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A N I N T E R N A T I O N A L J O U R N A L

Evaluation of Oxidative Stability of Compound Oils under Accelerated Storage Conditions

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ABSTRACT

The oxidative stability of linseed (L), cotton (A), and coconut (C) oils, as well as of linseed:cotton (LA), linseed:coconut (LC), and linseed:cotton:coconut (LAC) compound oils was evaluated under accelerated storage at 60°C/20 days. Coconut oil showed to be rather stable, mainly due to low levels of peroxides, conjugated dienes, ρ-anisidine, and long induction period. In addition, along with cotton oil, it improved the stability of linseed oil in the formulation of LAC compound oil. As to fatty acid profile, the compound oils showed to be composed mainly by unsaturated fatty acids. Cotton and coconut oils presented higher retention of total phytosterols, 78.87 and 76.16%, respectively, after 20 days of storage, when compared to linseed oil. The highest retention of total tocopherols at the end of storage was observed in LA (90.81%). In relation to antioxidant activity, by the DPPH method, with the increase in storage time, a reduction in the antioxidant substances of linseed, LC, and LAC oils was observed. Through the FRAP method, oscillations were observed, especially in linseed and compound oils. Although the oils were degraded over time, it was possible to verify that cotton and coconut oils contributed to increase the stability of linseed oil, which, in turn, raised the levels of coconut oil bioactive compounds.

Keywords: linseed, cotton, coconut, lipid oxidation, vegetable oils

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INTRODUCTION

Vegetable oils are widely consumed all over the world, being of great importance in human diet. These oils are sources of energy and essential fatty acids, carry liposoluble vitamins, and participate in the formation of steroid hormones[1,2]. In addition, they contribute to increased palatability, providing pleasant taste, aroma, and texture to foods[3].

However, vegetable oils are rather susceptible to lipid oxidation, due to their composition, especially the presence of unsaturated molecules, which can undergo degradation reactions, leading to nutritional losses and formation of compounds that are toxic to human health. Such degradation, although relatively slow, may occur during heating and/or storage of the final product, affecting its shelf life[4].

It is possible to evaluate the stability of oils by their storage under accelerated storage conditions, in which periodic analyses are performed to monitor chemical, physical, or sensorial changes. The Schaal Oven Test is one of the most widely used methods[5]. This test makes it possible to know the oil shelf life, since the results provided have a good correlation with the evaluation carried out in storage at room temperature[6].

In order to avoid oxidation problems and increase stability in the oils, it is necessary to eliminate traces of metals such as iron, copper, and chromium. Additionally, it is important to prevent contact with oxygen and high temperatures, as well as to eliminate the pro-oxidants and block the formation of free radicals through antioxidants, which, in small amounts, act to inhibit or retard the oxidation process of lipids[7].

The formulation of compound oils has emerged as an alternative to increase stability, in addition to raising the content of vegetable oil bioactive compounds. These oils are products obtained from the mixture of oils from two or more plant species, which have been studied as an economical way to modify fatty acid composition and physicochemical characteristics[2].

The investigation of compound oils, which are formulated by the combination of vegetable oils, is a field of emerging research that has not yet been explored. In this context, the objective of this study was to evaluate the oxidative stability and antioxidant activity of compound oils formulated with linseed, cotton, and coconut oils stored at 60°C for 20 days.

MATERIAL AND METHODS

Oils

Linseed (L) oil was extracted by cold continuous pressing (< 60°C) in Scott Tech Equipamentos industries, located in Vinhedo, São Paulo, Brazil. The seeds were previously submitted to a dryer (model SMR 610-G, Scott Tech, Vinhedo, SP, Brazil) with a rotary system and LPG gas at 50°C for 25 minutes, to reduce moisture. Subsequently, they were taken to a vegetable oil extractor (model ERT 60 III, Scott Tech, Vinhedo, SP, Brazil) with a system of tubular radial extraction. Then, the oil went through filter press (model FP 240-N2, Scott Tech, Vinhedo, SP, Brazil), was bottled in amber bottles, and kept under freezing temperature (-18°C) until analysis.

Cotton (A) and coconut (C) refined oils from Triângulo Alimentos industries, located in Itápolis, São Paulo, Brazil, were used to formulate the compound oils: linseed and cotton (LA), 1:1 (v:v); linseed and coconut (LC), 1:1 (v:v); and linseed, cotton, and coconut (LAC), 2:1:1 (v:v:v).

Accelerated storage test

The accelerated storage test was performed at 60ºC for 20 days, using beakers containing 30 mL of each oil with a surface/volume ratio of 0.3/cm. The samples were collected in 0, 10, and 20 days, inertized with nitrogen gas, and stored at -18°C until analysis.

Physicochemical properties

Physicochemical analysis were performed using the compound oils The following parameters were analysed: peroxide value, conjugated dienes, and ρ-anisidine indexes, according to the AOCS method[8]. The total oxidation value (Totox) was calculated by using the equation: Totox = 2 (peroxide index) + (ρ-anisidine value)[9]. Oxidative stability was performed using Rancimat (Model 743, Metrohm Ltd., Herisau, Switzerland) at 110°C, with 20 L/h air flow[8].

In the sequence, the fatty acid profile was determined by means of gas chromatography of the esterified oils according to the AOCS method[8]. A gas chromatograph (Model 3900, Varian, Walnut Creek, CA, USA) was used with a flame ionization detector, split injection system, and fused silica capillary column (CP-Sil 88, Microsorb, Varian, Walnut Creek, CA, USA). The initial oven temperature was 90°C for 4 min, heated at 10°C/min up to 195°C, then maintained at the same temperature for 16 min. The temperatures used in the injector and the detector were 230 and 250ºC, respectively. Hydrogen was used as carrier gas. Fatty acids were identified according to their retention times, comparing them to the standard composed of 37 fatty acid methyl esters of C4:0 to C24:1, with purity between 99.1 and 99.9% (Supelco, Bellefonte, USA).

The phytosterol profile was determined by gas chromatography from the unsaponifiable matter. Saponification was performed according to Duchateau[10]. For the determination, a gas chromatograph (Model 2010 Plus-Shimadzu, Chiyoda-ku, Tokyo, Japan) was used with flame ionization detector, split injector and fused silica capillary column (Restek RTX 5, Shimadzu, Chiyoda-ku, Tokyo, Japan). The column temperature was maintained at 260°C for 35 min. The temperatures used in the injector and the detector were 280 and 320°C, respectively. Hydrogen was used as carrier gas. The quantification of each isomer was performed by internal standardization (cholestane-5α-3β-ol), based on peak areas, and expressed as mg/kg.

The analysis of tocopherols was conducted in a high performance liquid chromatograph (Model 210-263 Varian Inc., Walnut Creek, CA, USA), with fluorescence detector, silica packed stainless steel column (100 Si, Microsorb, Varian, Walnut Creek, CA, USA) with a 290 nm excitation wavelength and a 330 nm emission wavelength. The concentration values were calculated based on the peak excitation areas and expressed as values for each separate isomer. A standard curve of α-, β-, γ- and δ-tocopherols (Supelco, Bellefonte, USA) was prepared with a high purity level to express the tocopherol contents in mg/kg. Vitamin E was calculated according to the method described by McLaughlin and Weihrauch[11]. The conversion values were: α-tocopherol x 1.0; β-tocopherol x 0.40; γ-tocopherol x 0.10; and δ-tocopherol x 0.01.

Phenolic compounds and antioxidant activity

The extraction of total phenolic compounds was performed by the procedure described by Parry[12] and they were quantified using the Folin-Ciocalteu reagent, according to a

methodology described by Singleton and Rossi[13]. Total phenolic compounds were detected at λ = 765 nm (UV-VIS mini model 1240, Shimadzu, Chiyoda-ku, Tokyo, Japan). Gallic acid was used to plot the standard curve (R2 = 0.9999), and the results were expressed as mg EAG/kg.

The evaluation of antioxidant activity was carried out by two different spectrophotometer methodologies (Model UV-VIS mini 1240, Shimadzu, Chiyoda-ku, Tokyo, Japan). The DPPH analysis, which consists of evaluating the scavenging activity of the free radical 2,2-diphenyl-1-picrylhydrazyl, was carried out according to the method of Kalantzakis[14], through which the sample absorbance was measured at λ = 517 nm and the result expressed as percentage.

The antioxidant activity analysis was also conducted through the FRAP method, which is based on the ability of phenols to reduce Fe+ $TPTZ^{-3}$ (tripyridil-s-triazine ferric iron) complex into Fe+ $TPTZ^{-2}$ (tripyridil iron-s-ferrous triazine) complex at pH 3.6. This methodology has been described by Szydlowska-Czerniak[15] and the results were expressed as μM trolox/100 g.

Statistical analysis

The results obtained from the analytical determinations, in triplicate, were submitted to analysis of variance and the differences between means were tested at 5% probability by the Tukey test[16], through the ESTAT program, version 2.0.

RESULTS AND DISCUSSION

Physicochemical properties

According to *Codex Alimentarius*[17], refined and crude vegetable oils should have a maximum of 10 and 15 meq/kg of peroxides, respectively, in order to be considered good quality oils. Thus, it was possible to observe that, initially, all the oils presented peroxide value within the established limits (Table 1).

Table 1-Physicochemical properties of oils under storage.

Properties	Oils	Days of storage		
		0	10	20
Peroxide (meq/kg)	L	1.80 ± 0.04^{cA}	31.22 ± 3.80^{bA}	47.71 ± 2.45^{aA}
	A	3.30 ± 0.27^{bA}	4.06 ± 0.02^{abC}	6.73 ± 0.06^{aE}
	C	0.66 ± 0.04^{aA}	1.20 ± 0.04^{aC}	1.75 ± 0.21^{aF}
	LA	1.17 ± 0.06^{cA}	4.87 ± 0.43^{bC}	21.94 ± 1.91^{aD}
	LC	0.70 ± 0.03^{cA}	14.89 ± 0.06^{bB}	40.50 ± 0.04^{aB}
	LAC	1.11 ± 0.06^{bA}	2.70 ± 0.05^{bC}	28.59 ± 1.97^{aC}
Conjugated dienes (%)	L	0.14 ± 0.01^{cD}	0.40 ± 0.01^{bB}	0.61 ± 0.002^{aA}
	A	0.42 ± 0.01^{cA}	0.54 ± 0.01^{aA}	0.52 ± 0.002^{bB}
	C	0.09 ± 0.001^{bE}	0.11 ± 0.002^{aF}	0.12 ± 0.01^{aE}
	LA	0.25 ± 0.01^{cB}	0.34 ± 0.01^{bC}	0.46 ± 0.01^{aC}
	LC	0.14 ± 0.01^{cD}	0.17 ± 0.002^{bE}	0.42 ± 0.004^{aD}
	LAC	0.23 ± 0.01^{cC}	0.22 ± 0.003^{bD}	0.45 ± 0.003^{aC}
ρ-anisidine	L	0.33 ± 0.03^{cD}	11.81 ± 0.41^{bB}	28.19 ± 0.16^{aA}
	A	5.77 ± 0.18^{bA}	6.33 ± 0.11^{bC}	7.33 ± 0.06^{aD}
	C	2.99 ± 0.11^{bC}	3.20 ± 0.19^{bD}	5.73 ± 0.05^{aE}
	LA	4.40 ± 0.33^{cB}	5.71 ± 0.03^{bC}	6.84 ± 0.44^{aDE}
	LC	0.73 ± 0.01^{cD}	13.76 ± 0.03^{bA}	24.06 ± 1.06^{aB}
	LAC	1.42 ± 0.01^{cD}	11.43 ± 0.84^{bB}	21.88 ± 0.50^{aC}
Totox	L	3.93	74.25	123.61
	A	12.37	14.45	20.79
	C	4.31	5.60	9.23
	LA	6.74	15.45	50.72
	LC	2.13	43.54	105.06
	LAC	3.64	16.83	79.06
Oxidative stability (h)	L	0.9 ± 0.03^{aD}	0.71 ± 0.03^{bE}	0.77 ± 0.03^{bC}
	A	15.40 ± 0.07^{aB}	12.94 ± 0.25^{bB}	9.85 ± 0.17^{cB}
	C	53.79 ± 0.02^{aA}	45.49 ± 0.69^{bA}	45.66 ± 0.98^{bA}
	LA	5.80 ± 0.14^{aC}	5.31 ± 0.05^{aC}	2.06 ± 0.13^{bC}
	LC	1.01 ± 0.01^{aD}	2.28 ± 0.04^{bD}	0.76 ± 0.01^{cC}
	LAC	5.84 ± 0.16^{aC}	6.16 ± 0.01^{aC}	1.13 ± 0.03^{bC}

The mean ± standard deviation followed by lowercase letters in the lines do not differ by Tukey test ($p > 0.05$). Means ± standard deviation followed by upper case letters in the columns do not differ by Tukey test ($p > 0.05$).

However, as the storage time increased, linseed oil showed high oxidation, reaching 47.71 meq/kg of peroxides in 20 days of storage. This increase may have influenced the compound oils peroxide content, as cotton (6.73 meq/kg) and coconut (1.75 meq/kg) oils remained stable, contributing to a lower peroxide formation compared to linseed oil.

Conjugated dienes are primary oxidation products of polyunsaturated fatty acids formed by the displacement of double bonds[18]. The oils showed low values of conjugated dienes at the beginning of storage. However, there was significant increase during storage at 60ºC, except in coconut oil, which was stable after 10 days of storage. It was also possible to verify, when comparing the compound oils, that LC showed lower formation of conjugated dienes, possibly due to the influence of coconut oil.

According to Guillén and Cabo[19] and Marina *et al*.[20], oils of good quality must have ρ-anisidine index below 10. Thus, it is possible to infer that, initially, all the oils presented good quality. However, the values of ρ-anisidine increased during storage. In 20 days, only cotton, coconut, and LA oils remained below 10, while linseed oil

showed the lowest quality, 28.19 of ρ-anisidine. This fact is possibly due to the fact that linseed oil is crude and constituted by a large amount of α-linolenic acid.

It is also considered that well-preserved fat must have a Totox value below 10[21]. According to Table 1, initially, all oils presented good conservation status, except for cotton oil (12.37). However, after 10 days of storage, only coconut oil remained below this limit, indicating that it is a stable oil.

In the oxidative stability test, coconut (53.79 h) and cotton (15.40 h) oils showed the highest rates. According to Michotte *et al.*[22], the high content of unsaturated fatty acids of linseed oil makes it extremely sensitive to oxidative reactions. This information is confirmed in this study, since linseed oil presented the lowest oxidative stability during storage, in relation to the other oils. When comparing the compound oils, it was found that LA and LAC remained stable up to 10 days of storage, probably due to the synergism between linseed and cotton oils.

Ten different types of fatty acids were identified (Table 2). Initially, coconut oil showed higher value of α-linolenic acid (47.9%) than linseed oil (47.1%). With heating, lauric, palmitic, and stearic fatty acids were found to remain stable for up to 20 days of storage. However, α-linolenic acid showed significant reduction in compound oils, especially in LC, in which it was reduced by 47.41%.

Table 2-Fatty acid composition of oils under storage.

Fatty acids (%)	Oils	Days of storage	
		0	**20**
Caproic (6:0)	L	nd	nd
	A	nd	nd
	C	2.07 ± 0.02^{bA}	2.24 ± 0.01^{aA}
	LA	nd	Nd
	LC	0.58 ± 0.01^{bB}	1.36 ± 0.01^{aB}
	LAC	0.32 ± 0.01^{bC}	0.55 ± 0.01^{aC}
Caprylic (8:0)	L	nd	nd
	A	nd	nd
	C	2.44 ± 0.01^{bA}	2.50 ± 0.01^{aA}
	LA	nd	nd
	LC	0.70 ± 0.01^{bB}	1.56 ± 0.02^{aB}
	LAC	0.36 ± 0.01^{bC}	0.61 ± 0.01^{aC}
Lauric (12:0)	L	nd	nd
	A	0.27 ± 0.02^{aD}	0.29 ± 0.02^{aD}
	C	47.10 ± 0.01^{aA}	47.11 ± 0.01^{aA}
	LA	0.18 ± 0.0^{aE}	0.19 ± 0.01^{aE}
	LC	13.62 ± 0.02^{bB}	29.87 ± 0.03^{aB}
	LAC	7.14 ± 0.01^{bC}	11.56 ± 0.02^{aC}
Myrístic (14:0)	L	nd	nd
	A	0.83 ± 0.01^{aD}	0.83 ± 0.02^{aD}
	C	13.33 ± 0.01^{bA}	14.92 ± 0.02^{aA}
	LA	0.24 ± 0.01^{bE}	0.54 ± 0.01^{aE}
	LC	4.44 ± 0.01^{bB}	9.62 ± 0.03^{aB}
	LAC	2.59 ± 0.01^{bC}	4.05 ± 0.01^{aC}
Palmitic (16:0)	L	11.44 ± 0.01^{aF}	11.64 ± 0.01^{aF}
	A	40.31 ± 0.01^{aA}	40.04 ± 0.01^{aA}
	C	12.75 ± 0.01^{aD}	12.54 ± 0.01^{aD}
	LA	25.19 ± 0.02^{bB}	29.15 ± 0.01^{aB}
	LC	11.93 ± 0.03^{aE}	12.31 ± 0.01^{aE}
	LAC	20.51 ± 0.01^{aC}	20.13 ± 0.01^{aC}
Stearic (18:0)	L	5.34 ± 0.02^{aA}	5.09 ± 0.01^{aB}
	A	3.21 ± 0.01^{aE}	3.22 ± 0.02^{aF}

Cont.

	C	3.76 ± 0.01^{aD}	3.66 ± 0.02^{aE}
	LA	3.22 ± 0.03^{aE}	3.99 ± 0.02^{aD}
	LC	4.92 ± 0.02^{aB}	4.35 ± 0.01^{aC}
	LAC	4.33 ± 0.04^{bC}	5.36 ± 0.03^{aA} Cont.

Fatty acids (%)	**Oils**	**Days of storage**	
		0	**20**
Oleic (18:1n9c)	L	26.98 ± 0.01^{aA}	27.37 ± 0.02^{aA}
	A	18.72 ± 0.02^{aE}	18.87 ± 0.01^{aE}
	C	15.40 ± 0.01^{aF}	15.11 ± 0.01^{aF}
	LA	19.29 ± 0.04^{bD}	22.06 ± 0.01^{aB}
	LC	23.56 ± 0.01^{aB}	19.02 ± 0.03^{bD}
	LAC	23.07 ± 0.04^{aC}	21.54 ± 0.02^{bC}
Linoleic (18:2n6c)	L	13.31 ± 0.01^{aD}	13.34 ± 0.01^{aD}
	A	36.23 ± 0.01^{aA}	36.23 ± 0.04^{aA}
	C	1.29 ± 0.01^{aF}	1.28 ± 0.02^{aF}
	LA	23.50 ± 0.03^{bB}	26.71 ± 0.01^{aB}
	LC	9.81 ± 0.01^{aE}	5.77 ± 0.02^{bE}
	LAC	18.49 ± 0.01^{aC}	16.33 ± 0.04^{bC}
Arachidic (20:0)	L	nd	nd
	A	0.15 ± 0.01aC	0.15 ± 0.01aB
	C	1.89 ± 0.02aA	0.67 ± 0.04bA
	LA	nd	nd
	LC	0.57 ± 0.02aB	0.13 ± 0.03bB
	LAC	0.14 ± 0.04aC	nd
α-Linolenic (18:3n3)	L	42.93 ± 0.01^{aA}	42.56 ± 0.01^{aA}
	A	0.30 ± 0.01^{aE}	0.37 ± 0.01^{aE}
	C	nd	nd
	LA	28.39 ± 0.02^{aC}	17.34 ± 0.05^{bC}
	LC	30.48 ± 0.06^{aB}	16.03 ± 0.11^{bD}
	LAC	23.06 ± 0.03^{aD}	19.89 ± 0.09^{bB}
Σ Saturated	L	16.78 ± 0.03^{aF}	16.73 ± 0.02^{aF}
	A	44.62 ± 0.01^{aB}	44.38 ± 0.01^{aC}
	C	81.45 ± 0.01^{aA}	82.97 ± 0.01^{aA}
	LA	28.83 ± 0.03^{aE}	33.87 ± 0.02^{aE}
	LC	36.19 ± 0.05^{bC}	59.07 ± 0.03^{aB}
	LAC	35.25 ± 0.04^{bD}	42.26 ± 0.03^{aD}
∑ Monounsaturated	L	26.98 ± 0.07^{aA}	27.37 ± 0.02^{aA}
	A	18.72 ± 0.02^{aE}	18.87 ± 0.01^{aE}
	C	15.40 ± 0.01^{aF}	15.11 ± 0.01^{aF}
	LA	19.29 ± 0.03^{bD}	22.06 ± 0.01^{aB}
	LC	23.56 ± 0.01^{aB}	19.02 ± 0.03^{bD}
	LAC	23.07 ± 0.04^{aC}	21.54 ± 0.02^{bC}
Σ Polyunsaturated	L	56.24 ± 0.03^{aA}	55.90 ± 0.04^{aA}
	A	36.68 ± 0.01^{aE}	36.75 ± 0.01^{aC}
	C	3.18 ± 0.01^{aF}	1.95 ± 0.02^{bF}
	LA	51.89 ± 0.01^{aB}	44.05 ± 0.04^{bB}
	LC	40.86 ± 0.04^{aD}	21.93 ± 0.06^{bE}
	LAC	41.69 ± 0.01^{aC}	36.22 ± 0.06^{bD}

The mean ± standard deviation followed by lowercase letters in the lines do not differ by Tukey test ($p > 0.05$). Means ± standard deviation followed by upper case letters in the columns do not differ by Tukey test ($p > 0.05$). nd: not detected.

The compound oils showed to be constituted mainly of unsaturated fatty acids, representing 64 to 71% of the totality (Table 2). These oils can help lower cholesterol and triglyceride levels, regulate blood pressure, and reduce chronic inflammation and the development of cancer, heart diseases, and stroke[23,24]. During storage, saturated

fatty acids did not change significantly, except in LC and LAC, probably due to the influence of linseed and coconut oils. On the other hand, the compound oils showed reduction in relation to unsaturated fatty acids, especially in LC (46.32%).

As for the phytosterols profile, the oils showed four different isomers: campesterol, stigmasterol, β-sitosterol, and stigmastanol (Table 3). The presence of β-sitosterol was higher than the other isomers in all oils, and stigmastanol was not detected in cotton and coconut oils.

Initially, linseed oil had higher amounts of campesterol (6.51 mg/kg) and stigmastanol (56.09 mg/kg), while cotton had higher amount of β-sitosterol (90.27 mg/kg), and coconut oil had higher stigmasterol content (8.50 mg/kg).

According to Ferrari *et al.*[25], phytosterols are susceptible to oxidation by reaction with oxygen, light, metal ions, and high temperature, depending on their degree of unsaturation. Thus, it was observed that all phytosterol isomers decreased during storage. However, linseed oil presented greater degradation of total phytosterols than cotton and coconut oils, which remained stable after 10 days of storage. When comparing the compound oils, it was verified that LC retained greater amount of total phytosterols, 95.1%, in 20 days.

Table 3-Phytosterol profile of oils under storage.

Phytosterol (mg/kg)	Oils	Days of storage		
		0	10	20
Campesterol	L	6.51 ± 0.04^{aA}	5.38 ± 0.04^{bA}	4.86 ± 0.14^{cA}
	A	4.81 ± 0.06^{aD}	3.53 ± 0.04^{bE}	3.31 ± 0.27^{bB}
	C	5.71 ± 0.03^{aB}	4.94 ± 0.05^{bB}	4.89 ± 0.04^{bA}
	LA	4.13 ± 0.04^{aF}	3.93 ± 0.04^{aD}	3.59 ± 0.12^{bB}
	LC	5.54 ± 0.02^{aC}	5.46 ± 0.09^{abA}	5.23 ± 0.04^{bA}
	LAC	4.61 ± 0.01^{aE}	4.25 ± 0.06^{bC}	3.54 ± 0.06^{cB}
β-sitosterol	L	58.13 ± 0.03^{aC}	55.44 ± 0.05^{bB}	43.25 ± 0.07^{cE}
	A	90.27 ± 0.05^{aA}	72.11 ± 0.02^{bA}	71.72 ± 0.59^{bA}
	C	27.07 ± 0.04^{aF}	19.75 ± 0.11^{bF}	19.82 ± 0.04^{bF}
	LA	58.42 ± 0.04^{aB}	54.07 ± 0.04^{bC}	53.57 ± 0.04^{cB}
	LC	49.37 ± 0.04^{aE}	49.33 ± 0.33^{aD}	47.37 ± 0.04^{bC}
	LAC	50.49 ± 0.04^{aD}	46.44 ± 0.06^{bE}	44.64 ± 0.06^{cD}
Stigmasterol	L	4.28 ± 0.04^{bC}	4.10 ± 0.03^{cC}	4.87 ± 0.04^{aB}
	A	6.57 ± 0.04^{aB}	5.27 ± 0.05^{bB}	5.15 ± 0.22^{bB}
	C	8.50 ± 0.03^{aA}	6.75 ± 0.04^{bA}	6.74 ± 0.03^{bA}
	LA	3.68 ± 0.04^{aD}	3.65 ± 0.01^{aD}	3.12 ± 0.02^{bD}
	LC	4.31 ± 0.04^{aC}	4.22 ± 0.03^{aC}	3.83 ± 0.06^{bC}
	LAC	3.50 ± 0.06^{aE}	2.80 ± 0.15^{bE}	2.15 ± 0.07^{cE}
Stigmastanol	L	56.09 ± 0.02^{aA}	48.08 ± 0.03^{bA}	24.39 ± 0.16^{cD}
	A	nd	nd	nd
	C	nd	nd	nd
	LA	37.04 ± 0.03^{aC}	34.19 ± 0.02^{bC}	31.32 ± 0.12^{cB}
	LC	47.97 ± 0.04^{aB}	46.13 ± 0.16^{bB}	45.44 ± 0.09^{cA}
	LAC	36.89 ± 0.04^{aD}	30.74 ± 0.23^{bD}	27.45 ± 0.64^{cC}
Total	L	124.99 ± 0.01^{aA}	112.99 ± 0.14^{bA}	77.37 ± 0.13^{cD}
	A	101.65 ± 0.05^{aD}	80.91 ± 0.03^{bE}	80.17 ± 1.07^{bC}
	C	41.28 ± 0.03^{aF}	31.44 ± 0.11^{bF}	31.44 ± 0.03^{bE}
	LA	103.26 ± 0.01^{aC}	95.83 ± 0.01^{bC}	91.66 ± 0.06^{cB}
	LC	107.17 ± 0.01^{aB}	105.14 ± 0.04^{bB}	101.87 ± 0.04^{cA}
	LAC	95.49 ± 0.04^{aE}	84.22 ± 0.26^{bD}	77.78 ± 0.83^{cD}

The mean ± standard deviation followed by lowercase letters in the lines do not differ by Tukey test ($p > 0.05$). Means ± standard deviation followed by upper case letters in the columns do not differ by Tukey test ($p > 0.05$). nd: not detected.
Limits of detection: campesterol ≤ 52 mg/kg; stigmasterol ≤ 56 mg/kg e stigmastanol ≤ 42,5 mg/kg.

The oils presented α-, γ-, and δ-tocopherol isomers (Table 4). Initially, regarding α-tocopherol, cotton oil (86.05 mg/kg) stood out, whereas in LA and linseed oils, γ- and δ-tocopherol were higher, 79.60 and 17.65 mg/kg, respectively.

Table 4-Quantification of tocopherols and vitamin E of oils under storage.

Tocopherols (mg/kg)	Oils	Days of storage		
		0	10	20
α-tocol	L	29.53 ± 0.11^{aC}	27.50 ± 0.14^{bC}	8.20 ± 0.14^{cD}
	A	86.05 ± 0.36^{aA}	85.35 ± 0.35^{aA}	83.90 ± 0.28^{bA}
	C	nd	nd	nd
	LA	55.00 ± 0.14^{aB}	48.25 ± 0.07^{bB}	47.35 ± 0.07^{cB}
	LC	24.70 ± 0.57^{D}	nd	nd
	LAC	21.80 ± 0.14^{aE}	18.45 ± 0.07^{bD}	17.45 ± 0.07^{cC}
γ-tocol	L	76.05 ± 0.49^{aB}	71.20 ± 0.14^{bB}	39.65 ± 0.49^{cD}
	A	59.85 ± 0.07^{aE}	59.50 ± 0.28^{aC}	53.25 ± 0.21^{bB}
	C	nd	nd	nd
	LA	79.60 ± 0.14^{aA}	78.90 ± 0.14^{aA}	75.35 ± 0.77^{bA}
	LC	63.15 ± 0.35^{aD}	9.25 ± 0.21^{bD}	nd
	LAC	65.15 ± 0.21^{aC}	59.85 ± 0.21^{bC}	42.45 ± 0.21^{cC}
δ-tocol	L	17.65 ± 0.07^{aA}	16.55 ± 0.35^{bA}	nd
	A	12.55 ± 0.07^{aD}	12.15 ± 0.21^{aB}	12.45 ± 0.07^{aA}
	C	nd	nd	nd
	LA	11.25 ± 0.07^{aE}	10.40 ± 0.14^{bC}	9.75 ± 0.21^{cB}
	LC	16.60 ± 0.28^{B}	nd	nd
	LAC	14.50 ± 0.14^{aC}	9.45 ± 0.21^{bC}	9.05 ± 0.07^{bC}
Total	L	123.22 ± 0.67^{aC}	115.25 ± 0.35^{bC}	47.85 ± 0.35^{cD}
	A	158.44 ± 0.50^{aA}	157.00 ± 0.42^{aA}	149.60 ± 0.01^{bA}
	C	nd	nd	nd
	LA	145.85 ± 0.21^{aB}	137.55 ± 0.07^{bB}	132.45 ± 0.49^{cB}
	LC	104.45 ± 0.64^{aD}	9.25 ± 0.21^{bE}	nd
	LAC	101.45 ± 0.07^{aE}	87.75 ± 0.35^{bD}	68.95 ± 0.07^{cC}
Vitamin E*	L	39.98 ± 0.28^{aC}	37.36 ± 0.13^{bC}	13.61 ± 0.07^{cD}
	A	94.16 ± 0.44^{aA}	93.47 ± 0.39^{aA}	91.16 ± 0.26^{bA}
	C	nd	nd	nd
	LA	65.96 ± 0.16^{aB}	59.11 ± 0.09^{bB}	57.57 ± 0.03^{cB}
	LC	33.46 ± 0.61^{aD}	1.26 ± 0.03^{bE}	nd
	LAC	30.82 ± 0.11^{aE}	26.70 ± 0.04^{bD}	23.32 ± 0.04^{cC}

The mean ± standard deviation followed by lowercase letters in the lines do not differ by Tukey test ($p > 0.05$). Means ± standard deviation followed by upper case letters in the columns do not differ by Tukey test ($p > 0.05$). nd: not detected. Limits of detection: δ-tocol $\leq$ 2,30 mg/kg.*Expressed as α-tocol.

It can be observed that γ-tocopherol isomer was the one in highest amount in all oils. Tocopherols and vitamin E were not detected in coconut oil. Concerning vitamin E, the highest amount was found in cotton (94.16 mg/kg) and LA (65.96 mg/kg) oils, mainly due to the amount of α-tocopherol present in cotton oil.

It was observed that the isomers of tocopherols and vitamin E decreased during storage. According to Lampi *et al.*[26], oxidizing agents, especially in the presence of heat, light, metals, and alkali, easily oxidize tocopherols. In relation to total tocopherols, cotton oil remained stable, with retention of 94.42% at the end of the process. Among compound oils, LA retained higher amount of total tocopherols (90.81%) and vitamin E (87.28%) at the end of storage.

Phenolic compounds and antioxidant activity

In relation to phenolic compounds (Table 5), at the beginning of storage, coconut oil stood out with 220.40 mg/kg.

Table 5-Phenolic compounds and antioxidant activity of oils under storage.

	Oils	Days of storage		
		0	**10**	**20**
Phenolic compounds (mg/kg)	L	214.14±15.41^{bA}	235.77 ± 16.32^{bD}	218,.7 ± 10.01^{aCD}
	A	142.84 ± 4.44^{cB}	371.07 ± 19.23^{aA}	213.51 ± 19.02^{bE}
	C	220.40 ± 2.00^{cA}	378.85 ± 4.02^{bA}	515.29 ± 4.33^{aB}
	LA	213.95 ± 20.00^{bA}	278.62 ± 1.54^{aC}	238.84±28.07^{abDE}
	LC	150.40 ± 1.76^{cB}	371.29 ± 5.67^{bA}	667.29 ± 9.05^{aA}
	LAC	211.51 ± 1.68^{bA}	318.85 ± 15.67^{aB}	330.84 ± 24.24^{aC}
DPPH (%)	L	57.20 ± 1.19^{aB}	55.79 ± 1.69^{aC}	47.07 ± 0.73^{bC}
	A	92.76 ± 1.13^{aA}	88.05 ± 2.26^{aA}	88.64 ± 2.19^{aA}
	C	26.01 ± 0.67^{aD}	26.66 ± 0.52^{aD}	25.0 ± 0.18^{aDE}
	LA	59.18 ± 7.12^{cB}	80.91 ± 1.98^{aB}	71.75 ± 2.12^{bB}
	LC	42.42 ± 7.20^{aC}	27.99 ± 1.66^{bD}	29.46 ± 0.67^{bD}
	LAC	38.71 ± 1.77^{aC}	19.51 ± 0.96^{bE}	19.51 ± 0.41^{bE}
FRAP (µM/100 g)	L	97.23 ± 6.69^{bA}	107.95 ± 0.39^{aA}	103.05 ± 0.13^{abA}
	A	102.59 ± 2.44^{aA}	74.09 ± 0.45^{bC}	71.27 ± 3.15^{bB}
	C	89.73 ± 2.51^{aB}	66.68 ± 3.21^{bC}	57.0 ± 0.32^{cC}
	LA	75.55 ± 0.32^{bC}	90.14 ± 1.67^{aB}	76.55 ± 1.61^{bB}
	LC	99.09 ± 1.22^{bA}	109.41 ± 0.51^{aA}	109.86 ± 0.64^{aA}
	LAC	74.32 ± 3.34^{cC}	84.64 ± 0.19^{bB}	109.0 ± 1.74^{aA}

The mean ± standard deviation followed by lowercase letters in the lines do not differ by Tukey test ($p > 0.05$). Means ± standard deviation followed by upper case letters in the columns do not differ by Tukey test ($p > 0.05$).

Throughout storage, there was an increase in the levels of phenolic compounds in all oils. Such increase may have occurred due to the reaction of Folin-Ciocalteu reagent with phenol groups resulting from the degradation of tocopherols, since the presence of these easily oxidizable compounds results in the formation of blue complexes, causing overestimation of the total phenolic compounds[27].

In the test for the reduction of DPPH radical, cotton oil (92.76%), followed by LA (59.18%) and linseed (57.20%) oils, stood out showing higher antioxidant activity at the beginning of storage. This may be due to the greater presence of natural antioxidants in these oils. Cotton and coconut oils showed stable antioxidant activity up to 20 days of storage. Yet, it was possible to detect a decrease in antioxidant substances in the other oils after 10 days of storage.

Regarding the FRAP method, cotton (102.59 µM/100 g), LC (99.09 µM/100 g), and linseed (97.23 µM/100 g) oils showed higher antioxidant power. However, oscillations occurred during storage, possibly due to the presence of pro-oxidant compounds from crude linseed oil.

CONCLUSIONS

The oils underwent degradation during storage at 60°C/20 days, as peroxide, conjugated dienes, ρ-anisidine, and Totox values increased. However, it was found that the formation of the degradation compounds was lower in the compound oils, especially in LA. In the compound oils, unsaturated fatty acids predominated, and α-linolenic acid stood out. The oils showed reduction of phytosterols and tocopherols during storage, with higher retention in the compound oils, especially LC, with 95.1% of phytosterols, and LA, with 90.81% of tocopherols, at the end of storage. The oils showed significant antioxidant activity, probably due to the presence of phenolic compounds, phytosterols, and tocopherols. It is possible to conclude that cotton and

coconut oils can be used in the formulation of compound oils, adding oxidative stability to linseed oil and, consequently, helping in the retention of compounds such as phytosterols and tocopherols.

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