



Preservation of the Polyphenolic Content and Antioxidant Properties of *Rubus Glaucus* Benth

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Abstract

The Andes berry (*Rubus glaucus* Benth) has an ongoing production in Colombia and is considered a competitive market product with the potential for exportation and broad applications in agroindustry because of its high content of antioxidants, nutrients, and proteins, which are keys to a balanced diet. In this study, the effect of an *Aloe vera* gel coating on the preservation of the nutraceutical properties of the Andes berry was evaluated by measuring the antioxidant capacity and the total phenolic content of the fruit using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) and Folin-Ciocalteu methods, respectively. The antioxidant activities in fruits without and with coating were 95.14 ± 3.28 mmol AAE 100 g⁻¹ sample and 135.94 ± 1.09 mmol AAE 100 g⁻¹ sample, respectively, after ten day of storage. The total phenolic content of the fruits with and without coating did not present significant differences, i.e., the phenol concentrations remained constant over time. Implementation of the gel coating is recommended as a low-cost and highly available option for the preservation of the Andes berry and its nutraceutical properties.



Article History

Received: 4 September 2019
Accepted: 10 December 2019

Keywords

Antioxidant;
Edible Coating;
Mucilage;
Shelf Life;
Total Phenols.

Introduction


The Andes berry (*Rubus glaucus* Benth) is from America high tropical zone; it was growth in Colombia, Ecuador, Panamá, Guatemala, Honduras, México and Salvador. This fruit plays an important role in the human diet due to its high content of

antioxidants, nutrients, and proteins, all of which are key to a balanced diet.¹ It has an essential economic role as it is one of the most produced crops in the department of Risaralda, Colombia, with a 12% share in the national market.² The rapid maturation of Andes berry fruits causes large losses for farmers

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Doi: <http://dx.doi.org/10.12944/CRNFSJ.7.3.27>

due to difficulties in transportation and exportation and also affects traders and consumers. Research on the preservation of Andes berry fruits in Colombia is scarce, necessitating further research on ways to increase the shelf life of this fruit.

Various technologies, such as the use of modified atmospheres, biodegradable packaging and freezing, have been implemented for fruit conservation in the industry; however, these methods require high monetary investment and energy consumption, and/or some cause damage to the fruit or changes in its sensory, textural and nutritional properties.³ Cold storage is the most commonly used technology and, although results in loss of firmness and freshness, effectively delays physiological processes such as respiration and vital heat production that lead to fruit deterioration. Decreased respiration intensity reduces losses of aroma, flavour, colour and texture and improves the quality of other attributes of the fruit.⁴ Although edible coatings such as wax, lard, and chitosan are already available on the market, it is important to seek other alternatives that are economical and easily accessible and do not modify the nutritional properties of the fruits. One such alternative is *Aloe vera* gel, which minimizes fruit respiration and acts as a barrier to moisture and oxygen, slowing dehydration.⁵

Several studies established the use of *Aloe vera* gel as an effective post-harvest technology that can be used to maintain the functional properties of the fruit.⁶ It is due the *Aloe vera* gel can produce biodegradable films,⁷ and it presents antimicrobial activity because of its chemical composition.⁸ The coating could protect the fruit through different action mechanism. The first one, it is forming a protection layer of oxygen and humidity, preventing the oxidation and strange losses. The second way controls the respiratory frequency and disable the microorganism action because of antibacterial chemical compounds presents. It was used *Aloe vera* gel coating in peaches, plums, strawberries, grapes and pomegranate arils in order to reduce the microorganism proliferation.⁹⁻¹²

Research conducted by Castillo *et al.*, (2010)¹⁰ y Yaman and Bayoindirli, (2002)¹³ on the effects of applying an *Aloe vera* gel coating to table grapes (*Vitis vinifera*) and cherries (*Prunus avium* L.)

demonstrated the effectiveness of the coating in preserving functional properties and slowing maturation, weight loss and respiration; these studies established the use of *Aloe vera* gel as an effective post-harvest technology that can be used to maintain the functional properties of these fruits.

In Colombia, Ramírez *et al.*, (2013)⁵ evaluated the use of an edible coating based on a mucilaginous gel derived from *Aloe vera* leaf to increase the shelf life of the Andes berry at refrigeration temperatures, the results suggest that *Aloe vera* is an effective conservation method because it slows physiological processes such as respiration and transpiration. Villegas *et al.*, (2016)¹⁴ was found that with a mixture of *Aloe vera* gel, carnauba wax y glycerol it was possible to attenuate changes in Brix grades (total soluble solids content), acidity and pH, preserving the quality of the fruit until ten days of refrigerated storage of the *Rubus glaucus*. Quiñonez *et al.*, (2014)¹⁵ apply a coating of chitosan, alginate and glycerol, in order to increase the shelf life and to preserve the active biological compounds presents in the Andean berry fruits.

Ayala Sánchez *et al.*, (2014)¹⁶ studied coatings based on sodium alginate and ions of calcium, in combination with vacuum packaging, watching the increase in shelf life conservation of up to 7 days for the fruits of *Rubus glaucus* Benth.

In general, the Andean berry fruits have been studied through different types of edible coating in order to preserve the bioactive and physicochemical properties. However few studies have addressed how this coating affects the antioxidant activity and phenolic content of the fruit, properties that are affected by rapid maturation. Therefore, the objective of this study was to estimate the effect of *Aloe vera* gel coating on the nutraceutical properties (total phenolic content and antioxidant activity) of Andes berries cultivated in Risaralda, Colombia.

Materials and Methods

Plant Material

Andes berries (*Rubus glaucus* Benth) cultivated in the department of Risaralda, Colombia were used at maturity stages 4 and 5 as established in NTC 4106.¹⁷ *Aloe vera* leaves were washed with TEGO 51 (Merck, Germany), distilled water to remove dirt,

and allowed to stand to eliminate the exudate; the mucilage was then separated from the epidermis and washed with copious amounts of potable water.

The coating was prepared at several concentrations of *Aloe vera* gel (40, 50 and 60%) by a modification of the method described by Restrepo and Aristizábal (2010).¹⁸ Briefly, the coating was the coating was heated to 95°C for 5 minutes followed by rapid cooling to 25°C and homogenization at 3500 rpm for 15 minutes.

Coating Application

Each Andes berry batch consisted of 20 fruits, previously washed with TEGO 51 (Merck, Germany) and 2% sodium hypochlorite. Coating was applied manually to the epidermis of fruit by immersion of fruit for 30 seconds followed by drying at room temperature. Fruits were stored in plastic containers under refrigeration at 5°C. Non-coated fruits were used as control for comparison purposes measurements of coated and control fruits were performed in duplicate on days 1, 5 and 10.

Parameters Evaluated

Sample Preparation

Fruits were passed through a mill, and subjected to processing by cutting and impact. The extracts were collected using with 1% acidified methanol according to modifications made to the methodology proposed by Milosevic *et al.*, 2012.¹⁹

Antioxidant activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) spectrophotometric method was used according to modifications made to the method described by other authors.²⁰ In this procedure, 2 mL of the DPPH solution at 20 mg L⁻¹ and 30 µL of the extract or sample to be evaluated were mixed and incubated

for 30 minutes at room temperature, and the absorbance of the sample at 517 nm was then read. A calibration curve was standardized using ascorbic acid as standard (concentration range 5–200 mg L⁻¹, R² = 0.9969), and the results were expressed in mmol ascorbic acid equivalents per 100 g of sample.

Total Phenols

The Folin-Ciocalteu spectrophotometric method was used with the modifications described by Waterhouse (2003).²¹ To a 5-mL flask, 200 µL of distilled water, 50 µL of the extract or sample to be evaluated and 250 µL of Folin-Ciocalteu working solution were added. After 1 minute, 750 µL of 20% sodium carbonate was added, and the volume was brought to 5 mL with distilled water. The mixture was incubated for 30 minutes at room temperature, and the absorbance was then read at 760 nm. A calibration curve was standardized using gallic acid as the standard (concentration range 50–800 mg L⁻¹, R² = 0.9999), and the results were expressed in mg gallic acid equivalents per gram of sample.

Statistical Analysis

All data were expressed as mean ± standard deviation. The treatments were applied in a 3² factorial arrangement. Each treatment was replicated twice. An analysis of variance (ANOVA) was conducted followed by the Tukey's test. P values of <0.05 were considered to indicate statistically significant differences in the evaluated parameters and the time of application. The Infostat program, version 2008, was used for all statistical analyses.

Results

Coatings were applied to the Andes berry fruits by immersion and their visual appearance after the application is shown in Figure 1.

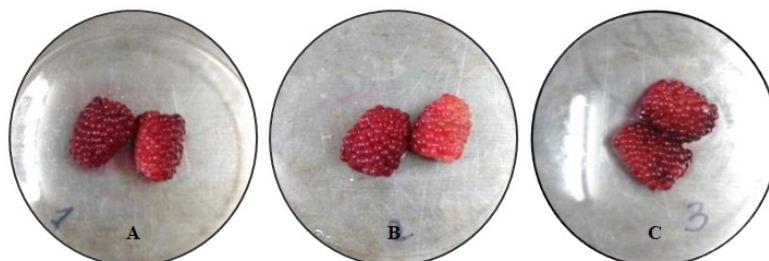


Fig.1: Visual appearance of Andes berries coated with *Aloe vera* at different concentrations (a) 40%, (b) 60%, (c) 50%, first day of the experiment

Antioxidant activity and total phenolic content of Andes berry fruits with and without coating were measured on three different days. The results

obtained are presented in Figures 2 and 3, respectively.

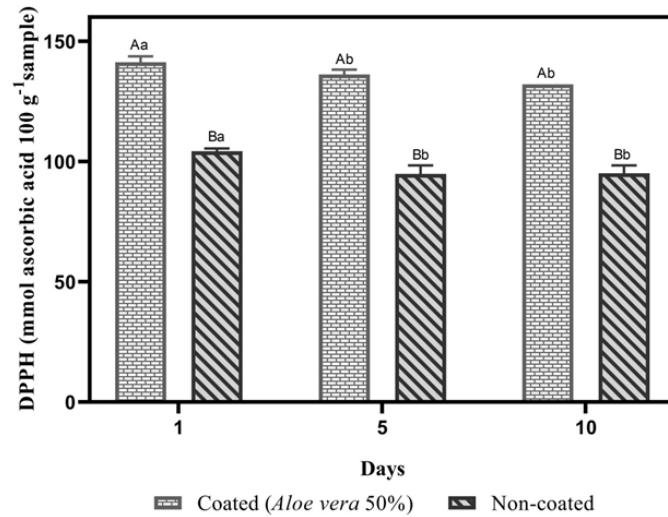


Fig. 2: Antioxidant activity of fruits with *Aloe vera* gel coated and non-coated (Values are expressed as the arithmetic mean ± the standard deviation and do not differ by 5% of probability as assessed using the Tukey’s test. Mean values with similar letters do no significantly differ according to the Tukey’s test)

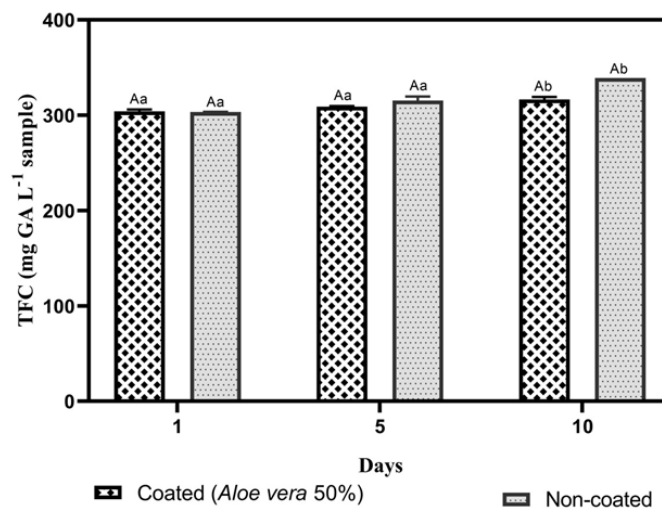


Fig. 3: Total phenolic content of fruits with *Aloe vera* gel coated and non-coated (Values are expressed as the arithmetic mean ± the standard deviation and do not differ by 5% of probability as assessed using the Tukey’s test. Mean values with similar letters do no significantly differ according to the Tukey’s test)

Discussion

The results obtained in Figure 1 at concentrations of 40% (a) and 60% (b), there was poor adherence

of the coating to the epidermis of the fruits, whereas at a concentration of 50% (c), greater coating adherence and better coating consistency were

observed. Therefore, the latter concentration was used throughout the experiment.

The chemical composition has been reported that the *Aloe vera* plant is made up of a complex mixture of compounds. The gel consists mainly of water, mucilage and other carbohydrates, organic acids and salts, saponin enzymes, tannins, anthrax heteroxides.²²

The presence of low or high concentrations of Aloe gel in the formulation of the coating can lead to different behaviors against adhesion on the fruit. Due to the high abundance of hydroxymethyl groups in the case of polysaccharides like cellulose, in the polymer chain, this can give rise to crystallization phenomena in the applied mixture.²³ In turn, proteins vary in molecular weight, load, conformation, flexibility and thermal behavior.²³ And the molecular characteristics within the system will determine its ability and coating properties.

On the other hand, lipids due to their hydrophobic properties, and their non-polymeric character, make their mechanical properties lower to hydrocolloids, forming more brittle coatings. Due to the complex system established in the formulation, it is possible that the properties of adhesion of the coat was less in 60% of concentration than 50% concentrations of Aloe in the mixture.

According to the data shown in Figure 2, the coated fruits showed higher antioxidant activity than the uncoated ones; on the 1st day, the measured values were 141.24 ± 1.21 and 104.28 ± 1.13 mmol ascorbic acid 100 g^{-1} , respectively. This finding was confirmed by ANOVA, in which significant differences were observed between the evaluated treatments ($p < 0.05$). These results are consistent with the results reported by Serrano *et al.*, (2006),²⁴ who found that the antioxidant capacity of table grapes (*Vitis vinifera*) treated with *Aloe vera* gel was higher than that of the uncoated control, and by Hassanpour (2015)²⁵ for raspberry (*Rubus* spp.).

The preservation of antioxidant activity by the coating is mainly due to the composition of the *Aloe vera* gel, which includes glucomannan, mannan, glucan, arabinogalactan and galacto gluco arabino mannan polysaccharides^{26,27} that act as a barrier to water and

oxygen diffusion during storage, thus increasing the resistance of the tissues to decomposition.²⁵

There were no significant differences in the total phenolic content of coated and non-coated Andes berry fruits ($p > 0.05$), whereas during storage the differences became statistically significant, where the phenol concentration on the first day was 304.00 ± 2.12 mg gallic acid eq. g^{-1} , and it increased significantly to 339.05 ± 0.08 mg gallic acid eq. g^{-1} after ten days of storage.

The results shown in Figure 3 indicate that although the total phenolic content of the fruits in the two treatment groups (coated and non-coated) was similar higher total phenolic content was observed on the last day. It was found statistically significant differences of total phenolic content between storage days. The ten-day storage presented the higher content in relationship the first day. This behaviour has been previously observed in raspberry, strawberry, and blueberry; when stored at 4°C .²⁸ The accumulation of phenolic compounds can be attributed mainly to the abiotic stress that the fruits undergo, through phenylalanine lyase activity, a key enzyme in the metabolism of phenylpropanoids.²⁵

Another aspect related to the increased content of total phenols at day 10 may be attributed to the presence of endophytic fungi or other fungi such as *Botrytis cinerea*, *Colletotrichum* sp. and *Peronospora* sp. that cause diseases in these fruits. In response to these organisms, the plant activates physiological and biochemical processes that generate an increase in phenolic compounds that act in various ways.²⁹

Regarding the use of *Aloe vera* in edible coatings, a number of studies have addressed how to improve the quality and shelf life of products such as dried plums,³⁰ papayas,³¹ grapes and cherries,^{24,32} apples,³³ kiwi fruits³⁴ and blueberries.³⁵ As well as in the preservation of physicochemical and antioxidants proprieties of zapote³⁶ y strawberry fruits.³⁷

One advantage of using natural edible coatings for fruit preservation is that they, as in the case of *Aloe vera* gel, often contain a variety of chemical compounds such as proteins,³⁸ polysaccharides,³⁹ vitamins, and sterols⁴⁰ that have various biological activities that are

beneficial to health. These properties may include antidiabetic, anti-inflammatory, immunomodulatory, antioxidant, anticancer, antibacterial and antifungal activities.^{26,39} In addition, these coatings help reduce moisture loss, and decrease respiration rates, the growth of microorganisms and oxidative browning in some fruits (Andrade-Pizano *et al.*, 2016).

Given the various properties of *Aloe vera* and the interest of consumers in quality foods with good nutritional, microbiological and organoleptic properties that may be affected by the conditions to which the fruits are exposed to during harvest, storage and sale,⁴¹ the use of *Aloe vera* in the food industry has been implemented for the conservation of fruits and vegetables.⁴²

In this study, a low-cost and highly available 50% *Aloe vera* coating was produced. The coating showed great potential for preserving Andes berry fruits based on the measurement of the antioxidant activity and total phenolic content of the coated

fruits. Further studies are required to establish the coating quality and properties by evaluating other parameters.

Acknowledgements

The authors thank the Technological University of Pereira and the research programme "Development of scientific and technological capabilities in biotechnology applied to the health and agroindustry sectors in the department of Risaralda" of the General Royalty System of Colombia.

Funding

The General Royalty System of Colombia (BPIN 2012000100050), and Vice-Rector of Research of the Technological University of Pereira, Internal Call to Finance the Publication of Scientific Research Articles year 2019.

Conflict of Interest

The authors do not have any conflict of interest.

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