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Effect of an edible crosslinked coating and two types of packaging on antioxidant capacity of castilla blackberries

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Abstract

In order to increase the shelf life and maintain the quality and stability of the biological compounds with antioxidant activity present in Castilla blackberry fruits, a sodium alginate-based edible crosslinked coating was applied, and the fruits were packed in two different plastic containers and stored under refrigeration (3 ± 1 °C). Total antioxidant capacity and its relationship to physicochemical variables such as pH, Brix, and acidity were evaluated in six treatments: uncoated blackberry stored in a macroperforated container (T1) and thermosealed container (T2), without crosslinked coating in a macroperforated container (T3) and thermosealed container (T4), with crosslinked coating (calcium ions) packed in macroperforated container (T5) and thermosealed container (T6). The results indicated that factors such as gas permeability in the coatings, the packaging used, and physicochemical parameters significantly affected the fruit total antioxidant capacity, with the highest level in T1 (0.22 μgEAA/ml) at the end of the essay, which is related to the lowest levels of pH and direct exposure to air. On the other hand, the lowest value was obtained in T6 (0.16 μgEAA/ml) due to the crosslinked coating, packaging in the thermosealed container, and higher pH value. Variations in acidity, Brix, and pH indicate the presence of degenerative processes in the crosslinked coating treatments, which limited the physicochemical changes.

Keywords: fruit preservation; food packaging; functional compounds.

1 Introduction

Rubus glaucus Benth known as "Mora de Castilla" is a type of blackberry widely grown in Colombia, specifically at higher altitudes with cold temperate climate zones. This type of blackberry is prized by the agro-industrial business and the fresh products market for its sensory qualities (acidity, juiciness, and flavour) and attractive dark red to purple colour (Garzón et al., 2009), giving it the recognition as a source of antioxidant compounds (Samec & Piljac-Zegarac, 2011). Their morphological and physiological characteristics make it a highly perishable fruit and lixiviates' generator. In addition, this fruit is sold at an advanced stage of maturity and ripeness, which affects its nutritional quality and bioactive compounds. Therefore, it is necessary to implement alternative technologies that allow easy access to all of the different links of the production chain, which will help maintaining fruit freshness while keeping its functional compounds, such as pigments, and antioxidants (Garzón et al., 2009).

Recently, the food and medical industry has increased its interest in the identification and characterization of phytochemical compounds with antioxidant activity, usually present in plant tissues, for their important role as effective scavengers of free radicals and for providing protection against oxidative DNA damage (Arumugam et al., 2006; Benvenuti et al., 2004; Manach et al., 2005). Several studies indicate the importance of eating fruits and vegetables because they reduce the risk of chronic diseases such as cancer, diabetes, and cardiovascular disease (Cerón et al., 2012). Compounds such as polyphenols, flavonoids, and carotenoids are recognized as natural antioxidants present in fruits and vegetables. Likewise, vitamins such as ascorbic acid have an antioxidant function as thus preventing damage induced by free radicals (Benvenuti et al., 2004). The flavonoid pigments and anthocyanin, responsible for the colour in a variety of fruits, ranging from shades of red-orange through blue to purple, are associated with anti-inflammatory, antioxidant, and anticarcinogenic activity. The six anthocyanins most commonly found in nature are Pelargonidin, Cyanidin, Peonidin, Delphinidin, Petunidin, and Malvidin; which have been reported as prevalent in fruits of the gender Rubus – cyaniding-based anthocyanins – specially in "Mora de Castilla" in mono- or di-acylated forms of cyanidin (Lee et al.., 2008, 2012; Kuskoski et al.., 2004; Rodríguez-Saona & Wrolstad, 2001; Cerón et al.., 2012).

Many methodologies have been applied concerning the interest in measuring antioxidant activity in various food matrixes. However, ABTS is one of the spectrophotometric methods that have been applied for this purpose in biological materials, pure compounds, or natural hydrophilic or lipophilic plant extracts (Kuskoski et al., 2004). ABTS chromogenic compound has a blue/green colour and it is best suited for trials with coloured compounds such as anthocyanin. It was chosen for having absorption maximum at near-infrared wavelength (734nm) reducing the possibility of interferences from coloured compounds that could be absorbed in the visible region or other compounds resulting from side reactions. In addition, it is also very soluble in water and its stability and reproducibility were

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validated since it is produced chemically (potassium persulfate) (Re et al., 1999).

Additionally, the development and application of edible films and coatings (EF and EC) has provided advances in the conservation of fresh horticulture and minimally processed products. Their synergistic effect with various packaging materials and control of environmental factors contribute to the physical, chemical, bioactive, and microbiological stability of products (Quintero et al., 2010). Various polysaccharides, lipids, and proteins are used as base in the formulation of coatings forming solutions that not only generate a modification of the gas atmosphere in function of their barrier properties, but also can carry additives with specific functions (antifungal, antibacterial, antioxidant vitamins, minerals, etc.). Thus, films based on sodium alginate (alginic acid polysaccharide) improve texture, reduce water loss, maintain the quality of physicochemical, microbiological, and antioxidant properties in melon, papaya, and minimally processed apples (Oms-Oliu et al.., 2008; Olivas et al.., 2007; Tapia et al.., 2008). Recent studies on "Mora de Castilla" blackberry show that the application of EC based on Sodium Alginate, Potassium Sorbate (antifungal), and glycerol (plasticizer) cross-linked with calcium ions can prevent physicochemical decay by acting as a barrier to water loss providing stability to bioactive compounds (phenols, anthocyanin) for a period of 7 days under commercial refrigeration conditions in thermo-sealed packaging (BOPP-PEBD-ANTIFOG-56μ) (Ayala et al.., 2012).

This research focused on the assessment of total antioxidant stability, its relationship to physicochemical variables such as pH, Brix, and acidity and on the shelf life of Castilla blackberry fruit subjected to treatments involving the application of an edible coating (crosslinked with calcium ions) based on Sodium Alginate, Glycerol, and Potassium Sorbate, packaged in plastic containers and refrigerated for 11 days.

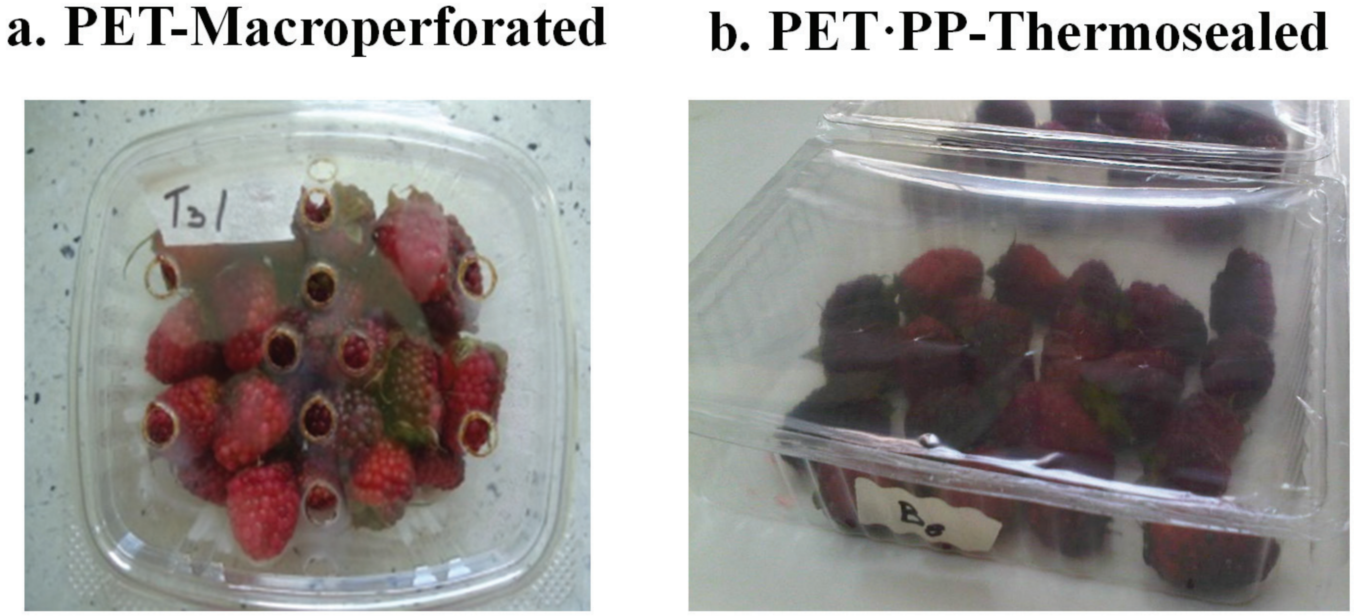
2 Materials and methods

2.1 Plant Material

"Mora de Castilla" blackberry fruits (*Rubus glaucus* Benth) were collected from the "El Retiro" village, located in The Combeima Canyon (1800-2300 meters above sea level), Municipality of Ibagué (Colombia), (Bohórquez, 2006) at a ripeness degree of 5 (NTC 4106) (Instituto Colombiano de Normas Técnicas y Certificación, 1997); they were harvested meeting physical and phytosanitary requirements and were placed in Polyethylene Terephthalate (PET) in plastic containers and Expanded Polystyrene coolers and transported to the laboratory, where they were coated, packed, and stored under refrigeration (3 ± 1°C and 65 to 85% relative humidity) for further antioxidant capacity analysis.

2.2 Treatments and storage conditions

The blackberry fruits with a ripeness degree of 5 (Instituto Colombiano de Normas Técnicas y Certificación, 1997) were washed (sprayed) and disinfected by immersion in a 1% citric acid solution for 3 minutes; the excess of moisture was then removed by airflow (21°C ± 0.2°C). The edible coatings based on Sodium Alginate (2%), Glycerol as plasticizer (2%), and Potassium Sorbate (300ppm) were applied with and without crosslinking with Calcium Chloride 2% based on the "*dipping*" technique (Raybaudi-Massilia et al.., 2008; Rojas-Graü et al.., 2007; Oms-Oliu et al.., 2008). An average of 250g samples of treated fruits were placed in: macroperforated (10% of its area) thermoformed Polyethylene Terephthalate (PET) containers and PET containers laminated with polypropylene (PP) thermosealed with superior flexible bi-oriented polypropylene film (BOPP) plus + Low Density Polyethylene (LDPE) + ANTIFOG 56 microns thick (Figure 1). A total of six different treatments were applied, as shown in Table 1. All treated samples were stored



a. PET-Macroperforated

b. PET·PP-Thermosealed

Figure 1. Storage Systems.

Table 1. Treatments applied to the Castilla blackberry.

Treatment	Fruit	Container
T1	Uncoated	PET-Macroperforated
T2	Uncoated	PET·PP-Thermosealed
T3	Coated	PET-Macroperforated
T4	Coated	PET·PP-Thermosealed
T5	Crosslinked coating	PET-Macroperforated
T6	Crosslinked coating	PET·PP-Thermosealed

under conventional refrigeration conditions at temperatures of 3 ± 1°C and relative humidity of 65-85% for 11 days. This time period was established considering the positive results obtained in other studies on fruits of the genus Rubus, including (Garzón et al.., 2009) conservation of physicochemical and physiological properties, (Samec & Piljac-Zegarac, 2011) stability of bioactive compounds (phenols and anthocyanins monomeric), and (Arumugam et al.., 2006) reduction of weight loss and incidence of fungi, through the use of active/passive modified atmospheres and edible coatings during refrigerated storage for periods of 6-18 days (Ayala et al.., 2012; Oliveira et al.., 2012; Sora et al.., 2006).

2.3 Determination of physicochemical parameters

These parameters were assessed in the juice extracted from whole fruit. The content of total soluble solids (TSS) was determined directly using a manual refractometer ATAGO (Master PM, Japan), the titratable acidity (TA) was quantified with potentiometric titration method and expressed as percentage of malic acid, and the pH was assessed using an potentiometer SCHOTT (CG810, Germany); for all parameters were taken as reference ICONTEC standards for fruit and vegetable products (Gómez, 2004).

2.4 Extraction of compounds with antioxidant capacity

For this purpose, the juice was extracted from the fruit using a Black & Decker (JE1500 juice extractor model - 400W, China) juicer, this juice was subjected to centrifugation at 5000 rpm x 10 minutes in a Hettich (EBA 20 model, Germany) centrifuge and vacuum filtered. Fractions of obtained juice (spin-filtered) were stored frozen at a temperature of –15°C ± 2°C for further analysis.

2.5 Determination of antioxidant capacity

As an estimate of the total antioxidant capacity of the bioactive components of the fruits, the bleaching ABTS radical method was used (Kuskoski et al.., 2004, 2005; Sánchez et al.., 2010). ABTS Radical (Diammonium Acid 2,2'-Azino-Bis(3-ethylbenzothiazoline)-6-sulfonic; Sigma-Aldrich - Dorset, UK) was obtained after reaction (7 mM) with Potassium Persulphate (Merck KGaA - Darmstadt, Germany; 2.45 mM final concentration) and incubation at room temperature (±25°C) in the dark for 16 hours. Once formed, the ABTS radical was diluted in ethanol to obtain an absorbance value of 0.70 ± 0.02 at 754 nm (wavelength of maximum absorption). The samples were prepared by diluting the extracted juice in distilled water. The absorbance of ABTS (control at time 0) and samples were read directly in a Thermo Electron Scientific Genesys 6 UV-Vis spectrophotometer (United States) after adding 3.9 ml of ABTS radical diluted in 0.1 ml of the sample after 6 minutes. A seven-point calibration curve (5-100 mg/L) of ascorbic acid (Merck KGaA - Darmstadt, Germany), a natural antioxidant of reference, was used under the same test conditions. Total antioxidant capacity was expressed in µgEAA/ml (equivalent micrograms to ascorbic acid per millilitre).

2.6 Statistical analysis

The results presented as the average values obtained in triplicates were tabulated and statistically analysed by Multifactorial analysis of variance MANOVA; the treatments and storage time varied, and the least significant difference (LSD) proposed by Fisher was used as a method of multiple comparison, with a confidence level of 95.0%. These analyses were performed using the statistical package Statgraphics Centurion, Version XV.II (StatPoint Technologies Inc., Warrenton, VA, USA).

3 Results and discussion

Total Antioxidant Capacity (TAC), measured by ABTS radical stabilization (Figure 2), showed, in general, an increasing tendency determined by the type of the treatment applied, which generated different levels of stress in the fruits. Thus, T1 (uncoated fruit stored in a PET-Macroperforated container) had the highest TAC value at the end of the experiment, which makes it possible to infer that in such treatment, an increased synthesis of secondary metabolites (such as polyphenols) occurred as a result of the protective effect of plant tissues against biotic and abiotic stresses (Hager et al.., 2008), taking into account the important content of anthocyanin in the blackberry fruit (Garzón et al.., 2009), and that this synthesis continues after harvesting, particularly when the fruits are stored without refrigeration (Holcroft & Kader, 1999). It is important to note that there is a significant correlation between the phytochemical compounds and the antioxidant activity (Sariburun et al.., 2010; Hung & Yen, 2002); strong evidence, provided by studies on berry fruit varieties such as raspberries and blackberries, suggests that the predominant source of the antioxidant activity is derived from flavonoids, phenols, and anthocyanin present in these fruits.

LSD-Fisher's least significant difference, a multiple comparison procedure, identified significant differences in the average levels of (T6) in contrast with those of the other treatments at 95.0% confidence level since the TAC average values in such treatment are lower than those in the other treatments. In (T6), TAC values of 0.14 and 0.16 µgEAA/ml for 7 and 11 days of storage, respectively, indicate a possible slowdown of metabolic reactions and senescence in fruits because the deleterious effect of oxygen (oxidation) on fresh and minimally processed horticultural products is effectively reduced by the use of films and coatings with limited oxygen permeability. According to Bonilla et al. (2012), hydrophilic EF and EC (based on polysaccharides or proteins) generally provide a good barrier to oxygen transfer. The treatment (T6) demonstrates the aforementioned because low oxygen permeability was generated by sodium alginate combined with the reduction in oxygen transport produced by the incorporation of calcium into the structure (crosslinking), as suggested by the decrease in water vapour permeability of the sodium alginate coatings reported in other studies (Miller & Krochta, 1997; Rhim, 2004). Moreover, the flexible packaging film contributes to the modification of the gas atmosphere by greatly reducing the oxygen availability, consequently increasing CO_2 concentration affecting the antioxidant capacity by decreasing anthocyanin biosynthesis, as previously reported in other studies on red berries (Holcroft & Kader, 1999). In addition, it was observed (Figure 2) that some treatments show a reduction in the CAT on the seventh day of storage, followed by a gradual increase up to the end of the experiment. These slight variations are explained by several factors, mainly by chemical changes and the correlation between TAC and the content of phenols and anthocyanins in the fruit, which in turn are affected by the stress levels to which the fruit is subjected, specifically, storage conditions and concentration of gases (especially oxygen) (Ayala et al.., 2012; Sariburun et al.., 2010; Fan-Chiang & Wrolstad, 2005; Castañeda-Ovando et al.., 2009). However, it is important to highlight that during refrigerated storage of red fruits, there is an increase in the content of anthocyanins (mainly responsible for the TAC), especially in high oxygen packaging atmosphere (Holcroft & Kader, 1999; Ayala-Zavala et al.., 2004).

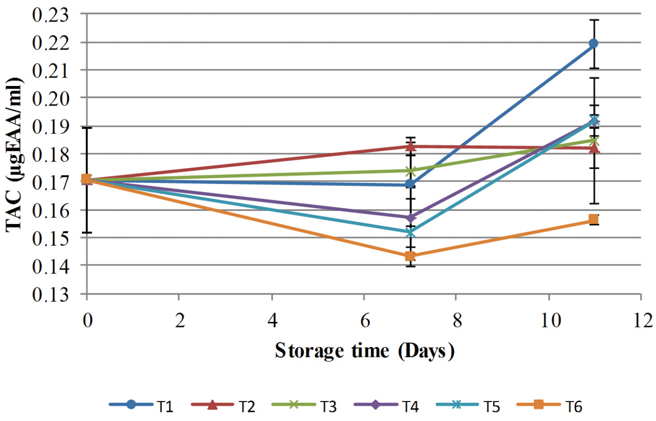


Figure 2. Effect of the treatments on TAC of blackberry fruits during cold storage (11days, 3 ± 1°C).

The statistical analysis showed that the treatments and storage time have a statistically significant effect on the total antioxidant capacity ($P < 0.05$) at 95% confidence level. In addition, variables such as acidity, Total Soluble Solids concentration (°Brix), and pH levels (Table 2) were also probably related to the behaviour of the TAC. The acidity showed a decreasing trend in all treatments, in accordance with the findings of Sora et al.. (2006) in cold storage of "Mora de Castilla" blackberry under modified atmospheres. Total Soluble Solids varied during the storage period depending on the treatment applied. However, (T4) and (T6) were the only treatments that had °Brix values below the lower limit of the standard NTC 4106 (Instituto Colombiano de Normas Técnicas y Certificación, 1997) at a ripeness degree of 5 (7.2 °Brix) at the end of the trial, which is attributable to the metabolic slowdown.

There was an increase or decrease of only 0.1 units (or less) in the pH values during storage in all treatments, which was negligible after 11 days. However, there were two trends related with the type of packaging because the pH values were slightly increased in the PET·PP-Thermosealed containers (T2, T4, and T6), which normally happens during storage of fruits, consistent behaviour with the decrease in acidity. However, it should be noted that the application of the coating had an impact on the changes in the pH level of the fruits because higher values were

Table 2. Behaviour of physicochemical parameters in the fruit juice subjected to six treatments during storage.

Parameter	Day			
	Treatment	0	7	11
pH	T1	2.86±0.017^{a}	2.69±0.000^{a}	2.74±0.023^{a}
	T2		2.89±0.006^{a}	2.78±0.000^{a}
	T3		2.80±0.000^{a}	2.89±0.010^{a}
	T4		2.97±0.006^{a}	2.78±0.000^{a}
	T5		2.73±0.000^{a}	2.79±0.006^{a}
	T6		2.99±0.006^{a}	2.71±0.006^{a}
Acidity (% Malic A.)	T1	3.61±0.021^{a}	3.17±0.041^{b}	3.26±0.036^{b}
	T2		3.03±0.035^{b}	2.92±0.000^{b}
	T3		2.80±0.026^{b}	3.17±0.037^{b}
	T4		2.68±0.031^{b}	2.47±0.025^{b}
	T5		2.74±0.028^{b}	3.00±0.029^{b}
	T6		2.82±0.032^{b}	3.13±0.036^{b}
°Brix	T1	7.00±0.000^{a}	7.87±0.116^{a}	8.00±0.000^{a}
	T2		7.13±0.116^{a}	7.20±0.000^{a}
	T3		7.93±0.116^{a}	9.13±0.116^{a}
	T4		7.07±0.116^{a}	6.53±0.116^{a}
	T5		7.60±0.000^{a}	7.53±0.116^{a}
	T6		6.93±0.116^{a}	6.80±0.000^{a}

^{a,b}Mean values n=3±SD. Superscript letters in each column indicate significant differences ($p < 0.05$).

obtained in T6 and T4, followed by T2, indicating that both the packaging material and the crosslinked coating in these treatments limited the fermentation degradation processes up to the 7th day of storage.

On the other hand, treatments packed in PET-Macroperforated containers (T1 and T5) showed a slight decrease in pH and therefore, higher antioxidant capacity influenced by oxygen availability, which in this case was modified only by the presence or absence of crosslinked coating. This is important since stability of some phenolic compounds, mainly anthocyanins, is affected by several factors, among which the pH is considered the most important. Horbowicz et al. (2008) mentioned the fact that in aqueous solutions, the anthocyanins undergo structural transformations that are pH dependent and were found to be more stable in acidic media (pH <2) than in neutral or alkaline media. Similarly, statistical analysis identified differences in the TAC levels between the treatments only on the 11th day of storage at 95.0% level of confidence. The values obtained in the beginning and on the 7th day of the experiment were identified as belonging to the same homogeneous group by Fisher's LSD test. These results show that even a pH <3 influences positively the stability of the TAC.

Finally it should be noted that recent studies on "Mora de Castilla" have evidenced a TAC average value of 9.04 ± 0.30 mgEQV ascorbic acid/100g of fresh fruit (FF), determined in the juice extract and expressed in fresh fruit mass (Quintero-Cerón et al.., 2012), indicating that there is most antioxidant capacity in the fruit treated in this experiment is neglected, which could be related with the difference in the extraction methodology. Nonetheless, "Mora de Castilla" has been recognized to contain significant amounts of phenolic compounds, ascorbic acid, and anthocyanins that exhibit high antioxidant activity measured by ABTS and FRAP (Garzón et al.., 2009).

4 Conclusions

Variations in total antioxidant capacity of fruits were identified, depending on the treatment, packaging, and metabolic and physicochemical evolution of the product. A TAC value of 0.22 μgEAA/ml was found in the treatment T1 (PET-macroperforated) at the end of the trial. However, Physicochemical signs of damage evidenced by levels of acidity and concentration of soluble solids in this treatment were also identified; on the other hand, a lower TAC value (0.16 μgEAA/ml) was found in fruits packed in PET•PP-Thermosealed (T6), which, due to its low permeability, influenced the slowing senescence and lower levels of physicochemical changes.

The results obtained with the application of edible coatings based on sodium alginate and calcium ions, in combination with heat-sealed packaging as a quality conservation system, should be standardized since it prolongs the shelf life of the product up to 7 days under refrigeration conditions without significant effects on TAC and contributes to the preservation of the physicochemical properties and antioxidant potential of "Mora de Castilla" blackberry fruit.

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