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Composition and oxidative stability of soybean oil in mixtures with jojoba oil

Improvement of the oxidative stability of soybean oil (SBO) by blending with jojoba oil (JO) was investigated. SBO in the presence of 5, 10, 15 and 20 wt-% of JO was subjected to accelerated storage at 60 °C. Peroxide values (PV), anisidine values (AV), UV absorption characteristics (K_{232} and K_{270} values), and headspace volatiles were determined to monitor the oxidative stability of oil samples. JO was effective in reducing the formation of hydroperoxides and volatile compounds in SBO. The effect was remarkable in SBO/JO blends containing 15 and 20% JO, which showed significant reductions in PV, AV and volatile content with respect to pure SBO. The increased oxidative stability of SBO/JO blends could not be attributed to JO tocopherols, since the addition of JO to SBO significantly reduced the tocopherol content of SBO. Besides the tocopherol content and unsaturation degree of SBO and JO, the effect of the JO ester structure on the oxidative stability of the blends is discussed. The enhanced chemical and flavor stabilities of SBO/JO blends with respect to pure SBO may make a significant contribution to improve the shelf life of SBO by reducing the deterioration reactions related to lipid peroxidation.

Keywords: Soybean oil, jojoba oil, blends, chemical composition, oxidative stability.

1 Introduction

At present, soybean oil (SBO) is the largest-volume industrial vegetable oil. Natural SBO is a complex mixture of triacylglycerols in which fatty acids form esters with glycerol. The fatty acid composition of SBO has been extensively investigated [1–4]. Large amounts of linoleic and linolenic acids are found in SBO, compared with those in other vegetable oils. Although this fact is considered to be nutritionally favorable, the high content of polyunsaturated fatty acids (PUFA) plays a crucial role in the oxidation of oils [5, 6]. PUFA, such as linoleic and linolenic acids, are extremely susceptible to peroxidation with molecular oxygen, and they are the most important precursors for off-flavor development in SBO.

Several methods have been proposed to enhance SBO resistance to oxidation, including addition of antioxidants, partial hydrogenation, interesterification with palm oil and genetic improvement devoted to reduce linolenic acid concentration [2, 7, 8]. In recent years, there has been some concern about the possible toxicity of *trans* isomers generated during hydrogenation and the safety of synthetic antioxidants has been questioned [9, 10]. On the other hand, new soybean breeding lines with low linolenic acid content have not yet appeared in the world oil market, and interesterification of SBO with other oils increases processing costs dramatically. Therefore, researchers are starting to consider non-conventional natural lipid sources, as well as more simple processes such as mixing, in order to obtain pre-designed fat products with the desired physical, chemical, and nutritional characteristics [11–13]. In this respect, the jojoba plant may be of interest because its seeds contain about 50% of a light yellow, odorless wax commonly referred to as jojoba oil (JO). It is a narrow mixture of straight-chain esters of monounsaturated long-chain fatty acids and long-chain primary fatty alcohols, in particular two ester molecules containing 40 and 42 carbon atoms which make up to 80% of the oil [14, 15]. Interest in JO stems from its unusual properties differing from all other known seed oils. It has been shown to have an extraordinary thermal and oxidative stability [16, 17]. JO is extensively used in the cosmetics industry due to its dermatological properties. Other uses include pharmaceuticals, lubricants, foam control agents, plasticizers and foods [16, 18–20].

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This work examines the influence of adding JO on the compositional and oxidative parameters related to SBO stability.

2 Materials and methods

2.1 Oil sources

Refined, bleached and deodorized SBO was provided by Aceitera General Deheza (Córdoba province, Argentina), and it was stored under nitrogen at −10 °C until used. Jojoba seeds were collected from commercial plantations at Aimogasta, La Rioja province, Argentina. After homogenization and cleaning, the seeds were extracted using a manually operated pilot-plant hydraulic press as described previously [17]. The oil obtained was passed through Whatman N° 1 filter paper and stored at −10 °C, without further treatment. Four oil blends were prepared by mixing different ratios (wt/wt) of the parent oils (Tab. 1).

2.2 Oil analyses

Peroxide, acidic, anisidine, K_{232}, K_{270} and iodine values from parent oils and blends were determined according to standard methods of the AOCS [21]. All chemicals used were of analytical grade.

Fatty acids and alcohols were analyzed by gas chromatography (GC) (Shimadzu GC-R1A, Kyoto, Japan). Briefly, oils were subjected to alkaline saponification (1 N potassium hydroxide in methanol), and unsaponifiable matter was extracted with *n*-hexane. The fatty acid methyl esters were obtained using 1 N sulfuric acid in methanol and analyzed by GC according to Maestri and Guzmán [22]. Unsaponifiable material from JO was fractionated on preparative TLC (silica gel 60 G, 0.5 mm), developed with *n*-hexane/diethyl ether (50 : 50, vol/vol). After developing, the alcohol fraction was extracted from the plate with chloroform and then purified further by repeated preparative silica gel TLC for subsequent GC analysis. The

Table 1. Chemical and oxidative parameters of SBO, JO and their blends.

Parameter	SBO	Oil blends SBO/JO wt/wt				JO
		95 : 5	90 : 10	85 : 15	80 : 20	
Palmitic (C16:0)	10.9^{a}	10.8^{a,b}	10.6^{a,b}	10.4^{b}	10.0^{c}	1.08^{d}
Stearic (C18:0)	4.21^{a}	4.04^{a,b}	3.91^{a,bç}	3.84^{bç}	3.65^{c}	Tr
Oleic (C18:1)	20.6^{a}	20.7^{a}	20.5^{a,b}	20.1^{bç}	19.9^{c}	10.1^{d}
Linoleic (C18:2)	55.7^{a}	53.2^{b}	52.0^{b}	50.5^{c}	49.2^{c}	Nd
Linolenic (C18:3)	7.84^{a}	7.60^{b}	7.39^{bç}	7.32^{bç}	7.05^{c}	Nd
Cis-11-eicosenoic (C20:1)	0.38^{a}	3.04^{b}	4.90^{b}	6.41^{c}	8.43^{d}	72.5^{e}
Cis-13-docosenoic (C22:1)	0.37^{a}	0.55^{b}	0.70^{b}	1.10^{bç}	1.48^{c}	14.8^{d}
Cis-15-tetracosenoic (C24:1)	Nd	Nd	Nd	Tr	Tr	1.42
SFA	15.11^{a}	14.84^{a,b}	14.51^{a,b}	14.24^{b}	13.65^{c}	1.08^{d}
MUFA	21.35^{a}	24.29^{b}	26.1^{c}	27.61^{c,d}	29.81^{d}	98.82^{e}
PUFA	63.54^{a}	60.8^{b}	59.39^{b}	57.82^{c}	56.25^{c}	Nd
Iodine value	141.6^{a}	138.8^{b}	137.5^{b}	135.7^{c}	134.4^{c}	81.2^{d}
Acidic value [% oleic]	0.06^{a}	0.07^{a}	0.07^{a}	0.08^{b}	0.09^{b}	0.11^{c}
Peroxide value [meq O_2/kg]	4.44^{a}	6.00^{a}	5.94^{a}	6.00^{a}	5.00^{a}	5.00^{a}
Induction period [h]†	76.4^{a}	80.7^{a,b}	86.2^{b}	108.0^{c}	152.7^{d}	
Stabilization factor§		1.05^{a}	1.13^{a}	1.41^{b}	2.0^{c}	
Anisidine value	4.22^{a}	4.21^{a}	4.20^{a}	4.15^{a}	3.90^{a}	0.12^{b}
K_{232}	4.02^{a}	3.93^{a}	4.05^{a}	3.96^{a}	3.96^{a}	2.21^{b}
K_{270}	1.01^{a}	0.91^{b}	0.91^{b}	0.84^{c}	0.82^{c}	0.07^{d}
α-Tocopherol	151.0^{a}	146.5^{a,b}	137.9^{b}	127.3^{c}	121.5^{c}	22.7^{d}
β-Tocopherol	27.5^{a}	25.7^{a,b}	23.8^{b}	21.9^{c}	20.9^{c}	Nd
γ-Tocopherol	545.1^{a}	519.0^{b}	489.2^{b}	457.9^{c}	436.8^{c}	40.6^{d}
δ-Tocopherol	207.2^{a}	190.3^{b}	177.8^{bç}	165.5^{c,d}	155.7^{d}	Nd
Total tocopherols	930.5^{a}	881.5^{b}	828.7^{c}	772.6^{d}	734.9^{e}	63.3^{f}

Fatty acids (C16:0–C24:1) are expressed as percentages of total fatty acids. Tocopherols are expressed as μg/g oil.
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Tr, trace (<0.3%); Nd, not detected.

† The time needed for the PV of the sample to become 20 meq O_2/kg oil.

§ $F = IP_{jo}/IP_{o}$, where IP_{jo} is the induction period of SBO in the presence of JO, and IP_{o} is the induction period of SBO alone.

Mean values (n = 3). Values in the same row followed by different superscript letters present significant differences at $p < 0.05$.

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identification of fatty acids and alcohols was carried out by GC-mass spectrometry [23] and by comparison of the retention times with those of reference compounds (Sigma-Aldrich, St. Louis, MO, USA).

Tocopherols were analyzed by HPLC (gradient pump model 2249 and variable-wavelength monitor model 2249; Pharmacia LKB, Bromma, Sweden) according to the procedure of Pocklington and Dieffenbacher [24]. Individual tocopherols were identified by comparison of their retention times with those of authentic standards (ICN, Costa Mesa, CA, USA) and published data [12, 13, 17].

Volatile compound analysis was carried out from 5-mL oil samples put into 15-mL headspace vials, fitted with silicon septa. Volatiles were sampled for 30 min at 50 °C from the headspace of the vial, with a 100-μm fiber coated with divinylbenzene/carboxene on polydimethylsiloxane, conditioned prior to use as recommended by the producer. After sampling, the fiber was immediately inserted into the injection port of a HP 5890 II gas chromatograph coupled to a HP 5972 A mass-selective detector (Hewlett-Packard, Palo Alto, CA, USA). The GC separations were performed using a HP 5 fused-silica capillary column (30 m long × 0.25 mm i.d.), coated with a 0.25-μm layer of 5% phenyl methyl siloxane, and helium (flow rate 1 mL/min) as carrier gas. The injector temperature was kept at 250 °C and the GC oven temperature was initially maintained at 50 °C (2 min) and then increased at 5 °C/min to 250 °C. Volatile compounds were identified by comparison of the mass spectral data with those of authentic reference compounds (Sigma-Aldrich). When standards were not available, the components were identified by mass spectrum matching using the Wiley mass spectra search library.

An accelerated stability test (Schaal Oven Test) was performed to evaluate the oxidative stability of the oils. Two replicates of each oil and oil blend sample (50 g each) were stored in 100-mL beakers without covers at 60 °C in the dark for 10 days. Every day, each individual oil sample was removed from the oven and used to measure the peroxide, anisidine, K_{232}, K_{270} and total tocopherol values. Headspace volatiles were monitored at the initial time (day 0) and the final time (day 10).

2.3 Statistical analyses

Statistical differences among treatments were estimated from ANOVA test at the 5% level ($p = 0.05$) of significance for all the parameters evaluated. Whenever ANOVA indicated a significant difference, a pairwise comparison of means by least significant difference (LSD) was carried out.

3 Results

Tab. 1 presents the chemical data of the parent oils (SBO and JO) and blends used in this study. Linoleic acid was the predominant fatty acid in SBO, followed by oleic, palmitic and linolenic acids in a decreasing order. JO was exceptionally rich in *cis*-11-eicosenoic acid. Besides this fatty acid, oleic and *cis*-13-docosenoic acids were detected in similar amounts. As expected, SBO was characterized by a high proportion of PUFA, whereas monounsaturated fatty acids (MUFA) were almost 99% of the fatty acids present in JO. As a consequence, SBO had the highest iodine value.

Eight fatty alcohols were found in JO: 0.7% *cis*-9-octadecenol, 42% *cis*-11-eicosenol, 48% *cis*-13-docosenol, 9% *cis*-15-tetracosenol, together with trace amounts (<0.3%) of hexadecanol, octadecanol, eicosanol and docosenol.

The combination between acids and alcohols in JO led to esters with 38, 40, 42, 44 and 46 as total number of carbon atoms, representing 5.4, 24.1, 55.1, 13.1 and 2.0% of the wax esters, respectively. These results were consistent with those reported by Busson-Breysse *et al.* [14].

The total tocopherol content in SBO was considerably higher than in JO (Tab. 1). The type and the amount of individual tocopherols present in SBO agreed with those of the literature [12, 13]. γ-Tocopherol was predominant, together with minor levels of δ-, α- and β-tocopherols. Only α- and γ-tocopherols were found in JO, and their contents were in agreement with those reported earlier [17]. Data for individual and total tocopherol contents showed statistically significant differences among oil samples. The SBO/JO blends had tocopherol values that decreased as the proportion of JO in the mixture increased.

The addition of JO to SBO increased the MUFA content and decreased the PUFA content and the iodine value of SBO (Tab. 1). Blending SBO with JO caused small but statistically significant increases in the acidic values of the oil blends. Peroxide values (PV) of pure oils and blends were unchanged. Pure JO had an anisidine value (AV) significantly lower than those of SBO and the oil blends (Tab. 1).

PV, AV, and conjugated dienes (K_{232}) and trienes (K_{270}) were used to measure the level of oxidation in the oils during storage at 60 °C (Figs. 1–4). All SBO/JO blends were more stable than pure SBO; the resultant PV from every oil blend were situated between the values of the constituent single oils (Fig. 1). Oil oxidation initially proceeded at a lower rate. The PV of all oil samples were not significantly different from each other until 72 h of storage. From this moment, the blends containing 15 and 20% JO

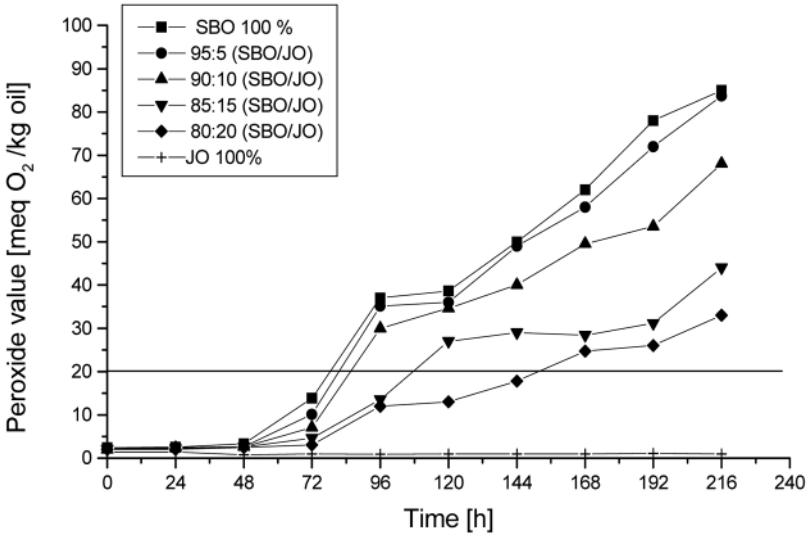

Figure 1. Kinetic curves of peroxide accumulation during oxidation of SBO, JO and their blends at 60 °C in the dark. All kinetic curves were the average result of two independent experiments.

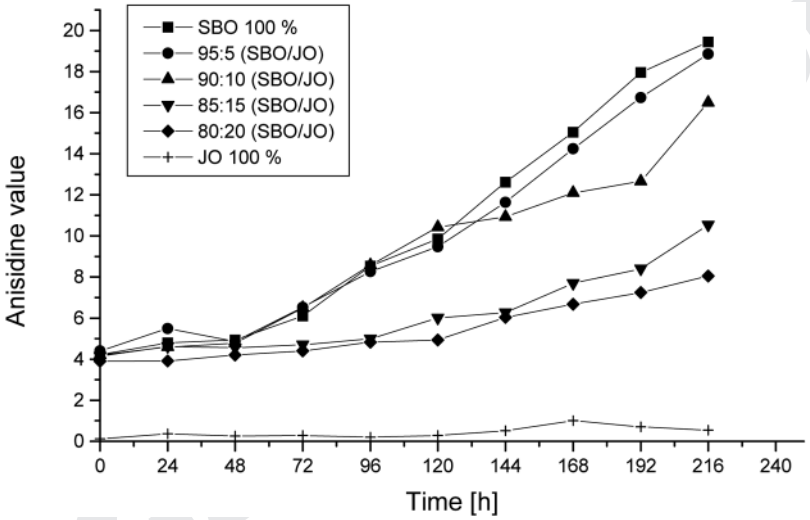

Figure 2. Kinetic curves of AV during oxidation of SBO, JO and their blends at 60 °C in the dark. All kinetic curves were the average result of two independent experiments.

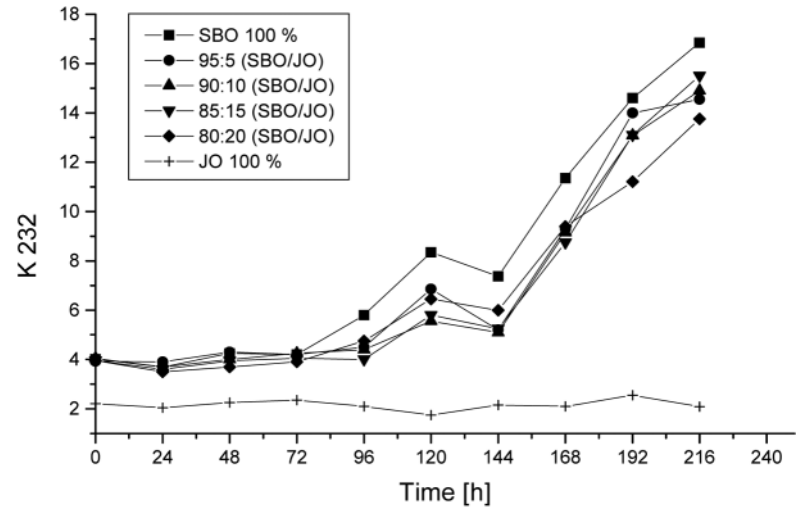

Figure 3. Kinetic curves of K_{232} values during oxidation of SBO, JO and their blends at 60 °C in the dark. All kinetic curves were the average result of two independent experiments.

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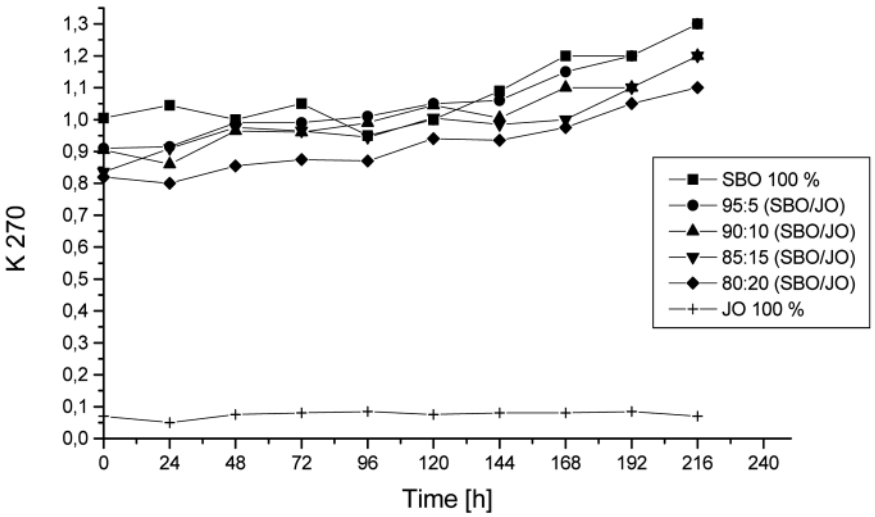

Figure 4. Kinetic curves of K_{270} values during oxidation of SBO, JO and their blends at 60 °C in the dark. All kinetic curves were the average result of two independent experiments.

had PV significantly lower compared with the other blends. The blend 80 : 20 (SBO/JO) had the largest impact on the oxidative stability performance of SBO. It increased the induction period (the time needed for the PV of the sample to become 20 meq O_2/kg oil) of SBO in about 76 h ■pls check■. The PV of SBO increased 19 times from 0 to 10 days of storage, whereas that of the blend 80 : 20 (SBO/JO) increased about eight times.

A measure for the effectiveness of JO to enhance the oxidative stability of SBO could be the stabilization factor:

$F = IP_{jo}/IP_{o}$

where IP_{jo} is the induction period of SBO in the presence of JO, and IP_{o} is the induction period of SBO alone. The results obtained (Tab. 1) showed a non-rectilinear dependence of F on the JO concentration (p value = 0.061, not significant for a confidence level of 95%). The blend 80 : 20 (SBO/JO) had an F value that duplicated that of the blend 95 : 5 (SBO/JO). This means that the oxidation of SBO was proportionally more retarded when it was blended with 20% JO than when it was mixed with JO at lower concentrations.

The AV is a relative measure of the secondary products of oxidation. It specifically measures conjugated dienals, particularly 2-alkenals. Kinetic curves of AV from pure SBO and blends (Fig. 2) were similar to those of PV. After 72 h of storage, the blends containing 15 and 20% JO oxidized at significantly lower rates than did the other blends and pure SBO. PV and AV from pure JO did not show significant variations during the storage period.

With respect to the UV absorption characteristics, K_{232} and K_{270} (Figs. 3 and 4, respectively), the differences observed among oil blends and among these ones and pure SBO were less remarkable. Nevertheless, values from the SBO/JO blends were smaller than those of pure SBO. The K_{232} values increased abruptly from day 6 (144 h of storage at 60 °C), whereas the K_{270} values rose gradually along all the storage period. K_{232} and K_{270} values from pure JO were unchanged throughout the course of the experiment.

The changes in total tocopherol content during accelerated storage are shown in Fig. 5. As expected, the tocopherols decreased as the storage time increased. It is known that tocopherols may be oxidized during storage to form oxidized tocopherols [25], which have been reported to be pro-oxidants in the oxidation of SBO [26]. The degradation of tocopherols in SBO and oil blends was more pronounced from day 4 (96 h of storage) and the pattern of degradation was similar in all samples.

Tab. 2 shows the composition of the headspace volatiles from the parent oils and blends. At time 0 (fresh oils), the volatile content of SBO and the blends was very low. No volatile compounds were detected in JO. 2,4-Heptadienal was quantitatively the largest compound in SBO. This aldehyde is produced by oxidative breakdown of 12-hydroperoxide derived from linolenic acid, the most unstable fatty acid in SBO. After 10 days of oxidation at 60 °C, the major volatile compounds were aldehydes: hexanal, 2-heptenal, 2,4-decadienal (produced by breakdown of different linoleate hydroperoxide isomers), nonanal (derived from 9-oleate hydroperoxide), and 2,4-heptadienal. Some hydrocarbons, such as dodecane and tridecane, were present in high quantities in oxidized samples; however, their contribution to SBO off-flavors is negligible because their flavor thresholds are significantly higher than those of aldehydes [27]. 2-Pentilfuran and

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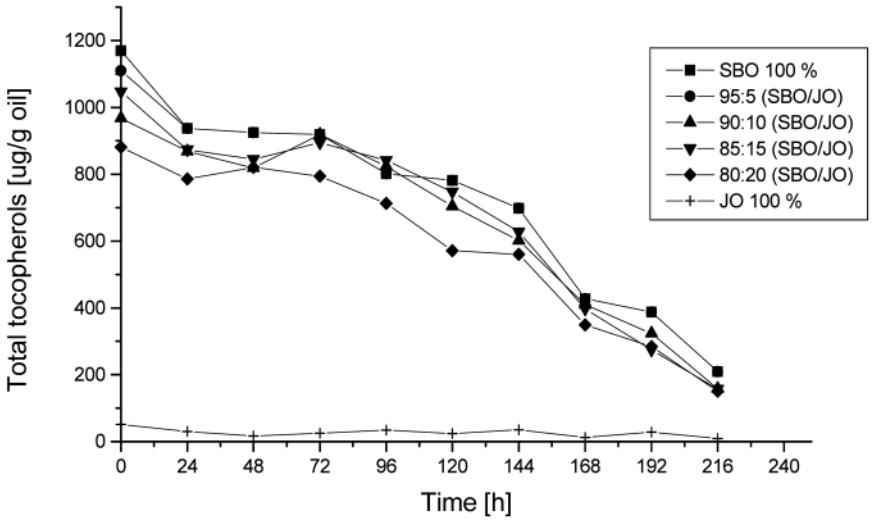

Figure 5. Kinetic curves of total tocopherol values during oxidation of SBO, JO and their blends at 60 °C in the dark. All kinetic curves were the average result of two independent experiments.

Table 2. Headspace volatiles (% normalized areas) of SBO and SBO/JO blends.

Compounds	SBO		Oilblends SBO/JO wt/wt							
			95 : 5		90 : 10		85 : 15		80 : 20	
	IV	EV	IV	EV	IV	EV	IV	EV	IV	EV
Hexanal	0.47^{a}	6.37^{A}	0.28^{b}	2.37^{B}	0.26^{b}	1.47^{C}	0.19^{b}	1.10^{C,D}	Tr	0.73^{D}
2-Heptenal	0.63^{a}	5.27^{A}	0.33^{b}	4.20^{B}	0.20^{c}	5.11^{A}	Tr	1.50^{C}	Tr	1.70^{C}
1-Octen-3-ol	Nd	2.30^{A}	Nd	2.34^{A}	Nd	2.21^{A}	Nd	1.37^{B}	Nd	Nd
2-Pentylfuran	Nd	1.42^{A}	Nd	0.66^{B}	Nd	Nd	Nd	Nd	Nd	Nd
2,4-Heptadienal	1.17^{a}	10.33^{A}	0.76^{b}	10.67^{A}	0.57**bç**	5.06^{B}	0.38**c,d**	3.41^{C}	0.23^{d}	3.72^{C}
Undecane	0.42^{a}	2.46^{A}	0.25^{b}	2.22^{A}	0.20^{c}	3.60^{B}	Tr	0.55^{C}	Nd	0.89^{C}
Nonanal	0.81^{a}	4.88^{A}	0.65^{a}	4.47^{A,B}	0.37^{b}	3.81^{B}	0.16^{bç}	2.08^{C}	Tr	0.96^{D}
Dodecane	0.82^{a}	4.92^{A}	0.50^{b}	4.83^{A}	0.27^{bç}	5.87^{B}	Tr	2.14^{C}	Tr	2.18^{C}
2,4-Nonadienal	Tr^{a}	1.80^{A}	Tr	1.17^{BÇ}	Tr	1.21^{BÇ}	Tr	1.55^{C}	Tr	0.53^{D}
Tridecane	0.63^{a}	5.54^{A}	0.48^{a,b}	2.85^{B}	0.30^{b}	5.45^{A}	Tr	1.03^{C}	Nd	Nd
2,4-Decadienal	0.68^{a}	6.92^{A}	0.67^{a}	4.71^{B}	0.58^{a,b}	6.45^{A}	0.36^{bç}	1.60^{C}	0.20^{c}	3.03^{D}
Total	5.67^{a}	53.57^{A}	3.90^{b}	42.72^{B}	2.69^{c}	42.75^{B}	1.09^{d}	17.04^{C}	0.43^{e}	14.58^{C}

Tr, trace (<0.3%); Nd, not detected.
Mean values (n = 3).
Initial values (IV) in the same row with different small letters present significant differences at $p < 0.05$.
Ending values (EV) in the same row with different capital letters present significant differences at $p < 0.05$.

1-octen-3-ol were also found in some oxidized samples and they may make a small contribution to SBO off-flavor. The addition of JO to SBO produced a significant reduction of total volatile compounds, in particular saturated and unsaturated aldehydes having sensory properties and odor thresholds low enough to contribute to SBO off-flavors. The effect was noteworthy in the treatments containing 15 and 20% JO, which showed a reduction of 68 and 73%, respectively, of total volatile content with respect to that of pure SBO.

4 Discussion and conclusions

The oxidative stability of refined SBO can be improved by blending it with JO.

The addition of relatively small amounts of JO to SBO may effectively reduce the formation of hydroperoxides and, consequently, may decrease the generation of secondary oxidation products such as the volatiles mentioned previously, resulting in more stable flavor quality.

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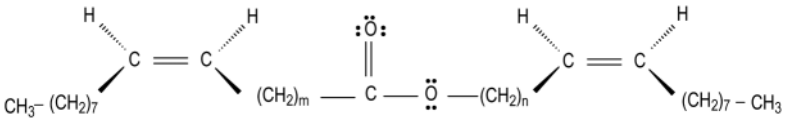

m = 7–13, n = 8–14

Figure 6. A typical jojoba ester molecule.

As compared with SBO, JO has a reduced unsaturation level. Notwithstanding, JO oxidative susceptibility is much lower than it could be predicted from its unsaturation degree. Hence, other factors besides unsaturation should be considered.

Increasing evidence shows that the tocopherol content of an oil has a dramatic impact on its oxidative stability ([28] and references therein). With this concept in mind, we observed that even though SBO contained a significantly higher amount of tocopherols, its oxidative stability was markedly lower than that of JO. In SBO, PUFA esterified to glycerol have their double bonds separated only by two single covalent bonds. Due to this close proximity, reactions at these double bond sites are complex and interdependent, resulting in double bond sites that are very weak to attack by oxidizing agents because they cannot hold their shared electrons [5].

On the other hand, an examination of a typical jojoba ester molecule (Fig. 6) shows that double bonds are more or less equidistant from the central ester linkage (the combination of alcohol/acid chain lengths is quite symmetric). These bonds are considered isolated and their shared electrons are well protected against oxidation.

In addition, the presence of unique substances – such as ferrulates and simmondsin phytochemicals or some other unknown antioxidants – found in jojoba seeds, but not characterized in this study, and their possible synergistic action with SBO tocopherols may be also contributing to the improved oxidative stability of SBO/JO blends.

A thermodynamic analysis of the surface mixing properties [29] showed that SBO and JO molecules are laterally mixed and that their blends form non-ideal mixtures stabilized by attractive interactions. These blends are soluble at all proportions.

In summary, we think that due to the low cost and accessibility of SBO and the stability of its blends with JO, they can be explored through experimental research for the production of oils with improved performance characteristics.

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