

DEVELOPMENT AND APPLICATION OF ANTIOXIDANT COATING ON *Fragaria* spp. STORED UNDER ISOTHERMAL CONDITIONS

Sarah Finardi¹, Tuany Gabriela Hoffmann¹, Betina Louise Angioletti¹, Eduarda Mueller¹, Raissa Souza Lazzaris¹, Sávio Leandro Bertoli¹, Miroslava Hlebová², Mars Khayrullin³, Natalia Nikolaeva³, Mohammad Ali Shariati^{3,*}, Carolina Krebs de Souza^{1*}

Address(es):

¹University of Blumenau, Department of Chemical Engineering, 89030-000, Blumenau, Santa Catarina, Brazil.

²University of SS. Cyril and Methodius, Department of Biology, Faculty of Natural Sciences, Nám. J. Herdu 2, SK-91701 Trnava, Slovak Republic.

³K.G. Razumovsky Moscow State University of technologies and management (The First Cossack University), 73, Zemlyanoy Val St., Moscow, 109004, Russian Federation.

*Corresponding author: carolinakrebs@furb.br ; shariatymohammadali@gmail.com

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ABSTRACT

Strawberry (*Fragaria* spp.) is a non-climacteric fruit, widely consumed in several countries, but is extremely sensitive and susceptible to mechanical damage during harvest, transport and storage, reducing the shelf life. In order to prolong the shelf life of this product, edible starch-based coatings were developed, with propolis extract (MSP) and without the addition of ethanolic propolis extract (MS) and uncoated samples, used as control (MC). All samples were stored under isothermal conditions ($4\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$), for 10 days. Physicochemical analyzes (pH, mass variation, vitamin C, color and phenolic compounds) were carried out to characterize and identify the coating efficiency in the conservation of strawberries. The formulations of the coatings with and without the addition of propolis had a significant ($p \leq 0.05$) effect on the variation of fruit weight. The propolis coating showed greater stability in the vitamin C content and pH levels, up to the 10th day of cold storage, in comparison to the samples with starch coating and the control. The treatments (MS, MSP and MC) did not significantly influence ($p > 0.05$) the color and the content of total polyphenols.

Keywords: strawberry, propolis coating, physicochemical, shelf life

INTRODUCTION

Strawberry (*Fragaria* spp.) is a non-climacteric fruit, among the most consumed in the world (Thomas *et al.*, 2016; Schmitz *et al.*, 2019). Despite having several nutritional components, such as vitamins (A, C, E, B5 e B6), anthocyanins and minerals (Calcium, Iron, Magnesium, Potassium and Selenium), important for a healthy diet, with several health benefits, strawberry is a fruit sensitive to mechanical damage, during harvest, and high senescence in the post-harvest storage (Martínez-González *et al.*, 2020; Hoffmann *et al.*, 2021a). For this reason, preservation technologies, combined with refrigeration systems, are needed to expand the shelf life of strawberries (García *et al.*, 2012; Angioletti *et al.*, 2020a; Pavinatto *et al.*, 2020; Finardi *et al.*, 2021; Hoffmann *et al.*, 2021b). Edible coatings are technologies developed to slow down the decay process of fresh food produce. A thin coating layer, produced from edible materials (protein and polysaccharides) (Roy & Rhim, 2021), with plasticizing capacity, provides the shelf-life extension of coated food (Fakhouri *et al.*, 2015). The edible coating works by preventing moisture loss from the food to the environment and reducing the oxidative process (Khodaei & Hamidi-Esfahani, 2019). In addition, gas exchange between the food and the environment is also controlled (Wong *et al.*, 1994), which maintain the firmness and delay the senescence of fresh fruit and vegetables (Mannozi *et al.*, 2017).

Starch is a natural biopolymer, based on amylose and amylopectin, widely used in edible film/coating formulations, due to its low cost, easy handling and complete biodegradability (Hoffmann *et al.*, 2019; Hoffmann *et al.*, 2021c). In order to enhance the coating effect, active compounds (antioxidants and/or antimicrobials) can be added to the coating solution (Khodaei & Hamidi-Esfahani, 2019). Coatings containing active compounds can provide antimicrobial and antioxidant properties to coated foods, contributing to an increase in their shelf life (Angioletti *et al.*, 2020b). Several researchers confirmed the positive effects of the addition of active compounds in food coatings, such as propolis extract (Ali *et al.*, 2014), chitosan and lemon essential oil (Perdones *et al.*, 2012), and potassium sorbate (García *et al.*, 2012).

Propolis is a viscous resin produced by bees (*Apis mellifera* L.), composed of 50% resin (containing flavonoids, phenolic acids and esters), 30% waxes, 10% essential oils and 5% pollen (Pastor *et al.*, 2011). This composition is directly dependent on geographic origin, time of collection and climatic conditions (Vasilaki *et al.*, 2019). Propolis possess antimicrobial and antioxidant properties, in other words, possess the ability to donate electrons to free radicals, reducing them to more stable and non-reactive species, protecting food against oxidation (Thomas *et al.*, 2016). Edible coating, using propolis extract, are able to prolong the shelf-life of post-harvest fruits, such as pitaya, papaya and strawberry, improving their physicochemical properties (Zahid *et al.*, 2013; Barrera *et al.*, 2015; Thomas *et al.*, 2016).

In the light of these considerations, this study aimed to investigate the influence of an edible coating, produced with starch, glycerol, water and the addition of propolis extract, on the quality of fresh hydroponic strawberries, during the post-harvest storage period (10 days), in a refrigerated environment ($4\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$).

MATERIAL AND METHODS

Material

The materials used, for the edible coating development and their respective physicochemical analyzes, were: ethyl alcohol (Reatec, CAS [64-17-5]), green propolis alcoholic extract (Apis Flora, CAS N/A), oxalic acid (Anidrol, CAS [144-62-7]), starch (Dinâmica, CAS [9005-25-8]), glycerol (Dinâmica, CAS [56-81-5]), 2,6-dichlorophenol indophenol (Dinâmica, CAS [6520-45-1]), sodium carbonate (Anidrol, CAS [144-55-8]), sodium carbonate (Reatec, CAS [497-19-8]) and Folin-Ciocalteu reagent (Alphatec, CAS N/D).

Propolis coating production

For the development of the edible coating, propolis active compounds (MSP), starch, glycerol, propolis alcoholic extract and distilled water were used. The solution was prepared using starch (5% m/v), glycerol (1.5% v/v), as described by

Hoffmann et al. (2021c), with slight modifications; and extract (3% v/v). Starch gelatinization was carried out to obtain a thermoplastic starch, in this procedure, the soluble starch was initially heated (52 °C) in a thermostatic bath under mechanical manipulation for 15 minutes, with the addition of glycerol and distilled water. Then the temperature was increased to 82 °C and maintained for 50 minutes to complete gelatinization and form the filmogenic solution. After cooling the solution, half of the total coating solution generated was transferred and added the propolis extract. Coating formulation without propolis addition (MS), was developed and studied, as well as uncoated samples (control: MC), for comparison purposes.

Coating application on strawberries (Fragaria ssp.)

Strawberries (3 kg) with greater color homogeneity, size similarity and without apparent damage on their surface were selected, in a hydroponic, located in Indaial, Santa Catarina, Brazil. After harvesting, the strawberries were transported in a thermal container to LabFood (Campus II / University of Blumenau). The samples were sanitized, with the aid of a brush, to remove dirt from the strawberries surface. The strawberries were submerged in the film-forming solution, for 5 seconds: strawberries coated with plasticizer solution (MS) and strawberries coated with the film-forming solution, with propolis extract addition (MSP). Still, uncoated strawberries (MC: control condition) were used. After the coating was dried at a constant temperature, 20 °C ± 1 °C (24h), the samples were stored in a refrigerator (TE-371, Tecnal), as showed in Figure 1, at constant temperature and relative humidity, 4 °C e 60%, respectively, for 10 days.

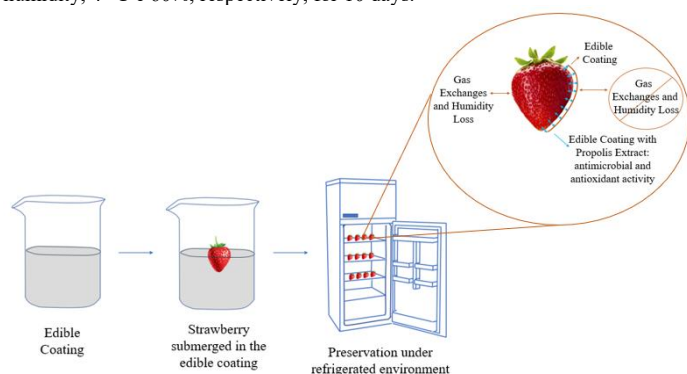


Figure 1 Coating application process and effect on strawberries.

Fresh mass variation

The fresh mass variation of the samples was measured on a scale (A1000, Marte) and the results were expressed as a percentage (%), according to equation (1):

$$MS (\%) = 100 - \left(\frac{CM}{M0}\right) * 100 \tag{Eq. 1}$$

MS is the fresh mass variation percentage, CM is the current mass, in grams (g) and M0 is the mass on the first day, in grams (g).

Quantification of vitamin C

The vitamin C content was quantified by titration, with 2,6-dichloroindophenol, according to AOAC (2000). The vitamin C extraction, from the samples, was performed with oxalic acid (1%), according to Zhang, Zhang and Yang (2015). The results are expressed in mg of ascorbic acid per 100 g of dry mass.

Color analysis

The color was measured in the CIE-Lab, where L* is luminosity, a* is red to green coordinate and b* is yellow to blue coordinate. To this end, a sphere spectrophotometer (SP30, Lovibond) was used. The analyzes were carried out on the samples color homogeneity points. To calculate the luminosity index (LI) and the red index (RI), Equations 2 and Equations 3 were used, respectively.

$$LI = 1 - \left(\frac{L^*}{L0^*}\right) \tag{Eq. 2}$$

L* is the luminosity on the reading day and L0* is the luminosity of the first day of storage.

$$RI = 1 - \left(\frac{a^*}{a0^*}\right) \tag{Eq. 3}$$

Where a* is red to green coordinate, on the analysis day, and a0* is red to green coordinate on the first day of storage.

Determination of pH

To the pH reading, 1 g of the sample was ground and diluted in 29 mL of distilled water. The pH was measured with the aid of a pH meter (Tec-3MP, Tecnal), at room temperature (25 °C).

Total phenolic compounds

The total phenolic compounds determination was carried out according to the Folin-Ciocalteu method (Samec et al., 2016). For quantification, 5 g of pulp samples (crushed), stirred in ethylic solution (96% v/v), were used. Firstly, 0.1 mL of the pulp solution was transferred to a test tube, with 0.2 mL of Folin-Ciocalteu reagent and 2 mol of distilled water. After 3 minutes of rest, 1 mL of calcium carbonate was added to the solution, to reach the blue color. The samples total polyphenols quantification was performed in a spectrophotometer (Cirrus 80 ST, Femto), at a wavelength of 765 nm, and compared to the standard curve, which was developed using a standard solution of gallic acid. The concentration results are expressed in mg of gallic acid per 100 g of fresh fruit.

Statistical analysis

The fresh mass variation analyzes, vitamin C, color, pH and total phenolic compounds were carried out in triplicate and the results were statistically evaluated using the software Statistica (version 7.0, StatSoft Inc., Tulsa, USA). The results obtained, during the 10 days of storage were evaluated at a 5% (p ≤ 0.05) level of significance, by one-way analysis of variance (ANOVA) and with Tukey’s test.

RESULTS AND DISCUSSION

Fresh mass variation

In Figure 2, it is possible to observe the fresh mass variation of strawberries, over the 10 days of storage. Continuous mass loss was observed in all treatments, however, the edible coating, with the addition of propolis (MSP), showed a more satisfactory result in mass stability, with a reduction of 31.76%, after 10 days of storage, followed by MS treatment (38.12% mass reduction) and MC, with 60.18% reduction, compared to the first day of storage. The MSP treatment retained 29.05% more mass than the control, presenting a statistically significant difference (p ≤ 0.05). The samples treated with MS, had significant differences (p ≤ 0.05), compared to MC, retaining 22.69% more mass than the control. In addition, the three treatments obtained excellent linear approximation (Table 1), as already reported by Hoffmann et al. (2021a) and Hoffmann et al. (2021b), when evaluating the cold storage of strawberries and other fresh food produce. For this reason, the high MC angular coefficient value, explains the fast mass loss in the control samples (with no coating).

Table 1 Trendline equation and R² values of treatments (MC, MS e MSP)

| | Trendline | | | R ² | | |
|----------------|------------------|------------------|------------------|----------------|-------|-------|
| | MSP | MS | MC | MSP | MS | MC |
| Mass variation | y = 3.57x - 3.91 | y = 4.24x - 3.90 | y = 6.71x - 4.77 | 0.999 | 0.999 | 0.993 |

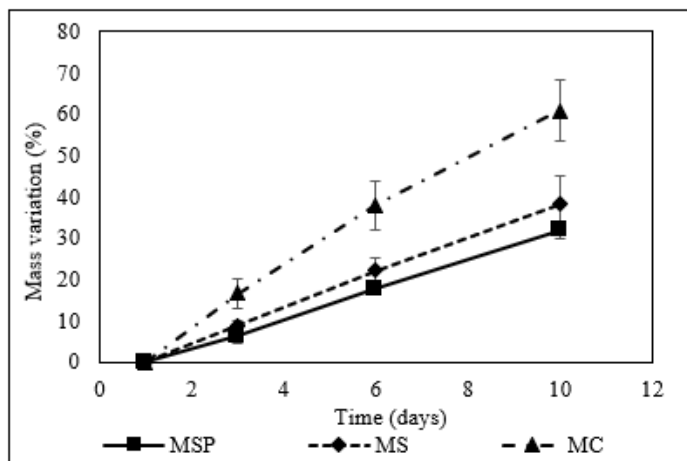


Figure 2 Strawberry mass variation treated with starch and propolis (MSP), starch (MS) and control (MC)

The coating on the strawberry, acted as a barrier for mass transfer, preventing the food from transferring moisture to the environment. Similar behavior was observed by Garcia et al. (2012), in tests with cassava starch coating (3%) and potassium sorbate (0.05%), on minimally processed strawberries, which showed a smaller reduction in mass loss, (approximately 2.4%), at the end of storage. This result is in response to the coating presence, where the water permeation from the fruit to the environment was reduced. Colla et al. (2006) found a maximum mass loss reduction, of approximately 23% in strawberries coated with a biodegradable edible coating, after 18 days of storage. Different from MC, the MSP and MS presented reduced mass loss due to coating presence. However, the MSP and MS treatments did not show a statistical difference ($p > 0.05$), concerning the propolis addition.

Quantification of vitamin C

Table 2 shows the vitamin C behavior, in strawberry samples, under preservation conditions (MS, MC e MSP), in this study. Statistically significant differences ($p \leq 0.05$), in the vitamin C reduction, were observed in MSP and MS treatments, during the 10 days of storage. The MSP treatment showed higher vitamin C retention, over time than the other treatments, which can be explained by the fact that the coating differs in gas permeability, resulting in a greater vitamin C retention (Mditshwa et al., 2017).

Table 2 Vitamin C strawberry content under different preservation conditions (MC, MS e MSP), during 10 days of storage

| | Vitamin C (mg/100 g de fresh mass) | | | |
|-----|------------------------------------|-----------------------------|------------------------------|-----------------------------|
| | Day 1 | Day 3 | Day 6 | Day 10 |
| MC | 1430 ± 156.96 ^a | 1287.2 ± 70.43 ^a | 1227.8 ± 223.56 ^a | 670.5 ± 72.55 ^{ab} |
| MS | 1419.8 ± 83.79 ^a | 1077.9 ± 221.7 ^a | 1354 ± 179.02 ^a | 587.6 ± 88.82 ^b |
| MSP | 1405.4 ± 54.45 ^a | 1335.4 ± 93.75 ^a | 1335.4 ± 120.08 ^a | 902.1 ± 149.93 ^a |

Different lowercase letters denote a significant difference between the rate values in a column ($p \leq 0.05$).

Hong et al. (2012) observed a slower behavior in decreasing vitamin C content, in coated samples with 1.0 and 1.2% chitosan, showing approximately 13% and 10.7% reduction, at the end of storage, respectively. Vitamin C is one of the most important components in food and directly affects the nutritional quality factor of strawberries (Koyuncu & Dilmacı, 2010), as it is a fundamental antioxidant in citrus fruit. However, the content can be influenced by several factors, such as storage, post-harvest treatment and water loss, which accelerates the reduction of this vitamin C content (Mditshwa et al., 2017).

Color analysis

The luminosity indices and red indices, of the analyzed samples, did not show significant differences ($p > 0.05$) between them, during the 10 days of storage. The control (MC) showed less luminosity and red index reduction (Table 3), demonstrating that the treatment with propolis was the one that best preserved the color, related to the first day of storage.

Table 3 Luminosity index (LI) and red index (RI) in strawberries, subjected to treatments (MC, MS e MSP).

| | LI | | | | RI | | | |
|-----|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| | Day 1 | Day 3 | Day 6 | Day 10 | Day 1 | Day 3 | Day 6 | Day 10 |
| MC | 0.00 ± 0.00 ^a | 0.05 ± 0.02 ^a | 0.07 ± 0.04 ^a | 0.08 ± 0.08 ^a | 0.00 ± 0.00 ^a | 0.03 ± 0.03 ^a | 0.16 ± 0.20 ^a | 0.11 ± 0.15 ^a |
| MS | 0.00 ± 0.00 ^a | 0.03 ± 0.02 ^a | 0.10 ± 0.04 ^a | 0.10 ± 0.04 ^a | 0.00 ± 0.00 ^a | 0.09 ± 0.05 ^a | 0.16 ± 0.01 ^a | 0.25 ± 0.08 ^a |
| MSP | 0.00 ± 0.00 ^a | 0.05 ± 0.02 ^a | 0.07 ± 0.01 ^a | 0.09 ± 0.01 ^a | 0.00 ± 0.00 ^a | 0.08 ± 0.03 ^a | 0.14 ± 0.03 ^a | 0.21 ± 0.07 ^a |

Different lowercase letters denote a significant difference between the rate values in a column ($p \leq 0.05$).

As presented by Garcia et al. (2012), L^* , used to calculate the luminosity index, which represents the fruit darkening coordinate, showed that coated and uncoated strawberries obtained similar results, which indicate that cassava starch-based coating did not exert influence on the fruit brightness. In the study conducted by Valenzuela et al. (2015), the strawberry color was not differentiated by the coating presence, corroborating with studies that demonstrate that the application of plasticizer solutions may cause changes in the coated strawberries opacity (Vargas et al., 2006). Therefore, the results of this study are consistent with those found in the literature, indicating that these coatings do not influence strawberry color maintenance.

Determination of pH

According to Table 4, the pH of the sample, in the different treatments, shows a continuous tendency to increase, with statistically significant differences ($p \leq 0.05$) between the samples over time, due to fungi growth. Strawberries coated with propolis (MSP) showed greater pH stability, reducing significant changes over time.

Table 4 Strawberry pH variation in different treatments (MC, MS and MSP) during 10 days of storage

| | pH | | | |
|-----|---------------------------|---------------------------|---------------------------|---------------------------|
| | Day 1 | Day 3 | Day 6 | Day 10 |
| MC | 3.55 ± 0.021 ^a | 3.59 ± 0.015 ^a | 3.72 ± 0.015 ^b | 3.76 ± 0.025 ^b |
| MS | 3.52 ± 0.026 ^a | 3.55 ± 0.035 ^a | 3.72 ± 0.036 ^b | 3.77 ± 0.006 ^b |
| MSP | 3.54 ± 0.012 ^a | 3.58 ± 0.010 ^a | 3.64 ± 0.010 ^a | 3.68 ± 0.015 ^a |

Different lowercase letters denote a significant difference between the rate values in a column ($p \leq 0.05$).

The pH values are associated with changes in the organic acid content of fresh fruit, during storage (Jiang et al., 2020). Alharaty and Ramaswamy (2020) analyzed the influence of sodium alginate-calcium chloride edible coating, applied on strawberries, and observed a significant increase in the pH of the control samples, while the coated samples did not show important changes during storage.

Total phenolic compounds

The phenolic compounds content in MC (control) samples showed a statistically significant difference ($p \leq 0.05$) on the third day, concerning the other samples, stored at 4 °C. However, all samples showed an increase in the phenolic compounds content (Table 5), with interesting results in the MSP sample (with propolis).

Table 5 Phenolic compounds content in strawberries during storage at constant temperature (4 °C).

| | Phenolic compounds (mg gallic acid/100 g fresh fruit) | | | |
|-----|---|----------------------------|-----------------------------|----------------------------|
| | Day 1 | Day 3 | Day 6 | Day 10 |
| MC | 160.26 ± 4.55 ^a | 185.15 ± 2.90 ^b | 175.01 ± 10.99 ^a | 177.69 ± 0.97 ^a |
| MS | 155.66 ± 3.12 ^a | 166.75 ± 3.11 ^a | 162.72 ± 4.32 ^a | 178.41 ± 4.89 ^a |
| MSP | 164.25 ± 4.83 ^a | 161.15 ± 0.82 ^a | 175.66 ± 3.64 ^a | 179.25 ± 2.63 ^a |

Different lowercase letters denote a significant difference between the rate values in a column ($p < 0.05$).

Strawberries have phenolic compounds in the form of anthocyanins, which in turn are responsible for their red color (Aaby, Ekeberg & Skrede, 2007). Furthermore, phenolic compounds are classified as secondary fruit metabolites (Haminiuk et al., 2012). According to Apriyanti et al. (2018), strawberries coated with green tea extract and chitosan coating obtained an increase in the content of phenolic compounds. Similarly, Petriccione et al. (2015) verified that strawberries samples coated with chitosan and refrigerated storage, showed constant results of phenolic compounds, over the storage.

CONCLUSION

Strawberry samples (MSP and MS) showed results that prove efficiency in reducing mass loss compared to uncoated strawberries. Strawberries coated with starch formulation and added propolis (MSP) showed better results in vitamin C content and stability of pH values, during storage. The coatings did not influence the color values and total phenolic compounds. In general, it was verified that the coating with the propolis addition (3%) is a promising and sustainable alternative for extending the shelf life of strawberries.

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