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Abstract

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Keywords	Vitamin C; total phenolics; lycopene; CIE L*a*b*; mechanical compression test; Citrus paradise.
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Stability of the physical properties, bioactive compounds and antioxidant capacity of spray-dried grapefruit powder

Running title: Properties of spray-dried grapefruit powder with storage

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15 **Abstract**

16 Spray-drying may be an interesting alternative means of offering consumers high quality,

17 stable, and easy-to-handle fruit. The stability of grapefruit powder formulated with gum Arabic,

18 maltodextrin and whey protein isolate was studied. The changes during powder storage at 20ºC

19 of the vitamin C (VC), total phenolics (TP), lycopene (Lp) , antioxidant activity (AOA), color

20 and mechanical properties were studied at different relative humidities (RH), from 0 to 56% for

21 up to 9 month, either exposed to light or in darkness. Results showed that TP were the most

22 stable compounds and Lp the most unstable. The properties studied with grapefruit powder

23 were relatively stable when stored at 20ºC, in darkness or light, at RH $\leq$23.1% and for no more

24 than 6 months. With these conditions, losses of 32, 3, 23-68 and 90% were observed for TP,

25 VC, AOA and Lp, respectively, and the powder maintained its flowability and color.

26

29

1. Introduction

The production of powdered food and food ingredients is an increasingly important industrial activity, given the high stability and ease of handling they provide (Fitzpatrick and Ahrné, 2005). The consumption of fresh fruit is declining in part due to its short lifespan and/or the sometimes special handling needed, which decreases their convenience, barely compatible with the current lifestyle. Fruit powder may be an interesting alternative means of promoting fruit consumption among consumers, easy to store and use. Nevertheless, the process used to obtain the powder should ensure the maximum quality of the product obtained. Despite the stability of the healthy components, it is important to know more about the powders' physical properties. High quality powder products can be obtained in terms of their sensory, nutritional and functional properties using spray-drying (Nandiyanto and Okuyama, 2011). In addition, these powders are very fine, with a homogeneous particle size, low water activity and, in the case of fruit powders, with good reconstitution properties. Furthermore, this technique is easy to industrialize and permits continuous production (Igual et al. 2014).

As regards the physical properties of the fruit powder obtained, color is of great importance when choosing a food and the flowability is important in handling and processing operations (Teunou et al. 1999). Both the color and, to a greater extent, the mechanical properties, will be influenced by the water content of the powder, depending on the relative humidity (RH) of the surrounding environment (Roos, 1995). If the water activity (a_w) of the food is lower than the RH/100, the food will gain water and if it is higher, it will lose it. On the other hand, with rapid dehydration processes, such as spray-drying, it is very common to obtain an amorphous matrix, glassy or rubbery, depending on the final water content of the product and the temperature at which it is stored (Roos, 1995). The matrix in the glassy state is much more viscous than in the rubbery state, which affects the diffusional and mechanical properties of the product.

54   Powdered food in the rubbery state can undergo structural collapse and show stickiness and

55   caking problems (Roos, 1995). In dehydrated fruits, with a high content of organic acids and

56   low molecular weight sugars, the rubbery state prevails with the usual storage conditions (Telis

57   and Martínez-Navarrete, 2009). To promote the easy handling and stability of the glassy state,

58   some compounds can be added to the product before drying. The use of high molecular weight

59   biopolymers capable of increasing the glass transition temperature such as maltodextrins,

60   modified starches or gums, for instance, or biopolymers with a steric role, such as fibers,

61   proteins or some inorganic compounds, has been reported (Telis and Martínez-Navarrete, 2009;

62   Ghosal et al. 2010). These biopolymers prevent the adhesion of powder particles, not only to

63   each other but also to the equipment itself, increasing the yield and avoiding operational

64   problems. In addition, at the same time they may act as encapsulating agents, helping to prevent

65   the degradation of some bioactive compounds (Rascón et al. 2011).

66   Grapefruit has been reported to be a rich source of bioactive phytochemical constituents with

67   antioxidant properties that, independently or jointly, could be responsible for the health-

68   protective effects of this fruit (Igual et al. 2010; La Cava and Sgroppo, 2015; Cristóbal-Luna et

69   al. 2017; Zou et al. 2015). Ascorbic acid (AA) is the main citrus fruit compound with

70   antioxidant capacity and may prevent oxidative stress mediated diseases (Gardner et al. 2000).

71   Flavonoids are phenolic compounds associated with a reduced risk of coronary heart disease,

72   anti-inflammatory and anti-tumor effects (Fujita et al. 2008; Kim et al. 2008; García-Martínez

73   et al. 2018). Naringin is the main flavonoid in grapefruit juice and it is responsible for its bitter

74   taste. In pink grapefruit varieties, β-carotene and lycopene, the latter being the most abundant,

75   are responsible for the color and contribute to the health benefits by decreasing the risk of some

76   cancers and eye diseases (Jomova and Valko, 2013).

77   The objective of this study was to learn more about the effects of spray-drying on some of the

78   bioactive compounds of the liquidized grapefruit (L), formulated with gum Arabic (GA),

79 maltodextrin (MD) and whey protein isolate (WPI), and the powder stability with storage.

80 Different storage conditions were tested by varying RH, exposure to light and time. The changes

81 of vitamin C (VC), total phenolics (TP), lycopene (Lp), antioxidant activity (AOA), color and

82 mechanical properties were measured.

83

84 **2. Material and methods**

85 **2.1 Raw material**

86 Pink grapefruit (*Citrus paradisi* var. Star Ruby) from Murcia (Spain) was purchased from a local

87 supermarket in Valencia (Spain). The selection of the fruit pieces was made by visual

88 appearance on the basis of a similar size (80-90 mm diameter), color and the absence of any

89 physical damage on the surface. The samples were formulated by incorporating GA (Scharlau

90 SL, Barcelona, Spain), MD (Sigma Aldrich, Darmstadt, Germany) and WPI Lacprodan® DI-

91 9213, with both fat and lactose <0.2 and protein around 90% (d.m.) (Arla, Viby, Denmark), as

92 carriers for the drying process and to stabilize the products.

93 **2.2 Sample preparation**

94 Grapefruits were washed with tap water, manually peeled with the careful removal of the albedo

95 and liquidized at speed 1 in an electrical food processor of 180 W and 500 g capacity

96 (DeLonghi, Barcelona, Spain). The liquid was sieved through 0.7 mm mesh (CISA, 200/50,

97 Barcelona, Spain), to ensure the absence of any pulp. To 100 g of L, 9.4 g of GA, 1.44 g of WPI

98 and 1.25 g of MD were added, according to a formulation optimized in a previous study (Egas

99 et al. 2015). The solutes were incorporated slowly using a stirrer (Heidolph, RZR2020,

100 Schwabach, Germany), working at between 800-1200 rpm, until visual homogeneity was

101 achieved. To obtain the fruit powder, this sample (LS) was spray-dried in a Büchi mini spray-

102 dryer (B-290, Flawil, Switzerland) with the following operating conditions: aspirator rate 35

103 m^3/h, feed rate 9 ml/min, atomization air rotameter 473 l/h with co-current flow, drying air inlet

temperature 148°C and pressure $5 \cdot 10^5$ Pa. The powder sample obtained (P_0) was collected from the product collection vessel, weighed, analyzed and stored as described below.

The powder was conditioned in different environments using hermetic bisphenol A free polypropylene containers (EMSA, Emsdetten, Germany), with a capacity of 3.7 l, acquired in a department store in Valencia (Spain). In each vessel, a glass with a saturated salt solution was arranged to ensure a controlled and constant RH. The salts used (Scharlab SL, Barcelona, Spain) and the RH obtained, at 20ºC, were: lithium chloride (RH = 11.3%), potassium acetate (RH = 23.1%), magnesium chloride (RH = 33.1%), potassium carbonate (RH = 43.2%) and magnesium nitrate (RH=55.9%) (Greenspan, 1977). A series with each of these 5 containers was placed in a Binder chamber (KBF720, Hechingen, Germany) to ensure darkness and another series was placed in a Nüve Test Gabinet chamber (TK120, Istanbul, Turkey) with artificial 6500K daylight emitted by 6000 Lx fluorescent tubes (Feilo Sylvania Europe LTD, Newhaven, UK), both at 20ºC. In each hermetic container, 6 aluminum plates (55 mm diameter, 1 mm height) were placed with approximately 10 g of P_0. In addition, another plate with 10 g of P_0 that was vacuum packaged (Edesa machine vac-20 SL, Guipúzcoa, Spain) with a transparent polyethylene bag (Productos Pilarica SA, Paterna, Spain), was included in both the dark and light series.

All of the samples of each series were analyzed at 30, 90, 180 and 270 days for TP, VC, Lp and AOA, as well as their mechanical properties and color, as described below.

2.3 Analytical determinations

The water content of L and P_0 was determined, in triplicate, using the gravimetric method in a vacuum oven (Vaciotem, JP Selecta, Barcelona, Spain) at 60°C, p <100 mm Hg until constant weight. Six replicates were made (bioactive extractions in triplicate, analyses in duplicate) for the chemical analyses described below. They were carried out on L, LS, P_0 and on the powders stored using the different conditions. As these samples have a different composition as regards

129   water and added solutes, they were standardized to the grapefruit's own solutes, GS (Equations

130   1 and 2) to make the results comparable (Agudelo et al. 2016).

131

132
$$m_i = \frac{m_i^p}{(1-x_w^p) \cdot x_{GS/TS}}$$
(1)

133

134
$$x_{SP/ST} = \frac{m_L \cdot (1-x_w^L)}{(m_{GA}+m_{MD}+m_{WPI}+m_L) \cdot (1-x_w^L)}$$
(2)

135

136   where m_i is the mass of each analyzed compound standardized to grapefruit solutes (mg/g_{GS}),

137   m_i^p is the mass of each compound analyzed in the powder (mg/g), following sections 2.3.1 to

138   2.3.4, x_w^p is the water content of the powder (g_{water}/g_{powder}) analyzed as previously described,

139   $x_{GS/TS}$ is the mass fraction of GS to TS (total solids), m_L, m_{GA}, m_{MD} and m_{WPI} are the mass of

140   L, GA, MD and WPI, respectively, in the sample and x_w^L is the water content of L

141   (g_{water}/$g_{liquidized}$) analyzed as previously described.

142   The losses of each analyzed compound due to the addition of solutes (comparing sample L with

143   LS), spray-drying process (comparing sample LS with P_0) or storage (comparing sample P_0

144   with the powder stored at each condition) were calculated and expressed as % loss (Equation

145   3).

146
$$\% \, loss = 100 \frac{(m_i^B - m_i^A)}{m_i^B}$$
(3)

147

148   where m_i^B is the mass of each analyzed compound standardized to grapefruit solutes

149   (mg/g_{GS}) before the corresponding process, m_i^A is the mass of each compound standardized

150   to grapefruit solutes (mg/g_{GS}) analyzed after the corresponding process.

151   **2.3.1 Total phenolics**

152   Extracts for TP were prepared by mixing 1 g of sample with 9 ml of methanol:water (70:30)

153   using a magnetic multistirrer at 400 rpm (Velp Scientifica, Usmate Velate, Italy) in the dark at

154 20ºC for 30 min. The homogenates were centrifuged at 5870 x g at 4ºC for 10 min (Eppendorf

155 5804 R, Wesseling-Berzdorf, Germany). The supernatants were collected and TP was analyzed

156 using the Folin–Ciocalteu colorimetric method (Benzie and Strain, 1999). An aliquot of 250 µl

157 extract was mixed with 15 ml of distilled water and 1.25 ml of Folin–Ciocalteu reagent (Sigma-

158 Aldrich). After 8 min, 3.75 ml of 7.5% anhydrous Na_2CO_3 (Scharlab SL) aqueous solution were

159 added and water was added to adjust the final volume to 25 ml. Absorbance was measured at

160 765 nm (UV-visible V-1200 VWR International Eurolab S.L, Barcelona, Spain) after 2 h of

161 incubation at room temperature in the dark. The TP content was expressed as mg of gallic acid

162 equivalents (GAE)/100 g_{GS}) (Equations 1 and 2) using a standard curve range of 0–1000 ppm

163 of gallic acid (Sigma-Aldrich).

164 **2.3.2 Vitamin C**

165 VC was determined using high-performance liquid chromatography (HPLC) (Jasco, Cremella,

166 Italy). To quantify the total VC content, dehydroascorbic acid was reduced to ascorbic acid

167 (AA) by mixing 0.5 g powder with 2 ml of a 20 g/l DL-dithiothreitol solution (Scharlab SL) for

168 2 h at room temperature and in the dark (Sánchez-Mata et al., 2000; Sánchez-Moreno et al.,

169 2003). Afterwards, 1 g of this mixture was extracted with 9 ml 0.1% oxalic acid (Scharlab SL)

170 with manual stirring for 3 min and filtered through a 0.45 µm nylon membrane filter (VWR,

171 Radnor, PA, USA) before injection (Xu and Chang, 2007). The HPLC conditions were:

172 Kromaphase100-C18, 5 mm (4.6 x 250 mm) column (Scharlab SL); mobile phase 0.1% oxalic

173 acid, volume injected 20 µL, flow rate 1 ml/min, detection at 243 nm (detector UV-visible

174 MD-1510) at 25ºC. A standard solution of L(+) ascorbic acid (Scharlab SL) in the range of 10-

175 530 ppm was prepared. The VC content was calculated as mg AA/100 g_{GS} (Equations 1 and 2).

176 **2.3.3 Lycopene**

177 Lp was extracted using the methodology recommended by Olives et al. (2006) with some

178 modifications. Briefly, 1 g of the powder was mixed with 9 ml of hexane/acetone/ethanol

179 (50:25:25, v/v/v) for 30 min with magnetic stirring (400 rpm) in the dark. The homogenates

180 were centrifuged at 5870 x g at 4ºC for 10 min and the supernatants were collected. Distilled

181 water was added (15 ml/10 ml of supernatant) and mixed with manual stirring for 2 min in the

182 dark. An upper layer aliquot was taken for spectrophotometric analysis (AOAC, 1990), at 501

183 nm. The Lp content was expressed as mg Lp/100 g_{GS}) (Equations 1 and 2). A standard solution

184 of Lp (Cayman Chemicals, Ann Arbor, MI, USA) in the range of 0.5-10 ppm was prepared.

185 **2.3.4 Antioxidant capacity determinations**

186 The AOA of the methanolic extract obtained for the quantification of TP was determined with

187 the DPPH and FRAP tests. AOA was measured using the free radical scavenging activity with

188 the stable radical 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) (Puupponen-pimiä et al.

189 2003). Briefly, the absorbance at 515 nm of 3.9 ml of the DPPH reagent (0.030 g/L, Sharlau

190 S.L, Barcelona, Spain) (absorbance at initial time, $A_{control}$) was measured. Then 0.1 ml of the

191 extract was added and the absorbance was measured again at 5 min, when the reaction had

192 reached the steady state (A_{Sample}).

193 The percentage of DPPH was calculated using Equation 4. The final results were converted to

194 mmol trolox equivalents (TE)/100 g_{GS} (Equations 1 and 2) using a trolox (Sigma-Aldrich)

195 calibration curve in the range of 8-125 ppm.

196

197
$$\%DPPH = \frac{(A_{Control} - A_{Sample})}{A_{Control}} \times 100 \tag{4}$$

198

199 For the ferric reducing ability of samples, the FRAP assay was used (Benzie and Strain, 1999).

200 The FRAP solution was prepared by mixing 2.5 ml 10 mM TPTZ (2,4,6-tripyridyl-s-triazine)

201 solution (in 40 mM HCl), 2.5 ml 20 mM $FeCl_3 \cdot 6H_2O$ and 25 ml 0.3M acetate buffer (pH 3.6).

202 For the analysis 30 µL extract, 30 µL water and 900 µL of the FRAP solution (kept at 37ºC

203 throughout the whole analysis) were mixed and allowed to react for 30 min at 37ºC in the dark.

204   Absorbance of the colored product (ferrous tripyridyltriazine complex) was then measured at

205   593 nm. Results were expressed as mmol TE/100 g_{GS} (Equations 1 and 2), using a trolox

206   (Sigma-Aldrich) calibration curve in the range of 8-125 ppm.

207   **2.3.5 Mechanical properties**

208   Each of the samples conditioned at the different RH and the vacuum-packed sample were

209   placed, at established times, in a circular aluminum sample holder of 11 mm in diameter and

210   5.5 mm in height, which was completely filled. Mechanical compression tests were done using

211   a universal texture analyzer TA- TXT2 (Stable Micro Systems, Ltd., Godalming, UK). A

212   cylindrical probe of 10 mm in diameter at a deformation rate of 0.1 mm/s for 3 mm was used

213   for this purpose. The maximum force attained during the test (Fmax) was selected as the

214   characteristic mechanical parameter. This assay was done in quintuplicate.

215   **2.3.6 Color**

216   The sample holder containing the compressed sample was then used to measure the color by

217   placing a low reflectance glass plate CR-A51 (Konica Minolta, Valencia, Spain) in between the

218   sample and the spectrophotomer CM 3600-D (Konica Minolta) and providing a measurement

219   window of 5 mm in diameter. CIE L*a*b* color coordinates were obtained by using the D65

220   illuminant and a 10º observer. In this color space, L* indicates the sample light/darkness, a*

221   and b* being the chromatic coordinates on a green (−) to red (+) and blue (−) to yellow (+) axis,

222   respectively. These coordinates allowed for the calculation of the color attributes, hue angle

223   (h^*_{ab}, Equation 5) and the chrome or color purity (C^*_{ab}, equation 6). The global color difference

224   (ΔE, Equation 7) of the samples stored under the different conditions commented on above and

225   the newly-obtained one was also calculated. This assay was done in triplicate.

226

227   (5)

$$h^*_{ab}=arctg\frac{b^*}{a^*}$$

228

229

$$C^*_{ab} = \sqrt{a*^2 + b*^2} \qquad (6)$$

231

$$\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \qquad (7)$$

234

2.3.7 Statistical analysis

Data are expressed as the mean value and standard deviation of the different replicates. Multifactor analyses of variance (MANOVA) were carried out at 0.05 significance level to evaluate the differences between the samples brought about by storage conditions: RH, time and presence or absence of light. Furthermore, a Pearson's correlation analysis between the bioactive compounds, AOA and ΔE was carried out at a 95% significance level. All of the statistical analyses were done using Statgraphics Centurion XVI.II (® 2010 StatPoint Technologies, Inc., Warrenton, VA, USA).

3. Results and discussion

3.1 Bioactives and antioxidant activity of grapefruit samples

The water content of L batch was x_w 91 ± 1 g/100 g and that of P_0 1.5 ± 0.7 g/100 g. The concentration of each bioactive compound analyzed and the AOA of L, LS and P_0 are shown in Table 1. The TP, VC and Lp content of the L are consistent with those reported in other grapefruit studies (Toh et al. 2013; Igual et al. 2015). The addition of solutes produced a significant reduction ($p < 0.05$) in Lp (10% loss). The instability of carotenoids is due to the fact that they are highly unsaturated compounds, whose degradation is fundamentally due to oxidative processes. Other factors, such as temperature, light or pH, can also produce important qualitative changes in these compounds as a consequence of isomerization reactions (Meléndez-Martínez et al. 2010). In the case of citrus juices, some studies stated that

255 isomerization is favored by the loss of compartmentation brought about by the squeezing of the

256 citrus, which brings together organic acids and carotenoids (Vanamala et al. 2005; Meléndez-

257 Martínez et al. 2009). It has been shown that oxidation processes are more pronounced when

258 the cellular integrity is lost, so that in crushed plant foods, the loss of cellular compartmentation

259 brings into contact substances that can structurally modify and even destroy the pigments

260 (Meléndez-Martínez et al. 2010). During the stirring process for the purposes of blending the

261 carriers, the incorporation of oxygen into the samples and the additional cellular breakage may

262 have caused the direct exposure of carotenoids to oxygen and other substances that may

263 accelerate their loss. The addition of solutes did not affect TP and VC content even though it

264 produced a significant reduction ($p < 0.05$) in the AOA of LS, regardless of the measurement

265 method used.

266 With respect to the spray-drying process, neither VC nor TP were significantly affected (>90%

267 retention). Different authors concluded that one disadvantage of spray-drying is the high

268 operation temperature, as many bioactive compounds are sensitive to heating (Đorđević et al.

269 2016). However, it may be possible to increase the stability of sensitive compounds during

270 processing and to improve the quality of the finished product by adding carriers or drying

271 excipients like those selected in this study (Murugesan and Orsat, 2012). Nevertheless,

272 obtaining the powder product caused a significant ($p < 0.05$) drop in the Lp content to 71%. A

273 significant ($p < 0.05$) loss in the AOA of the powder was also observed.

274 **3.2 Changes in phytochemical compounds and antioxidant capacity as a function of**

275 **relative humidity, light condition and storage time**

276 Figures 1 to 5 show the changes of each bioactive compound and AOA with different storage

277 conditions. The MANOVAS carried out with each of the three factors considered determined a

278 significant effect ($p < 0.05$) of RH, time and light condition in all the parameters studied, except

279 for Lp which were not affected by this last factor. For each significant factor, the result of

280   MANOVAS Multiple Range Tests, showing which means are significantly different from

281   which others, is shown in Figures 1 to 5. Nevertheless, in all the cases the MANOVAS also

282   showed significant interactions ($p < 0.05$) among the factors, which are discussed below.

283   Interaction plots are not shown to not increase the number of figures shown.

284   The TP were better preserved in darkness. Except for the vacuum stored sample, the phenolic

285   content decreased until 90 or 180 days, depending on the RH. After this time, the TP remained

286   constant at the lowest RH, while at RH >33.1% they seemed to increase until the end of the

287   storage, regardless of light. Taking into account that phenolic compounds act as substrates in

288   various types of reactions, their stability or degradation will depend on the molecular complexes

289   that they can form. In this sense, autoxidation reactions caused by exposure to light or oxygen

290   may result in the formation of phenol radicals that may subsequently react with other radicals,

291   forming dimers or new structures, depending on the precise location of the electrons in the

292   reaction over time (Fraga et al. 2010). The vacuum-packed grapefruit powder that was stored

293   in darkness was the only sample that preserved its TP content the entire time.

294   During storage and with increasing RH, the Lp showed a declining trend. Storage conditions

295   have an essential role in preserving the carotenoid content of processed food (Nagarajan et al.

296   2017). In this case, losses occurred mostly during the first month of storage, regardless of light

297   and RH (losses of 75-95%). Other studies showed that light exposure had minor effects on the

298   loss of total Lp in tomato puree (Shi et al. 2008). After 30 days of storage, the Lp remained

299   constant when stored under vacuum conditions and at 11.3% RH until 90 days. A certain Lp

300   decrease was observed from 23.1% RH upwards and it became completely degraded at the

301   highest RH (43.2 and 55.9%) after 6 months of storage. As commented on above, the

302   degradation of carotenoids is mainly due to oxidation reactions. Water availability, which

303   increases as the RH rises, is also a parameter that greatly affects these compounds (Nagarajan

304   et al. 2017). The influence of water content on the degradation of lipophilic compounds seems

305 to be related to the availability of water, from a determined water content, to participate in

306 degradative reactions or to act as a vehicle that allows the mobility of the different substrates

307 involved, oxygen among others (Lavelli et al. 2007; Moraga et al. 2012).

308 As regards VC, regardless of the light condition and at a RH <33.1%, its content remained

309 stable throughout the 180 days of storage, but decreased over the range of RH from this value

310 up to 55.9%. The greatest losses of this bioactive component occurred in environments with the

311 higher RH. The water content of the grapefruit powder with this level of a_w could be considered

312 high enough to provoke an increase in the degradation reactions of AA at the storage time

313 studied. The various reactions involved in the final phases of ascorbate degradation are

314 important in the formation of flavor compounds and as precursors of non-enzymatic browning

315 (Belitz et al. 2009). In this case, as with carotenoids, light does not seem to affect the

316 degradation of this vitamin. AA is easily oxidized, especially in aqueous solutions, and greatly

317 favored by the presence of oxygen, heavy metal ions, especially Cu^{2+}, Ag^+, and Fe^{3+}, and by

318 alkaline pH and high temperatures (Du et al. 2012).

319 The grapefruit maintained its AOA measured using the DPPH assay until 90 days of storage at

320 RH <33.1% and V, regardless of light. From that moment on, the AOA decreased until the end

321 of storage. Similar results were observed from the FRAP analysis, but in this case, the AOA

322 values were stable only during the first month and in darkness.

323 There is some controversy about the influence of the phytochemicals present in fruits and

324 vegetables with their AOA (Guo et al. 2003). Polyphenols have been reported as responsible

325 for the antioxidant activity of citrus fruits due to their redox characteristics, which allow them

326 to act as reducing agents, hydrogen donors, singlet oxygen quenchers, and even metal chelators

327 (Carocho and Ferreira, 2012). On the other hand, carotenoids show antioxidant characteristics

328 through quenching $^1O_2$ and eliminating harmful free radicals, while VC can effectively

329 scavenge a variety of reactive oxygen species (ROS) and give off semi dehydroascorbic acid,

330 removing $^1O_2$ and reducing sulfur radicals (Zou et al. 2016). Chemical interactions affecting

331 free radical scavenging properties between hydrophilic and lipophilic compounds have not been

332 extensively reported in fruits and vegetables, yet both synergistic and antagonistic interactions

333 may affect antioxidant capacity (Talcott et al. 2003). In this study a statistical correlation was

334 carried out to determine the relationship among the bioactive compounds quantified in the

335 samples and with AOA (Table 2). The results showed that all the bioactive compounds analyzed

336 in the grapefruit powder, both the hydrophilic and the lipophilic ones, showed a positive

337 significant ($p < 0.05$) correlation among them and with the AOA measured both by using the

338 DPPH and FRAP assays, indicating a complementary and synergistic antioxidant behavior (Zou

339 et al. 2016). The greatest contribution to the AOA was provided by the Lp and TP, followed by

340 VC. The results obtained in this work are consistent with other studies (Igual et al. 2015).

341 **3.3 Changes in mechanical properties and color as a function of relative humidity, light**

342 **condition and storage time**

343 The mechanical properties of a powdered product are related to its ability to be compacted and

344 its negative influence on flow capacity. Compression tests have been widely used in the field of

345 food powders as a simple and convenient method to measure some physical properties such as

346 powder compressibility and flowability (Barbosa-Cánovas et al. 2005). In powder technology,

347 great attention has been paid to the general behavior of powders under compressive stress. The

348 force–distance relationship obtained from the compression test carried out on the different

349 samples was similar to that shown in Figure 6. The compression process of a food powder takes

350 place in two stages: filling voids with particles equal in size or smaller than the voids brought

351 about by particle movement, and filling smaller voids with the particle's elastic and/or plastic

352 deformation, or fragmentation. If the free flow of the particles predominates during

353 compression, it will be necessary to apply greater force to achieve their compaction, as it occurs

354 in samples stored at RH 11.3 and 23.1% (Figure 6). However, when the deformation of the

355   particles predominates (samples stored at RH 55.9%, Figure 6), the force observed during

356   compression will be weaker.

357   The Fmax values of the powder, stored as previously described, are shown in Figure 6. A

358   significant effect ($p < 0.05$) of both RH and time was observed, although, as expected, there

359   were no differences between samples whether stored in the presence or absence of light. The

360   Fmax value of the sample stored for 30 days at RH 33.1% is in the order of that shown by the

361   newly spray-dried powder, which means that $P_0 a_w$ may be ~0.331. In general terms, Fmax was

362   greater at each storage time in the samples stored under vacuum and at RH $\leq$23.1%, and

363   decreased at higher RH. The increase may be related to a certain water loss at the lower RH,

364   which increases the powder flowability. The decrease in the Fmax value may be related to the

365   transition of the amorphous matrix from the glassy to the rubbery state, this being responsible

366   for the structural collapse of the powder associated with the development of stickiness and a

367   softening of the product (Telis and Martínez-Navarrete, 2010). In this way, the a_w of the powder

368   has to be equal to or lower than 0.231 to ensure the flowability of the powder. Despite this, as

369   the glass transition is a time dependent phenomenon (Roos, 1995), the Fmax decreases over

370   storage time at any RH so that no more than 6 months of storage are recommended for the

371   purposes of maintaining the initial mechanical properties of the powder.

372   As regards the color of the grapefruit powder (Table 3), on the whole it was affected more by

373   the storage time and the RH than by the presence or absence of light. In every case, a darkening

374   of the powder (decrease in L*) was measured after 3 months of storage, especially at the highest

375   RH, as was an evolution towards more yellowish tones (increase in hue angle) from the first 30

376   days onwards when RH was higher than 22.1%. The chrome, or color purity, was the only color

377   attribute that was dependent on the presence or absence of light, the samples stored in the

378   absence of light being purer. The chrome of the samples also increased at the highest RH, as

379 much as the storage time increases. An increase in chrome above powder collapse has also been

380 described by Telis and Martínez-Navarrete (2009); as related to porosity loss.

381 The aforementioned color evolution resulted in a global color change of the powder with respect

382 to the newly obtained one (Figure 7), which is evident ($\Delta E \geq 3$, Bodart et al. 2008), before 90

383 days only if the RH of the sample environment is $\geq 43.2\%$ and, from 6 months of storage

384 onwards, at any other RH. All of this takes place regardless of the presence or absence of light.

385 The color stability at low a_w and the increase in color changes at intermediate a_w values have

386 been related to the enzymatic browning of the samples, maximum at a_w of around 0.5 at which

387 level the conditions of diffusion and concentration of oxidized phenols are optimal (Venir et al.

388 2007; Telis and Martínez-Navarrete, 2009). Nevertheless, the diffusion of reactants requires a

389 certain time before they coincide and the reaction occurs.

390 The color change was related to the bioactive compound content by means of the Pearson's

391 correlation (Table 2). A significant correlation was found with VC and Lp, but not with TP.

392 The carotenoid structure, the length of the chromophore, the arrangement of conjugated

393 double bonds in the end ring and the geometrical (cis/trans) isomers of carotenoids all

394 influence its perceived color (Meléndez-Martínez et al. 2010). Carotenoids show yellowish

395 to reddish colors, with Lp contributing more to the yellow hue and carotene more to the red.

396 The increase in hue could be justified by assuming not only the observed Lp loss but also the

397 β-carotene loss (Agudelo, 2017). The storage time related to the presence of oxygen,

398 especially in combination with light and temperature, can lead to oxidative degradation

399 (Rodriguez-Amaya and Kimura, 2004). As the vacuum-stored grapefruit also showed Lp loss,

400 both with light and in darkness, storage temperature can be assumed to exert a great influence.

401 Agudelo (2017) observed that the β-carotene loss at 4ºC was half of that at 20ºC. On the other

402 hand, both carotenoid isomerization and oxidation reactions, together with AA oxidation

403 products, lead to color change (Du et al. 2012; Sant'Anna et al. 2013).

404

4. Conclusions

The TP, VC and Lp content of grapefruit contributed to its antioxidant capacity. Spray-drying operations, when used with the same conditions as in this study, together with the added solutes led to an overall preservation of TP and VC, while Lp was found to be very unstable both during processing and storage. Bioactive compounds, together with the mechanical properties and color of the spray-dried grapefruit powder achieved a high degree of stability when stored at 20ºC, being the recommended choice using a surrounding RH equal to or lower than 23.1% and for no more than 6 months. In these conditions, the global color change of the powder will not be evident to the human eye, the powder flowability will be ensured, none of the VC would be lost, despite more than 68% of the TP and only 10% of Lp would remain.

Conflict of interest

None.

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573 **Table 1.** Mean values (with standard deviation) of total phenolic content (TP), vitamin C (VC),
574 lycopene (Lp) and antioxidant capacity (DPPH, FRAP) of liquidized grapefruit (L), liquidized
575 grapefruit with solutes mixture (LS) and spray-dried powder (P_0).

Sample	L	LS	P_0
TP [1]	590 (70)[a]	590 (50)[a]	570 (10)[a]
VC [2]	740 (40)[a]	750 (10)[a]	710 (30)[a]
Lp [3]	32 (1)[a]	29 (1)[b]	8.3 (0.2)[c]
DPPH [4]	1.8 (0.1)[a]	1.51 (0.05)[b]	1.1 (0.1)[c]
FRAP [4]	5.0 (0.4)[a]	4.0 (0.4)[b]	1.33 (0.04)[c]

576 Different letters within each row indicate significant differences ($p < 0.05$).
577 [1] mg of gallic acid equivalents/100 g grapefruit solutes, [2] mg ascorbic acid
578 /100 g grapefruit solutes, [3] mg lycopene/100 g grapefruit solutes, [4] mmol trolox
579 equivalents/100 g grapefruit solutes.

580

581 **Table 2.** Pearson's correlation coefficients among total phenols (TP), lycopene (Lp), vitamin
582 C (VC), antioxidant capacity (DPPH and FRAP analysis) and color differences (ΔE) of the
583 powder samples with respect to the initial powder P_0.

584 .

	DPPH	FRAP	TP	VC	ΔE
TP	0.7728*	0.7813*	-	0.5514*	-0.2223
Lp	0.9251*	0.9729*	0.8382*	0.5967*	-0.7642*
VC	0.5990*	0.5737*	-	-	-0.6222*

585 *Correlation is significant at a significance level of $p < 0.05$

586

Table 3. Luminosity (L*), hue angle (h*) and chroma (C*) of grapefruit powder stored for different time periods (t, days) at different relative humidities (RH).

	t \ RH	V[(2)]	11.3%	23.1%	33.1%	43.2%	55.9%
L*[(1)]	30	79 ^{b,A}	79 ^{b,A}	78 ^{c,A}	80 ^{b,AB}	81 ^{c,B}	79 ^{c,AB}
	90	78 ^{b,A}	79 ^{b,A}	78 ^{c,A}	80 ^{b,A}	79 ^{c,A}	81 ^{c,A}
	180	71 ^{a,C}	71 ^{a,C}	73 ^{b,C}	69 ^{a,B}	68 ^{b,B}	66 ^{b,A}
	270	72 ^{a,C}	73 ^{a,C}	67 ^{a,B}	71 ^{a,C}	72 ^{a,C}	62 ^{a,A}
h*[(1)]	30	72.3 ^{a,A}	72.4 ^{a,A}	73 ^{a,A}	75.1 ^{b,A}	80.5 ^{c,A}	74.3 ^{b,A}
	90	74 ^{a,A}	73.2 ^{a,A}	74.4 ^{a,A}	80 ^{b,B}	85 ^{c,B}	78 ^{b,B}
	180	79 ^{b,B}	76 ^{a,B}	77.3 ^{a,B}	83 ^{b,C}	85 ^{c,B}	80 ^{a,C}
	270	79 ^{c,B}	76.3 ^{b,B}	78 ^{b,B}	85 ^{d,D}	84 ^{d,B}	75 ^{a,A}
C* **(light)**	30	10.4 ^{b,A}	11 ^{b,A}	11.2 ^{c,A}	11 ^{b,A}	11.5 ^{b,A}	17 ^{a,B}
	90	10 ^{b,AB}	9.4 ^{a,A}	10 ^{b,A}	9.4 ^{ab,A}	11.2 ^{ab,BC}	13 ^{a,C}
	180	8.5 ^{a,A}	9 ^{a,A}	9 ^{ab,A}	9.2 ^{ab,A}	11 ^{a,A}	15 ^{a,B}
	270	10 ^{ab,AB}	9.3 ^{a,AB}	8.0 ^{a,A}	8.1 ^{a,A}	11.4 ^{b,B}	16 ^{a,C}
C* **(darkness)**	30	11 ^{b,A}	12 ^{c,AB}	12 ^{c,AB}	12 ^{b,AB}	13 ^{b,B}	15.4 ^{b,C}
	90	11.0 ^{b,A}	11.0 ^{bc,A}	11 ^{b,A}	11.5 ^{b,A}	14.1 ^{c,B}	18.1 ^{c,C}
	180	9.1 ^{a,A}	9.1 ^{a,A}	9.3 ^{a,A}	10.2 ^{ab,A}	11 ^{a,A}	13.4 ^{a,B}
	270	8.3 ^{a,A}	9.4 ^{ab,A}	9 ^{a,A}	9.4 ^{a,A}	14 ^{bc,B}	18 ^{c,C}

Standard deviations: L* between 1 and 6; h* 0.4-5; C*+light: 0.2-4; C*-light: 0.3-6. [(1)]No significant differences were observed between samples exposed to light or in darkness. Mean values appear in the table. [(2)]Vacuum storage. Different superscripts within the same row indicate significant differences ($p < 0.05$) between RH (A-C) and within the same column indicate significant differences ($p < 0.05$) between time (a-d).

595

596

597 **Figure 1** Mean values and standard deviation of total phenolics of grapefruit powder, mg gallic

598 acid equivalents/100 g grapefruit solutes (mg GAE/100 g_{GS}), as a function of storage

599 conditions: 11.3, 23.1, 33.1, 43.2 and 55.9% relative humidity and vacuum packed (V) after 30,

600 90, 180 and 270 days of storage, with and without light. The dotted line marks the phenolic

601 content of the newly spray-dried powder. Different letters indicate significant differences ($p <$

602 0.05) between relative humidity (A-F), time (a-d) and light condition (X-Y).

603 **Figure 2** Mean values and standard deviation of lycopene of grapefruit powder, mg

604 lycopene/100 g grapefruit solutes (mg Lp/100 g_{GS}), as a function of storage conditions: 11.3,

605 23.1, 33.1, 43.2 and 55.9% relative humidity and vacuum packed (V) after 30, 90, 180 and 270

606 days of storage, with and without light. The dotted line marks the Lp of the newly spray-dried

607 powder. Different letters indicate significant differences ($p < 0.05$) between relative humidity

608 (A-F), time (a-d) and light condition (X-Y).

609 **Figure 3** Mean values and standard deviation of vitamin C of grapefruit powder, mg ascorbic

610 acid/100 g grapefruit solutes (mg AA/100 g_{GS}), as a function of storage conditions: 11.3, 23.1,

611 33.1, 43.2 and 55.9% relative humidity and vacuum packed (V) after 30, 90, 180 and 270 days

612 of storage, with and without light. The dotted line marks the vitamin C of the newly spray-dried

613 powder. Different letters indicate significant differences ($p < 0.05$) between relative humidity

614 (A-F), time (a-d) and light condition (X-Y).

615 **Figure 4** Mean values and standard deviation of antioxidant capacity of grapefruit powder

616 measured by DPPH assay, mmol trolox equivalents (TE)/100 g grapefruit solutes (mmol

617 TE/100 g_{GS}), as a function of storage conditions: 11.3, 23.1, 33.1, 43.2 and 55.9% relative

618 humidity and vacuum packed (V) after 30, 90, 180 and 270 days of storage, with and without

619 light. The dotted line marks the antioxidant capacity of the newly spray-dried powder. Different

620 letters indicate significant differences (p < 0.05) between relative humidity (A-F), time (a-d)

621 and light condition (X-Y).

622 **Figure 5** Mean values and standard deviation of antioxidant capacity of grapefruit powder

623 measured by FRAP assay, mmol trolox equivalents (TE) /100 g grapefruit solutes (mmol

624 TE/100 g_{GS}), as a function of storage conditions: 11.3, 23.1, 33.1, 43.2 and 55.9% relative

625 humidity and vacuum packed (V) after 30, 90, 180 and 270 days of storage, with and without

626 light. The dotted line marks the antioxidant capacity of the newly spray-dried powder. Different

627 letters indicate significant differences (p < 0.05) between relative humidity (A-F), time (a-d)

628 and light condition (X-Y).

629 **Figure 6** Mean values and standard deviation of the maximum force attained during the

630 grapefruit powder compression test (Fmax) as a function of storage conditions: 11.3, 23.1, 33.1,

631 43.2 and 55.9% relative humidity and vacuum packed (V) after 30, 90, 180 and 270 days of

632 storage, with and without light. The dotted line marks the newly spray-dried powder value. The

633 inner graph shows an example of the force–distance relationship obtained from the compression

634 test carried out on the samples stored for 180 days, in the dark, at the different relative

635 humidities and vacuum packed. Different letters indicate significant differences (p < 0.05)

636 between relative humidity (A-F), time (a-d) and light condition (X-Y).

637 **Figure 7** Evolution of the color change of grapefruit powder with respect to the newly obtained

638 one, as a function of storage conditions: 11.3, 23.1, 33.1, 43.2 and 55.9% relative humidity and

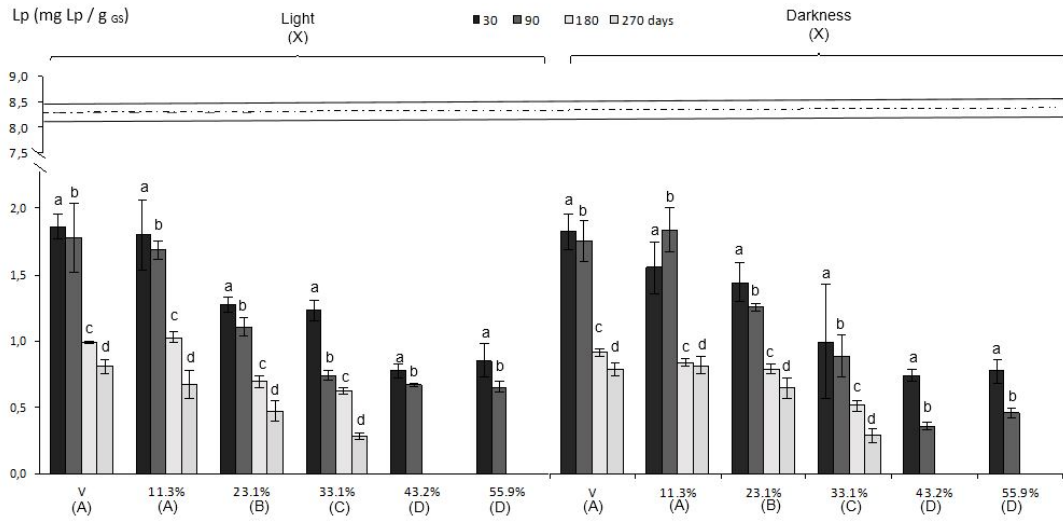639 vacuum packed (V), after 30, 90, 180 and 270 days of storage, with and without light
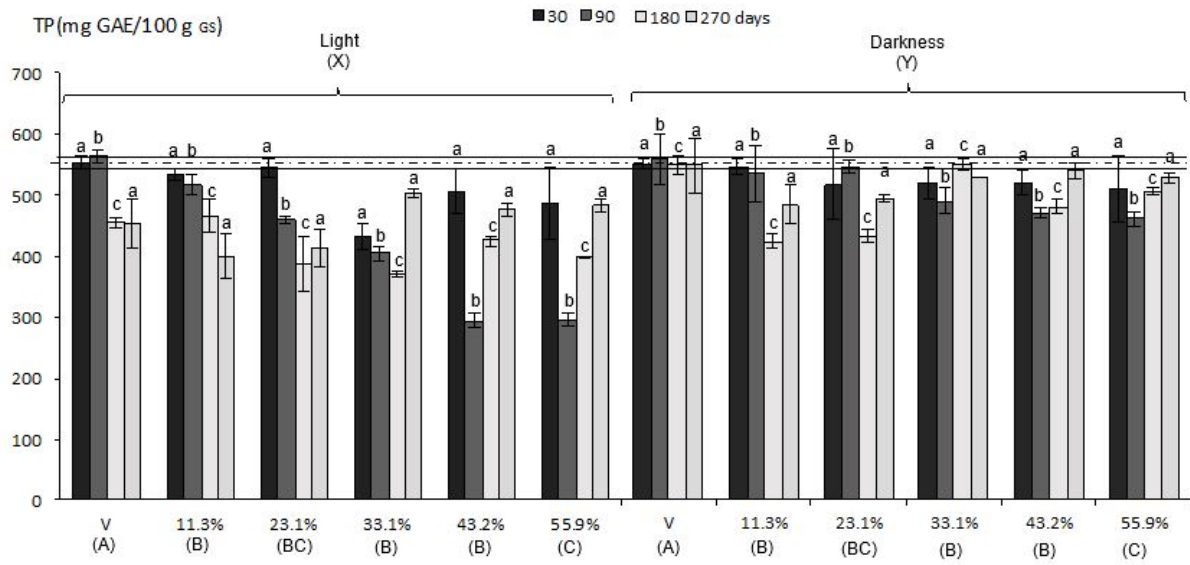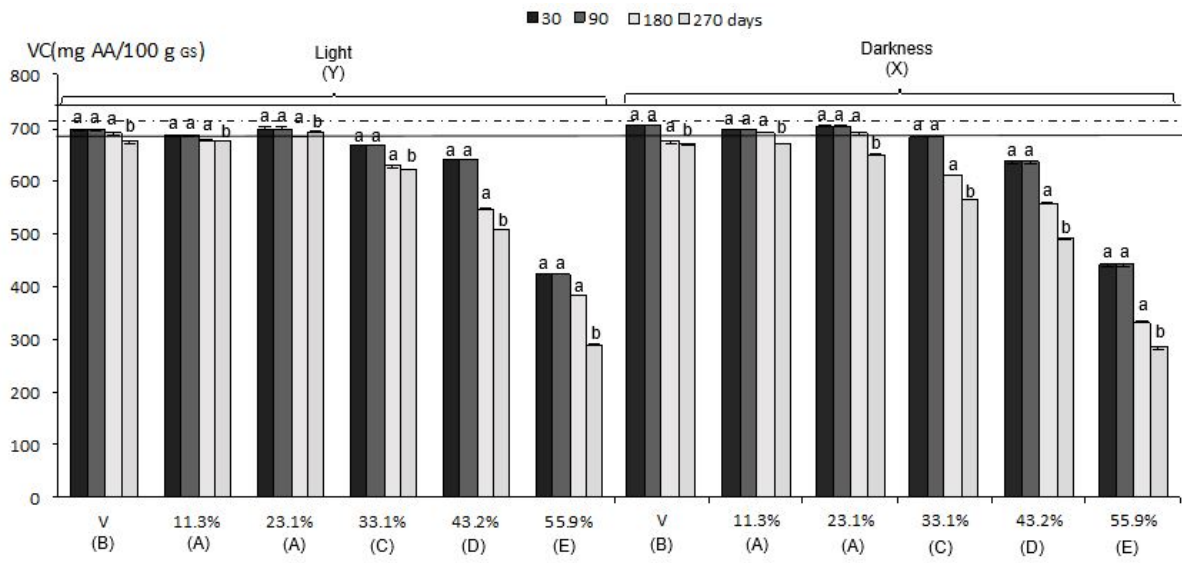
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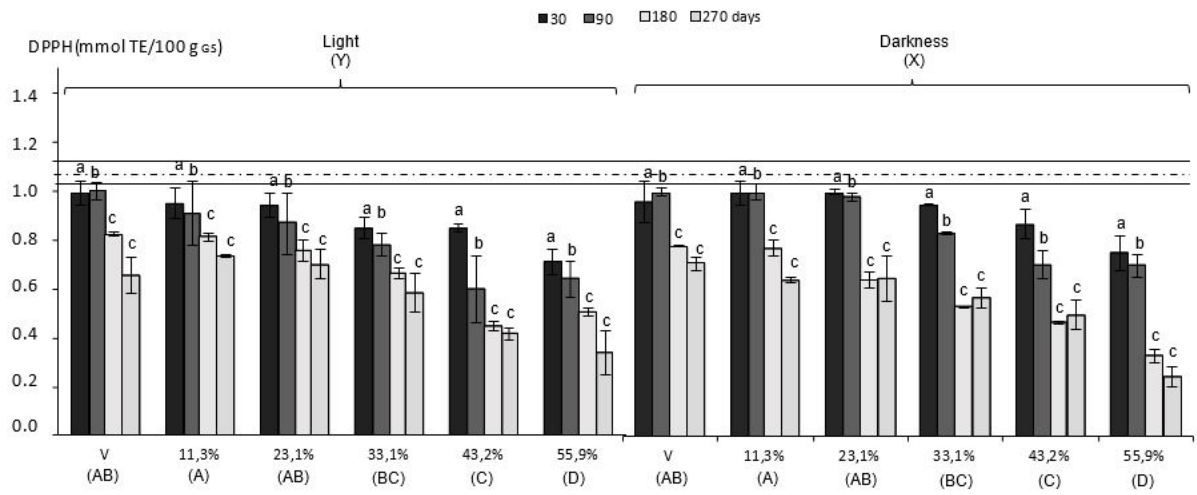
642 **Fig. 1.**

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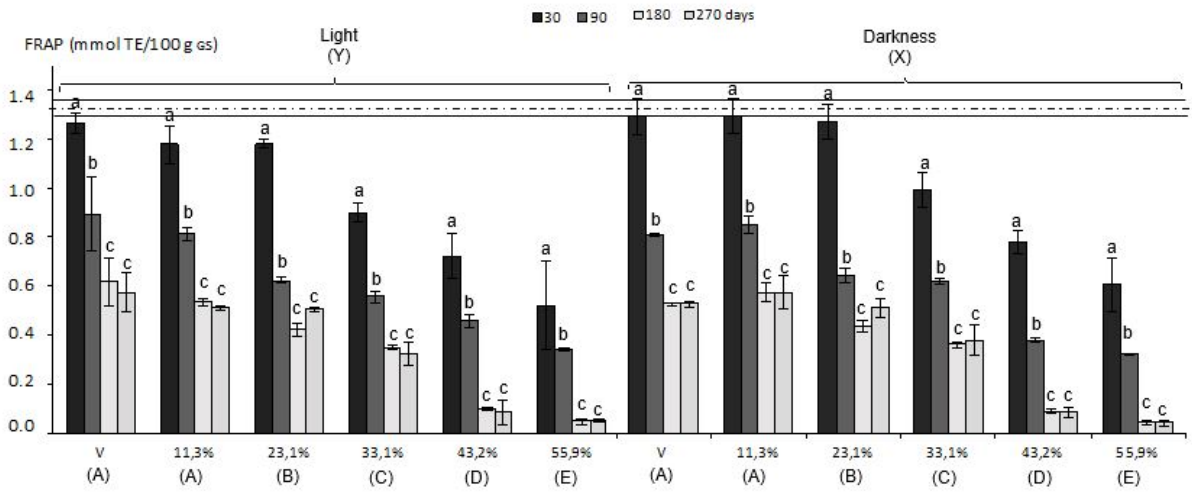645 **Fig. 2.**

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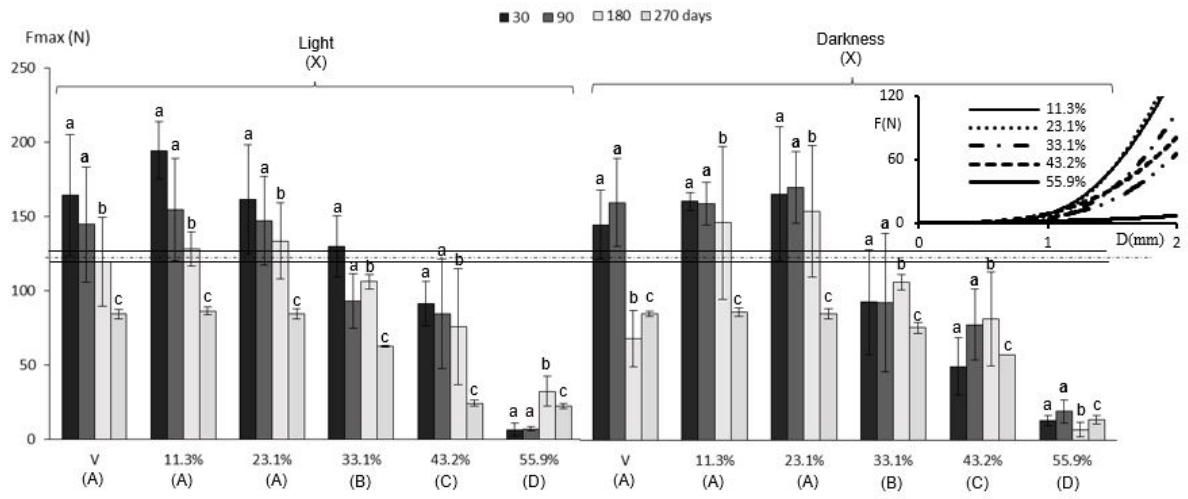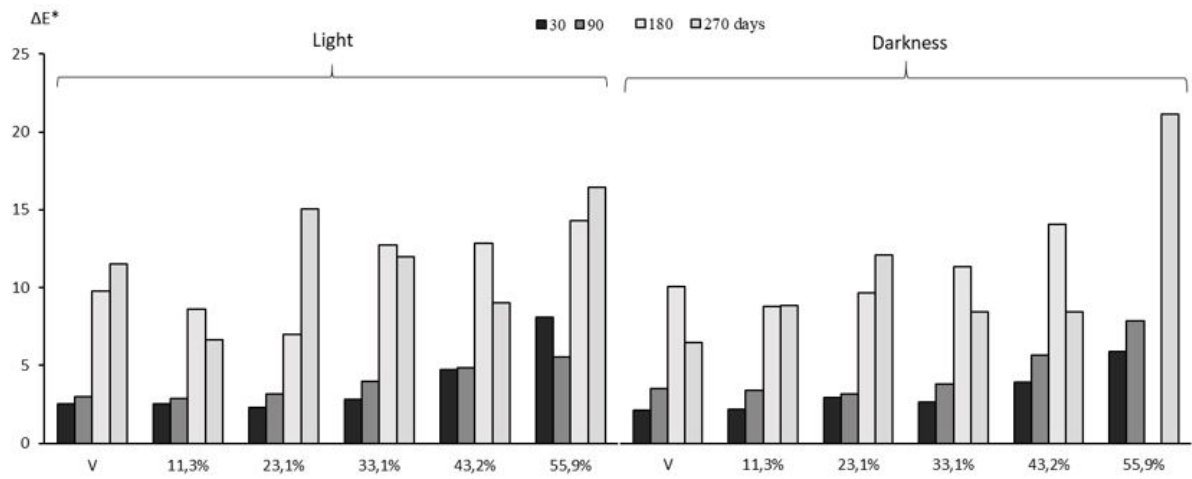
649 **Fig. 3.**

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652 **Fig. 4.**

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655 **Fig. 5.**

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657 **Fig. 6**

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660   **Fig. 7.**

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