Postharvest Biology and Technology 129 (2017) 118–128

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

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

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ARTICLE INFO

Keywords:
Allium
Controlled atmosphere
Roasting
Antioxidant activity
Total phenolic content
In vitro digestion

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 °C 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 *calçots* 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· 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*.

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 6–8 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).

The vast majority of available studies are focus on TPC and AA of non-digested samples. However, in practice, fruit and vegetables are subjected to simulated digestion to measure antioxidant potentially

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http://dx.doi.org/10.1016/j.postharvbio.2017.03.013
Received 16 November 2016; Received in revised form 21 March 2017; Accepted 22 March 2017

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

Therefore, the aim of this study was to evaluate the effect of CA storage on the physicochemical, nutritional and sensory quality of *calçots* at different reduced O_2 and elevated CO_2 concentrations. Study of simulated *in vitro* digestion (gastric and intestinal phase) of the roasted samples was carried out to evaluate the bioaccessibility of total phenols and the 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 (41°13′47″N, 01°13′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 8000 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× 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

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

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

2.4. Morphological analysis

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

2.5. Colour

The colour of the white shaft was measured as described by Altisent et al. (2014). Nine 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):

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

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

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 individual *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.