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WHEY BASED BIOPOLYMERIC COATING AS AN ALTERNATIVE TO IMPROVE QUALITY OF FRESH FRUITS (*MALPIGHIA EMARGINATA D.C.)* FROM SOUTHERN BRAZIL

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ABSTRACT

Refrigeration and coating of active biopolymers are two potential approaches to extending the shelf life of fresh fruits. Thus, the aim of this study was to test the influence of storage temperature (5 and 10 °C ± 1 °C) and whey concentration (edible coating), on functional, physical-chemical and microbiological characteristics of acerola stored for 12 days. Edible coatings were produced using whey (30% and 60%) and glycerol. Samples were compared to a control (no coating), during storage at both temperatures. Storage at 10 °C improved the maintenance of the acerola colour, while storage at 5 °C increased the stability of vitamin C and decreased microbial growth ($p \leq 0.05$). The application of edible coating reduced the total colour variation and degradation (a*) at both temperatures. The concentration of whey influenced significantly ($p \leq 0.05$) on the vitamin C content (A10-60), and on the mesophilic (A5-60 and A10-60) and psychrotrophic bacteria (A10-60) counts.

Keywords: acerola; whey; coating; biodegradable; refrigeration

INTRODUCTION

Acerola (*Malpighia emarginata* D.C.) is a fruit noted for its high content of vitamin C and bioactive compounds, such as ascorbic acid, organic acids, anthocyanins, carotenoids, polyphenols and glycosinolates (**Silva *et al*., 2016; Rezende *et al*., 2018; N. Carvalho Gualberto *et al*., 2021**). It is also rich in vitamins, pectin, fibres, proteins and some salts (iron, calcium, and phosphorus) (**Assis *et al.*, 2008; Poletto *et al*., 2021**). Some bioactive compounds, such as anthocyanins (polyphenols), give acerola antioxidant properties and change the colour of the acerola epidermis, giving it its characteristic colour (**Souza *et al*., 2014**).
Since the acerola is a perishable food, different methods that prolong its shelf life can be applied, such as refrigeration (**Hoffmann *et* Hoffmann *et al*., 2021a**), freezing **(Alhamdan *et al*., 2018)**, ozonation (Soares et al., 2018), intelligent and active packaging **(Roy and Rhim, 2021; Hoffmann *et.al*, 2021b**), ultraviolet-LED lights application (**Finardi *et al.*, 2021**), etc. Refrigeration is the method most commonly applied to extend the shelf life of fruits and vegetables (**Hoffmann *et al*., 2021a)**, since low temperatures decrease microbial growth, reduce the respiration rate and delay ripening (**Chitarra *et al*., 2005; Hoffmann *et al*. 2021c, Schlei *et al*., 2020**). However, acerola is susceptible to cold damage when stored at temperatures below 6 °C, which can cause internal darkening, surface depressions and ripening failures (**Yahia, 2011; Oliveira *et al*., 2015**). According to **Alves *et al.* (1995)**, the ideal temperature for maintaining acerola quality is 8 °C. Edible biodegradable coatings can also be effective in extending the shelf life of acerola fruit (**Azeredo *et al*., 2012**). Coverings obtained from whey are transparent and flexible, and they have no odour or taste, which favours consumer acceptability (**Alleoni *et al*., 2006; Dinika *et al*., 2020**). Moreover, studies have shown that dairy protein derivatives have antimicrobial effects (***Damodaran et al*., 2010; Rantamaki *et al*., 2019**). **Galietta *et al.* (2004)** noted that the application of biofilms formulated with whey and glycerol delayed the deterioration of the red colour of tomatoes stored for 4 weeks. **Martin-Diana *et al.* (2006)** observed a reduction in the microbial count of lettuce and carrots after using whey as a sanitizing agent.
Given the relevance of refrigeration and coating with regard to the shelf life of acerola, the influence of storage temperature (5 and 10 °C) and the whey content of edible coatings (30 and 60%), on functional, physical, chemical and microbiological characteristics of acerola, were investigated over a 12-day period. Control samples (without coating) were prepared and stored at both temperatures, for the same period.

MATERIAL AND METHODS

Sample preparation and storage

Mature acerolas, after being harvested from trees located in the city of Blumenau (Santa Catarina, from southern Brazil), were immediately sent (in thermal boxes) to the Laboratory of Food Processing (University of Blumenau, Brazil). Then, they were sanitized with chlorinated water (200 ppm), for 15 min. Whey was obtained from artisanal cheese production, using pasteurized milk and commercial rennin as coagulant. After the whey extraction, the biopolymers solutions were prepared. Edible coatings were formulated using glycerine (5%), whey (30 and 60%) and potable water (65 and 35%).
Polypropylene plastic trays (14.5×13×5.3 cm), with perforations in the lids, were used to store the acerola. Sixteen acerola berries were placed in each package. The samples were submitted to storage (5 ± 1 °C and 10 ± 1 °C) in a refrigerated BOD incubator (TECNAL, model TE-371) for 12 days. The relative humidity of the air inside the refrigerator was monitored using a Klima Logg device (INCOTERM, 30.3180).
Analysis to determine the vitamin C and anthocyanins contents, colour and microbial growth were performed on test days 0, 4, 8 and 12.

Vitamin C content

Vitamin C content was determined by titulometry with 2,6-dichloroindophenol solution (AOAC, 2000). Results are express in mg ascorbic acid per 100 g of fruit.

Total anthocyanins

Total anthocyanins were determined using a Cirrus 80 ST spectrophotometer (FEMTO, São Paulo, Brazil), at a wavelength of 535 nm. Samples were prepared using 95% alcohol and 1.5 N hydrochloric acid. Results are express in mg 100 g^{-1} (Francis, 1982).

Colour analysis

Colour analysis was performed using the SP60 series sphere spectrophotometer, in a three-dimensional system: L* (brightness), a* (red-green) and b* (yellow-blue). Results are expressed as a* variation (Δa), b* variation (Δb) and total colour variation (ΔE), according to equation (1). Changes were considered by comparison with day 0 of storage.

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (1)$$

Microbial growth

In the microbiological analysis, mesophilic and psychrotrophic aerobic bacteria were determined. Ten (10) g of acerola were diluted in 90 ml of sterile peptonized water. Dilutions were prepared in series (10^1) with the same diluent, and inoculation was carried out by deep plating with 1 ml of sample. The total count was performed using plate count agar (PCA, NEOGEN Culture Media) and incubation at 35 °C for 48 h (mesophilic bacteria) and 7 °C for 10 days (psychrotrophic bacteria). Counting was performed on plates containing between 30 and 300 CFU at the end of incubation. Results are presented in log (CFU g^{-1}) (APHA, 1992).

Experimental matrix

The experimental matrix, with the runs and their identification, can be observed in Table 1. The analysis was performed in duplicate.

Table 1 Experimental Matrix.

Sample Identification	Temperature (°C)		Whey Concentration (%)	
	T (real value)	X_T (coded value)	Wc (real value)	X_{wc} (coded value)
A5-30	5	-1	30	-1
A5-60	5	-1	60	1
A10-30	10	1	30	-1
A10-60	10	1	60	1

Control samples (no biopolymer solution) were also stored at 5 °C (A5-0) and 10 °C (A10-0).

Statistical analysis

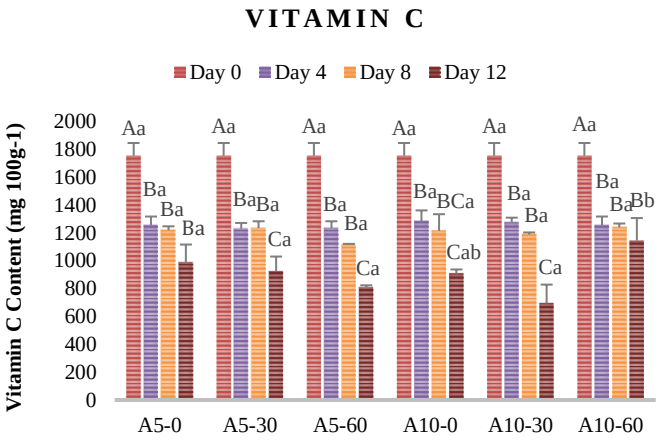
The experimental data were analyzed by analysis of variance (ANOVA), to generate linear regression models and averages were compared applying the Tukey test (5%), using the *Statistica* 7.0 software (Box et al., 2005), where the model parameters (temperature and whey Concentration) were estimated. The difference was considered statistically significant if $p \leq 0.05$.

RESULTS

Vitamin C content

The ANOVA results showed that only the temperature-whey concentration interaction significantly influenced ($p<0.05$) the vitamin C value, at the end of storage. On day 0, the vitamin C content was 1755 ± 200 mg 100 g^{-1}. In the case of the acerola samples kept at 5 °C, the results for vitamin C, for all samples, showed no statistical difference, according to the whey concentration ($p\geq0.05$), at the end of storage, with values ranging between 810 and 990 mg 100 g^{-1} (A5-60 and A5-0, respectively).

Thus, the application of the coating with 60% whey improved the retention of vitamin C in acerola samples stored, at 10 °C ($p\leq0.05$), although it has no effect on acerolas stored at 5°C ($p\geq0.05$). In summary, the use of active packaging containing whey, in high concentrations was effective at 10°C.

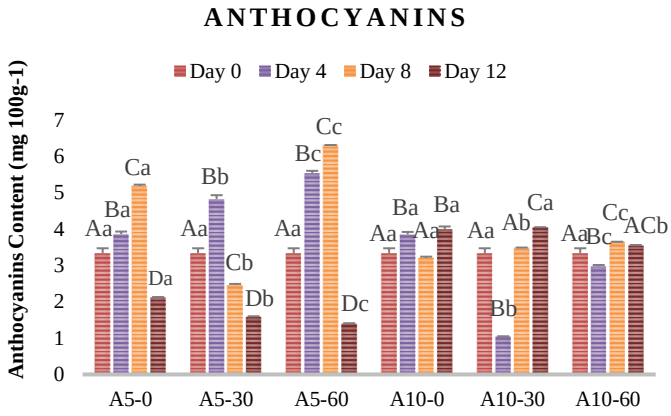


Model: Vitam C content (mg 100g^{-1}): $893.750 - 83.750\, x_c - 26.250\, x_T + 141.250\, x_c x_T$ *(R^2: 0.7982)*

Figure 1 Vitamin C content in acerola samples, during storage at 5 and 10 °C, packaged in films with different concentrations of whey: A5-0 = 5 °C and control sample (no coating); A5-30 = 5 °C and 30% whey; A5-60 = 5 °C and 60% whey; A10-0 = 10 °C and control sample; A10-30 = 10 °C and 30% whey; A10-60 = 10 °C and = 60% whey; upper case compares days of storage, lower case compares whey concentrations. The same letters indicate that there was no significant difference ($p\geq0.05$), between samples.

Total anthocyanins

The content of anthocyanins in the samples (Figure 2), on the first day of storage (day 0) was 3.33 ± 0.4 mg 100 g^{-1}. On the 12th day, the values for samples kept at 5 °C ranged from 1.40 to 2.12 mg 100g^{-1} (A5-60 and A5-0), while those for samples kept at 10 °C ranged from 3.54 to 4.04 mg 100 g^{-1} (A10-60 and A10-30).



Model: Anthocyanins (mg 100g^{-1}) = $2.642 + 0.172\, x_c - 1.150\, x_T - 0.080\, x_c x_T$ *(R^2: 0.9999)*

Figure 2. Anthocyanins content in acerola samples, during storage at 5 and 10 °C, packaged in films with different concentrations of whey: A5-0 = 5 °C and control sample (no coating); A5-30 = 5 °C and 30% whey; A5-60 = 5 °C and 60% whey; A10-0 = 10 °C and control sample; A10-30 = 10 °C and 30% whey; A10-60 = 10 °C and 60% whey; upper case compares days of storage, lower case compares whey concentrations. The same letters indicate that there was no significant difference ($p\geq0.05$), between samples.

There was a significant ($p\leq0.05$) decrease in the anthocyanins content of acerola samples stored at 5 °C, comparing days 0 and 12 of storage. However, it was observed that there was an increase of anthocyanins up to the 8th day at this temperature, with values of 2.46 and 6.31 (A5-30 and A5-60, respectively), followed by a decrease on the 12th day. For samples stored at 10 °C, there was a small, but significant increase in anthocyanins content at the end of storage, this being higher for samples A10-0 and A10-30 and slightly lower for A10-60.

Therefore, at the end of storage, the best results were obtained at 10 °C. The use of edible coating had no significant influence ($p\geq0.05$) on the anthocyanins content of acerola, at the end of storage.

Colour analysis

The results obtained for the colour analysis can be seen in Table 2.

Table 2 Total colour variation, red colour degradation and yellow colour development in acerola samples packaged in coating containing different concentrations of whey, during storage at 5 and 10 °C, for 12 days.

Total Colour Variation

Concentration	A5-0	A5-30	A5-60	A10-0	A10-30	A10-60
Day 0	0.00± 0.00$^{a}_{A}$	0.00± 0.00$^{a}_{A}$	0.00± 0.00$^{a}_{A}$	0.00± 0.00$^{a}_{A}$	0.00± 0.00$^{a}_{A}$	0.00± 0.00$^{a}_{A}$
Day 4	9.78± 5.01$^{a}_{AB}$	8.97± 0.73$^{a}_{AB}$	9.34± 0.23$^{a}_{BC}$	7.89± 2.55$^{a}_{A}$	9.09± 3.04$^{a}_{A}$	5.75± 1.32$^{a}_{B}$
Day 8	12.22± 2.56$^{a}_{AB}$	17.80± 3.92$^{a}_{B}$	7.45± 0.85$^{a}_{AB}$	4.97± 2.14$^{a}_{A}$	5.85± 5.54$^{a}_{A}$	6.88± 1.07$^{a}_{B}$
Day 12	20.80± 3.09$^{a}_{B}$	15.33± 6.60$^{a}_{AB}$	15.29± 3.60$^{a}_{C}$	5.89± 3.49$^{a}_{A}$	12.82± 4.38$^{a}_{A}$	10.66± 1.89$^{a}_{B}$

Red colour degradation (a* parameter)

Concentration	A5-0	A5-30	A5-60	A10-0	A10-30	A10-60
Day 0	0.00± 0.00$^{a}_{A}$	0.00± 0.00$^{a}_{A}$	0.00± 0.00$^{a}_{A}$	0.00± 0.00$^{a}_{A}$	0.00± 0.00$^{a}_{A}$	0.00± 0.00$^{a}_{AB}$
Day 4	-1.27± 3.02$^{a}_{A}$	-0.97± 0.42$^{a}_{A}$	-3.07± 1.18$^{a}_{AB}$	-2.64± 0.64$^{a}_{A}$	-6.83± 0.20$^{b}_{B}$	2.30± 1.24$^{c}_{A}$
Day 8	-5.34± 1.53$^{a}_{A}$	-8.03± 2.45$^{a}_{A}$	-2.59± 0.95$^{a}_{A}$	-4.14± 1.41$^{a}_{A}$	3.03± 2.59$^{a}_{A}$	1.99± 2.26$^{a}_{A}$
Day 12	-14.50± 1.56$^{a}_{B}$	-9.95± 4.66$^{a}_{A}$	-12.96± 4.71$^{a}_{B}$	-0.37± 2.27$^{a}_{A}$	-6.22± 0.22$^{b}_{B}$	-4.04± 0.03$^{ab}_{B}$

Yellow colour development (b* parameter)

Concentration	A5-0	A5-30	A5-60	A10-0	A10-30	A10-60
Day 0	0.00± 0.00$^{a}_{A}$	0.00± 0.00$^{a}_{A}$	0.00± 0.00$^{a}_{A}$	0.00± 0.00$^{a}_{AB}$	0.00± 0.00$^{a}_{AB}$	0.00± 0.00$^{a}_{A}$
Day 4	3.030± 11.08$^{a}_{A}$	4.525± 0.17$^{a}_{A}$	7.840± 0.59$^{a}_{B}$	4.035± 0.54$^{a}_{A}$	-4.105± 3.27$^{a}_{AB}$	3.215± 2.47$^{a}_{A}$
Day 8	-8.775± 1.92$^{ab}_{A}$	-14.090± 2.52$^{a}_{B}$	-3.320± 1.55$^{b}_{A}$	-2.455± 1.57$^{a}_{B}$	4.800± 4.66$^{a}_{A}$	4.990± 0.13$^{a}_{A}$
Day 12	0.865± 0.74$^{a}_{A}$	3.535± 2.92$^{a}_{A}$	-5.875± 3.37$^{a}_{A}$	2.160± 1.39$^{a}_{A}$	-10.695± 4.38$^{b}_{B}$	-7.685± 2.01$^{ab}_{B}$

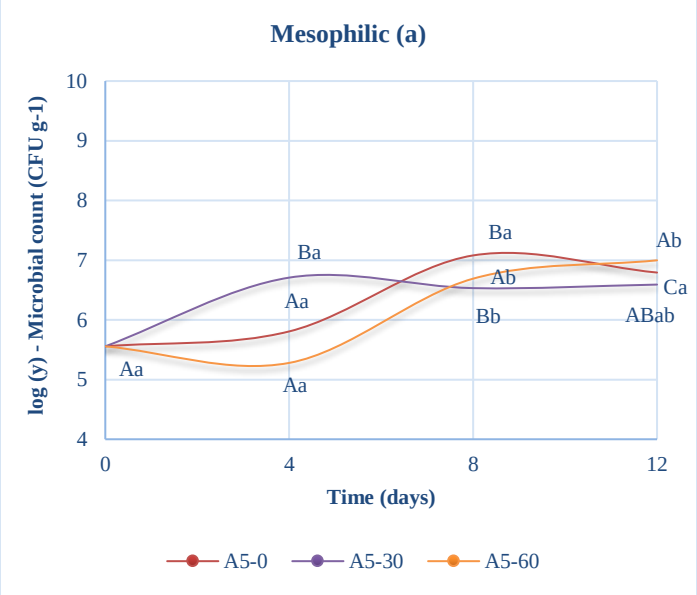
Model: Yellow Colour Development = $-5.180 + 1.600\ x_C + 4.010\ x_T + 3.105\ x_C x_T$ *(R^2: 0.8401)*

A5-0 = 5 °C and control sample (no coating); A5-30 = 5 °C and 30% whey; A5-60 = 5 °C and 60% whey; A10-0 =10 °C and control sample; A10-30 = 10 °C and 30% whey; A10-60 = 10 °C and 60% whey; upper case compares days of storage and lower case compares whey concentrations. The same letters indicate that there was no significant difference (p≥0.05), between samples.

On analysing the total colour variation, on the last day of storage at 5 °C, higher values were found when compared to 10 °C, with values ranging from 15.29 to 20.80 (A5-60 and A5-0, respectively) (no statistical significance). At 10 °C, at the end of storage, a smaller (not statistically significant) variation was observed, with higher values for the sample A10-0 (5.89) compared to A10-30 (12.82) and A10-60 (10.66).

With regard to the degradation, it can be noted that at the end of storage at 5 °C, there was a significant decrease (compared to day 0) in a*, with values of -9.95 and -14.50, for A5-30 and A5-0, respectively (no statistical significance). On comparing the storage temperatures, the samples kept at 10 °C had lower (no statistical significance) degradation values on the last day of storage, with the lowest degradation for the sample A10-0 (-0.37) and the highest for A10-30 (-6.22) and A10-60 (-4.04).

The yellow colour (b*), at the end of storage at 5 °C, showed values of -5.875 and 3.535, for A5-60 and A5-30, respectively, while at the end of storage at 10 °C, the values were -10.695 and 2.160, for A10-30 and A10-0, respectively. At 5 °C, a small increase (no statistical significance) was observed in the values for A5-0 (0.865) and A5-30 (3.535), demonstrating the development of a yellow colour. On the other hand, a significant decrease in b* was observed for A10-30 (-10.695) and A10-60 (-7.685).



Microbial growth

The behaviour of microbiological growth, of mesophilic and psychrotrophic bacterial, are presented in Figure 3.

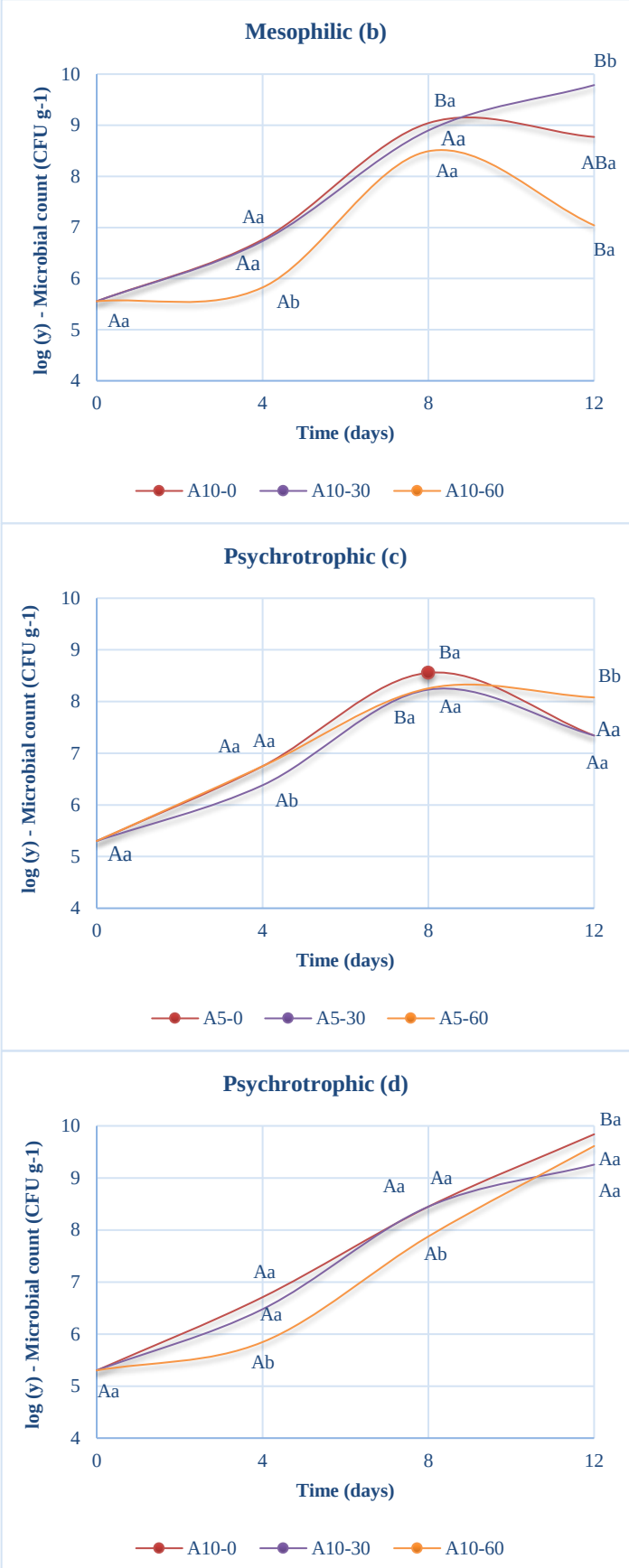


Figure 3 Microbial growth curve, for mesophilic and psychrotrophic, at temperatures of 5 °C (a) and 10 °C (b), and packaged in coating containing different concentrations of whey (30% and 60%), where A5-0 = 5 °C and control sample (no coating); A5-30 = 5 °C and 30% whey; A5-60 = 5 °C and 60% whey; A10-0 = 10 °C and control sample; A10-30 = 10 °C and 30% whey; A10-60 = 10 °C and 60% whey; upper case compares days of storage, lower case compares whey concentrations. The same letters indicate that there was no significant difference ($p \geq 0.05$), between samples.

For the mesophilic microorganisms (Fig. 3a and 3b), it can be seen that samples A5-0, A5-60 and A10-60, up to day 4 showed stability, and A5-60 even presented a slight decline in the lag phase (with no statistically significant variation in the initial concentration of microorganisms). The mesophilic bacteria then started to grow exponentially until day 8. Stability was noted for the samples A5-30 and A5-60, with mesophiles concentrations below 10^{-7} CFU g^{-1}, considered within the limit for food safety. For sample A5-60, the stationary phase was maintained until the end of storage.

Samples A5-30, A10-0 and A10-30 showed an exponential growth from the beginning (without latency phase), while sample A10-30 remained in the exponential growth phase until the end of storage. The values for the A5-30 sample remained practically stable, from day 4 until the end of storage (around 10^{-6} CFU g^{-1}). All samples at 10 °C exceeded the concentration of 10^{-7} CFU g^{-1} (mesophiles), on the 5th day of storage.

In the results for psychrotrophic microorganisms (Fig. 3c and 3d), samples A5-0, A5-30 and A5-60 presented exponential growth, from the first day up to the 8th day of storage, while the other samples (A10-0, A10-30 and A10-60) showed exponential growth, from day 0 up to the last day of storage. Only sample A10-60 showed a latency phase, up to the 3th day of tests, followed by the beginning of exponential growth. All samples kept at 5 °C reached 10^{-7} CFU g^{-1}, between the 4th and 5th days, indicating the need for them to be discarded due to food safety issues (Brasil, 2019). Samples stored at 10 ºC (control and 30% whey) behaved in the same way, except sample A10-60, which reached 10^{-7} CFU g^{-1}, after the 7th day of storage.

DISCUSSION

Vitamin C content

The content of vitamin C decreases as the senescence of the fruit occurs (**Alves *et al.*, 1995; Mditshwa *et al.*, 2017**). According to **Bobbio *et al.* (2003)** and **Mercali *et al.* (2012)**, the stability of vitamin C increases as the temperature decreases, as shown in Figure 1.

At 10 °C, there were significant differences ($p \leq 0.05$) in the vitamin C content, according to the whey concentration. A lower retention was found for samples A10-30 (695 mg 100 g^{-1}) compared with A10-60 (1145 mg 100 g^{-1}) at the end of storage. This lower retention occurs because vitamin C is easily oxidized when exposed to heat, light and oxygen and during handling **(Mditshwa *et al.*, 2017; Hong *et al.*, 2014; Chitarra *et al.*, 2005**).

Studies (**Poletto *et al.*, 2021; Jaeschke *et al.*, 2016**) with other active agents showed positive effects on the maintenance of vitamin C content in acerola. **Maciel *et al.* (2004)** employed cassava starch biofilm in acerola concentrations of 1, 2, 3 and 4% and at temperatures of 10 and 22 °C. They found higher vitamin C contents in fruits treated with concentrations of 1% and stored at 10 °C, prolonging shelf life by up to 15 days.

Besides improving the vitamin C stability, many authors have studied other uses for whey film (***Dinika et al.*, 2020**). **Galietta *et al.* (2004)** applied films of whey (10%) and acetylated glyceryl monostearate (10%) to tomatoes, and observed a decrease in weight loss, firmness and the development of red colour. In this case, the film acted as a barrier to oxygen, carbon dioxide and water.

Total anthocyanins

Anthocyanins are very unstable pigments, which can be degraded under the action of vitamin C, oxygen, temperature and pH (**Brouillard, 1982; De Rosso and Mercadante, 2007**). According to Moura et al. (2002), the higher the anthocyanins content the better the acceptance of the product will be, since yellowish acerola berries are likely to be rejected by consumers and the importing markets (**Araújo *et al.*, 2007**).

According to the ANOVA results, the storage temperature and whey concentration significantly ($p<0.05$) influenced the value of anthocyanins at the end of storage (Fig. 2). **Ribeiro (2017)** observed stability in the anthocyanins content during storage of acerola at 12 °C. However, during storage at 8 and 10 °C for 14 days, the author observed losses in the development of the red coloration, due to cold damage and/or rotting symptoms, as noted in this study for acerolas stored at low temperatures (5 °C).

Color analysis

Colour is one of the most important characteristics for consumers and is closely linked to the market value of the product. Due to the visual impact, colour is a particularly important aspect for the acceptance and consumption of red fruits (**Brunini *et al.*, 2004; Lima *et al.*, 2003; Mditshwa *et al.*, 2017; Silva, 2007**).

Acerola is susceptible to cold damage, which can be observed for the samples stored at 5 °C (Fig. 4). In general, besides symptoms such as surface depressions and loss of odour and taste, there are also failures in the ripening process, affecting the normal development of the pulp colour (**Oliveira *et al.*, 2015; Kim *et al.*, 2021**).

Thus, these results are consistent with those obtained for the anthocyanin content (Fig. 2), i.e., that a significant decrease (Table 2) was noted for storage at 5 °C, the acerola samples presenting a yellowish colour, according to a study conducted by **Ren and Giusti, (2021)**. At 10 °C, there was less colour degradation compared with the samples stored at 5 °C. **Shao-Qian *et al.* (2011)** analysed orange juice and reported that the colour variation decreased with the reduction in anthocyanins, highlighting the relation between anthocyanin content and the berry colour.
According to the statistical analyses (table 2), only the temperature significantly influenced ($p<0.05$) the development of the yellow colour (b*) at the end of storage and no factor significantly ($p<0.05$) influenced the total colour variation and red colour degradation (a*).
In summary, for storage at 5 °C there was an increase in the colour variation and, for storage at 10 °C, there was a reduction. With the application of the edible coating, the stability of the samples kept at both storage temperatures increased. The same occurred for the degradation of a* and the development of the yellow colour (b*). After storage at 5 °C, the samples presented a yellowish colour while after storage at 10 °C the acerola did not show a great loss of the reddish colour, as seen in Figure 4.

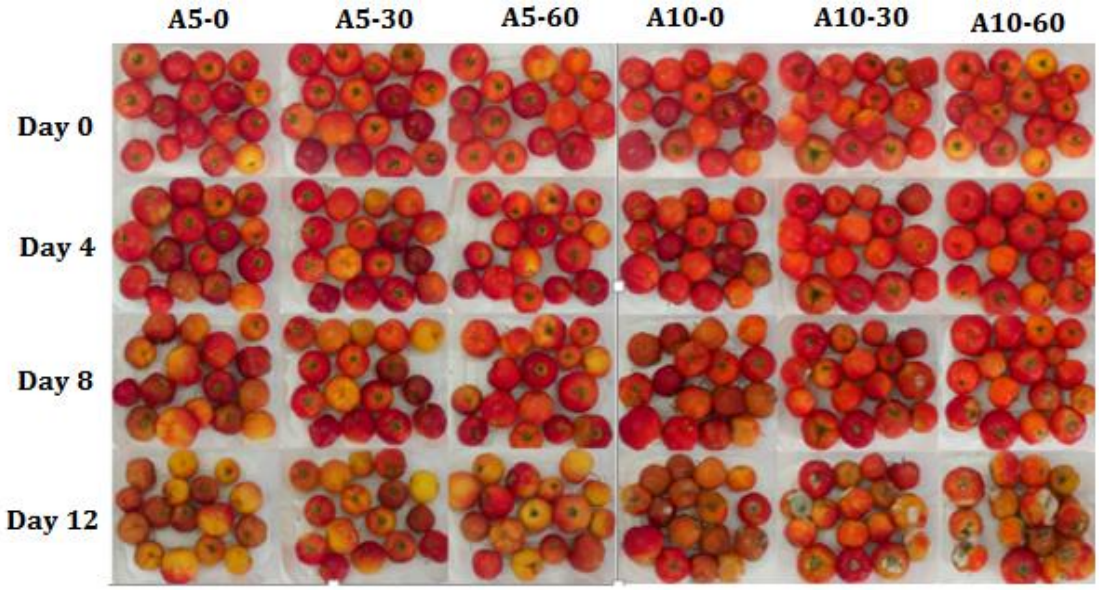


Figure 4 Senescence of the acerola during the 12-day storage period. Lines represent the respective days of analysis (0, 4, 8 and 12) and the columns represent the samples (A5-0, A5-30, A5-60, A10-0, A10-30 and A10-60).

Microbial growth

Large post-harvest losses of fruits are common at high storage temperatures, which favors microbial growth, leading to rotting (**Chitarra *et al.*, 2005; Chen *et al.*, 2020**). To reduce this phenomenon, low temperatures can be employed (**Oliveira *et al.*, 2015; Chaomuang *et al.*, 2019**).
Temperature is probably the most important factor affecting the growth of microorganisms (**Brackett, 1997; Mascheroni, 2012**) and the lower the temperatures applied the slower the chemical reaction rate, enzymatic activity and microbial growth will be (**Chaomuang *et al.*, 2019**).
Observing the microbial curves (Fig. 3) can be noted that a whey concentration of 60% increased the lag phase of mesophilic (5 and 10 °C) and psychrotrophic (10 °C) microbes. According to **Muratore *et al.* (2005), Hung *et al.* (2018) and Hoffmann *et al.* (2021b)** to avoid microbial contamination in fresh food products, coating containing adequate permeability can be applied. According to **Damodaran *et al.* (2010)**, whey compounds derived from the proteins β-lactoglobulin, α-lactoalbumin, lactoferrin and lactoperoxins have antimicrobial effects.
Some studies have shown that the antimicrobial effect of whey in acerolas (**Angioletti *et al.*, 2020**) and its use as sanitizing agent for lettuce and carrots, achieving a reduction in the microbial load of these foods **(Martin-Diana *et al.*, 2006**). Moreover, some studies propose a relation from the antimicrobial effect of the whey with low pH. The death of microorganisms would occur due to the presence of lactic acid that penetrates the cells in the decoupled form **(Brink; Šipailienė and Leskauskaitė, 2019).**
However, in the present study, the coating presented neutral pH (6.92 for films with 30% whey and 6.90 for films with 60% whey). **Amaral (2014)** found that the microbial growth observed for apples was statistically higher ($p\leq0.05$) using whey proteins, glycerol and water, when compared to the control sample. The author suggested that the higher growth could be explained by the absence of acids in this coating.

CONCLUSION

In this study of refrigerated storage of acerola with and without biopolymeric coating, vitamin C levels decreased over time for both storage temperatures tested. However, there was greater stability of this vitamin in samples stored at 5 °C. The edible coating containing 60% whey was effective (statistical significance) in reducing vitamin C loss in acerola, stored at 10 °C. At 5 °C, it was observed that the content of anthocyanins decreased and the total degradation of colour and a* increased, with a yellowish colour indicating cold damage. At 10 °C, less colour degradation was observed, with a redder colour being evident at the end of storage.
In the microbiological analysis, it was observed that the use of a coating with 60% whey increased the lag phase of mesophilic (5 and 10 °C) and psychrotrophic (10 °C) microorganisms. Lower microbial growth was observed at 5 °C at the end of storage. Thus, storage at 10 °C improved the maintenance of the acerola colour, while storage at 5 °C increased the stability of vitamin C and decreased the microbial growth. The application of an edible coating containing whey, showed a positive effect, that was statistically significant ($p\leq0.05$), in terms of vitamin C content (A10-60) and levels of mesophilic (A5-60 and A10-60) and psychrotrophic (A10-60) microorganisms. The results of this research demonstrate the potential application of edible biopolymeric coatings, using whey (industrial waste), as a sustainable and economical alternative method for the preservation of fresh chilled acerola.

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could appear to influence the work reported in this paper. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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