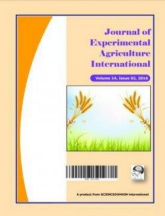

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Biodegradable Coatings on Blueberries Postharvest Conservation Refrigerated in a Modified Atmosphere

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Authors' contributions

This work was carried out in collaboration between all authors. Authors IPO and PMF designed the study. Authors IPO and ARR performed the statistical analysis and wrote the manuscript. Authors MBM, MRGM and CSPL managed the study analyses. Authors IPO, ARR, PMF, MBM, MRGM and CSPL managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the present study was to evaluate the effect of edible coatings based on manioc starch, kefir and chitosan on the physicochemical and microbiological characteristics of blueberry subjected to refrigerated storage in a modified atmosphere.
Study Design: The experimental design was completely randomized in a two-factorial scheme.
Place and Duration of Study: Department of Crop Science, Fruit Science Laboratory, Faculty of Agronomy Eliseu Maciel and Center for Chemical Sciences, Pharmaceutical and Food, Federal University of Pelotas, between October 2015 and September 2016.
Methodology: The treatment factors were composed of edible coating (cassava starch, Kefir, chitosan and uncoated, which corresponded to the control), and the periods of refrigerated storage in a BOD chamber simulating the shelf life (9, 18, 27, 36 days). The physicochemical analyses were a mass loss; texture; color parameters (L, a*, b* and Hue); soluble solids (SS); pH; titratable acidity

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(TA); and SS/TA ratio. In addition, total and thermotolerant coliforms were counted and the presence or absence of *Escherichia coli* and *Salmonella* spp. were observed.
Results: Edible coatings based on kefir grains and chitosan maintained the physicochemical characteristics of blueberry during refrigerated storage in modified atmosphere. Fruits are in compliance with the microbiological standards established by the legislation.
Conclusion: Extending storage time of chilled blueberries under a modified atmosphere promotes an increase in mass loss after application of the coatings and after 27 days.

Keywords: Chitosan coating; post-harvest storage; vaccinium ashei, kefir, cassava starch.

1. INTRODUCTION

Blueberries are known for their bioactive compounds, including flavonoids, phenolic acids, tannins and anthocyanins, which help individually or synergistically protect against cardiovascular diseases, cancer, inflammation, obesity, diabetes and other chronic diseases [1]. However, fresh fruit deteriorates rapidly by loss of water, juice leakage, gray mold and/or ripe rot [2]. Blueberry deterioration is usually caused by fungi, such as anthracnose (*Colletotrichum acutatum*) the most common fungal disease, followed by rot caused by *Alternaria* spp. and gray mold (*Botrytis cinerea*) [3].

In this context, cold storage, edible coatings, UV irradiation, modified atmosphere packaging, ozonation and fumigation with sulfur dioxide are among the post-harvest preservation technologies used to reduce post-harvest deterioration, prolong shelf life and preserve the nutritional quality of fresh blueberries [4,5,1].

Chitosan, which acts as a bioactive compound, is a biocompatible and biodegradable polysaccharide, with excellent film-forming properties and antimicrobial function, being among the most studied edible coatings. This biodegradable polysaccharide can be used to form edible coatings, thereby controlling the internal gas in the fruit atmosphere and serving as a water vapour barrier to reduce moisture loss and delay fruit dehydration [5,6]. Studies have shown that chitosan coatings can effectively reduce fungal growth, extending the shelf life of strawberries, blueberries and raspberries [7,8,6].

Cassava starch is one of the agents that can be used in the edible coatings formation, this biofilm looks good, is not sticky, is shiny and transparent, improves the fruits visual appearance, and can be removed with water [9]. Kefir grains have also been used for this purpose. These grains are irregular and gelatinous masses, composed of acetic acid bacteria and yeasts, which contribute to the fermentation. The use of a coating made from kefir grains, associated with a temperature of 5ºC, could be an efficient alternative for preserving and increasing the shelf life of organic blueberry [10]. The edible coatings, glycerol and sorbitol, act on the hydrogen bonds, reducing the intermolecular forces along the polymer chains, improving packaging mechanical properties, like flexibility, strength and resistance [11].

When combined with adequate temperature control, modified packing atmosphere can prolong the shelf life of fresh produce while maintaining the nutritional and sensory qualities of the product [12,1,13], the fungi are also significantly reduced by the limited levels of O_2 and high CO_2 in the package [14,15]. Therefore, combining edible coating to modified atmosphere can further extend the shelf life of fresh products. The objective of this study was to evaluate the effect of edible coatings based on manioc starch, kefir and chitosan on the physicochemical and microbiological characteristics of blueberry subjected to refrigerated storage in a modified atmosphere.

2. MATERIALS AND METHODS

The blueberries (*Vaccinium ashei* Reade) used as raw material were of the Powderblue cultivar of the rabbiteye group from the 2015/16 productive cycle, cultivated in the southeast mesoregion of Rio Grande do Sul (RS), in a commercial orchard located in the municipality of Morro Redondo (31º39'05''S, 52º30'01''W and 87 m in altitude). According to the Köppen and Geiger [16] classification, the climate of the region is of type Cfb, temperate humid. During the 2015/16 production cycle, the average minimum temperature was 14.9ºC and the average maximum was 24ºC.
The fruits were harvested at the stage of complete maturation [17], with violet staining and

presence of bloom, in polyethylene trays and then taken to the Post-Harvest Fruit Physiology and Technology Laboratory, of the Plant Engineering Department of the Federal University of Pelotas (UFPel) and placed in a refrigeration chamber for temperature stabilization. At the harvest stage the fruits presented 0.43 N of texture; 30.99 for L; 0.67 for a*; -2.18 for b*; 287.19 Hue; 16.53 °Brix; pH 3.06; titratable acidity of 0.74 g of citric acid 100 g^{-1}; and 22.42 SS/TA ratio.

The experimental design was completely randomized in a two-factorial scheme with three replicates. Factor A was composed of edible coating (cassava starch, Kefir, chitosan and uncoated, which corresponded to the control), and factor B the periods of refrigerated storage in B.O.D. (biochemical oxygen demand) type chamber, simulating shelf life (9, 18, 27, 36 days). Each replicate consisted of three trays of polyethylene terephthalate with 125 g of fruits.

Preparation of cassava starch (2.5%) was obtained from 50 g dissolved in two liters of distilled water, followed by heating (70ºC) with constant stirring of the suspension until gelation (20 to 30 minutes) and rest until cooling. 20 mL of glycerol and 10 mL of glycerol/10 g sorbitol were mixed with the aid of a blender for two minutes to homogenize the coating [18].

For the kefir-based edible coating, the grains were drained from the liquid, 400 g were weighed and disintegrated. Thereafter, 1.5 liters of distilled water was added, kept under heating (50ºC) for 30 minutes with gentle stirring. After cooling, 10 mL of glycerol and 10 g of sorbitol were added to the solution, which had its volume measured to two liters and was kept under stirring for two minutes for homogenization of the coating [19].

To prepare the chitosan-based coating, 30 g of chitosan (Polymar), with deacetylation degree of 86.5% in 2 L of acidified 0.8% ascorbic acid solution was used, with mixing by agitation for 2 minutes. 10 mL of glycerol was added and stirred again until complete homogenization [18].

The fruits were immersed in the coating solutions for three minutes and then placed in nylon screens to dry at room temperature. After drying, the fruits were weighed, packed in polyethylene terephthalate trays and subjected to refrigerated storage in B.O.D. (temperature of 3 ± 1ºC and relative humidity between 85-95%). The control had fruits immersed in distilled water, without cover and stored under the same conditions. The physicochemical evaluations of the fruits took place in the period of 36 days, with samples taken at intervals of nine days. The physicochemical analyses were mass loss, expressed as a percentage; texture; colour parameters (L, a*, b* and Hue); soluble solids (SS); pH; titratable acidity (TA); and SS/TA ratio.

The texture was determined in the equatorial region of each fruit by the Texture Analyzer (TA.XT plus, Stable Micro Systems Texture Technologies®) with a 2 mm probe (diameter). Each fruit was penetrated by 50%, with a velocity of 2.5 mm s^{-1} and the results were expressed in Newton (N). The colour parameters of the epidermis were measured by a reading at two points of each fruit by repetition with a Minolta 450 colourimeter, illuminant D65, and 8 mm aperture in the system registered by the Commission Internationale de l'Eclairage L, a* and b* (CIE-Lab). The values of Hue (h° angle), expressed in degrees, were obtained by the formula h° = $\tan^{-}1$ b*/a*.

Soluble solids (SS) were obtained with PAL-1 digital refractometer (Atago, Tokyo, Japan) with automatic temperature correction and results were expressed as °Brix. The pH reading was performed directly on the juice using a Mettler Toledo digital pH meter (model 320), with a Mettler Toledo electrode (Inlab 413). To determine the titratable acidity (TA), 10 mL of juice was used in 90 mL of distilled water; this dilution was titrated with NaOH solution (0.1 N). Mettler Toledo digital pH meter (model 320) was used with Mettler Toledo electrode (Inlab 413) until pH 8.1 (turning point) and the results were expressed as grams of citric acid per 100 g^{-1} of pulp. The SS/TA ratio was obtained through the quotient between the two variables.

For microbiological determinations, 25 g of blueberry pulp were aseptically transferred into vials with 225 mL of sterile peptone water (10^{-1} dilution). From this dilution, serial dilutions were made up to 10^{-4} with the same diluent, all evaluations being done according to Silva et al. [20] and Downes and Ito [21]. For the counting of total and thermotolerant coliforms, the Most Probable Number (MPN) technique was used. The presumptive analysis of coliforms was carried out in Sodium Lauryl Sulphate (SLS) broth with incubation at 35ºC for 48 hours. The enumeration of total coliforms was carried out in Brilliant Green Bile Lactose Broth (BGBLB),

incubated at 35ºC for 24 hours. The enumeration of thermotolerant coliforms was carried out in *Escherichia coli* broth, incubated at 45.5ºC for 24 and 48 hours. The results were expressed in NMP of total coliforms and thermotolerant coliforms per gram of sample.

Seeding was carried out with Eosin Methylene Blue Agar (EMB) culture medium from the positive *Escherichia coli* broth tubes. The plates were incubated at 37ºC for 24 hours. The colonies with characteristic morphology of *Escherichia coli* were identified through the tests of indole production, methyl red reactions and Voges-Proskauer, and citrate use.

Regarding *Salmonella* spp. isolation, pre-enrichment was performed in buffered peptone water at 37ºC for 24 hours, followed by selective enrichment in Rappaport-Vassiliadis Broth, at 42ºC for 24 hours and Tetrathionate Broth at 37ºC for 24 hours. Seeds were then plated with deoxycholololysolysin-xylose (XLD) and Hektoen-enteric (HE) agar plates, both incubated for 24 hours at 37ºC. Typical colonies were submitted to biochemical identification in Triple Agar, Iron Lysine Agar and Urease Agar, at 37ºC for 24 hours. Samples that presented a characteristic biochemical reaction were submitted to serological identification, using the polyvalent anti salmonella sera somatic and flagellar (Probac). The results for *Escherichia coli* and *Salmonella* spp. were analyzed in the form of presence or absence of the microorganism in colony forming units per gram of sample (UFC g^{-1}).

The data obtained were analyzed for normality by the Shapiro Wilk test; homoscedasticity by the Hartley test; and residue independence by graphic analysis. Subsequently, the data were submitted to analysis of variance through the F test ($p \leq 0.05$). Being statistically significant, the effects of the coatings were compared by the Waller-Duncan test ($p \leq 0.05$) while the comparison with the control was performed with the Dunnett test ($p \leq 0.05$). The storage periods (days) were compared by regression models ($p \leq 0.05$) as follows: $y = y_o + ax$; $y = y_o + ax + bx^2$; $y = y_o + a/x + b/x^2$, where: y = response variable; y_o = response variable corresponding to the minimum point of the curve; a = estimated maximum value for the response variable; b = slope of the curve; x = storage period (days). The selection of the model was based on low residue; low p-value; and high R^2 and R^2adj. The presence of correlations between the dependent variables in the study was analyzed using the Pearson correlation coefficient (r).

3. RESULTS AND DISCUSSION

The assumptions of the mathematical model were all met and data transformation was not necessary for all variables. In the analysis of variance, the variables mass loss ($F = 6.69$, $p < 0.0001$), L ($F = 4.63$, $p = 0.0006$), b* ($F = 2.71$, $p = 0.0188$) and Hue ($F = 5.65$, $p = 0.0003$) showed significance for the interaction between the treatment factors tested (Table 1 and Fig. 1).

For mass loss, significant differences were only verified between the coatings at 27 and 36 days of storage. In both, the kefir characterized the largest mass loss. At 27 days of storage, differences were observed in relation to the control for both cassava starch and chitosan. For the 36 days of storage, only the cassava starch differed from the control (Table 1). The mass loss data were adjusted appropriately to the quadratic polynomial regression model for control ($F = 19.0656$, $p = 0.0009$), kefir ($F = 20.5018$, $p = 0.0012$) and chitosan ($F = 11.1106$, $p = 0.0049$). For cassava starch, it was not possible to adjust the regression model (Fig. 1A). Increases in mass loss values of 123 and 167% were observed for the kefir and chitosan coatings, respectively, when comparing the 27 and 36 days of storage.

Chitosan associated with modified atmosphere storage reduced mass loss compared to control, this result was also confirmed in chitosan coated blueberries incorporated with blueberry extracts [22]. Chitosan-based coatings have demonstrated superior efficacy to control mass loss in pears and apples as a water transport barrier [23], in the same way as in chitosan-coated blueberries associated with *Aloe vera* [24].

For texture, there was no significance for interaction between the factors ($F = 0.66$, $p = 0.7340$) and neither for the main coating effect ($F = 2.91$, $p = 0.06$) 2.19, $p = 0.1079$). Generally, water loss leads to higher texture during post-harvest blueberry storage [25]. In this study, the mass loss was reduced by the action of the coatings tested and, consequently, it avoided the loss of water, maintaining the texture of the fruits, this is justified because there was no significance for blueberries texture (Table 1). In contrast to other studies in which blueberries stored under a CO_2 effect [14,26] and/or coated with chitosan incorporated with extracts of blueberry leaves

[19] obtained a reduction of the texture throughout the storage. Loss of firmness is related to enzymatic hydrolysis of cell wall substances and softening is often associated with loss of water, which is responsible for the loss of turgor and crispness of fresh fruits.

As for the values of L, the fruits submitted to the chitosan-based coating only presented a difference in relation to cassava starch after 9 days of storage. In this same storage period, no differences were observed for chitosan in relation to the control. The cassava starch and chitosan did not present differences when compared to the control at 27 and 36 days of storage (Table 1). The L data corresponded well to the quadratic polynomial regression model for the control ($F = 18.1988$, $p = 0.0007$), cassava starch ($F = 30.07$, $p = 0.0001$), kefir ($F = 12.4794$, $p = 0.0025$) and chitosan ($F = 10.6311$, $p = 0.0043$) (Fig. 1B). In the comparison between the storage periods, the chitosan coating presented the lowest percentages of increases in the L values, these being 4.3; 6.8; and 7.50% respectively, when 18, 27 and 36 days of storage were compared to 9 days.

Table 1. Mass Loss (%), texture (N) and colour parameters (L, a*, b* and Hue) of blueberry fruits cv. Powder blue as a function of different edible coatings in four storage periods

Coating	Storage period (days)							
	9		18		27		36	
	Mass loss (%)							
Control	0.00±0.00		2.13±0.27		2.13±0.27		2.80±0.40	
Cassava starch	0.00±0.00	a[1/ns]	0.00±0.00	a *	0.40±0.40	b *	0.40±0.40	b *
Kefir	0.00±0.00	a ns	0.00±0.00	a *	1.60±0.00	a ns	2.40±0.46	a ns
Chitosan	0.00±0.00	a ns	0.00±0.00	a *	0.27±0.27	b *	1.20±0.40	ab ns
	Texture (N) NS							
Control	0.35±0.02		0.35±0.03		0.30±0.00		0.31±0.02	
Cassava starch	0.33±0.01		0.30±0.02		0.32±0.02		0.31±0.02	
Kefir	0.33±0.02		0.33±0.04		0.32±0.04		0.29±0.02	
Chitosan	0.31±0.01		0.30±0.01		0.25±0.00		0.28±0.00	
	L							
Control	27.69±0.52		29.94±0.05		30.14±0.26		29.99±0.28	
Cassava starch	21.66±0.93	b *	28.11±0.65	a *	28.99±0.64	a ns	28.31±0.69	a ns
Kefir	24.38±1.04	ab*	27.58±0.22	a *	28.09±0.52	a *	28.14±0.41	a *
Chitosan	27.13±0.49	a ns	27.93±0.49	a *	29.31±0.26	a ns	29.20±0.06	a ns
	a* NS							
Control	1.10±0.09		0.89±0.17		1.36±0.13		1.07±0.11	
Cassava starch	1.72±0.10		0.91±0.06		1.15±0.06		1.10±0.25	
Kefir	1.13±0.25		0.97±0.33		1.12±0.12		1.03±0.13	
Chitosan	1.41±0.25		1.55±0.15		1.14±0.12		0.99±0.09	
	b*							
Control	-1.40±0.08		-2.35±0.04		-2.19±0.18		-2.28±0.13	
Cassava starch	-0.76±0.30	ab ns	-2.25±0.20	c ns	-2.02±0.19	a ns	-1.63±0.14	a *
Kefir	-0.25±0.31	a *	-1.00±0.05	a *	-1.48±0.20	a ns	-1.26±0.13	a *
Chitosan	-1.39±0.14	b ns	-1.47±0.13	b *	-1.71±0.18	a ns	-1.68±0.16	a *
	Hue							
Control	308.11±3.36		290.57±3.26		298.39±0.67		294.97±1.12	
Cassava starch	336.03±7.33	a ns	292.47±3.30	b ns	299.88±1.18	a ns	303.70±6.82	a ns
Kefir	327.52±14.6	a ns	302.53±5.12	ab ns	307.39±4.33	a ns	309.19±3.59	a ns
Chitosan	314.93±7.97	a ns	312.92±5.73	a *	303.98±4.50	a ns	301.09±4.59	a ns

[1/] Means (± standard error) accompanied by the same letter in the column do not differ by the Waller-Duncan test (p≤0.05) comparing the coatings in each period. [, ns] Significant and not significant, respectively, in relation to the control by the Dunnett test (p≤0.05). [NS]: not significant by the F test (p≤0.05) of the analysis of variance. L (0 = black, 100 = white); a* (+a = red, -a = green); b* (+b = yellow, -b = blue); Hue (0° = red, 90° = yellow, 180° = green, 360° = blue).*

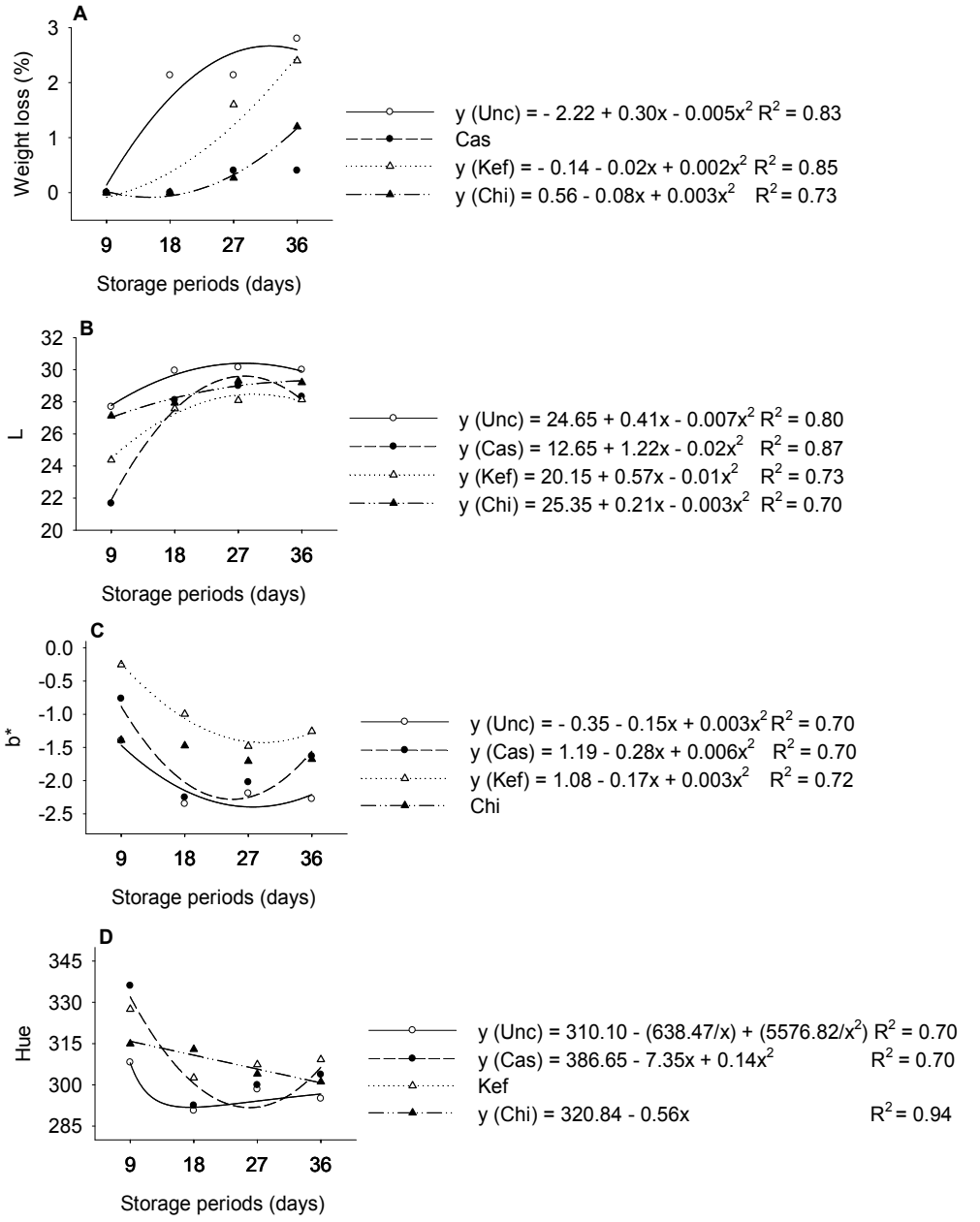

Fig. 1. Mass Loss (%) (A), L (B), b* (C) and Hue (D) of blueberry fruits cv. Powder blue as a function of the edible coatings cassava starch (Cas), Kefir (Kef), chitosan (Chi), and the control (Unc), in four storage periods (9, 18, 27, 36 days)

The variable a* was not significant for the interaction between the treatment factors (F = 1.86, p = 0.0959) and neither for the main coating effect (F = 1.39, p = 0.2635) and storage period (F = 2.45, p = 0.0812) (Table 1). For the values of b*, all the samples treated with the coatings presented negative values, characterizing the blue tint of blueberry fruits. Both at 9 and 18 days after storage, fruits treated with kefir obtained higher values of b*. After 9 days of storage, only kefir differed from the control, and at 18 days, kefir and chitosan presented differences compared to the control (Table 1). The data of b* fit adequately to the quadratic polynomial regression model for the control (F = 10.7912, p = 0.0041), cassava starch

(F = 9.3986, p = 0.0079) and kefir (F = 11.8765, p = 0.003). For chitosan, it was not possible to adjust to the regression model (Fig. 1 C). The highest percentages increases of b* were verified for kefir, being higher than 300%, during the storage periods, which represented a higher intensity of blue colour in the fruits. This behaviour was confirmed by the correlation between b* and Hue (r = 0.82, p <0.0001), demonstrating that increases in the blue hue (b*) guaranteed increases in the violet/blue hue of the fruits.

The colouring of the blueberry fruit epidermis was characterized as violet, although differences between the coatings occurred only at 18 days of storage. The chitosan showed a greater violet/blue colour hue (h°) with a presence of red, confirmed by the positive values of a* and, by the correlation between a* and Hue (r = 0.65, p <0.0001). Only chitosan also showed a difference in relation to the control (Table 1) after 18 days of storage. Hue data were fitted to the second-order inverse polynomial regression model for control (F = 9.4721, p = 0.0078), quadratic for cassava starch (F = 9.1167, p = 0.0086) and linear for chitosan (F = 29.6119, p = 0.0322). For kefir, it was not possible to adjust the regression model (Fig. 1D). In the comparison of storage periods, chitosan was responsible for the lowest losses of characteristic colour, of 1.6; 3.2; and 4.8%, respectively, for 18, 27 and 36 days, when compared to 9 days of storage.

In contrast to the results obtained in this work, both in blueberry [22] and other fruits such as raspberry and strawberry [7,5] submitted to edible chitosan-based coatings, no significant differences were observed for the colour parameters. The chitosan coating showed satisfactory control of the blueberry colour during storage, suggesting delay in maturation. This can be explained by different reactions of anthocyanins with chitosan [27]. Anthocyanins carry positive charges in their stable form, the same is true for chitosan. The positive charge of chitosan can stabilize the positive form of the anthocyanins, leading to the maintenance of the colour of the fruit.

In the analysis of variance, the variables pH (F = 2.50, p = 0.0272), titratable acidity (F = 2.90, p = 0.0180) and SS/TA ratio (F = <0.0001) showed significance for the interaction between the treatment factors tested (Table 2 and Fig. 2). On the other hand, for soluble solids there was no significance for interaction between the factors (F = 1.29, p = 0.2804) and neither for the main coating effect (F = 1.20, p = 0.2771) and period of storage (F = 2.39, p = 0.0869). Similar results were reported in studies with blueberry, where SS values remained unchanged throughout the storage [24,25]. The loss of water caused an apparent increase in the concentration of SS that can be incorrectly interpreted as a change in the amount of acids or sugars present in fruits [28]. In addition, the lower mass loss (Table 1) can be translated by the constant soluble solids over time.

pH values characterized differences between coatings at 18 and 27 days, where the highest averages were found for Kefir and chitosan. In relation to the control, a difference was only verified for cassava starch after 27 days of storage (Table 2). For all coatings, second-order inverse polynomial regression models were used (control: F = 29.9169, p <0.0001; cassava starch: F = 12.3698, p = 0.0026; kefir: F = 23.3741, p = 0.0003 and chitosan: F = 16.0660, p = 0.0011) (Fig. 2A).

From 9 to 18 days of storage increases in pH values were observed in all coatings, 2.7% for cassava starch, 6.4% for kefir and 3.8% for chitosan. This increase is related to the depletion of starch in reducing sugars and its conversion to pyruvic acid caused by fruit respiration [29]. This increase may also be associated with the slight deterioration of the blueberry, with formation of alkaline compounds, for example, nitrogenous compounds [24]. From 27 days of storage, there were decreases in pH values for all coatings, varying from 1.4% for kefir at 27 days to 7.3% at 36 days for cassava starch, decreasing with respect to time (9 days of storage). These decreases were responsible for the conservation of blueberries over time, avoiding the presence of bacteria and fungi [24,30].

The blueberry fruits submitted to the kefir coating obtained lower acidity when compared to cassava starch and chitosan after 9 days of storage. After 18 and 27 days storage, chitosan differed from cassava starch. Still, in these two storage periods, only the cassava starch showed difference in relation to the control (Table 2).

The titratable acidity data were adjusted to the second-order inverse polynomial regression model for cassava starch (F = 10.2090, p = 0.0084) and kefir (F = 5.5981, p = 0.0425). For the control and chitosan, it was not possible to

adjust to regression models (Fig. 2 B). The highest increases in acidity were verified with the use of Kefir, with 45.5% after 18 days; 21.9% after 27 days; and, 2.8% after 36 days, when each period was compared to 9 days of storage. Again, the coatings associated with the modified atmosphere aided in the retention of the titratable acidity of the fruits, preventing gas exchange. The high acidity of blueberries stored in controlled atmosphere has been previously reported [26]. Literature reports that chitosan-coated blueberries associated with *Aloe vera* showed decreases in TA values throughout storage time [24].

Regarding the SS/TA ratio, kefir and chitosan characterized the highest averages only after 27 days storage, with no difference between each other. The values of this ratio are a reflection of the titratable acidity, since there was no significance in the soluble solids contents. This behavior is justified by the negative correlation between SS/TA ratio with TA ($r = -0.93$, $p < 0.001$), where decreases in TA values resulted in increases in the SS/TA ratio, giving a sweeter taste to the fruits. Over the storage periods, all coatings did not differ from the control (Table 2). The SS/TA ratio data were adjusted to the second-order inverse polynomial regression model only for cassava starch ($F = 11.2778$, $p = 0.0065$), for the others it was not possible to adjust to regression models (Fig. 2 C). For cassava starch, there were decreases in the SS/TA ratio of 19.7 and 4.6% for 18 and 27 days, respectively, when compared to the initial period.

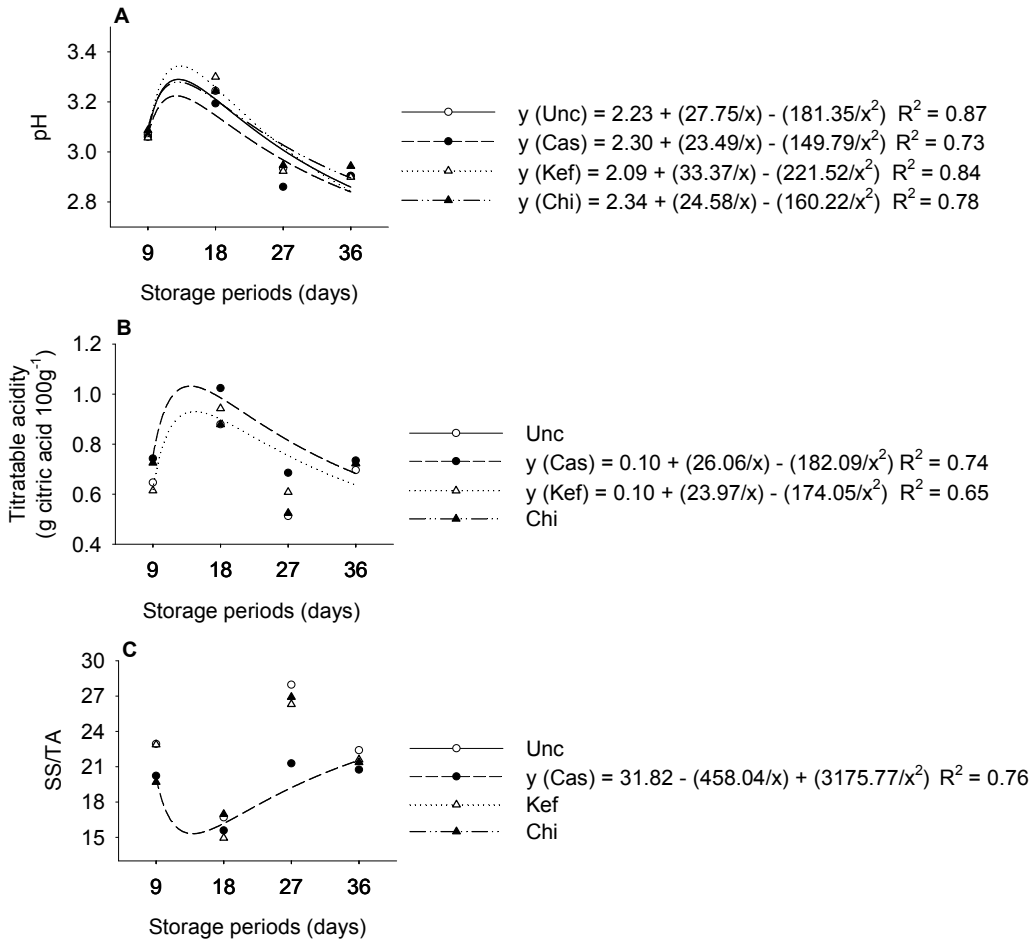

Fig. 2. pH (A), titratable acidity (TA - g citric acid 100g^{-1}) (B) and SS/TA ratio (C) of blueberry fruits cv. Powderblue as a function of the edible coatings of cassava starch (Cas), Kefir (Kef), chitosan (Chi), and the control (Unc) in four storage periods (9, 18, 27 and 36 days)

Table 2. Soluble solids (SS - °Brix), pH, titratable acidity (TA - g citric acid 100 g^{-1}) and SS/TA ratio of blueberry fruits Powderblue cv. as a function of different edible coatings in four storage periods

Coating	Storage period (days)							
	9		18		27		36	
	Soluble solids (SS - °Brix) ^{NS}							
Control	13.90±0.90		14.60±0.93		14.17±0.49		15.57±0.03	
Cassava starch	14.47±0.61		14.93±0.58		14.07±0.49		15.20±0.06	
Kefir	15.00±0.58		14.03±1.05		15.43±0.35		15.60±0.00	
Chitosan	14.27±0.54		14.87±0.28		13.53±0.63		14.70±0.25	
	pH							
Control	3.07±0.01		3.24±0.01		2.93±0.00		2.90±0.00	
Cassava starch	3.06±0.00	a ^{ns}	3.19±0.00	b ^{ns}	2.86±0.01	b *	2.90±0.02	a ^{ns}
Kefir	3.06±0.02	a ^{ns}	3.30±0.00	a ^{ns}	2.92±0.00	a ^{ns}	2.90±0.03	a ^{ns}
Chitosan	3.09±0.02	a ^{ns}	3.24±0.04	ab ^{ns}	2.95±0.00	a ^{ns}	2.94±0.01	a ^{ns}
	Titratable Acidity (TA - g citric acid 100 g^{-1})							
Control	0.65±0.00		0.88±0.05		0.51±0.03		0.70±0.00	
Cassava starch	0.74±0.01	a *	1.02±0.02	a *	0.68±0.02	a *	0.73±0.01	a ^{ns}
Kefir	0.61±0.03	b ^{ns}	0.94±0.01	ab ^{ns}	0.61±0.03	ab ^{ns}	0.72±0.00	a ^{ns}
Chitosan	0.72±0.01	a *	0.88±0.03	b ^{ns}	0.52±0.00	b ^{ns}	0.72±0.00	a ^{ns}
	SS/TA ratio							
Control	22.91±0.37		16.68±1.09		27.95±2.31		22.39±0.19	
Cassava starch	20.21±0.32	a ^{ns}	15.57±0.41	a ^{ns}	21.27±0.39	b ^{ns}	20.73±0.43	a ^{ns}
Kefir	22.87±1.41	a ^{ns}	14.93±1.34	a ^{ns}	26.30±1.07	a ^{ns}	21.59±0.19	a ^{ns}
Chitosan	19.69±0.82	a ^{ns}	16.96±0.53	a ^{ns}	26.91±0.95	a ^{ns}	21.37±0.98	a ^{ns}

[1] Means (± standard error) accompanied by the same letter in the column do not differ by the Waller-Duncan test (p≤0.05) comparing the coatings in each period. $^{*, ns}$ Significant and not significant, respectively, in relation to the control by the Dunnett test (p≤0.05). ^{NS}: not significant by the F test (p≤0.05) of the analysis of variance.

As for the counts of total and thermotolerant coliforms, all the samples were within the standards established by the legislation [31,32]. The presence of *E. coli* bacteria was determined in all samples and did not exceed $1x10^2$ CFU g^{-1}, being in accordance with the Brazilian Legislation, ANVISA - Resolution RDC-12/2001, which establishes a maximum *E. coli* count of $5x10^2$ UFC g^{-1} for fresh, *in natura*, prepared (peeled or selected or fractionated), sanitized, refrigerated or frozen fruits, fruit products and the like for direct consumption [32]. The presence of the bacteria of the genus *Salmonella* spp. was not detected in any of the analyzed samples, thus being in accordance with the current legislation. The edible coatings tested showed antibacterial properties, characterizing the protective action of blueberries [30].

4. CONCLUSION

Extending storage time of chilled blueberries under a modified atmosphere promotes an increase in mass loss after application of the coatings and after 27 days. Edible coatings based on kefir and chitosan grains maintain the physicochemical characteristics of blueberry during refrigerated storage under a modified atmosphere. The fruits are in compliance with the microbiological standards established by the legislation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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