

FULL PAPER

Evaluation of Antioxidant Potential and Chemical Composition Blends of Sunflower Oil (*Helianthus annuus* L.) with Coconut Oil (*Cocos nucifera* L.)

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Abstract:

Coconut oil has more than 90% of saturated fatty acids. Of this percentage, about 47% of lauric acid, an important saturated acid that gives great stability to oxidation, in addition to benefic properties such as anti-inflammatory. Sunflower oil also presents important properties for daily balance of the body, however, the high amount of unsaturated fatty acids provides low oxidative stability. Knowing the nutritional values of both raw materials under study, the aim of this study was to mix them in two different proportions: 50% sunflower + 50% coconut (blend 1) and 70% sunflower + 30% coconut (blend 2), in order to evaluate the antioxidant potential and chemical composition, by analysis of fatty acids content; antioxidant activity (DPPH); phenolic compounds; oxidative stability, by accelerated oxidation test, Rancimat method. The acidity values for both pure oils and blends are within the values established by Anvisa and Codex Alimentarius, and phenolic compounds were proportional to the amount of sunflower oil incorporated into the coconut oil. The values of oil stability index (OSI), determined by Rancimat, were high, especially for coconut oil, and in the mixtures, the increase in oxidative stability was proportional to the amount of coconut oil added to sunflower oil.

Keywords: oxidative stability; OSI; phenolic compounds; Rancimat.

1. Introduction

Vegetable oils are obtained from different natural sources and are essential in human diet, since they are among the main raw materials composed of edible lipids. Their application is diversified and can be used in industries, food and cosmetics [1]. With the increasing demand for healthier food, different vegetable oils have gained notable attention due to protective functions in health and disease prevention, such as: coconut oil (*Cocos nucifera* L.), sunflower oil (*Helianthus annuus* L.), linseed oil (*Linum usitatissimum* L.), among others [2,3].

Vegetable oils are formed by different

chemical compounds, such as monoglycerides, diglycerides, triglycerides and phospholipids, as well as, significant sources of essential fatty acids, including omega-3 and omega-6. Some fatty acids are not produced by the body, thus it is important to include them in the diet. Among these, some have anti-inflammatory function, in addition to beneficial effects on cholesterol levels. The fatty acids can also be categorized into different groups, with different functions, including saturated fatty acid, monounsaturated fatty acid and polyunsaturated fatty acid [4].

The properties of vegetable oils are determined by their composition of fatty acids and one of the features that is affected by this

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composition is oxidative stability: oils with saturated fatty acids are more stable than unsaturated. Therefore, mix vegetable oils with different properties is one of the simplest methods to create new products with nutritional and oxidative properties. A pure vegetable oil may present unsatisfactory physical and/or chemical and/or nutritional properties for a particular use in addition to low oxidative stability. For instance, the use of pure sunflower or sesame oil has some disadvantages, such as low concentration of linolenic acid ($\omega 3$, essential fatty acid) and high content of linoleic acid (unsaturated), which make them more susceptible to oxidation, while coconut oil, rich in lauric acid (saturated), with ability to enhance the immune system and act as anti-inflammatory, is more stable to oxidation [5,6].

It is possible to evaluate the oxidative stability of different vegetable oils, by accelerated oxidation test, Rancimat method, which determines the Oil Stability Index. This method was adopted as a standard by the AOCS Cd 12b-92 - sampling and analysis of commercial fats and oils: Oil Stability Index [7,8].

In addition to being formed by different fatty acids, vegetable oils have, in different concentrations, natural antioxidants that cooperate to maintain oil quality. These antioxidants have ionizable hydrogens in their structure that are donated to free radicals formed in the beginning of oxidative process, stopping the oxidation mechanism [9].

Phenolic compounds are important metabolites found in different foods and are involved in prevention of several diseases, such as cancer. A high content of phenolic compounds

can contribute to a strong antioxidant activity, which results in excellent health benefits [10]. In oils, these metabolites are found in different amounts and also act as antioxidants.

Although some studies have shown the benefits of different vegetable oils, there are still few studies with blends, mostly of coconut oil with sunflower oil. Therefore, due to the growing demand for healthier food and knowing the nutritional potential of sunflower and coconut oils, the aim of this study was to evaluate the physico-chemical characteristics of blends of sunflower oil with coconut oil, in the following proportions: 50% sunflower + 50% coconut (blend 1) and 70% sunflower + 30% coconut (w: w) (blend 2), by analysis of fatty acids content; antioxidant activity (DPPH); phenolic compounds and oxidative stability by accelerated oxidation test, Rancimat method.

2. Results and Discussion

The composition and physico-chemical characteristics of vegetable oils determine their quality and nutritional value (Table 1). Acidity value (AV) measures the content of free fatty acids (FFA) in vegetable oils. It is considered one of the main parameters for measuring the quality and state of degradation. In this study, the values found for acidity are below established by ANVISA [11] and Codex Alimentarius [12], which establish maximum acidity value of 0.6 mg KOH/g for cold pressed and refined oils, which is the case of sunflower oil, and non-refined oil, such as coconut oil, maximum acidity value of 4.00 mg KOH/g [12].

Table 1. Physico-chemical characteristics of sunflower oil, coconut oil, blend 1 and blend 2.

Properties	Vegetable oils			
	Sunflower Oil	Coconut Oil	Blend 1	Blend 2
Acidity Value (mg KOH/g)	0.51 ± 0.01	0.24 ± 0.02	0.12 ± 0.05	0.21 ± 0.01
Acidity in oleic acid (%)	0.25 ± 0.02	0.12 ± 0.03	0.06 ± 0.01	0.10 ± 0.02
Phenolic compounds (mg/g)	267.54 ± 1.03	132.15 ± 0.81	159.37 ± 0.53	183.54 ± 0.17
DPPH IC ₅₀ (µg/mL)	47.96 ± 0.07	>1000	143.57 ± 0.46	125.78 ± 0.29

Data in triplicate with mean ± standard deviation.

Although acidity value was within the rules established by regulatory agencies, mixing coconut oil with sunflower oil decreased the acidity values. Blend 1, with equal proportions of sunflower and coconut oil (50:50), presented value of 0.12 mg KOH/g and blend 2, where the

amount of sunflower oil is higher (70:30), presented value of 0.21 mg KOH/g, yet lower values compared to pure oils. The highest value of acidity presented by Blend 2, can be explained by a possible breakage of unsaturations, since this one has greater amount of sunflower oil. The

breakdown of unsaturations results in the presence of free fatty acids that, consequently, increase the value of acidity. Therefore, based on the acid value, Blend 1 is better for human consumption.

Regarding phenolic compounds and antioxidant activity, sunflower oil showed the best values 267.54 ± 1.03 mg/g and 47.96 ± 0.07 μ g/ml, respectively. The lower the IC_{50} value, the greater the ability to capture free radicals. Blends 1 and 2, 143.57 ± 0.46 μ g/ml and 125.78 ± 0.29 μ g/ml, show antioxidant activity, however, low compared to pure sunflower oil. Blends 1 and 2 presented high values of phenolic compounds 159.37 ± 0.53 mg/g and 183.54 ± 0.17 mg/g due to the presence of sunflower oil, when compared to the pure coconut oil, 132.15 ± 0.81 mg/g. The value of phenolic compounds for the sunflower oil was close to that found by Redondo-Cuevas et al. [13], 329 mg/g. During the process of extraction and refining, there is a decrease in the amount of compounds, such as phenolic and tocopherols [13,14], which might explain the variation of value found in this study in relation to literature. For coconut oil, phenolic content is low if compared to the same study [13], 681 mg/g, however, Marina et al. [15], in their review about coconut oils, concluded that the phenolic content is highly variable depending on the varieties of coconut cultivated, type of soil used in cultivation and process of oil extraction, which might explain the low values of the present study.

Studies with blends of oils [16] indicate that mixtures of oils can enhance nutritional values, increasing their quality. The authors studied four edible vegetable oils (oils of sunflower, linseed, grape seed and coconut) and nine blends of these oils, using refined sunflower oil as base. The physico-chemical properties showed superior features for oil mixtures, such as lower acidity value found for mixtures of sunflower oil and coconut oil, from 0.48 mg KOH/g decreased to 0.24 in the proportion of 70:30 (sunflower:coconut) consistent with the value found in this study. The authors concluded that the best combination for sunflower oil was with coconut oil (lower acidity).

The chromatographic analysis of the blends presented variations, depending on the proportion of sunflower oil incorporated (Figure 1). Lauric acid, for instance, is one of the main fatty acids

present in coconut oil. In the pure sample, its percentage was 47.3% and in the mixtures with sunflower, blends 1 and 2, the values were 27.1% and 14.3%, respectively.

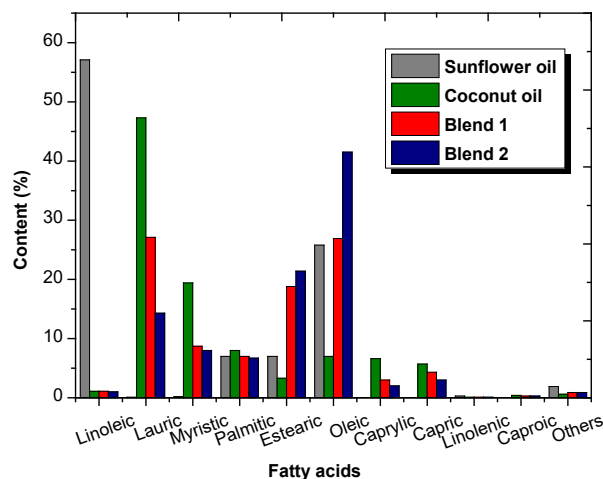


Figure 1. Chromatographic data of samples of sunflower oil, coconut oil, blend 1 and blend 2.

Study of two samples of coconut oil, both cold pressed, being one unrefined and the other refined [13], showed values of saturated fatty acids close to those found in the present study, 94.3% and 92.7%, respectively. The value of lauric acid also corroborated the one found in the present study, 52.2% and 47.2%, respectively. Whether refined or not, the values were close. Regarding blends, a study with a mixture of coconut oil and sunflower oil, in the same proportions as the Blend 2, showed 53.4% saturated fatty acids, results that corroborate those found in this study, 56.3% [17]. Research conducted by Bhatnagar et al. [18] with mixtures of coconut oil with different vegetable oils, such as sunflower, soybean, rice oil, among others, described that produce blends of coconut oil with other vegetable oils results in mixtures with higher amount of saturated fatty acids, which provides greater oxidative stability, while for coconut oil, which is being enriched with polyunsaturated fatty acids, monounsaturated, provides greater amount of natural antioxidants and a greater activity of free radical scavenging, DPPH, as well as the results found for blend 1 and 2. In the study by Bhatnagar et al., Coconut oil blends with sunflower oil in the proportions 80:20, 50:50, 40:60, 30:70 and 20:80 (w: w), were analyzed only when their oxidation stability (OSI) and for their

peroxide value after storage for a period of 6 weeks. The authors affirmed that the addition of coconut oil to vegetable oils inhibited the formation of peroxides, which are the result of an oxidative process, which corroborated with the positive results of increased oxidative stability found for blends 1 and 2, however obtained by other analyzes besides OSI: chromatography (fatty acid profile,) antioxidant activity and phenolic compounds.

3.1. Oil Stability Index (OSI) - Rancimat method

The thermal stability of an oil depends on the amount of saturations and unsaturations present in its structure. Saturated fatty acids and other substances, such as carotenoids, sterols, phospholipids, phenolic substances and tocopherol, are pointed out as responsible for maintaining, for longer time, the stability to oxidation. Table 2 describes the OSI values for oil samples and their blends, in function of temperature.

Table 2. Oil stability index for samples of sunflower oil, coconut oil, blend 1 and blend 2, in function of temperature.

Temperature (°C)	OSI (hours)			
	Sunflower Oil	Coconut Oil	Blend 1	Blend 2
100	11.48 ± 0.31 ^{aA}	55.31 ± 0.19 ^{bA}	43.43 ± 0.15 ^{cA}	37.02 ± 0.04 ^{dA}
110	5.77 ± 0.05 ^{aB}	35.34 ± 0.04 ^{bB}	21.92 ± 0.02 ^{cB}	18.58 ± 0.02 ^{dB}
120	1.48 ± 0.02 ^{aC}	32.91 ± 0.07 ^{bC}	9.04 ± 0.06 ^{cC}	8.60 ± 0.02 ^{cC}
130	1.47 ± 0.02 ^{aC}	32.87 ± 0.59 ^{bC}	5.47 ± 0.05 ^{cD}	4.65 ± 0.02 ^{cD}

Means and standard deviations followed by the same letters, lowercase in lines, and uppercase in columns do not differ. Tukey Test, $p < 0.05$ and $R^2 = 0.9962$.

There was significant difference between oils and blends at all temperatures, except between blend 1 and 2 at temperatures of 120 °C and 130 °C. The same is observed, within the same sample, for sunflower oil and coconut oil: the OSI of these samples at the temperatures of 120 °C and 130 °C were 1.48 ± 0.02 and 1.47 ± 0.02 ; 32.91 ± 0.07 and 32.87 ± 0.52 , respectively. Studies performed with sunflower oil by Rancimat method [19], also found low OSI value, 1.89 at temperature of 120 °C. This low value of OSI can be attributed to the high amount of unsaturated fatty acids and refining process by which the oil passes, resulting in a significant reduction in the amount of natural antioxidants [13]. The addition of coconut oil has significantly improved the OSI value in both levels of mixtures at the four temperatures tested. This can be attributed to the stabilizing role of high saturation of coconut oil, due to the presence of lauric acid, 47.3%, than the presence of phenolic compounds in sunflower oil.

According to Heidarpour & Farhoosh [20], although sunflower oil present high value of phenolic compounds, which act as natural antioxidants, increasing the positive effect on the oxidative stability, the amount of saturations and unsaturations exert greater influence when it comes to thermal stability. In the study in

question, the authors made blends of olive oil (18.4% saturations), sunflower oil (16.2% saturations) and palm oil (42.7% saturations) in different proportions. In olive oil, 10-30% of sunflower oil or palm oil were added. In blends of 70:30 proportion, with sunflower oil, OSI values were lower than those found in our study, 5.2 ± 0.2 and 2.5 ± 0.1 , at temperatures of 120 °C and 130 °C, respectively, regardless of the presence of phenolic compounds, and in blends with palm oil, which has high amounts of saturations as well as in coconut oil, the OSI values were higher when compared to blends with sunflower oil, 7.8 ± 0.1 and 3.9 ± 0.2 , at temperatures of 120 °C and 130 °C, respectively.

In Figure 2 is possible to observe, with greater emphasis, the increase in thermal stability with addition of coconut oil. Blend 1, with higher proportion of coconut oil (50:50), showed greater stability compared to blend 2, whose proportion of sunflower oil is higher, 70%. Studies with different olive oils, regarding the OSI [7] assigned greater thermal stability to the presence of phenolic compounds. That statement considered the lowest percentage of saturated fatty acids of the oils studied, which ranged from 18.8% to 19.8%. The data of the aforementioned authors justify the positive trend found in our study, regarding the

greater thermal stability associated with the higher percentage of saturated fatty acids of coconut oil.

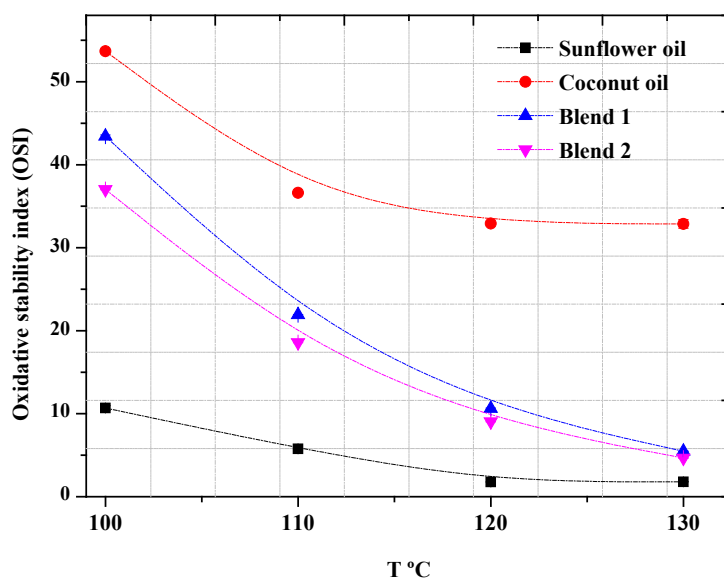


Figure 2. Oil stability index for samples of sunflower oil, coconut oil, blend 1 and blend 2, at four different temperatures.

3. Material and Methods

Sunflower oil was acquired in natural products company, located in São Paulo/SP and coconut oil in a supermarket in Dourados/MS. Both, according to the label, were extracted by cold pressing, followed by filtration and refining for sunflower oil, and not refined for coconut oil.

Acidity analysis were performed according to the American method ASTM D664 [21] in a Potentiometric Titration Titrino Plus 848/Metrohm. In this methodology, 20 g of oil sample were weighed and 125 mL of the mix toluene alcohol/isopropyl alcohol/water (1.0:0.95:0.5 v:v:v) were added, performing titration with 0.1 mol/L KOH solution in isopropyl alcohol. It is defined as the mass in milligrams of potassium hydroxide required to neutralize the free fatty acids of 1 g of sample. The content of free fatty acids can also be expressed as percentage of oleic acid, dividing the acidity value by 1.99, as specified by American Oil Chemists' Society and adopted by international bodies such as the Codex Alimentarius [12,22,23].

The samples prepared at a concentration of 100 µg/mL, were subjected to the assessment of phenolic compounds content. For the analysis, it was employed the Folin-Ciocalteu reagent [24].

To calculate the content of phenolic compounds an analytic curve (1; 5; 10; 15; 30; 40 µg) employing gallic acid was used as standard. The analysis was performed in triplicate.

The antioxidant activity test with the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) was carried out with samples at concentrations of 10-1000 µg/mL. The results were expressed in relation to the percentage of inhibition, which was calculated using the following equation $(\% \Delta 0) = 100 \times (A_0 - A) / A_0$, in which, $(\% \Delta 0)$ is the percentage of scavenging, A_0 is the absorbance of the reference solution and A is the absorbance of the sample after 30 min of reaction [25]. The analysis was performed in triplicate. With the data obtained a linear regression was performed and the IC_{50} was calculated.

The analysis of fatty acid profile was performed in a gas chromatograph GC 17 A Shimadzu. The chromatographic separation was carried out in a column of fused silica Restek RTX-5MS (30 m long x 0.25 mm internal diameter, 0.25 µm film thickness), with injection volume of 1 µL of sample in split mode (1:20), helium as carrier gas (5 mL/min), and heating ramp starting at 130 °C for 1 minute, reaching 170 °C at a rate of 6.5 °C/min, from 170 to 215 °C at a rate of 2.75 °C/min remaining at this temperature for 12 minutes, and

finally from 215 to 230 °C at 40° C/min. The injector temperature was 220 °C. The identification of methyl esters of fatty acids was performed by comparison with retention times of fatty acids of standard acids: C6:0; C8:0; C10:0; C12:0; C14:0; C16:0; C16:1; C18:0; C18:1; C18:2; C18:3; C20:0; C20:1; C22:0; C22:1; C24:0 (98 – 99%, Sigma-Aldrich).

Determination of OSI was performed in triplicate, at four different temperatures, 100, 110, 120 and 130 °C in an equipment for analysis of oxidative stability, model 893 Professional Rancimat. In this method, 3 g of sample are placed in a reaction vessel sealed. An air flow (20 L/h) passes through the sample causing oxidation of the fatty acid molecules. During the process, there is the formation of peroxides followed by volatile organic acids. The organic acids are transported through an air stream to a second vessel containing deionized water, the conductivity of which is continuously recorded. The formation of organic acids rises water conductivity and when there is a rapid increase in formation of these products derived from oxidative process the OSI is determined from the maximum of the second derivative. The data are automatically calculated by the equipment software StabNet.

The results were compared by analysis of variance, employing the Tukey test at 95% significance level, Action Stat software, 2018.

4. Conclusions

The blends showed high or low values of antioxidant activity and phenolic compounds proportional to the amount of sunflower oil incorporated. Regarding the addition of coconut oil, the main factor in the increase of oxidative stability can be attributed to the high amount of lauric acid, an important saturated fatty acid. The mixture of coconut oil with sunflower oil, provided greater oxidative stability to blends, as well as higher content of phenolic compounds, which act as natural antioxidants and improve antioxidant capacity, obtained by analysis of DPPH.

It was expected that the high amount of natural antioxidants in sunflower oil would have stand out in terms of thermal stability, however, the differential was the high concentration of

saturated fatty acids from coconut oil in blends.

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