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Article

Study of the Oxidative Stability of Chia Oil (*Salvia hispanica* L.) at Various Concentrations of Alpha Tocopherol

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Abstract

Chia oil has a high content of linoleic and linolenic acid, which are prone to oxidation and isomerization processes that can affect the quality of the oil. Here, the oxidative stability of cold-pressed chia oil was evaluated using different concentrations of α -tocopherol. α -Tocopherol was added to the extracted oil at concentrations ranging from 0 to 0.10% in increments of 0.025% (TC, T1, T2, T3, and T4) with a randomized block design with three replicates was used. Lipid oxidation was estimated using the induction period with an Oxitest. Both the best treatment and the control were stored for 15 months at 15 °C, and the fatty acid content was quantified using a gas chromatograph with a single quadrupole mass detector. The fatty acid content in the freshly extracted oil was found to be 7.23% palmitic acid, 2.22% stearic acid, 5.61% oleic acid, 19.39% linoleic acid, and 65.03% linolenic acid. No significant differences were found in the oxidation induction period between treatments T2, T3, and T4, so T2 (0.05% α -tocopherol) was selected as the best treatment due to its lower amount of stabilizer. In conclusion, the addition of 0.05% α -tocopherol maintained the quality of the oil within the values permitted by Ecuadorian regulations.

Keywords: antioxidant; cold pressed; fatty acids; gas chromatography; Oxitest

1. Introduction

The nutritional qualities of foods are defined by their organoleptic attributes. Modern society demands nutritious, high-quality products that meet strict safety standards and possess an extended shelf life [1]. To improve the lipid profile in the production of meat products, specialty oils must exhibit specific physical properties and contain an appropriate combination of essential fatty acids that contribute to the overall well-being of consumers [2]. The demand for these distinctive oils, characterized by a high content of essential fatty acids, is increasing [3]. At the same time, the market for high-quality alter-native food options is expanding to ensure that the nutritional needs of the population are met [4].

Chia seeds are classified as a bioactive food due to their positive impact on improving the lipid profile in the bloodstream [5]. These benefits include properties such as reducing blood pressure and blood sugar levels, exhibiting antimicrobial activity, and promoting stimulation of the immune system [6,7]. Currently, scientists around the world are developing technologies that enable chia seeds to serve as a primary ingredient providing omega-3, proteins, and fiber in various products, including beverages, nutraceutical supplements, processed foods, and cosmetics [8–10].

These seeds, native to Mexico and Central America, are widely used in culinary preparations due to their rich content of vitamins, minerals, dietary fiber, healthy fats, proteins, and antioxidants. The quality of this diverse range of components in chia seeds can be influenced by several factors, such as geographical origin, agricultural practices, and environmental conditions [11,12]. Chia oil

contains 63.8% α -linolenic acid (ALA), 20% linoleic acid (LA), 6.9% palmitic acid, and 2.8% stearic acid [13]. Exposure of polyunsaturated fatty acids to air, light, and temperature can trigger oxidation reactions leading to the formation of undesirable flavors [14], rancid odors, discoloration, and other reactions that reduce the quality of the oil, even at the molecular level [15].

The oxidation process leads to a decrease in both the acceptability and the nutritional value of oils [16]. In this regard, several strategies have been explored to inhibit lipid oxidation in chia oil [17,18]. Antioxidants are essential to prevent these undesirable reactions, and vitamin E can serve as an effective alternative. This vitamin, also known as tocopherol and tocotrienol, has been widely recognized for its ability to donate hydrogen from its phenolic groups, thereby helping to protect cell membranes from free radicals and tissue damage caused by pathological processes [19].

High-quality chia oil extraction has been achieved using supercritical CO₂ fluids, yielding favorable results comparable to other extraction methods such as Soxhlet and cold pressing [20]. Although chia oil offers nutritional benefits due to its highly unsaturated fatty acid profile, it presents a drawback in terms of stability, which makes it susceptible to oxidation processes [21].

In this study, the oxidative stability of chia oil obtained through cold pressing was evaluated using a variety of vitamin E concentrations as antioxidant. Subsequently, the oils were stored for a period of 15 months to assess possible physicochemical alterations and changes in the fatty acid composition of chia oil.

2. Materials and Methods

2.1. Chia Seeds

Salvia hispanica L., or chia seeds, were purchased from a commercial company in the community of Saquisilí, Cotopaxi, Ecuador. To evaluate the oxidative stability of chia oil, vitamin E α -tocopherol (1000 U.I., brand Toco Vit-E) was used as an antioxidant. In addition, a fatty acid methyl ester standard (FAME MIX) from Sigma-Aldrich–Supelco was used.

Using a Florapower Expeller, the chia oil was cold-pressed at 50–60 °C (Figure 1) [22]. Once extracted, the oil was filtered, decanted, and stored at 15 °C in 60 mL amber glass bottles to protect it from sunlight.



Figure 1. Oil extraction equipment (Expeller).

To calculate the yield, Equation 1 was used, based on the amount of oil obtained per 100 kg of chia seeds processed.

$$\%RA = (PA/PC) * 100, \quad (1)$$

where:

%RA = Oil extraction yield (percentage).

PA = Weight (in grams) of chia oil extracted by pressing.

PC = Weight (in grams) of chia seeds used for oil extraction.

The five chia oil treatments, with and without antioxidant, were: TC (0% vitamin E, control treatment), T1 (0.025% vitamin E), T2 (0.050% vitamin E), T3 (0.075% vitamin E), and T4 (0.10% vitamin E). These were used to determine oxidative stability. Three replicates were applied following a randomized block design.

2.2. Oxidative Stability

Oxidative stability was evaluated using an Oxitest reactor (Velp Scientifica, Usmate, Milan, Italy) (Figure 2), according to the methodology described in previous studies [22–24]. The Induction Period (IP), expressed in hours, was determined at 80 °C and a pressure of 6 bar using grade 5 oxygen supplied by Linde—Ecuador.



Figure 2. Oxitest test reactor (VELP SCIENTIFIC).

The hydrogen potential (pH) was measured using a Mettler Toledo Seven Compact potentiometer [25]. Moisture content was determined with a Mettler Toledo HX 204 infrared balance [26], and the Acid Value was assessed using the Official Method [27] and the Ecuadorian Technical Standard INEN 0038 [28], expressed in terms of oleic acid.

To determine the acidity of the oil, Equation 2 was used.

$$A = \frac{M \cdot V \cdot N}{10 \cdot m}, \quad (2)$$

where:

A = Acid value of the oil, expressed as a percentage of oleic acid.

M = Molecular mass of oleic acid, 282 g/mol.

V = Volume of the hydroxide solution consumed in the titration, in mL.

N = Molarity of the sodium hydroxide solution, determined daily against a primary standard.

m = Mass of the sample analyzed, expressed in grams.

10 = Conversion factor to percentage.

2.3. Peroxide Value

It was determined using the Official Method No. 965.33 [27]. This is expressed in milliequivalents of oxygen per kilogram of oil (meq O₂/kg oil), for which Equation 3 was used.

$$Pv = \frac{V \cdot M \cdot 1000}{P}, \quad (3)$$

where:

Pv: Peroxide value, expressed in milliequivalents of O₂ per kilogram of oil.

V: Volume of sodium thiosulfate titrated, appropriately corrected to account for the blank, in mL.

M: Exact molarity of the sodium thiosulfate solution.
P: Mass of the sample, in grams.

2.4. Fatty Acid Determination

The fatty acid composition of chia oil was evaluated through esterification, generating two phases of fatty acid methyl esters. The lower layer contained the aqueous phase with additional esterification reaction products, while the upper layer consisted of the organic phase composed of hexane and fatty acid esters [29].

A 0.5 µL aliquot of the organic phase containing fatty acid methyl esters was injected into an Agilent Technologies 7890B GC System gas chromatograph. This gas chromatograph was coupled to an Agilent Technologies MSD 5977A mass spectrometer and used an HP-88 column (60 m × 0.25 mm, 0.20 µm). The oven temperature program was as follows: initial temperature at 80 °C, followed by ramps of 10 °C/min to 120 °C, 20 °C/min to 140 °C, 2 °C/min to 200 °C, held for 10 min, and finally increased at 5 °C/min to 240 °C, where it was maintained for 4 min. Helium (99.999% purity, Linde, Ecuador) was used as the carrier gas at a flow rate of 1.4 mL/min. The NIST 14.L library was used for qualitative identification of fatty acid esters.

Additionally, Supelco fatty acid methyl esters (FAME Mix C8–C22) were used as reference material to identify and quantify the fatty acids present in chia oil. This was done by comparing retention times and integrating peak areas obtained from the chromatographic analysis [30].

For statistical analysis, Statgraphics Centurion XVII (Statpoint Technologies Inc., Warrenton, VA, USA) was used. Treatment comparisons were performed using analysis of variance (ANOVA). Significant differences and mean values were compared using Tukey’s or Duncan’s multiple range tests at a 95% confidence level and a probability of ≤ 0.05.

3. Results and Discussion

3.1. Extraction Yield

The yield of chia oil extraction by cold pressing [26] is presented in Table 1.

Table 1. Chia oil extraction yield.

Seeds used (g)	Oil extracted (g)	Oil extraction percentage (%)
16,000	3,900	24.37
16,200	4,000	24.69
17,680	4,250	24.03
Mean ± standard deviation for 3 replicates		24.42 ± 2.18

Table 1 reports a yield of 24.42 ± 2.18% for chia oil obtained by cold pressing. These results are consistent with those reported in other studies, which indicated oil yields ranging from 26 to 34% [6,31] and 30 to 33% fat content [32]. The fat content in chia seeds is influenced by various factors, including plant variety, climatic conditions, agronomic practices, fertilization programs, and irrigation methods [33]. Additionally, the extraction percentage is related to the technique employed during the oil extraction process [31], which affects sensory attributes and ultimately oil quality, including viscosity, color, texture, appearance, shape, odor, and taste [34]. Oils extracted from chia seeds native to Ecuador display a light green color with a slight yellow hue and an oily appearance [35].

3.2. Accelerated Shelf-Life Study

The induction period (IP) from the VELP SCIENTIFIC Oxitest Reactor was used to evaluate the oxidative stability of chia oil. The longer the induction period, the more stable the oil is against oxidation. The results are presented in Figure 3.

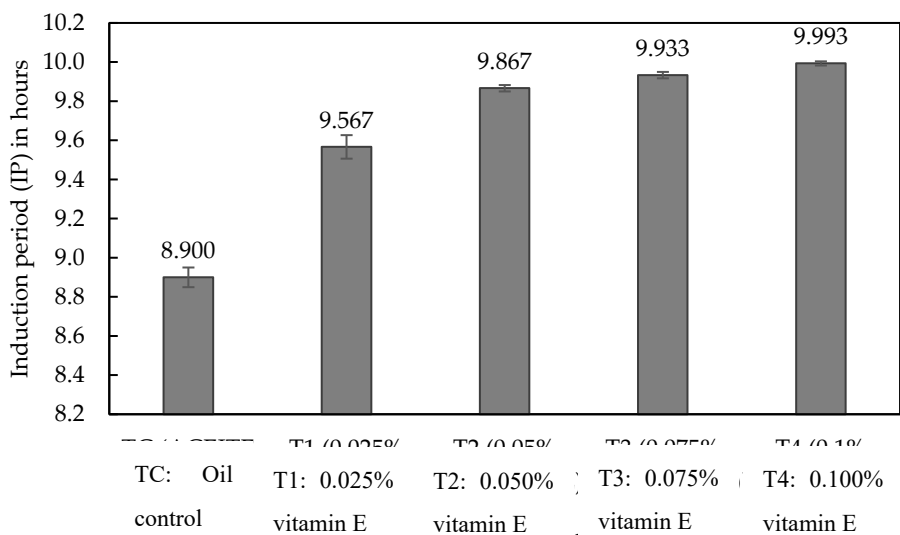


Figure 3. Induction period (IP) in hours for oils analyzed with the Oxitest equipment at 80 °C. Different letters within the bars indicate significant differences (p < 0.05).

According to Figure 3 and the statistical analysis, it can be concluded that the treatments with the highest Induction Period values correspond to the following groups: T2 (oil with 0.05% vitamin E), T3 (oil with 0.075% vitamin E), and T4 (oil with 0.1% vitamin E). These groups are statistically similar (P < 0.05), followed by T1 (oil with 0.025% vitamin E) and, finally, the control treatment TC (control oil). Therefore, T2 was considered the best treatment since it used a lower amount of stabilizer (vitamin E) while achieving a higher IP value.

On the other hand, the physicochemical properties of chia oil and treatment T2 were determined before and after 15 months of storage. Table 2 reports the physicochemical characteristics of the different oils with and without vitamin E.

Table 2. Physicochemical analysis of chia oil at 0 and 15 months of storage.

Physicochemical parameter	Control treatment, CT		With 0.05% vitamin E, T2	
	t = 0 months	t = 15 months	t = 0 months	t = 15 months
Moisture (%)	0.116±0.015	0.860±0.006	0.067±0.003	0.176±0.004
Hydrogen potential (pH)	4.560±0.036	3.070±0.070	4.200±0.050	3.883±0.015
Acidity index (% oleic acid)	0.308±0.056	0.509±0.007	0.279±0.004	0.465±0.005
Peroxide value (meq O ₂ /Kg)	3.460±0.159	10.267±0.115	2.042±0.010	5.543±0.004

Values are expressed as the mean of three replicates ± standard deviation.

Regarding the moisture percentage, a high moisture content increases the tendency of the oil to hydrolyze, resulting in a high content of free fatty acids, unpleasant odor, and rancid taste [36]. This requirement was affected in the TC, as its moisture content increased by a factor greater than 7 during storage time, in contrast to the T2 treatment, where it increased by a factor of approximately 2.6.

Chia oil (control treatment) showed the following values: an acidity index of 0.308 (% oleic acid) and a peroxide index of 3.46 (meq O₂/kg oil). Since there is no specific standard for chia oil, the Ecuadorian Technical Standard NTE INEN 29:2012 for olive oil was used as a reference, which establishes maximum values of 0.5% acidity and a peroxide index of 10 meq O₂/kg.

It is important to note that the lower the acidity index of an oil, the lower its potential for oxidative degradation of free fatty acids [37]. Therefore, the obtained chia oil meets both requirements regarding acidity and peroxide index.

When comparing data from other studies, a peroxide index of 2.56 meq O₂/kg and an acidity index of 0.13±0.0031 were reported for chia oil, which are lower than those obtained in the present

study [38]. Another study demonstrated that chia oil extracted by cold pressing has a peroxide index of 1.35 meq O₂/kg [37]. The reported results are comparable to the acidity index of 3.0 mg KOH/g and peroxide index of 2.3 meq O₂/kg found in chia oil obtained by cold pressing [39].

In contrast, the control treatment showed values of 0.509±0.007 for the acidity index and 10.267±0.115 for the peroxide index after 15 months of storage at room temperature, exceeding the limits established by the Ecuadorian Standard NTE INEN 29:2012. This indicates that the chia oil has oxidized and is no longer suitable for human consumption. In another study, peroxide index values of 15 meq O₂/kg were obtained after 135–150 days of storage of chia oil without preservatives and exposed to light [37].

On the other hand, after the storage period, the T2 treatment showed acidity index values of 0.465±0.005 and peroxide index values of 5.543±0.0043, which fall within the limits established by the cited Technical Standard, indicating that vitamin E contributed to its preservation. Moreover, after 15 months, both acidity and peroxide index values were higher than those of freshly cold-pressed chia oil that had not been preserved, indicating that the oil gradually deteriorated over the study period.

3.3. Fatty Acid Profile

Table 3 reports the fatty acid profile of freshly extracted chia oil without storage. It was found to contain saturated fatty acids (palmitic and stearic acids) at 9.45±0.30%; monounsaturated fatty acids (oleic acid) at 5.61±0.25%; and polyunsaturated fatty acids (linoleic and linolenic acids) at 84.95±0.08%. Regarding the ratio of saturated to unsaturated fatty acids, values ranged from 0.10 in freshly extracted chia oil to 0.13 in chia oil with and without vitamin E after 15 months of storage.

Table 3. Fatty acid profile of chia oils with and without vitamin E.

Compound number	Common name	Abbreviation	[31]	[36]	[37]	[40]	Control oil, 0 months	Control oil, 15 months	0.05% vitamin E, 15 months
1	Palmitic acid	C16:0	7.226	7.22	7.46	8.54	7.23±0.14 a	8.34±0.03 b	8.60±0.10 b
2	Stearic acid	C18:0	2.91	3.54	-	3.37	2.22±0.16 a	2.80±0.02 b	2.56±0.06 b
3	Oleic acid	C18:1n9c	7.395	7.10	7.18	10.24	5.61±0.25 b	6.26±0.03 a	6.10±0.01 a
4	Linoleic acid	C18:2n6c	19.708	18.74	20.1	18.69	19.39±0.17 a	17.88±0.06 b	17.71±0.07 b
5	Linolenic acid	C18:3n3	62.76	63.23	61.8	54.08	65.55±0.09 a	64.72±0.07 c	65.03±0.19 b
Saturated fatty acids							9.45±0.30 a	11.14±0.05 b	11.16±0.12 b
Monounsaturated fatty acids							5.61±0.25 b	6.26±0.03 a	6.10±0.01 a
Polyunsaturated fatty acids							84.95±0.08 a	82.60±0.05 b	82.74±0.12 b
Ratio of saturated to unsaturated fatty acids							0.10±0.00 a	0.13±0.00 b	0.13±0.00 b

4. Conclusions

The addition of α-tocopherol in treatment T2 proved to be the most effective approach for in maintaining the oxidative stability of cold-pressed chia oil, as it preserved its quality within the limits established by Ecuadorian regulations. This treatment was sufficient to prevent the oxidation of unsaturated fatty acids and maintained the omega 3 content even after the evaluated storage period.

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Data Availability Statement: The original data supporting the results of this research are included in the article. Additional information can be obtained from the corresponding author.

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