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Research Article

Alginate Edible Coating and Cold Storage for Improving the Physicochemical Quality of Cape Gooseberry (*Physalis Peruviana* L.)

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Abstract

The Cape gooseberry is an exotic tropical fruit and, nowadays, is the second most exported fruit from Colombia. Therefore, the high demand for quality required research for a better understanding of fruit behavior. Furthermore, postharvest quality properties play an important role in meeting consumer demands. Cold storage and edible coatings are reported as efficient technologies for extending shelf life and preserve the quality of fruits in postharvest. As there are no reports of studies about the effect of these technologies on shelf life, quality and antioxidant activity in Cape gooseberry, this work aim to evaluate the use of alginate 1% during 21 days of storage at 2ºC as an alternative for postharvest handling of this fruit. Cape gooseberry exhibits a high respiration rate and ethylene production at 20ºC. Alginate coat decreased significantly the metabolism activity of fruit during the cold storage without change significantly the fruit organoleptic quality and showing total phenolic, carotenoid contents and antioxidant activity. Alginate is an efficient edible coat for preserve the quality and bioactivity of Cape gooseberry during 21 days of storage at 2ºC.

Keywords: Alginate; Antioxidant activity; *Physalis peruviana*; Phytochemicals; Postharvest; Quality

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Introduction

Cape gooseberry (*Physalis peruviana* L.) is a tropical fruit of the Solanaceous family, native of the Andean region that bears cherry tomato-like fruit, being Colombia and South Africa the biggest producers and exporters and Germany and Netherlands the principal importers. *Physalis* have few cultivars and rather genotypes that has been selected in different countries and adapted to the different climates of the specific regions (ecotypes). The main ecotypes commercialized are associated to the production country: 'Colombia', 'Kenya', 'South Africa' and 'Ecuador'. This fruit is one of the most promising exotic fruits and many interesting functional products could be developed from these berries [1].

Nevertheless, is necessary to implement appropriate technologies and improve postharvest handling operations, in order to obtain fruit of excellent quality and guarantee it for marketing, avoiding high product losses [2].

Normally, Cape gooseberry fruit is exported in fresh to Europe with the calyx because this protect the fruit and enhanced the shelf life, but USA import this fruit without the calyx because of the cold quarantine treatment (T-107-b) required, being necessary to replace this protection for enhanced the shelf life. In this sense, edible coatings are known to increased storage period and preserve de quality of many fruits [3]. Alginate is a natural polysaccharide extracted from brown sea algae (Phaeophyceae) and it is composed of two uronic acids: β-D-mannuronic acid and α-L-guluronic acid. Sodium alginate is composed of block polymers of sodium poly (L-guluronate), sodium poly (D-mannuronate), and alternating sequences of both sugars. Alginate is known as a hydrophilic biopolymer that has a coating function because of its well-studied unique colloidal properties, which include its use for thickening, suspension forming, gel forming, and emulsion stabilizing [4]. As edible coating, sodium-alginate has been effective on maintaining postharvest quality of tomato [5] and plum cultivars [6].

Cape gooseberry has a high nutritional composition and biologically active health-promoting components [7]. It has been used as a good source of provitamin A, minerals, vitamin C and vitamin B complex. It also contains high levels of antioxidant compounds as well as minerals such phosphorous and iron. In traditional Colombian medicine is widely used as an anti-inflammatory medicinal plant. Cape gooseberry fruit is a climacteric fruit and its ripening is regulated by ethylene [8]. Reports indicate that the fruit contains high level of antioxidant compounds [7,9]. Due to a high antioxidant capacity of this fruit species, its popularity above all as a promising raw material, which can be used for human nutrition.

Storage of fruit for consumption exposes the physicochemical, color, antioxidant capacity and sensory characteristics to detrimental factors that may lead to alterations in concentrations and health-related quality, being important to investigate the effect of alginate coating and cold storage on the bioactive compounds that are present in the fruit. Since there is no literature on the use of alginate on Cape gooseberry quality and antioxidant properties, the aim of this work was to evaluate the effect of an edible coating based on alginate

on the quality and antioxidant activity of Cape gooseberry during cold storage.

Materials and Methods

Plant material and edible coating

Cape gooseberry (*Physalis peruviana* L.) fruit with the calyx, Colombian ecotype, was imported from Colombia to Spain by Verdefresh. The experimental work was done in Spain at the Department of Food Technology of Miguel Hernández University and at the Department of Food Science and Technology, Department of Edaphology and Applied Biology Center of Segura (CEBAS-CSIC) in the framework of collaboration between research groups of the CYTED thematic network 112rt0460 CORNUCOPIA.

Once at laboratory, 21 homogeneous lots (based on color and size) of ten fruits each were performed at random. Three lots were used to determine the fruit properties at harvest (day 0) and the 18 remaining were split into two groups for the following treatments in triplicate: 0% (control) and 1% (w/v) alginate coating.

Alginate (alginic acid sodium salt from brown algae purchased from Sigma, Madrid, Spain) was prepared according to a previous paper [5] (at 1% concentration w/v, by dissolving alginate in hot water (45ºC) with continuous shaking until the solution became clear. After cooling to 20ºC, glycerol at 20% (v/v) was added as a plasticiser, and treatments were performed by dipping the fruit twice in fresh coating solutions for 1min to ensure the uniformity of the coating of the whole surface. Control fruit were dipped in distilled water. After treatments, fruit were dried for 30 min using an air-flow heater at 25ºC. After drying, the lots were weighed, and then stored at 2ºC during 21 days. Three lots for control and treated fruits were sampled at random after 7, 14 and 21 days of storage.

Respiration rate and ethylene production

Ethylene production was measured by placing each lot of ten fruit in a 0.5L glass jar hermetically sealed with a rubber stopper for 30min and 1mL of the holder atmosphere was withdrawn with a gas syringe, and the ethylene was quantified using a ShimadzuTM GC-2010 gas chromatograph (Kyoto, Japan), equipped with a Flame Ionisation Detector (FID) and a 3m stainless steel column with an inner diameter of 3.5mm containing activated alumina of 80/100 mesh. Results were the mean ± SD of determinations for three replicates of ten fruit and expressed as nL g^{-1} h^{-1} (g of fresh weight). For respiration rate, 1mL of the same atmosphere was used to quantify CO_2 concentration by using a ShimadzuTM GC-2010 with Thermal Conductivity Detector (TCD). Results were the mean ± SD (n = 3) and expressed as mg CO_2 kg^{-1} h^{-1} (g of fresh weight).

Quality parameters

Fruit color was measure with a Minolta colorimeter (CRC200, Minolta Camera Co., Japan) in the CIE L*a*b*color space and the Index Color (1.000 x a*) / (L* x b*) was determined. Three measures per fruit were made in 10 fruits of each replicate. Fruit firmness was determined independently in 10 fruits of each replicate using a TX-XT2i® Texture Analyzer (Stable Microsystems, Godalming, UK) interfaced to a personal computer, with a maximum force of 25kN test, a flat steel plate with a lowering speed of 18mm min^{-1} and a measurement accuracy of 0.5-1%. For each fruit, the equatorial diameter was measured and then a force that achieved a 3% deformation of the fruit diameter was applied. The average diameter of the *Physalis* fruits used in this study was of 20mm. Results were expressed as the ratio between the force necessary to achieve the deformation and the deformation distance (N mm^{-1}) and were the mean ± SD.

After that the 10 fruits of each replicate were cut in small pieces to obtain a homogeneous sample. Total Soluble Solids (TSS) were determined in duplicated in the juice obtained from 5g of each sample with a digital refractometer Atago PR-101 (Atago Co. Ltd., Tokyo, Japan) at 20°C, and expressed as ºBrix (mean ± SD. Total Titratable Acidity (TTA) was determined in duplicated in the same juice by automatic titration (785 DMP Titrino, Metrohm) with 0.1N NaOH up to pH 8.1, using 1mL of diluted juice in 25mL distilled H_2O, and results (mean ± SD) expressed as g citric acid equivalent $100g^{-1}$ fresh weight. The maturity index was calculated as the quotient TSS/TTA. The remaining samples from each replicate were quickly frozen in liquid N_2 and stored at-20ºC until the following determinations were performed.

Antioxidant analysis

Total Antioxidant Activity (TAA) was quantified according to Serrano et al., [10] which enables to determine TAA due to both hydrophilic and lipophilic compounds in the same extraction. Briefly, for each sub-sample, five grams of tissue were homogenized in 5mL of 50mM phosphate buffer pH = 7.8 and 3mL of ethyl acetate, and then centrifuged at 10,000 x g for 15min at 4°C. The upper fraction was used for total antioxidant activity due to Lipophilic compounds (L-TAA) and the lower for total antioxidant activity due to Hydrophilic compounds (H-TAA). In both cases, TAA was determined using the enzymatic system composed of the chromophore 2,2'-Azino-Bis-(3-ethylbenzothiazoline-6-sulfonic acid) Diammonium Salt (ABTS), the Horse Radish Peroxidase enzyme (HRP) and its oxidant substrate (hydrogen peroxide), in which ABTS•+ radicals are generated and monitored at 730nm. The decrease in absorbance after adding the extract was proportional to TAA of the sample. A calibration curve was performed with Trolox ((R)-(+)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) (0-20nmol) from Sigma (Madrid, Spain), and results were the mean ± SD and expressed as mg of Trolox equivalent $100g^{-1}$ fresh weight.

Phytochemical analysis

Total carotenoids were estimated in the lipophilic extract [11] by reading the absorbance at 450nm in a UNICAM Helios-α spectrophotometer (Cambridge, UK). An ethyl acetate solution (98%) was used as blank. To calculate the amount of total carotenoids expressed as beta-carotene the specific absorption coefficient of $\varepsilon^{1\%}_{cm}$ = 2560 was used, which represents the theoretical absorbance of the solution of 1.0g of pigment in 100mL of solvent (C) measured in a cell thickness of 1cm, according to the Lambert-Beer Law [12]. The results were expressed as mg of α-carotene $100g^{-1}$ fresh weight, and the results were the mean ± SD.

Total phenolics were extracted as previously reported [10], using water:methanol (2:8) containing 2mM NaF (to inactivate polyphenol oxidase and prevent phenolic degradation) and quantified in duplicated in each extraction by using the Folin-Ciocalteu reagent [13]. Results (mean ± SD) were expressed as mg gallic acid equivalent $100g^{-1}$ fresh weight.

Statistical analysis

Experimental data were subjected to ANOVA analysis. Sources of variation were treatments and storage. The overall least significant

differences (Fisher's LSD procedure, $p<0.05$) were calculated and used to detect significant differences among treatments and storage time. All analyses were performed with SPSS software package v. 11.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Figure 1, shows the ethylene production and respiration rate of Cape gooseberry during ripening at 20°C without coating, showing that this fruit has a climacteric ripening-pattern reaching the ethylene peak (ca. 350nL g^{-1} h^{-1}) after 2 days of storage at 20°C. Respiration rate increased at the end of storage (day 9) until ca. 200mg kg^{-1} h^{-1}, maybe due to overripe of the fruit.

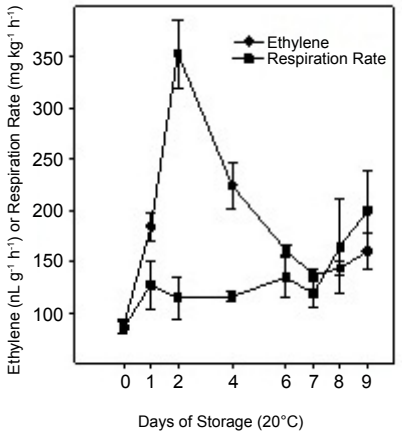

Figure 1: Ethylene production and respiration rate of Cape gooseberry during storage at 20°C without coating. Data are the mean ± SD (n = 3) and vertical bars represent the standard deviation.

The ethylene production and respiration rate of control and alginate-coated Cape gooseberry stored during 21 days at 2°C are presented in figure 2. At day 0 in control fruits, ethylene production was 88.0nL g^{-1} h^{-1} and decreased during the first 7 days of storage although ethylene production peaked at day 14 which could be associated to occurrence of climacteric peak, as has been observed at 20°C at day 2 (Figure 1). Respiration rate was 85.6mg kg^{-1} h^{-1} at day 0 and remained without significant changes during the first 14 days of storage. In alginate-coated fruits, the same behavior was observed, although ethylene production and respiration rate were significantly lower in all sampling dates (Figure 2).

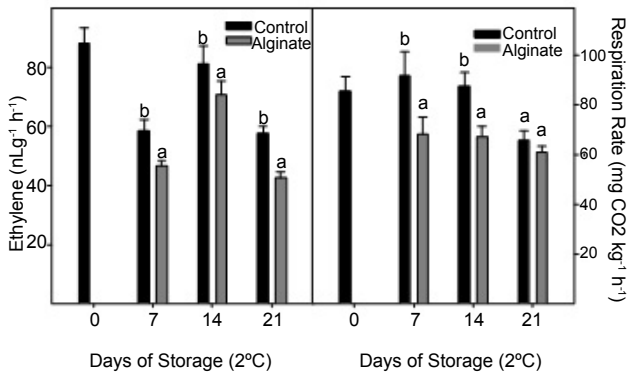

Figure 2: Ethylene production and respiration rate of Cape gooseberry coated with alginate and storage during 21 days at 2°C. Data are the mean ± SD (n = 3) and vertical bars represent the standard deviation. Same letters represent non significant differences (p>0.05) between treatments.

Fruit color index did not change significantly after 21 days of cold storage in control fruit storage, observing no significant differences between coated and non-coated fruit (Figure 3).

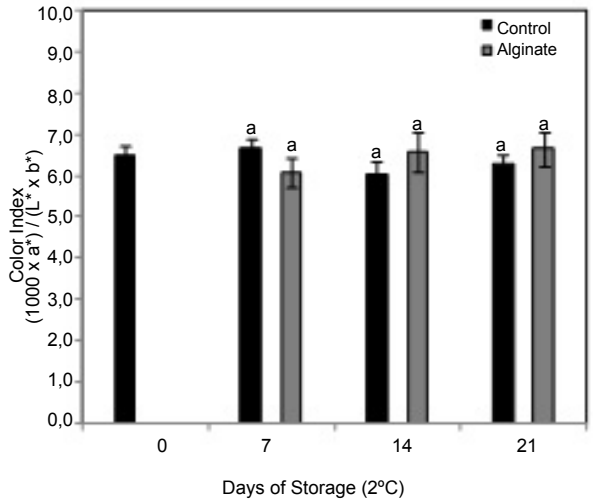

Figure 3: Color evolution of Cape gooseberry coated with alginate and storage during 21 days at 2°C. Data are the mean ± SD (n = 3) and vertical bars represent the standard deviation. Same letters represent non-significant differences (p>0.05) between treatments.

No significant changes were observed for total soluble solids and total acidity during storage and generally neither between treatments (Figure 4).

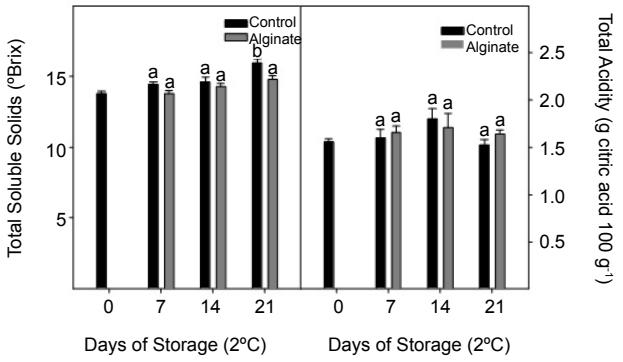

Figure 4: Total soluble solids and acidity evolution of Cape gooseberry coated with alginate and storage during 21 days at 2°C. Data are the mean ± SD (n = 3) and vertical bars represent the standard deviation. Same letters represent non significant differences (p>0.05) between treatments.

Maturity index did not change significantly during cold storage and no differences were observed between treatments (Figure 5). Fruit firmness was significantly reduced in the first week of storage, nevertheless no significant differences between alginate edible coat and control fruit were registered.

No significantly changes were observed for total phenolic content during storage in the control fruit, while the fruit coated with alginate

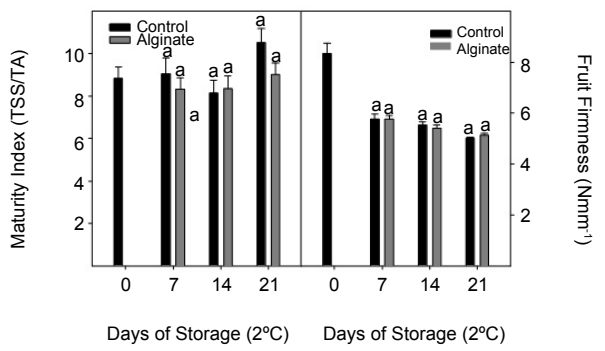

Figure 5: Maturity index and fruit firmness evolution of Cape gooseberry coated with alginate and storage during 21 days at 2ºC. Data are the mean ± SD (n = 3) and vertical bars represent the standard deviation. Same letters represent non significant differences (p>0.05) between treatments.

presented values significantly higher during storage for all sampling dates (Figure 6). Carotenoids content did not change significantly in control fruits during 21 days of cold storage. Alginate coated fruits had significantly higher carotenoid content at the end of the storage (Figure 6).

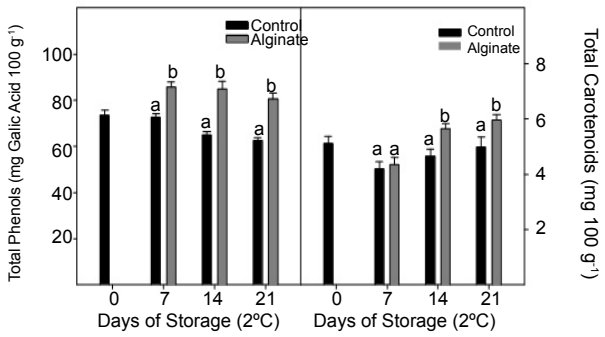

Figure 6: Total phenolics and carotenoids evolution of Cape gooseberry coated with alginate and storage during 21 days at 2ºC. Data are the mean ± SD (n=3) and vertical bars represent the standard deviation. Same letters represent non significant differences (p>0.05) between treatments.

Hydrophilic antioxidant activity was higher than the lipophilic fraction in Cape gooseberry, but both decreased significantly during storage, especially during the first week (Figure 7). Fruits coating with alginate registered a higher hydrophilic antioxidant activity compared to control fruits after 21 days of storage.

Discussion

The ethylene production and respiration rate of Cape gooseberry results confirm previous report in which this fruit can be classified as a fruit with extremely high climacteric rise in both ethylene (peak of 350nL g^{-1} h^{-1}) and CO_2 (peak of 134.5mg kg^{-1} h^{-1}) production [8,14] when compared with other climacteric fruits, such as apricot with a variation of 4-6nL ethylene g^{-1} $h^{-1}$and 30-50mg CO_2 kg^{-1} h^{-1} during ripening at 20°C [15], avocado reach near 100nL ethylene g^{-1} h^{-1} [16], or plum with a variation of 0.10 to 200nL ethylene g^{-1} h^{-1} and 16 to

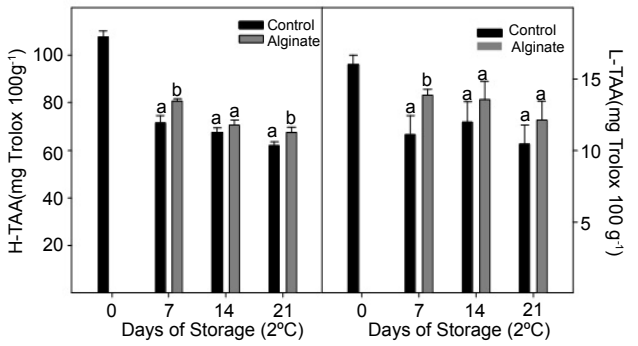

Figure 7: Hydrophilic and lipophilic antioxidant activity of Cape gooseberry coated with alginate and storage during 21 days at 2ºC. Data are the mean ± SD (n = 3) and vertical bars represent the standard deviation. Same letters represent non significant differences (p>0.05) between treatments.

14mg CO_2 kg^{-1} h^{-1} [17]. According to Gutiérrez et al., [18] the Cape gooseberry fruits are considered climacteric fruit, because after physiological maturity have an increased respiratory rate.

Alginate coating significantly reduced the ethylene and respiration rates in Cape gooseberry fruits during cold storage, has been previously reported in other climacteric fruits such as tomato [5] and 4 plum cultivars [19]. This can be explained by the fact that alginate coatings increase the skin resistance to gas diffusion by blocking the pores on the fruit surface, resulting in a modified internal atmosphere of relatively high CO_2 and low O_2 [20]. The elevated internal CO_2 could be responsible for the ethylene inhibition by a reduction of the 1-Aminocyclopropane-1-Carboxylic Acid (ACC) synthase activity [5,21]. Additionally, alginate has a moderate permeability to CO_2 (500cm^3 CO_2 m^{-2} bar^{-1} day^{-1}) probably reducing the pass of CO_2 through the fruit skin [22].

Although, the fruit color index was not significant different between alginate coating fruit and control fruit, this parameter tended to be higher in coated fruit at the end of cold storage (day 14 and 21), as observed by Díaz-Mula et al., [6] where alginate coating were effective on delaying the evolution of color for sweet cherry fruits. Rojas-Graü et al., [23] reported alginate and gellan-based coatings as good carriers for anti browning agents in fresh-cut Fuji apples. Ali et al., [24] reports a significant delay in changes of color development for tomato fruit coated with 10% gum arabic. Balaguera et al., [25] observed an evolution of IC from 4 to 7 in Cape gooseberry at 16°C during 22 days of storage, while in our study the values remain between 6 and 7.

The TSS was near 15° Brix and the TA near to 1.5 during the 21 days at 2°C, similar to the values observed by Balaguera et al., [25] for *Physalis*, nevertheless they observed a marked decreased of MI (near 7 to near 4) during storage at ambient temperature because the decreased in TA. As these parameters remain constant at 2°C the MI did not show significant differences during cold storage period and the fruits of this study showed a higher MI of near 9. Garzón-Acosta et al., [26] observed lower maturity index in Cape gooseberry fruits stored at 1°C compared to room temperature probably due to the retarding effect of low temperature in the fruit metabolism.

The fruit firmness is regarded as one of the main attributes of quality and often limits the postharvest life. It reflects the changes in cell structure, cell cohesion and some biochemical changes [27].

Alginate coating did not retain significantly the cape gooseberry fruit firmness during cold storage respect to control fruit, although

the firmness decline drastically from 8.3 to 5.7Nmm^{-1} in 7 days of cold storage maybe do to the high MI of fruit (8.87). Gutiérrez et al., [18] observed a continuous decrease in Cape gooseberry firmness during the postharvest, however fruits with low MI at harvest retain higher values of firmness during storage than fruits harvested with greater MI. Nevertheless, fruit firmness in this study decreased but remain high reaching values of 5Nmm^{-1} after 21 days in cold storage, because according the same authors after eight days of storage at 20°C, the firmness of the fruits of Cape gooseberry can reach about 3 or 4N.

Majumder and Mazumdar [28], found high activity of the enzyme Poligalacturonase (PG) in *Physalis* fruits at 30 days after anthesis with a continuous increase during the maturation process, coinciding this with ethylene synthesis and high respiratory rate. The PG apparently plays an important role in the solubilization of pectin substances that lead to gradual softening in fruit ripening in *Physalis*.

In other fruits, such as plums and tomatoes firmness retention and delayed acidity loses have been observed in alginate-coated fruits during storage [5,6], which could be related that these fruits had lower values of ethylene production at the climacteric peak (8-20nL g^{-1} h^{-1}) compared with Cape gooseberry (≅ 350nL g^{-1} h^{-1}).

Phenolic compounds are secondary metabolites, widely distributed in plants. They are important components of many fruits and vegetables not only for their major influence on sensory qualities of the fruit (color, flavour, taste), but also for their antioxidant, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory properties [29].

As observed by different authors the total phenols in Cape gooseberry decreased significantly during cold storage in control fruits. The composition of the antioxidant phenolics in fruit increases during ripening, meanwhile during shelf life, antioxidants are rapidly reduced [30,31].

Total carotenoids and phenols content did not change significantly during storage but was significantly higher for alginate coated fruits at the end of storage, with higher hydrophilic antioxidant activity in day 21 of storage respect to control fruits. However, Fischer et al., [32] and Severo et al., [33] observed a significantly increase of carotenoids and phenols content during cold storage at Passive Modified Atmospheres (PMA) in Cape gooseberry fruits, although antioxidant capacity decreased after the second day of storage and remained lower in fruits as observed in our study for control fruits. Strawberries treated with chitosan also maintained better fruit quality with higher levels of phenolics, anthocyanins, flavonoids [34]. Tomatoes fruit coated with 10% gum arabic maintained total antioxidant capacity, total phenolics and total carotenoids during storage as compared to the uncoated control and fruit treated with 5% gum arabic concentration [24].

Chitosan coatings significantly increased the content of total phenolics and antioxidant activity in apricot fruits, as 0.5% chitosan showed maximum total phenolics (82.65mg GAE/100g), content similar to those reached by Cape gooseberry with alginate in this study [35]. According to Benhamou [36], chitosan also has a potential of inducing phenolic contents in plants. The phenols content decreased at the end of storage as reported by Macheix et al., [37] which might be due to breakdown of cell structure in order to senescence phenomena during storage.

The carotenoids content significantly increased in alginate coating fruit although it maintained constant in control fruit. No significant change in color fruit were observed for control fruit and for alginate coating fruit the CI increased slightly without significant differences. According to Balaguera et al., [38] the color change during postharvest fruit of *Physalis* depends, among other factors, on the stage of maturity at harvest. As the MI of the fruits studied was high the fruit color and the carotenoid content evolution will be expected to be low. Studies realized by Fischer et al., [39] indicate that β-carotene increased in Cape gooseberry fruit until the fruit purchased the orange color and then dropped and rise again in the state of over ripeness. High antioxidant capacity has been demonstrated for Cape gooseberry juice [7], and the synergistic effect of different antioxidants has also been suggested. Furthermore, a high level of phenols was reported for the fruit [9]. In general, alginate coating preserved a higher antioxidant activity respect to control fruit in Cape gooseberry during cold storage as reported by Ali et al., [24] for gum arabic (10%) edible coating, where the antioxidant capacity of tomato fruits was preserved for up to 20 days during storage at 20°C without any negative effects on postharvest quality.

The total content of antioxidants in a fruit depends on the species and cultivar and can be affected by many factors, such as environmental growing conditions, harvest time, ripening stage, storage and processing conditions [7].

In our studies Cape gooseberry showed high content of total phenols (76mg Gallic Acid 100g^{-1}), carotenoids (5.6mg 100g^{-1}) and hydro-antioxidant capacity of (110mg Trolox 100g^{-1}) similar to values reported by other authors [9,40].

The antioxidant activity of Cape gooseberry seems to be related to hesperidin, tannic acid, quercetin and gallic acid [41]. Gironés et al., [42] only found quercetin and Kaempferol in this fruits. Maturity degree and fruit size affect the fruit's chemical characteristics and antioxidant activity [43].

Both antioxidant activities decreased markedly in the first 7 days of cold storage, maybe due to the high MI of fruit and the high production of ethylene and respiration rate. Valdenegro et al., [31] observed that unripe fruit of Cape gooseberry presented a high antioxidant level, and a clear increment in antioxidant capacity and polyphenol contents was observed throughout ripening with maximum values at the ripe stage. Nevertheless, after harvest the antioxidant capacity was rapidly reduced during the shelf-life period (20°C) and ethylene treatment increased this reduction.

Conclusion

Cape gooseberry exhibits a high respiration rate and ethylene production at 20°C compared with other fruits and the alginate coat decreased significantly the metabolism activity of fruit during the cold storage. Alginate did not changed significantly the organoleptic quality of Cape gooseberry and total phenols, carotenoids and antioxidant activity was higher for this treatment, during 21 days of storage at 2°C. Antioxidant activity decreased drastically after one week of storage. The edible coating preserves the total phenolic and carotenoid content during cold storage. Alginate is an efficient edible coat for preserve the quality and bioactivity of Cape gooseberry during storage. These findings represent an alternative for postharvest handling of fresh Cape gooseberry fruit preserving their natural and health contents.

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