 <sup>b</sup>	0.20 ± 0.00 <sup>c</sup>	1.54 ± 0.00 <sup>d</sup>	1.37 ± 0.00 <sup>e</sup>	1.12 ± 0.00 <sup>f</sup>	0.92 ± 0.01 <sup>g</sup>	0.91 ± 0.01 <sup>g</sup>	0.85 ± 0.00 <sup>h</sup>	0.72 ± 0.00 <sup>i</sup>	0.63 ± 0.01 <sup>j</sup>

Values (mean ± SD, n = 3) with different superscripts within a row differ significantly ( $p < 0.05$ ) among samples. MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; SFA: Saturated fatty acids; UFA: Unsaturated fatty acids; DFA: Index of desirable fatty acids; OFA: Sum of hypercholesterolemic fatty acids; HH: Ratio of hypocholesterolemic to hypercholesterolemic; AI: Index of atherogenicity; TI: Index of thrombogenicity; ODR: Oleic desaturation ratio; LDR: Linoleic desaturation ratio; COX: Calculated oxidizability value; S/P: Saturation fat index; OL: oleic acid; LA: Linoleic acid; ALA: Linolenic acid.

(C18:0), and behenic acid (C22:0); however, VAO has a higher SFA content (21.66 %) in comparison to VIO (17.74 %). MUFA content was higher in VIO, accounting for 52.32 %. On the other hand, PUFAs were found in greater quantities in VAO (38.62 %) due to their high C18:2 content. As regards WO, the predominant FAs were C18:2 (61.32 %), followed by C18:1 (15.08 %), and linolenic acid (C18:3; 12.94 %). WO contains an extremely high level of PUFA (74.26 %), primarily attributed to its high C18:2 and C18:3 content, which is significantly greater than that of VAO and VIO. Therefore, the MUFA content in WO was relatively low (15.50 %) compared to VIO and VAO. These results were consistent with the literature and confirmed the distinct FA profile of the three pure oils, which represents the basis of the present work (El Idrissi

et al., 2023; Idrissi et al., 2022; Eloufy et al., 2022).

The blending of VIO and VAO with WO significantly altered the composition of the analyzed FAs. The major changes were observed for palmitic, oleic, linoleic, and linolenic FAs content. These results were consistent with those of other authors (Sharma et al., 2023; Hashempour-Baltork et al., 2016). The C18:1 content of the VIO:WO and VAO:WO mixtures decreases as the concentration of WO increases, compared with pure VIO and VAO, leading to a reduction in MUFA content. For instance, blending with WO resulted in significant decreases from 52.32 % (VIO) to 50.62, 48.71, 44.86, and 41.22 % in the MUFA content of VIO:WO blends in (95:5), (90:10), (80:20) and (70:30), respectively. Therefore, a greater decrease in C16:0 content was also

observed in the blended oils, leading to a reduction in SFA content, ranging from 17.35 to 15.22 % for VIO:WO blends and from 21.05 to 18.01 % for VAO:WO blends compared to the pure oils.

On the other hand, mixing VIO and VAO with WO leads to an increase in the level of C18:2 and C18:3 as the proportion of WO in the oils rises. As a result, the C18:2 and C18:3 contents in the final oil blends were significantly higher than in the parent oils (VIO and VAO). For example, adding WO to VIO at (95:5), (90:10), (80:20), and (70:30) resulted in a gradual increase, with values of 0.72, 1.40, 2.73, and 3.94 %, respectively, in the C18:3 proportion of the resulting blends compared to pure VIO (0.08 %). In addition, the VAO:WO mixture showed a notable increase in PUFA content, rising from 40.58 % at a (95:5) ratio to 49.56 % at a (70:30) ratio. This is mainly due to the enrichment of VAO in C18:2 and C18:3 after the incorporation of WO.

Several studies mentioned the effect of blending oils on the FA profile. Torres et al. (2011) reported comparable results, indicating that the addition of WO to virgin olive oil resulted in a progressive rise in PUFA levels and a decline in MUFA and SFA levels. In addition, Bordón et al. (2019) stated that the incorporation of chia seed oil into walnut, almond, virgin, and roasted sesame oils at 20, 30, and 40 % caused a gradual increase in C18:3 and a decrease in C18:2 and C18:1 proportions of the resulting binary blends compared to the pure oils. In contrast to our findings, Pan et al. (2020) revealed that as the proportion of almond oil increased (from 5 to 30 %) in the almond:walnut blend oils, the proportion of MUFA also gradually increased, while the PUFA and SFA levels decreased. Therefore, all the blends of groundnut oil with other minor oils (red palm olein, rice bran oil, and sesame oil) showed a notable elevation in MUFA and PUFA contents (Sunil et al., 2015).

There was a significant negative correlation between MUFA and PUFA ( $r^2 = -0.777$ ,  $p < 0.05$ ), and PUFA showed a strong negative association with SFA ( $r^2 = -0.619$ ,  $p < 0.05$ ) according to Pearson's correlation analysis (Tables 2S and 3S, S1).

### 3.1.3. Nutritional quality indices of pure oils and binary blends

FAs can either contribute positively or negatively to the prevention and treatment of diseases. The quality of lipids' health in parent oils and their blends was evaluated by nutritional indices based on their FAs composition, as presented in Table 2.

**3.1.3.1.  $\omega 6/\omega 3$ , MUFA/PUFA, UFA/SFA, PUFA/SFA.**  $\omega 3$  PUFAs are thought to influence several health conditions, including cardiovascular disease, diabetes, Alzheimer's disease, and visual and neurological development (Pattnaik & Mishra, 2022). It is nutritionally important to consider changes in the quantity of SFAs, as well as raising the total of  $\omega 3$  PUFAs and reducing the ratio of  $\omega 6/\omega 3$  PUFAs (Chen & Liu, 2020). As per the FAO/WHO guidelines, it is recommended to maintain a specific ratio of  $\omega 6/\omega 3$  in daily intake, such as 5–10:1 (WHO, 2008). The  $\omega 6/\omega 3$  ratio is regarded as a crucial factor for achieving a balanced synthesis of eicosanoids and is of great nutritional importance (Hashempour-Baltork et al., 2016). A low intake of saturated fat and an elevated PUFA/SFA ratio is linked to a reduced risk of coronary heart disease (CHD) in humans (Chen & Liu, 2020). Hence, the PUFA/SFA ratio serves as a key measure for evaluating the nutritional value of dietary lipid components, with guidelines requiring a ratio above 0.45.

The  $\omega 6/\omega 3$  ratio was 374.92 and 430.61 in VIO and VAO, respectively. Therefore, these pure oils do not exhibit an optimal  $\omega 6/\omega 3$  ratio. However, the latter decreased as the percentage of WO increased, reaching optimal values of 10.02 in the VIO:WO (70:30) blend. The  $\omega 6/\omega 3$  ratio level was balanced and consistent with the literature (Hashempour-Baltork et al., 2016). In fact, reducing the risk of various diseases is associated with a more desirable lower ratio of  $\omega 6/\omega 3$  FAs.

Regarding MUFA/PUFA, VIO:WO (95:5) blend showed a significantly higher value of 1.58, which is a favorable indicator of fat quality and its positive impact on human health compared to the other blends. This is mainly due to the reduction in MUFA and an increase in PUFA as

the WO concentration increases, which directly promotes a decrease in  $\omega 6/\omega 3$  and MUFA/PUFA ratios. The incorporation of WO, owing to its high levels of C18:2 and C18:3, led to an effective enhancement of UFA/SFA and PUFA/SFA in the parent oils. It is worth noting that a diet with a low PUFA/SFA ratio, specifically below 0.45, poses a risk for elevated blood cholesterol levels. The VIO:WO and VAO:WO (70:30) blends displayed the highest PUFA/SFA ratio, with values of 2.85 and 2.75, respectively. Thus, these findings affirm that these blended oils exhibit a well-balanced and suitable FA composition. Hashempour-Baltork et al. (2018) observed comparable outcomes, noting a markedly elevated PUFA/SFA ratio of 7.42 in linseed oil. The substantial increase in its concentration within the blends resulted in a notable rise in this ratio for the oil mixtures, with the highest PUFA/SFA ratio reaching 2.41 in the 55:30:15 olive:sesame:linseed tertiary oil blends. Pan et al. (2020) also showed that almond:walnut blended oils (ranging from 5 to 30 %) were in compliance with WHO regulations regarding  $\omega 6/\omega 3$  ratio. Therefore, as the proportion of almond oil increased in the blended oil, the PUFA/SFA ratio gradually decreased.

As depicted in Tables 2S and 3S (S1), the  $\omega 6/\omega 3$  ratio showed a negative association with OV ( $r^2 = -0.699$ ,  $p < 0.05$ ). In addition, the PUFA/SFA ratio displayed a strong positive correlation with PUFA and COX ( $r^2 = 0.937$  and  $0.964$ , respectively,  $p < 0.05$ ) and a negative correlation with MUFA and SFA ( $r^2 = -0.740$  and  $-0.822$ , respectively,  $p < 0.05$ ).

**3.1.3.2. DFA, OFA, HH indices.** The desirable FAs index (DFA) reports the hypocholesterolemic properties of the lipids studied, indicating their capacity to reduce total cholesterol levels (Szpunar-Krok & Wondolowska-Grabowska, 2022). Conversely, an increase in the hypercholesterolemic index (OFA), representing the sum of hypercholesterolemic SFAs, has the potential to increase cholesterol levels (Hashempour-Baltork et al., 2018). The hypocholesterolemic/hypercholesterolemic ratio (HH), serving as an indicator of the cholesterol effect of a fat source, may more accurately reflect the impact of FA profile on cardiovascular disease, compared to the PUFA/SFA ratio.

As shown in Table 2, the DFA of pure WO had the highest value (92.59) compared to VIO (84.95) and VAO (81.72), reflecting positively on the DFA values of blended oils, which increased with WO proportion. Thus, the DFA yielded the most favorable results in VIO:WO (70:30) blends, reaching the highest value of 87.55. In contrast, the incorporation of WO, owing to its low levels of SFAs, resulted in low values of OFA in the blended oils, with minimum values in the ratio (70:30) of VIO:WO (8.44) and VAO:WO (9.70) blends. This observation aligns with Pearson's correlation analysis (Tables 2S and 3S, 1S), which revealed a positive correlation between DFA and PUFA ( $r^2 = 0.679$ ,  $p < 0.05$ ) as well as a positive association between OFA and SFA ( $r^2 = 0.979$ ,  $p < 0.05$ ). These results were confirmed by the HH values, which presented a strong positive correlation with DFA and PUFA/SFA ratio ( $r^2 = 0.989$  and  $0.828$ , respectively,  $p < 0.05$ ) and a negative correlation with OFA ( $r^2 = -0.979$ ,  $p < 0.05$ ). This indicates that VIO:WO blends, particularly at (70:30), were the most desirable in terms of reducing the occurrence of CHD compared to the other blends. Hashempour-Baltork et al. (2018) demonstrated significantly higher HH values of 6.88, 7.2, and 8.03 in the tertiary oil blend of olive:sesame:linseed with a linseed oil concentration of 5, 10, and 15 %, respectively. Likewise, blending perilla seed oil with palm olein, groundnut oil, and coconut oil improved the HH value of the binary blended oils (Dhyani et al., 2022).

**3.1.3.3. AI, and TI indices.** The atherogenicity index (AI) indicates the correlation between the sum of SFAs and the sum of UFAs. Only SFAs with chain lengths of 12, 14, or 16 carbon atoms are deemed pro-atherogenic, as they promote lipid adhesion to circulatory and immune system cells (Khalili Tilami & Kourimská, 2022). UFAs are recognized for their anti-atherogenic properties, as they hinder plaque accumulation and lower the levels of phospholipids, cholesterol, and

esterified FAs. The thrombogenicity index (TI) defines the thrombogenic potential of FAs, reflecting their propensity to form blood clots (Chen & Liu, 2020). It delineates the contribution of various FAs, elucidating the association between pro-thrombogenic FAs (C12:0, C14:0, and C16:0) and anti-thrombogenic FAs (MUFAs and the  $\omega$ 3 and  $\omega$ 6 families). Both the AI and the TI may be used to evaluate the potential impacts of FA composition on cardiovascular health.

In relation to AI, the addition of WO did show a slight impact on the value of AI in the blended oils (Table 2). There was a slight decrease in the blends of VIO:WO and VAO:WO from 0.11 to 0.10 and from 0.14 to 0.12, respectively. Regarding TI, a noticeable reduction was observed with an increase in WO proportion compared to the parent oils, reaching a minimum value of 0.22 in VIO:WO (70:30). Lower AI and TI values suggest better nutritional quality of FAs; therefore, diets featuring such lower values may potentially contribute to reducing total cholesterol and LDL-C levels in human blood plasma (Szpunar-Krok & Wondolowska-Grabowska, 2022). A similar AI and TI values were provided by Hashempour-Baltork et al. (2018) for all three tertiary blends (lower than 1) owing to their high PUFA level. Additionally, Motamedzadegan et al. (2020) reported that with the addition of palm olein (from 50 to 90 %), the AI (1.08–2.76) and TI (1.59–2.14) values drastically reduced in the blends of virgin coconut oil and palm olein. In a recent study by Dhyani et al. (2022), it was found that incorporating perilla seed oil into blends with palm olein, groundnut oil, and coconut oil resulted in a decrease in the AI and TI values of the blended oils.

As evidenced in Tables 2S and 3S (S1), AI and TI presented a strong positive correlation with OFA ( $r^2 = 0.987$  and  $0.951$ , respectively,  $p < 0.05$ ) and a significant ( $p < 0.05$ ) negative association with DFA ( $r^2 = -0.955$  and  $-0.986$ , respectively) and HH ( $r^2 = -0.963$  and  $-0.961$ , respectively). Furthermore, a significant ( $p < 0.05$ ) negative correlation of TI and COX ( $r^2 = -0.713$ ) was observed.

**3.1.3.4. ODR, LDR, COX, S/P and OL/ (LA + ALA) ratio.** The ODR, LDR, and COX ratios offer insights into the structural changes of oleic acid (ODR), linoleic acid (LDR), and the oxidation levels of C18:1, C18:2, and C18:3 FAs in oxidized ester mixtures (COX) (El Idrissi et al., 2023). The saturation fat index (S/P) serves as an indicator of dietary appropriateness for humans, with higher S/P ratios in fats indicating a more favorable balance of FAs. The recommended S/P ratio, ideally close to 0.45, is considered suitable for the human diet (Pattnaik & Mishra, 2022). The OL/(LA + ALA) ratio serves as a well-established indicator of oil quality. Higher OL (C18:1) content and lower LA (C18:2) and ALA (C18:3) contents are advantageous for fat stabilization.

Table 2 demonstrates a significant increase in ODR, LDR, and COX and a decrease in the OL/(LA + ALA) ratio as the WO proportion increases in the blended oils, compared to the parent oils (VIO and VAO). However, VAO:WO blends exhibited the highest average ODR value, ranging from 52.21 to 61.16, whereas the highest LDR average values were recorded for VIO:WO blends, ranging from 2.25 to 9.07. The elevated ODR values suggest an efficient biosynthetic pathway in the production of PUFA (C18:2 and C18:3) from MUFA (C18:1). Furthermore, the substantial sums of LDR indicate the effectiveness of this process in the formation of C18:3 through C18:2 desaturation.

With regard to the COX value, WO had the greater value of 9.26, followed by VAO (4.37) and VIO (3.59); therefore, the VAO:WO blends were characterized by a high COX value, varying from 4.63 to 5.86. This could be attributed to the prevalence of PUFAs. Compared to C18:2 and C18:3, C18:1 undergoes oxidation at a slower rate and, therefore, has a lower weight in the calculation of the COX index. However, the oil's oxidative stability diminishes as the COX values increase. A reverse trend in COX value was observed in the findings reported by Dhyani et al. (2022) and Abdel-Razek et al. (2011). Pearson's correlation data revealed a strong positive correlation between COX and PUFA ( $r^2 = 0.995$ ,  $p < 0.05$ ), as well as a negative correlation with MUFA ( $r^2 = -0.696$ ,  $p < 0.05$ ) (Table 2S and 3S, S1). In the case of S/P, there were

slight, though unchanged, differences in the blended oils compared to the parent oils. Additionally, the highest value of the OL/(LA + ALA) ratio was recorded in the VIO:WO blends, varying between 0.92 and 1.54.

### 3.1.4. Tocopherol analysis of pure oils and binary blends

**3.1.4.1. Total and individual tocopherols profile.** Tocopherols, which are lipid-soluble antioxidants, have been linked to the prevention of CVD and specific forms of cancer (Hashempour-Baltork et al., 2016). These essential components primarily protect oils by serving as radical scavengers, thereby delaying the propagation stages of oxidative degradation. Table 3 summarizes the results of the qualitative and quantitative analysis of tocopherols, vitamin E activity, and the  $\alpha$ -tocopherol equivalent to PUFA ratio.

The highest concentration of total tocopherol was observed in VAO with a value of 804.47 mg/kg, followed by WO and VIO with 797.37 and 793.39 mg/kg, respectively. In VIO and VAO, the primary isomers were  $\alpha$ - and  $\gamma$ -tocopherols, whilst  $\gamma$ - and  $\delta$ -tocopherols dominated in WO. The analysis of tocopherols did not reveal the presence of  $\beta$ -tocopherol in WO, whereas all tocopherol isomers were identified in VIO and VAO. However, the level of  $\beta$ -tocopherol in VIO (13.75 mg/kg) was comparatively lower than that in VAO (35.98 mg/kg).  $\alpha$ -tocopherol, which is known for its superior ability to scavenge peroxy radicals, was found to be significantly higher in both VIO and VAO, registering 355.82 and 323.98 mg/kg, respectively, compared to WO, which had a concentration of only 16.10 mg/kg. Conversely, WO exhibited elevated levels of  $\gamma$ - and  $\delta$ -tocopherols (697.11 and 84.14 mg/kg, respectively) compared to VAO (399.43 and 34.52 mg/kg) and VIO (396.82 and 27.01 mg/kg). These results are consistent with the findings of other authors (El Idrissi et al., 2023; Idrissi et al., 2022; Elouafy et al., 2022).

Noticeable alterations were observed in the tocopherol levels of the blended oils due to the addition of WO. The total tocopherol content in the blended oils decreased compared to the pure oils, except for the VIO:WO blends at (95:5) which had a tocopherol content of 823.99 mg/kg. However, the tocopherol level in all blends was found to be greater compared to the findings of Siddeeg and Xia (2015), who reported a range of 580–693 mg/kg for the total tocopherol content in Seinat seed oil and peanut oil blends. Likewise, Al-Farga et al. (2020) claimed a range of total tocopherol levels from 580 to 719 mg/kg in the blends of alhydwan seed oil and peanut oil.

The blended oils were enriched with  $\gamma$ - and  $\delta$ -tocopherols as the proportion of WO increased from 5 to 30 %. The blends of VIO:WO and VAO:WO, with a ratio of (70:30), recorded the highest levels of  $\gamma$ -tocopherol at 475.33 and 449.53 mg/kg, respectively. Similarly, these blends also showed the highest levels of  $\delta$ -tocopherol at 44.22 and 46.20 mg/kg, respectively, in comparison to the other mixtures. Conversely, a notable gradual reduction in  $\alpha$ -tocopherol levels was observed from 343.57 to 252.52 mg/kg in VIO:WO blends and from 356.37 to 267.74 mg/kg in VAO:WO blends. Furthermore, as  $\beta$ -tocopherol was absent in WO, its level also decreased with increasing WO proportion. Previously, Pan et al. (2020) reported an increase in  $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocopherols in the blended walnut and almond oils with an increase in the proportion of almond oil from 5 to 30 %. In addition, Rudzińska et al. (2016) claimed that the incorporation of 5, 10, and 20 % black cumin oil into rapeseed oil led to a progressive elevation in  $\alpha$ - and  $\gamma$ -tocopherols levels. Conversely, when rapeseed oil was blended with rice bran oil at the same proportions, a marginal reduction in the levels of  $\alpha$ - and  $\gamma$ -tocopherols was observed. In our study, Pearson's correlation analysis revealed that  $\alpha$ -tocopherol correlated negatively with  $\gamma$ - and  $\delta$ -tocopherols, and COX ( $r^2 = -0.990$ ,  $-0.984$ , and  $-0.958$ , respectively,  $p < 0.05$ ), while  $\gamma$ -tocopherol correlated positively with  $\delta$ -tocopherol and COX ( $r^2 = 0.959$  and  $0.924$ , respectively,  $p < 0.05$ ) (Table 2S and 3S, 1S).

**Table 3**

Total and individual tocopherols content, vitamin E activity, and the  $\alpha$ -TE/PUFA ratio of pure Virginia (VIO), Valencia (VAO), and walnut (WO) oils, and their binary blends.

Tocopherols (mg/kg)	Pure oils			VIO:WO blends (v/v)				VAO:WO blends (v/v)			
	VIO	VAO	WO	(95:5)	(90:10)	(80:20)	(70:30)	(95:5)	(90:10)	(80:20)	(70:30)
$\alpha$ -tocopherol	355.82 $\pm$ 0.14 <sup>a</sup>	323.98 $\pm$ 0.33 <sup>b</sup>	16.10 $\pm$ 0.00 <sup>c</sup>	343.57 $\pm$ 0.14 <sup>d</sup>	319.56 $\pm$ 0.13 <sup>e</sup>	286.41 $\pm$ 0.12 <sup>f</sup>	252.52 $\pm$ 0.10 <sup>g</sup>	356.37 $\pm$ 0.14 <sup>a</sup>	335.51 $\pm$ 0.14 <sup>h</sup>	302.95 $\pm$ 0.12 <sup>i</sup>	267.74 $\pm$ 0.11 <sup>j</sup>
$\beta$ -tocopherol	13.75 $\pm$ 0.01 <sup>a</sup>	35.98 $\pm$ 0.03 <sup>b</sup>	0.00 $\pm$ 0.00 <sup>c</sup>	31.28 $\pm$ 0.01 <sup>d</sup>	20.50 $\pm$ 0.01 <sup>e</sup>	16.05 $\pm$ 0.00 <sup>f</sup>	12.86 $\pm$ 0.00 <sup>g</sup>	22.18 $\pm$ 0.00 <sup>h</sup>	14.40 $\pm$ 0.00 <sup>i</sup>	14.40 $\pm$ 0.00 <sup>j</sup>	16.37 $\pm$ 0.01 <sup>k</sup>
$\gamma$ -tocopherol	396.81 $\pm$ 0.16 <sup>a</sup>	399.43 $\pm$ 0.41 <sup>b</sup>	697.11 $\pm$ 0.28 <sup>c</sup>	419.97 $\pm$ 0.17 <sup>d</sup>	411.22 $\pm$ 0.17 <sup>e</sup>	442.18 $\pm$ 0.18 <sup>f</sup>	475.33 $\pm$ 0.19 <sup>g</sup>	375.15 $\pm$ 0.19 <sup>h</sup>	385.22 $\pm$ 0.16 <sup>i</sup>	423.24 $\pm$ 0.18 <sup>j</sup>	449.53 $\pm$ 0.18 <sup>k</sup>
$\delta$ -tocopherol	27.01 $\pm$ 0.01 <sup>a</sup>	34.52 $\pm$ 0.04 <sup>b</sup>	84.14 $\pm$ 0.03 <sup>c</sup>	29.17 $\pm$ 0.01 <sup>d</sup>	32.30 $\pm$ 0.01 <sup>e</sup>	39.24 $\pm$ 0.01 <sup>f</sup>	44.22 $\pm$ 0.01 <sup>g</sup>	33.54 $\pm$ 0.01 <sup>h</sup>	36.02 $\pm$ 0.01 <sup>i</sup>	41.98 $\pm$ 0.01 <sup>j</sup>	46.20 $\pm$ 0.01 <sup>k</sup>
Total tocopherols	793.39 $\pm$ 0.32 <sup>a</sup>	804.47 $\pm$ 0.84 <sup>b</sup>	797.37 $\pm$ 0.33 <sup>c</sup>	823.99 $\pm$ 0.34 <sup>d</sup>	783.58 $\pm$ 0.32 <sup>e</sup>	783.89 $\pm$ 0.32 <sup>eh</sup>	784.95 $\pm$ 0.32 <sup>eh</sup>	787.25 $\pm$ 0.32 <sup>f</sup>	771.16 $\pm$ 0.31 <sup>g</sup>	782.60 $\pm$ 0.32 <sup>h</sup>	779.84 $\pm$ 0.32 <sup>i</sup>
Vitamin E activity (mg $\alpha$ -TE/kg)	401.27 $\pm$ 0.16 <sup>a</sup>	378.66 $\pm$ 0.39 <sup>b</sup>	86.66 $\pm$ 0.04 <sup>c</sup>	398.37 $\pm$ 0.16 <sup>d</sup>	369.20 $\pm$ 0.15 <sup>e</sup>	337.44 $\pm$ 0.13 <sup>f</sup>	305.65 $\pm$ 0.13 <sup>g</sup>	403.09 $\pm$ 0.16 <sup>h</sup>	380.15 $\pm$ 0.15 <sup>i</sup>	351.46 $\pm$ 0.14 <sup>j</sup>	319.70 $\pm$ 0.13 <sup>k</sup>
$\alpha$ -TE/PUFA ratio (mg/g)	1.34 $\pm$ 0.00 <sup>a</sup>	0.98 $\pm$ 0.00 <sup>b</sup>	0.12 $\pm$ 0.00 <sup>c</sup>	1.25 $\pm$ 0.00 <sup>d</sup>	1.07 $\pm$ 0.00 <sup>e</sup>	0.86 $\pm$ 0.00 <sup>f</sup>	0.70 $\pm$ 0.00 <sup>g</sup>	0.99 $\pm$ 0.00 <sup>b</sup>	0.89 $\pm$ 0.00 <sup>h</sup>	0.76 $\pm$ 0.00 <sup>i</sup>	0.64 $\pm$ 0.00 <sup>j</sup>

Values (mean  $\pm$  SD, n = 3) with different superscripts within a row differ significantly ( $p < 0.05$ ) among samples.  $\alpha$ -TE:  $\alpha$ -tocopherol equivalent;  $\alpha$ -TE/PUFA: Ratio of  $\alpha$ -tocopherol equivalent to polyunsaturated fatty acids.

**3.1.4.2. Vitamin E activity and the ratio of vitamin E to PUFA.** The biological activity of vitamin E, defined as  $\alpha$ -tocopherol equivalents ( $\alpha$ -TE) was calculated on the basis of the tocopherol profile, which is a crucial factor in assessing the nutritional value of the oil (Romanić et al., 2021). This data provides insight into the overall E-vitamin activity, encompassing all tocopherols present.

In light of the results presented in Table 3, the highest E-vitamin activity was observed in VIO and VIO, with values of 401.27 and 378.66 mg  $\alpha$ -TE/kg, respectively, in comparison to WO, which recorded the lowest value of 86.66 mg  $\alpha$ -TE/kg. However, as the WO proportion increases from 5 to 30 %, the E-vitamin activity declines in the blended oils, mainly due to its low  $\alpha$ -tocopherol content, as discussed above. Among these blends, VIO:WO and VAO:WO at 95:5 recorded the highest E-vitamin activity amounting to 398.37 and 403.09 mg  $\alpha$ -TE/kg, respectively. The richness of VIO and VAO in all forms of vitamin E is crucial for considering the production of binary oil blend with WO, which has relatively low biological activity of vitamin E. These findings were in harmony with those reported by Choudhary et al. (2015), whereby the  $\alpha$ -TE decreased in the rice ban oil blends with groundnut oil and palm-olein oil.

The recommended optimal ratio of vitamin E to PUFA in the diet is typically between 0.4 and 0.6 mg of  $\alpha$ -TE per gram of PUFA, which is characteristic of a diet with average PUFA levels (Romanić et al., 2021). Results showed that the  $\alpha$ -TE/PUFA ratio for the blended oils ranged from 0.64 to 1.25 mg/g, which is double the recommended range. This suggests that these oils do not meet the vitamin E requirements for humans and animals. Likewise, the parent oils did not possess the favorable  $\alpha$ -TE/PUFA ratio in relation to the guidelines. In a previous study conducted by Romanić et al. (2021), it was noted that the E-vitamin activity and  $\alpha$ -TE/PUFA ratio exhibited a decline as the flaxseed oil proportion increased (from 10 to 90 %) in blends of sunflower and flaxseed oils. Our findings also revealed that E-vitamin activity correlated positively with  $\alpha$ -TE/PUFA ( $r^2 = 0.905$ ,  $p < 0.05$ ), while both of these parameters resented a significant negative correlation ( $p < 0.05$ ) with PUFA ( $r^2 = -0.932$  and  $-0.966$ , respectively) and COX ( $r^2 = -0.962$  and  $-0.961$ , respectively) (Tables 2S and 3S, S1).

### 3.2. Oil blends and their behavior during accelerated storage conditions

#### 3.2.1. Evolution of the overall quality of pure oils and binary blends during thermal oxidation

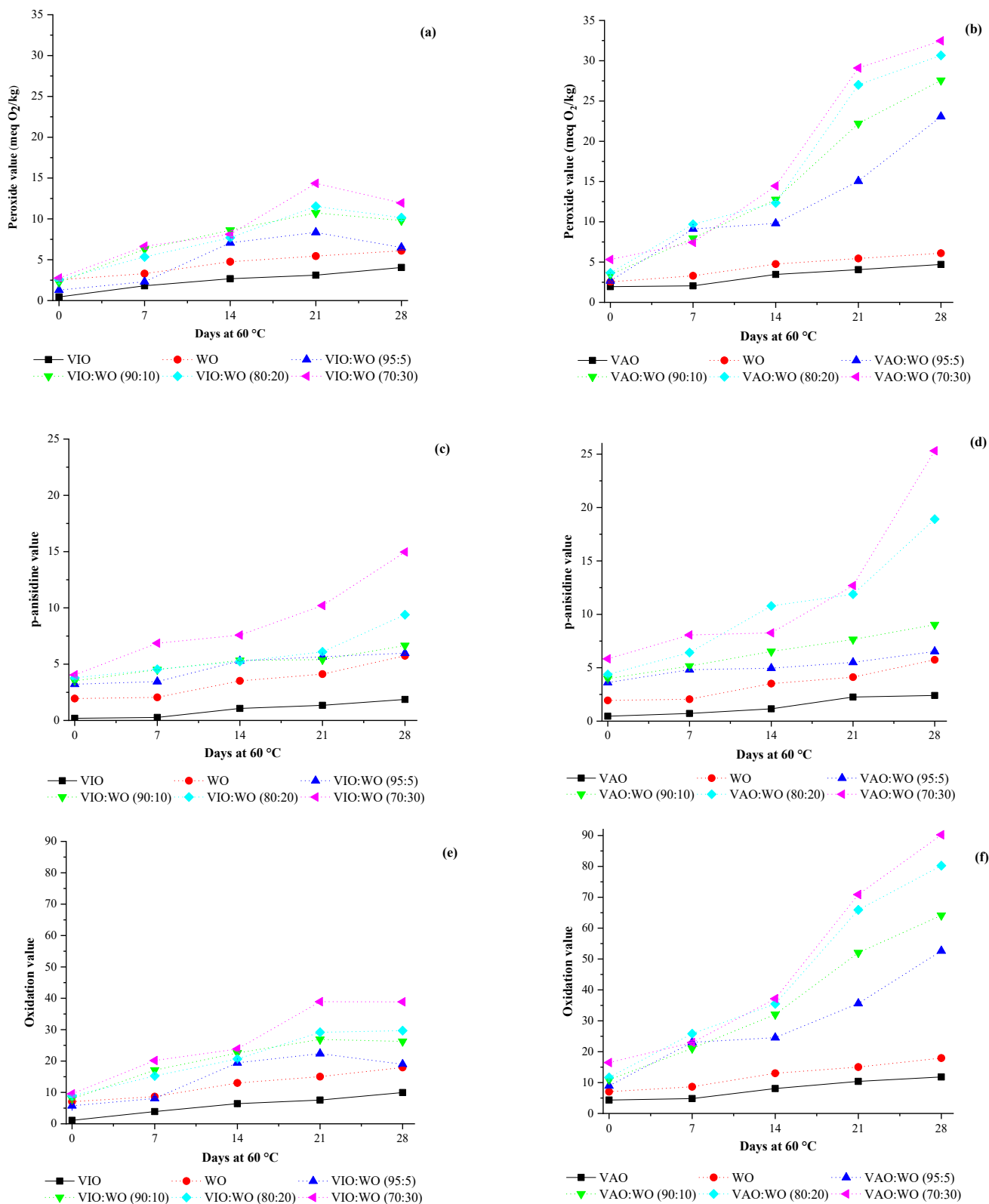
With regard to the evolution of AV, a slight increase was observed with blend proportion and storage time of VIO:WO and VAO:WO blends (Fig. 1S, Supplementary file 2; S2). However, VAO:WO (70:30) and WO recorded the highest AV of 1.01 mg KOH/g oil during 28 days of storage,

remaining less than 4 mg KOH/g. This phenomenon could potentially be attributed to an elevated rate of hydrolysis of triglycerides under thermal oxidation conditions. These observations do not differ from those obtained by Ndomou et al. (2023), who showed a similar pattern of increase in AV with the increase in groundnut oil and palm olein proportions in the quaternary blends during heat treatment. Indeed, the decrease in AV observed for VAO after 7 days of storage could be ascribed to the rapid transformation of the released FAs into primary and secondary oxidation products.

Hydroperoxide is the primary oxidation product of lipids and assessing the PV can serve as an indicator to evaluate the early oxidation stage of lipids (Idrissi et al., 2022). Results showed that adding WO to VIO and VAO resulted in a marked increase in their PV during storage (Fig. 1a and b). Following a storage period of 28 days, the PV values for the parent oils, VIO, VAO, and WO were 4.05, 4.70, and 6.10 meq O<sub>2</sub>/kg, respectively. In addition, VIO:WO blends, with ratios of (95:5), (90:10), (80:20), and (70:30) showed a notable increase in PV after 21 days of storage. The PV values recorded were 8.35, 10.74, 11.54, and 14.35 meq O<sub>2</sub>/kg, respectively (Fig. 1a). After this period, a decrease in PV levels was observed, which can be attributed to the phenomenon where initial oxidation products undergo a subsequent oxidation process, resulting in the formation of non-volatile and volatile secondary oxidation products. Regardless of their initial composition, VAO and its blends with WO in the proportion of (95:5), (90:10), (80:20), and (70:30), showed comparable patterns of oxidative deterioration. These enriched oils exceeded 15 meq O<sub>2</sub>/kg after 21 days of storage, reaching PV values of 15.05, 22.20, 27.01, and 29.10 meq O<sub>2</sub>/kg, respectively (Fig. 1b). However, the rise in PV with increasing in WO proportion is attributed to its high content of PUFA, which are more sensitive to oxidation than MUFA. These results agree with the statement of Pan et al. (2020), who found that the PV of the mixed oils combining walnut oil with almond oil was greater than that of the two pure oils during 24 days of storage at 60 °C. In addition, Bordón et al. (2019) demonstrated that pure almond, virgin, and roasted sesame oils, and their respective blends with chia seed oil, generated fewer primary oxidation products than walnut oil blends during 12 days' storage at 40 °C.

The p-AnV quantifies the secondary product generated as a result of the decomposition of hydroperoxide into non-volatile carbonyls during the process of lipid oxidation which ultimately contributes to the development of a rancid taste in the oil (El Idrissi et al., 2023). VIO, VAO, and WO, as well as their respective blends, showed a significant ( $p < 0.05$ ) increase in p-AnV with the length of storage time as illustrated in Fig. 1c and d. Nevertheless, parent oils showed a low generation of secondary oxidation products. As for the studied blends, p-AnV rose with increasing WO proportion. Among these blends, VAO:WO blends





**Fig. 1.** Kinetic curve of peroxide value (a,b), p-anisidine value (c,d), and oxidation value (e,f) during thermal oxidation of VIO, VAO, and WO and their binary blends in the Schaal oven test. Plotted values are the average of three individual replicates  $\pm$  standard deviation. WO: (a), (b), (c), (d), (e) and (f). VIO and VIO:WO blends: (a), (c), and (e). VAO and VAO:WO blends: (b), (d), and (f).

recorded the highest p-AnVs, reaching 6.52, 9.03, 18.92, and 25.31 for proportions (95:5), (90:10), (80:20), and (70:30), respectively, after 28 days of storage (Fig. 1d). This result corroborates that obtained by Li et al. (2014) who observed an increase in p-AnV in soybean oil and oil blends during 24 days of storage under oxidizing conditions. In addition, the incorporation of chia seed oil into walnut oil substantially enhanced the generation of secondary oxidation components (Bordón et al., 2019). Indeed, the observed elevation in p-AnV could be ascribed to the lower production of  $\alpha$ - and  $\beta$ -unsaturated aldehydes resulting from the oxidative degradation of C18:1 compared to C18:2 and C18:3 (Ndomou et al., 2023).

The OV provides insight into the early and advanced stages of lipid oxidation. It measures both hydroperoxides and their degradation products, offering a more accurate estimation of the oil's gradual degradation (Idrissi et al., 2022). As seen in Fig. 1e and f, the OV of the studied oils exhibited substantial variation, in line with the trends observed in primary and secondary oxidation parameters (PV and p-AnV) during accelerated storage conditions. However, VAO:WO blends exceeded 30, industry-approved limits for edible oil, between days 14

and 28, reaching final OV values of 52.65, 64.16, 80.24, and 90.23 for the proportions (95:5), (90:10), (80:20), and (70:30), respectively (Fig. 1f). Conversely, the OV values of pure oils and VIO:WO blends [(95:5), (90:10), and (80:20)] remained consistently below 30 throughout the entire storage period (Fig. 2e), thus suggesting a slower oxidation process and higher oil quality. This trend is consistent with the literature report (Ndomou et al., 2023).

Results also showed a typical decrease in flavor score values during storage at 60 °C, indicating that the examined oils would receive relatively low acceptance as edible oil without further refinement (Table 4S, S1). This could be due to the fact that storage at elevated temperatures negatively affects flavor stability, and results in poor oxidation properties (Al-Farga et al., 2020).

The formation of hydroperoxides occurs simultaneously with the conjugation of double bonds in PUFA, which can be quantified by measuring their absorptivity in the UV spectrum. The conjugated dienes show a strong absorption at 232 nm, while the conjugated trienes exhibit absorption at 270 nm. Results of the UV-spectrophotometric indexes are presented in Fig. 2S (S2). The plotted curve for the accumulation of

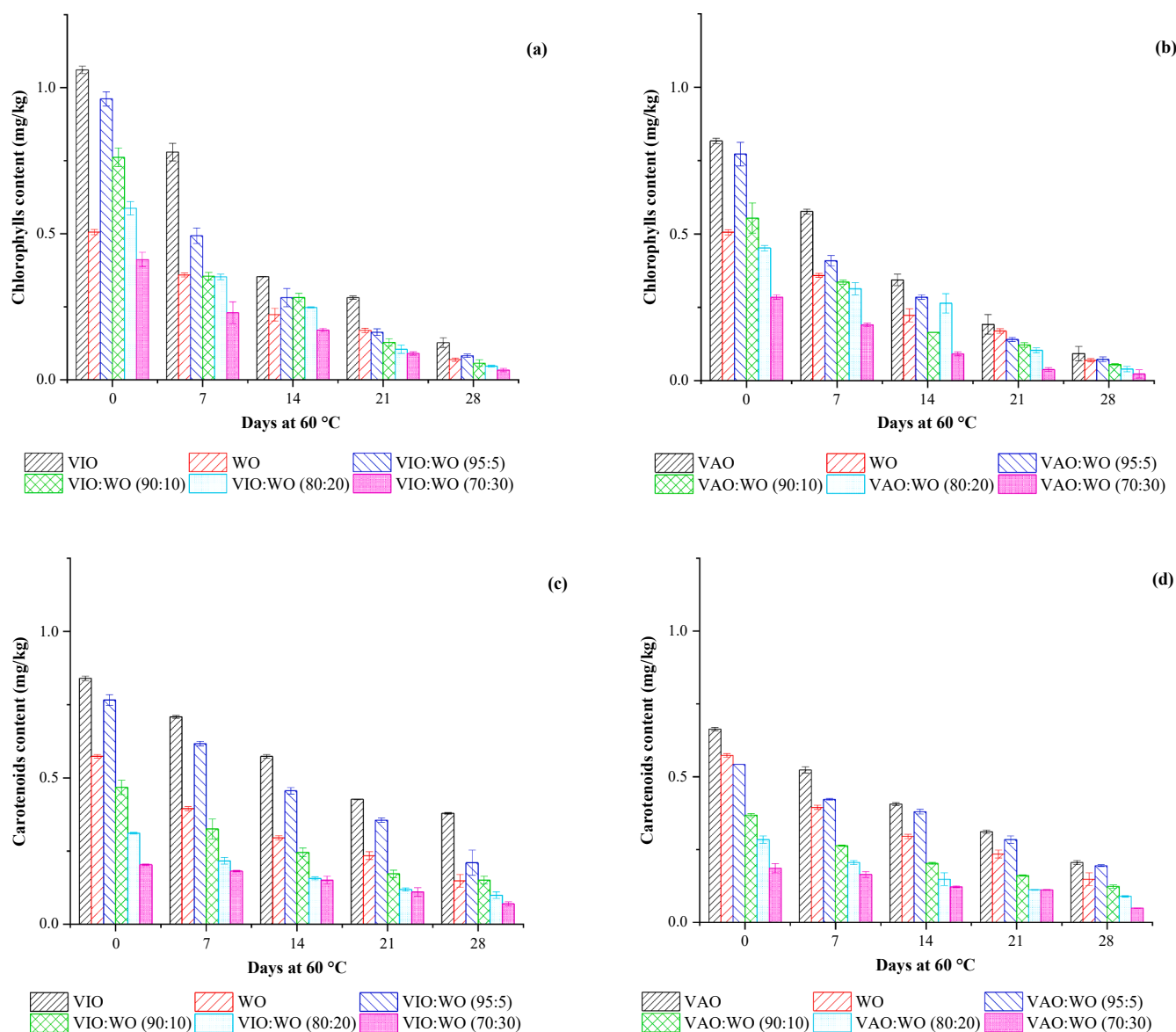


Fig. 2. Changes in chlorophylls (a,b) and carotenoids (c,d) content during thermal oxidation of VIO, VAO, and WO and their binary blends in the Schaal oven test. Plotted values are the average of three individual replicates  $\pm$  standard deviation. WO: (a), (b), (c), and (d). VIO and VIO:WO blends: (a) and (c). VAO and VAO:WO blends: (b) and (d).

primary and secondary oxidation products (PV and p-AnV) in each treatment showed a close correspondence with the curves for E<sub>232</sub> and E<sub>270</sub>, suggesting a correspondence between the formation of lipid hydroperoxides and conjugated double and triple-bonded FAs. The significant presence of conjugated oxidative products in WO and VAO:WO blends can be attributed to their substantial content of C18:2 and C18:3, which undergo rapid decomposition to form conjugated hydroperoxides under thermal oxidation. These data align with the statement of Pan et al. (2020), who observed a linear increase in conjugated diene content in all samples of walnut oil and almond oil blends throughout a 24-day period of accelerated storage. Furthermore, Li et al. (2014) observed a steady increase in the E<sub>232</sub> value as the storage time (0–24 days) prolonged in soybean oil and its blend.

Fig. 2 shows the changes in pigment content in parent oils and their binary blends during accelerated storage. As anticipated, all oil samples experienced a gradual decrease in both chlorophyll and carotenoid content during storage. The most marked decline was observed in VAO:WO blends, with chlorophyll content varying from 0.02 to 0.07 mg/kg and carotenoid content from 0.05 to 0.19 mg/kg after 28 days of storage. Moreover, the depletion of pigment was comparatively more significant than that observed in VIO:WO mixtures. Generally, carotenoids effectively protect the oil from oxidation but are vulnerable to oxygen, contributing to their degradation during storage, whilst chlorophyll, being thermolabile, is influenced by heat treatment and may act as an antioxidant in darkness, potentially leading to its breakdown through hydrogen donation to free radicals (El Idrissi et al., 2023).

### 3.2.3. Changes in chemical profile and nutritional properties during accelerated storage

The changes in the FA profile of the investigated oils and their blends following a 28-day period of dark storage at 60 °C are summarized in Table 5S (S1). Subtle differences in the proportions of SFA, MUFA, and PUFA were observed for all blended oils compared to pure oils, indicating that duration of storage has a minor impact on the FA composition. As expected, the PUFA level decreased, with greater losses of 1.38 % for VIO:WO (70:30) and VAO:WO (80:20) blends, compared to VIO (0.07 %) and VAO (0.77 %). Based on the findings, it was observed that the PUFA underwent degradation during the accelerated oxidation process, potentially due to the oxidation and polymerization-induced breakdown of C=C bonds (Pan et al., 2020). In contrast, an increase in the MUFAs level was observed in all oil samples, mainly as a result of the increase in C18:1. Besides, VIO:WO and VAO:WO blends with a (95:5) ratio showed a reduction in SFAs content. The recorded losses were 1.07 and 0.80 %, respectively. On the other hand, the remaining oils recorded a rise in the level of SFAs. Similar findings were reported by Pan et al. (2020) for walnut oil and almond oil blends.

In terms of nutritional quality indices, a slight variation was observed as a function of storage conditions, similar to those observed for FAs as indicated in Table 6S (S1), given that the latter are calculated on the basis of the FA profile. After 28 days of storage, the MUFA/PUFA and ω6/ω3 ratios increased slightly in all oil samples, whereas the UFA/SFA and PUFA/SFA ratios decreased, except for VIO:WO and VAO:WO blends at 95:5. Additionally, the DFA and HH indices showed a decrease with storage, whilst the OFA values exhibited an increase. Nonetheless, AI and TI remained unchanged during storage. Therefore, the values of ODR, LDR, and COX gradually declined in all blended oils, while the values of S/P and OL/(LA + ALA) ratios remained unaltered throughout the storage period. The thermal oxidation of the binary blended oils resulted in acceptable nutritional fat quality, thereby having a positive effect on health.

The behavior of parent oils and their binary blends, based on the different tocopherols composition after 28 days of storage in the dark at 60 °C are presented in Table 7S (S1). Results showed that tocopherol losses occurred during thermal storage, wherein α- and γ-tocopherols significantly dropped in all examined oils, with a greater reduction in α-tocopherol compared to the other homologs. Our findings were

slightly in line with those observed by Pan et al. (2020), wherein the tocopherol content of walnut oil and almond oil blends decreased during 24 days of storage at 60 °C, with α-tocopherol being completely depleted after 8 days of storage.

In the present study, the highest rate of tocopherol loss was observed in VAO (28.92 %). WO, on the other hand, showed a moderate loss of 15.14 %, while VIO had the lowest loss at 1.84 %. However, VIO:WO blends at ratios of (90:10) and (95:5) demonstrated greater stability among oil blends, with loss rates of 10.06 and 13.42 %, respectively. In contrast, VIO:WO and VAO:WO blends at (70:30) exhibited more pronounced tocopherol losses of 55.36 and 46 %, respectively. It was noticed that the tocopherol loss of VAO:WO at the ratio of (95:5), (90:10), and (80:20) were lower than the pure VAO. Interestingly, γ-tocopherol exhibits a limited capacity for hydrogen donation and possesses high oxidative stability, suggesting its superior antioxidant effectiveness compared to α-tocopherol (Pan et al., 2020). In food systems, the reduction in antioxidant activity occurs in the following order: γ > δ > β > α isomers. In a previous study, it was found that the blends of sunflower oil and black cumin oil in a ratio of (80:20) demonstrated a strong oxidation resistance compared to other oil blends (Kiralan et al., 2017). This can be attributed to the significant level of tocopherols, especially of γ-tocopherol, present in black cumin oil. Besides, all oils experienced a significant decrease in E-vitamin activity and α-TE/PUFA during storage, primarily attributable to α-tocopherol retention. However, VAO:WO at a ratio of (90:10) and (70:30) blends had the most favorable α-TE/PUFA ratio (0.56 and 0.50 mg/g, respectively) (Table 7S, S1).

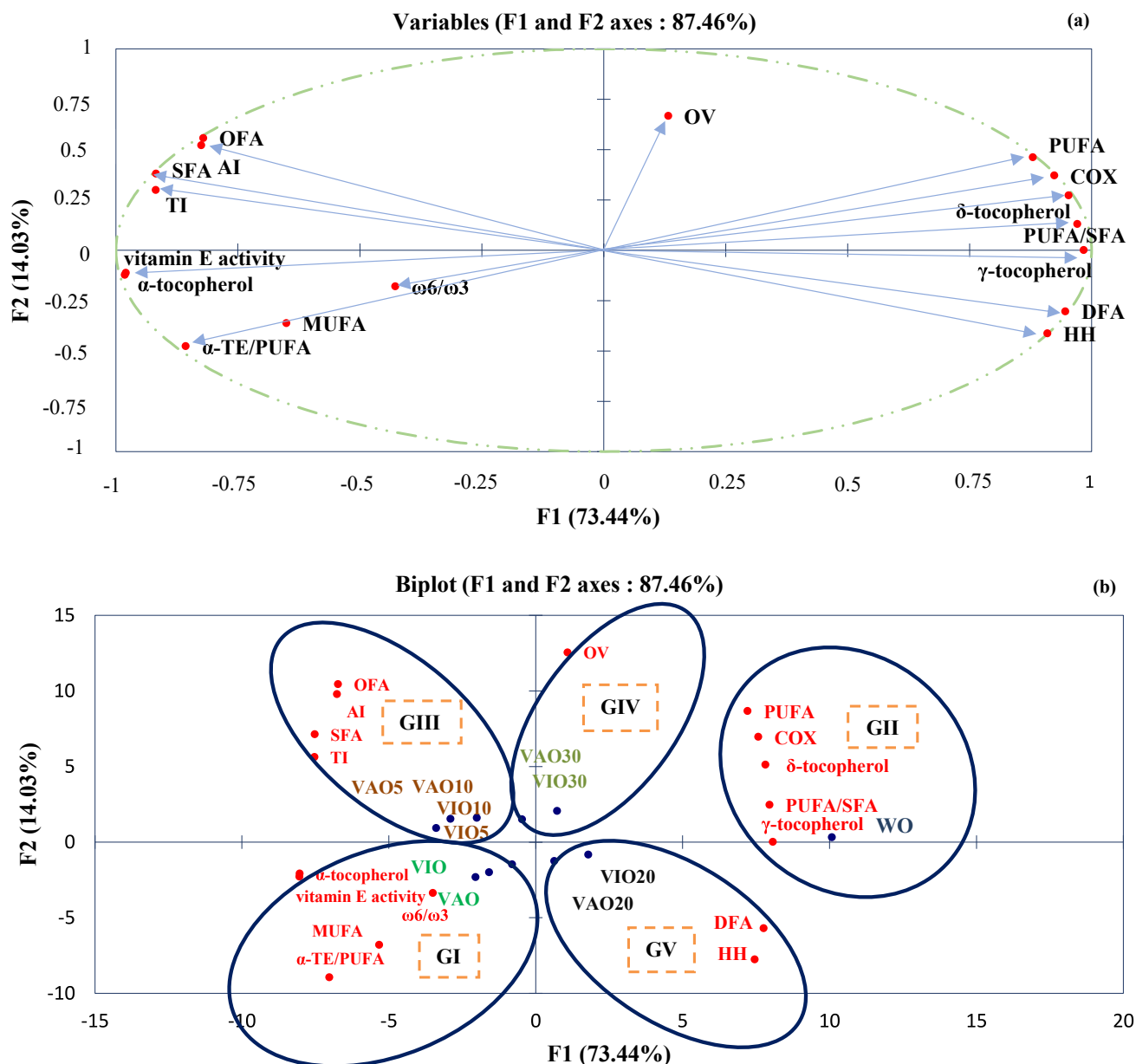
Thus, it can be inferred that the oxidative stability of pure VIO and VAO significantly ( $p < 0.05$ ) decreased with the addition of WO. This reduction could also be attributed to the incorporation of oxygen during the blending process, particularly while stirring the oils. Henceforth, incorporating natural antioxidants recovered from food waste or by-products of food processing industries is a promising approach for stabilizing these oil mixtures and protecting them from oxidation (Fadda et al., 2022). This approach aligns with circular economy principles and meets consumers' preferences for natural and safer foods, while also simplifying the production and marketing of these oil blends.

### 3.3. Principal component analysis (PCA)

PCA was performed using the following variables: FA contents (MUFA, PUFA, and SFA), nutritional quality indices (PUFA/SFA, ω6/ω3, DFA, OFA, HH, AI, TI, and COX), tocopherol isomer content (α-tocopherol, γ-tocopherol, and δ-tocopherol), vitamin E activity, the ratio of α-TE to PUFA, and oxidation value (OV).

Fig. 3a illustrates the projection of variables using the F1 and F2 factorial schemes in PCA. The first main component (F1) accounted for 73.44 % of the variance, while the second main component (F2) represented the remaining 14.03 %. The cumulative percentage of the first two principal components amounted to 87.46 % of the overall variance. Therefore, their linear combination serves as a reliable indicator of the underlying elements, since it surpasses 50 %. Moreover, Fig. 3a depicts the plane formed by the F1 and F2 axes, elucidating the correlation between the variables. The F1 axis primarily reflects the positive correlation between PUFA, COX, DFA, HH, PUFA/SFA, δ-tocopherol, and γ-tocopherol. The F2 axis, on the other hand, is formed by the positive association between the OFA, AI, TI, and SFA. Additionally, the variables vitamin E activity and α-tocopherol are dispersed along the positive side of the F2 axis. These findings are consistent with the results of the correlation analysis. Fig. 3b displays the biplot derived from the first two principal components of the PCA. It indicates that the eleven individuals, representing the pure and the blended POs, are categorized into five groups.

Group I, comprising VIO and VAO, showcases elevated levels of MUFA, ω6/ω3, α-tocopherol, vitamin E activity, and α-TE/PUFA. Additionally, they exhibit moderate values of DFA, HH, SFA, TI, AI, OFA, and



**Fig. 3.** Principal component analysis (a) and projection on the factorial plan (F1  $\times$  F2) of the individual's variable (b). GI: Group I; GII: Group II; GIII: Group III; GIV: Group IV; and GV: Group V. The blended POs designations are as follows: VIO5 and VAO5: VIO:WO and VAO:WO (95:5); VIO10 and VAO10: VIO:WO and VAO:WO (90:10); VIO20 and VAO20: VIO:WO and VAO:WO (80:20); VIO30 and VAO30: VIO:WO and VAO:WO (70:30), respectively.

OV, while demonstrating lower values of PUFA, PUFA/SFA, COX,  $\delta$ -tocopherol, and  $\gamma$ -tocopherol. Group II, represented by WO, is distinguished by high levels of PUFA, PUFA/SFA, COX,  $\delta$ -tocopherol, and  $\gamma$ -tocopherol, alongside moderate values of OV, HH, and DFA. Furthermore, Group II displays lower levels of MUFA, SFA,  $\omega$ 6/ $\omega$ 3, TI, AI, OFA,  $\alpha$ -tocopherol, vitamin E activity, and  $\alpha$ -TE/PUFA. Group III consists of four individuals (VIO5, VIO10, VAO5, and VAO10), recognized for their high content of SFA, TI, AI, and OFA. They also exhibit moderate levels of MUFA,  $\omega$ 6/ $\omega$ 3, OFA, DFA, HH,  $\alpha$ -tocopherol, vitamin E activity, and  $\alpha$ -TE/PUFA. Moreover, they demonstrate lower values of PUFA, PUFA/SFA, COX,  $\delta$ -tocopherol, and  $\gamma$ -tocopherol compared to Group II. Group IV encompasses two individuals, VAO30 and VIO30, distinguished by their high OV and medium levels of SFA, PUFA/SFA, DFA, HH, TI, AI, OFA, COX,  $\delta$ -tocopherol, and  $\gamma$ -tocopherol. Moreover, the MUFA,  $\omega$ 6/ $\omega$ 3,  $\alpha$ -tocopherol, vitamin E activity, and  $\alpha$ -TE/PUFA of this group have lower values compared to Group I. Group V comprises two individuals

(VIO20 and VAO20), characterized by a high content of DFA, and HH, along with average values of MUFA, SFA,  $\omega$ 6/ $\omega$ 3, TI, AI, OFA,  $\alpha$ -tocopherol, vitamin E activity, and  $\alpha$ -TE/PUFA and OV. Moreover, this category has lower values of SFA, PUFA/SFA, TI, AI, OFA, COX,  $\delta$ -tocopherol, and  $\gamma$ -tocopherol compared to Group II.

#### 3.4. Hierarchical cluster analysis (HCA)

The pure and binary blended oils in this study underwent classification using Ward's method and the Euclidean square to assess similarity. HCA was employed to measure correlation and visualize similarity among the eleven oil samples based on the same variables as described in PCA earlier. The resulting dendrogram is depicted in Fig. 3S (S2). According to these findings, the eleven oil samples were classified into five distinct clusters.

Cluster I comprises two individuals, namely VIO and VAO,

representing 18.18 % of the overall samples. These samples are characterized by strong average values of SFA (19.71 %),  $\omega_6/\omega_3$  (402.77), OFA (9.94), AI (0.13), TI (0.33),  $\alpha$ -tocopherol (339.9 mg/kg), vitamin E activity (389.965 mg  $\alpha$ -TE/kg), and  $\alpha$ -TE/PUFA (1.16 mg/g). Additionally, they exhibited a moderate mean value of MUFA (34.64 %) and lower mean values of PUFA (34.23 %), PUFA/SFA (1.73), DFA (83.33), HH (8.02),  $\gamma$ -tocopherol (398.12 mg/kg),  $\delta$ -tocopherol (30.77 mg/kg), COX (3.98), and OV (2.73).

Cluster II consists of a single sample (WO), which accounted for 9.09 % of the total samples. The mean values of PUFA (74.26 %), PUFA/SFA (7.29), DFA (92.59), HH (12.62),  $\gamma$ -tocopherol (697 mg/kg),  $\delta$ -tocopherol (84.14 mg/kg), and COX (9.26) were all significantly elevated in this sample. Moreover, this cluster displayed a moderate mean value of OV (7.05) and low mean values of MUFA (15.51 %), SFA (10.18 %),  $\omega_6/\omega_3$  (4.74), TI (0.13),  $\alpha$ -tocopherol (16.1 mg/kg), vitamin E activity (86.66 mg  $\alpha$ -TE/kg),  $\alpha$ -TE/PUFA (0.12 mg/g).

Cluster III contains four samples (VIO5, VIO10, VAO10, VAO5), representing 36.36 % of the total samples. They are identified by their high mean MUFA value (43.66 %). Furthermore, they showed a moderate average value of SFA (18.84 %), PUFA/SFA (1.98),  $\omega_6/\omega_3$  (37.24), DFA (84.26), OFA (9.75), HH (8.26), AI (0.12),  $\alpha$ -tocopherol (338.75 mg/kg), vitamin E activity (387.70 mg  $\alpha$ -TE/kg), and OV (8.38). In addition, their average  $\gamma$ -tocopherol content was low (397.89 mg/kg).

Cluster IV includes two samples (VIO30 and VAO30), which accounted for 18.18 % of the total samples. This cluster demonstrated a moderate mean value of the following: MUFA (39.65 %), PUFA (42.71 %), SFA (17.56 %), PUFA/SFA (2.43),  $\omega_6/\omega_3$  (14.48), DFA (85.53), OFA (9.32), HH (8.78), AI (0.11), TI (0.25),  $\alpha$ -tocopherol (294.68 mg/kg),  $\gamma$ -tocopherol (432.71 mg/kg),  $\delta$ -tocopherol (40.61 mg/kg), vitamin E activity (344.45 mg  $\alpha$ -TE/kg),  $\alpha$ -TE/PUFA (0.81 mg/g), COX (5.09), and OV (10.28).

Cluster V, comprising two samples (VIO20 and VAO20), accounts for 18.18 % of the total samples. They are characterized by a high mean value of OV (13.01). Further, this cluster showed moderate mean values of MUFA (36.78 %), PUFA (46.52 %), SFA (16.62 %), PUFA/SFA (2.81),  $\omega_6/\omega_3$  (10.84), DFA (86.44), OFA (9.07), HH (9.13), AI (0.11), TI (0.24),  $\alpha$ -tocopherol (260.13 mg/kg),  $\gamma$ -tocopherol (462.43 mg/kg),  $\delta$ -tocopherol (45.21 mg/kg), vitamin E activity (312.67 mg  $\alpha$ -TE/kg),  $\alpha$ -TE/PUFA (0.67 mg/g), and COX (5.59). The score plot indicates a consistent tendency in the distribution of all oil samples, which aligns with the findings of the PCA. Consequently, the PCA results were consistent with those of the HCA.

### 3.5. Chi-squared automatic interaction detector (CHAID)

The CHAID is a highly effective method for identifying the key factors that contribute to the splitting of examined samples into distinct sets. Herein, the CHAID method was utilized to analyze a set of eleven oil samples based on their PUFA and COX content, thereby yielding a model that effectively distinguishes between these samples. Fig. 4S (S2) depicts the tree produced by the CHAID model, with the node numbers within the small frames indicating the variable domains utilized for the split. The method employed a basic algorithm for building non-binary trees, relying on the Chi-squared test to identify the most suitable split.

In light of the findings, it should be emphasized that the samples (WO) exhibited a noteworthy level of PUFA, and COX compared to the other samples. Thus, the CHAID analysis is of significant interest due to its ability to accurately classify samples based on the adjacent grouping of samples according to PUFA and COX. These findings validate the established association between PUFA and COX ( $r^2 = 0.9950$ ,  $p < 0.05$ ).

## 4. Conclusion

This work was primarily aimed to investigate the feasibility of enriching VIO and VAO with  $\omega_3$  FAs by adding WO with the intention of improving the nutritional attributes. By increasing the proportion of WO

from 5 to 30 %, PUFA levels rose (31.91–49.56 %), whereas MUFA (32.35–50.63 %) and SFA (15.22–21.06 %) contents declined in the blended oils. Additionally, the VIO:WO and VAO:WO blends at a (70:30) ratio showed the highest concentrations of  $\gamma$ -tocopherol (475.34 and 449.53 mg/kg, respectively) and  $\delta$ -tocopherol (44.22 and 46.21 mg/kg, respectively). There was a strong positive correlation between  $\alpha$ -TE/PUFA ratio and E-vitamin activity, alongside a negative correlation with PUFA levels. From a nutritional point of view, VIO:WO at (70:30) ratio had a more desirable  $\omega_6/\omega_3$ , PUFA/SFA, S/P ratios, DFA, OFA, HH, AI, and TI, compared to other blends.

During storage at 60 °C, there was a significant increase in hydrolytic (AV) and oxidative degradation indicators (PV, p-AnV, OV,  $E_{232}$ , and  $E_{270}$ ) in the blended oil, particularly in the VAO:WO blends. However, the VIO:WO blends retained acceptable physicochemical profiles. Subtle alterations in the FA profile of the blended oils were also recorded during storage. Additionally, the VIO:WO and VAO:WO blends at a (70:30) ratio exhibited more significant tocopherol losses, with reductions of 55.35 and 40 %, respectively.

Additional research is required to determine the actual shelf-life of the formulated blends in both static conditions (such as those found in retail markets) and dynamic conditions (where the head-space volume increases over time), as well as their performance during frying. In addition, further research on various types of peanuts, such as high oleic peanuts, can help to expand the existing database. In summary, the development of VIO and VAO blends with WO presents an opportunity to acquire lipid matrices that are abundant in  $\omega_3$  FAs, resulting in enhanced nutritional value compared to pure oils.

### CRedit authorship contribution statement

**Zineb Lakhlifi El Idrissi:** Writing – original draft, Investigation, Formal analysis, Data curation. **Chakir El Guezzane:** Software, Data curation. **Ihssan Boujemaa:** Writing – original draft, Resources, Investigation, Data curation. **Sara El Bernoussi:** Writing – original draft, Resources, Investigation, Data curation. **Aicha Sifou:** Writing – review & editing, Methodology, Data curation. **Hamza El Moudden:** Investigation, Data curation. **Riaz Ullah:** Writing – review & editing, Supervision, Investigation, Funding acquisition. **Ahmed Bari:** Writing – review & editing, Investigation, Data curation. **Khang Wen Goh:** Writing – review & editing, Supervision, Investigation, Funding acquisition. **Bey Hing Goh:** Investigation, Visualization, Writing – review & editing. **Abdelhakim Bouyahya:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Hicham Harhar:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Conceptualization. **Mohamed Tabyaoui:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization.

### Declaration of competing interest

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

### Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2024.101453>.

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