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Effect of Storage Conditions and Antioxidants on the Oxidative Stability of Sunflower–Chia Oil Blends

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Abstract The mixture of different proportions of sunflower with chia oil provides a simple method to prepare edible oils with a wide range of desired fatty acid compositions. Sunflower–chia (90:10 and 80:20 wt/wt) oil blends with the addition of rosemary (ROS), ascorbyl palmitate (AP) and their blends (AP:ROS) were formulated to evaluate the oxidative stability during storage at two temperature levels normally used, cool (4 ± 1 °C) and room temperature (20 ± 2 °C) for a period of 360 days. Peroxide values (PV) of the oil blends with antioxidants stored at 4 ± 1 °C showed levels ≤10.0 mequiv O_2/kg oil; the lowest levels of PV were found for blends with AP:ROS. Values higher than 10.0 mequiv O_2/kg were observed between 120–240 days for oil blends stored at 20 ± 2 °C. Similar trends were observed with *p*-anisidine and Totox values. The oxidative stability determined by the Rancimat method and differential scanning calorimetry showed a greater susceptibility of the oils to oxidative deterioration with increasing unsaturated fatty acids content. The addition of antioxidants increased the induction time and decreased the Arrhenius rate constant, indicating an improvement in the oxidative stability for all the oil blends. Temperature had a strong influence on the stability of these blends during storage.

Keywords Oil blends · Oxidative stability · Storage conditions · Differential scanning calorimetry · Rancimat

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Introduction

Sunflower (*Helianthus annuus* L.) oil is one of the most consumed vegetable oils in Argentina. The oil obtained from traditional hybrids contains 65–70 % of linoleic acid (ω-6) [1]. Chia (*Salvia hispanica* L.) seed oil is an interesting source of polyunsaturated fatty acids (PUFA), containing the highest content of α-linolenic acid (~60 %) of any known vegetable source. This fatty acid (FA) belongs to the ω-3 family, which is essential for normal growth and development in the human body. PUFA oxidation generates volatile compounds that impart undesirable flavors and aromas, and compromise the nutritional quality of the oil limiting its shelf life [2, 3]. The products formed in this degradation process have been associated with physiological disorders such as aging and diseases (atherosclerosis and carcinogenesis) [4].

The nutritional aspects of edible oils associated with the presence of minor and major components play an important role in preventing diseases and improving health. It is important to formulate vegetable oil blends with special composition in order to enhance their stability and nutritional value [5, 6]. FAO/WHO have recommended that the essential ω-6:ω-3 FA balance in the diet should be between 5:1 and 10:1. Individuals who consume a ratio in excess of 10:1 should be encouraged to eat more ω-3 rich foods [7].

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Blending of vegetable oils has emerged as an economical way of modifying the physicochemical characteristics of vegetable oils and of enhancing their oxidative stability.

Oilseeds produce natural substances with antioxidant properties, among which tocopherols stand out. Tocopherols exist in four different naturally-occurring forms (α-, β-, γ- and δ-tocopherol) that differ in the location of the methyl groups on the chromanol ring. There are differences among the four types of tocopherols in relation to their antioxidant activity *in vitro* and *in vivo*. Thus, α-tocopherol is characterized by a maximum effectiveness as *in vivo* antioxidant or vitamin E, but its *in vitro* activity is low in comparison with other tocopherols; γ-tocopherol, in contrast, has high *in vitro* antioxidant activity. Total tocopherol concentration in sunflower oils from traditional hybrids (mainly α-tocopherol) varies widely [1]. On the other hand, the total amount of tocopherols in chia oils (mainly γ-tocopherol) is lower than that reported for sunflower oils [8].

In recent years, various natural and synthetic antioxidants, such as rosemary extract (ROS) and ascorbyl palmitate (AP), have appeared on the market. The main compounds responsible for the antioxidant activity of ROS are phenolic diterpenes, including carnosic acid, carnosol, rosmanol, epirosmanol, isorosmanol, methyl carnosate, and phenolic acids, such as rosmarinic acid. AP is a synthetically-derived oil-soluble ester of ascorbic acid. The mechanisms by which these antioxidants are involved in the control of the autoxidation process are different: ROS acts as a radical scavenger, whereas AP acts as an oxygen scavenger [2].

Previous reports on oil blends have focused on storage stability in relation to the content of minor components [6] and FA composition [4, 5]. However, little information regarding oxidative stability as a function of storage conditions of sunflower–chia oil blends with and without the addition of antioxidants is available in the literature.

Numerous methods using accelerated oxidation conditions have been developed for the evaluation of oxidative stability. The Rancimat method and differential scanning calorimetry (DSC) are based on the generation of volatiles and thermal release, respectively [9].

However, accelerated tests have a disadvantage because the oxidation process takes place under drastic conditions, quite unlike those typically occurring in oil storage tanks or even during the commercialization of these products. As a consequence, the methods selected to determine the endpoint of the stability assays and the changes observed in the oils could not have a satisfactory correlation with the autoxidation process that takes place at room temperature [2].

Taking the above background into account, the present study was performed in order to obtain a product with an appropriate balance of essential ω-6:ω-3 FA and oxidative stability using blends with different proportions of sunflower and chia oils, with the addition of antioxidants (ROS, AP and 1:1 AP:ROS).

The objective of this work was to study the oxidative deterioration of oil blends using different methods (DSC, Rancimat) in order to evaluate the influence of temperature and time, and the effectiveness of antioxidants during the storage of sunflower–chia oil blends, determining the evolution of primary and secondary oxidation products.

Experimental Procedures

Materials

The oils used in this work were chia seed oil (Nutracéutica Sturla S.R.L., Argentina) obtained by cold pressing, and refined sunflower oil (Molinos Río de la Plata S.A, Argentina). Rosemary extract (Guardian™ 201, oil-soluble) and ascorbyl palmitate (Grindox™ 562) were obtained from Danisco (Denmark). The content of the main rosemary antioxidative component (diterpene phenols) was 4 % (wt/wt), and Grindox™ 562 contained 10 % of ascorbyl palmitate. All the antioxidants used are classified as GRAS additives (generally recognized as safe) in the United States. All the chemicals and solvents used were of analytical grade.

Blending of Vegetable Oils

The oil blends were formulated by blending sunflower with chia seed oil in proportions of 80:20 and 90:10 (wt/wt). The oils were thoroughly mixed for 5 min to obtain uniform blends. Rosemary extract (ROS), ascorbyl palmitate (AP) and their blends AP:ROS (1:1) were added to the oil blends in the following concentrations: 5,000, 2,000 and 2,000:2,000 ppm of the commercial products, respectively. Samples without antioxidants were used as control treatments.

Once prepared, the oil blends were packaged in amber glass bottles (30 mL each), flushing with nitrogen for 30 s immediately before closing the bottles. Half of the bottles from each treatment were stored in a cold chamber at 4 ± 1 °C, while the rest were placed in a temperature-controlled room at 20 ± 2 °C, both batches for 360 days. Two bottles corresponding to each treatment in each storage condition were taken periodically for analysis.

Analytical Methods

Physicochemical Characteristics of Oils and Their Blends

Fatty acid composition was analyzed by GC using a Hewlett Packard 6890 chromatograph with a flame

ionization detector (FID) according to IUPAC 2.302 standard method. Fatty acids were transesterified into fatty acid methyl esters (FAME) using the BF_3-methanol reagent following IUPAC method 2.301 [10]. Free fatty acid content was determined according to AOCS recommended practice Ca 5a-40 [11]. The results were expressed as the relative percentage of each individual fatty acid (FA) presents in the sample.

The tocopherol content in the oil was determined by normal phase HPLC using a Hewlett Packard chromatography system (HPLC Hewlett Packard 1050 Series, Germany) equipped with an Agilent 1100 Series fluorescence detector (Agilent Technology, USA) following the procedures described in IUPAC 2.432 [10] and AOCS Ce8-89 [11].

The oxidative stability of the oil blends during storage was monitored by measuring periodically the peroxide value (PV) expressed as milliequivalents of peroxides per kilogram of oil (mequiv O_2/kg of oil) and the *p*-anisidine value (*p*-AV) according to AOCS methods Cd 8-53 and Cd 18-90, respectively [11]. The total oxidation value (Totox) was calculated from the PV and *p*-AV as Totox = 2PV + *p*-AV.

Differential Scanning Calorimetry

Thermal-oxidative decomposition of the vegetable oil blends was studied by a differential scanning calorimetry method (DSC). A non-isothermal DSC study was carried out in a Q 100 (TA Instruments, USA) differential scanning calorimeter [12]. Oil samples of 3–5 mg were placed in an aluminium pan and then heated at constant heating rates $\beta = 2.5$, 5.0, 10.0, 15.0 and 20.0 °C/min from 10 to 350 °C in an oxygen flow of 100 mL/min. Temperatures of the extrapolated start of oxidation (T_e) and temperatures of maximum heat flow (T_{p1} and T_{p2}) were determined with each DSC thermogram using the TA Universal Analysis 2000 software (v. 4.2E) (TA Instruments, USA).

Calculation of Kinetic Parameters Using DSC

T_e, T_{p1} and T_{p2} were used to calculate E_a, the pre-exponential factors (A) and the Arrhenius rate constant (k) by the Ozawa–Flynn–Wall (OFW) method [2, 12].

The plot of log β vs. $1/T$, found by the OFW method, shows the linear dependence described by the following equation:

$$\log \beta = aT^{-1} + b \tag{1}$$

Arrhenius plots with a slope ($a = \mathrm{d}\log \beta/\mathrm{d}T^{-1}$) calculated by means of the method of least squares were used to obtain E_a and the A from Eqs. 2 and 3 as follows:

$$E_a = -2,19R\frac{\mathrm{d}\log \beta}{\mathrm{d}T^{-1}} \tag{2}$$

$$A = \frac{\beta E_a \exp\left[E_a/RT\right]}{RT^2} \tag{3}$$

where R is the gas constant and T the absolute temperature [12].

Values of E_a and A can be used to calculate k given by the Arrhenius Eq. 4:

$$k = A\mathrm{e}^{-E_a/RT} \tag{4}$$

Rancimat Analysis

The oxidative stability of each oil and their blends during storage was evaluated by the Rancimat method Mod 743 (Metrohm AG, Herisau, Switzerland) [11]. The assays were carried out using 5 g of oil sample at 98 ± 0.5 °C with an air flow of 20 L/h. Oil stability was expressed in terms of induction time (t_i, h).

Statistical Analysis

Statistical analysis was performed by ANOVA at the 5 % significance level ($p \leq 0.05$). Means were separated according to Tukey's multiple comparison test ($p \leq 0.05$) in all cases. Data were processed using the Statgraphics Plus statistical package (Version 4.0 for Windows, Manugistics Inc., USA) [13].

Results and Discussion

Characterization of Oils and Their Blends

The initial physicochemical characteristics of the sunflower and chia oils and their blends are given in Table 1. The results of chia oil (obtained by cold pressing) are similar to those reported in the literature [2]. The antioxidant and prooxidant minor components of chia seed oil were not removed in order to obtain information for industrial applications. The fatty acid profile of refined sunflower oil was characterized by high amounts of linoleic (C18:2, ω-6) and oleic (C18:1, ω-9) acids. Chia oil had a high content of α-linolenic acid (C 18:3, ω-3) representing 65 % of total fatty acids. The 80:20 and 90:10 wt/wt sunflower–chia blends contained approximately 17.4 and 9.0 % of C18:3, corresponding to a ω-6:ω-3 ratio of 2.7:1 and 5.3:1, respectively. The total tocopherol concentrations of the pure oils were 411 mg/kg (chia) and 502 mg/kg (sunflower). Other authors reported ranges from 447 to 900 mg/kg of tocopherols (mainly α-tocopherol) in sunflower oil

Table 1 Physicochemical characteristics of sunflower, chia oil and their blends

	Sunflower oil	Chia oil	Sunflower–chia 80:20 wt/wt	Sunflower–chia 90:10 wt/wt
Fatty acids (relative area %)				
$C_{16:0}$	6.6 ± 0.1	7.1 ± 0.3	7.6 ± 1.5	7.3 ± 1.4
$C_{18:0}$	2.3 ± 0.1	2.1 ± 0.2	2.3 ± 0.1	1.1 ± 0.4
$C_{18:1}$	36.6 ± 0.1	6.3 ± 0.3	26.1 ± 0.3	34.6 ± 2.3
$C_{18:2}$	54.4 ± 0.1	19.4 ± 0.1	46.7 ± 1.4	48.0 ± 1.8
$C_{18:3}$	nd	65.2 ± 0.9	17.4 ± 0.1	9.0 ± 0.8
Free fatty acids (% oleic acid)	0.06 ± 0.01	0.55 ± 0.02	0.18 ± 0.01	0.10 ± 0.01
Tocopherols (mg/kg)				
Total	502 ± 19	411 ± 19	454 ± 19	433 ± 19
α	498 ± 19	nd	376 ± 1	422 ± 1
β	4 ± 1	nd	6 ± 17	2 ± 17
γ	nd	404 ± 3	72 ± 3	9 ± 3
δ	nd	7 ± 2	nd	nd
Metal content (mg/kg)				
Cu	0.01 ± 0.00	0.19 ± 0.01	0.03 ± 0.01	0.01 ± 0.00
Fe	0.64 ± 0.05	0.30 ± 0.01	0.28 ± 0.06	0.61 ± 0.00
Peroxide value (mequiv O_2/kg)	1.5 ± 0.2	0.8 ± 0.1	1.5 ± 0.3	1.0 ± 0.2
p-Anisidine value	5.1 ± 0.2	0.5 ± 0.1	3.6 ± 0.1	4.5 ± 0.2
Induction time (h)	13.0 ± 0.3	3.0 ± 0.1	7.6 ± 0.3	9.2 ± 0.4

Mean values ± standard deviation of two independent batches ($n = 2$)

nd not detected

from traditional hybrids [1], with extreme values varying from 389 to 1,873 mg/kg [14, 15]. On the other hand, total tocopherols in chia oils (mainly γ-tocopherol) varied from 238 to 427 mg/kg [8].

Free fatty acid content, PV and *p*-AV were low, indicating the high quality of the starting oils used. The induction time (Rancimat) showed that chia oil was extremely susceptible to oxidation. Blending it with sunflower oil enhanced markedly this indicator of oxidative stability. Traces of metals, particularly copper and iron ions, are known to be effective prooxidants in lipid oxidation, therefore they are undesirable in oils. Contents of both metals in the oil blends were lower than those reported in the literature for chia oils [8].

Differential Scanning Calorimetry

Several authors reported that DSC is a good technique to determine the kinetic parameters of fatty acid oxidation which are necessary to control and predict oxidation reactions in lipid-based products [12]. Non-isothermal DSC methods are of practical analytical value because they are simple, not time consuming and may be applied to the analysis of small samples (2–10 mg).

One example of DSC oxidation curves obtained for sunflower oil, chia oil and their blends at one of the heating rates studied ($\beta = 10$ °C/min) is shown in Fig. 1 The point at which the heat flow signal separates from the baseline is considered to be the start of oxidation or initiation stage where the exothermic changes are minor. A sudden increase in the heat flow signal is related to the propagation stage, associating the maximum value (T_{p1}) with the termination stage, where stable products are formed. The second maximum heat flow, T_{p2}, may be associated with secondary oxidation processes (i.e., hydroperoxide degradation, cross-linking and polymerization). This suggests that at least two principal exothermic processes are occurring. Ideally, each peak can be analyzed and the kinetic parameters being associated with the respective oxidative process [16].

All the thermograms presented two main peaks, which were more or less pronounced depending on the type of oil or blend studied. The oxidation curve obtained for chia oil showed the highest first peak, whereas sunflower oil had the lowest one. Oil blends presented intermediate heights (Fig. 1). According to Litwinienko [12], an approximate model of the sequential reactions with autocatalytic onset is the best explanation for the DSC signal shape. This model is in accordance with the chain reaction scheme of free radical oxidation of lipids. It shows that the first process observed in the non-isothermal DSC curve is caused by the formation of peroxides. The second peak results from the

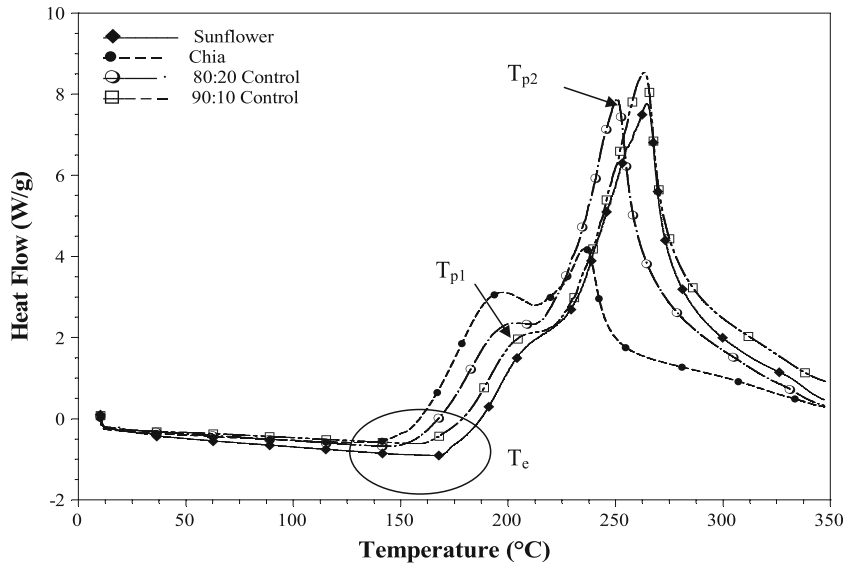

Fig. 1 DSC curves of thermal oxidation of sunflower oil, chia oil and their blends at a heating rate of $\beta = 10$ °C/min

decomposition of the peroxides to further products. Litwinienko suggests that the Arrhenius activation energies (E_a) and rate constants (k) calculated from the onset and first peak may be useful in assessing the susceptibility of edible oils to oxidation. This reaction can be represented by the scheme shown in Eq. 5.

$$a \xrightarrow{b} b \longrightarrow c \tag{5}$$

where the first step, a → b, is the process catalyzed by b. The above interpretation was confirmed experimentally by several other studies including oxidation of chia oils [2].

The evaluation of the stability of edible oils using DSC to calculate the kinetic parameters of thermoxidation should consider onset temperatures (T_e) of the process and temperatures of the first peak (T_{p1}) rather than the second (T_{p2}). Some authors suggest that, from an analytical point of view, the start of the thermal effect of autoxidation (T_e) is the most accurate point for the calculation of kinetic parameters because the first and second exothermal peaks can overlap (and usually do) [17]. Besides, different studies [12, 18] reported that the addition of antioxidants delayed the start of the oxidation and the first peak, whereas the second peak was not affected.

The E_a value obtained from T_e for chia oil was 69.5 ± 1.2, similar to that reported by Ixtaina *et al.* [2] and to that of pure α-linolenic acid, which is the most abundant FA in this oil. On the other hand, the E_a value for sunflower oil (106.3 ± 3.4 kJ/mol) was higher than that of chia oil and similar to that of corn oil (104.3 kJ/mol) [18], both oils containing similar percentages of 18:2 (from 34 to 65.6 % according to the Codex alimentarius [19]). This kinetic parameter indicates that the oxidative stability of sunflower oil is greater than that of chia oil, and this is associated with the lower content of PUFA present in sunflower oil.

All the thermograms of the treatments with and without the addition of antioxidants for a given heating rate were similar, showing two main peaks, but the calculated kinetic parameters were different (Tables 2, 3). The values of E_a and A obtained from T_e were on average about 7 % higher than those calculated from T_{p1} and 35 % higher than T_{p2}. Oxidation is a very complex process leading to numerous oxidation products involving various intermediates. These intermediate compounds have their own rate constant. The overall E_a is the cumulative effect of all the E_a available in the system during the period of oxidation [12, 18]. According to the Arrhenius principle, oil with a high E_a value oxidizes faster at high temperatures, while oil with a low E_a value oxidizes faster at low temperatures. However, calculated values of E_a should not be used as a single parameter to compare the oxidation stability of different lipid systems. Value of k is another important kinetic parameter for this type of comparison [20].

Regarding k, the differences between values calculated from T_e and T_{p1} were 0.033 and 0.015 s^{-1} for control 80:20 and 90:10 wt/wt blends. The addition of antioxidants decreased the k value obtained from T_{p1}, but not from T_{p2}, such that the differences between k calculated from both temperatures are lower. This finding suggests that these antioxidants act mainly in the start of the oxidation. Thus, the onset of the thermoxidation process may be a very useful point for the determination of the effects of additives, such as antioxidants.

It can be noted that kinetic parameters E_a and A calculated from T_e of the 80:20 wt/wt sunflower–chia oil blends were lower than those of the 90:10 wt/wt blends. The value

Table 2 Statistic and kinetic parameters of thermoxidation of sunflower–chia 80:20 (wt/wt) oil blends with the addition of different antioxidants calculated from temperatures T_e, T_{p1} and T_{p2} by the Ozawa–Flynn–Wall method

	T_e	T_{p1}	T_{p2}	T_e	T_{p1}	T_{p2}
	Sunflower–chia 80:20 control			*Sunflower–chia 80:20 + 2,000 ppm AP*		
Slope (*a*)	-4.370 ± 0.110^{c}	-4.224 ± 0.377^{a}	-3.338 ± 0.358^{a}	-4.564 ± 0.054^{b}	-4.475 ± 0.265^{a}	-2.954 ± 0.093^{a}
Constant (*b*)	11.170 ± 0.039^{a}	9.951 ± 0.807^{a}	7.329 ± 0.684^{a}	$11.414 \pm 0.118^{a,b}$	10.418 ± 0.596^{a}	6.604 ± 0.179^{a}
R^2	0.962	0.972	0.984	0.985	0.982	0.995
E_a (kJ/mol)	79.6 ± 0.2^{a}	76.9 ± 0.9^{a}	60.8 ± 6.5^{a}	$83.1 \pm 1.0^{a,b}$	81.5 ± 4.8^{a}	53.8 ± 1.7^{a}
A (s^{-1})	$3.19 \times 10^{12} \pm 2.78 \times 10^{11\ a}$	$1.98 \times 10^{11} \pm 4.36 \times 10^{10\ a}$	$9.59 \times 10^{9} \pm 8.56 \times 10^{8\ a}$	$5.44 \times 10^{12} \pm 1.40 \times 10^{12\ b}$	$8.09 \times 10^{11} \pm 8.35 \times 10^{10\ a}$	$1.33 \times 10^{8} \pm 4.95 \times 10^{7\ a}$
$k_{25\ °C}$ (s^{-1})	0.036 ± 0.002^{c}	0.003 ± 0.001^{a}	0.028 ± 0.003^{a}	0.012 ± 0.002^{b}	0.003 ± 0.002^{a}	0.049 ± 0.015^{a}
	Sunflower–chia 80:20 + 5,000 ppm ROS			*Sunflower–chia 80:20 + 2,000:2,000 ppm AP:ROS*		
Slope (*a*)	-5.027 ± 0.079^{a}	-4.861 ± 0.041^{a}	-3.061 ± 0.038^{a}	-4.823 ± 0.045^{ab}	-4.297 ± 0.316^{a}	-2.959 ± 0.063^{a}
Constant (*b*)	12.467 ± 0.162^{c}	11.262 ± 0.126^{a}	6.834 ± 0.086^{a}	11.952 ± 0.117^{b}	10.024 ± 0.644^{a}	6.615 ± 0.099^{a}
R^2	0.993	0.978	0.994	0.979	0.978	0.996
E_a (kJ/mol)	$91.5 \pm 1.4^{b,c}$	88.5 ± 0.7^{a}	55.7 ± 0.7^{a}	87.8 ± 0.8^{b}	78.2 ± 5.76^{a}	53.9 ± 1.14^{a}
A (s^{-1})	$5.67 \times 10^{13} \pm 1.98 \times 10^{13\ c}$	$3.62 \times 10^{12} \pm 1.00 \times 10^{12\ a}$	$2.12 \times 10^{8} \pm 3.93 \times 10^{7\ a}$	$1.78 \times 10^{13} \pm 4.59 \times 10^{12\ c}$	$3.57 \times 10^{11} \pm 1.17 \times 10^{11\ a}$	$1.33 \times 10^{8} \pm 2.72 \times 10^{7\ a}$
$k_{25\ °C}$ (s^{-1})	0.005 ± 0.001^{a}	0.002 ± 0.001^{a}	0.036 ± 0.003^{a}	0.007 ± 0.001^{a}	0.005 ± 0.004^{a}	0.048 ± 0.012^{a}

Mean values ± standard deviation of two independent batches

R^2 determination coefficient, E_a activation energy, A pre-exponential factor, k rate constant calculated for 25 °C, T_e temperature of extrapolated start of the oxidation, T_{p1} *and* T_{p2} maximal points of the two peaks

Different superscript letters indicate significant differences ($p \leq 0.05$) between treatments for each temperature (T_e, T_{p1}, T_{p2}) according to Tukey's (HSD) test

Table 3 Statistic and kinetic parameters of thermoxidation of sunflower–chia 90:10 (wt/wt) oil blends with the addition of different antioxidants calculated from temperatures T_e, T_{p1} and T_{p2} by the Ozawa–Flynn–Wall method

	T_e	T_{p1}	T_{p2}	T_e	T_{p1}	T_{p2}
	Sunflower–chia 90:10 control			*Sunflower–chia 90:10 + 2,000 ppm AP*		
Slope (a)	-4.595 ± 0.020^{c}	-4.425 ± 0.049^{a}	-2.987 ± 0.090^{b}	$-4.835 \pm 0.109^{b,c}$	-4.142 ± 0.109^{a}	-3.886 ± 0.176^{a}
Constant (b)	11.628 ± 0.054^{a}	10.345 ± 0.107^{a}	6.636 ± 0.183^{a}	$11.949 \pm 0.221^{a,b}$	9.647 ± 0.207^{a}	8.259 ± 0.329^{a}
R^2	0.992	0.990	0.991	0.966	0.986	0.961
E_a (kJ/mol)	83.7 ± 0.4^{a}	80.6 ± 0.9^{a}	54.4 ± 1.6^{a}	$88.0 \pm 2.0^{a,b}$	75.4 ± 2.0^{a}	70.7 ± 3.2^{b}
A (s^{-1})	$8.75 \times 10^{12} \pm 1.04 \times 10^{12\ a}$	$4.78 \times 10^{11} \pm 1.11 \times 10^{11\ a}$	$1.42 \times 10^{8} \pm 5.40 \times 10^{7\ a}$	$1.84 \times 10^{13} \pm 8.59 \times 10^{12\ a,b}$	$1.06 \times 10^{11} \pm 4.63 \times 10^{10\ a}$	$4.98 \times 10^{9} \pm 5.40 \times 10^{8\ a}$
$k_{25\ °C}$ (s^{-1})	0.019 ± 0.002^{c}	0.004 ± 0.001^{a}	0.041 ± 0.013^{a}	0.009 ± 0.001^{b}	0.004 ± 0.001^{a}	0.038 ± 0.010^{a}
	Sunflower–chia 90:10 + 5,000 ppm ROS			*Sunflower–chia 90:10 + 2,000:2,000 ppm AP:ROS*		
Slope (a)	-5.250 ± 0.015^{a}	-4.411 ± 0.256^{a}	$-3.370 \pm 0.393^{a,b}$	$-5.017 \pm 0.053^{a,b}$	-4.723 ± 0.120^{a}	$-3.030 \pm 0.100^{a,b}$
Constant (b)	12.880 ± 0.017^{c}	10.195 ± 0.191^{a}	7.312 ± 0.746^{a}	12.301 ± 0.095^{b}	10.901 ± 0.276^{a}	6.711 ± 0.033^{a}
R^2	0.978	0.981	0.992	0.994	0.995	0.975
E_a (kJ/mol)	95.6 ± 0.27^{c}	80.3 ± 4.7^{a}	$61.4 \pm 7.2^{a,b}$	$91.3 \pm 1.0^{b,c}$	86.0 ± 2.2^{a}	$55.2 \pm 0.2^{a,b}$
A (s^{-1})	$1.36 \times 10^{14} \pm 4.94 \times 10^{12\ c}$	$4.54 \times 10^{11} \pm 4.33 \times 10^{10\ a}$	$9.86 \times 10^{8} \pm 1.13 \times 10^{8\ a}$	$3.80 \times 10^{13} \pm 7.88 \times 10^{12\ b}$	$1.74 \times 10^{12} \pm 9.98 \times 10^{11\ a}$	$1.60 \times 10^{8} \pm 4.56 \times 10^{5\ a}$
$k_{25\ °C}$ (s^{-1})	0.002 ± 0.000^{a}	0.003 ± 0.001^{a}	0.033 ± 0.009^{a}	0.004 ± 0.001^{a}	0.001 ± 0.000^{a}	0.034 ± 0.011^{a}

Mean values ± standard deviation of two independent batches

R^2 determination coefficient, E_a activation energy, A pre-exponential factor, k rate constant calculated for 25 °C, T_e temperature of extrapolated start of the oxidation, T_{p1} *and* T_{p2} maximal points of the two peaks

Different superscript letters indicate significant differences ($p \leq 0.05$) between treatments for each temperature (T_e, T_{p1}, T_{p2}) according to Tukey's (HSD) test

of k for 25 °C increased with the % of chia oil. This suggests that, as the percentage of unsaturated fatty acids increased due to the addition of chia oil, the oxidation reaction was faster. For both oil blends the addition of ROS and AP:ROS increased the E_a and A values and decreased k with respect to the control treatments ($p \leq 0.05$). For the 80:20 sunflower–chia blend, the addition of AP, AP:ROS and ROS caused a decrease in k of 66.6 80.5 and 86.1 % vs. the control treatment, respectively. Regarding the 90:10 wt/wt oil blends, these changes were of 52.6, 78.9 and 89.4 %. This fact could be associated with the ability of these compounds to delay the autoxidation process. Thus, according to the DSC study, ROS and AP:ROS showed the best antioxidant activity.

Storage of Sunflower–Chia Oil Blends

Hydroperoxides, the primary products of lipid oxidation, were determined by PV while the secondary oxidation products (mainly 2-alkenals and 2,4-dienals) were monitored by *p*-AV. The Totox value provides a measurement of both primary and secondary oxidation products. For practical purposes, the prediction of the oxidative stability in oils and foods is related to the product shelf life. Therefore, it is important to note that the experimental conditions used should be as similar as possible to those under which the product will be stored [21].

Ixtaina *et al*. [2] evaluated the effectiveness of different antioxidants and concentrations on the oxidative stability of chia seed oil. This was taken into account in the present work in order to choose the most effective antioxidants and their concentrations.

The evolution of the PV, *p*-AV, and Totox values for the different treatments studied during storage at 4 ± 1 and 20 ± 2 °C are shown in Figs. 2 and 3, respectively. As expected, storage temperature was a relevant factor that affected the evolution of the oxidative process of the oils blends, similarly to what Ixtaina *et al*. [2] reported for chia oil.

After 360 days of storage at 4 ± 1 °C, none of the sunflower–chia oil blends (90:10 and 80:20 wt/wt) with the addition of different antioxidants exceeded the upper limit of PV (10.0 mequiv O_2/kg oil) established by the Codex Alimentarius for human consumption of oils not covered by individual standards (Fig. 2a) [19]. Oil blends without antioxidants and chia oil reached the legal limit after 150 days of storage at 4 ± 1 °C. After 30 days of storage, both control treatments showed an increase in the primary oxidation rate, with significant differences ($p \leq 0.05$) with respect to oil blends with the addition of antioxidants (Fig. 2a). The 90:10 and 80:20 wt/wt oil blends with AP:ROS had the lowest levels of PV, being significantly lower ($p \leq 0.05$) than the control treatments during all the storage time. A similar trend was observed with the addition of AP, which showed no significant differences ($p > 0.05$) with the samples with AP:ROS until 180 and 270 days for the 90:10 and 80:20 sunflower–chia blends, respectively.

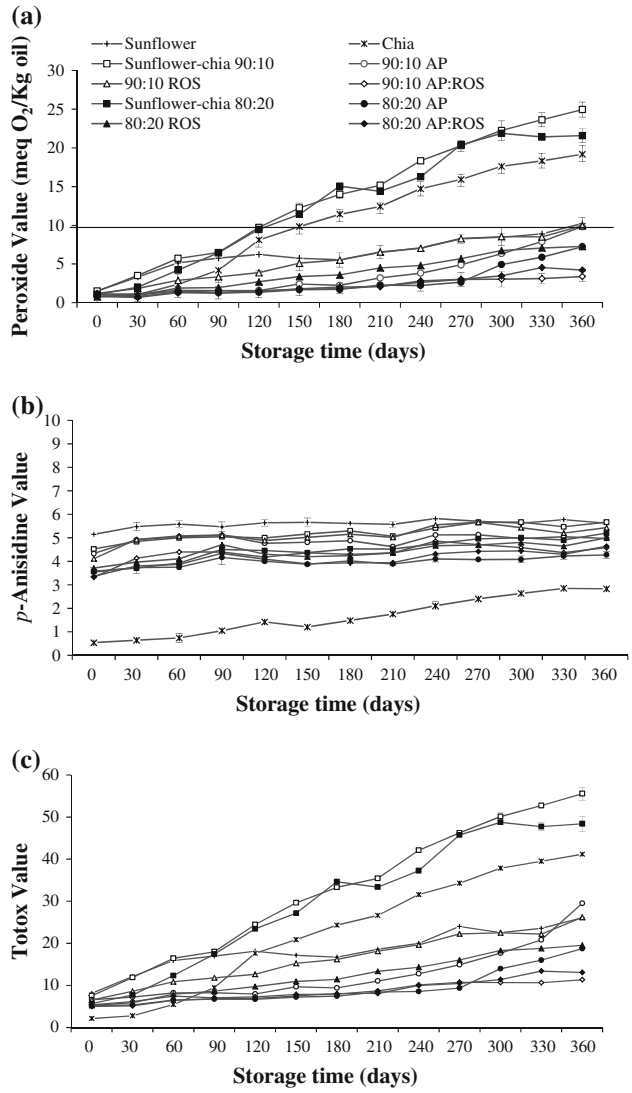

Fig. 2 Peroxide (**a**), *p*-anisidine (**b**) and Totox (**c**) values of sunflower oil, chia oil and their blends with and without the addition of antioxidants, stored at 4 ± 1 °C. Values are the mean of the two independent batches ($n = 2$) and *vertical bars* indicate standard deviation

In contrast, during storage at 20 ± 2 °C, the legal upper limit was reached between 120 and 240 days of storage in the following order: control oil blends (120 days), oil blends with ROS (150 days), oil blends with AP (180 days for 90:10 wt/wt, 210 days for 80:20 wt/wt), oils with AP:ROS for both oil blends (240 days) (Fig. 3a). PV values corresponding to the 90:10 and 80:20 wt/wt oil blends with AP:ROS presented the lowest increase after 360 days of storage, being AP:ROS the most effective antioxidant to

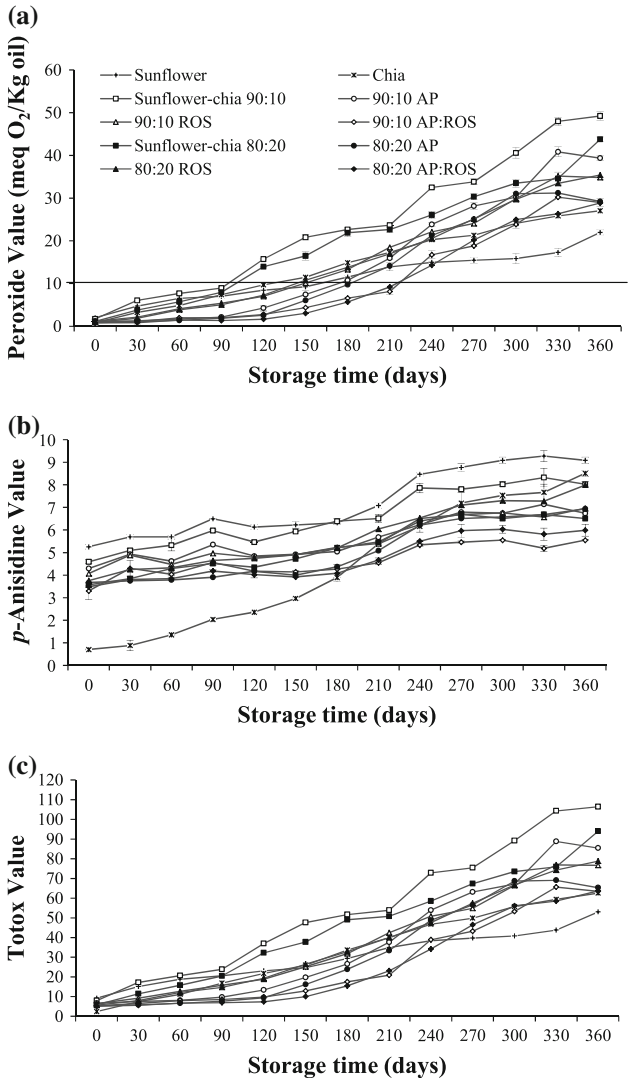

Fig. 3 Peroxide (**a**), *p*-anisidine (**b**) and Totox (**c**) values of sunflower oil, chia oil and their blends with and without the addition of antioxidants, stored at 20 ± 2 °C. Values are the mean of the two independent batches ($n = 2$) and *vertical bars* indicate standard deviation

retard the formation of hydroperoxides at room temperature (Fig. 3a).

With respect to *p*-AV at the initial stage of storage ($t = 0$), sunflower oil showed the highest value (5.1 ± 0.2), whereas chia oil (0.5 ± 0.1) presented the lowest one with the oil blends showing intermediate values according to the sunflower:chia oil ratio. Previous results [22] have shown that the induction period depends markedly on the *p*-AV of the starting oil.

The *p*-AV of oils was low during storage at 4 ± 1 °C (Fig. 2b), while at 20 ± 2 °C all the treatments varied widely (Fig. 3b). Chia oil showed the highest formation of secondary oxidation products for both storage temperatures, which may be attributed to its high content of PUFA.

Regarding oil blends stored at 4 ± 1 °C, the addition of AP produced a variation in *p*-AV of 15 and 21 % for the 90:10 and 80:20 wt/wt blends, respectively, whereas the treatments with ROS presented an increase of 32 and 35 %. For both oil blends with AP:ROS, changes of 38 % were observed. In contrast, after 180 days of storage at 20 ± 2 °C, the treatments studied showed marked increases (57–113 %) with the addition of different antioxidants.

Totox values exhibited an evolution similar to that found for PV for both storage temperatures (Figs. 2c, 3c).

The highest Totox values at 4 ± 1 °C were recorded at the end of the storage period, when the control oil blends presented values significantly higher ($p \leq 0.05$) than those with the addition of antioxidants (Fig. 2c). This difference could be mainly associated with the primary oxidation products (hydroperoxides), determined from the PV. Oil blends with the addition of antioxidants stored at 4 ± 1 °C presented Totox levels <30.0. In contrast, at 20 ± 2 °C the oil blends showed high levels of total oxidation, reaching values between 63.5 (90:10 sunflower–chia blend with AP:ROS) and 85.5 (90:10 sunflower–chia blend with AP) (Fig. 3c). These results show the influence of temperature on the preservation of oil blends with and without antioxidants.

In spite of the differences between oil blends in the fatty acid composition and tocopherol content, few differences were found between the oxidative stability of these treatments at the different conditions studied. One explanation to these results is related to the different types of natural tocopherols present in the samples. Sunflower oil contains mainly α-tocopherol (99.2 % of total tocopherols), which is the major vitamin E component, whereas chia oil has a high content of γ-tocopherol (98.3 % of total tocopherols) which has a high "*in vitro*" antioxidant activity Thus, oil blends with high proportions of chia oil and γ-tocopherol presented a similar behavior to that of oils with a high content of sunflower oil and α-tocopherol. The 80:20 wt/wt sunflower–chia oil blends contained 14.0–16.0 % of γ-tocopherol and 82.8 % of α-tocopherol relative to the total tocopherol content; the 90:10 wt/wt oils blends showed a lower content of γ-tocopherol (<6.0 %) and higher content of α-tocopherol (>94.0 %) (Table 1). However, additional factors could affect the antioxidant-prooxidant balance of oils blends, mainly metal and phospholipid content and other minor components.

The fatty acid composition of oil blends with and without the addition of antioxidants did not vary significantly ($p > 0.05$) during storage at both temperatures (data not shown).

Rancimat Analysis

The Rancimat method was used to evaluate the oxidative stability of sunflower oil, chia seed oil and their blends

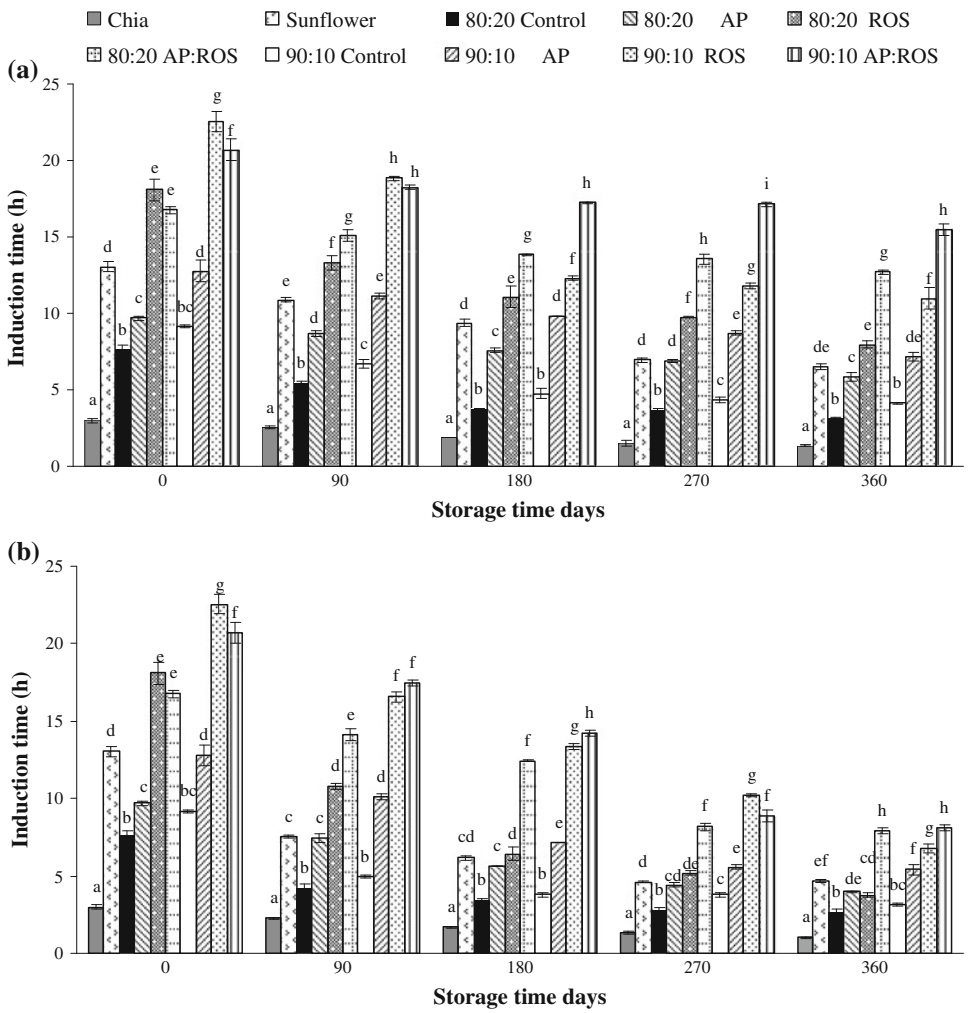

Fig. 4 Induction time measured by the Rancimat method for sunflower oil, chia oil and their blends, with and without the addition of antioxidants, stored at 4 ± 1 °C (**a**) and 20 ± 2 °C (**b**). Values are the mean of the two independent batches ($n = 2$) and *vertical bars* indicate standard deviation. *Different letters* indicate significant differences ($p \leq 0.05$) between treatments for each time according to Tukey's (HSD) test

with the addition of different antioxidants (AP, ROS and AP:ROS) during storage at 4 ± 1 and 20 ± 2 °C and the results were expressed in terms of induction time (t_i). The t_i of the oil blends with and without the addition of antioxidants is shown in Fig. 4. The t_i value of every oil blend was between the two values of the constituent oils. The stability was affected by the fatty acid composition of the oils, decreasing as a function of increasing amounts of unsaturated fatty acids. The addition of antioxidants increased significantly ($p < 0.05$) the t_i of the oil blends. The stability of the 90:10 and 80:20 oil blends showed great variations of the t_i value treated with ROS (146 and 138 %) and AP:ROS (126 and 120 %) observed at the initial time ($t = 0$).

During storage at 4 ± 1 °C, AP:ROS was the most effective antioxidant. This can be observed from 90 days of storage for the 80:20 sunflower–chia oil blend and from 180 days for the 90:10 sunflower–chia oil blend (Fig. 4a). At 20 ± 2 °C, AP:ROS was the most effective antioxidant for the 80:20 sunflower–chia oil blend, while both AP:ROS and ROS were effective for the 90:10 blend, varying during storage. The results obtained using this accelerated test are in agreement with those found by monitoring oxidation indices (PV, *p*-AV and Totox) during storage of the oils previously reported in this paper.

Conclusions

The fatty acid composition of the sunflower–chia oil blends studied in this work indicates that the essential ω-6/ω-3 fatty acid balance can be achieved with a low proportion of chia oil (10 and 20 % wt/wt).

Differential scanning calorimetry thermograms at different heating rates were used to obtain the kinetic parameters of oils and their blends against lipid oxidation. E_a and A values calculated from T_e increased and k decrease with the addition of antioxidants for both oil blends, mainly with the addition of ROS and AP:ROS, indicating an improvement in the oxidative stability.

PV of oil blends (80:20 and 90:10 wt/wt) stored at 4 ± 1 °C with the addition of antioxidants recorded lower

PV values than the legal limit of 10 mequiv O_2/kg oil, indicating that relatively low oxidation rate occurred at low temperature. In contrast, most samples stored at 20 ± 2 °C achieved the legal limit of PV at between 120–240 days of storage. The addition of antioxidants resulted in marked decrease in the oxidation rate during storage. AP:ROS exhibited the most effective antioxidant activity for the conservation of the oil blends studied.

A decrease in induction times was observed in parallel with increasing PUFA content (mainly $C_{18:3}$), temperature and storage time. The addition of antioxidants increased the t_i in both oil blends. The addition of AP:ROS was more effective, improving the oxidative stability of the 80:20 and 90:10 sunflower–chia oil blends during storage at 4 ± 1 °C after 90 and 180 days, respectively. Oil blends stored at 4 ± 1 °C exhibited higher t_i values than those stored at 20 ± 2 °C. Temperature had a strong influence on oil oxidation. DSC study showed that ROS had the best antioxidant effect, whereas AP:ROS was the most effective antioxidant during storage at 4 ± 1 °C according to Rancimat tests and PV, *p*-AV and Totox values.

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