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Carolea olive oil enriched with an infusion of *Capsicum annum* and *C. chinense* dried pepper powders to produce an added value flavoured olive oils

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1 **CAROLEA OLIVE OIL ENRICHED AN INFUSION OF *CAPSICUM ANNUUM* AND *C.***
2 ***CHINENSE* DRIED PEPPER POWDERS TO PRODUCE A FLAVOURED OLIVE OIL WITH**
3 **ENHANCED OXIDATIVE STABILITY**

4
5 **Running title**

6 **OLIVE OIL ENRICHED AN INFUSION OF DRIED PEPPER**

7
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29

30 **Abstract**

31 The effects on the quality and oxidative stability of flavoured virgin olive oils (FVOOs) obtained from
32 Carolea extra virgin olive oli (EVOO) was investigated. The oils were prepared by adding dried pepper
33 powder from *Capsicum annuum* L. “Amando”, “Mirasol”, “Topepo rosso” and *C. chinense* Jacq. “Aji
34 limo” and “Red mushroom” cultivars. The total phenol, flavonoid, and carotenoid content was
35 monitored in pepper extracts, EVOO, and FVOOs phenolic fractions as well as their oxidative
36 stability. DPPH, ABTS, and FRAP assays were applied to test the antioxidant activity. Interesting
37 results were obtained from FVOO formulated with Aji limo with IC₅₀ of 18.8 and 27.6 µg/mL in
38 DPPH and ABTS test, respectively. Moreover, this FVOO showed an induction time of 17.40 h
39 compared to 12.30 h for EVOO.

40

41 **Practical applications**

42 Consumers are taking greater responsibility for their own health and they are increasingly turning to
43 their diet to improve it. Virgin olive oil, the main fat of the Mediterranean diet, is per se considered as
44 a functional food—as stated by the European Food Safety Authority (EFSA)—due to its content in
45 healthy compounds. The daily intake of endogenous bioactive phenolics from virgin olive oil is
46 variable due to the influence of multiple agronomic and technological factors. Thus, a good strategy to
47 ensure an optimal intake of polyphenols through habitual diet would be to produce enriched virgin
48 olive oil with well-known bioactive polyphenols.

49

50 **Keywords** *Capsicum*; flavoured olive oil; phenols; carotenoids; capsaicinoids; antioxidant; oxidative
51 stability.

52

53 **1. INTRODUCTION**

54 The EVOO used was obtained from the Carolea cultivar, widely grown for oil production in the south
55 of Italy, including Calabria, together with other cultivars that characterise the biodiversity of this
56 Region (Giuffrè, 2017; Giuffrè, 2018). It gives a medium fruity oil with hints of bitterness. EVOO is
57 known not only for its shelf-life, but also for its pharmaceutical properties and as an aid against some
58 chronic diseases.

59 Chilli pepper (genus *Capsicum*) is a widely-consumed spice worldwide. It contains many
60 phytochemicals with antioxidant properties, including carotenoids, flavonoids, phenols, terpenoids,
61 saponins, stilbenes, and nitrogenous compounds (Wahyuni et al., 2013).

62 The addition of chilli pepper to EVOO has become more popular in recent years, due to consumer
63 demand. The resulting oils, in addition to being flavoured, can also have an extended shelf-life.

64 The addition of spices or other flavourings means the resulting oil no longer satisfies the European
65 Union Commission definition for extra virgin olive oil, but can be defined as a Flavoured Olive Oils
66 (FVOO).

67 The main technique to produce FVOO is infusion. In this case powdered chilli pepper was added to
68 EVOO and left to steep in amber bottles, after which it was filtered.

69 The main objective of this study was to evaluate the effect of 30 days' infusion in Carolea
70 extravirgin olive oil (EVOO) of powdered *C. annuum* L. Mirasol, Amando, and Topepo rosso, *C.*
71 *chinense* Jacq. Aji limo and Red mushroom cultivars.

72 For this purpose: *i*) total phenol, flavonoid, and carotenoid contents were assessed in pepper
73 extracts, EVOOs, and FVOOs; *ii*) capsaicin, dihydrocapsaicin, vitamin C, and vitamin E were
74 quantified in all pepper samples; *iii*) the EVOO fatty acid profile was studied; *iv*) the protective effect
75 of pepper extracts on FVOOs' oxidative stability was investigated; *vi*) the antioxidant potential of
76 pepper extracts, EVOO, and FVOOs' phenolic fraction was investigated by different *in vitro*
77 techniques.

78

79 **2. MATERIALS AND METHODS**

80 **2.1 Chemicals and reagents**

81 Chemicals, reagents and solvents were purchased from Sigma-Aldrich S.p.a. (Milan, Italy).

82

83 **2.2 Plant materials, EVOO and FVOOs formulation**

84 The olive fruits of *Olea europea* Carolea cultivar were collected in Calabria (Italy) during the
85 2018/2019 season. Carolea EVOO was obtained using continuous mills at the “Meringolo” olive oil
86 mill (Corigliano Calabro, Cosenza, Italy). The EVOO sample received UNI10939, 2001 certification.

87 *Capsicum* fruits were obtained from the “Miceli” farm. (39°48'21 N, 15°47'46 E) (Scalea,
88 Calabria, Italy). Table S1 reports their main characteristics. *Capsicum* fruits were collected at complete
89 maturation and dried in the sun for 2 weeks. Subsequently, the dry product was powdered and 50 mg
90 was added to 5 g of Carolea EVOO and stirred to obtain the corresponding FVOO. FVOO were stored
91 for 30 days in amber bottles at -20 °C until analysis.

92

93 **2.3 Extraction procedure**

94 *Capsicum* fruits (250 g) were subjected to maceration using ethanol (350 mL) as a solvent (3 × 72 h).
95 The extracts were combined and stored -20 °C until analysis.

96

97 **2.4 Determination of phytochemical content**

98 The total phenol content (TPC) and the total carotenoid content (TCC) were determined following the
99 procedure previously reported (Gao et al., 2000). Chlorogenic acid equivalents (CAE)/100 g of fresh
100 weight (FW) and mg β -carotene equivalents (β CE)/100 g FW were used to express results on TPC and
101 TCC, respectively. For the total flavonoid content (TFC) the protocol of Yoo et al. (2008) was applied.
102 mg Quercetin equivalents (QE)/g FW were used to express the obtained results.

103 Gas chromatography (GC) (Shimadzu GC17A, Shimadzu, Milan, Italy) equipped with a flame
104 ionisation detector (FID) was used for capsaicin and dihydrocapsaicin determination in $\mu\text{g/g}$ FW
105 (Menichini et al., 2009).

106

107 **2.5 Vitamin C and E content**

108 The pepper's vitamin C content was determined according to the method of Klein and Perry, (1982)
109 and expressed as mg/100 g DW. Gas chromatography-mass spectrometry (GC-MS) analysis (Agilent,
110 Milan, Italy) was used for vitamin E quantification in mg/100 g DW.

111 EVOO quality parameters (acidity, peroxide index, and UV light absorption) were determined
112 according to the procedures described by EC Regulation (EUC, 2013). The oxidative stability index as
113 defined in AOCS Official methods (1993) was investigated by using Rancimat apparatus (Metrohm,
114 Basel, Switzerland). The curve inflection point was defined by induction time and expressed in hours
115 (Karakuş et al., 2017).

116

117 **2.6 Extraction of phenolic fraction**

118 The EVOO phenolic fraction was obtained following the procedure of Montedoro et al. (1982) using
119 hydroalcoholic solution (7:3 v/v) and then *n*-hexane. After centrifugation, the residue was taken up
120 with hydroalcoholic solution (1:1 v/v) and stored at -20°C until analysis.

121

122 **2.7 Determination of TPC, TFC, and TCC content in Carolea phenolic fraction**

123 The procedure for spectrophotometric determination of TPC and TFC was the same as that applied to
124 the pepper extract (see paragraph 2.4). In EVOO the TCC was determined as described by Minguez-
125 Mosquera et al. (1991). Concisely, EVOO (5 mL) was mixed with *n*-hexane (1:1, v/v). Results are
126 expressed as ppm.

127

128 **2.8 Fatty acid analysis**

129 Carolea EVOO fatty acids were determined by GC-MS analyses (Agilent, Milan, Italy) following the
130 procedure previously reported (Leporini et al., 2018).

131

132 **2.9 Antioxidant activity of pepper extracts and EVOO phenolic fraction**

133 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl
134 (DPPH) radical scavenging assays were applied to examine the radical scavenging activity of
135 *Capsicum* extracts and EVOO phenolic fraction using the procedure previously described by Loizzo et
136 al.^[14] In both cases ascorbic acid was used as a positive control.

137 Moreover, both pepper extracts and EVOO phenolic fraction (at concentration of 2.5 mg/mL)
138 were tested, also, to evaluate the ability of samples to protect iron from redox reaction (Loizzo et al.,
139 2016). Butylated hydroxytoluene (BHT) was used as control.

140

141 **2.10 Statistical analysis**

142 Data are expressed as mean \pm standard deviation (S.D.). Prism GraphPad Prism version 4.0 for
143 Windows (GraphPad Software, San Diego, CA, USA) was used to calculate IC₅₀ value and to perform
144 ANOVA test followed by a multicomparison Dunnett's test ($\alpha= 0.05$). *Pearson's correlation*
145 *coefficient* (*r*) and Tukey's multiple range test were also done. Principal component analysis (PCA)
146 was applied by SPSS software for Windows, version 15.0 (Chicago, IL, USA).

147

148 **3. RESULTS AND DISCUSSION**

149 **3.1 Phytochemical content of peppers**

150 Extraction yields in the range of 6.2-6.5% were obtained for *C. annuum* Aji limo, Topepo rosso, and
151 Amando. Highest yields were found for Mirasol and Red mushroom (8.2 and 7.1%, respectively).

152 Extracts were analysed in order to evaluate their TPC, TFC, TCC, and capsaicinoid content (Table 1).

153 *C. chinense* Red mushroom exhibited the highest TPC value of 623.6 mg CAE/100 g FW, followed by
154 Topepo rosso and Aji limo. Aji limo pepper was characterized by the highest TFC (64.5 mg QE/ 100 g
155 FW), followed by Amando pepper (54.5 mg QE/ 100 g FW).

156 Carotenoids are responsible for *Capsicum* colour. Except for Aji limo pepper (98.1 mg β CCE/100
157 g FW), the carotenoid content was in the range 227.4-328.1 β CCE/100 g FW.

158 The most well-known phytochemicals of *Capsicum* are capsaicinoids (capsaicin and
159 dihydrocapsaicin) that are responsible for *Capsicum* pungency (Estrada et al., 1998). *C. chinense* Red
160 mushroom showed the highest capsaicin content with value of 2504.4 μ g/g FW followed by *C.*
161 *chinense* Aji limo pepper (2234.5 μ g/g FW). The lowest capsaicin content was found in *C. annuum*
162 (410.2-510.2 μ g/g FW). The same trend was observed for dihydrocapsaicin. *C. chinense* peppers are
163 characterized by the highest vitamin C and E content with values of 5.6 and 6.0 mg/g FW for Red
164 mushroom, and 5.9 and 6.3 mg/g FW Aji limo, respectively.

165 Our data on *C. chinense* Red mushroom agree with those reported for *C. chinense* Habanero
166 (Menichini et al., 2009). Several varieties of *C. annuum* have been investigated. Among them, in
167 agreement with our obtained data on *C. annuum* species, are values of *C. annuum* var. *acuminatum*
168 with TPC 970.2 mg CAE/100 g FW, TFC 56.0 mg QE/100 g FW TCC of 324.2 β CCE/100 g FW,
169 respectively (Tundis et al., 2016). Significantly lower TFC (5.6 mg QE/100 g FW) and TCC (133.9
170 β CCE/100 g FW) values were found in *C. annuum* var. *cerasiferum*. In another work, Tundis et al.
171 (2016) investigated the evolution of phytochemical content during ripening of *C. annuum* cv Cayenne
172 Golden, *acuminatum*, Orange Thai and Fiesta. When mature, TPC ranged from 648.6 to 679.6 mg
173 CAE/100 g FW. *C. annuum* cayenne golden showed the highest TPC value. TFC values ranged from
174 34.9 to 61.5 mg CAE/100 g FW. *C. annuum* *acuminatum* showed the highest TCC (414.1 β CCE/100 g
175 FW). *C. annuum* Orange Thai, *acuminatum*, and Fiesta are spicier than *C. annuum* Mirasol, Amando,
176 and Topepo rosso with capsaicin content about 3-times higher.

177

178 **3.2 EVOO quality parameters and chemical profile**

179 The analysis of Carolea EVOO quality parameters showed a free acidity value of 0.37%, a peroxide
180 level of 7.98 meq O₂/kg of oil, and a ΔK value of 0.0024. A free acidity of 0.47%, and a peroxide level
181 of 6.91 meq O₂/kg of oil were found for Carolea EVOO by Piscopo et al. (2016) whereas a mean value
182 of 0.3 g oleic acid/100 g oil was previously, recorded for Frantoio EVOO by Leporini et al. (2018).
183 Our values are in agreement with those reported by Lavelli et al. (2005) for EVOO obtained by
184 Pendolino, Leccino, Moraiolo, and Taggiasca cultivars.

185 The quantity of phenols in EVOO is not only responsible for the perception of pungency but
186 above all for EVOO resistance to the normal oxidative process. Carolea EVOO showed a TPC of
187 851.3 ppm, this value is 2-times higher than that found by Piscopo et al. (2016) for the same cultivar
188 (317.44 ppm) (Table 2). Values in the range from 286.73 to 305.65 ppm were found for Ottobratica
189 and Sinopolese EVOO, respectively (Loizzo et al., 2016). Previously, Leporini et al. (2018) showed
190 that TPC varied significantly in EVOO from Frantoio cultivars from different areas of Calabria.
191 However, our data are in agreement with those found for Campania's Frantoio EVOO (Lavelli et al.,
192 2005). A higher TPC value was recorded for Bosana EVOO from Sardinia (Italy) (Del Caro et al.,
193 2006). A TFC value of 28.5 ppm was found for Carolea EVOO phenolic fraction (Table 3). An
194 increase in all phytochemical contents was observed in all FVOOs. In particular, FVOO enriched with
195 Aji limo pepper showed the highest TPC, TFC and TCC with values of 912.1 mg CAE/100 g FW, 42.6
196 mg QE/100 g FW, 33.1 mg βCE/100 g FW, respectively.

197 The first qualitative parameter observed by consumers is colour, hence the attention to the
198 EVOO pigment content (Loizzo et al., 2009; Loizzo et al., 2012). Among them carotenoids occupy an
199 important role since they are strong protectors against light induced EVOO oxidation. Previously,
200 Šarolić et al. (2015) investigated Croatian EVOO carotenoid content and found values ranging from
201 3.86 to 4.75 ppm. Lower values were recorded by Zegane et al. (2015) in Algerian EVOO (0.67-1.70
202 mg/kg). The TPC, TFC and TCC content was monitored also in FVOOs (Table 3). As it is possible to

203 see, all phytochemical contents are higher in FVOO compared to EVOO. Red mushroom FVOO
204 showed the highest TPC with a value of 912.1 ppm followed by Topepo rosso FVOO (905.6 ppm).
205 The following trend was observed for TFC content in FVOOs Aji limo > Topepo rosso > Red
206 mushroom > Amando > Mirasol. With regard to the TCC, it could be observed that oils flavoured with
207 Red mushroom and Aji limo peppers are characterized by the highest content in carotenoids with
208 values of 28.8 and 25.1 ppm.

209 Carolea EVOO possessed a high content of oleic acid (Table 3). Among saturated fatty acids
210 (SFA) C16:0 was detected in a significant amount with a value of 13.7% while C18:2 with a
211 percentage of 6.5% was the most abundant polyunsaturated fatty acid. A high oleic/linoleic *ratio* of
212 11.4 was found for Carolea EVOO, which indicates the high stability of the EVOO (Zegane et al.,
213 2015).

214 Leporini et al. (2018) previously recorded values ranging from 9.0 to 12.2% for Frantoio
215 EVOOs. Our data are in line with Sicilian EVOO (Biancolilla, Cerasuola, Nocellara Etnea, Nocellara
216 del Belice, and Moresca) (Patumi et al., 2003). More recently, Blasi et al. (2019) reported the fatty acid
217 composition of Frantoio, Dolce Agogia, Leccino, and Moraiolo. Oleic acid was the most abundant
218 with percentages from 76.2 to 78% for Leccino and Dolce Agogia, respectively followed by palmitic
219 acid. Linoleic acid was identified in the range of 6.0 to 7.1% for Dolce Agogia and Frantoio,
220 respectively.

221

222 **3.3 Antioxidant activity**

223 The antioxidant potential of Carolea EVOO and FVOO phenolic extract as well as pepper extracts
224 was reported in Table 4. Carolea EVOO exhibited a good radical scavenging potential with IC₅₀ of
225 26.6 and 33.5 µg/mL for DPPH and ABTS test, respectively. Previously, Baiano et al. (2009)
226 investigated the evolution of Italian EVOO antioxidant activity during 12 months' storage. The
227 TPC of the investigated Italian EVOO is strictly dependent on the cultivar, area, and time of fruit

228 collection. The analysis of pepper extracts showed that *C. chinense* Aji limo had the highest
229 antioxidant potential with IC₅₀ of 11.8 and 18.2 µg/mL, in DPPH and ABTS tests, respectively. A
230 promising FRAP value was also observed (78.8 µM Fe(II)/g). A notable antioxidant potential was
231 observed with Topepo rosso pepper, which showed IC₅₀ of 18.9 and 28.4 µg/mL in DPPH and
232 ABTS tests, respectively. This test was positively correlated with TPC ($r = 0.68$) and TCC (r
233 $= 0.92$). A positive correlation was observed also for the TCC and DPPH assay with r value of
234 0.77. The antioxidant activity of fresh and processed *C. annuum* and *C. chinense* peppers was
235 investigated by Loizzo et al. (2015). Samples characterized by the highest antioxidant activity are
236 also richest in TPC and capsaicinoids. The promising *C. annuum* antioxidant potential was
237 confirmed also by the investigation of Loizzo et al. (2017) who demonstrated how both Pellegrino
238 and Idealino dried pepper samples exhibited ABTS radical scavenging activity with IC₅₀ of 45.2
239 and 45.7 µg/mL, respectively.

240 The highest radical scavenging activity was recorded with FVOO enriched with Aji limo
241 pepper that showed IC₅₀ values of 18.8 and 27.6 µg/mL for DPPH and ABTS test respectively,
242 followed by FVOO enriched with Red mushroom (IC₅₀ values of 19.3 and 28.9 µg/mL for DPPH
243 and ABTS test, respectively). The same trend was observed, also in reduction of iron with FRAP
244 values in the range 129.8-139.5 for FVOO with Topepo rosso and Red mushroom, respectively.
245 Correlation analysis on EVOO and FVOOs phytochemical content and bioactivity showed that
246 TCC is positively correlated with both ABTS and FRAP test with r value of 0.75. From the
247 analysis of the literature on antioxidant activity of FVOOs a controversial data emerges. This may
248 depend on the matrix used for enrichment (spices, herbs, fruits) and on the different techniques
249 used for obtaining it (infusion or co-processing) (Reboredo-Rodríguez et al., 2017).

250 According to Caporaso et al. (2013) the radical scavenging potential of a mixture of virgin
251 olive oil and refined olive oil enriched with hot *C. annuum* from Campania (Italy) (20% w/w)
252 showed a greater ABTS radical scavenging activity than the starting olive oil even after only 7

253 days of infusion. Moreover, the antioxidant activity was correlated to the capsaicinoids and
254 carotenoids released by the pepper during infusion time.

255 256 **3.4 Effect of the addition of peppers on FVOOs oxidative stability**

257 The effect of the addition of different peppers on FVOOs was investigated by simulating oxidation
258 process using Rancimat apparatus. Carolea EVOO had an induction time of 12.3 h. Generally, all
259 FVOOs are characterized by a higher induction time even if there is a difference depending on the
260 pepper cultivar added to the oil. In particular, the addition of *C. chinense* Aji limo and Red
261 mushroom cultivars peppers to oil resulted in FVOOs with induction times of 17.4 and 15.2 h,
262 respectively. A lesser effect on FVOO's oxidative stability was observed with the addition of *C.*
263 *annuum* peppers that prolonged the induction time to 14.9, 14.2 and 14.0 h for Mirasol, Amando
264 and Topepo rosso, respectively (Figure 1).

265 The result of the quotient of the induction time of FVOO and EVOO, namely protection
266 factor (PF), was used as an index of oxidative stability. By using this parameter, the protective
267 activity was demonstrated for all applied pepper extracts with PF values in the range 1.4-1.1 for
268 Aji limo and Topepo rosso or Amando, respectively.

269 *Pearson's correlation coefficient* showed that all quantified phytochemicals in FVOOs (TPC,
270 TFC, TCC, vitamins and capsaicinoids) are positively correlated with the oxidative stability
271 measured as induction time. In particular, FVOOs enriched with peppers characterized by high
272 vitamin C and E are more resistant to oxidation (r values of 0.87 and 0.82 for induction time and
273 vitamin C and E content, respectively).

274 Piscopo et al. (2016) investigated the resistance to oxidation of Calabrian EVOO and found
275 the following order: Carolea > Ottobratica > Sinopolese > Grossa di Gerace. The literature on the
276 effect of the addition of herbs and spices on FVOO oxidative stability is controversial. Previously,
277 Caporaso et al. (2013) showed that the addition of 10-20% w/w of dried *C. annum* to EVOO

278 determined a reduction of olive oil oxidative stability over a 30-day period. Conversely,
279 Gambacorta et al. (2007) reported an increased oxidative stability in FVOO over 30 days when hot
280 pepper (10-20%) was added by infusion using Dauno EVOO.

281

282 **3.5 PCA analysis**

283 Principal Component Analysis (D'Agostino et al., 2014). was applied to oils flavoured by an infusion
284 of *Capsicum annuum* and dried *C. chinense* peppers. By choosing eigenvalues greater than one (>1),
285 the dimensionality was reduced from 11 variables to two principal components (PC). PCA results
286 revealed that the first two principal components explained 92.77 % of total variance. The loadings of
287 first and second principal components (PC1 and PC2) accounted for 59.20 and 33.57 % of the
288 variance, respectively (Fig. 2).

289 Figures 2 and Table S2 illustrate the strong correlation that exists between the analyzed variables.

290 The first component (PC1) is highly positively correlated with FRAP, TPC and OSI; while the
291 second component (PC2) is positively correlated with DPPH and ABTS. Total flavonoids (TFC) show
292 negative correlation for PC1 and PC2. The bi-dimensional PCA analysis clearly classifies the
293 similarities or differences of the enriched extra virgin olive oil. The scores plot analysis clearly
294 classifies the enriched extra virgin olive oils in the upper region of the PCA score plot. The analysis
295 demonstrated that among the enriched oils analyzed they were located in the top right quadrant, which
296 represents the highest FRAP, DPPH, TPC and OSI.

297 Infusion of *Capsicum annuum* and *C. chinense* dried peppers in EVOO enriched the oil
298 antioxidant compounds and significantly influenced the chemical composition of these new products
299 (FVOO).

300 PCA confirmed that all the enriched oils analyzed possess the highest bioactive capacity. Thus, the
301 present results provided the basic data for choosing extra virgin olive oils with higher antioxidant
302 activity for direct consumption.

303

304 **4. CONCLUSIONS**

305 In recent years, flavoured oil has gained attention to not only flavour meat, fish or salad but also for
306 the potential health benefits of the phytochemicals contained in herbs and spices. In this context, we
307 decided to test the effects of the addition by infusion of different pepper cultivars of *C. annuum* and *C.*
308 *chinense* to Carolea EVOO. All pepper extracts are rich in bioactive compounds. Several quantified
309 phytochemicals (capsaicin, vitamins C, E TPC and TCC) exert a protective action on the oxidative
310 process, which the oil spontaneously undergoes.

311 Among peppers tested in the EVOO infusion, *C. chinense* Aji limo and Red mushroom cultivars are
312 the most active. Based on the obtained results FVOOs could be proposed as functional oils
313 characterized by high stability and health properties due their antioxidant potential.

314

315 **Conflicts of Interest**

316 The authors declare no conflict of interest.

317

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420 **TABLE 1. GAS PERCENTAGE OF ORANGES PACKED IN FILM**

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Storage Conditions	Sample Typology	Time (Days)	O ₂ (%)	CO ₂ (%)
		0	17.27±1.3a	3.54±1.04d
6°C and 80% U.R.	Orange fruits wrapped in Nanoactive film	15	15.16±4.97c	7.33±4.71b
		30	14.8±5.22b	8.86±4.61c
		45	12.7±3.66d	9.10±3.79a

	Sig.	**	**
	0	19.30±0.42c	1.84±0.45b
6°C and 80% U.R. Orange fruits wrapped in BOPP film	15	20.18±0.28a	0.97±0.49d
	30	19.67±0.32b	1.59±0.59c
	45	18.97±0.25d	2.33±0.38a
	Sig.	**	**

422 Significance at P<0.05. Means in each column with the same letter do not differ
423 significantly.

424 U.R. = relative humidity

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442 **TABLE 2.** CHANGES IN THE CONTENT OF PH, TOTAL SOLUBLE SOLIDS AND TOTAL
443 ACIDITY OF THE JUICE FROM ORANGES WRAPPED IN BOPP FILM, NANOACTIVE
444 FILM AND NON-WRAPPED

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Analysis	Time (Days)	Control	N.A. Film	BOPP Film	Sig.
Total Soluble Solids (°Brix)	0	13.10±0.10b	13.10±0.10b	13.10±0.10a	
	15	13.30±0.10a	13.40±0.06a	12.70±0.20b	**

	30	13.80±0.10a	13.00±0.06b	13.00±0.10a	*
	45	13.30±0.00a	12.97±0.58b	13.10±0.06a	**
Total Acidity (g/L Citric Acid)	0	11.38±0.14b	11.38±0.14a	11.38±0.14a	
	15	10.09±0.02d	9.31±0.12b	10.91±0.83a	**
	30	11.63±0.02a	9.57±0.19c	9.12±0.19b	**
	45	10.58±0.01c	9.61±0.13d	9.29±0.17b	**
pH	0	3.27±0.04d	3.27±0.04c	3.27±0.04c	
	15	3.39±0.01c	3.54±0.03ab	3.42±0.01b	**
	30	3.45±0.02b	3.48±0.06b	3.49±0.03a	*
	45	3.61±0.02a	3.57±0.01a	3.48±0.01a	**

446 Significance at P<0.05. Means in each column with the same letter do not differ significantly

447 Sig. - Film, Time, Film*Time

448 N.A.- nanoactive

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467 **TABLE 3. CHANGES IN THE CONTENT OF TOTAL FLAVONOIDS, POLYPHENOLS AND ASCORBIC**
468 **ACID OF THE JUICE FROM ORANGES WRAPPED IN BOPP FILM, NANOACTIVE FILM AND**
469 **NON-WRAPPED**

470

Analysis	Time (Days)	Control	N.A. Film	BOPP Film	Sig.
Total Flavonoids (mg/L hesperidin)	0	1209±12.78b	1209±12.78a	1209±12.78c	
	15	987±26,02d	1086±30,05b	1171±23,94c	**
	30	1126±51,11c	1082±57,44b	1285±69,65b	**
	45	1248±59,79a	1179±63.91a	1599±11,84a	**
Total Polyphenols (mg/L gallic acid)	0	5229±151.71a	5229±151.71a	5229±151.71a	
	15	5272±132.12b	5685±152.58b	5396±218.23b	**
	30	5140±110.82c	5284±115.13a	5231±101.89a	**
	45	4501±100.10d	4926±79.54c	4917±114.12b	**
Ascorbic Acid (mg/L)	0	606.65±4.75a	606.65±4.75a	606.65±4.75a	
	15	493.20±4.12c	538.08±1.59b	581.17±1.66b	**
	30	505.83±5.20b	536.19±8.39b	548.99±19.21c	**
	45	498.06±0.00c	550.40±4.14c	501.43±12.43bc	**

471 Significance at P<0.05. Means in each column with the same letter do not differ significantly

472 Sig. - Film, Time, Film*Time

473 N.A. - nanoactive

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487 **TABLE 4.** CHANGES IN THE CONTENT OF HESPERIDIN AND NARIRUTIN OF THE JUICE FROM
488 ORANGES WRAPPED IN BOPP FILM, NANOACTIVE FILM AND NON-WRAPPED
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Analysis	Time (Days)	Control	N.A. Film	BOPP Film	Sig.
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Hesperidin (mg/Kg hesperidin)	0	265.21±8.36a	265.14±11.03d	265.11±2.45c	
	15	171.02±6.31b	271.12±10.96b	222.28±3.41a	**
	30	99.33±1.54c	229.11±8.46a	221.31±3.58d	**
	45	58.35±2.64d	272.08±4.31c	164.09±4.62b	**
Narirutin (mg/Kg hesperidin)	0	174.45±4.55a	174.45±7.74c	174.45±4.85a	
	15	37.23±1.15d	185.22±5.60a	112.20±8.36b	**
	30	40.07±1.89b	173.14±11.11c	86.23±1.88d	**
	45	34.89±1.06c	206.21±5.29b	89.07±2.48c	**

Significance at P<0.05. Means in each column with the same letter do not differ significantly

Sig. - Film, Time, Film*Time

N.A. - nanoactive

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TABLE 5. ANTIOXIDANT ACTIVITY OF THE JUICE FROM ORANGES WRAPPED IN BOPP FILM, NANOACTIVE FILM AND NON-WRAPPED

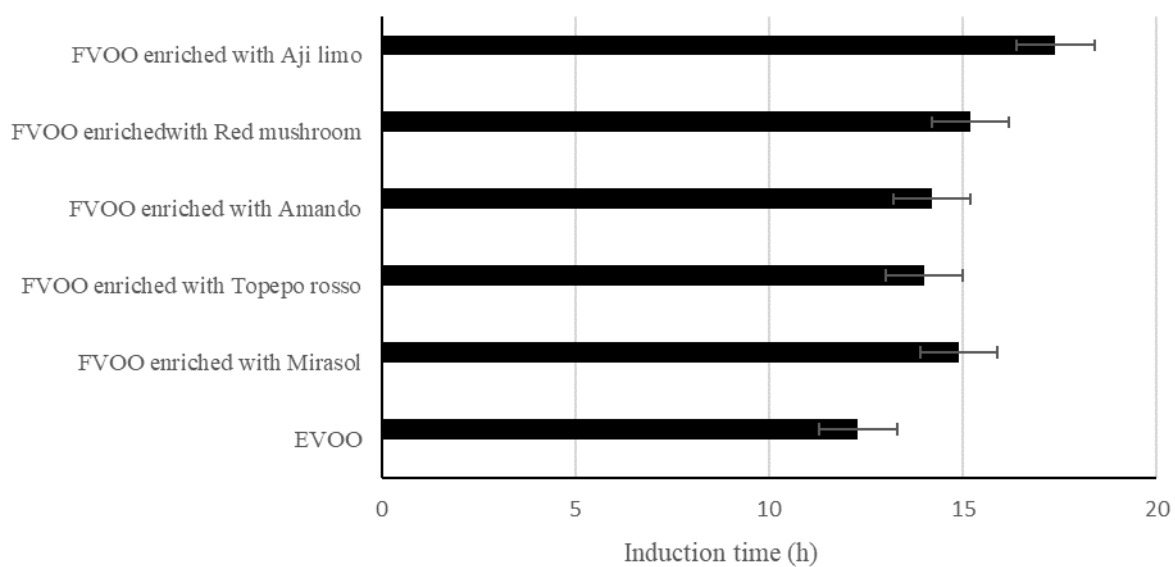
Analysis	Time (Days)	Control	N.A. Film	BOPP Film	Sig.
	0	2.54±0.20a	2.54±0.20a	2.54±0.20a	
Antioxidant Capacity DPPH (-OD ⁻³ min ⁻¹ g dm ⁻¹)	15	1.64±0.62b	1.58±0.51d	1.68±0.25d	**
	30	2.22±0.25a	1.76±0.47b	1.72±0.57c	**
	45	1.70±0.26b	2.73±0.56c	2.38±0.17b	**

535 Significance at P<0.05. Means in each column with the same letter do not differ significantly

536 Sig. - Film, Time, Film*Time

537 N.A. - nanoactive

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FIGURE 1 Induction time of Carolea EVOO and FVOO

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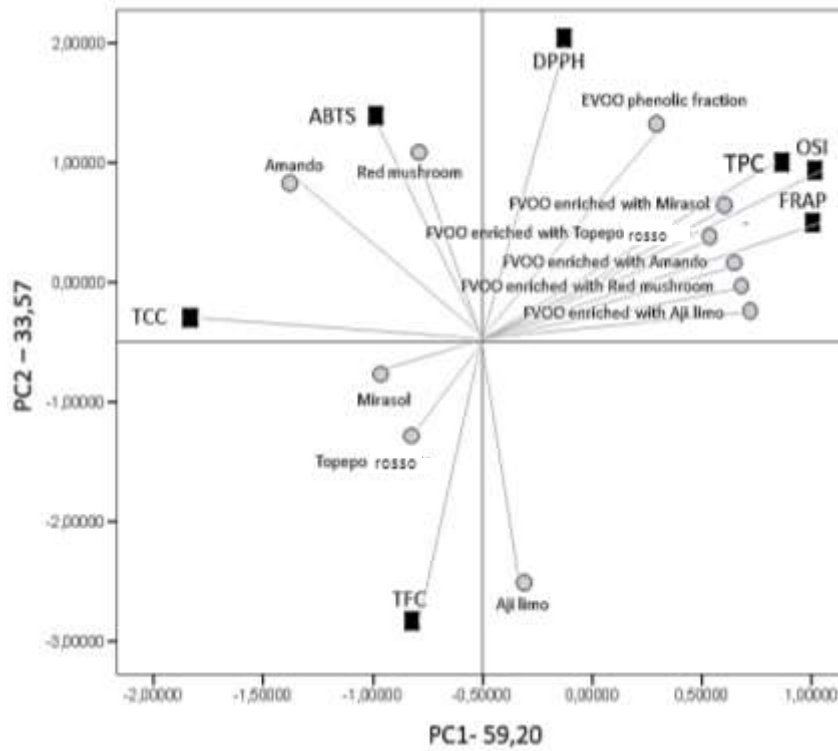
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561 FIGURE 2 Factor loadings for principal components (PC) PC1 and PC2 and scatter plot of all oil
562 samples