Food Sci Biotechnol (2018) 27(4):987–996
https://doi.org/10.1007/s10068-018-0326-0

Effect of alginate coating on the physico-chemical and microbial quality of pansies (*Viola* × *wittrockiana*) during storage

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Received: 3 November 2017 / Revised: 23 January 2018 / Accepted: 25 January 2018 / Published online: 12 February 2018

Abstract Edible flowers, such as pansies, are becoming more popular, but they are highly perishable. So, postharvest technologies are needed, being edible coatings a good alternative. Thus, the aim of this study was to evaluate the effect of alginate coating on physico-chemical and microbiological quality of pansies during cold storage (4 °C for 0, 7, 14, 21 days). Coated pansies maintained good appearance until 14 days of storage, 7 days more than uncoated ones. Flavonoids, hydrolysable tannins and monomeric anthocyanins, as well antioxidant activity, were higher in coated pansies when compared to uncoated ones, on all assayed storage times. Furthermore, after 14 days of storage, uncoated pansies presented microorganism counts higher than coated, namely yeasts and moulds, suggesting an effective barrier protection of the alginate coating treatment. In summary, alginate coating has potential for extending shelf-life and improving physico-chemical and microbiological quality of pansies.

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Keywords *Viola* × *wittrockiana* · Alginate coating · Antioxidant activity · Bioactive compounds · Microbial load

Introduction

Edible flowers have been eaten for thousands of years, as evidenced in old writings. Nowadays, the demand for edible flowers has increased because consumers search for unique culinary experiences and they want to make a return to earlier lifestyles, in which edible flowers played an important role [1].

Pansies (*Viola* × *wittrockiana*) are edible flowers with an intense flavor being used in soups, salads and drinks, and to give shape and color to dishes. In addition, pansies contain healthy components such as anthocyanins, carotenoids, flavonoids, potassium and phosphorus, with recognized bioactivity in terms of antioxidant and free radical-scavenging properties [1–3]. Nowadays, pansies are marketed fresh, suitably packed in bunches, boxes, etc. and sold either directly in farm shops or through various specialized outlets. However, pansies have a limited shelf-life because flowers are susceptible to petal abscission, discoloration, wilting, dehydration and tissue browning soon after harvest. The most common methods used to improve postharvest storage of fresh pansies flowers quality include refrigeration, drying, canning in sugar and preservation in distillates. However, these methods may cause undesirable biochemical and nutritional changes in the processed product that may affect its overall quality.

Edible coatings can be used to protect perishable food products from deterioration by providing a selective barrier to moisture, oxygen and carbon dioxide, delaying dehydration, suppressing respiration, improving textural quality,

while helping to retain volatile flavor compounds and reducing microbial growth [4]. The use of coatings derived from proteins, lipids and polysaccharides for this purpose, has received increased interest over recent years, particularly regarding the preservation of important characteristics as texture [5]. Therefore, the application of edible coatings can be a suitable method for preserving pansies. Thus, the objective of this study was to evaluate the effect of alginate coating on the quality of white pansies during cold storage (4 °C). Thus, the following physicochemical characteristics were evaluated: visual appearance, weight loss, water activity (a_w), pH and acidity, as well as several bioactive compounds (monomeric anthocyanins, flavonoids, carotenoids, total phenolic content and hydrolysable tannins) and antioxidant activity (Reducing power and DPPH radical scavenging activity). Furthermore, pansies' microbial quality was also evaluated.

Materials and methods

Samples

Fresh white pansies (*Viola* × *wittrockiana*) were collected in full ripening stage at the greenhouse of School of Agriculture, Polytechnic Institute of Bragança (Bragança, Portugal). After harvest, fresh flowers were immediately transported to the laboratory under refrigeration.

Edible coatings

Edible coating treatment was applied according to the method used by Tay and Perera [6]. Commercial sodium alginate (Panreac Química SA, Barcelona, Spain) solution was prepared by solubilizing 2.0 g of its powder in 100 mL of water under stirring. Pansies were immersed in the alginate solution for 30 min at room temperature and afterwards allowed to drip off. Then, pansies were immersed in a calcium chloride solution (1%, w/v) for 5 min to induce spontaneous cross-linking reactions. When sodium alginate is put into a solution of calcium ions, the calcium ions replace the sodium ions in the polymer, as each calcium ion can attach to two of the polymer strands. Alginate coating was selected because it has good film-forming properties and it produces uniform, transparent and water-soluble films [7]. It also enhances the coating adhesion to the surface of vegetables [8]. Furthermore, alginate coatings are good oxygen barriers [9], and reduce the weight loss and the microflora counts [10]. Even though alginate is not such a good barrier to water loss as chitosan, alginate will not cause allergy to sensitive persons to seafood, from which chitosan is obtained.

Storage

Approximately 2 kg of fresh and coated pansies were stored under refrigeration (4 °C) during 21 days. After 7, 14 and 21 days of storage, photos of the flowers were taken and some physico-chemical properties were evaluated. A portion (300 g) was frozen and freeze-dried (Scanvac, Coolsafe, Lynge, Denmark) for later evaluation of bioactivity and antioxidant activity, as detailed below.

Physico-chemical analyses

Moisture was determined by weight loss at 105 °C until constant weight [11]. Water activity (a_w) was determined with a portable water activity meter (Novasina, LabSwift-aw, Lachen, Switzerland). Weight was measured in a digital balance (Kern ACJ/ACS, Balingen, Germany). Weight loss (WL) was determined according to Eq. 1:

$$WL = \frac{M_0 - M}{M_0} \times 100 \quad (1)$$

where M_0 is the initial mass of pansies (fresh or coated) in day 0, M is the mass of pansies after storage.

pH and titratable acidity (TA) were determined following standard methods [12]. Briefly, 0.5 g sample was homogenized in 50 mL of distilled water, filtered and the pH measured with a potentiometer (Hanna Instruments, HI8417). TA was measured by titrating 10 mL of this solution with a 0.01 N NaOH solution using phenolphthalein as an indicator. Results were express in g acid citric/100 g of dry weight (DW).

Carotenoids

The carotenoid contents were determined according to the method used by Aquino-Bolaños et al. [13]. One gram of freeze-dried powder of uncoated and coated pansies was extracted twice with 20 mL acetone:hexane solution (1:1, v/v). Both extracts were combined in a separation funnel, being added 200 mL of distilled water to eliminate acetone. The acetone-free phase was mixed with 5 g anhydrous sodium sulphate to eliminate any residual water, being the remaining solution filtered and completed to 100 mL with hexane. Total carotenoid content was determined by reading the absorbance at 450 nm and comparing the results to a β-carotene calibration curve (0.22–8.8 μg/mL). Results were expressed in μg β-carotene equivalents/g DW.

Extraction conditions for monomeric anthocyanins and bioactivity determination

Extraction was based on the method described by Li et al. [14] with slight modifications. Freeze-dried powders (1 g) of uncoated and coated pansies were extracted with 50 mL of water:acetone (6:4, v/v) at 37 °C for 30 min, under agitation (IKA, RCT Model B, Staufen, Germany) at 1000 rpm. The water:acetone extracts were filtered and placed in a rotary evaporator (Stuart, RE300DB, Stone, UK) to remove the solvent. Then, all extracts were frozen and placed in the freeze drier (Coolsafe, Lynge, Denmark) for 2 days. The extracts obtained were redissolved within the same solvent to a concentration of 50 mg extract/mL and covered with aluminium foil under freezing until further analysis.

Monomeric anthocyanins

The total monomeric anthocyanin contents on the extracts of uncoated and coated pansies during storage were estimated by the pH differential method, following the methodologies used by Bchir et al. [15.] and Rajasekar et al. [16]. The method consisted on using two buffer systems: potassium chloride buffer at pH 1.0 (0.025 M) and sodium acetate at pH 4.5 (0.4 M). Extracts portions were diluted on both buffers, and allowed to stand for 30 min at room temperature. Subsequently, the absorbance readings were made on a UV–Visible spectrophotometer (Thermo, Genesys 10 UV, Waltham, USA) at the wavelengths of 510 and 700 nm, being the absorbance difference (A) determined by the equation:

$$A = (A_{510\,nm} - A_{700\,nm})_{pH\,1.0} - (A_{510\,nm} - A_{700\,nm})_{pH\,4.5} \quad (2)$$

The monomeric anthocyanin pigment concentration was expressed on cyanidin-3-glucoside, determined by the equation:

$$\text{Monomeric anthocyanin pigment}\,(\text{mg Cy 3} - \text{glu/L}) = A \times \text{MW} \times \text{DF} \times 1000/(\varepsilon) \quad (3)$$

where MW = molecular weight (449.2), DF = dilution factor and ε = Molar absorptivity (26,900). All measurements were performed in triplicate. The results were expressed in mg of cyanidin-3-glucoside/g fresh weight (mg Cy 3-glu/g FW).

Total flavonoids

The total flavonoid content was determined by the method described by Viuda-Martos et al. [17], with slight modifications. To fresh and coated pansies extracts (1 mL) were added 0.3 mL of $NaNO_2$ (5%, m/v) and, after 5 min, 0.3 mL of $AlCl_3$ (10%, m/v) were mixed. After 6 min, 2 mL of NaOH (1 M) were added. Absorbance was read at 510 nm and flavonoids were quantified using a standard curve of quercetin (10–160 µg/mL). Results were expressed in mg of quercetin equivalent/g fresh weight (mg QE/g FW).

Hydrolysable tannins

The content of hydrolysable tannins was determined by the method described by Elfalleh et al. [18]. To one mL of uncoated and coated pansies extracts, 5 mL of 2.5% KIO_3 was added and stirred for 10 s. Absorbance was measured at 550 nm. Different concentrations of tannic acid (0.025–1.6 g/L) were used for calibration. Results were expressed in mg of tannic acid equivalent/g fresh weight (mg TAE/g FW).

Total phenolic content

The total phenolic content (TPC) of each sample was determined by the Folin-Ciocalteu method as described by Falcão et al. [19]. To 8 mL of uncoated and coated pansies extracts solutions were added 500 µL of Folin-Ciocalteu reagent. After 5 min, 1.5 mL of saturated sodium carbonate solution was added. After 2 h the absorbance values were read at 765 nm. A calibration curve was obtained with gallic acid (0.25–5 mg/L) and the results expressed in mg gallic acid equivalent/g fresh weight (mg GAE/g FW).

Antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

DPPH radical scavenging activity was determined by the procedure described by Delgado et al. [20] with some modifications. A 0.0024 g amount of DPPH was dissolved in 100 mL of methanol to obtain a 6.09×10^{-5} mol/L solution. Pansies extract diluted solutions (300 µL) were added to 2.7 mL of the DPPH methanolic solution. After 1 h in the dark at room temperature, absorbance was read at 517 nm. Antioxidant activity was expressed by the percentage of scavenging effect according to the formula in Eq. 4:

$$DPPH\ radical\ scavenging\ effect\ (\%) = \frac{A_{DPPH} - A_{Sample}}{A_{DPPH}} \times 100 \quad (4)$$

A_{DPPH} was the absorbance of the DPPH solution and A_{Sample} the absorbance in the presence of the sample. The extract concentration providing 50% of DPPH radical

scavenging effect (EC_{50}) was calculated from the graph of DPPH radical scavenging effect percentage *versus* extract concentration.

Reducing power

The reducing power of each extract was determined by the procedure described by Delgado et al. [20]. To 1.0 mL of uncoated and coated pansies extracts solutions at different concentrations were added 2.5 mL of phosphate buffer 0.2 M (pH 6.6) and 2.5 mL of $K_3[Fe(CN)_6]$ 1% (m/v). After shaking, the mixtures were incubated at 50 °C for 20 min after which 2.5 mL of 10% trichloroacetic acid (m/v) was added with further stirring. A volume of 2.5 mL of the mixture was transferred to another test tube, to which 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ 0.1% (m/v) were added. The absorbance values were read at 700 nm. From the graph $Abs_{700\ nm}$ *versus* concentration, the EC_{50} values were determined corresponding to the concentration that gave an absorbance of 0.5.

Microbial quality

Uncoated (3 g in triplicate) and coated (3 g in triplicate) pansies at the beginning of storage (0 days), as well as, after 14 days of cold storage (4 °C) were analyzed for total aerobic mesophilic, yeast and molds, lactic acid bacteria, total coliforms, *Escherichia coli* and psychrotrophic bacteria counts. All samples were diluted in 27 mL physiological peptone water. Samples were placed in sterile stomacher bags and homogenized in a Stomacher 400 (Seward, UK) for 2 min. The homogenates were subjected to serial dilutions with peptone water and then 1 mL of each dilution was pipetted into the surface of plate count agar (PCA, Merck, Algés, Portugal), Rose Bengal Chloramphenicol Agar (RBC-Agar, Merck) and Man, Rogosa and Sharpe Agar (MRS-Agar, Merck). The PCA plates were then incubated for 2 days at 30 °C for total aerobic mesophilic count and 5 days at 10 °C for psychrotrophic bacteria count. Lactic acid bacteria were determined in MRS-Agar plates, at 37 °C for 2 days. Yeast and molds were determined in RBC-Agar plates, incubated at 27 °C for 3–5 days. Total coliforms and *E. coli* were determined according to the SimPlate method. All counts were expressed as log10 cfu/g fresh sample.

Statistical analysis

SPSS Statistic software, version 18.0 (SPSS Inc., Chicago, USA), was used for the statistical treatment of the data. Analyses of variance (ANOVA) or ANOVA Welch were carried out to evaluate if there were significant differences ($p < 0.05$) between samples. ANOVA was applied when homogeneity of variances was observed, while ANOVA Welch was applied for the other cases. Additionally, significant post hoc analyses were performed (Tukey HSD test if variances in the different groups were identical or Games-Howell test if they were not). The homogeneity of variance was tested by Levene's test. The correlations between variables were determined by Pearson correlation coefficients. All analyses were performed in triplicate.

Results and discussion

Visual appearance

The visual appearance of the uncoated and coated pansies during storage (4 °C) is shown in Fig. 1. The uncoated pansies showed good appearance until 7 days, but after this period the petals were shrived and smaller than at 0 days. On the other hand, coated pansies showed good appearance until 14 days, similar to fresh samples. After 21 days, although the majority of coated pansies preserved a good appearance, some began to present brown spots on the petals, as those develop under moist conditions [21].

Weight loss, a_w, pH and titratable acidity

Weight loss increased during cold storage for both uncoated and coated pansies (Table 1), with masses at 21 days of storage corresponding to losses 85.9% for uncoated and 81.8% for coated. Even though coated pansies had always lower mean weight loss values than uncoated ones, there were no statistically differences during the storage period.

Low a_w values are important not only to prevent microbial growth but also to avoid texture degradation and to minimize deteriorative chemical and enzymatic reactions. Coated (0.97) and uncoated (0.91) pansies maintained high values of a_w until 14 days of storage (Table 1), despite some visual differences after 14 days of storage, with uncoated pansies showing drier and more shriveled petals than coated pansies. Only after 21 days of storage, both samples showed a_w values (0.50 and 0.59 for uncoated and coated pansies, respectively) that are known to prevent pathogenic microorganisms ($a_w < 0.86$) and yeasts and moulds ($a_w < 0.62$) growth [22], resulting in a hurdle to microbial development.

Regarding pH, some variability was observed, without any special trend, varying the results between 5.56 and 6.09 for uncoated, and 5.42–5.67 for coated pansies. The pH of uncoated pansies after 7 days decreased slightly when compared to fresh ones, while for coated pansies the pH increased. This pH increase may be due to the break-up of acids with respiration during storage. However, at 14 and

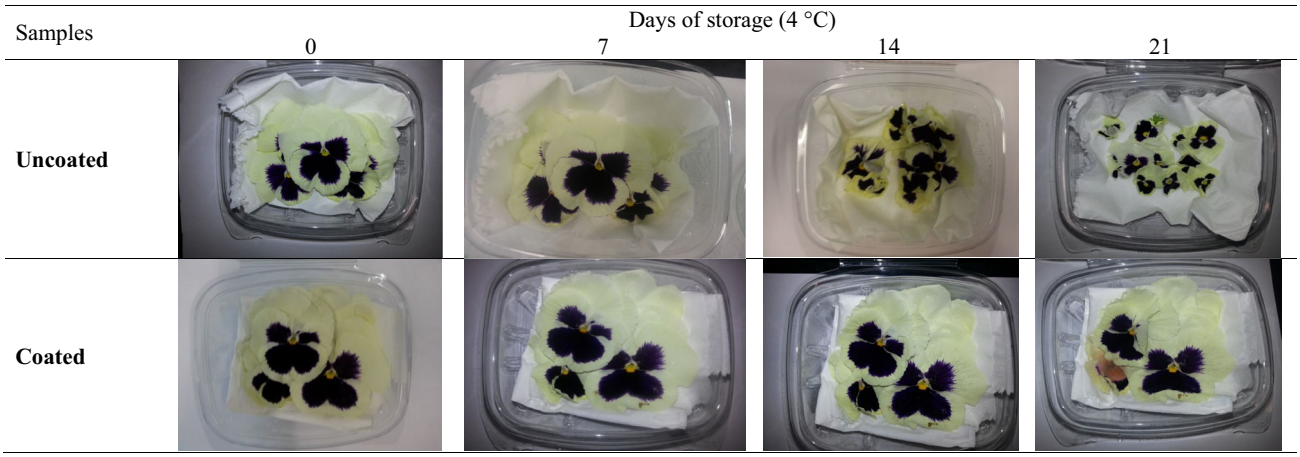

Fig. 1 Visual appearance of uncoated and coated pansies during storage (4 °C)

Table 1 Weight loss, a_w, pH, TA and total carotenoids of uncoated and coated pansies during storage (4 °C)

Properties	Storage days	Uncoated	Coated
Weight loss (%)	0	–	− 18.0 ± 5.5^{a}
	7	43.7 ± 6.9^{a,A}	29.6 ± 6.7^{b,A}
	14	74.9 ± 6.7^{b,A}	66.8 ± 5.2^{c,A}
	21	85.9 ± 3.6^{b,A}	81.8 ± 2.5^{d,A}
a_w	0	0.98 ± 0.01^{c,A}	1.00 ± 0.00^{b,B}
	7	0.96 ± 0.02^{c,A}	0.97 ± 0.01^{b,A}
	14	0.91 ± 0.02^{b,A}	0.97 ± 0.04^{b,B}
	21	0.50 ± 0.04^{a,A}	0.59 ± 0.03^{a,A}
pH	0	6.09 ± 0.29^{b,B}	5.42 ± 0.09^{a,A}
	7	5.56 ± 0.06^{a,A}	5.67 ± 0.27^{b,A}
	14	6.04 ± 0.06^{b,B}	5.56 ± 0.06^{a,b,A}
	21	6.00 ± 0.08^{b,B}	5.48 ± 0.07^{a,b,A}
TA (g citric acid/100 g FW)	0	0.10 ± 0.01^{b,A}	0.11 ± 0.04^{a,A}
	7	0.12 ± 0.02^{b,B}	0.07 ± 0.02^{a,A}
	14	0.04 ± 0.01^{a,A}	0.08 ± 0.01^{a,B}
	21	0.05 ± 0.01^{a,A}	0.06 ± 0.02^{a,A}
Total carotenoids (mg β-carotene/g FW)	0	93.0 ± 4.3^{c,A}	107.4 ± 6.4^{c,B}
	7	45.0 ± 3.6^{b,A}	73.5 ± 3.8^{b,B}
	14	39.7 ± 0.4^{a,b,A}	69.6 ± 4.2^{b,B}
	21	33.0 ± 1.9^{a,A}	31.7 ± 3.3^{a,A}

Lowercase letters—values with the same letter in the same column are not statistically different ($p > 0.05$); uppercase letters—values with the same letter in the same line are not statistically different ($p > 0.05$)

21 days of storage, the pH values for the coated and uncoated pansies were not significantly different to fresh.

Concerning TA of uncoated pansies, our results suggest that TA decreased after 14 days (Table 1), probably due to the use of organic acids as substrates for the respiratory metabolism in vegetables during postharvest storage [23]. After 7 days, an increase on TA content of uncoated pansies was observed, which was in line with the decrease of pH. On the other hand, no significant differences on TA values of coated pansies were observed along storage. In general, the TA content changed more slowly in coated than in uncoated pansies. So, alginate coating delayed the reduction of TA in pansies. This may be attributed to the modification of endogenous levels of O_2 and CO_2 imposed by the coating presence, inhibiting the respiratory activities and reducing ethylene biosynthesis [24, 25]. Still, our results were similar to those reported by Varasteh et al. [25], who reported a reduction of TA during storage (45, 90 and 135 days) in uncoated and coated (chitosan) pomegranate fruits.

Total carotenoids

Total carotenoids decreased during storage on both coated and uncoated pansies (Table 1), from 93.0 to 33.0 (uncoated) and 107.4 to 31.7 (coated) mg β-carotene/g FW, probably due to carotenoids' degradation. After treatment (day 0), 7 and 14 days, coated pansies showed significantly higher ($p < 0.05$) values than uncoated pansies (aprox. 1.6 times more). Thus, coating had a positive effect in preserving total carotenoids content until 14 days, probably by reducing oxygen's exposure of the product, since β-carotene is rapidly oxidized when exposed to light and oxygen [26]. Similar results were observed with alginate coating and cold preservation of different plum cultivars [27].

However, in our work no significant differences were observed between samples at the end of storage (21 days), being obtained the lowest total carotenoids' content (around 3-fold lower than at the beginning of storage), besides the unsatisfactory visual appearance for both coated and uncoated pansies.

Total phenolic content

Figure 2(A) shows the TPC of uncoated and coated pansies extracts, over 21 days of storage at 4 °C. Significant differences among uncoated and coated pansies ($p < 0.05$) were observed. Coated pansies always showed higher values of TPC than uncoated ones along the storage period, probably because the alginate edible coating produces an abiotic stress on tissue plants, modifying their metabolism and affecting the production of some secondary metabolites such as phenolics [28, 29].

After 21 days of storage, coated pansies (0.91 mg GAE/g fresh weight) showed a TPC content 3-fold higher than uncoated ones (0.37 mg GAE/g fresh weight). No significant differences on the TPC of uncoated pansies were observed along 21 days of storage (from 0.27 to 0.37 mg GAE/g fresh weight for 0 and 21 days, respectively). On contrary, the phenolic content in coated pansies decreased initially (from 2.06 to 1.24 mg GAE/g fresh weight for 0 and 7 days, respectively), but after that period the TPC remained relatively constant (from 1.24 to 0.91 mg GAE/g fresh weight, for 7 and 21 days). Similar results were reported by Robles-Sánchez et al. [30], who detected that phenols content also decreased significantly during 12 days in alginate coated fresh-cut Kent mangoes. This initial decrease can be attributed to an increase in the activity of some enzymes that may cause the oxidation of phenolics [31], as well as to chemical degradation that can occur

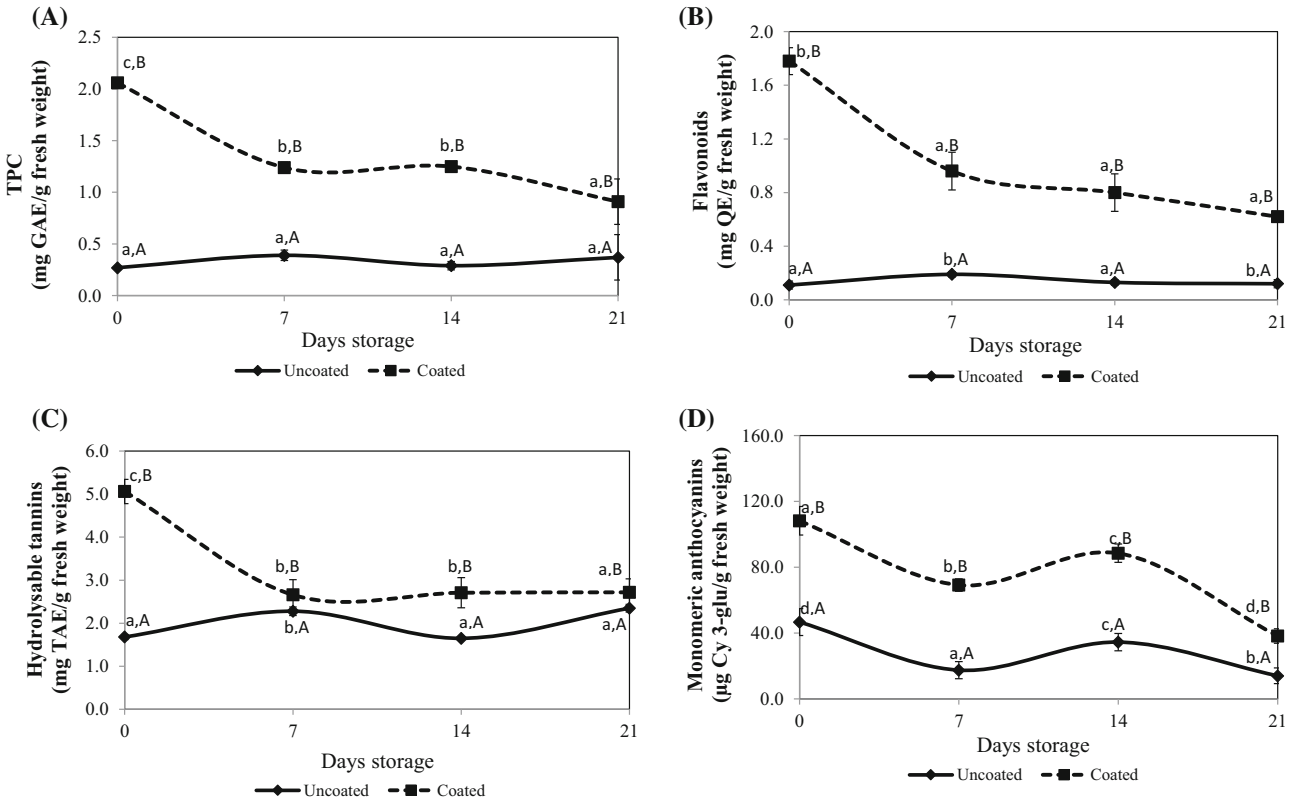

Fig. 2 TPC (**A**), flavonoids (**B**), hydrolysable tannins (**C**) and monomeric anthocyanins (**D**) contents in uncoated and coated pansies during storage (4 °C)

during storage, depending on the available oxygen and exposure to light [32].

Flavonoids

The total flavonoids contents in uncoated and coated pansies are presented in Fig. 2(B). In coated pansies a pronounced reduction in total flavonoids was observed during the first 7 days of storage. After that period the decrease in total flavonoids was lower (0.96–0.62 mg QE/g fresh weight at 7 and 21 days, respectively). A different behavior was reported in alginate coated fresh-cut Kent mangoes, when a reduction in total flavonoids was observed only after 6 days of storage [30]. In the case of uncoated samples, the contents of total flavonoids remained constant until 21 days of storage (0.11–0.12 mg QE/g fresh weight at 0 and 21 days, respectively). Furthermore, coated pansies showed always higher flavonoids content than uncoated samples during all storage period, probably because the production of these compounds, which are a class of phenolics, may be promoted in order to protect the plant tissues against biotic and abiotic stresses, as reported previously in "Total phenolic content" section relative to TPC.

Hydrolysable tannins

Figure 2(C) shows the changes in the hydrolysable tannins contents of alginate coated and uncoated pansies over 21 days of storage at 4 °C. The hydrolysable tannins contents of coated pansies were always significantly higher than uncoated, being this difference more pronounced at day 0 (5.06 versus 1.68 mg TAE/g fresh weight for coated and uncoated pansies, respectively). In coated pansies the hydrolysable tannins contents decreased significantly from the beginning until 7 days of storage (2.66 mg TAE/g fresh weight, approx. 1.9 fold), remaining constant afterwards. Concerning uncoated pansies, no significant differences were detected on hydrolysable tannins contents along the storage period, but these were always smaller than coated ones.

Monomeric anthocyanins

The amount of monomeric anthocyanins in pansies stored during 21 days is represented in Fig. 2(D). Again, coated pansies showed always higher values of monomeric anthocyanins than uncoated pansies, with the lowest anthocyanins contents being observed after 21 days of storage for both samples, 14.1 and 38.3 μg Cy 3-glu/g fresh weight for uncoated and coated pansies, respectively. This reduction of anthocyanins during storage has been reported for other coated fruits such as peel of litchi fruits coated with 1.0 and 2.0% chitosan during storage at 4 °C [33] and strawberry fruit coated with 0.5–1.0% (w/v) carboxymethyl cellulose (CMC) along storage under refrigerated conditions for 21 days [34]. Furthermore, in general terms, the behavior of hydrolysable tannins (Fig. 2C) and monomeric anthocyanins (Fig. 2D) was very similar to flavonoids (Fig. 2B), probably because both are subclasses of flavonoids [35].

Antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The EC_{50} values of DPPH radical scavenging activity for uncoated and coated pansies are shown in Fig. 3(A). As expected, coated samples had always lower EC_{50} values of DPPH radical scavenging activity than uncoated pansies, indicative of higher antioxidant activity, probably associated with the accumulation of phenolic compounds (ex. flavonoids) as mentioned in previous sections and similarly to reported by Reyes and Cisneros-Zevallos [36] and Frusciante et al. [37]. Furthermore, until 14 days of storage, the values of EC_{50} of coated samples increased, indicative of an antioxidant activity reduction. This fact may be due to the decrease observed on phenolics contents as stated in Fig. 2(A.)

Reducing power

Figure 3(B) shows the changes in reducing power of uncoated and alginate coated pansies over 21 days of storage at 4 °C. Pansies treated with alginate coating showed significant differences ($p < 0.05$) on their reducing power, increasing the EC_{50} values along storage, indicative of a decrease in the antioxidant potential of coated pansies. Regarding uncoated samples, no changes values were observed throughout storage (1.32 and 1.22 μg extract/mL at 0 and 21 days, respectively). As observed in DPPH assay, the EC_{50} values of the reducing power of uncoated pansies were always higher than coated pansies. So, our results show that alginate coating increases the antioxidant potential of pansies.

Correlations between monomeric anthocyanins, flavonoids, hydrolysable tannins, total phenolic content, DPPH radical scavenging activity and reducing power

Table 2 shows the correlations among monomeric anthocyanins, flavonoids, hydrolysable tannins, total phenolic content, DPPH radical scavenging activity and reducing power of uncoated and coated pansies. It was found that the

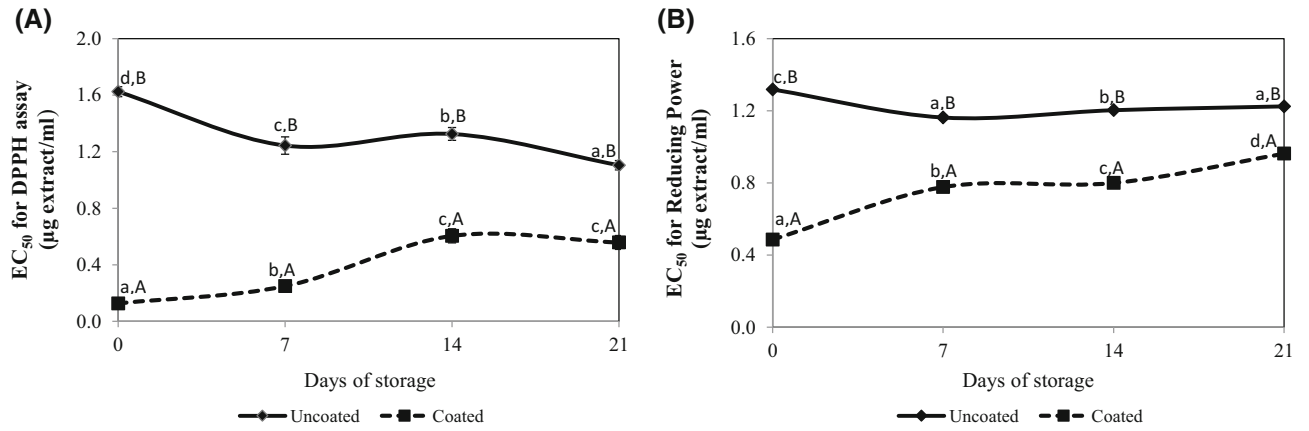

Fig. 3 EC_{50} values for DPPH (**A**) and reducing power (**B**) assays for uncoated and coated pansies during storage (4 °C)

Table 2 Pearson correlation coefficients for total phenolic content, monomeric anthocyanins, flavonoids, hydrolysable tannins and EC_{50} values of DPPH and reducing power assays

	Monomeric anthocyanins	Flavonoids	Hydrolysable tannins	EC_{50} DPPH	EC_{50} reducing power
Total phenolic content	− 0.951**	0.911**	0.965**	− 0.751**	0.850**
Monomeric anthocyanins	–	− 0.876**	− 0.958**	− 0.836**	− 0.886**
Flavonoids	–	–	0.936**	− 0.697**	0.684**
Hydrolysable tannins	–	–	–	− 0.836**	0.794**
EC_{50} DPPH	–	–	–	–	− 0.531**

Correlation is significant at $^{**}p < 0.01$

contents of flavonoids and hydrolysable tannins showed significantly positive correlations with total phenolic content, namely 0.911 and 0.965, respectively. These results were expected because flavonoids and hydrolysable tannins are phenolic compounds. Negative correlations of the EC_{50} values of DPPH with monomeric anthocyanins (− 0.836), hydrolysable tannins (− 0.836), flavonoids (− 0.697) and total phenolic content (− 0.751) were obtained. These results indicated that bioactive compounds, such as flavonoids, monomeric anthocyanins, hydrolysable tannins and phenolic compounds, have an important role in the antioxidant properties of pansies. A higher content of these compounds implies higher antioxidant activity, corresponding to a lower EC_{50} value. Regarding, the EC_{50} of reducing power assay, a negative correlation was only detected with monomeric anthocyanins (− 0.886), showing again the antioxidant potential of these compounds.

Microbial quality

The results of microbial quality of uncoated and coated pansies are shown in Table 3. There were no significant differences between uncoated and coated pansies in day 0. Even though no significant differences were observed between both samples along the storage period, after 14 days of storage uncoated pansies showed higher microorganism counts than coated ones, namely yeasts and moulds, suggesting some protection of the alginate coating treatment. *E. coli* and lactic acid bacteria were not detected in any sample.

According to the guidelines of microbiological quality for ready-to-eat foods [38], and including pansies in level 3 (this level applies to foods such as fresh fruits and vegetables, including salad vegetables), our results suggest that coated (0 and 14 days) and uncoated (0 day) pansies were regarded as being of satisfactory and acceptable quality for all microorganisms analyzed. After 14 days of storage, uncoated pansies presented high levels of moulds ($> 10^3$), having an unacceptable quality.

In summary, pansies coated with alginate showed good appearance until 14 days of storage, 7 days more than uncoated. Furthermore, after 14 days of storage, coated pansies also showed higher TA, higher values of some bioactive compounds (carotenoids, total phenols, total flavonoids, hydrolysable tannins and monomeric anthocyanins) and antioxidant activity (DPPH and reducing power assays) than uncoated pansies along storage. Furthermore, coated pansies presented a significant reduction

Table 3 Mean counts (log cfu/g ± standard deviation) of total aerobic mesophilic, yeasts, moulds, total coliforms, *E. coli*, psychrotrophic bacteria, and lactic acid bacteria examined in uncoated and coated pansies at 0 and 14 days of storage

Conditions		Microbial groups						
Samples	Days	Total aerobic mesophilic	Yeasts	Moulds	Total coliforms	*E. coli*	Psychrotrophic bacteria	Lactic acid bacteria
Uncoated	0	4.83 ± 0.73^{a}	5.95 ± 0.30^{c}	$< 2^{a}$	1.15 ± 0.22^{a}	< 1	$< 2^{a}$	< 2
	14	5.48 ± 0.34^{a}	4.20 ± 0.28^{b}	4.42 ± 0.60^{b}	$< 1^{a}$	< 1	6.40 ± 0.14^{b}	< 2
Coated	0	5.12 ± 0.26^{a}	$5.34 \pm 0.01^{b,c}$	$< 2^{a}$	1.30 ± 0.01^{a}	< 1	$< 2^{a}$	< 2
	14	5.08 ± 0.96^{a}	2.85 ± 0.53^{a}	$< 2^{a}$	$< 1^{a}$	< 1	5.76 ± 0.35^{b}	< 2

Values with the same letter in the same column are not statistically different ($p > 0.05$)

of yeasts and moulds counts compared with uncoated pansies after 14 days of storage, suggesting some protection of the alginate coating treatment. So, according to these results, it can be concluded that alginate coated pansies can be stored 14 days at 4 °C, without damages on the appearance and quality. The use of alginate coating in this type of flowers could be considered as a safe and effective treatment. Future research should be focused on evaluating the effect of pansies treated with edible coatings on sensory quality.

Acknowledgements The authors acknowledge the Portuguese Foundation for Science and Technology (FCT, Portugal) for the financial support provided by the research Grant SFRH/BD/95853/2013 and FCT/MEC for the financial support to QOPNA research Unit (FCT UID/QUI/00062/2013), through national funds and when applicable co-financed by the FEDER, within the PT2020 Partnership Agreement and REQUIMTE through the Project PEst/UID/QUI/50006/2013. The authors are also grateful to FCT (Portugal) and FEDER under Programme PT2020 for financial support to CIMO (UID/AGR/00690/2013).

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