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**CHIA (*Salvia hispanica* L.) OIL STABILITY: STUDY OF THE EFFECT OF NATURAL
ANTIOXIDANTS**

Romina M. Bodoira^a, María C. Penci^{bc}, Pablo D. Ribotta^{bc} and Marcela L. Martínez^{ac}

^a Instituto Multidisciplinario de Biología Vegetal (IMBIV, CONICET-UNC), Av. Vélez Sarsfield 1611, Córdoba Argentina.

^b Instituto de Ciencia y Tecnología de los Alimentos Córdoba (ICYTAC, CONICET – UNC), Juan Filloy S/N, Córdoba , Argentina.

^c Instituto de Ciencia y Tecnología de los Alimentos (ICTA – FCEFyN – UNC). Av. Vélez Sarsfield 1611, Córdoba Argentina.

*Corresponding author: María Cecilia Penci

Email: cpenci@gmail.com

Romina Mariana Bodoira email: rominabodoira@hotmail.com

Marcela Lilian Martínez email: marcelamartinez78@hotmail.com

María Cecilia Penci email: cpenci@gmail.com

Pablo Daniel Ribotta email: pribotta@agro.unc.edu

37 **ABSTRACT**

38 The chia seed (*Salvia hispanica* L.) is globally popular and valued for its nutritional and
39 health attributes. Chia oil is mainly composed of triglycerides, in which polyunsaturated fatty
40 acids (PUFAs, linoleic and α -linolenic acids) are found in high amounts. Although it seems
41 evident that such fatty acid composition is favorable from a nutritional point of view, a higher
42 content of linoleic and linolenic acids results in poorer oxidative stability and shorter shelf
43 life of the oil. The aim of this study was to evaluate the combined effects of the storage
44 condition (300 days under fluorescent light - 800 Lux - or in the dark, both at room
45 temperature) with the addition of natural antioxidants (rosemary extract, RE; tocopherol,
46 TOC; ascorbyl palmitate, AP). In the dark, the combined addition of AP and TOC
47 significantly reduced lipid oxidation and improved oil shelf life. Moreover, this combination
48 maintained an acceptable quality of at least up to 300 storage days. Results from this work
49 highlight the influence of illumination condition on chia oil oxidative stability, suggesting that
50 this oil should be stored in containers with light-barrier properties, and probably added to
51 the antioxidants examined in the current study.

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55 **KEY-WORDS:** chia oil, oxidative stability, natural antioxidant, storage.

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63 **1.INTRODUCTION**

64 Chia (*Salvia hispanica* L.) is an annual herbaceous plant that belongs to the Lamiaceae
65 family, which is native from southern Mexico and northern Guatemala. Chia seed contains
66 about 0.32 g oil /g seed; 0.28 g fiber /g seed, 0.21 g protein /g seed and 0.05 g ash /g seed,
67 and chia oil contains the highest proportion of α -linolenic acid (0.6 g /g oil) from any known
68 vegetable source (Ayerza & Coates 2004). The antioxidant capacity of this oil is relatively
69 low because the phenolic compounds present in the seed are mostly hydrophilic in nature
70 (Da Silva Marineli *et al.* 2014). Polyunsaturated fatty acids (PUFA) play a major role in the
71 prevention and treatment of non-communicable diseases (NCDs), also known as chronic
72 diseases, such as: hypertension, coronary artery disease, diabetes and cancer (Fereidoon
73 2009; Poudyal *et al.* 2012). Although it seems clear that such fatty acid composition is
74 favorable from a nutritional point of view, a higher content of PUFAs results in poorer
75 oxidative stability and shorter shelf life of the oil. When PUFAs are exposed to
76 environmental factors such as air, light and temperature, oxidation reactions produce
77 undesirable flavors, rancid odors, discoloration and other forms of spoilage. Natural or
78 synthetic antioxidants can increase the shelf life of food products by retarding lipid oxidation
79 through different action mechanisms. The synthetic antioxidants of the food industry, such
80 as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertbutyl
81 hydroquinone (TBHQ), have widespread use as food additives; yet, their effects on human
82 health and metabolic routes are being questioned (Shahidi & Zhong 2005; Iqbal & Bhangar
83 2007). As a result, there is a great interest in obtaining and using antioxidants from natural
84 sources, such as simple phenols, phenolic acids, carotenoids, anthocyanins, flavonoids,
85 vitamins and spice extracts (Maestri *et al.* 2006). Among these, rosemary extract
86 (*Rosmarinus officinalis* L) has been evaluated in many studies on different lipid matrices
87 (Hras *et al.* 2000; Erkan *et al.* 2008; Ixtaina *et al.* 2012; Martinez *et al.* 2013^a; Martinez *et al.*

88 2013^b; Chen *et al* 2014). This is a GRAS additive and its major active antioxidant
89 component is carnosic acid (Terpinc *et al* 2009). Ascorbic acid has strong reducing
90 properties due to the presence of the enediol group. The tocopherols abound naturally in
91 unrefined vegetable oil and have been extensively tested. Numerous studies have
92 evaluated antioxidant effectiveness in different lipid matrices by accelerated oxidation tests;
93 however, work carried out under real storage conditions is scarce (Gómez Alonso *et al*
94 2007; Let *et al* 2007; Olmedo *et al* 2008; 2009 Arcoleo *et al* 2009; Ixtaina *et al* 2012;
95 Martínez *et al* 2013^a).

96 This work aimed at evaluating the effectiveness of natural antioxidants alone and/or in
97 combination during ten-month storage in the dark and at room temperature in the oxidative
98 stability of chia oil obtained by cold pressing.

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100 **2. MATERIALS AND METHODS**

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102 **2.1 Materials**

103 Chia seeds were obtained from commercial plantations in the province of Salta. Chia oil
104 extraction was carried out in a single step with a Komet screw press, pilot plant scale type
105 (Model CA 59 G, IBG Monforts, Germany). The moisture content of the seeds was adjusted
106 to 0.11 g/g dry basis, the pressing temperature was 30 °C, the screw speed was 20 rpm
107 and the restriction die was 6 mm (Martínez *et al* 2012). The oil obtained was filtered through
108 a filter press and stored until use in amber glass bottles at -20 °C under nitrogen
109 atmosphere.

110 Natural mixed tocopherols (GUARDIAN™ TOCO 70 IP A), rosemary extract (GUARDIAN™
111 12, fat soluble), ascorbyl palmitate (GRINDOX™ 562), citric acid (GRINDOX™ 373) and a
112 commercial mixture of ascorbyl palmitate (10%) and tocopherols (10%) (GRINDOX™ 497)

113 were obtained from Danisco (Copenhagen, Germany). The content of the main rosemary
114 antioxidative components (carnosic acid) was 1% and the relative percentage of tocopherol
115 isomers was analysed by HPLC (purity 70%: α = 8,8%, β = 1,55%, γ = 61,82% and δ =
116 27,82%). Tertbutyl hydroquinone (TBHQ), a synthetic antioxidant, was used as a positive
117 control due to its known ability to stabilize edible oils and consequent wide use in the food
118 industry.

119

120 **2.2 Methods**

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122 *2.2.1 Total seed oil content*

123 Three samples (10 g each) of dry chia seeds were used to determine total oil content in
124 accordance with AOCS official method Ba 3-38 (AOCS, 2009).

125 *2.2.2 Oil analysis*

126 Acidity index (AI), peroxide (PV), K_{232} (conjugated dienes, CD) and K_{270} (conjugated trienes,
127 CT) values were evaluated using standard AOCS (2009) methods. Chlorophyll and
128 carotenoid content was determined at 670 and 470 nm, respectively, in cyclohexane via
129 specific extinction values using the method of Mínguez- Mosquera *et al.* (1991).

130 The oxidative stability (OSI) was measured following the Rancimat (Metrohm, Switzerland)
131 method (Cd 12b-92 AOCS, 2009) by using 3g oil aliquots. Airflow rate was set at 20L/h and
132 temperature of the heating block was maintained at 100 °C. Results corresponded to the
133 break points in the plotted curves and were expressed as induction time (IT) in hours.

134 To evaluate radical scavenging capacity (RSC), 100 mg of chia oil in 1 mL toluene was
135 vortexed (20 s, ambient temperature) with 3.9 mL toluene solution of the free stable DPPH
136 (2,2-diphenyl-1-picrylhydrazyl) radical (DPPH \cdot) at a concentration of 10^{-4} mol/L according to
137 Martinez *et al* (2008). Against a blank of pure toluene, the absorption at 515 nm was

138 measured in 1 cm quartz cells using an UV-visible spectrophotometer (Perkin-Elmer
139 Lambda 25, Shelton, CT, USA). RSC toward DPPH[·] was estimated by mean of the
140 following equation:

141

142 $DPPH^{\cdot} = \{1 - [(Absorbance\ of\ control - Absorbance\ of\ test\ sample) / Absorbance\ of$
143 $control]\} \times 100$

144

145 Where DPPH[·] expresses the amount of the radical that remains in the medium after the
146 antioxidants in the oil sample are depleted (Espín *et al.* 2000). RSC was expressed as
147 IC50, reflecting the depletion of the free radical to 50%. A lower IC50 value indicates higher
148 antiradical activity.

149 For fatty acid (FA) composition determinations, 0.5 g of oil was subjected to alkaline
150 saponification by reflux (45 min) using 30 mL 1 N KOH in methanol. Unsaponifiable matter
151 was extracted with n-hexane (3 x 30 mL). FA were converted to methylesters (FAME) by
152 reflux (45 min) using 50 mL 1 N H₂SO₄ in methanol and analysed by gas chromatography
153 (GC) (Perkin-Elmer, Shelton, CT, USA) using a fused silica capillary column (30 m x 0.25
154 mm i.d.x 0.25 lm film thickness) CP Wax 52 CB (Varian, Walnut Creek, CA, USA); carrier
155 gas N₂ at 1 mL/min; split ratio 100:1; column temperature programmed from 180 °C to 240
156 °C (10 min) at 4 °C/min; injector and detector temperatures at 250 °C, FID. The
157 identification of FAME was carried out by comparison of their retention times with those of
158 reference compounds (Sigma–Aldrich, St. Louis, MO, USA) (Martinez *et al.* 2013 a).

159 Iodine value (IV) was calculated from fatty acid percentages (Torres & Maestri, 2006) by
160 using the formula:

161

162 $IV = (\% \text{ Palmitoleic acid} \times 1.001) + (\% \text{ Oleic acid} \times 0.899) + (\% \text{ Linoleic acid} \times 1.814)$

163

164 The relationship between monounsaturated fatty acids (MUFA) and polyunsaturated fatty
165 acids (PUFA) was also calculated:

166

167
$$\text{MUFA/PUFA} = (\% \text{ Palmitoleic acid} + \% \text{ Oleic acid}) / (\% \text{ Linoleic acid} + \% \text{ Linolenic}$$

168 acid)

169 Tocopherols were analyzed by HPLC (Perkin-Elmer, Shelton, CT, USA) according to the
170 procedure of Lazzez *et al.* (2008) with some modifications. Samples of 1 g oil were placed
171 into 10 mL volumetric flasks. A quantity of n-hexane was added, swirling to dissolve the
172 sample and making up to volume with the same solvent. An aliquot of 20 μL of this solution
173 was injected onto a Supelcosil LC-NH₂-NP column (25 cm x 4.6 mm, Supelco, Bellefonte,
174 PA, USA). The mobile phase was n-hexane/ethyl acetate (70/30 v/v) with a flow rate of 1
175 mL/min. UV detection at 295 nm was performed. Individual tocopherols were identified by
176 comparison of their retention times with those of authentic standards (CN Biomedicals,
177 Costa Mesa, CA). Individual tocopherols were quantified by the external standard method.
178 The linearity of the response was verified by fitting to line results of each tocopherol
179 individual of twenty standard solutions with known concentrations. The concentration was
180 expressed as mg tocopherol / kg of oil.

181 The total phenol content (TPC) in oil was determined by the Folin-Ciocalteu method
182 according to Torres *et al* (2009). Briefly, phenolic compounds were analysed in 20 g
183 aliquots of oil. They were dissolved in 10 mL of n-hexane and extracted three times with
184 12.5 mL of methanol/water (60 : 40 v/v) by stirring over a magnetic plate for 15 min. The
185 pooled extracts were washed twice with 10 mL of n-hexane, and solvents were removed in
186 a rotating evaporator (Buchi, Flawil, Switzerland) at 30 °C under vacuum. To

187 a suitable dilution of the extracts, Folin–Ciocalteu reagent (Fluka, Buchs, Switzerland) was
188 added and the absorbance values of the solutions at 725 nm (total phenols) were
189 measured. Total phenol content is given as mg gallic acid /kg oil. In the case of σ -diphenols,
190 concentration was calculated from the reaction with $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ and reading at 350 nm
191 (Gutfinger 1981) expressed as mg caffeic acid/kg oil

192 Squalene determinations were done from 200 mg oil aliquots according the procedure of
193 Maestri *et al.* (2015). The unsaponifiable fractions in oil were determinate according
194 Martinez et al. 2006. An aliquot of oil (1g) was saponificated by reflux (45 min, 20 mL KOH
195 in methanol 1M) and then extracted with n-hexane (3 × 30 mL). Preparative TLC plates (0.5
196 mm silica gel; Merck, Darmstadt, Germany) were used to separate fractions
197 (toluene/acetone (95:5, vol/vol). Sterols and methylsterols were run without further
198 treatment using a VF-5ms (Varian, Walnut Creek, CA) capillary column (30 m × 0.25 mm
199 i.d.) coated with a 0.25 μm layer of 5% phenyl, 95% polydimethylsiloxane; carrier gas N_2 at
200 1 mL/min¹; column temperature programmed from 240°C (1 min) to 290°C at 2°C min⁻¹;
201 injector and detector temperatures 300°C; FID. GC–MS used an HP 5 (Hewlett-Packard,
202 Palo Alto, CA) fusedsilica capillary column (30 m × 0.25 mm i.d.) coated with a 0.25 μm
203 layer of 5% phenyl methyl siloxane, and helium (flow rate 1 mL/min) as carrier gas. The
204 column, injector, and detector temperatures were as for GC analysis. Sterols and
205 methylsterols were identified by comparison of the mass spectral data with those of
206 authentic reference compounds. Hydrocarbons were analyzed by GC and GC–MS. Briefly,
207 hydrocarbons purified by TLC as described above were analyzed by GC using a VF-5ms
208 capillary column. The column temperature was programmed from 70 to 300°C at 4°C min⁻¹–
209 1, injector and detector temperatures 320°C, carrier gas N_2 at 1 mL/min, FID. GC–MS used
210 an HP 5 capillary column and helium (flow rate 1 mL/min) as carrier gas. The column,
211 injector, and detector temperatures were as for GC analysis. Hydrocarbons were identified

212 by their retention times and comparison of the mass spectral data with those of authentic
213 reference compounds.

214

215 2.2.3 Rancimat analysis

216 Rosemary extracts (RE), tocopherols (TOC), ascorbyl palmitate (AP), citric acid (CA) and
217 blends were added separately to chia oil aliquots at different concentrations. The additives
218 were dissolved in oil by using a shaker 5 min (Martinez *et al* 2013^a). Tocopherols were in
219 range of 50 to 800 mg/kg; rosemary extract ranged from 1000 to 16000 mg/kg; ascorbyl
220 palmitate from 50 to 700 mg/kg; citric acid 100 mg/kg and TBHQ 100 and 200 mg/kg.
221 Several combinations were evaluated: ascorbyl palmitate and tocopherols (50-700 mg/kg),
222 rosemary extract (8000 mg/kg) and citric acid (100 mg/kg), rosemary extract (8000 mg/kg)
223 and ascorbyl palmitate (200 mg/kg), rosemary extract (8000 mg/kg) and tocopherols (200
224 mg/kg). Oil oxidative stability was evaluated by the Rancimat method, using 3 g of oil
225 sample warmed at 100 °C with an air flow of 20 L/h. Oil stability was expressed in terms of
226 induction time (IT) (h) and the effectiveness of all tested antioxidants and their blends was
227 expressed as the protection factor (PF):

228

229

230

$$PF = \frac{IT_{ant}}{IT_0}$$

231

$$IT_0$$

232

233 where IT_{ant} is the induction time of the samples treated with antioxidant, and IT_0 is the
234 induction time of the control system (without antioxidant).

235 Upon combining two antioxidants, the resulting IT were used for each concentration to
236 calculate synergism between antioxidants using the equation proposed by Bishov *et al.*
237 (1977).

238

$$\%Syn: \frac{(IT_{blend} - IT_0) - [(IT_1 - IT_0) + (IT_2 - IT_0)]}{[(IT_1 - IT_0) + (IT_2 - IT_0)]} \times 100$$

241

242 where IT_1 and IT_2 are the induction time of the individual antioxidants, IT_{blend} is the induction
243 time of the mixture of antioxidant 1 and 2 in the same concentration as that alone and IT_0 is
244 the induction time of the control system (without antioxidant).

245

246 2.2.4 Experimental design for storage stability test

247 Antioxidants (RE, AP, TBHQ, TOC, CA) or their mixtures were added to oil samples
248 selected on the basis of Rancimat analyses, and taking into account Argentine Food Code
249 and Codex Alimentarius standards. Briefly, the additives (RE, AP, TBHQ, TOC, CA) were
250 dissolved in 50 mL-oil aliquots by using a shaker (approximately 5 min) until a
251 homogeneous oil was achieved. The mixtures (oil plus additive) were transferred separately
252 to transparent glass bottles (250 mL) each containing 200 mL chia oil. The bottled oils (final
253 volume 250 mL) were mixed thoroughly and then placed in a thermostated chamber at $25 \pm$
254 1°C kept in the dark by wrapping each bottle with an aluminum foil. For each treatment
255 three sets of bottled oils were prepared. For control treatments, oil samples without added
256 antioxidants were used, three of them in the dark (OC) and three without aluminum foil to
257 evaluate effect of photooxidation on chia oil (LC). Bottled oils were stored for ten months
258 under illumination (800 Lux). Every fifteen days, each individual oil sample was withdrawn
259 from the chamber for scheduled analysis. This is a typical dynamic storage assay,

260 compared with static storage assay (Ixtaina *et al.* 2012). In dynamic assays the head space
261 volume increases over time. Acidity (A), peroxide value (PV), K₂₃₂ and K₂₇₀ values were
262 evaluated (AOCS 2009) every fifteen days in each treatment and control. Additionally, with
263 the aim of analyzing possible changes in the characteristic profile of oil, the following
264 determinations were performed on controls (LC and DC) at 0, 165 and 300 days of storage:
265 fatty acid (FA) composition and Iodine value, pigments, quantification of tocopherols and
266 radical scavenging capacity (DPPH).

267 2.2.5 Statistical analysis

268 Statistical differences among treatments were estimated from ANOVA test at the 5% level
269 (P < 0.05) of significance, for all parameters evaluated. Whenever ANOVA indicated a
270 significant difference, a pair wise comparison of mean by least significant difference (Fisher
271 LSD) was carried out.

272

273 3. RESULTS AND DISCUSSION

274

275 3.1 Chia oil analysis

276 Table 1 shows results from chia oil analysis. Acidity (A), peroxide value, conjugated dienes
277 and trienes values (K₂₃₂ and K₂₇₀) are similar to those observed in cold-pressed chia oil
278 (Martínez *et al.* 2012), much lower than the maximum values established by the Codex
279 Alimentarius (2001) for non-refined oils, indicating that the oil extraction method employed
280 did not affect adversely those indicators of hydrolytic and oxidative rancidity. Linolenic acid
281 is the major fatty acid (61.8 g/100 g oil) followed by linoleic (20.1 g/100 g oil) and oleic (7.18
282 g/100 g oil) acids. Such composition leads to high unsaturation degree (iodine value, 212).
283 This fact, together with a relatively low tocopherol and phenolic compound concentration ,
284 accounts for the low oxidative stability of chia oil (< 3.04 h). According to Wong (1995),

285 vegetable oils are susceptible to photooxidation during storage under light, especially when
286 photosensitizers, such as chlorophylls, are present. Although chia oil has very low
287 chlorophyll content (4.66 mg/kg oil), such concentration may be sufficient to induce
288 photochemical oxidation (Suzuki *et al.* 1984; Martinez *et al.* 2013a).

289 Regarding carotenoid pigments, Warner and Frankel (1987) have shown that, in soybean
290 oil, the presence of β -carotene at concentrations between 5 and 20 mg/kg oil has a
291 protective effect against oxidative damage induced by light. Considering the fatty acid
292 composition of chia oil, it can be assumed that carotenoid content (5.41 mg/kg oil) is not
293 enough to provide protection against photooxidative degradation.

294 The unsaponifiable fraction analysis shown that sterols were represented by β -sitosterol (60
295 mg/100 mg), 4-6-cholestadien-3 β -ol (30 mg/100 mg) and campesterol (9,7 mg/100 mg), the
296 triterpene alcohols and methylsterols were not detected. The hydrocarbon fraction was
297 represented mostly by alkanes, the main were octadecane (36,42 mg/100 mg), hexadecane
298 (29,25 mg/100 mg), docosane (16,33 mg/100 mg) tetradecane (7,96 mg/100 mg) and
299 nonadecane (2,68 mg/100 mg). Regarding squalene, since there are not previous studies in
300 chia oil, it was analyzed but no significant levels of this hydrocarbon were detected.

301

302 *3.2 Natural and synthetic antioxidant performance on chia oil*

303 In order to evaluate the antioxidant efficiency of natural and synthetic substances (individual
304 or in combination) in chia oil, oxidative stability (Rancimat test) was studied under different
305 conditions. Table 2 shows a summary of antioxidant conditions for chia oil and their
306 protection factors (PF). Considering RE, significant differences can be found ($p \leq 0.05$)
307 between PF of all concentrations tested, but from 10000 (mg/kg oil) a marked decrease is
308 evidenced in the solubility of the extract in oil (turbidity). In addition to this technological
309 drawback, the increase in oxidative stability was not proportional to the increase in the

310 concentration of ER, hence we used 8000 (mg/kg oil) of RE. A similar behavior was
311 reported when RE was incorporated to walnut and almond oils (Martinez *et al.* 2013^a,
312 Martinez *et al.* 2013^b). Tocopherols showed the lowest antioxidant performance (PF 1.39,
313 800 mg/kg oil). For PA, the highest FP was achieved at 800 mg/kg oil; however, the
314 maximum level has been set to be 200 mg/kg oil (Codex Alimentarius). At this
315 concentration, PF was 3.55 ± 0.08 . When RE was combined with CA 100 (mg/kg oil), the
316 blend showed a significant additional protective effect ($p \leq 0.05$) compared to that of RE
317 alone. When achieved, synergism was relatively low despite being positive (17.25%). Some
318 authors reported that the combination of PA and TOC could be positive for some oils
319 (Marinova & Yanishlieva 1990). In this work an equal part blend of each antioxidant was
320 evaluated. For 250 mg/kg oil TOC and 250 mg/kg oil PA, the maximum PF (9.49 ± 0.05)
321 was achieved. The synergism effect varied from 141 per cent (300 mg/kg oil TOC + 300
322 mg/kg oil PA) to 42 per cent (400 mg/kg oil TOC + 400 mg/kg oil PA). In order to compare
323 natural and synthetic antioxidants, TBHQ was employed at 100 and 200 mg/kg oil in chia
324 oil. PF were 3.79 ± 0.07 and 6.64 ± 0.01 respectively, showing that the antioxidant capacity
325 of synthetic additives could be higher than that of natural antioxidants for chia oil.

326

327 3.3 Storage stability test of chia oil

328 Considering the results obtained from the study of antioxidant performance in chia oil and
329 regulations in force on the level of additives, Table 3 shows concentration of each
330 antioxidant and storage condition. The oxidative stability of chia oil stored in the dark
331 without adding any antioxidant shows a similar oxidation rate than that stored under light.
332 Carotenoids do not show a statistically significant difference between both lighting
333 conditions; yet, chlorophylls show a decrease of 80 % and 63 % under light and dark
334 conditions, respectively. The antioxidant activity of this edible oil decreased at 300 days of

335 storage in both lighting conditions. This could be attributed to the fact that tocopherol
336 content in chia oil decreased by approximately 30 %. The relative proportion of the different
337 fatty acids was not significantly affected during storage. Particularly, acidity did not differ
338 significantly ($p \leq 0.05$) between treatments, showing no significant increase in time.
339 Considering that the maximum allowed for this parameter in chia oil is around 1 g oleic
340 acid/100g oil, in this test, at 165 days of storage, CL condition presented a 0.22 value. This
341 evidences that chia oil is stable against the hydrolytic degradation of glycerides, even under
342 exposure to light. Figure 2 shows that the combination of ascorbyl palmitate (AP) and
343 tocopherols (TOC) at 200 mg/kg oil each is more effective in the stabilization of chia oil than
344 the addition of TBHQ at 200 mg/kg oil stored in the dark (0.66 and 2.35 meq O₂/kg oil at
345 300 days of storage, respectively). However, the addition of ascorbyl palmitate (AP) at 200
346 mg/kg oil, tocopherols (TOC) at 200 mg/kg oil and rosemary extract (RE) at 8000 mg/kg oil
347 did not show good antioxidant capacity, exceeding 15 meq O₂/kg oil (Codex Alimentarius,
348 2001) from approximately 135-150 days of storage (Figure 1). Although found in static tests,
349 similar trends were reported by Ixtaina *et al.* 2012 for rosemary extract and for the addition
350 of tocopherol in the preservation of chia oil. It should be noted that light exerts a significant
351 effect on the generation of primary oxidation products, accelerating photooxidation probably
352 due to the presence of photosensitizers such as chlorophylls (Wong, 1995; Frankel, 2005)
353 in sufficient quantity to promote photochemical production of singlet oxygen (Suzuki *et al.*
354 1984). Regarding K232 and K270, parameters show the same trend as that found in PV. In
355 addition, pigment content in chia oil decreased significantly at 165 days of storage.

356

357 **4. CONCLUSIONS**

358 The nutritional benefits gained by consuming chia oil are mainly ascribed to the high
359 content of ω 3 and ω 6 fatty acids; yet, they also show a technological disadvantage in terms
360 of product stability. Although chia oil contains naturally antioxidant substances to prevent

361 oxidation, when exposed to environmental factors such as light and the oxygen, its
362 chemical quality could be altered. In response to this, it was found that the protective effect
363 of the combination of natural antioxidants PA and TOC (50:50) was more marked than that
364 achieved with TBHQ, one of the most widely used synthetic antioxidants. This result
365 represents an interesting alternative for these unconventional oils from crops being
366 reexamined in the country and in the continent as a novel alternative to the oil industry.

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368

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