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Quality of minimally processed 'Monalisa' apples with antioxidants and edible toppings

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Abstract
Minimally processed fruits are an alternative to add value to products that are difficult to sell, in addition to facilitateconsumer consumption. The objective is to research pulp browning inhibition in a simulated commercialization of minimally processed 'Monalisa' apples, stored in a refrigerated environment. After being sanitized with sodium hypochlorite, the apples were cut and immersed in the following treatments: the control being sodium erythorbate (ES) + A (distilled water); ES + FM (cassava starch 3%); ES + AS (3% sodium alginate); ES + AM (6% waxy maize starch) and ES + AP (3% pine nut starch), all together with 1% calcium chloride. Afterwards, 10 slices were placed in a polyethylene tray, wrapped in stretchable PVC film and stored in a refrigerated chamber at 4 °C ± 1 °C and relative humidity of 90 - 95%. Phytochemical evaluations were performed at 0, 3, 6 and 9 days of storage. The applied treatments maintained fruit quality. The treatment with cassava starch and pine nut starch were efficient in preserving the darkening of the fruit pulp on day 9. As for the waxy corn starch treatment, the enzyme activity was lower in the period of 0 and 6 days, maintaining quality and delaying darkening. The control sample (ES + A + CC) stood out during the 9-day period, as there was a decrease in the activity of polyphenoloxidase and peroxidase enzymes.

Keywords: antioxidant agent, edible toppings, enzymatic browning, malus domestica

Introduction

In Brazil, minimally processed (MP) products have gained increasing popularity in the market due to changes in people's daily lives, with a demand for quick food preparation and a desire for fruits with the same characteristics as fresh ones. This type of product is presented to consumers in a ready-to-eat form. Consequently, growing demand for MP fruits requires improvement of knowledge in this area and production of high-quality and appealing products (Mendonça et al., 2019).

Furthermore, fruits that do not meet the desired standards for fresh sale, such as size, color, appearance, and nutritional value, particularly apples (*Malus domestica* Borkh), face difficulties in commercialization. To reduce waste and minimize these problems, an alternative is to sell MP apples, which adds high value to the product, provides convenience in consumption, and utilizes leftover products.

The production of MP products involves several stages that can lead to rapid deterioration (Teixeira et al., 2022) and enzymatic browning. Currently, there are strategies to inhibit enzymatic activity of Polyphenoloxidase (PPO) and Peroxidase (PO) without using chemical agents, but they are not sufficiently efficient (Kumar et al., 2012). Therefore, to treat MP products by immersing them in agents (antioxidants and edible coatings) after the production stages is necessary. Some antioxidants, such as erythorbic acid and calcium chloride, can be used to inhibit enzymatic browning and also retard the growth of spoilage and pathogenic microorganisms in MP apples (Barreto et al., 2016; Ribeiro et al., 2019). Edible coatings, in conjunction with antioxidants, aim to increase MP applesshelf life. Examples of edible coatings include starch (Chen et al., 2019; Galindez et al., 2019; Pajak et al., 2019), sodium alginate (Salama et al., 2018;

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Ruan et al., 2019), and cassava starch (Rodríguez et al., 2020; Costa et al., 2022).

Therefore, the objective of this study was to evaluate pulp browning inhibition caused by enzymatic activity of polyphenoloxidase and peroxidase, during simulated commercialization of minimally processed ‘Monalisa’ apples, stored in a refrigerated environment.

Material and Methods

This study was carried out at the Food Center/ Postharvest Physiology Laboratory of Embrapa Clima Temperado, located at BR392 km78, in Pelotas - RS, Brazil. The experiment was conducted using 'Monalisa' apples from the commercial orchard of “Rasip Agropastoril S/A”, located at Rodovia BR116, Km33, N/A – Countryside, Vacaria–RS, Brazil (50°56'02" S, 28°30'14" W, and 900m above sea level).

Apples were selected to ensure uniform ripeness and absence of mechanical damage or visible rot. After selection and application of 1-Methylcyclopropene (1-MCP), fruits were packed in 18 kg boxes. Subsequently, they were transported in a refrigerated truck, protected from direct sunlight, for approximately 6 hours to “Embrapa Clima Temperado” facility, where apples were stored in a cold room at 4°C ± 4°C and 90% ± 5% relative humidity (RH) for approximately 48 hours before preparation and analysis.

The experiment represented post-processing during the storage period, simulating commercial shelf life (0, 3, 6, and 9 days), with chemical additives (treatments) [distilled water + sodium erythorbate (SE) as control; sodium erythorbate (SE) + cassava starch (CS), sodium erythorbate (SE) + sodium alginate (SA), sodium erythorbate (SE) + modified waxy corn starch (MS), and sodium erythorbate (SE) + pine nut starch (PS), all together with calcium chloride (CC)].

Firstly, apples were sanitized by immersing them in a solution of 200 ppm sodium hypochlorite, at pH 6.5 (pH was adjusted with 1 N HCl) for 10 minutes at room temperature. Then, they were removed and longitudinally sliced (in wedge-shaped slices), and the central part containing the seeds was removed, leaving only the pulp.

Subsequently, apple slices were immersed again for one minute in each of the following treatments: SE + W: (Control) 5% sodium erythorbate + distilled water + 1% calcium chloride; SE + CS: 5% sodium erythorbate + 3% cassava starch + 1% calcium chloride; SE + SA: 5% sodium erythorbate + 3% sodium alginate + 1% calcium chloride; SE + MS: 5% sodium erythorbate + 6% modified starch + 1% calcium chloride; and SE + PS: 5% sodium erythorbate + 3% pine nut starch + 1% calcium chloride.

Then, fruits were removed and placed in strainers for five minutes to remove excess solution. Afterward, ten fruit slices were placed in polyethylene trays (15 x 15 cm), covered with stretch PVC film (9 μm), and the slices were stored at a temperature of approximately 4°C ± 4°C and a relative humidity of 90-95% for four periods (0, 3, 6, and 9 days), simulating the product's commercial shelf life.

Temperature and chambers RH were monitored using a computerized system. Three replicates were performed for each treatment, resulting in a total of 60 trays with 600 pieces of 'Monalisa' apples over the four periods. Only one intact fruits chamber exit was chosen, as storage time in the cold room alters apples physiological characteristics, as reported by Moreno et al. (2016). The authors removed intact apple fruits from the cold room at 30, 60, 90, and 120 days for further minimally processed preparation.

The determination of Polyphenoloxidase (PPO) and Peroxidase (PO) enzymatic activity in apple fruits was performed using a Molecular Devices-spectramax 190 spectrophotometer, according to the protocol adapted by Campos & Silveira (2003). The extract for these analyses was prepared by diluting 6 grams of fresh sample in 20 mL of pH 7.0 phosphate buffer containing 1% polyvinylpyrrolidone, and, then, it was triturated using an Ultra Turrax homogenizer. The extract was centrifuged, and the supernatant was collected for enzymatic analysis.

PPO enzyme was quantified as follows;in 15 mL Falcon tubes, 1 mL of apple fruit enzymatic extract and 3.6 mL of pH 6.5 phosphate buffer + 1 mL of 0.1 M pyrocatechol were added. The samples were incubated at 30°C for 30 minutes and immediately cooled in an ice bath for 10 minutes. Then, it was measured at 395 nm of absorbance using a spectrophotometer, and the results were expressed as grams of fresh pulp per minute (UAE.g^{-1}.min^{-1}).

PO enzyme was quantified as follows;in 15 mL Falcon tubes, a 3 mL aliquot of enzymatic extract was taken, and a solution containing 5 mL of 0.02 M citrate-phosphate buffer (pH 5), 0.5 mL of 30% hydrogen peroxide and 0.5 mL of guaiacol were added. Then, the solution was incubated at 30°C for 5 minutes and immediately cooled in an ice bath for 10 minutes. Absorbance was measured at a wavelength of 470 nm using a spectrophotometer, and the results were expressed as grams of fresh pulp per minute (UAE.g^{-1}.min^{-1}).

The experimental design was completely randomized, with a factorial scheme, composed of five treatments with antioxidant agents, edible coatings and four storage periods, resulting in a 5 x 4 scheme. The experimental unit consisted of a tray with ten apple slices, with three replicates, and the data for phytochemical variables were subjected to analysis of variance using R Studio 7.0 software. When a significant effect was observed (p≤0.05), a Tukey's test was conducted for antioxidant comparison at a 5% probability of error, and polynomial regression analysis was performed for evaluation periods.

Results and Discussion

A significant interaction among the factors of storage time and antioxidant associated with edible coatings was observed for enzymatic activity of polyphenol oxidase (PPO).

Regarding storage time factor, a significant difference was observed for PPO activity in all applied treatments (**Table 1**). There was a linear increase in enzymatic activity as storage time increased for the control treatment (SE+ W) as well as SE + SA and SE+ MS.

This enzymatic increase of PO and PPO during storage period was also observed by Cantillano et al. (2017) in minimally processed 'Rayal Gala' apples, as these enzymes are responsible for apple pulp enzymatic browning.

For SE + CS and SE + PS treatments, quadratic equations were observed, showing values of x (storage time) and y (PPO enzymatic activity in UAE.g^{-1}.min^{-1}). The maximum values for x were 3.83 for SE + CS and 5.33 for SE + PS, while the maximum values for y were 1955.75 for SE + CS and 2036.45 for SE + PS. Both treatments, SE + CS and SE + PS, showed an increasing trend up to six days of storage, followed by a decrease at nine days of storage. This is an important finding, as the reduction in PO enzymatic activity reduces oxidation of phenolic compounds and consequently minimizes browning in the minimally processed fruits.

For the treatment factor with antioxidants associated with edible coatings, a significant difference was observed among the applied treatments for PPO enzymatic activity within each storage time of 0, 6, and 9 days (Table 1). On day zero, SE + MS and SE + PS treatments showed high PPO enzymatic activity, indicating a higher pulp browning compared to the control treatment (SE + W) with the lowest value. In relation to the sixth day, all treatments exhibited high PPO enzymatic activity except for the control treatment (SE + W), demonstrating an effectiveness of 5% sodium erythorbate antioxidant. There was no significant difference observed with the use of concurrently applied edible coatings. Ribeiro et al. (2019), who evaluated minimally processed 'Gala' apples treated with 5% sodium erythorbate and 3% erythorbic acid, found that these compounds are anti-browning agents for 'Royal Gala' apples, similar to the findings in this research.

For the ninth day, SE + SA, SE + MS, and SE + W treatments showed higher enzymatic activity, with a linear growth trend model. However, SE + MS and SE + PS treatments exhibited a reduction in PPO, indicating that both cassava and pine nut starch reduced PPO enzymatic activity, thereby reducing enzymatic browning compared to the other applied coatings. Starches have the characteristic of forming thermoreversibleof water in a gel, providing a favorable and useful composition for food industry (Freitas & Zambrano, 2022). This happens especially with pine nut starch, which is stable, white, odorless, and has a gelatinization at low temperatures. Cassava starch is widely used by food industries in developing biodegradable edible films and coatings, due to its barrier properties in gas transmission and flexibility, promoting a reduction in food deterioration rates (Travalini et al., 2019). Assis & Brito (2014) found that

Table 1 - Quantification of polyphenol oxidase enzymatic activity (UAE.g^{-1}.min^{-1}) in minimally processed 'Monalisa' apples, treated with antioxidants and edible coatings, and stored in a refrigerated chamber (4 °C and 90-95% relative humidity). Embrapa Clima Temperado, Pelotas, RS

	Storage days				P	R^2
	0	3	6	9	(≤0.05)*	Value
SE+ W	806.11 b	1262.77 b	1435.55 b	2293.33 ab	0.001	0.92
SE+ CS	1277.22 ab	1470.55 ab	2136.66 a	300.33 c	0.001	0.744
SE+ SA	1215.0 ab	1559.44 ab	2093.33 a	2675.61 a	0.001	0.98
SE+ MS	1507.22 a	1611.66 a	2072.22 a	2548.33 a	0.001	0.94
SE+PS	1516.11 a	1786.66 a	2168.88 a	1721.11 b	0.01	0.80
	Regressionequation			x ma	y ma	
SE + W	y= 154.48x + 754.28			-	-	
SE + CS	y= -56.38x² +431.93x + 1128.5			3.83	1955.75	
SE+ SA	y= 163.86x + 1148.5			-	-	
SE+ MS	y= 119.46x + 1397.3			-	-	
SE +PS	y= -19.954x² + 212.82x + 1469			5.33	2036.45	

Note: Means in the same row, p<= 0.05* (significant), p<= 0.01 (highly significant), p<= 0.001 (extremely significant). Values, followed by different letters in the column, indicate significant differences among treatments (T) on the storage day. Values of x and y followed by the letter "ma" (maximum) refer to maximum values of x or y.

raw materials used in the formulation of edible coatings aim to extend the fruits' storage time, which corroborates with some results in this study, thus providing a longer product shelf life.

A significant interaction was observed among storage time and antioxidants associated with edible coatings for PO enzyme activity in minimally processed 'Monalisa' apples (**Table 2**).

Considering the storage time factor, there was a significant difference for SE + W, SE + SA, SE + MS, and SE + PS treatments, except for cassava starch treatment (SE + MS) (Table 2). There was a linear increase in PO activity for SE + SA as storage time increased. For the other treatments, quadratic equations were analyzed, with x (storage time) and y (peroxidase enzymatic activity in UAE.g-1.min-1) values. Thus, maximum x values were obtained for SE + W (5.59), SE + MS (5.61), and SE + PS (5.31), and maximum y values were obtained for SE + W (157.68), SE + MS (170.83), and SE + PS (303.94).

Regarding treatments with antioxidants associated with edible coatings, a significant difference was observed amongapplied treatments for PO variable within each storage time (Table 2). The combination of antioxidants with coatings of minimally processed products demonstrated greater efficiency in controlling oxidation and in reducing enzymatic browning (Barreto et al., 2020). At day zero, only SE + CS treatment showed the highest value compared to other treatments. At daythree, SE + PS and SE + MS treatments exhibited high PO values, while the lowest value was found for the control treatment (SE + W). On the sixth day of storage, pine nut starch treatment (SE + PS) maintained the highest PO enzymatic activity, while SE + MS showed the lowest value for this period, followed by other treatments. Enzymatic activity promotes enzymatic browning, which can vary depending on the studied plant (Moreno et al., 2016; Barreto et al., 2020).And it can be estimated that the lower the activity, the more efficient the prevention of enzymatic browning is.

Throughout this experiment, treatment with pine nut starch combined with 5% sodium erythorbate and 1% calcium chloride exhibited the highest PO enzymatic activity, which promotes enzymatic browning. At day nine, SE + CS, SE + SA, and SE + PS treatments showed higher values, negatively influencing changes in flavor, browning, and quality.The control treatment, at the same storage time, showed the lowest value for PO enzyme activity, not differing statistically from SE + MS, indicating lower enzymatic activity. The use of sodium erythorbate reduces the activity of PO and PPO enzymes, as observed by Barreto et al. (2020), and thus the treatment that combined this antioxidant with cassava starch was more efficient.

At six and nine days of storage, corn starch coating exhibited low PO values, not differing from the control when combined with 5% sodium erythorbate and 1% calcium chloride. The effect of using edible coatings to inhibit PO enzyme activity becomes ineffective over storage days when comparing its low efficiency to the control, supporting findings from Ribeiro et al. (2019). They evaluated minimally processed apples at different storage times and observed an increase in peroxidase activity over storage days.

Recent advances in controlling enzymatic browning in post-harvest, as suggested by Tinello & Lante (2018), can be achieved through the incorporation of agents in edible coating formulations or the immersion in solutions containing synthetic additives. However, edible coatings are essential to promote the maintenance of quality and safety of these products. In a study conducted

Table 2 - Quantification of peroxidase enzymatic activity (UAE.g^{-1}.min^{-1}) in minimally processed 'Monalisa' apples, treated with antioxidants and edible coatings, and stored in a refrigerated chamber (4 °C and 90-95% relative humidity). Embrapa Clima Temperado, Pelotas, RS

	Storage days				P	R^2
	0	3	6	9	(≤0,05)*	Value
SE + W	103.51b	103.66c	196.85b	119.44b	0.001	0.42
SE + CS	259.81a	203.51b	218.51ab	229.62a	ns	-
SE+ SA	131.66b	208.70b	182.22b	203.33a	0.01	0.4802
SE+ MS	97.77b	228.33ab	102.22c	175.18ab	0.001	0.11
SE +PS	163.51b	293.88a	286.11a	244.25a	0.001	0.9494
	Regression equation			x ma	y ma	
SE + W	$y= -2.1543x^2 + 24.088x + 90.337$			5.59	157.68	
SE + CS	-			-	-	
SE+ SA	$y= 6.284x + 153.2$			-	-	
SE+ MS	$y= -1.5998x^2 + 17.935x + 120.56$			5.61	170.83	
SE +PS	$y= -4.784x^2 + 50.87x + 168.72$			5.31	303.94	

Note: Means in the row, ns = not significant, p<= 0.05* (significant), p<= 0.01 (highly significant), p<= 0.001 (extremely significant). Values followed by different letters in the column indicate significant differences among treatments (T) on the storage day. Values of x and y followed by the subscript "ma" (maximum) refer to the maximum values of x or y.

by Santos et al. (2018), when analyzing the effect of enzymatic browning in minimally processed 'Gala' apples using edible coatings based on sodium alginate, a reduction in enzymatic browning was observed in coated samples.

Conclusions

The applied treatments maintained the quality of minimally processed apple fruits. The best treatment for reducing the activity of both enzymes (polyphenol oxidase and peroxidase) was distilled water combined with 5% sodium erythorbate and 1% calcium chloride over a 9-day period. The 3% cassava starch and 3% pine nut starch treatments were efficient in preserving polyphenol oxidase enzyme activity on day 9. As for peroxidase enzyme activity, the treatment using waxy corn starch associated with SE and CC was effective in delaying enzymatic activity on days 0 and 6, thus maintaining quality and delaying browning.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.