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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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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 read-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, polypenol oxidase.

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

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

experimental design with four replicates, consisting of twenty plants per each plot. Plants were planted 0.8 m apart within a row and 1.2 m apart among close rows, adopting a plant density of 1.0 plant m⁻². Crop management (fertilization, irrigation, weed and pest control) was performed according to the standard commercial practice. Giberellic acid was not supplied to the plants during 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. The slices were then immersed in sanitizing solution (0.23 g L^{-1} active chlorine) for 5 min, rinsed 99 100 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.

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

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 dryng 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 $85\pm 2^{\circ}$ 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 ± 2 °C with sucrose to 125 determine the fructose from sucrose and a third aliquot was incubated for 60 min at 60±2 °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.

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

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

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

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

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169 2.4. Surface colour analysis

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

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178 2.5. Statistical analysis

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

- 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 globe artichokes harvested in April compared to those collected in March (22.9 and 18.3 g kg⁻¹ of 191 192 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 ($P \le 0.001$ and $P \le 0.01$, respectively) (**Table 1**). The inulin content changed from 118.2 ('Explorer') to 230.6 ('Spinoso di Palermo') g kg⁻¹ of DW, in the range of previously reported values (Schütz, 198 199 Muks, Carle, & Schieber, 2006; Pandino, Lombardo, & Mauromicale, 2011). A similar trend was found for sucrose, with 'Apollo' showing the highest content (32.2 g kg⁻¹ of DW) and 'Exploter' the 200 lowest (20.7 g kg⁻¹ of DW) (**Table 2**). According to Vijn & Smeekens (1999), the prerequisite to 201 202 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 $(P \le 0.01, \text{ Table 1})$ by the interaction genotype \times 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 through the synthesis of short-chain fructans (Van den Ende & Van Laere, 1996). In this study, the 223 224 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 ($P \le 0.001$) 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. 0.9 g kg⁻¹ of DW) (Figure 2). Previously, Lombardo, Lo Monaco, Pandino, Parisi, & Mauromicale 230 231 (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 O_2 . 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 ≤ 0.001). The

240 TPC showed the highest variation after 7 days of storage, ranging from 15.1 ('Exploter') to 26.0 g kg⁻¹ of DW ('Spinoso di Palermo') (Figure 1). In particular, the TPC increased in 'Spinoso di 241 242 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 \times 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 \times 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 globe artichoke after cutting and dipping, whereas the above authors measured the TPC content in 250 251 the intact globe artichoke.

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

257 3.1.3. Polyphenol oxidase (PPO) activity. The significant genotype \times 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 lower endogenous PPO concentration at processing time (2.3 and 2.2 U mmol min⁻¹ g⁻¹, 269 respectively) compared to the 'Exploter' (3.0 U mmol min⁻¹ g⁻¹). In addition, 'Apollo' and 'Spinoso 270 271 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 3.1.1.1) 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.

Storage time resulted the predominant factor influencing colour changes of ready-to-use globe artichoke slices, showing the highest percentage of total variation for all the measured colour parameters (L*, a*, b*) and for the calculated ones (C* and *h*), with higher percentages for L* (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 × 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 'Explorer', 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 'Explorer' 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

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

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

Source of veriation	Degree	Biochemical parameter							
Source of variation	freedom	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***	$1.7^{\rm NS}$
(H) x (S)	3	8.5 ^{NS}	8.3 ^{NS}	$0.8^{ m NS}$	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

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

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 $P \le 0.001$, $P \le 0.01$ and $P \le 0.05$, and NS, not significant.

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	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)	$\mathbf{AsA} (g kg^{-1} DW)$	PPO (U mmol $\min^{-1} g^{-1}$)
Genotype								
'Apollo'	$22.9^{a}\pm2.3$	$72.3^{a}\pm6.5$	$21.7^{a}\pm2.5$	$16.1^{a}\pm2.6$	$32.2^{a}\pm4.5$	224.3 ^a ±31.3	0.6^{a}	$6.7^{a}\pm0.6$
'Exploter'	$19.9^{b}\pm2.5$	$74.9^{a}\pm 6.8$	$19.5^{a}\pm2.0$	$13.5^{a}\pm2.4$	$20.7^{b}\pm2.2$	$118.2^{b} \pm 12.4$	0.4°	2.7°
'Spinoso di Palermo'	$22.6^{a} \pm 1.9$	$72.1^{a} \pm 7.2$	$20.4^{a}\pm2.3$	$15.1^{a}\pm1.9$	$30.8^{a} \pm 3.5$	$230.6^{a} \pm 35.7$	0.6^{b}	$5.6^{b} \pm 0.4$
<u>Storage time (days)</u>				L. L.	\mathbf{O}			
0	$22.2^{a}\pm2.3$	$76.6^{a} \pm 7.4$	$25.0^{a} \pm 3.0$	13.7 ^b ±1.9	$29.7^{a}\pm4.4$	$266.8^{a} \pm 30.4$	1.0^{a}	2.5 ^c
4	$21.4^{a}\pm 2.6$	$75.1^{ab}\pm5.5$	$20.7^{b} \pm 3.0$	$14.9^{b}\pm2.4$	$29.6^{a}\pm4.0$	$215.4^{b}\pm 26.5$	0.4^{b}	$5.6^{b}\pm0.2$
7	$21.3^{a}\pm2.4$	$70.7^{b} \pm 7.1$	$18.9^{bc} \pm 1.5$	$13.4^{b}\pm2.0$	$25.1^{a}\pm3.3$	$173.0^{\circ} \pm 15.3$	0.3 ^c	$6.2^{a}\pm0.9$
11	$22.3^{a}\pm2.5$	$70.1^{b}\pm 5.3$	$17.8^{\circ}\pm2.2$	$17.7^{a}\pm2.2$	27.1 ^a ±3.9	$109.0^{d} \pm 6.4$	0.3 ^c	$5.7^{b}\pm0.6$
Harvest time								
March 2014	$23.3^{a}\pm2.7$	$71.0^{b} \pm 6.4$	18.3 ^b ±2.8	$11.1^{b} \pm 1.6$	$24.3^{b}\pm3.1$	$153.4^{b}\pm21.4$	0.4^{b}	$5.0^{a}\pm0.7$
April 2014	$20.3^{b} \pm 3.0$	$75.2^{a}\pm 6.6$	22.9 ^a ±2.7	$18.7^{a}\pm0.8$	$31.5^{a}\pm 5.2$	$228.7^{a} \pm 30.3$	0.7^{a}	$5.0^{a}\pm0.7$

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* \leq 0.05).

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

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Source of variation	Degree	Colour parameter						
Source of variation	freedom	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		

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

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

	Colour parameter					
	L*	a*	b*	C*	h	
<u>Genotype</u>						
'Apollo'	$36.8^{b}\pm2.4$	0.2°	$10.9^{a} \pm 1.1$	$11.0^{a} \pm 1.1$	86.8 ^b ±3.7	
'Exploter'	$37.7^{a}\pm1.8$	0.4^{b}	$10.3^{b} \pm 1.7$	$10.4^{b} \pm 1.7$	87.3 ^a ±1.7	
'Spinoso di Palermo'	37.0 ^b ±2.8	0.6 ^a	$10.6^{b}\pm0.9$	$10.6^{b}\pm0.9$	$86.7^{b} \pm 1.5$	
<u>Storage time (days)</u>						
0	$40.1^{a}\pm2.0$	0.8^{a}	13.1 ^a ±1.3	$13.2^{a}\pm1.4$	84.5 ^d ±3.4	
4	$37.2^{b}\pm1.4$	0.2°	10.2 ^b ±0.9	10.2 ^b ±0.9	88.0 ^b ±1.6	
7	35.8°±1.0	0.2°	9.7°±1.3	9.7°±1.3	88.5 ^a ±0.8	
11	$35.6^{c} \pm 1.5$	0.4^{b}	$9.4^{c}\pm1.6$	$9.5^{\circ} \pm 0.6$	86.8 ^c ±1.1	
<u>Harvest time</u>						
March 2014	$36.4^{b}\pm1.9$	0.4^{a}	$9.9^{b} \pm 0.9$	$9.9^{b}\pm0.9$	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	$87.1^{b} \pm 1.8$	

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*, $P \le 0.05$).

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' **A**'Explorer' 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.

 \blacksquare = March; \blacksquare = 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.

[.] $\bullet = March; \bullet = April.$

- 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.