Quality and bioaccessibility of total phenols and antioxidant activity of *calçots* (*Allium cepa* L.) stored under controlled atmosphere conditions

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Abstract

Calçots are the floral stems of the second-year onion (*Allium cepa* L.) resprouts with economic importance in Spain, where they are usually consumed roasted. The effect of two controlled atmospheres (CA) of 2.0 % O_2 + 3.5 % CO_2 (CA1), 1.0 % O_2 + 2.0 % CO_2 (CA2) and air at 1 ºC for 60 d on the physicochemical, nutritional and sensory quality of *calçots* were studied. In addition, the total phenolic content (TPC) and the antioxidant activity (AA) of roasted *calçot*s were evaluated after an *in vitro* gastrointestinal (GI) digestion. Both CA regimes reduced the respiration rate of the stored product without causing physiological disorders. The TPC and AA of *calçots* increased during storage. Storage for 60 d in CA2 resulted in the highest AA by $DPPH^{\cdot}$ assay, whereas *calçots* stored in air for 60 d showed the highest TPC and AA by FRAP assay. *Calçots* stored in air for 30 d and fresh harvested sample presented the highest total flavonoids values. After 30 d of storage, *calçots* stored in CA had a higher liking degree than *calçots* stored in air. The AA of digested *calçots* decreased drastically after *in vitro* GI digestion in comparison to the non-digested samples. However, TPC increased after digestion. Roasted *calçots* stored in CA1 for 30 d showed the highest TPC and AA retention in the intestinal phase. CA could be a postharvest strategy for the storage of *calçots*.

Keywords: *Allium*; Controlled atmosphere; Roasting; Antioxidant activity; Total Phenolic Content; i*n vitro* digestion.

1. Introduction

Calçots (*Allium cepa* L.) are the floral stems of second-year onion resprouts of the 'Blanca Tardana de Lleida' landrace with an economical importance in Catalonia (northeast Spain), where they are usually consumed roasted. 'Calçot de Valls' was awarded with a Protected Geographical Indication (PGI) (EC No 905/2002) by the European Union (Simó et al., 2013). The demand and interest in *calçots* worldwide has motivated producers to explore postharvest techniques to extend the storage life of this seasonal crop while maintaining their nutritional and organoleptic characteristics. Controlled atmosphere (CA) is used to prolong the quality of fresh fruit and vegetables by modifying the atmospheric composition different from air while supplementing proper temperature and relative humidity management during storage (Kader, 1996). However, no data are currently available regarding the effect of CA storage conditions in the nutritional, morphologic and quality parameters of fresh *calçots.* According to the commercial storage recommendations by U.S. Department of Agriculture (Gross et al., 2016), bunched green onions can be stored for 6 to 8 weeks in 2.0 % of O_2 and 5.0 % CO_2 at 0 °C, tolerating storage conditions up to 1.0 % of O_2 and 5.0 % of CO_2.

Epidemiological studies have stand out that routine consumption of fruit and vegetables provides benefits to the organism and those could be due to the content of antioxidant compounds such as phenols (Akhmadieva et al., 1993; Hertog et al., 1995). *Allium* genus vegetables such as leek, onions or garlic are good sources of nutrients beneficial to human health (Santas et al., 2008; Vandekinderen et al., 2009). For example, onions contain high amount of compounds without nutritional value but with high antioxidant capacity, which could have protective effect against different types of diseases based on oxidative stress (Pérez-Gregorio et al., 2010). Culinary treatments such as frying, boiling and roasting, and length of exposure, could be important factors in the reduction of total flavonoid content (Rodrigues et al., 2009).

78 The vast majority of available studies are focus on TPC and AA of non-digested samples.
79 However, in practice, fruit and vegetables are subjected to simulated digestion to measure
80 antioxidant potentially available for absorption in intestine (Ryan and Prescott, 2010; Wootton-
81 Beard et al., 2011). This is referred to as bioaccessibility, which is the amount of antioxidants or
82 nutrients available in the intestinal brush border for transport into the cell (Garrett et al., 1999).
83 *In vitro* digestion has been often used to simulate gastrointestinal conditions because *in vivo*
84 models have some disadvantages such as safety and ethical restrictions (Soriano Sancho et al.,
85 2015). Previous studies have confirmed that an *in vitro* model system simulating human
86 digestion could support reliable prediction of bioaccessibility of bioactive compounds and total
87 antioxidant capacity in plant products (Carbonell-Capella et al., 2015). In recent years the
88 interest in *in vitro* digestion studies with analysis of antioxidant capacity and bioactive
89 compounds (phenols and flavonoids) has increased in beverages (Carbonell-Capella et al.,
90 2015), juices (Gil-izquierdo et al., 2001; Rodríguez-Roque et al., 2015, 2013; Stanisavljevic et
91 al., 2015; Wootton-Beard et al., 2011), coffee (Campos-Vega et al., 2015), apple (Bouayed et
92 al., 2012, 2011), fruit extracts (Pavan et al., 2014), cooked cauliflower (Girgin and El, 2015),
93 grape (Tagliazucchi et al., 2010), chokeberry (Bermúdez-Soto et al., 2007), strawberry
94 (Kosińska-Cagnazzo et al., 2015) and broccoli (Vallejo et al., 2004). However, no studies are
95 available regarding the effect of digestion on the bioaccessibility of antioxidant compounds of
96 roasted *calçots*.

97 Therefore, the aim of this study was to evaluate the effect of CA storage on the
98 physicochemical, nutritional and sensory quality of *calçots* at different reduced O_2 and elevated
99 CO_2 concentrations. Study of simulated *in vitro* digestion (gastric and intestinal phase) of the
100 roasted samples was carried out to evaluate the bioaccessibility of total phenols and the
101 antioxidant activity at different phases.

2. Materials and methods

2.1 Plant material

Calçots (*Allium cepa* L.) were provided by '*Cooperativa de Valls'* (Tarragona, Spain) at commercial size. Those *calçots* had the European quality label PGI 'Calçot de Valls'. They were cultivated in northeast of Spain (41º13'47''N, 01º13'12''E), during the crop growing season of 2014 and 2015. In August 2014, the bulbs of 'Blanca Tardana de Lleida' onion were transplanted at a density of 8,000 plants per hectare. The resprouts arising in the autumn were covered with soil three times to increase the length of the edible white part. The plants were manually harvested in February.

2.2 Reagent and chemicals

Sodium hydroxide, methanol, sodium acetate trihydrate pure, acetic acid glacial pure, ethanol, iron (III) chloride 6-hydrate, potassium chloride, sodium chloride and magnesium chloride hexahydrate were obtained from Panreac (Barcelona, Spain). 2,2-diphenyl-1-picylhydrazyl (DPPH), L-ascorbic acid, 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), sodium carbonate, gallic acid, aluminium chloride hexahydrate, potassium acetate, quercetin, sodium bicarbonate, ammonium carbonate, calcium chloride dehydrate, sodium hydroxide (ACS reagent), as well as the enzymes for digestion [α-amylase from *Bacillus* sp. (≥ 400 U mg^{-1} protein), pepsin from porcine gastric mucosa (≥ U 400 mg^{-1} protein), pancreatic from porcine pancreas (4 x USP spc) and bile bovine], were purchased from Sigma-Aldrich (Steinheim, Germany). Hydrochloric acid (35 %), potassium dihydrogen phosphate and Folin-Ciocalteu's reagent were obtained from VWR (Llinars del Vallès, Spain). All chemicals and reagents were of analytical grade.

2.3 Controlled Atmosphere storage conditions and processing

126 The *calçots* were immediately cooled to 1 °C at arrival and kept at these conditions until
127 they were completely cooled. The time between harvesting and cooling was around 3 hours.
128 *Calçots* were then stored in Paliflex400 pallet storage system (Van Amerongen, Biezenwei,
129 Netherlands) (volume of 2 m^3) under controlled atmosphere (CA) system or air at 1 °C with 85
130 % of relative humidity (RH) for 60 d. The Palliflex unit comprised a cover and a special plastic
131 pallet on which the *calçot* packing cases were placed. Bunches of 50 *calçots* each were placed
132 in vertical position. A gastight, transparent cover was pulled over the product. The study was
133 comprised by three treatments: control (air), CA1: 2.0 % O_2 + 3.5 % CO_2 and CA2: 1.0 % O_2 +
134 2.0 % CO_2. The system measured the gas conditions in the cover and automatically corrected
135 them when was necessary with either CO_2, N_2 or air, several times a day.

136 190 *calçots* were randomly removed from each storage regime (air, CA1 or CA2) at
137 each storage time (30 or 60 d) of which 70 were used for roasting. The physicochemical,
138 nutritional and sensory quality of samples were evaluated. In addition, *calçots* were roasted at
139 270 ºC for 8 min using a Self Cooking Center (Mod SCC WE 101, Rational AG, Landsberg am
140 Lech, Germany) and then, cooled into a blast chiller (Infrico, Cordoba, Spain) until they reached
141 3 °C. The nutritional and sensory quality of roasted samples was also assessed. After conducting
142 the physicochemical and sensory assays, both fresh and roasted samples were crushed, powered
143 and frozen with liquid nitrogen and stored at -80 °C for nutritional analysis.

144 2.4 Morphological analysis

145 The largest, smallest and medium diameter of the samples, measured 5 cm from the
146 beginning of the root, and the length of the white shaft were determined in fifteen *calçots*
147 randomly selected from each treatment at each storage period. Fresh weight was also measured
148 and data was expressed as percentage of fresh weight loss (FWL).

149 2.5 Colour

150 The colour of the white shaft was measured as described by Altisent et al. (2014). Nine
151 random individual *calçots* per treatment at each sampling time were evaluated. The values a*,

b* and L* were used to calculate the browning index (BI) (Eq. (1)) according to Liu et al. (2016):

$$BI = \frac{100(x - 0.31)}{0.172} \quad (1)$$

where $x = \frac{a^* + 1.75 \times L^*}{5.645 \times L^*} + (a^* - (3.012 \times b^*))$.

2.6 Firmness

To assess changes on texture, firmness (N) was measured at 5 cm from the roots set in transversal position using the TA.TX2 Texture Analyzer (Stable Micro Systems Ltd., Surrey, England) attached with Warner-Blatzler blade (HDP/BSK: Blade set with knife). Samples were placed into the press holder, and then the blade moved down at different rates: pre-test rate: 5 mm s^{-1}; test rate: 1 mm s^{-1}; post-test rate: 10 mm s^{-1} to 60 mm below the bottom of the holder. Data acquisition rate was 200 pulses per s. Eight random individual *calçots* per treatment at each sampling time were evaluated.

2.7 pH, soluble solids content (SSC) and titratable acidity

pH, SSC and titratable acidity were measured in the juice of ten random invidual *calçots* per treatment at each sampling time, extracted by grinding *calçots* pieces in a blender and were determined as described by Plaza et al. (2016). Soluble solids were expressed as % and titratable acidity as g of malic acid L^{-1}. Three determinations were performed per each treatment at each sampling time.

2.8 Respiration rate

The respiration rate of *calçots* was measured using the static method in a closed system with an O_2/CO_2 gas analyzer (Checkmate 3, PBI Dansensor, Ringsted, Denmark). The gas analyzer was equipped with a solid-state zirconia ion-selective electrode for O_2 determination.

174 To measure CO_2, the gas analyzer used a full-scale temperature compensated IR sensor. The
175 determination of 12 individual fresh-cut *calçots* (500 g) per treatment at each sampling time was
176 carried out according to the method described by Altisent et al. (2014) at 4 °C for 4 h. The
177 respiration rate of samples was measured in duplicate and expressed in mg CO_2 kg^{-1} s^{-1}.

178 2.9 Sensory assessment

179 Forty *calçots* per treatment at each sampling time were used. A portion of 3 cm of
180 roasted *calçot* of each sample was presented to an untrained panel of 40 consumers immediately
181 after they were heated for 10 s in the microwave at a 900 W. The assessment was carried out
182 following the same methodology previously described by Altisent et al. (2014).

183 2.10 Determination of antioxidant activity

184 Antioxidant activity was determined using two different methods: 2,2-diphenyl-1-
185 picrylhydrazyl ($DPPH^{\bullet}$) radical scavenging assay and ferric reducing antioxidant power (FRAP)
186 assay. The extraction and assays were carried out according to the methods described by Plaza
187 et al. (2016). Results were expressed on a weight basis as mol of ascorbic acid equivalents per
188 kg.

189 2.11 Determination of total phenolic content

190 The extraction and determination of total phenolic content were determined by the Folin
191 Ciocalteu method (Singleton et al., 1999), following the modifications described by Altisent et
192 al. (2014). Results were expressed on a weight basis as g of gallic acid equivalent per kg.

193 2.12 Determination of total flavonoids

194 The extraction and determination were performed according to the method described by
195 Santas et al. (2008) with some modifications as described below.

196 The flavonoids were extracted by homogenization of 5 g of frozen powdered samples
197 with 10 mL of ethanol (80 %, v/v). The resulting mixture was centrifuged at 13,523 × g for 20

198 min at 4 °C and filtered. The supernatant was collected and the precipitate was mixed with 5 mL
199 of ethanol (80 %, v/v). The process was repeated twice. Finally, the supernatants were combined
200 and diluted up to 25 mL with ethanol (80 %, v/v).

201 The aluminium chloride method was used for the determination of the total flavonoid
202 content of the sample extracts. Aliquots of extract solutions (1.5 mL) were mixed with ethanol
203 (95%, v/v) to a final volume of 2 mL. Then, 0.1 mL of $AlCl_3$ (10 %), 0.1 mL of potassium
204 acetate (1 mol L^{-1}) and 2.8 mL of distilled water were added sequentially. The test solution was
205 vigorously shaken and its absorbance at 415 nm was recorded after 30 min of incubation at
206 room temperature in darkness. A standard calibration plot was generated at 415 nm using
207 known concentrations of quercetin (ranging from 10 to 100 mg L^{-1} final volume). The results
208 were expressed on a weight basis as g quercetin equivalent per kg.

209 2.13 *In vitro* simulated gastrointestinal (GI) digestion of roasted *calçot*

210 *In vitro* simulated gastrointestinal digestion was performed according to the method
211 described by Minekus et al. (2014) with some modifications. That method is an international
212 consensus which consists of three sequential stages (Figure 1): oral (pH 7, containing α-
213 amylase), gastric (pH 3, containing pepsin) and intestinal (pH 7, containing pancreatin and fresh
214 bile). The digestion was performed in triplicate for each storage regime at each storage period.
215 A blank was prepared using only distilled water instead of sample following the same
216 procedure. Results were compared with non-digested roasted *calçots*.

217 2.13.1. Oral phase

218 Five g of frozen roasted *calçots* were mixed with 3.5 mL of simulated salivary fluid
219 (SSF) and minced together. Then, 0.5 mL of salivary α-amylase solution of 1500 U (enzymatic
220 activity unit) mL^{-1} were added followed by 25 µL of 0.3 mol L^{-1} $CaCl_2$ and 975 µL of water.
221 The mixture was thoroughly mixed (150 rpm) and incubated at 37.0 °C for 2 min.

222 2.13.2 Gastric phase

223 Ten mL of oral bolus were mixed with 7.5 mL of simulated gastric fluid (SGF), 1.6 mL
224 porcine pepsin stock solution of 25000 U mL^{-1}, 10 µL of 0.15 mol L^{-1} $CaCl_2$, 0.2 mL of 1 mol
225 L^{-1} HCl to reach pH 3.0 and 0.690 mL of water. The mixture was incubated with gentle shaking
226 (150 rpm) at 37.0 °C for 2 h. After 1 h of gastric phase, 10 µL of NaOH (1 mol L^{-1}) were added
227 to maintain the pH. The pH was measured before and after the gastric phase. After the gastric
228 phase, 10 mL of mixture were collected and centrifuged at 13,523 × g for 15 min at 4 °C. The
229 supernatant was frozen with liquid nitrogen and stored at -80 °C for further analyses.

230 2.13.3 Intestinal phase

231 Ten mL of gastric chyme were mixed with 5.5 mL of simulated intestinal fluid (SIF),
232 2.5 mL of a pancreatin solution 800 U mL^{-1} based on trypsin activity, 1.25 mL bile bovine (160
233 mmol L^{-1} in bile bovine), 20 µL of 0.3 mol L^{-1} $CaCl_2$, 0.145 mL of 1 mol L^{-1} NaOH to reach pH
234 7.0 and 0.585 mL of water. The mixture was incubated with gentle shaking (150 rpm) at 37 °C
235 for 2 h. Every 30 min of intestinal phase, 25 µL of HCl (1 mol L^{-1}) were added to maintain the
236 pH. The pH was measured before and after the intestinal phase. After the intestinal phase, the
237 mixture was collected and centrifuged at 13,523 × g for 15 min at 4 °C. The supernatants were
238 collected, frozen and stored at -80 °C for further analyses.

239 Determinations of antioxidant activity (FRAP and $DPPH^{\cdot}$) and total phenolic content using
240 previously described methods were performed after gastric and intestinal phases. The
241 percentage of retention was calculated as:

242 $$\text{Retention (\%)} = \frac{\text{values of gastric or intestinal digesta}}{\text{values of non-digested}} \times 100$$

243 2.14 Statistical analysis

244 All data were tested by analysis of variance (ANOVA) with JMP 8 software (SAS
245 Institute Inc., Cary, NC, USA). Means were separated by t-test and Tukey's test at $P \leq 0.05$.
246 Samples were characterised by the average measurement (instrumental analyses) or by the

247 average score over judges (sensory analyses) and standard deviation for each storage regime at
248 each storage period.

249 **3. Results and discussion**

250 3.1 Changes on morphological and quality parameters

251 The morphological parameters of *calçot* samples were affected by the storage treatment
252 and time in comparison with the harvested samples without visual impact (Table 1). The
253 morphological parameters were in the same range than those established by the PGI 'Calçot de
254 Valls', which establish that diameter of *calçots* must be between 1.7 and 2.5 cm and the white
255 shaft around 15 cm (D.A.R.P., 2009).

256 The lowest percentage of FWL was observed in samples stored under air after 30 d of
257 storage at 1 °C (Table 1). However, the FWL decreased by 23 % in those samples after 60 d of
258 storage. In contrast, *calçots* stored under the CA2 showed a FWL that did not exceed a 9.3 %
259 throughout storage. These results are in line with those observed previously in other vegetables
260 stored under CA. For example, Praeger et al. (2003) and Özden and Bayindirli (2002) reported
261 that onion bulbs and peppers stored in CA, respectively, retained weight compared with samples
262 stored in air. *Calçots* stored in lower O_2 atmospheres (CA2) lost less weight than those stored in
263 higher O_2 atmospheres (CA1). This might be due to their lower respiration rate (Table 2).

264 Firmness of *calçots* was maintained during the first 30 d of storage except in those
265 stored under CA1 (Table 2). Chope et al. (2006) reported that in three cultivars of onion (cvs.
266 Renate, Ailsa Craig and SS1) the greatest softening occurred between the beginning of CA
267 (3.03 % CO_2 and 5.05 % O_2; 2 ± 1 °C) storage and the first sampling date (40 d, 26 d and 14 d,
268 respectively). Yamashita et al. (2010) reported that the firmness of 'Super-Kitamomiji' onion
269 bulbs during CA storage (1 % O_2 and 1 % CO_2; -0.5 °C, 80 % RH; 92, 135 and 196 d) was
270 greater than that of bulbs stored under air. The application of CA decreased the activity of cell
271 wall degrading enzymes involved in softening and enzymes involved in lignification, leading to
272 toughening of vegetables (Gross et al., 2016).

273 Respiration rate of all stored *calçots* increased compared with those samples studied at
274 harvest (Table 2). Physical stress might stimulate the respiration rate of fresh vegetables (Kader
275 and Saltveit, 2003). *Calçots* stored under 1 % O_2 and 2 % CO_2 (CA2) had the lowest respiration
276 rates (18.04 mg CO_2 kg^{-1} s^{-1}) after 60 d of storage. This suggests that CA postharvest strategy
277 could represent an alternative to extend storage life of *calçots*. Results obtained in this study
278 differed from those reported by Praeger et al. (2003), who observed CO_2-production values in
279 onions (*Allium cepa* L. var. *cepa*) around 8.3 10^{-4} mg CO_2 kg^{-1} s^{-1} after stored under 1.0 % O_2
280 and <3.0 % CO_2 for 60 d at 2 ºC, suggesting the strongly effect of onion cultivar to CA
281 postharvest treatment.

282 In addition, an increase in the titratable acidity of all samples and a decrease in both pH
283 and SSC was observed at the end of the 60-day storage period. Chope et al. (2006) reported that
284 SSC changed during storage in onion bulbs (cvs. Renate, Ailsa Craig and SS1) with a maximum
285 concentration after 40 d followed by a decrease until the end of the storage period (230, 129 and
286 81 d, respectively) under CA storage (3.0 % CO_2/5.0 % O_2; 2 °C).

287 Browning index (BI) of *calçots* stored under air was higher compared with the fresh
288 harvested samples after 30 d of storage (Table 3). However, *calçots* stored under both CA1 and
289 CA2 strategies had similar BI as those obtained at harvest. Onion (*Allium* cepa L. var. *cepa*)
290 skin became darker under all atmospheres (0.5, 1 and 21 % O_2 and <0.3 % CO_2) after 252 d of
291 storage at 2 ºC but without treatment effects among them (Praeger et al., 2003). The
292 maintenance of the BI in CA storage could be due to the inhibition of PPO (Galvis-Sánchez et
293 al., 2004), delay of chlorophyll loss (green colour) or biosynthesis and oxidation of phenolic
294 compounds (brown colour) (Gross et al., 2016) caused by the low O_2 and high CO_2
295 concentrations.

296 3.4 Sensory assessment

297 After 30 d of storage, *calçots* stored in CA1 obtained higher liking degree than *calçots*
298 stored under air (Figure 2), but not with *calçots* stored in CA2. However, after 60 d of storage at

1 ºC, *calçots* stored in air obtained the highest liking degree average, although not different of that obtained for *calçots* stored under CA1 conditions. There were no liking degree differences in *calçots* stored in CA, but a higher liking degree value was observed in samples stored under air during 60 d when compared to those stored during 30 d under the same conditions. It is important to highlight that the score obtained in all cases was higher than 6 and *calçots* stored in air for 60 d obtained the highest score (around 7.5). These results suggest that CA storage treatment is a good storage strategy to maintain characteristic taste of *calçots*.

There is no information about the most relevant sensory characteristics of *calçots*. These products are eaten cooked, and Simó (2013) remarked that the importance of pungency disappear and that the most important sensory aspects of onions are sweetness.

3.5 Antioxidant activity

Figure 3 shows the antioxidant activity values obtained by the $DPPH^{\cdot}$ (Figure 3.A) and FRAP (Figure 3.B) methods in the studied fresh and roasted (270 ºC for 8 min) *calçots*. Among treatments, fresh samples stored under CA1 conditions had the lowest values along the 60 d of storage. Antioxidant capacity of fruit is believed to be caused by phenolic compounds. Therefore, higher $DPPH^{\cdot}$ values in CA-stored fruit may be due to suppressed oxidation of phenolic compounds (Ali et al., 2016).

Antioxidant activity of all the samples increased after cooking (Figure 3). This response was particular important in roasted harvested *calçots* with antioxidant activity values up to 1.26 and 1.33 10^{-3} mol kg^{-1} for $DPPH^{\cdot}$ and FRAP methods, respectively. *Calçots* stored under air also showed the same trend after 30 and 60 d of storage at 1 ºC. These results were in agreement with those reported by Juániz et al. (2016), where DPPH tended to be higher in cooked onions, especially in grilled onions (150 °C for 10 min + 110 ºC for 5 min). Increase of the antioxidant activity observed in *calçot* samples after cooking treatment might be attributed to the liberation of antioxidant compounds from insoluble portions, or to the formation of novel compounds such

as Maillard reaction products (MRPs) which possess antioxidant capacities (Hwang et al., 2012; Manzocco et al., 2001; Martins et al., 2001).

3.6 Total phenolic content

The TPC after cooking increased in fresh harvest samples (0.15 ± 0.01 g kg^{-1}) and *calçots* stored under air conditions (0.24 ± 0.01 and 0.26 ± 0.01 g kg^{-1}) (Figure 4) . This increase was also observed after 60 d of storage at 1 ºC in *calçots* stored at CA1 (0.22 ± 0.01 g kg^{-1}). Rawson et al. (2013) reported a reduction in the levels of antioxidant activity and phenols in roasted (160 °C, 15 min) fennel (*Foeniculum vulgare*) bulbs. Gorinstein et al. (2005) also observed that cooked garlic (100 °C, 40 or 60 min) had lower values of total polyphenols than fresh garlic. In the case of roasting as mentioned previously, the production of redox-active secondary metabolites (Maillard reaction and Amadori rearrangement products) or breakdown compounds at a very high temperature (160 °C) might affect the polyphenol levels and their structure (Dini et al., 2013; Palermo et al., 2014).

In general, the total phenolic content of stored fresh *calçots* increased respect to the harvest sample. Results showed an unclear relation between AA or TPC and the storage conditions. Nevertheless, Juániz et al. (2016) reported that the increment of phenolic compounds in stored onions corresponded with an increase in antioxidant capacity measured by DPPH assay. Furthermore, in the study carried out by Fernández-León et al. (2013) high values of AA were strongly associated (Pearsons's correlation coefficient $r = 0.65$, $p < 0.01$) with high TPC in 'Parhenon' broccoli after storage under 10 % O_2 + 5 % CO_2 at 1-2 °C for 21 d. Li et al. (2015) reported that the variation in total phenol content of kiwifruit during storage at 2 % O_2/3 % CO_2, 2 % O_2/6 % CO_2, 5 % O_2/3 % CO_2, 5 % O_2/6 % CO_2) at 2 °C had a strong negative correlation with PPO activity. Low O_2 atmospheres can delay the decrease in TPC perhaps due to inhibition of PPO activity.

3.7 Total flavonoids

In general, the total content of flavonoids in *calçots* was higher in fresh than in cooked samples because of their heat susceptibility, although in some cases there were no significant differences (Figure 5). In the study performed by Rodrigues et al. (2009) using two Portuguese onion cultivars ('branca da Póvoa' and 'vermelha da Póvoa'), oven roasting without water (180 °C for 15 min or 200 °C for 30 min) did not modify the total levels of some flavonoids (quercetin 3,4'-glucoside and quercetin 4'-glucoside). However, Sharma et al. (2015) reported that after heating onions at certain temperature (80 °C, 100 °C, 120 °C or 150 °C for 30 min), the total flavonoid content decreases, which indicates that some flavonoids were probably destroyed.

Fresh harvested and stored *calçots* under air had the highest total flavonoid values between 0.44 ± 0.02 and 0.58 ± 0.10 g kg^{-1}. The values were higher in fresh harvest samples and samples stored under air than in CA.

3.8 *In vitro* simulated gastrointestinal (GI) digestion of roasted *calçot*

3.8.1 Antioxidant activity

The $DPPH^{\cdot}$ and FRAP values of digested *calçots* are shown in Figure 6. Total AA in both gastric and intestinal digest were lower than those obtained by chemical extraction (non-digested). This suggests that the antioxidant components present in roasted *calçots* are potentially unstable to the pH change and enzymatic degradation.

A reduction in the antioxidant activity by the $DPPH^{\cdot}$ assay was observed in all samples after both gastric and intestinal phases. Chen et al. (2014) analysed antioxidant capacity of 33 fruit, where after the gastric phase of digestion, the $DPPH^{\cdot}$ values of some fruits were increased and after intestinal phase decreased. In our study the highest antioxidant retention was observed in samples stored under CA1 with concentration values of 5.60 10^{-4} ± 5.41 10^{-6} mol kg^{-1} (representing 43.6 % respect to the non-digested). Moreover, *calçots* stored under CA2 for 30 d and air for 60 d showed values of AA retention of 27.0 % and 29.4 %, respectively, respect to the non-digested. Those results indicated after 60 d the retention in the intestinal phase of AA

375 by the DPPH$^{\cdot}$ assay was lower in *calçots* stored in CA at 1 °C for 60 d than for 30 d. Therefore,
376 the storage time might have influenced in that retention.

377 The values of FRAP after gastric and intestinal phase were lower than in non-digested
378 as showed in DPPH$^{\cdot}$. The retention of AA by the FRAP assay after intestinal phase was similar
379 to DPPH$^{\cdot}$ assay, being between 21 and 40 %. The highest antioxidant retention was observed in
380 samples stored under CA1 for 30 d (representing 36.1 % respect to the non-digested) and air for
381 60 d (representing 38.7 % respect to the non-digested) as was showed in DPPH$^{\cdot}$ assay. Wootton-
382 Beard et al. (2011) observed that the FRAP values of 23 commercially available vegetable
383 juices increased after gastric phase with a subsequent decrease after the intestinal phase,
384 although remaining higher than non-digested. They also studied the correlation between DPPH$^{\cdot}$
385 and FRAP results and they were poorly correlated ($r^2 = 0.53$). Therefore, results can depend on
386 the type of fruit or vegetable studied.

387 The observed differences between the AA obtained after intestinal phase in the DPPH$^{\cdot}$
388 and FRAP assays could be attributed to the different principle of each assay. Both assays are
389 redox-linked colorimetric methods, but the DPPH$^{\cdot}$ assay is based on accept a hydrogen atom
390 (Mishra et al., 2012) and the FRAP assay is based on the acceptance of electrons from
391 antioxidants (Benzie and Strain, 1996). The differences in the results obtained in DPPH$^{\cdot}$ assay
392 might be associated with pH changes along the digestion (Bouayed and Bohn, 2010) because
393 the radical scavenger activity of polyphenols is strongly pH-dependent (Tagliazucchi et al.,
394 2010). According to Bouayed et al. (2011), the transition from acidic medium to basic or neutral
395 medium increase the AA of phenolic compounds by causing deprotonation of the hydroxyl
396 moieties present on their aromatic rings. Moreover, alterations in the structure of antioxidants
397 following digestion may affect their reactivity with the less biologically relevant nitrogen
398 radical formed in the DPPH$^{\cdot}$ assay (Wootton-Beard et al., 2011). Finally, the simulated fluids
399 contained different electrolytes which could release electrons during the digestion and produce
400 interferences in the FRAP assay.

401 3.8.2 Total phenolic content

402 The impact of *in vitro* simulated GI digestion in TPC is shown in Figure 7. The total
403 content increased after digestion for all samples. Polyphenols release was mainly achieved
404 during the gastric phase. Subsequently, a similar increase was obtained after the intestinal
405 phase. In other matrices such as vegetable juices (Wootton-Beard et al., 2011) and some fruit
406 (Chen et al., 2014) after the gastric phase of digestion a significant increase in TPC as well as
407 after intestinal phase was observed. Nevertheless, Rodríguez-Roque et al. (2013) reported that
408 after gastric digestion the TPC increased and after small intestinal digestion, decreased.
409 Regarding to the increment of TPC after intestinal phase, the highest increase was observed in
410 samples stored under CA1 with concentration values of 0.31 ± 0.01 g kg^{-1} (representing 47.5 %
411 respect to the non-digested), CA2 with concentration values of 0.21 ± 0.01 g kg^{-1} (representing
412 36.7 % respect to the non-digested) after 30 d at 1 °C and fresh harvested sample with
413 concentration values of 0.25 ± 0.05 g kg^{-1} (representing 38.9 % respect to the non-digested).
414 This increment observed in the TPC may be due to the additional time of extraction (13,523 x g,
415 15 min, 4 °C) along with the effect of gastric or intestinal enzyme on the complex food matrix,
416 which facilitates the release of phenolic bound to the matrix (Carbonell-Capella et al., 2014).

417 **4. Conclusions**

418 The quality and bioaccessibility of total phenolic content and antioxidant activity of
419 *calçots* was studied. Overall, the storage conditions did not affect the morphological parameters
420 of *calçots* obtained at harvest. Storage conditions of 1.0 % O_2 and 2.0 % CO_2 at 1 °C were the
421 most suitable to maintain weight, and lower respiration rate and browning index of the *calçots*
422 during storage. These conditions also resulted in a higher consumer acceptance and showed
423 potential for commercial use of *calçots* for a longer period.

424 Roasting *calçots* (270 ºC for 8 min) led to an increase in their antioxidant activity and
425 total phenolic content. However, AA values obtained after the *in vitro* simulated GI digestion of
426 roasted *calçot* were lower when compared with the non-digested sample. This reduction in the

AA did not depend on the CA used. In this way, around 30 % of the FRAP and DPPH· antioxidant activity was maintained in roasted *calçots* after GI simulated digestion. Nevertheless, TPC of roasted *calçots* increased between 15 and 47 % after *in vitro* simulated GI digestion perhaps due to the liberation of phenolic compounds from the matrix.

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640

641 **Figure Captions**

642 **Figure 1.** Diagram of *in vitro* simulated gastrointestinal digestion of roasted *calçots*. SSF:
643 Simulated Salivary Fluid; SGF: Simulated Gastric Fluid; SIF: Simulated Intestinal Fluid.

644 **Figure 2.** Liking degree of cooked *calçots* at harvest and after 30- and 60-day of storage at 1 ºC.
645 Values are expressed as mean ± standard deviation. Capital letters indicate significant
646 differences between storage conditions ($P<0.05$). Lower case letters indicate significant
647 differences ($P<0.05$) between storage time. CA: Controlled atmosphere; CA1: 2.0 % O_2 + 3.5 %
648 CO_2; CA2: 1.0 % O_2+ 2.0 % CO_2.

649 **Figure 3.** Antioxidant activity (A: $DPPH^{\cdot}$ assay, B: FRAP Assay) of fresh and roasted *calçots* at
650 harvest and after 30- and 60-day of storage at 1 ºC. Values are expressed as mean ± standard
651 deviation. Capital letters indicate significant differences ($P<0.05$) between fresh and roasted
652 samples. Lower case letters indicate significant differences ($P<0.05$) between storage treatments
653 at same storage time. CA: Controlled atmosphere; CA1: 2.0 % O_2 + 3.5 % CO_2; CA2: 1.0 %
654 O_2+ 2.0 % CO_2.

655 **Figure 4.** Total phenolic content of fresh and roasted *calçots* at harvest and after 30- and 60-day
656 of storage at 1 ºC. Values are expressed as mean ± standard deviation. Capital letters indicate
657 significant differences ($P<0.05$) between fresh and roasted samples. Lower case letters indicate
658 significant differences ($P<0.05$) between storage treatments at same storage time. CA:
659 Controlled atmosphere; CA1: 2.0 % O_2 + 3.5 % CO_2; CA2: 1.0 % O_2+ 2.0 % CO_2.

660 **Figure 5.** Total flavonoids of fresh and roasted *calçots* at harvest and after 30- and 60-day of
661 storage at 1 ºC. Values are expressed as mean ± standard deviation. Capital letters indicate
662 significant differences ($P<0.05$) between fresh and roasted samples. Lower case letters indicate
663 significant differences ($P<0.05$) between storage treatments at same storage time. CA:
664 Controlled atmosphere; CA1: 2.0 % O_2 + 3.5 % CO_2; CA2: 1.0 % O_2+ 2.0 % CO_2.

665 **Figure 6.** Antioxidant activity (A: $DPPH^{\cdot}$ assay, B: FRAP Assay) of roasted *calçots* after *In*
666 *vitro* simulated gastrointestinal (GI) digestion of samples stored under air and two different
667 controlled atmosphere (CA1 and CA2) after 30- and 60-day of storage at 1 ºC. Values are
668 expressed as mean ± standard deviation. Capital letters indicate significant differences ($P<0.05$)
669 between phases of *in vitro* simulated gastrointestinal (GI) digestion. Lower case letters indicate
670 significant differences ($P<0.05$) between storage treatments at same storage time and digestion
671 phase. CA: Controlled atmosphere; CA1: 2.0 % O_2 + 3.5 % CO_2; CA2: 1.0 % O_2+ 2.0 % CO_2.

672 **Figure 7.** Total phenolic content of roasted *calçots* after *In vitro* simulated gastrointestinal (GI)
673 digestion of samples stored under air and two different controlled atmosphere (CA1 and CA2)
674 after 30- and 60-day of storage at 1 ºC. Values are expressed as mean ± standard deviation.
675 Capital letters indicate significant differences ($P<0.05$) between phases of *in vitro* simulated
676 gastrointestinal (GI) digestion. Lower case letters indicate significant differences ($P<0.05$)
677 between storage treatments at same storage time and digestion phase. CA: Controlled
678 atmosphere; CA1: 2.0 % O_2 + 3.5 % CO_2; CA2: 1.0 % O_2+ 2.0 % CO_2.

679

680 **TABLES**

681 **Table 1.** Morphologic parameters of *calçots* at harvest and after 30- and 60-day of storage at 1 ºC. Values are the mean of independent determinations ±
682 standard deviation. Different lower case letters in the same column indicate significant differences between storage conditions ($P<0.05$) for each storage time.
683 CA: Controlled atmosphere; FWL: Fresh weight loss.

Storage time	Storage atmospheres	FWL (%)	Largest diameter (cm)	Lowest diameter (cm)	Medium diameter (cm)	Length of white shaft (cm)
Harvest			2.11 ± 0.30	1.78 ± 0.17	1.61 ± 0.21	15.13 ± 1.73
30 d	AIR	0.0 ± 0.0 c	2.10 ± 0.28 a	1.76 ± 0.24 b	1.88 ± 0.26 a	14.83 ± 1.99 b
	CA1 (2.0 % O_2 + 3.5 % CO_2)	6.3 ± 0.0 b	2.16 ± 0.29 a	1.98 ± 0.28 ab	1.98 ± 0.27 a	15.30 ± 2.08 b
	CA2 (1.0 % O_2 + 2.0 % CO_2)	9.3 ± 0.3 a	2.42 ± 0.51 a	2.05 ± 0.39 a	2.08 ± 0.34 a	17.17 ± 1.40 a
60 d	AIR	23.0 ± 8.2 a	2.20 ± 0.30 a	1.98 ± 0.24 a	2.20 ± 0.66 a	17.20 ± 2.09 a
	CA1 (2.0 % O_2 + 3.5 % CO_2)	23.4 ± 2.2 a	2.24 ± 0.26 a	1.98 ± 0.23 a	2.07 ± 0.27 a	14.47 ± 1.89 b
	CA2 (1.0 % O_2 + 2.0 % CO_2)	9.3 ± 0.3 b	2.13 ± 0.46 a	1.82 ± 0.39 a	1.95 ± 0.37 a	14.60 ± 2.20 b

684

685

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687 **Table 2.** Quality parameters of *calçots* at harvest and after 30- and 60-day of storage at 1 ºC. Values are expressed as mean ± standard deviation. Different

688 lower case letters in the same column indicate significant differences between storage conditions ($P<0.05$) for each storage time. CA: Controlled atmosphere.

Storage time	Storage atmospheres	Firmness (N)	Respiration rate (mg CO_2 kg^{-1} s^{-1})	Total acidity (g malic acid L^{-1})	Soluble Solids (%)	pH
Harvest		61.84 ± 10.45	6.70 ± 0.05	1.99 ± 0.01	10.0 ± 0.2	5.9 ± 0.0
30 d	AIR	79.00 ± 22.20 b	17.54 ± 0.41 a	1.36 ± 0.19 a	10.3 ± 0.2 a	5.8 ± 0.0 b
	CA1 (2.0 % O_2 + 3.5 % CO_2)	103.51 ± 15.35 a	16.27 ± 1.10 a	2.00 ± 0.52 a	8.4 ± 0.2 b	6.0 ± 0.0 a
	CA2 (1.0 % O_2 + 2.0 % CO_2)	73.37 ± 17.99 b	12.00 ± 0.39 b	1.88 ± 0.17 a	8.5 ± 0.1 b	6.0 ± 0.0 a
60 d						
	AIR	83.09 ± 19.23 a	26.79 ± 0.74 a	3.53 ± 0.04 a	9.5 ± 0.0 a	5.8 ± 0.0 b
	CA1 (2.0 % O_2 + 3.5 % CO_2)	93.53 ± 20.30 a	21.69 ± 0.00 b	2.19 ± 0.07 c	7.1 ± 0.0 c	5.9 ± 0.0 a
	CA2 (1.0 % O_2 + 2.0 % CO_2)	94.06 ± 15.62 a	18.04 ± 0.00 c	2.45 ± 0.08 b	7.7 ± 0.0 b	5.9 ± 0.0 a

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692 **Table 3.** Color parameters of *calçots* at harvest and after 30- and 60-day of storage at 1 ºC. Values are expressed as mean ± standard deviation. Different

693 lower case letters in the same column indicate significant differences between storage conditions ($P<0.05$) for each storage time. CA: Controlled atmosphere.

Storage time	Storage Atmospheres	L*	a*	b*	Browning Index
Harvest		80.08 ± 2.69	-0.91 ± 0.46	5.18 ± 1.24	5.7 ± 1.4
30 d	AIR	81.67 ± 4.09 a	-1.94 ± 0.91 b	7.11 ± 2.48 a	7.2 ± 2.8 a
	CA1 (2.0 % O_2 + 3.5 % CO_2)	80.10 ± 5.67 a	-1.52 ± 0.29 a	4.87 ± 0.96 b	4.7 ± 1.1 b
	CA2 (1.0 % O_2 + 2.0 % CO_2)	76.57 ± 6.11 b	-1.27 ± 0.36 a	5.12 ± 0.94 b	5.5 ± 1.1 b
60 d					
	AIR	80.07 ± 3.41 a	-2.66 ± 1.48 a	9.29 ± 3.85 a	9.6 ± 4.2 a
	CA1 (2.0 % O_2 + 3.5 % CO_2)	80.84 ± 2.92 a	-2.62 ± 1.18 a	8.26 ± 3.26 a	8.2 ± 3.6 a
	CA2 (1.0 % O_2 + 2.0 % CO_2)	80.70 ± 3.05 a	-2.24 ± 1.07 a	7.71 ± 2.38 a	7.7 ± 2.5 a

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