Title: Sacha inchi (Plukenetia volubilis) oil stability, chemical composition and antioxidant capacity changes during French fries deep-frying

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Abstract: Sacha inchi (Plukenetia volubilis) oil (SI), a novel food for European legislation, is appreciated for its nutritional and sensorial characteristics. The aim of this study was to evaluate its stability during French fries deep-frying at 170 °C or 180 °C; commercial soybean oil (SO) was tested as control. SI and SO differed for α-linolenic acid (53.8% vs. 7.1%), linoleic acid (33.4% vs. 58.2%) and total tocopherols (2540.1 vs. 1081.7 mg/kg). During frying tocopherol content, oil stability and antioxidant capacity (ABTS, DPPH) decreased following zero-order kinetics; g-tocopherol had the strongest antioxidant effect. Notwithstanding the high SI unsaturation and the presence of a commercial antioxidant (TBHQ) in SO, sacha inchi showed slightly higher (free fatty acids) or similar (diacylglycerols) hydrolysis, similar primary (K232, oxidized-triacylglycerols) and lower secondary (K268, triacylglycerol oligopolymers) oxidation. The very high tocopherol content of SI contributed to preserve the stability of the polyunsaturated fatty acids during deep-frying.

Dear Editor

please find enclosed our manuscript "**Kinetics of sacha inchi (*Plukenetia volubilis*) oil stability indices, chemical composition and antioxidant capacity changes during deep-frying**" which we wish to submit for consideration as a research article in Food Chemistry.

The interest in the utilization of sacha inchi oil as food is rapidly growing, because its high content in polyunsaturated fatty acids has several positive effects on human health. Its outstanding sensorial characteristics and health effects allowed its official introduction in the European market as a novel food in 2013. However, the high degree of unsaturation could also lead to a great susceptibility of sacha inchi oil to oxidation during food processing.

The aim of this study was to evaluate sacha inchi oil stability under real and severe processing conditions such as deep-frying.

Sacha inchi oil was tested during French fries deep-frying at 170 °C or 180 °C, using commercial soybean oil as control. We performed the following oils analyses after different frying times: colour, fatty acid composition, tocopherol content, antioxidant capacity (ABTS, DPPH tests of hydrophilic and lipophilic extracts), free fatty acids, K_{232}, K_{268}, triacylglycerol oligopolymers, oxidized triacylglycerols, and diacylglycerols.

During frying tocopherol content, oil stability and antioxidant capacity decreased following zero-order kinetics; γ-tocopherol had the strongest antioxidant effect. Notwithstanding the high sacha inchi oil unsaturation and the presence of a commercial antioxidant (TBHQ) in soybean oil, sacha inchi showed slightly higher (free fatty acids) or similar (diacylglycerols) hydrolysis, similar primary (K_{232}, oxidized-triacylglycerols) and lower secondary (K_{268}, triacylglycerol oligopolymers) oxidation. The very high tocopherol content of sacha inchi oil contributed to preserve the stabilty of the polyunsaturated fatty acids during deep-frying.

The main novelty of our paper is that we analysed the stability of sacha inchi oil by means of a demanding real-life approach, the deep-frying process. The kinetics study of the changes observed in the oil was also performed.

Looking forward to further word in due time, I remain

yours sincerely

Alyssa Hidalgo

Sacha inchi oil (SI) stability during deep-frying at 170 °C or 180 °C was tested

SI had more linolenic acid and tocopherols than a control commercial soybean oil (SO)

Tocopherols, oil stability and antioxidant capacity decreased in zero-order kinetics

SI showed similar primary but lower secondary oxidation than SO

The high tocopherol content preserved the stability of SI polyunsaturated fatty acids

1 **Sacha inchi (*Plukenetia volubilis*) oil stability, chemical composition and**

2 **antioxidant capacity changes during French fries deep-frying**

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18   ABSTRACT

19   Sacha inchi (*Plukenetia volubilis*) oil (SI), a novel food for European legislation, is appreciated for

20   its nutritional and sensorial characteristics. The aim of this study was to evaluate its stability during

21   French fries deep-frying at 170 °C or 180 °C; commercial soybean oil (SO) was tested as control. SI

22   and SO differed for α-linolenic acid (53.8% vs. 7.1%), linoleic acid (33.4% vs. 58.2%) and total

23   tocopherols (2540.1 vs. 1081.7 mg/kg). During frying tocopherol content, oil stability and

24   antioxidant capacity (ABTS, DPPH) decreased following zero-order kinetics; γ-tocopherol had the

25   strongest antioxidant effect. Notwithstanding the high SI unsaturation and the presence of a

26   commercial antioxidant (TBHQ) in SO, sacha inchi showed slightly higher (free fatty acids) or

27   similar (diacylglycerols) hydrolysis, similar primary (K_{232}, oxidized-triacylglycerols) and lower

28   secondary (K_{268}, triacylglycerol oligopolymers) oxidation. The very high tocopherol content of SI

29   contributed to preserve the stability of the polyunsaturated fatty acids during deep-frying.

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31   **Keywords**: kinetics, lipid hydrolysis, lipid oxidation, tocopherol, polar compounds, Rancimat.

1. Introduction

Sacha inchi (*Plukenetia volubilis*) is a perennial climbing plant of the *Euphorbiaceae* family traditionally cropped across the American tropical region. The oil extracted from the seeds is exceptionally rich in polyunsaturated fatty acids (82%), particularly ω-3 molecules, and in tocopherols (>2000 mg/kg) (Chirinos, Pedreschi, Domínguez & Campos, 2015; Cisneros, Paredes, Arana & Cisneros-Zevallos, 2014; Fanali et al., 2011), and its consumption may contribute to reduce some risk factors of cardiovascular diseases, such as inflammation and high serum level of LDL (Wang, Zhou & Kakuda, 2018). Sacha inchi oil has outstanding sensorial characteristics (in particular, a floral flavour), but is coveted also for cosmetic use as a nails and hair strengthener (Wang et al., 2018). Currently the oil is a niche product, officially introduced in the European market in 2013 and recognized as a novel food (EU, 2015). In the literature some information on the composition and food uses of sacha inchi oil is available (e.g. Chirinos et al., 2015; Fanali et al., 2011; Gutiérrez, Sanchez-Reinoso & Quiñones-Segura, 2019; Ramos-Escudero, González-Miret, Vinãs-Ospino & Ramos Escudero, 2919) but, to the best of our knowledge, nothing exists about its behaviour during the frying process. In fact, Takeyama and Fukushima (2013) studied sacha inchi oil oxidation under high-temperature conditions (to some extent comparable to frying) and observed a limited reduction in linolenic and linoleic acids after heating at 180 °C for 10 minutes. In other studies, Cisneros et al. (2014) observed a marginal decrease of linolenic acid and a slight increase (about 1%) of linoleic acid during storage at 60 °C, while Gutiérrez et al. (2019) did not record significant changes in fatty acids composition after a Rancimat accelerated stability test at 80 °C.

In deep-frying, the temperature of the oil is typically 165-190 °C. The heat is transmitted by convection between the oil and the surface of the product, and by conductivity within the product. The water vapour formed within the food migrates outwards, leading to the formation of a porous, dehydrated and lipophilic crust that absorbs part of the oil, acquiring characteristic colour and flavour as a result of Maillard's reaction. Because of all these changes and interactions, the oil goes

through numerous degradation reactions such as hydrolysis, autooxidation and polymerisation, catalysed by high temperature, water presence and airing (Firestone, 2004).

Different oils (canola, soybean, palm, corn, sunflower, safflower, etc.) are widely utilised for deep-frying, each with its specific characteristics of fatty acids profile, stability, taste, etc. Currently in the European Union the limit values for the control of the altered oils used for deep-frying are entrusted to national laws or other acts not having legal force (e.g., recommendations and guidelines); typically, the threshold for TPC lies between 25% and 27% (Firestone, 2004).

The purpose of this work was to analyse the stability of sacha inchi oil by means of a demanding real-life approach, the deep-frying process. To this end, French fries were manufactured using sacha inchi oil obtained by cold pressing; commercial soybean oil was tested as control. The analyses were carried out on oil samples from up to six cycles of four frying batches, each batch being performed at 170 °C for 3.5 min or at 180 °C for 2.5 min.

2. Materials and methods

2.1. Materials

The oil was extracted by cold pressing from sacha inchi seeds collected in the Lamas province, San Martín region, Peru (6°25'0" S, 76°32'0" W) and kindly provided by Amazon Health Products (Lima, Peru). Refined soybean oil and frozen, pre-fried French fries (Cocinero®, Alicorp, Peru) were purchased from a local market in Chimbote, Peru; the presence of an added synthetic antioxidant, tert-butyl-hydroquinone (TBHQ) was stated in the ingredients label of the soybean oil.

2.2. Methods

2.2.1 Frying protocol

The oil/potato ratio employed was 15:1 (1500 mL oil and 100 g potato chips). After heating the oil to the selected temperature with an electric fryer (Oster®, Cheadle, UK), 100 g batches of French fries were fried at 170 °C for 3.5 min or at 180° C for 2.5 min. After one cycle (four

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83 consecutively fried batches), 35 mL oil were sampled and dark-stored at -18 °C under nitrogen

84 atmosphere. The oil in the fryer was restored to the initial volume by adding 35 ± 1 mL and was

85 brought back to temperature. The cycles were repeated six times, for a total frying time of 119 min

86 at 170 °C and of 95 min at 180 °C (Supplementary Figure 1).

87

88 *2.2.2. Analyses*

89 The oil colour was measured in triplicate using a Chroma Meter CR-II Tristimulus colorimeter

90 (Minolta Italia SpA, Milan, Italy), using a standard-white reflector plate and illuminant C. The

91 coordinates scored were *L** (luminosity), *a** (red-green) and *b** (yellow-blue).

92 The fatty acids (FA) composition was determined as fatty acid methyl esters (FAME) by gas

93 chromatography after transesterification of the oils with 2N KOH in methanol, according to IUPAC

94 Standard Method 2.302 (IUPAC, 1987) The chromatographic analysis was performed with a GC-

95 2010 gaschromatograph (Shimadzu, Kyoto, Japan) including a flame ionization detector and an

96 AOC-20Si autosampler (Shimadzu, Kyoto, Japan). The capillary column was a SP®-2560 (100 m ×

97 0.25 mm, df 0.2 μm, Restek, Bellefonte PA, USA). The operative conditions were: carrier He at

98 261.5 kPa and at 30 mL/min; oven temperature 100 °C for 4 min, increased by 3 °C/min to 240 °C,

99 kept at 240 °C for 10 min; injection temperature 225 °C; flame ionization detector temperature 250

100 °C.

101 The tocopherols were determined according to Method 2.432 (IUPAC, 1987). Oil solutions in

102 hexane:isopropyl alcohol (90:10 v/v) at concentrations of 10 mg/mL and 100 mg/mL were filtered

103 through a 0.2 μm PTFE and immediately analysed by NP-HPLC as detailed in Brandolini, Hidalgo,

104 Gabriele, and Heun (2015). The tocols standard curves were built using eleven concentrations

105 (between 0.40 and 109.73 mg/L) of α-tocopherol standard (Fluka, St. Louis, MO, USA), sixteen

106 concentrations (between 0.20 and 23.20 mg/L) of γ-tocopherol standard (Supelco, Bellefonte, PA,

107 USA) and eleven concentrations (between 0.05 and 9.35 mg/L) of δ-tocopherol standard (Supelco,

108 Bellefonte, PA, USA). The calibration curves were linear in the concentration intervals assessed;

109 the detection limits were 0.39, 0.52 and 0.31 mg/L in the standard solution. The total tocopherols

110 were computed as the sum of the different homologues. The results are expressed as mg/kg DM.

111   To measure the antioxidant capacity, the 2-2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic

112 acid) (ABTS) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical cation scavenging capacity

113 tests were performed on hydrophilic (HF) and lipophilic (LF) fractions, recovered as follows:

114 exactly 2 g oil were weighed and 2 mL n-hexane were added, then vortexed until complete

115 dissolution. Subsequently, 2 mL 80:20 methanol-water were added and vortexed. The mixture was

116 centrifuged at 987 g for ten minutes. The methanolic phase (located in the lower part of the

117 centrifuge tube) and the n-hexane phase (found in the upper part of the tube) were recovered. ABTS

118 and DPPH tests were performed as described by Yilmaz, Brandolini and Hidalgo (2015). The

119 results are reported as mg Trolox equivalent (TE)/100 mL.

120   To evaluate the degree of lipid hydrolysis, the free fatty acids (FFA, % oleic acid) were

121 determined according to method Ca 5a-40 (AOCS, 2017). The specific extinction at 232 nm (K_{232})

122 was tested as an index of primary oxidation while the specific extinction at 268 nm (K_{268}) was

123 assessed as an index of secondary oxidation (ISO 3656 method; ISO, 2011).

124   The total polar compounds (PCs) were separated by silica gel column chromatography, as

125 reported in the IUPAC 2.507 method (IUPAC, 1987), and subsequently divided by high pressure

126 molecular exclusion chromatography (HPSEC) to determine the main polar classes: triacylglycerol

127 oligopolymers (TAGP), oxidized triacylglycerols (ox-TAG) and diacylglycerols (DAG). The

128 chromatography system consisted of a Perkin-Elmer series 10 pump; a 7125 S (Rheodyne) injector,

129 a 50 μL loop, a 5 cm × 0.75 cm i.d. and a series of 2 PL-gel columns 30 cm × 0.75 cm i.d. (Perkin-

130 Elmer Ltd., Beaconsfield, U.K.) packaged with PS-DVB (i.e., highly cross-linked styrene

131 divinylbenzene copolymers), with a 5 μm particles diameter and a 0.05 μm pore diameter. A

132 differential deflection refractometer (Perkin-Elmer, Beaconsfield, U.K.) was used as the detector.

133 The mobile phase consisted of tetrahydrofuran (THF, C_4H_8O) for HPLC stabilized with BHT (250

134 mg/L) at the flow rate of 1 mL/min. The procedure used to identify the peaks was described by

6

135 Gomes & Caponio (1999). The precision of the method, expressed as a coefficient of variation

136 (CV%), was 1.4% for polar compounds, 1.2% for triacylglycerol oligopolymers, 1.3% for oxidized

137 triacylglycerols and 1.6% for diacylglycerols.

138 The oxidation stability index (OSI), also known as induction time, was evaluated using a 743

139 Rancimat equipment (Metrohm Co., Herisau, Switzerland) following Cd 12b-92 method (AOCS,

140 2017) on 3.0 ± 0.1 g oil at 100 °C and an air flow of 20 L/h; the results were expressed in hours.

141 The shelf-life at 25 °C was extrapolated from the OSI results.

142 The induction period (IP; the time elapsed before a 10% decrease in the O_2 pressure due to

143 consumption by the sample) was measured with a RapidOxy reactor (Anton Paar, Blankenfelde-

144 Mahlow, Germany) and calculated by the OXISoft™ software integrated in the instrument,

145 according to the Cd 12c-16 method (AOCS, 2017).

146 All tests were performed on two independent samples.

147

148 *2.2.3. Kinetic analysis of lipid degradation*

149 The kinetic of oil degradation during frying was assessed following the evolution of tocopherol

150 content, antioxidant capacity, hydrolysis (FFA, DAG), primary oxidation (K_{232}, ox-TAG), and

151 secondary oxidation (K_{268}, tetramers, trimers, dimers, TAGP). The data were fitted with a zero-

152 order kinetics model:

153 $$C = C_0 + k\,t$$

154 where C_0 is the initial value of each parameter at time 0; C is the value of the parameter after a

155 certain time t at a given temperature; k is the rate constant of the reaction, i.e. the slope of the

156 regression line of the parameter with respect to time.

157

158 *2.5. Statistical analysis*

159 To evaluate the frying effect, the data were processed by two-way analysis of variance

160 (ANOVA) considering as factors the number of frying cycles and the temperature, as well as by

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one-way ANOVA, considering as factor the frying time. Before the ANOVA, the normal data distribution was verified; because of non-normal distribution, the colour coordinate a^* for the sacha inchi oil tested at 170 °C underwent power transformation, while the L^* colour coordinate for soybean oil tested at 180 °C was log-transformed. When significant differences were found, Fisher's least significant difference (LSD; $p \leq 0.05$) was computed. All the calculations as well as the comparison between the slopes of the regression lines were performed with the STATGRAPHICS® Centurion XVI v16.2.04 statistical software (Statpoint Technologies Inc., Warrenton VA, USA), while means and standard deviations were computed with the Microsoft® Office Excel 2016 software (Microsoft, USA).

3. Results and discussion

3.1. Fatty acids composition

Table 1 shows the fatty acids composition of the oils before frying. In fresh sacha inchi oil (SI) the fatty acids were, in decreasing order, α-linolenic (53.8%), linoleic (33.4%), oleic (7.6%), palmitic (3.0%) and stearic (2.2%); these results agree with those reported by Takeyama and Fukushima (2013). Such high percentages of α-linolenic acid are also found, among vegetable oils, in chia (61.8%; Bordón, Meriles, Ribotta, & Martinez, 2019) and linseed (55.9%; Varas Condori et al., 2020). The soybean oil (SO) had a much lower α-linolenic acid content (7.4%), but conversely higher percentages of linoleic (58.4%), oleic (18.6%), palmitic (9.5%) and stearic (4.2%) acids; limited quantities of other fatty acids (linolelaidic, 0.4%; arachidonic, 0.3%; eicosenoic, 0.8%; beenic, 0.3%) were also detected. These results fit into the composition interval reported by O'Brien (2004).

To better evaluate the changes in fatty acid composition during frying, the different unsaturated fatty acids/palmitic acid ratios are presented in Table 1. The one-way ANOVA, performed for every oil at each temperature, showed significant differences during frying. Similarly, the two-way ANOVA, carried out considering temperature and number of frying cycles as factors, showed that

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187   the C18:3/C16:0, C18:2/C16:0 and C18:1/C16:0 ratios decreased significantly as the two factors

188   increased. The decrease in polyunsaturated fatty acids, evident in both oils and more pronounced at

189   higher temperature, was stronger in SI than in SO probably for the lower palmitic acid content. The

190   comparison with literature results is difficult due to the huge number of variables involved in frying

191   tests, such as oil composition heterogeneity (even within species), frying protocol (different

192   preheating, cooking and interval times between tests), product fried (which can release part of its

193   lipids into the oil), frying temperature, etc. Nevertheless, a progressive decrease of polyunsaturated

194   fatty acids during frying has been observed in oils of soybean, canola, palm (olein and stearin),

195   sesame, various mixtures of these (Alireza, Tan, Hamed & Che Man, 2010; Zhang, Saleh & Shen,

196   2016).

197

198   *3.2. Colour*

199   Supplementary Table 1 shows the values of the colour coordinates L^*, a^* and b^*. Before

200   frying, sacha inchi oil showed $L^* = 43.8$, $a^* = -6.4$ and $b^* = 22.3$. These results differ from those

201   reported in the literature, especially in terms of brightness, because Gutiérrez et al. (2019) described

202   L^* ranging from 73.1 to 77.7, a^* from -0.4 to -3.1, and b^* from 10.5 to 42.1. Our soybean oil colour

203   indices (L^*: 41.9, a^*: = -0.9, b^*: 7.1) were also different from those (L^*: 75.0, a^*: -2.4, b^*: 6.4) of

204   Su and White (2004). Sacha inchi and soybean oil did not differ in luminosity, but SI was

205   significantly greener (a^* negative and inferior) and yellower (b^* positive and superior). A visual

206   appraisal of the two oils confirmed the colour differences: the soybean oil was pale, almost

207   colourless, while the sacha inchi oil was a more intense light yellow.

208   The two-way ANOVA showed temperature effect only for L^* in SI, while the number of frying

209   cycles was always significant. For both oils, frying led to a loss of brightness (faster at 180 °C) and,

210   in general, to a red component increase; b^*, instead, did not show a clear trend, apart from a

211   decrease from time 0 in sacha inchi oil and an increase, but only at 180 °C, in soybean oil.

212 Interestingly, the decrease in brightness and the increase of the yellow component in soybean oil

213 were also spotted by Yu, Cho and Hwang (2018).

214

215 *3.3. Tocols*

216   Figure 1 shows the tocopherols content and their variation during frying; no tocotrienols were

217 detected. The α-tocopherol was found only in soybean oil (106.6 mg/kg), while the γ-tocopherol

218 and the δ-tocopherol were present in both species (1643.6 mg/kg and 896.5 mg/kg, respectively, in

219 sacha inchi oil, 739.2 mg/kg and 235.9 mg/kg in soybean oil). Therefore, the total tocols content in

220 SI was 2540.1 mg/kg, i.e. 2.3 times that of SO (1081.7 mg/kg). The sacha inchi oil values were

221 within the variability reported by Chasquibol et al. (2016); additionally, similar values were found

222 by Liu et al. (2018) for γ-tocopherol, and by Fanali et al. (2011) for δ-tocopherol. In the case of

223 soybean oil, the tocopherols content agreed with Castelo-Branco and Torres (2012).

224   A linear decrease in total tocopherols content was observed during frying. After 119 min at 170

225 °C the remaining concentrations were 1101.5 mg/kg in SI and 371.3 mg/kg in SO, with a loss of

226 56.6% and 65.7%, respectively. This reduction was mainly due to the degradation of γ-tocopherol (-

227 72.9% in SI and -70.3% in SO) and δ-tocopherol (-26.9% in SI and -42.5% in SO). In soybean oil,

228 α-tocopherol decreased 84.7% but its initial content was much lower than the other homologues.

229   Tocopherol degradation followed a zero-order kinetics (Figure 1) whose *k* constants are

230 reported in Table 2: the degradation rates of the different tocopherols were γ> δ> α, thus suggesting

231 a superior antioxidant effect of γ-tocopherol. For soybean oil, the higher antioxidant capacity of γ

232 and δ-tocopherol compared to α-tocopherol agrees with Seppanen, Song, and Csallany (2010). At

233 both temperatures the degradation rates of γ-tocopherol and δ-tocopherol were higher in SI than in

234 SO, because the latter is enriched with the TBHQ antioxidant. The comparison of the regression

235 lines evidenced that the tocopherols degradation in sacha inchi oil was almost identical at both

236 temperatures, while in soybean oil the higher temperature led to a more rapid loss (Figure 1).

237

3.4. Antioxidant capacity

Figure 1 also reports the kinetics of antioxidant capacity during frying measured by the ABTS and DPPH tests in the lipophilic and hydrophilic fractions of the oils. In the hexane extracts, the higher antioxidant capacity of SI compared to SO was probably a consequence of the superior tocopherols concentration. On the other hand, the antioxidant capacity of the methanolic extract was low and similar in both oils. The decrease of the antioxidant capacity in the lipophilic extract followed a zero-order kinetics, with similar reaction rates in the ABTS test, while the DPPH rate constant was always slightly higher in SI than in SO (Table 2).

3.5. Hydrolysis and oxidation indices

Figure 2 shows the results of the hydrolysis (FFA) and oxidation (K_{232} and K_{268}) parameters. The free fatty acids, formed by hydrolysis of the triacylglycerols, are rapidly oxidized and promote further oxidation by solubilizing the metal catalysts (Paradiso, Summo, Pasqualone & Caponio, 2018). FFA at time 0 was $0.33 \pm 0.05\%$ in sacha inchi oil and $0.20 \pm 0.03\%$ in soybean oil. According to Ramos-Escudero et al. (2019), the acidity of sacha inchi oil, expressed as % linolenic acid, is very variable, ranging from 0.16 to 1.89% and with a median value of 0.45%. In commercial soybean oil, the free acidity is inferior thanks to refining, with values around 0.09–0.10% (Akil, Castelo-Branco, Magalhães Costa, do Amaral Vendramini, Calado & Guedes Torres, 2015; Naz, Siddiqi, Sheikh & Sayeed, 2005). For the kinetics study, the FFA values at time 0 were not considered because the initial value breaks the linear trend observed during frying. Similarly, Akil et al. (2015) and Naz et al. (2005) found that the increase in acidity was described by a broken line: from time 0 to the end of the first cycle (25–30 min) the acidity tripled, but in the subsequent tests the slope was reduced to one third of the first stretch. For both oils, frying increased the FFA which was 1.12% in SI and 0.65% in SO at the end of the 170 °C trials. The rate formation of FFA was slightly higher in sacha inchi oil (Table 2). The final values reached by SI were below the threshold established by the Codex Alimentarius for cold-pressed virgin oils, i.e. 4.0 mg KOH/g,

corresponding to about 2% free acidity expressed as oleic acid (Codex Alimentarius, 2019), although this restriction is not applicable for frying.

The extinction coefficients K_{232} and K_{268} are indicators of primary and secondary oxidation (conjugated dienes and trienes, respectively). High temperatures lead to isomerization in correspondence of the double bonds and formation of conjugated systems: the dienes are formed during rectification, while the degradation of the linoleic acid oxidation products, such as hydroxyl linoleate, produces the trienes (Marinova, Seizova, Totseva, Panayotova, Marekov & Momchilova, 2012). In fresh sacha inchi oil, K_{232} was 1.92 and K_{268} was 0.17; similar values were found by Ramos-Escudero et al. (2019), i.e. K_{232} was 2.10 (range 1.55–2.49) and K_{268} was 0.15 (range: 0.05–0.22). In soybean oil, K_{232} was 5.74, similar to the result (5.07) of Marinova et al. (2012) but higher than that (2.97) of Giuffrè, Caracciolo, Zappia, Capocasale and Poiana (2018); the K_{268} result (2.48) was between the values (2.17 and 4.29) reported for K_{270} by Giuffrè et al. (2018) and Marinova et al. (2012), respectively. Frying led to a linear increase of the extinction coefficients with increasing times: after 119 min at 170 °C K_{232} and K_{268} reached values of 11.58 and 4.20 in SI, and of 15.50 and 4.86 in SO, respectively. The comparison between the regression lines (Figure 2 and Table 2) showed that the temperature had a significant effect in accelerating the formation of conjugated dienes and trienes. Furthermore, while for K_{232} there were no differences in rate constant, K_{268} growth was sharper in sacha inchi oil. A rapid increase in the extinction coefficients at 232 and 268 nm during frying has been documented by Marinova et al. (2012).

3.6. Polar compounds

Figure 3 shows the content of total polar compounds as well as of each polar class before (point 0) and during frying. DAG content is an intrinsic characteristic of a properly stored product and, together with FFA, is an indicator of hydrolysis. Oxidized triacylglycerols are primary oxidation products which, during prolonged frying, are involved in polymerization and degradation or transformation reactions (Gomes, Caponio & Delcuratolo, 2003)

12

The fresh sacha inchi oil had 1.02% DAG and, because of the cold extraction process, a very low level of triacylglycerol dimers (0.04%) and ox-TAG (0.51%); overall, the total polar compounds were 1.89%. Before frying the commercial soybean oil contained 2.35% DAG, 1.67% ox-TAG and 0.57% TAGP (0.08% triacylglycerol trimers 0.49% triacylglycerol dimers), higher than SI probably because of the refining conditions, in particular of the deodorization. The PCs were 4.79%, similar to the data (4.50% and 5.90%) reported by Juárez, Osawa, Acuña, Sammán, Aparecida and Gonçalves (2011) and Rudzińska, Hassanein, Abdel-Razek, Kmiecik, Siger and Ratusz (2018), respectively. These same authors stated that the initial TAGP are mainly triacylglycerol dimers, while the oligomers are very scarce (about 0.1%). On the other hand, no comparable data are available in literature for sacha inchi oil, a virgin oil never previously used in frying.

The one-way ANOVA showed, overall, a significant effect of frying time on PCs increase. For TAGP and ox-TAG the differences among the frying cycles were all significant, while the DAG increase was less evident. The comparison of the regression lines showed that, except for DAG in sacha inchi oil, the formation rate of the polar compounds at 170 °C was always lower than at 180 °C. For example, at 170 °C the SI TAGP after 119 min (3.75%) was comparable to that observed at 180 °C after 50 min (3.80%). Similarly, ox-TAG in soybean oil after 119 min at 170 °C (5.15%) was almost identical to ox-TAG after 50 min at 180 °C (5.23%). Interestingly, in SO the final PCs values at the two temperatures were not statistically different. The comparison between the regression lines (Figure 3 and Table 2) showed that soybean oil had always higher reaction rate constants (k) than sacha inchi oil.

It must be emphasized that sacha inchi oil suffered significantly less degradation during frying: TAGP and PCs, the most important deterioration indices in frying oils, were 1.5-1.6 times higher in soybean oil than in sacha inchi oil, notwithstanding the presence of TBHQ in the former and the greater unsaturation degree of the latter. Finally, it should be mentioned that the maximum legal PCs value (25%; Firestone, 2004) was never reached in all these trials. In fact, even in soybean oil

316   this limit is normally exceeded only after long (14-15 h) intermittent frying times (Juárez et al.,

317   2011; Rudzińska et al., 2018).

318

319   *3.7. Oxidation stability*

320   The oxidative stability of the oils under accelerated conditions was assessed by Rancimat and

321   RapidOxy; the induction times determined are shown in Figure 4. Before frying, the induction times

322   determined with the Rancimat corresponded to 4.32 h (SI) and 11.02 h (SO), and those with the

323   RapidOxy were 27.6 min (SI) and 40.2 min (SO). The Rancimat SI value was similar to that (4.07

324   h) reported by Varas Condori et al. (2020) for linseed oil under the same analysis conditions, while

325   the SO result was within those (6.00 h and 16.79 h) reported by Ribeiro and Jorge (2017) and

326   Farhoosh (2007), respectively. Higher times (17.6 h) were described for sacha inchi oil by Gutiérrez

327   et al. (2019) for a Rancimat test performed at 80 °C. The high SO stability may be justified by the

328   presence of TBHQ which, even at low concentration (100 ppm), doubles the induction time of

329   soybean oil (Delfanian, Kenari & Sahari, 2016). At increasing frying times, the stability decreased

330   in both oils, following non-linear kinetics; to improve the fitting of the regression function, the

331   quadratic model *Induction time = $\beta_0 + \beta_1\ t + \beta_2\ t^2$* was implemented.

332   Both tests showed a greater stability of soybean oil, but the differences between the induction

333   times of the two oils progressively diminished with increasing frying times. In fact, the term β_1

334   (slope of the function) was progressively greater for soybean oil, in agreement with the slightly

335   higher SO degradation rate observed for the oxidation indices. Additionally, according to Gertz,

336   Klostermann and Kochhar (2000) the accelerated oxidation stability tests are suitable for defining

337   the shelf-life of an oil, but not for predicting its real frying performance, whose conditions are not

338   adequately simulated. During frying, for example, the steam and the stripping of volatile

339   degradation compounds limit the availability of oxygen; this does not happen with the Rancimat

340   test. In fact, as reported by Symoniuk, Ratusz and Krygier (2016) for linseed oil, the Rancimat

341   analysis conditions (high temperatures in air flow) lead to an early polymerization of oils rich in

14

342 polyunsaturated fatty acids (such as sacha inchi), releasing volatile compounds that can anticipate

343 the instrument endpoint.

344

345 **4. Conclusions**

346 Sacha inchi oil was very rich in α-linolenic (ω-3, 53.8%) and linoleic (ω-6, 33.4%) acids,

347 leading to a ω-3/ω-6 ratio of 1.6, while soybean oil had a lower α-linolenic acid content (7.1%),

348 partially compensated by the higher linoleic acid content (58.2%). Furthermore, sacha inchi oil had

349 abundant total tocopherols (2540.1 mg/kg; γ-tocopherol: 64.7% and δ-tocopherol: 35.3%), while

350 soybean oil concentration was far lower (1081.7 mg/kg; γ-tocopherol: 68.3%, δ-tocopherol: 21.8%

351 and α- tocopherol: 9.9%).

352 Frying caused a decrease in tocopherols and antioxidant (ABTS and DPPH) capacity. The

353 degradation rate of tocopherols was highest for γ-tocopherol, followed by δ- tocopherol and α-

354 tocopherol, suggesting a better antioxidant capacity of γ-tocopherol. A superior degradation rate

355 was found in sacha inchi that in soybean oil (k 170 °C = −11.79 mg/kg min vs. −6.53 mg/kg min),

356 probably because the control oil contained TBHQ, a strong antioxidant molecule.

357 During frying the sacha inchi oil had a slightly higher free acidity formation rate than the

358 soybean oil; the diacylglycerols formation rate was greater only at 170 °C. The formation rate of

359 conjugated dienes, conjugated trienes and oxidized triacylglycerols, superior at 180 °C than at 170

360 °C, was similar in both oils, but the polymerization speed was always higher in soybean oil.

361 Overall, sacha inchi oil had higher free acidity but significantly lower oxidation and

362 polymerization indices than soybean oil. In particular, the total polar compounds and

363 oligopolymers, main indicators of frying oil deterioration, were 1.5-1.6 times less than in soybean

364 oil. Hence, the very high tocopherol content of sacha inchi oil contributes to preserve its

365 polyunsaturated fatty acids better than the TBHQ added to soybean oil.

366

367 **Declaration of Competing Interest**

368 The authors declare that they have no known competing financial interests or personal relationships

369 that could have appeared to influence the work reported in this paper.

370

371

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Captions to Figures

Figure 1. Tocopherol content and antioxidant capacity (ABTS and DPPH) of lipophilic (hexane)

and hydrophilic (methanol 80%) extracts of sacha inchi (□, ■) and soybean (○, ●) oils during deep-

frying of French fries at 170 °C (white symbols) and at 180 °C (black symbols).

Figure 2. Evolution of free fatty acids (FFA), K_{232} and K_{268} in sacha inchi (□, ■) and soybean (○, ●)

oils during deep-frying of French fries at 170 °C (white symbols) and at 180 °C (black symbols).

Figure 3. Total polar compounds and single polar classes in sacha inchi (□, ■) and soybean (○, ●)

oils during deep-frying of French fries at 170 °C (white symbols) and at 180 °C (black symbols).

DAG, diacylglycerols; ox-TAG, oxidized triacylglycerols; TAGP, triacylglycerol oligopolymers.

Figure 4. Oxidation stability index (OSI), according to the Rancimat method, and induction period,

according to the RapidOxy method, of sacha inchi (□, ■) and soybean (○, ●) oils during deep-frying

of French fries at 170 °C (white symbols) and at 180 °C (black symbols).

*Declaration of Interest Statement

Conflict of interests

The authors declare no conflict of interests

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Table 1

Table 1. Fatty acid (FA) composition of the oils before deep-frying (%, average ± standard deviation) and ratios between unsaturated fatty acids and palmitic acid before (0) and during deep-frying (#, number of frying batches). Different letters in the same column indicate significant differences between the samples of each oil at 170 °C or at 180 °C, according to the LSD test (p <0.05).

FA composition				Sacha inchi			Soybean	
Palmitic (16:0)				3.0 ± 0.1			9.5 ± 0.0	
Stearic (18:0)				2.2 ± 0.1			4.2 ± 0.0	
Oleic (18:1)				7.6 ± 0.2			18.6 ± 0.0	
Linoleic (18:2)				33.4 ± 0.0			58.4 ± 0.0	
α-linolenic (18:3)				53.8 ± 0.3			7.4 ± 0.0	

FA ratios	#	Time (min)	18:1/16:0	18:2/16:0	18:3/16:0	18:1/16:0	18:2/16:0	18:3/16:0
170 °C	0	0	2.54[a]	11.13[a]	17.94[a]	1.96[a]	6.16[a]	0.78[a]
	4	24	2.43[b]	10.47[b]	16.45[b]	1.97[a]	6.04[b]	0.67[b]
	8	43	2.45[ab]	10.18[c]	15.50[c]	1.95[a]	5.95[c]	0.64[c]
	12	62	2.37[b]	9.66[d]	14.76[d]	1.89[b]	5.54[d]	0.61[d]
	16	81	2.25[c]	8.91[e]	13.59[e]	1.87[b]	5.41[e]	0.58[e]
	20	100	1.86[d]	7.06[f]	10.63[f]	1.79[c]	5.08[f]	0.54[f]
	24	119	2.00[e]	7.35[g]	11.01[g]	1.76[d]	4.89[g]	0.52[g]
180 °C	0	0	2.54[a]	11.13[a]	17.94[a]	1.96[a]	6.16[a]	0.78[a]
	4	20	2.37[b]	9.79[b]	15.46[b]	1.88[b]	5.62[b]	0.62[b]
	8	35	2.08[c]	8.31[c]	13.05[c]	1.97[a]	5.67[b]	0.47[c]
	12	50	2.07[c]	8.24[d]	12.84[d]	1.88[b]	5.17[c]	0.42[d]

Table 2

Table 2. Reaction rate constant (k) for the zero-order kinetics of the parameters analysed during the sacha inchi and soybean oils frying trials at 170 °C and 180 °C.

	Units	Sacha inchi		Soybean	
		170 °C	180 °C	170 °C	180 °C
α-tocopherol	mg/kg min			−0.79	−1.45
γ-tocopherol	mg/kg min	−9.83	−11.53	−4.81	−8.38
δ-tocopherol	mg/kg min	−1.96	−2.58	−0.92	−1.74
Total tocopherols	mg/kg min	−11.79	−14.11	−6.53	−11.57
$ABTS_{Hexane}$	mmol TE/kg min	-0.18	-0.19	-0.13	-0.20
$DPPH_{Hexane}$	mmol TE/kg min	-0.13	-0.16	-0.08	-0.10
Free fatty acids	$\times 10^{-2}$ %/min	0.45	0.46	0.28	0.27
K_{232}	$\times 10^{-2}$ /min	8.17	16.17	7.89	19.22
K_{268}	$\times 10^{-2}$ /min	3.49	4.66	2.08	4.49
DAG	$\times 10^{-2}$ %/min	0.40	0.57	0.23	0.63
ox-TAG	$\times 10^{-2}$ %/min	2.74	5.50	2.95	6.97
PCs	$\times 10^{-2}$ %/min	6.66	14.28	8.04	18.35
Tetramers	$\times 10^{-2}$ %/min	0.12	0.12	0.28	0.56
Trimers	$\times 10^{-2}$ %/min	0.53	1.05	0.92	2.16
Dimers	$\times 10^{-2}$ %/min	2.29	5.99	3.30	7.38
TAGP	$\times 10^{-2}$ %/min	2.94	7.15	4.50	10.10

DAG, diacylglycerols; ox-TAG, oxidized triacylglycerols; PCs, total polar compounds; TAGP, triacylglycerol oligopolymers.

Figure 1
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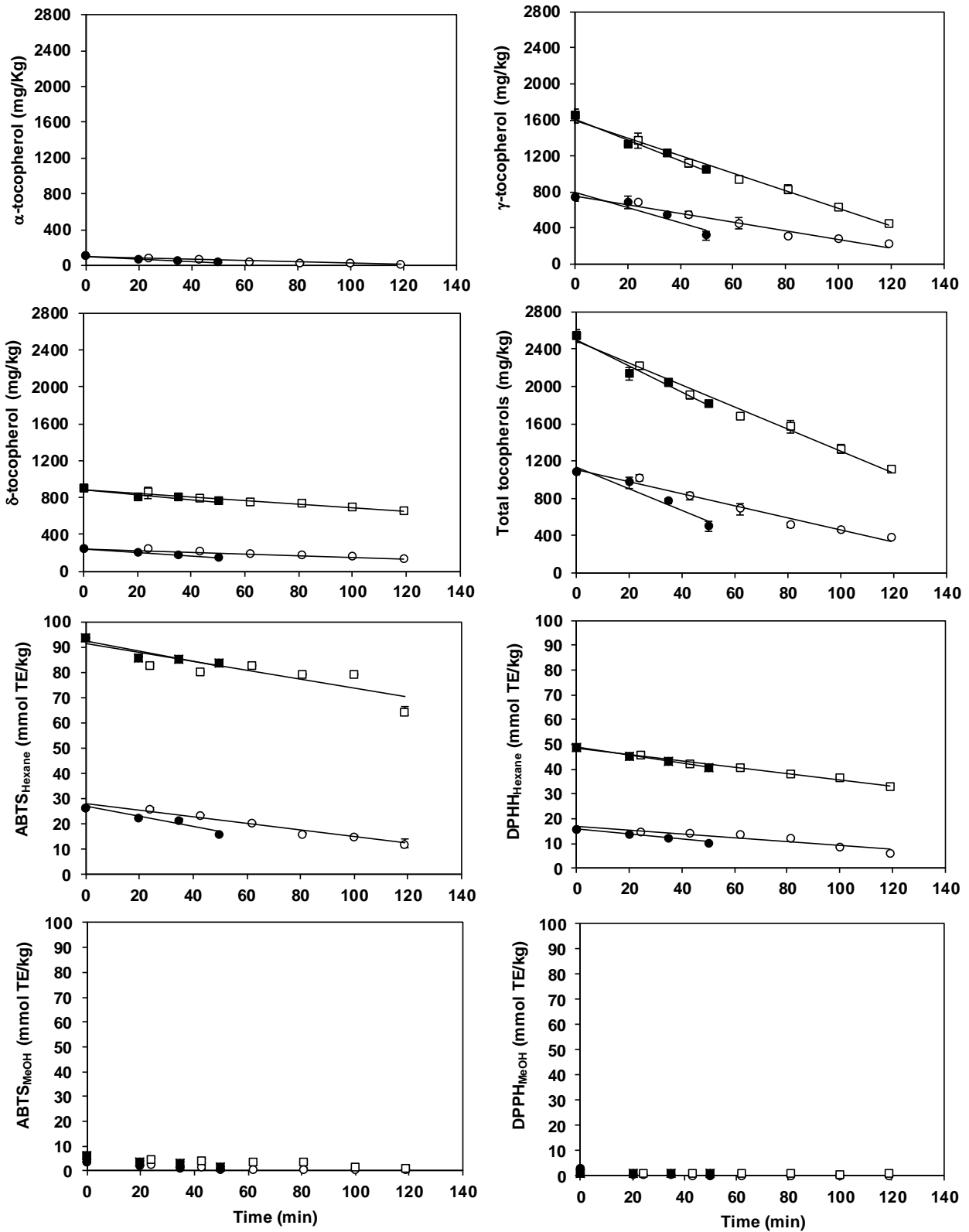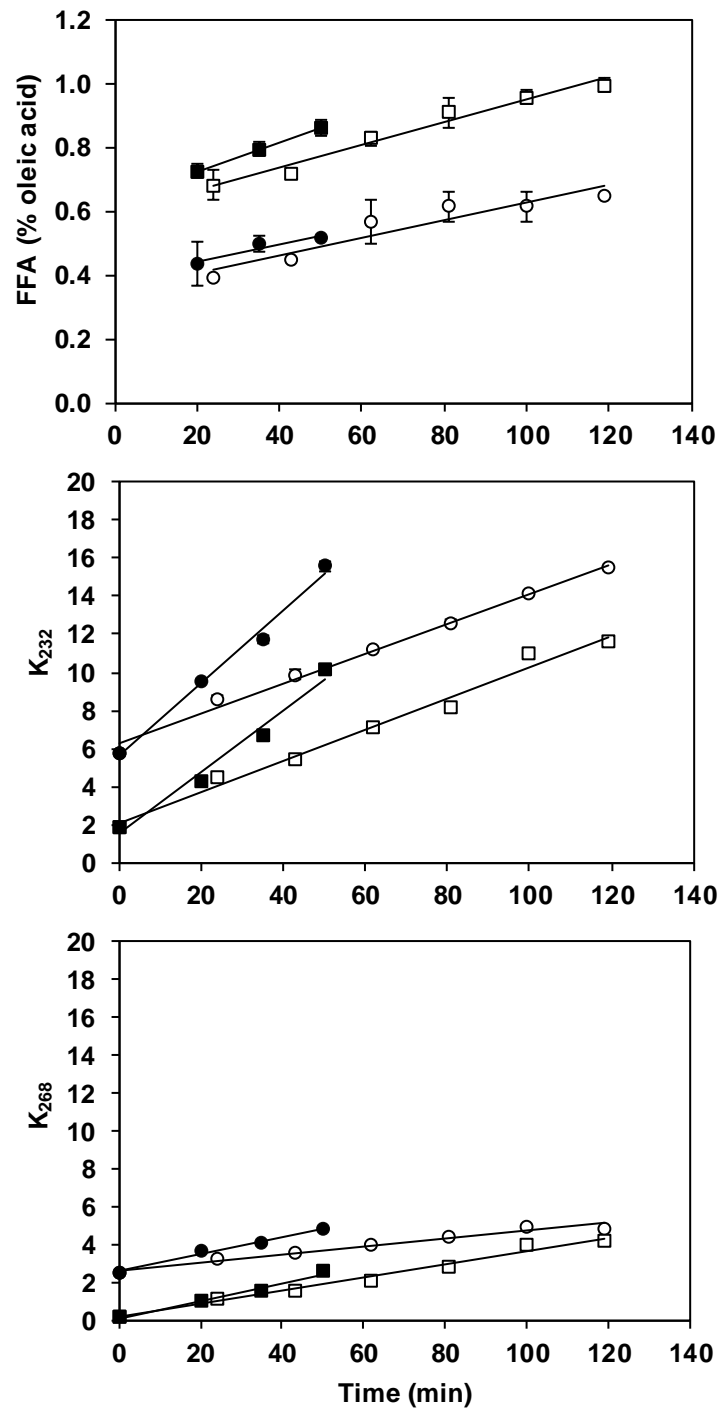
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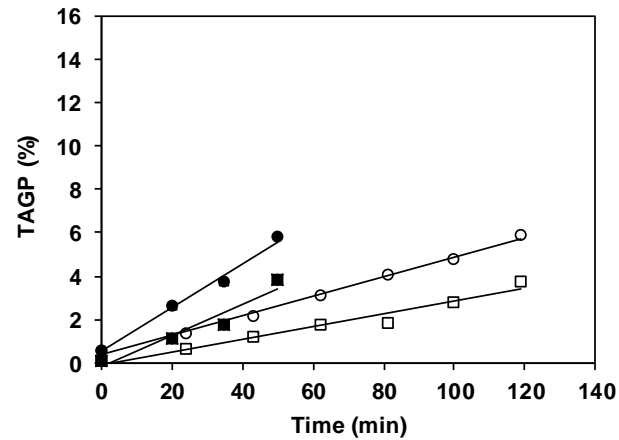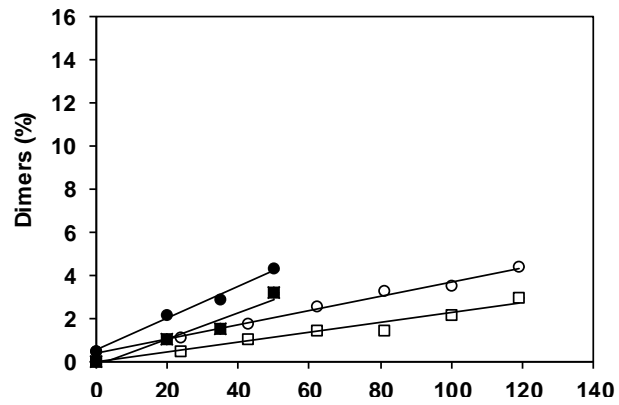
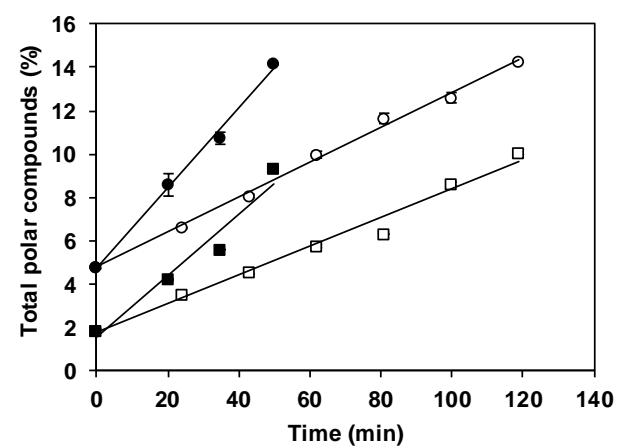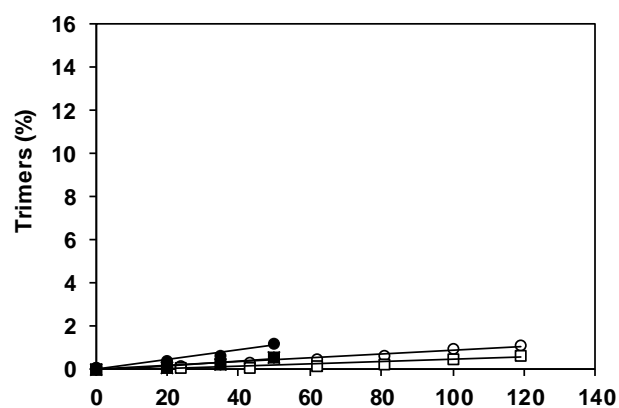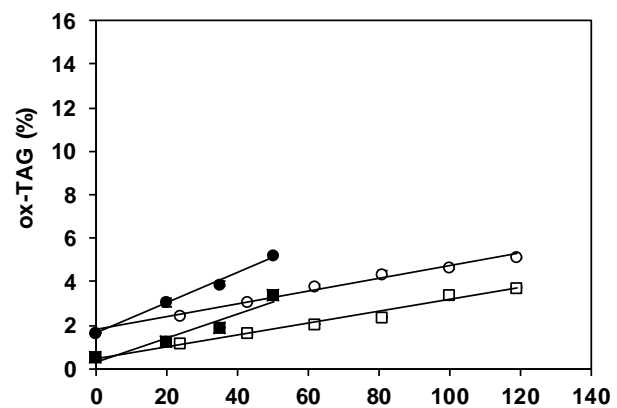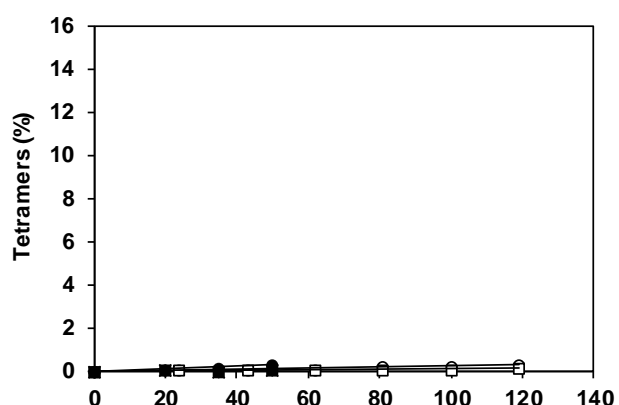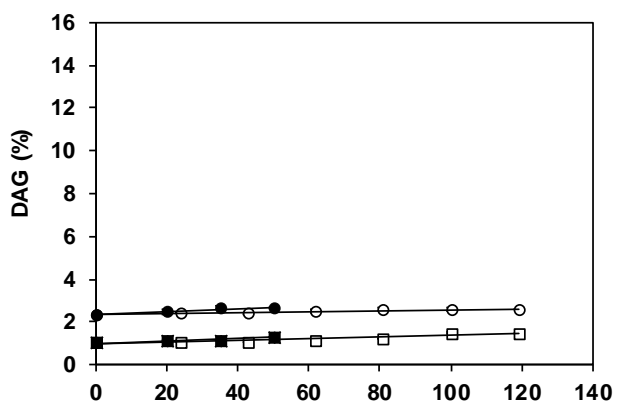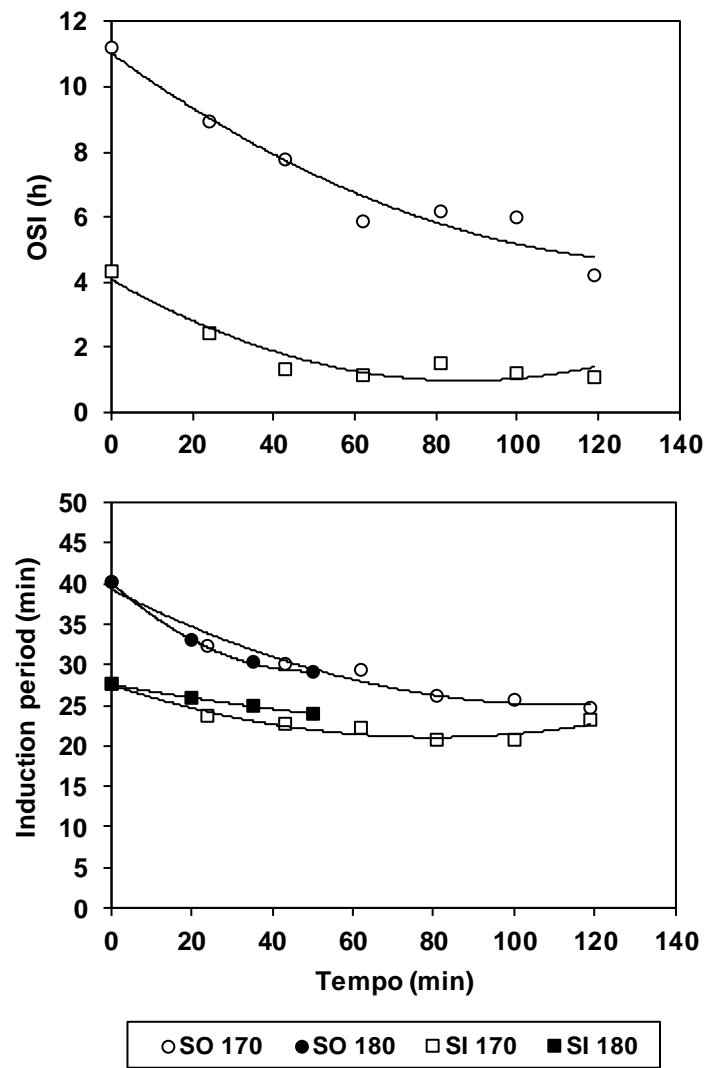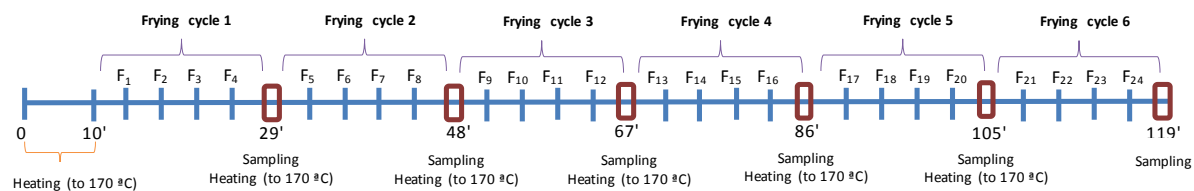**Supplementary Table 1**. Colour coordinates L^*, a^*, b^* (mean ± standard deviation) in sacha inchi and soybean oils before and during deep-frying (#, number of frying batches). Different letters in the same column indicate significant differences between the samples of each oil at 170 °C or at 180 °C, according to the LSD test ($p < 0.05$).

	#	Time (min)	L^*	a^*	b^*
Sacha inchi					
170 °C	0	0	43.8ª ± 0.8	-6.4ª ± 0.6	22.3ª ± 1.2
	4	24	41.5ᵃᵇ ± 1.3	-0.7ᶜ ± 0.6	9.4ᶜᵈ ± 1.4
	8	43	42.5ª ± 1.7	-0.5ᶜ ± 0.7	7.8ᵈ ± 1.2
	12	62	39.5ᵇᶜ ± 2.7	-0.3ᶜᵈ ± 1.4	12.4ᶜ ± 5.3
	16	81	37.6ᶜ ± 2.0	-1.9ᵇ ± 0.3	17.2ᵇ ± 3.2
	20	100	37.2ᶜᵈ ± 1.8	0.7ᵈ ± 0.6	7.5ᵈ ± 1.3
	24	119	34.8ᵈ ± 1.8	2.0ᵉ ± 0.7	6.6ᵈ ± 0.3
180 °C	0	0	43.8ª ± 0.8	-6.4ª ± 0.6	22.3ª ± 1.2
	4	20	42.4ª ± 1.9	-2.4ᵇ ± 1.6	9.6ᵇ ± 3.5
	8	35	37.8ᵇ ± 0.9	-1.8ᵇ ± 0.6	11.3ᵇ ± 0.3
	12	50	37.5ᵇ ± 0.8	0.6ᶜ ± 0.8	10.3ᵇ ± 3.2
Soybean					
170 °C	0	0	41.9ª ± 1.7	-0.9 ± 0.7	7.1 ± 2.9
	4	24	40.4ᵇ ± 1.1	-0.7 ± 1.2	11.1 ± 4.1
	8	43	40.8ᵃᵇ ± 0.6	-0.6 ± 0.9	10.5 ± 5.2
	12	62	37.3ᶜ ± 0.4	0.1 ± 0.5	10.1 ± 2.0
	16	81	38.0ᶜ ± 1.0	0.2 ± 0.8	9.6 ± 2.2
	20	100	37.0ᶜ ± 0.7	0.3 ± 0.6	9.4 ± 2.0
	24	119	36.7ᶜ ± 0.4	0.6 ± 0.2	12.4 ± 0.4
180 °C	0	0	41.9ª ± 1.7	-0.9 ± 0.7	7.1ª ± 2.9
	4	20	39.2ᵇ ± 1.2	-1.0 ± 0.8	10.2ᵃᵇ ± 2.6
	8	35	38.7ᵇ ± 0.8	-0.9 ± 0.5	12.7ᵇᶜ ± 1.4
	12	50	38.2ᵇ ± 0.4	-0.6 ± 0.2	13.9ᶜ ± 0.8

A

B

Supplementary Fig. 1. Experimental design of the deep-frying trials at 170 °C (A) and at 180 °C (B).