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Quality traits of ready-to-use globe artichoke slices as affected by genotype, harvest time and storage time. Part I: Biochemical and physical aspects

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1 **Quality traits of ready-to-use globe artichoke slices as affected by genotype, harvest time and**
2 **storage time. Part I: biochemical and physical aspects**

3

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14

15 **ABSTRACT**

16 Minimally processed globe artichoke products are not widespread due to speed with whom
17 biochemical and enzymatic damage occur. This article reports a study concerning the shelf life of
18 the ready-to-use globe artichoke slices based on principal traits, including phytochemicals content,
19 polyphenol oxidase activity, antioxidant activity and colour parameters. These traits were
20 monitored, during 11 days of storage at 4°C, for globe artichoke slices of three genotypes ('Apollo',
21 'Exploter' and 'Spinoso di Palermo'). Significant variations due to genotype, harvest time, storage
22 time and their interactions were found. For example, the harvest time markedly affected the level of
23 considered biochemical parameters. Results demonstrate that genotype and harvest time are key
24 factors for the extension of the shelf life of globe artichoke slices, but a compromise among
25 nutritional values can be achieved for 'Apollo' and 'Spinoso di Palermo'. The comparison among
26 the three genotypes analysed support the conclusion that 'Exploter' presents the best properties for a
27 commercial use as "minimally processed vegetable" (MPV). Furthermore, these results suggest that
28 ready-to-use globe artichoke slices maintained high nutritional quality and colour parameters for at
29 least 7 days of storage.

30

31 Keywords: globe artichoke slices, genotype, harvest time, phytochemicals, polyphenol oxidase.

32

33 1. Introduction

34 Many epidemiologic studies provide evidence on the health-promoting activity of fresh vegetables
35 and fruits and on the correlation between their consumption with a reduced risk of some types of
36 cancers (William & Hord 2005, Tyrovolas & Panagiotakos, 2009). These properties have been
37 associated with phytochemical compounds, such as micronutrient, vitamins and polyphenols, whose
38 content is affected by several pre- and post-harvest factors (genotype, environmental and agronomic
39 conditions, harvest time, food processing). Nowadays, there is a continuous increase in the demand
40 for fresh, ready-to-use products, such as minimally processed vegetables (MPV). Minimal
41 processing operations can cause undesirable changes in the sensorial, nutritional and health-
42 promoting properties by loss of soluble compounds or the formation of unstable components
43 (Shahidi, 1997). The main enzyme involved in the browning reaction is the polyphenol oxidase
44 (PPO; EC 1.14.18.1), which produces dark pigments (melanoidins), unacceptable in terms of
45 sensory and nutritional quality, which have also safety implications since they would provide an
46 excellent substrate for microbial spoilage (Lattanzio, Cardinali, Di Venere, Linsalata, & Palmieri,
47 1994; Barbagallo, Chisari, & Spagna, 2009). These negative effects have led, over the last twenty
48 years, to focusing research on the shelf life extension of fresh-cut fruits and vegetables. Globe
49 artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori] draws many scientific interests due to of its
50 established nutritional and antioxidant properties (Di Venere et al., 2004; Lattanzio, Kroon,
51 Linsalata, & Cardinali, 2009; Lombardo et al., 2012a; Sergio et al., 2016). The nutritional relevance
52 of globe artichoke is mainly due to its high polyphenol content (Lombardo, Pandino, Mauro &
53 Mauromicale 2009) which, on the other hand, makes it very susceptible to the browning
54 phenomenon. Many studies on minimally processed globe artichokes have been focused on efficient
55 ways to reduce this reaction and the growth of microorganisms, as well as on the use of innovative
56 packaging (Del Nobile et al., 2009; Amodio, Cabezas-Serrano, Peri, & Colelli, 2011; Restuccia et
57 al., 2014), but, to our knowledge, very few were focused on the ready-to-use globe artichoke slices.
58 These by-products might be used directly for salad or boiled or fried, and could represent a new

59 product with the potential to increase globe artichoke consumption over the Mediterranean Basin. In
60 a previous research, Cefola et al. (2012) regarded selection of globe artichoke genotypes as
61 fundamental pre-harvest step for the improvement of the fresh-cut vegetable processing. Obviously,
62 the level of biochemical compounds is also influenced by other factors, such as harvest time,
63 environmental conditions and agronomic management (Pandino, Lombardo, Lo Monaco, &
64 Mauromicale, 2013). On the basis of previous researches, the aim of this paper was to study the
65 influence of genotype, harvest time, storage time and their interactions on the quality maintenance
66 of ready-to-use globe artichoke slices by measuring their phytochemicals content, polyphenol
67 oxidase activity, antioxidant activity and colour changes.

68

69 **2. Materials and methods**

70 *2.1. Experimental field, plant material and management practices*

71 The experimental field was conducted at the farm of the University of Catania located in the Plain
72 of Catania (Sicily, Italy), which is a typical area for globe artichoke cultivation. The soil type is
73 vertic xerochrepts (USDA, Soil Taxonomy) and the local climate is semiarid-Mediterranean area,
74 with mild winters and hot, rainless summers. The mean 30-year maximum monthly temperature
75 ranges between 14.8 °C (January) and 30.6 °C (July) and minimum temperature between 7.8 °C
76 (January) and 22.3 °C (August).

77 Three globe artichoke genotypes were studied: ‘Apollo’, ‘Exploter’ and ‘Spinoso di Palermo’.
78 ‘Apollo’ a Romanesco-type genotype, features spherical-shaped heads with deep bracts that are
79 harvested from the end of February until May; ‘Exploter’ has been recently cropped in Sicily and is
80 characterized by deep violet bracts and ovoid shaped heads with harvest time in March-May;
81 ‘Spinoso di Palermo’ is an early reflowering multiclone genotype actually widespread in Sicily,
82 producing ovoidal, green heads with purple shades and yellow spines, which produces from
83 November to April. They were planted in the form of either semi-dormant offshoots (‘*ovoli*’) or
84 seedling with 3-4 leaves in August 2013. The plant material was arranged in a randomized block

85 experimental design with four replicates, consisting of twenty plants per each plot. Plants were
86 planted 0.8 m apart within a row and 1.2 m apart among close rows, adopting a plant density of 1.0
87 plant m⁻². Crop management (fertilization, irrigation, weed and pest control) was performed
88 according to the standard commercial practice. Gibberellic acid was not supplied to the plants during
89 the crop cycle.

90

91 *2.2. Head harvest, post-harvest treatments and sampling*

92 About 100 heads for each genotype and replicate were harvested in early March and early April at
93 marketable stage, when the length of central flower ranged from 2.0 to 3.0 mm, and transported to
94 the laboratories of the University of Catania under refrigerated conditions for the processing as
95 read-to-use vegetable. Three weeks before harvest, the maximum temperatures were 16 and 17 °C,
96 while minimum ones were 4 and 8.5 °C, respectively for the first and second harvest. After manual
97 removal of the inedible parts (leaves, floral stem and outer bracts) and trimming (about 2 cm) of
98 heads tips, the globe artichoke heads were sliced by a manual slicing machine at 5 mm thickness.
99 The slices were then immersed in sanitizing solution (0.23 g L⁻¹ active chlorine) for 5 min, rinsed
100 with tap water at 12°C for 1 min and immersed for 5 min in a solution of 2% ascorbic acid and 5%
101 citric acid. The excess aqueous solution was then eliminated by manual centrifugation.

102 A number of twelve slices (10±1 g for each one) were put into a PET trays (23×17.5×2 cm) and
103 packaged into a semi-permeable polyolefine film (SP/BY - System Packaging s.r.l., Siracusa, Italy;
104 thickness: 19 µm; oxygen permeability: 3700 cm³/m²/24h; carbon dioxide permeability: 11100
105 cm³/m²/24h).. The bags were hermetically sealed by a sealing bar. All samples were stored at 4±0.5
106 °C and 90-95% RH and analyzed after 0 (production day), 4, 7 and 11 days of storage, respectively.
107 At each storage time, the following physical, chemical and enzymatic determinations were
108 performed.

109

110 *2.3. Biochemical parameters analysis*

111 The colour parameters and polyphenol oxidase activity were assessed on the fresh tissues,
112 immediately after sampling for microbiological analyses (Licciardello et al., 2016). The other tissue
113 portion (about 1 kg) was freeze-dried (Christ freeze drier, Osterode am Harz, Germany) at the
114 following drying conditions: pressure, 15 atm; time, 4 days; shelf temperature, -50°C, and then
115 stored at -20°C until the determination of sugars, ascorbic acid, total polyphenol content and
116 antioxidant activity. All the reagents and solvents for the chemical determinations were purchased
117 from Sigma-Aldrich (Milan, Italy) and were of analytical or HPLC grade. Bi-distilled water was
118 used throughout this analytical trial.

119 *2.3.1. Soluble sugars and inulin.* Soluble sugars (glucose, fructose and sucrose) and inulin
120 content were determined following the method of Lombardo et al. (2016). Briefly, 1 g of freeze-
121 dried sample was added to 40 mL of boiling water and the pH was adjusted to 7.0 with 50 mM
122 KOH. The solution was kept at $85\pm 2^\circ\text{C}$ for 15 min. After cooling at room temperature, the volume
123 was made up to 100 mL with deionized water. One aliquot of this extract was used for the direct
124 analysis of free glucose/fructose, another was incubated for 30 min at $40\pm 2^\circ\text{C}$ with sucrose to
125 determine the fructose from sucrose and a third aliquot was incubated for 60 min at $60\pm 2^\circ\text{C}$ with
126 fructanase for the determination of total fructose. Absorbance was measured at 340 nm using a
127 Shimadzu 1601 UV-Visible spectrophotometer (Shimadzu Corp., Tokyo, Japan). All data presented
128 were expressed as g kg^{-1} of dry weight (DW).

129 *2.3.2. Ascorbic acid, total polyphenols and antioxidant activity.* The ascorbic acid
130 determination was carried out as previously described by Lombardo et. al, (2015a). Quantification
131 was performed at 245 nm by the external standard method, comparing the sample chromatographic
132 areas with a calibration curve obtained with suitable standard dilutions. The ascorbic acid content
133 was expressed as g kg^{-1} of DW.

134 Total polyphenol content was quantified using a modified Folin-Ciocalteu method (Cicco, Lanorte,
135 Paraggio, & Viaggiano, 2009). About 0.1 g of freeze-dried material was diluted in 1 mL ethanol
136 70% and stirred at room temperature for 1h, with shaking. The mixture was centrifuged at 5000g

137 for 5 min at 25°C; then, a suitably diluted aliquot was purified with a C-18 end-capped cartridge
138 Phenomenex-Strata (Castel Maggiore, Bologna, Italy) in order to avoid interference by other
139 reducing substances in the assay, and then mixed with Folin-Ciocalteu reagent at room temperature
140 for 2 min. Sodium carbonate (5%, w/v) was added and the mixture was allowed to rest at 40°C for
141 20 min in thermostatic bath. The absorbance was read at 760 nm. The content was determined on
142 the basis of a standard calibration curve generated with increasing concentrations of chlorogenic
143 acid and expressed as g of chlorogenic acid equivalent kg⁻¹ of DW.

144 The antioxidant activity of the extracts was evaluated as percentage inhibition of DPPH radical
145 (Brand-Williams, Cuvelier, & Berset, 1995). An aliquot (0.1 mL) of each extract used for total
146 polyphenol assay, was added to 3.9 mL of freshly prepared methanolic solution containing 0.24 g
147 L⁻¹ DPPH, and held in the dark for 30 min at room temperature. Then, the absorbance was
148 measured at 515 nm in the spectrophotometer. The percentage inhibition of DPPH was calculated
149 according to Brand-Williams et al. (1995).

150 *2.3.3. Polyphenol oxidase (PPO) activity.* The catecholase activity of PPO was determined
151 spectrophotometrically using a modified version of the method proposed by Espín, Tudela, &
152 García-Cánovas (1997), since other tested methodologies did not give the same reproducibility with
153 globe artichoke. Ten grams of fresh artichoke head homogenate were added to 20 mL cold acetone
154 (-20°C) and continuously stirred for 10 min. The homogenate was filtered through Whatman No. 42
155 paper (Milan, Italy) under vacuum on Buchner funnel and the obtained acetonetic powder, collected
156 and suspended in 15 mL 0.1 M citrate buffer (pH 5.8, which corresponds to the average pH of globe
157 artichoke heads), was kept overnight at 4°C, before being filtered again through Whatman No. 42
158 paper under vacuum on Buchner funnel. The clear solution was then ultrafiltered in a cell equipped
159 with a 50 kDa membrane (Millipore 8050, Milan, Italy). The enzymatic activity was assayed
160 spectrophotometrically at 505 nm using catechol as phenolic substrate. The standard reaction
161 mixture contained 0.9 mL of 0.04 M phenolic substrate, 0.1 mL of 0.093 M MBTH (3-methyl-2-
162 benzothiazolinone hydrazone) chromophore coupling agent in methanol, 0.05 mL of DMF (N,N-

163 dimethylformamide), 1.5 mL of 0.05 M sodium acetate buffer at pH 7.0 and 0.5 mL of enzymatic
164 extract. The reaction was stopped at different times with 0.5 mL of 0.9 M H₂SO₄. Blank was
165 prepared by inverting the order between the enzymatic extract and H₂SO₄. One unit of PPO activity
166 was defined as the amount of enzyme which produces an increase in absorbance of 0.001 per min at
167 25± 0.5°C under the conditions described above.

168

169 2.4. Surface colour analysis

170 Three colour measurements on different points of globe artichoke slices were performed using a
171 compact tristimulus chromameter (N-3000, Nippon Denshoku Ind. C. Ltd, Tokyo, Japan) with an 8
172 mm diameter viewing aperture and C illuminant (CIE, 2° observer). The equipment was calibrated
173 with a reference white tile ($x = 83.47$; $y = 84.43$; $z = 95.16$). According to CIElab standard, L*
174 (lightness), a* (from green to red) and b* (from blue to yellow) values were recorded. Then, the two
175 polar coordinates chroma (or saturation, C*) and colour intensity (or hue angle) h , were calculated
176 as follows: $C^* = (a^{*2} + b^{*2})^{1/2}$; $h = \tan^{-1} (b^*/a^*)$.

177

178 2.5. Statistical analysis

179 All data were subjected to analysis of variance (ANOVA) as a factorial combination 'genotype (3)
180 × storage time (4) × harvest time (2)'. Means were separated by a least significance difference
181 (LSD) test, when the F -test was significant. Percent values were transformed to $\arcsin \sqrt{x}$ (Bliss
182 transformation) prior to analysis and then subjected to ANOVA; untransformed data were reported
183 and discussed. All calculations and analyses were performed using CoStat[®] version 6.003 (CoHort
184 Software, Monterey, CA, USA).

185

186 3. Results and discussion

187 3.1. Biochemical parameters

188 3.1.1. *Soluble sugars and inulin.* Amongst the factors under study, harvest time explained
189 more than 50% of the total variation for fructose (68.4%) and glucose (52.3%) (**Table 1**). In
190 particular, glucose, as well as the other carbohydrates, showed a significantly higher content in
191 globe artichokes harvested in April compared to those collected in March (22.9 and 18.3 g kg⁻¹ of
192 DW, respectively) (**Table 2**). In agreement with our findings (Rosenfeld, Samuelsen, & Lea, 1998),
193 high carbohydrate levels have previously been associated with increasing temperatures, as recorded
194 in our experimental field from March to April. In addition, the more intense solar radiation might
195 have contributed to increased photosynthesis and higher accumulation of carbohydrates. Besides the
196 harvest time, sucrose and inulin concentration were also significantly affected by genotype
197 ($P \leq 0.001$ and $P \leq 0.01$, respectively) (**Table 1**). The inulin content changed from 118.2 ('Exploter')
198 to 230.6 ('Spinoso di Palermo') g kg⁻¹ of DW, in the range of previously reported values (Schütz,
199 Muks, Carle, & Schieber, 2006; Pandino, Lombardo, & Mauromicale, 2011). A similar trend was
200 found for sucrose, with 'Apollo' showing the highest content (32.2 g kg⁻¹ of DW) and 'Exploter' the
201 lowest (20.7 g kg⁻¹ of DW) (**Table 2**). According to Vijn & Smeekens (1999), the prerequisite to
202 start the fructooligosaccharides (FOS) accumulation is the availability of sucrose, specific substrate
203 for the activity of the 1-fructosyl transferase (1-SST), key enzyme for FOS synthesis. This suggests
204 that a higher sucrose level contributes to a genotype-specific inulin content, as observed in our
205 study.

206 The analysis of variance revealed a significant influence of the effect of storage time × harvest time
207 interaction for all considered carbohydrates, except for glucose, which was significantly influenced
208 ($P \leq 0.01$, **Table 1**) by the interaction genotype × storage time. Our results highlight a marked
209 glucose loss during the first 4 days of storage for 'Apollo' (-29%) and 'Spinoso di Palermo' (-25%),
210 whereas for 'Exploter' a slight increase was detected up to 7 days of storage (**Figure 1**). Leroy,
211 Grongnet, Mabeau, Le Corre, & Baty-Julien (2009) reported that the glucose content in a globe
212 artichoke variety changed in relation to storage conditions, as well as the inulin content. In this
213 study, the inulin content decreased during storage for both harvests (**Figure 3**). It is important to

214 note that in April, compared to March harvest, a higher loss of inulin was recorded during the 11
215 days of storage at 4°C. The different behaviour could be attributed to the activity and/or
216 accumulation of 1-fructan exohydrolase (1-FEH), responsible for the hydrolysis of inulin under
217 refrigerated conditions. In a previous study, changes in the activity of 1-FEH in burdock root during
218 storage at low temperature were observed (Imahori et al., 2010). Verhaest et al. (2007) reported that
219 this enzyme is directly regulated by sucrose level in the plant. The higher sucrose content registered
220 in April might explain our findings (**Table 2**). The fluctuant activity of 1-FEH affects sugars
221 composition, since the depolymerization process produces free sugars, such as fructose. However,
222 during storage an increase of the fructan 1-fructosyltransferase (1-FFT) activity was also observed
223 through the synthesis of short-chain fructans (Van den Ende & Van Laere, 1996). In this study, the
224 highest level of fructose was found after 4 and 11 days of storage, respectively in April and March,
225 while an opposite trend was recorded for the sucrose level (**Figure 3**).

226 *3.1.2. Ascorbic acid, total polyphenols and antioxidant activity.* ANOVA for the ascorbic
227 acid (AsA) content revealed high statistical significance ($P \leq 0.001$) for the interaction genotype \times
228 harvest time (**Table 1**). ‘Apollo’ and ‘Spinoso di Palermo’ were characterized by the highest
229 content of AsA when harvested in April respect to samples collected in March (on average, 0.3 vs.
230 0.9 g kg⁻¹ of DW) (**Figure 2**). Previously, Lombardo, Lo Monaco, Pandino, Parisi, & Mauromicale
231 (2012b) underlined the importance of genotype \times harvest time interaction on the AsA content in
232 potatoes. In relation to storage time, the ascorbic acid (AsA) content dropped by more than 70%
233 during 11 days of storage (**Table 2**), in agreement with what reported by Kevers et al. (2007) for
234 selected fruits and vegetables. The AsA content, which was increased with the antioxidant dipping
235 solution, is likely to undergo fast oxidation on the cut surface exposed to the headspace O₂.
236 Similarly, the antioxidant activity (AA) declined significantly after 11 days of storage (**Table 2**),
237 suggesting that the AsA content is the main compound affecting AA in globe artichoke slices. Our
238 assumption was confirmed by the not significant variation found for TPC during storage. ANOVA
239 revealed a significant influence of the genotype \times storage time interaction for TPC ($P \leq 0.001$). The

240 TPC showed the highest variation after 7 days of storage, ranging from 15.1 ('Exploter') to 26.0 g
241 kg⁻¹ of DW ('Spinoso di Palermo') (**Figure 1**). In particular, the TPC increased in 'Spinoso di
242 Palermo' and decreased in 'Exploter' during 7 days of storage. The different behaviour of these
243 genotypes is likely to be linked to their genetic background, which acts against the stress caused by
244 cutting and storage conditions. Furthermore, a significant interaction genotype × storage time was
245 observed by Muratore et al. (2015) in minimally processed globe artichoke heads. Conversely,
246 Cefola et al. (2012) indicated not significant influence of genotype × storage time on TPC. This
247 variation could be due to the differences in postharvest handling and storage conditions, as
248 reviewed by Soliva-Fortuny & Martín-Belloso (2003). Another possible explanation for the
249 observed differences is that the TPC content in the present experiment was determined in fresh-cut
250 globe artichoke after cutting and dipping, whereas the above authors measured the TPC content in
251 the intact globe artichoke.

252 The TPC was also affected by genotype × harvest time interaction (**Figure 2**). In particular,
253 'Exploter' heads harvested in March showed a higher TPC compared to those collected in April
254 (23.3 vs. 16.6 g kg⁻¹ of DW). These results are corroborated by our previous works, where the
255 genotype × harvest time interaction significantly influenced the level of polyphenols in globe
256 artichokes (Pandino et al., 2013; Lombardo et al., 2015b; Pandino et al., 2015).

257 *3.1.3. Polyphenol oxidase (PPO) activity.* The significant genotype × storage time
258 interaction confirmed the close interdependence between the suitability of globe artichoke
259 genotypes for minimal processing, and the shelf life of the commercial products. The different
260 initial content of endogenous PPO thus represents a selective parameter, that, in turn, is related with
261 the proneness to undergo browning after minimal operations of shredding and cutting. 'Apollo' and
262 'Spinoso di Palermo' showed comparable PPO activities. In relation to changes of this enzyme
263 during storage time (**Table 2**), its activity significantly increased during the first 7 days of storage,
264 to decrease then, showing comparable values to those observed after 4 days. The decline is to
265 attribute to the depletion of the endogenous PPO activity involved with phenolic components in the

266 browning phenomena and loss of nutritional value (Leoni, Calmieri, Lattanzio, & van Sumere,
267 1990), as well as to a molecular rearrangement of the phenolic compounds, which represent the
268 specific substrates for PPO. **Figure 1** shows that both ‘Apollo’ and ‘Spinoso di Palermo’ had a
269 lower endogenous PPO concentration at processing time (2.3 and 2.2 U mmol min⁻¹ g⁻¹,
270 respectively) compared to the ‘Exploter’ (3.0 U mmol min⁻¹ g⁻¹). In addition, ‘Apollo’ and ‘Spinoso
271 di Palermo’ had a similar pattern, showing an increase in the PPO activity up to 7 days of storage:
272 this trend is probably due to the activation of browning cascade reactions catalyzed by specific
273 phenolic substrates, available through the mediation of phenylalanine ammonia-lyase (PAL; EC
274 4.3.1.5). According to our previous laboratory experiences the impact of the peroxidase (POD, EC
275 1.11.1.7) isoenzymes in the Sicilian globe artichoke is lower or negligible compared to other plants
276 and succulent fruits, such as tomato or melon and strawberry, while Lattanzio, Linsalata, Calmieri,
277 & Van Sumere, 1989; Leoni et al. (1990) reported changes in phenolic compounds, PPO and PAL
278 activities and the formation of iron/chlorogenic acid complexes in fresh marketable globe artichoke
279 heads during refrigerated storage. The activation of the PPO enzyme could be induced by ageing
280 phenomena, and/or by stress conditions during storage. In addition, the browning increase could be
281 explained by a greater availability of PPO following its release from the cell structures by other
282 endogenous and degenerative enzymes, primarily polygalacturonase (EC 3.2.1.15), involved with
283 pectin methyl esterase (EC 3.1.1.11) in cell wall solubilization (Barbagallo et al., 2009). In both
284 ‘Apollo’ and ‘Spinoso di Palermo’ the PPO activity declined up to 11 days of storage, while a
285 different behaviour was shown by ‘Exploter’ which, despite a higher initial PPO activity, showed an
286 initial slight increase followed by a progressive decline until stabilization from 7 and 11 days of
287 storage. This behaviour is explained by the unavailability in this stage of phenolic substrates to
288 react, as evidenced by lower TPC values and by the higher levels of AA compared to the genotype
289 ‘Apollo’ and ‘Spinoso di Palermo’, although with a modest content of AsA.

290

291 *3.2. Surface colour parameters*

292 Storage time resulted the predominant factor influencing colour changes of ready-to-use globe
293 artichoke slices, showing the highest percentage of total variation for all the measured colour
294 parameters (L^* , a^* , b^*) and for the calculated ones (C^* and h), with higher percentages for L^*
295 (53%), C^* (40.7%) and h (47.4%) (**Table 3**).

296 **Table 4** shows a gradual decrease of all measured and calculated colour parameters, with a trend to
297 reach a steady-state between 7 and 11 days of storage for the L^* , b^* and C^* parameters, presumably
298 due to the inhibition of the degenerative reactions catalysed by the PPO isoenzymes. Due to the loss
299 of cell compartmentalization, fresh-cut globe artichoke is considered more susceptible to enzymatic
300 reactions (e.g. oxidases and pectinases) with respect to the whole vegetable (Barbagallo, Chisari,
301 Branca, & Spagna, 2008). ANOVA highlighted that the harvest time \times genotype interaction was
302 significant for all measured colour parameters, except for L^* . The colour change from green to red
303 tonality was evident in ‘Apollo’ which presented an average value of a^* (0.5) tending to 0 in the
304 April harvest, as well as in the ‘Spinoso di Palermo’, with a decrease of this colour parameter by
305 about 15% (**Figure 2**). An opposite behaviour was shown by the genotype ‘Exploter’, probably
306 because the higher temperatures and increased biochemical effect of solar radiation in April
307 increased the synthesis of some antioxidant compounds which do not contribute to the TPC, most
308 probably carotenoids (Ferracane et al. 2008). Relating to the change of colour from blue to yellow,
309 all genotypes highlighted an increase of the b^* values from the March harvest to April, though with
310 greater variations in the genotype ‘Exploter’, followed by ‘Spinoso di Palermo’ and ‘Apollo’. A
311 similar trend was observed for the polar coordinate C^* , although with greater variations for both the
312 genotypes ‘Exploter’ and ‘Apollo’. As for the h value, the total range of variation in the tested
313 genotypes appeared unchanged in the two harvest periods. The former was also affected by the
314 interaction genotype \times storage time. ‘Apollo’ showed (**Figure 1**) a lower intensity of the colour
315 perceived by the human eye (81.8) compared with ‘Spinoso di Palermo’ and ‘Exploter’ (85.5 and
316 86.1, respectively), an increase after 4 days (89.4), followed by a gradual decrease. This is
317 presumably due to phenomenon of tissue discolouration caused by an excessive presence of

318 colourless intermediates (quinones). The same behaviour shown for 'Apollo' was exhibited by
319 'Exploter' and 'Spinoso di Palermo', although delayed to 7 days of storage. In particular, the *h*
320 parameter for 'Spinoso di Palermo' was almost unchanged during the first 4 storage days.

321 The storage time \times harvest time interaction was significant only for the a^* parameter. Results
322 (**Figure 3**) suggest that the change of a^* followed an inverse trend in the early and late harvest:
323 samples harvested in March showed a progressive decrease in the a^* values, which corresponds to
324 the change from a green colour with reddish tones to a deeper green due to enzymatic browning
325 reactions catalyzed by PPO. On the other hand, the combined action of the high temperatures in
326 April and increased photosynthetic activity determine a more effective antioxidant activity against
327 the browning phenomena.

328

329 **4. Conclusion**

330 This study highlighted that some agronomic factors as genotype, harvest time and storage time are
331 of crucial importance for the shelf life of ready-to-use globe artichoke slices modifying some
332 chemical-physical and enzymatic parameters. In particular, harvest time was the predominant factor
333 affecting the nutritional quality of ready-to-use globe artichoke slices, showing the highest
334 percentage of total variation for six of the considered biochemical traits. On the contrary, colour
335 parameters appeared to be strongly influenced by storage time. Above all, ready-to-use globe
336 artichoke slices maintained high nutritional quality and colour parameters for at least 7 days of
337 storage. Nevertheless, the fluctuation observed for both harvest time and storage time would be
338 linked to the genotype, as demonstrated by the significant interactions among those factors.
339 Therefore, the food processors should be aware of the importance of genotype selection. Based on
340 such consideration, 'Exploter' was most suitable for processing as ready-to-use globe artichoke
341 slices, especially in the late harvest (April), when it showed better colour parameters and lower
342 content of TPC. Certainly, further extensive studies are required to clarify the contribution of other

343 pre-harvest factors, such as agronomic practices and environmental conditions, on the quality of
344 ready-to-use globe artichoke slices.

345

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351

ACCEPTED MANUSCRIPT

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- 468

469 **Figure captions**

470 **Fig. 1.** Total polyphenols content (TPC), glucose content (GLU), polyphenol oxidase (PPO) and
471 colour intensity (*h*) of globe artichoke slices ('Apollo', 'Exploter' and 'Spinoso di Palermo') as
472 affected by 'genotype x storage time' interaction.

473 **Fig. 2.** Total polyphenols content (TPC), ascorbic acid content (AsA), colour variation from green
474 to red (*a**), colour variation from blue to yellow (*b**), colour intensity (*h*) and chroma (*C**) of globe
475 artichoke slices ('Apollo', 'Exploter' and 'Spinoso di Palermo') as affected by 'harvest time x
476 genotype' interaction.

477 **Fig. 3.** Fructose content (FRU), sucrose content (SUC), inulin content (INU) and colour variation
478 from green to red (*a**) of globe artichoke slices ('Apollo', 'Exploter' and 'Spinoso di Palermo') as
479 affected by 'harvest time x storage time' interaction.

Table 1. Mean square per each source of variation (percentage of total) resulting from analysis of variance.

Source of variation	Degree of freedom	Biochemical parameter							
		TPC	AA	GLU	FRU	SUC	INU	AsA	PPO
Genotype (G)	2	12.5*	6.8 ^{NS}	4.2 ^{NS}	2.7 ^{NS}	27.8***	29.8***	3.0***	57.6***
Storage time (S)	3	0.9 ^{NS}	22.1*	25.1***	4.5**	2.6 ^{NS}	25.0***	37.3***	27.9***
Harvest time (H)	1	32.5**	37.4*	52.3***	68.4***	27.5***	31.7***	44.3***	0 ^{NS}
(G) x (S)	6	14.3***	5.2 ^{NS}	8.0**	3.2 ^{NS}	7.9 ^{NS}	1.9 ^{NS}	1.4 ^{NS}	11.6***
(H) x (G)	2	12.4*	2.7 ^{NS}	1.2 ^{NS}	5.5 ^{NS}	7.7 ^{NS}	4.7 ^{NS}	12.4***	1.7 ^{NS}
(H) x (S)	3	8.5 ^{NS}	8.3 ^{NS}	0.8 ^{NS}	11.9***	10.0**	5.9***	0 ^{NS}	0.2 ^{NS}
Total mean square		342.0	571.3	480.2	1024.3	2252.8	214467.1	3.7	181.6

Note: TPC: total polyphenols content; AA: antioxidant activity; GLU: glucose; FRU: fructose; SUC: sucrose; INU: inulin; AsA: ascorbic acid; PPO: polyphenol oxidase. ***, ** and * indicate significant at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$, and NS, not significant.

Table 2. Biochemical parameters of globe artichoke slices ('Apollo', 'Exploter' and 'Spinoso di Palermo') as affected by main factors. Different letters within the same parameter and main factor show significant differences (*LSD test, P*≤0.05).

	Biochemical parameter							
	TPC (g kg ⁻¹ DW)	AA (DPPH % inhibition)	GLU (g kg ⁻¹ DW)	FRU (g kg ⁻¹ DW)	SUC (g kg ⁻¹ DW)	INU (g kg ⁻¹ DW)	AsA (g kg ⁻¹ DW)	PPO (U mmol min ⁻¹ g ⁻¹)
Genotype								
'Apollo'	22.9 ^a ±2.3	72.3 ^a ±6.5	21.7 ^a ±2.5	16.1 ^a ±2.6	32.2 ^a ±4.5	224.3 ^a ±31.3	0.6 ^a	6.7 ^a ±0.6
'Exploter'	19.9 ^b ±2.5	74.9 ^a ±6.8	19.5 ^a ±2.0	13.5 ^a ±2.4	20.7 ^b ±2.2	118.2 ^b ±12.4	0.4 ^c	2.7 ^c
'Spinoso di Palermo'	22.6 ^a ±1.9	72.1 ^a ±7.2	20.4 ^a ±2.3	15.1 ^a ±1.9	30.8 ^a ±3.5	230.6 ^a ±35.7	0.6 ^b	5.6 ^b ±0.4
Storage time (days)								
0	22.2 ^a ±2.3	76.6 ^a ±7.4	25.0 ^a ±3.0	13.7 ^b ±1.9	29.7 ^a ±4.4	266.8 ^a ±30.4	1.0 ^a	2.5 ^c
4	21.4 ^a ±2.6	75.1 ^{ab} ±5.5	20.7 ^b ±3.0	14.9 ^b ±2.4	29.6 ^a ±4.0	215.4 ^b ±26.5	0.4 ^b	5.6 ^b ±0.2
7	21.3 ^a ±2.4	70.7 ^b ±7.1	18.9 ^{bc} ±1.5	13.4 ^b ±2.0	25.1 ^a ±3.3	173.0 ^c ±15.3	0.3 ^c	6.2 ^a ±0.9
11	22.3 ^a ±2.5	70.1 ^b ±5.3	17.8 ^c ±2.2	17.7 ^a ±2.2	27.1 ^a ±3.9	109.0 ^d ±6.4	0.3 ^c	5.7 ^b ±0.6
Harvest time								
March 2014	23.3 ^a ±2.7	71.0 ^b ±6.4	18.3 ^b ±2.8	11.1 ^b ±1.6	24.3 ^b ±3.1	153.4 ^b ±21.4	0.4 ^b	5.0 ^a ±0.7
April 2014	20.3 ^b ±3.0	75.2 ^a ±6.6	22.9 ^a ±2.7	18.7 ^a ±0.8	31.5 ^a ±5.2	228.7 ^a ±30.3	0.7 ^a	5.0 ^a ±0.7

See note to Table 1 for the list of abbreviations of the qualitative parameters under study.

Table 3. Mean square per each source of variation (percentage of total) resulting from analysis of variance.

Source of variation	Degree of freedom	Colour parameter				
		L	a	b	C*	h
Genotype (G)	2	3.5 ^{***}	9.0 ^{***}	1.6 ^{**}	1.6 ^{**}	2.0 ^{**}
Storage time (S)	3	53.0 ^{***}	10.8 ^{***}	40.0 ^{***}	40.7 ^{***}	47.4 ^{***}
Harvest time (H)	1	32.5 ^{***}	0 ^{NS}	31.4 ^{***}	30.3 ^{***}	1.7 [*]
(G) x (S)	6	2.9 ^{NS}	4.7 ^{NS}	4.7 ^{NS}	4.8 ^{NS}	12.9 ^{***}
(H) x (G)	2	0.9 ^{NS}	30.4 ^{***}	12.5 ^{***}	13.1 ^{***}	17.4 ^{***}
(H) x (S)	3	2.7 ^{NS}	18.0 ^{***}	4.4 ^{NS}	4.3 ^{NS}	7.9 ^{NS}
Total mean square		147.9	11.9	130.4	134.5	122.8

Note: L*:lightness; a*: from green to red; b*: from blue to yellow; C*: chroma; h: colour intensity.

Table 4. Physical parameters of globe artichoke slices ('Apollo', 'Exploter' and 'Spinoso di Palermo') as affected by main factors. Different letters within the same parameter and main factor show significant differences (*LSD test, $P \leq 0.05$*).

	Colour parameter				
	L*	a*	b*	C*	<i>h</i>
Genotype					
'Apollo'	36.8 ^b ±2.4	0.2 ^c	10.9 ^a ±1.1	11.0 ^a ±1.1	86.8 ^b ±3.7
'Exploter'	37.7 ^a ±1.8	0.4 ^b	10.3 ^b ±1.7	10.4 ^b ±1.7	87.3 ^a ±1.7
'Spinoso di Palermo'	37.0 ^b ±2.8	0.6 ^a	10.6 ^b ±0.9	10.6 ^b ±0.9	86.7 ^b ±1.5
Storage time (days)					
0	40.1 ^a ±2.0	0.8 ^a	13.1 ^a ±1.3	13.2 ^a ±1.4	84.5 ^d ±3.4
4	37.2 ^b ±1.4	0.2 ^c	10.2 ^b ±0.9	10.2 ^b ±0.9	88.0 ^b ±1.6
7	35.8 ^c ±1.0	0.2 ^c	9.7 ^c ±1.3	9.7 ^c ±1.3	88.5 ^a ±0.8
11	35.6 ^c ±1.5	0.4 ^b	9.4 ^c ±1.6	9.5 ^c ±0.6	86.8 ^c ±1.1
Harvest time					
March 2014	36.4 ^b ±1.9	0.4 ^a	9.9 ^b ±0.9	9.9 ^b ±0.9	86.8 ^a ±3.7
April 2014	38.0 ^a ±2.5	0.4 ^a	11.4 ^a ±0.2	11.0 ^a ±0.2	87.1 ^b ±1.8

See note to Table 3 for the list of abbreviations of the qualitative parameters under study.

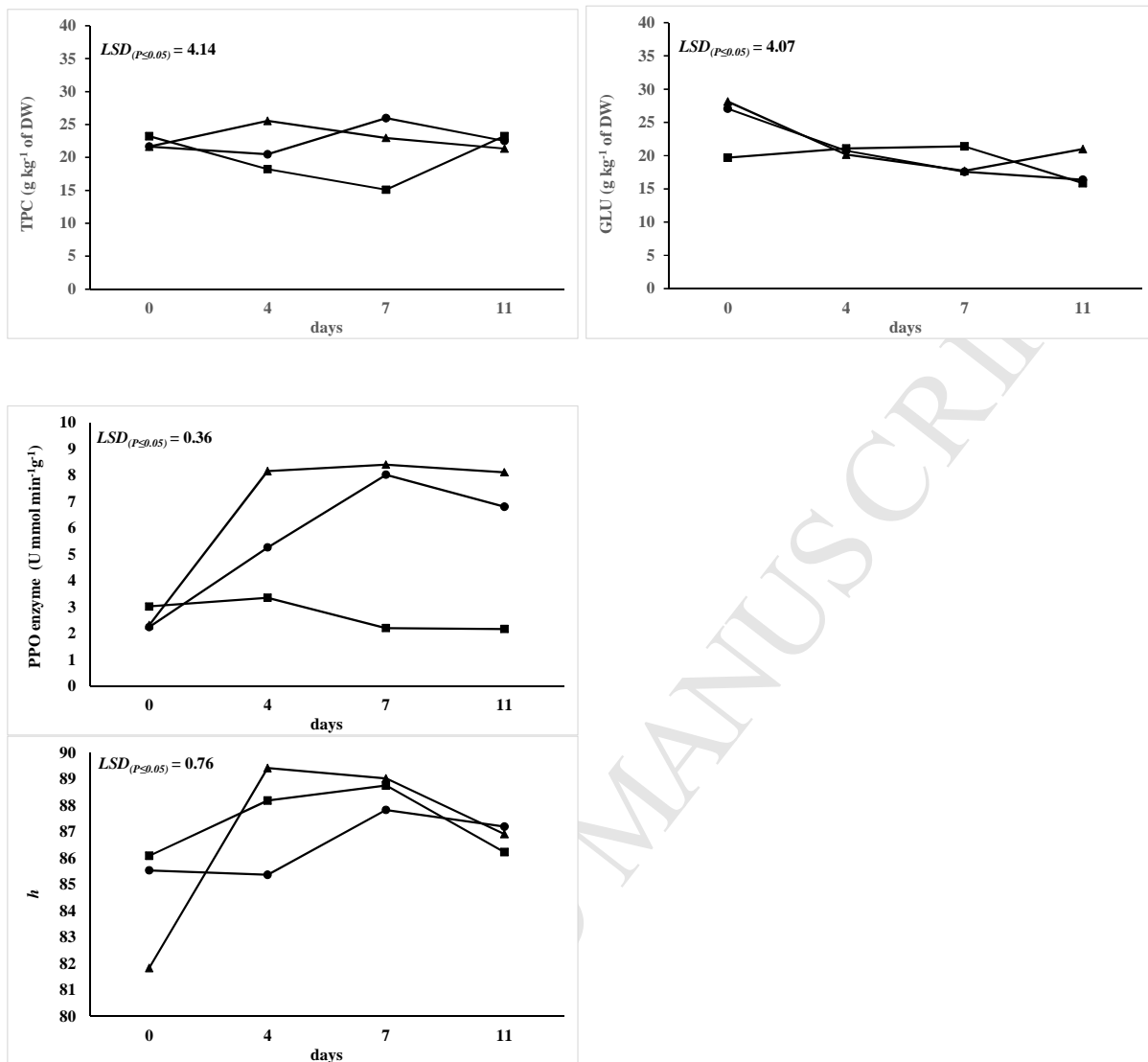


Fig. 1. Total polyphenols content (TPC), glucose content (GLU), polyphenol oxidase (PPO) and colour intensity (h) of globe artichoke slices ('Apollo', 'Exploiter' and 'Spinoso di Palermo') as affected by 'genotype x storage time' interaction.

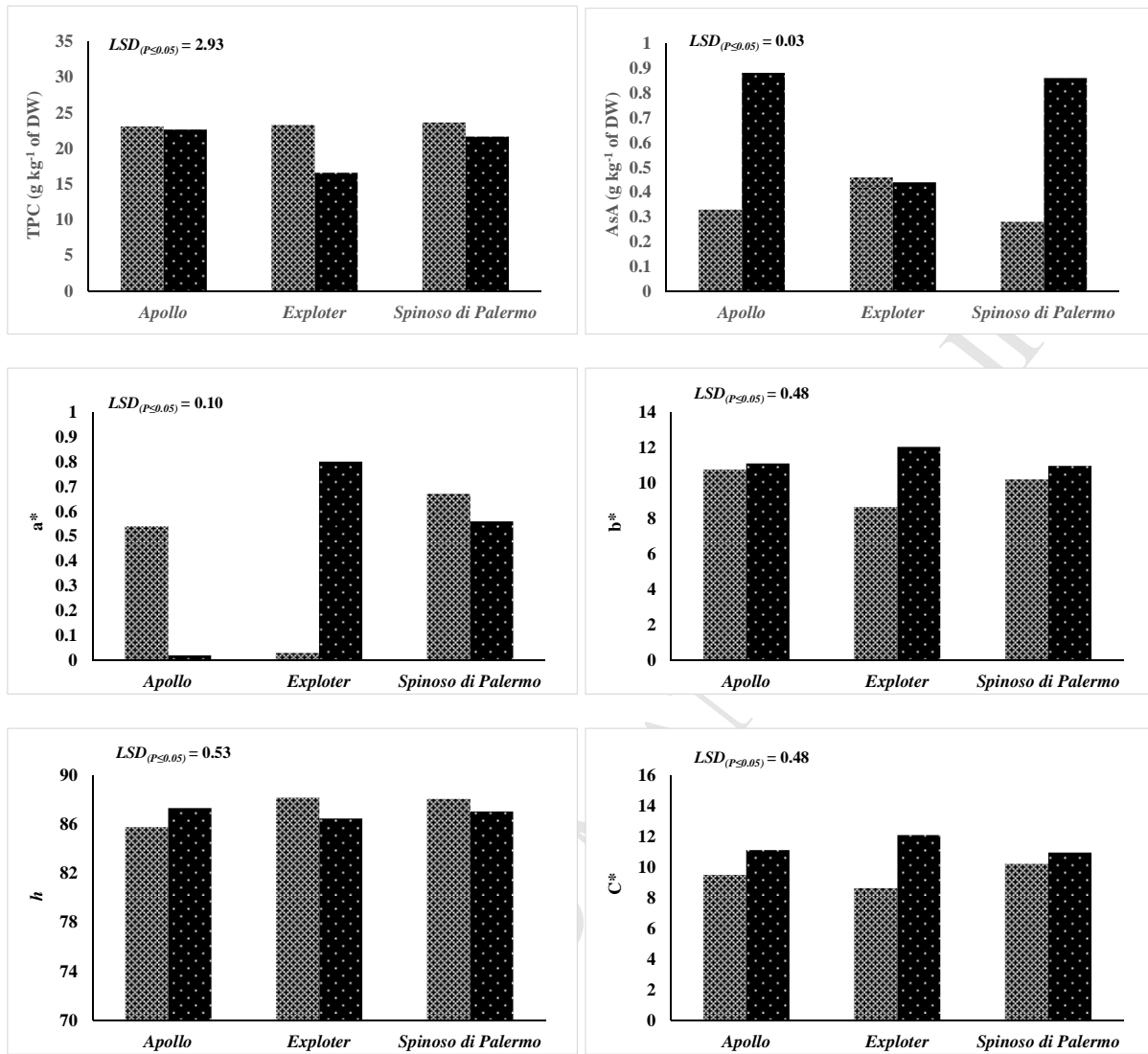


Fig. 2. Total polyphenols content (TPC), ascorbic acid content (AsA), colour variation from green to red (a*), colour variation from blue to yellow (b*), colour intensity (*h*) and chroma (C*) of globe artichoke slices ('Apollo', 'Exploter' and 'Spinoso di Palermo') as affected by 'harvest time x genotype' interaction.

▣ = March; ■ = April

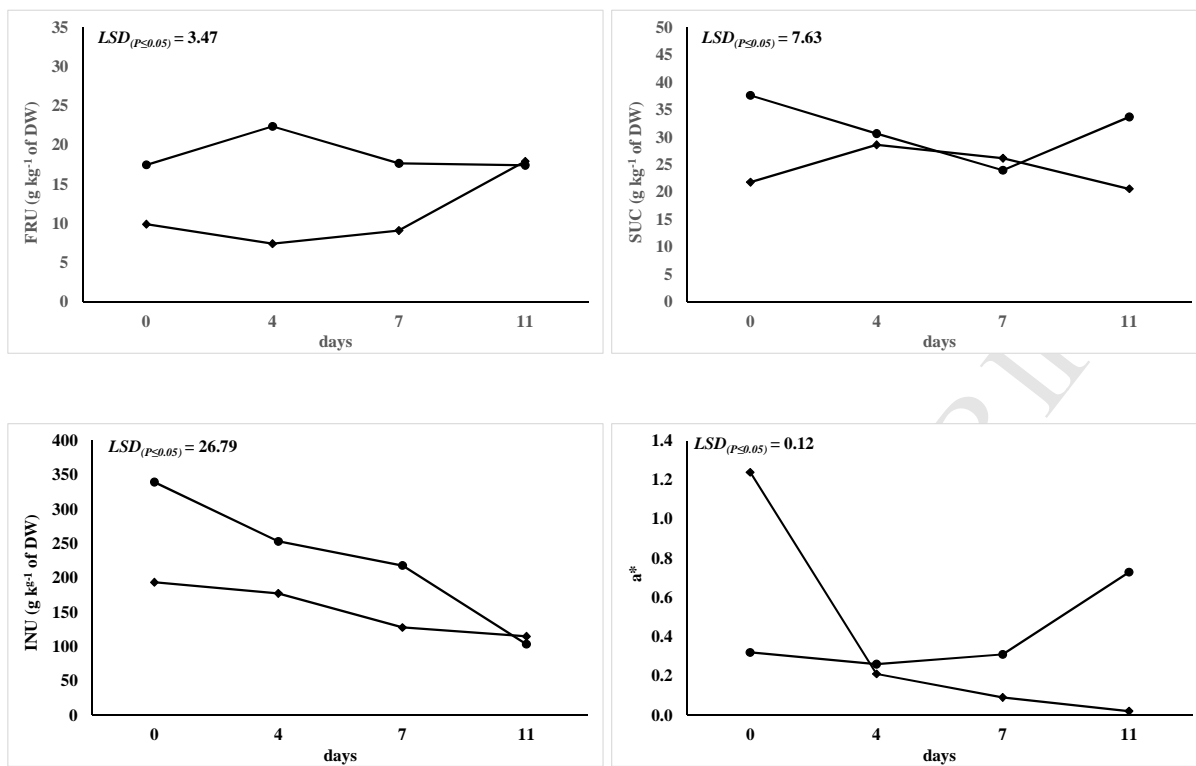


Fig. 3. Fructose content (FRU), sucrose content (SUC), inulin content (INU) and colour variation from green to red (a^*) of globe artichoke slices ('Apollo', 'Exploter' and 'Spinoso di Palermo') as affected by 'harvest time x storage time' interaction.

◆◆ = March; ●● = April.

1. Biochemical parameters on globe artichoke slices were studied.
2. ANOVA revealed a significant influence of storage time, harvest time and genotype.
3. Globe artichoke slices had high nutritional and colour quality for 7 storage days.
4. 'Exploter' was the most suitable for processing as globe artichoke slices.