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ORIGINAL PAPER

Mucilage of spineless cactus in the composition of an edible coating for minimally processed yam (*Dioscorea* spp.)

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Abstract

The objective of this study was to examine formulations of edible coatings based on spineless-cactus mucilage for the preservation of minimally processed yam. Cladodes of spineless cactus clone IPA-Sertânia were harvested, washed, sanitized, and subjected to mucilage extraction to be used as an edible coating. Yam roots were minimally processed and immersed into the following coating suspensions: cactus mucilage + cassava starch (3%) + glycerol (1%) or cactus mucilage solely. Control corresponded to immersion in water. Samples were dried, packed and stored at 5 ± 2 °C for 10 days. The biocoating containing cactus mucilage reduced dehydration and maintained the visual and sensory quality of the yam slices. Additionally, this hydrocolloid increased the amount of phenolic compounds and led to different responses between polyphenol oxidase and peroxidase. Therefore, the studied formulations containing cactus mucilage show to be promising for the composition of biofilms and application to minimally processed yam roots.

Keywords *Nopalea cochenillifera* L. Salm-Dyck · *Dioscorea* spp. · Edible coating · Polyphenol oxidase

Introduction

Minimal processing is a technology that consists of a number of physical alterations done to plants to maintain the characteristics of a fresh product, besides offering practicality to the consumer [1]. However, the operations that make up minimal processing lead to abiotic stresses, where responses vary according to the plant tissue. In sweet cassava, the action of cutting results in the formation of brown streaks due to the instability of total soluble phenols and the increased activity of enzymes such as polyphenol oxidase and peroxidase, which are found in the injured area [2]. Browning can also occur in carrot and yam as a result of surface drying caused by the reflection of light on the superficial cellular debris [3, 4] as well as deposition of starch grains [5].

An alternative to minimize browning and white blush in some minimally processed roots is the use of edible coatings, as seen in cassava [6] and carrot [3]. At present, hydrogel-based polymers are mostly used for the composition of biodegradable coatings as a sustainable alternative to conventional polymers [7]. Edible coatings of the most varied sorts can be found; e.g. mango kernel starch on tomato [8], oregano-containing basil seed gum on minimally processed apricot [9], essential cinnamon and lime oil on guava [10], flaxseed gum combined with lemongrass essential oil on pomegranate arils [11], and spineless-cactus mucilage on kiwi slices and whole strawberries [12, 13].

The mucilage from spineless cactus of the genus *Opuntia ficus-indica* is composed of heteropolysaccharide hydrocolloids with a wide range of physicochemical properties [14]. It is a viscous, colorless substance with shearing-induced wear in dispersion [15] as well as film-forming, impermeability, and plasticity properties, especially when some polyol-based plasticizers are added [16]. Additionally, it is a low-cost, safe, biodegradable source.

The efficiency of using cactus mucilage for biofilm formation, with or without addition of plasticizers [16], has been studied in whole fruits like kiwi and strawberry [12, 13] in an attempt to slow their ripening process. However, in

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roots that turn brown after being cut, such as yam, mucilage-containing coating may be a viable alternative to maintain their quality during preservation. Yam is a starch rich in dietary carbohydrates, fibers, fats, proteins, and minerals, which render it a recommended ingredient in healthy diets [17]. Furthermore, it is considered one of the cheapest energy sources able to nourish populations of developing countries [18].

It is evident that forage cactus mucilage is being applied to the fruit coating composition. In minimally processed roots, the present work will be one of the first to be found in the literature. Besides that, it is expected to develop a natural and sustainable biocoating from the forage cactus, which can be easily accessible, and also affordable to be widely used in yam minimally processed.

On these bases, the present study was undertaken to examine formulations of biocoatings based on spineless cactus mucilage, with and without the use of a plasticizer, for the preservation of minimally processed yam.

Materials and methods

Raw materials

Yam (*Dioscorea* spp.) roots were acquired from in the county of Custódia - PE, Brazil. These were selected and sanitized with active chlorine (100 mg L^{-1}, SUMAVEG®). Four raw materials were used as film-forming agents: distilled water, spineless-cactus mucilage, cassava starch, and glycerol (molecular weight: 92.09; Color (APHA): max. 15; dose: min. 99.5%; heavy metals (Pb): max. 0.0002%; neutrality: passed test; residue on ignition: max. 0.01%; sulfate (SO_4): max. 0.001%; appearance: clear liquid). The starch suspension (3%) was heated to approximately 70 °C for coating formulation.

Preparation of the edible coating suspension

Cladodes of spineless cactus (240–300 mm length and weight 250–300 g) clone IPA-Sertânia (*Nopalea cochenillifera* (L.) Salm-Dyck) were acquired from the experimental area of the Federal Rural University of Pernambuco/Academic Unit of Serra Talhada (UFRPE/UAST), Brazil, transported to the Laboratory of the Postgraduate Program in Plant Production of UFRPE/UAST, selected, weighed, washed in running water, and immersed in a sanitizing solution containing 200 mg L^{-1} active chlorine for 10 min. Next, the glochids were removed and the cladodes were diced into cubes measuring approximately 2 cm^3 and immersed in a solution containing 5 mg L^{-1} citric acid for 10 min to obtain the mucilage (Fig. 1).

Minimal processing

After being washed in running water, the yam roots were peeled, chopped into round slices approximately 1 cm thick, disinfected in 200 and 5 mg L^{-1} active chlorine solutions, both for 10 min, and then drained for 10 min and immersed into the following suspensions:

- Control: immersion in water for 1 min and drying for 10 min in forced air circulation.
- Spineless-cactus mucilage + 3% starch + 1% glycerol: immersion in the solution for 1 min and drying for 10 min in forced-air circulation.
- Spineless-cactus mucilage: immersion in the solution for 1 min and drying for 10 min in forced-air circulation.

Packaging

The minimally processed yams were packed into 15-µm multilayer Nylon packages. Each package contained two yam slices, totaling approximately 150 g per package. The products were maintained at 5 ± 2 °C for 10 days.

Fresh mass loss (FML)

Fresh mass loss was obtained as the difference between the initial mass (IM, measured right after processing) and the final mass (FM, measured hourly for unpackaged slices and daily for packaged slices) in accordance with the following relation: $[(IM - FM)/IM] \times 100\%$. Samples were weighed using an analytical balance (ARD110, Ohaus, Parsippany, NJ, USA) at each evaluation time [19].

Visual assessment

The visual assessment was carried out using a five-point Likert scale according to the intensity of the brown and/or whitish areas of the yam slices, according to Coelho-Júnior [19]. Score 5—slice with a typical white surface, with no signs of browning, excellent appearance and aroma for consumption and sale; Score 4—slice with white surface and appearance of some whitish streaks, but with excellent appearance and aroma for consumption and sale; Score 3—slice with white surface, increased whitish area and mild browning, threshold of acceptance for consumption and sale; Score 2—slice with surface area covered with whitish streaks, mild browning, but improper appearance for consumption or sale; and Score 1—slice with intensely

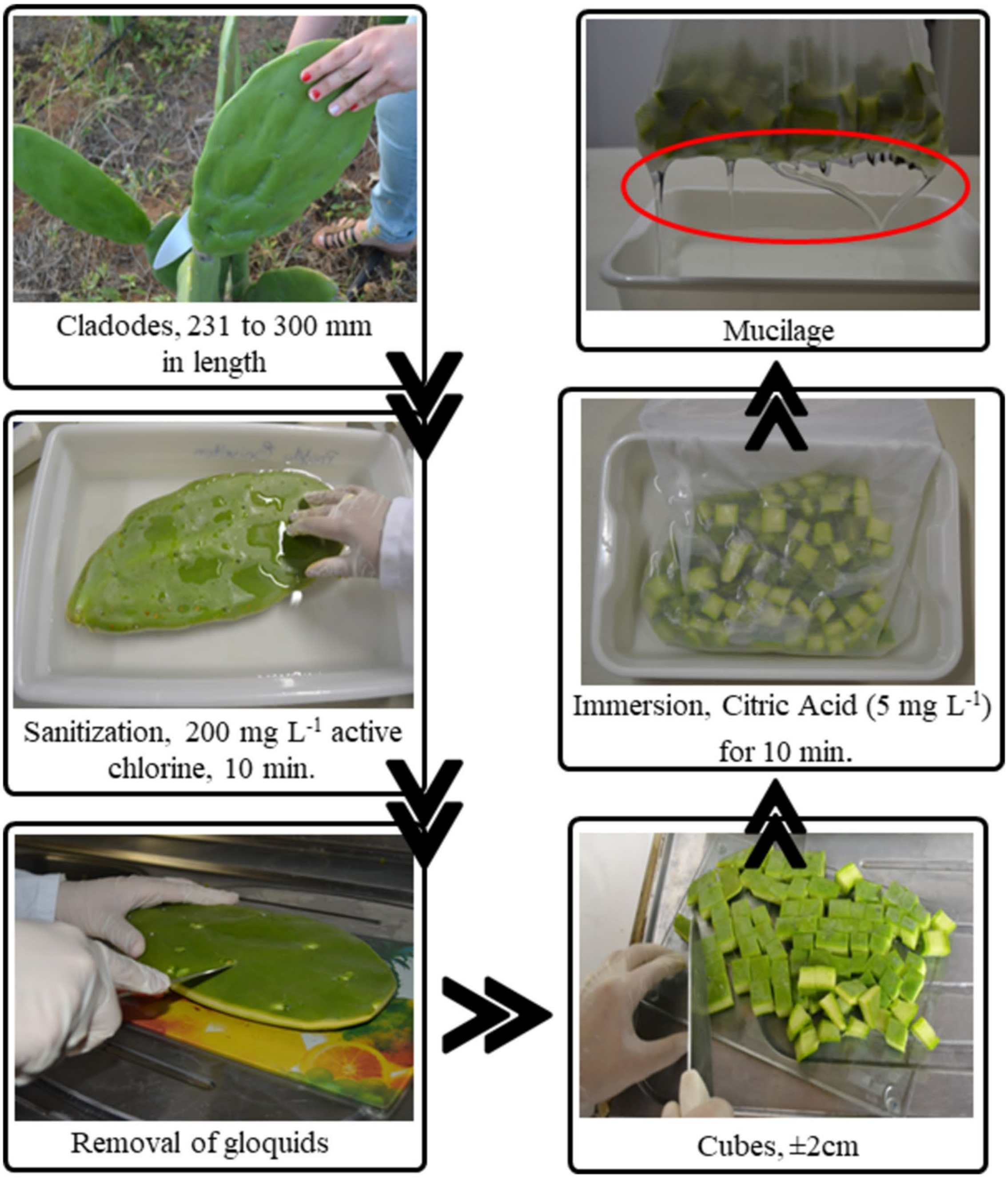

Fig. 1 Harvest and extraction of mucilage of forage cactus clone IPA-Sertânia, *Nopalea cochenillifera* (L.) Salm-Dyck

whitish and/or brown surface, in addition to alcoholic aroma, totally unsuitable for consumption or sale.

Sensory analysis

Sensory analysis was performed by 50 untrained evaluators. Samples of all treatments were placed separately in boiling water. The ideal cooking point was determined using a fork, which indicated the yam was cooked when easily penetrated into the slice (about 20 min). Soon afterwards, the samples were identified by three-digit numerical codes at random and served to the tasters, who also received an evaluation sheet where they assessed the sensory attributes of each biocoating treatment on a hedonic scale. The sensory test protocols were previously approved by the Ethics Committee on Research with Humans of the Federal Rural University of Pernambuco (Approval No. 1.969.344).

The attributes analyzed were: appearance, aroma, flavor and texture, using a numerical 9-point hedonic scale: 9—liked extremely; 8—liked very much; 7—liked moderately; 6—sightly; 5—liked nor disliked; 4—disliked sightly; 3—disliked moderately; 2—disliked very much; 1—disliked extremely, according to Feng and O'Mahony [20].

Total soluble phenols (TSP)

Determined using Folin-Ciocalteu reagents, following the method described by Singleton and Rossi [21]. 0.3 g of the tissue were macerated in a mortar containing 1.5 mL of pure methanol. The samples were then allowed to stand for 20 h in the dark at 4 °C. After this time, they will be centrifuged at 10,000 × g at 2 °C for 21 min. The assay was performed using 150 µL of the extract 2400 µL of distilled water and 150 µL of Folin-Ciocalteu (0.25 M). The mixture was homogenized for 3 min, and then 300 µL of sodium carbonate (1 M), kept in the dark at room temperature for 2 h. The control was made with 150 µL of methanol replacing the supernatant. The readings were carried out in a spectrophotometer (libra S8, Biochrom, Cambridge, England) at 725 nm and the results expressed in mg equivalent to Gallic acid kg^{-1} FM, quantified based on the standard curve of gallic acid.

Polyphenol oxidase (PPO) and peroxidase (POD) extraction and essay

Extraction was performed in accordance with the methodology described by Simões et al. [22], with adaptations. Using liquid nitrogen, 0.25 g yam were homogenized and macerated into 1.5 mL 0.1 M potassium phosphate buffer (pH 7.0) that was previously maintained at 5 ± 2 °C. The extract was centrifuged at 7960 × g for 23 min at 4 °C.

The PPO assay was determined by adding 40 µL of the supernatant to the reaction medium containing 1.5 mL 0.01 M phosphate buffer (pH 7.0) and 1.3 mL catechol (0.2 M). Readings were taken using a spectrophotometer (libra S8, Biochrom, Cambridge, England) at 425 nm, at a temperature of 25 °C, for 1 min. The PPO activity was calculated based on the molar extinction coefficient of 3.4 mM cm^{-1} for catechol and expressed as µmol g^{-1} FM min^{-1}.

The POD assay was determined by adding 150 µL of the supernatant to the reaction medium containing 1 mL 0.01 M phosphate buffer (pH 7.0), 100 µL guaiacol (5 g L^{-1}), and 100 µL hydrogen peroxide (0.8 g L^{-1}). Readings were taken using a spectrophotometer (libra S8, Biochrom, Cambridge, England) at 425 nm, at a temperature of 30 °C, for 3 min. The POD activity was calculated based on the molar extinction coefficient of 26.6 mM cm^{-1} for guaiacol and expressed in µmol g^{-1} FM min^{-1}.

Experimental design and statistical analysis

A completely randomized design (CRD) with two factorial arrangements was employed. The first was a 3 × 6 arrangement consisting of three treatments (control, water only; cactus mucilage + cassava starch (3%) + glycerol (1%); and cactus mucilage) and six evaluation days (0, 2, 4, 6, 8, and 10), with three replicates, for fresh mass loss and visual assessment. The second factorial arrangement was a 3 × 3 type, represented by three treatments (control, water only; cactus mucilage + cassava starch (3%) + glycerol (1%); and cactus mucilage) and three evaluation days (0, 4, and 10), with four replicates, for total soluble phenol content and enzymatic activity of polyphenol oxidase and peroxidase. Each experimental plot was composed of a package containing approximately 150 g of slices of minimally processed yam.

Data pertaining to fresh mass loss, visual assessment, and total soluble phenol content were analyzed by descriptive analysis, following Coelho-Júnior et al. [19], while the data referring to sensory analysis, polyphenol oxidase, and peroxidase were subjected to normality tests, analysis of variance, and Tukey's test at the 5% probability level in R software (R Development Core Team 2010).

Results and discussion

Fresh mass loss, visual and sensory quality

A gradual increase in fresh mass loss was observed throughout the cold storage period in the coated and uncoated slices. The uncoated slices showed higher fresh mass loss values until the sixth day, although there was no significant difference. The samples coated only with mucilage showed a lower fresh mass loss throughout the cold storage period (Fig. 2a).

In yam, fresh mass loss results in the perception of white blush on the tissues. According to Donégá [5], this phenomenon is also the result of the starch deposition resulting from leakage of cellular content caused by mass loss. Although the fresh mass loss values were minimal in the three formulations, immersion in mucilage alone led to the lowest percentage loss, suggesting that this formulation acted as a greater barrier to the loss of water vapor.

In this way, the mucilage applied as coating provided some protection against dehydration. By contrast, when it was mixed with starch and glycerol, less protection was obtained, which is possibly due to the hygroscopic effect of starch [23]. Besides, glycerol, despite being usually applied, can enhance the solubility and permeability to water vapor when mixed with mucilage, compared with other plasticizers such as polyethylene glycol (PEG) and sorbitol [16]. This

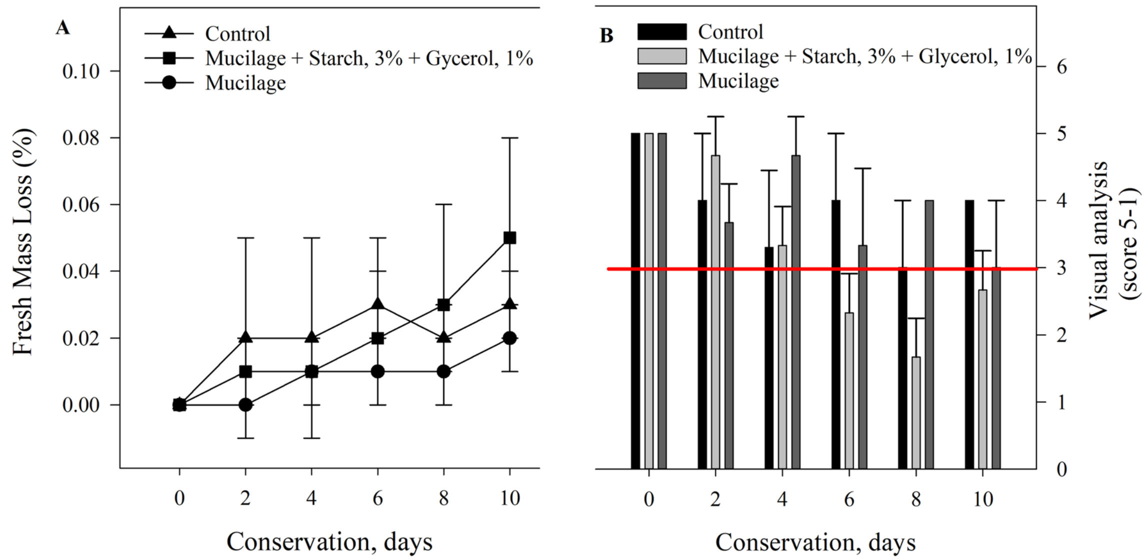

Fig. 2 **a** Cumulative fresh matter loss (FML) in minimally processed yam coated with the following formulations: control (water); cactus mucilage + cassava starch (3%) + glycerol (1%); or cactus mucilage. The slices were maintained at 5 ± 2 °C for 10 days. **b** Visual analysis of minimally processed yam coated with the following formulations: control (water); cactus mucilage + cassava starch (3%) + glycerol (1%); and cactus mucilage. The slices were maintained at 5 ± 2 °C for 10 days. Bars represent the standard deviation of the mean. The red line represents the threshold score for acceptance/consumption

may explain the greater dehydration of the pieces with the formulation containing mucilage and glycerol.

The slices termed control as well as those coated only with mucilage remained with score 3 or higher up to 10 days of preservation. This means that their appearance was acceptable for sale and/or consumption during that period, according to the Likert scale adopted (Fig. 2b).

From the 6th day of cold storage, the yam slices coated with cactus mucilage + cassava starch (3%) + glycerol (1%) showed average scores that were below the accepted limit (Fig. 2b). Thus, they were considered unsuitable for sale and/or consumption. This showed that the combination between the three components of this formulation resulted in a negative interaction, since it was not effective in maintaining the visual quality of minimally processed yam during the cold-storage period, thereby compromising the consumer's acceptance, considering that appearance is a critical factor in the purchase of a plant-based product [24].

According to the sensory evaluation performed on the slices immediately after minimal processing, there was no significant difference for the appearance, aroma, taste, and texture attributes between the coated and uncoated slices (Table 1). This means that the mucilage used as edible coating did not alter the sensory quality observed by the panel of tasters, rendering the spineless-cactus mucilage a component with potential for use as an edible coating for minimally processed yam.

Furthermore, the sensory attribute data associated with the question that involved sentences or words referring to taste (samples which they liked and disliked most) showed that, despite the lack of significant differences between the coated and uncoated slices, according to some comments written on the assessment sheet, the evaluators mostly disliked those coated with the cactus mucilage + cassava starch (3%) + glycerol (1%) coating, as they perceived a bitter taste at the end. This was indicated by comments like "Bitter, a different taste"; "A bitter taste at the end"; and "Tough texture, slightly bitter taste". In the slices coated with mucilage only, no such bitter taste was described.

Sensory analysis is an essential tool for the development of food products. It involves attributes that allow for evaluating the appearance, color, aroma, taste, and texture by using

Table 1 Sensory attributes of samples of minimally processed yam coated with the following formulations: control (water); cactus mucilage + cassava starch (3%) + glycerol (1%); or cactus mucilage, immediately after minimal processing, on day zero

Formulations	Attributes			
	Appearance	Aroma	Flavor	Texture
Control	7.32a	7.50a	6.92a	7.20a
Mucilage + starch, 3% + glycerol, 1%	7.42a	7.08a	6.60a	6.92a
Mucilage	7.42a	7.40a	6.82a	6.92a
MSD	0.75	7.36	0.82	0.82

Averages followed by equal letters in the column did not differ statistically from each other by the Tukey test at 5% probability

MSD minimal significant difference

hedonic scales that show the differences between samples for all sensory attributes evaluated [25].

In the present study, the results of the sensory attributes indicate that cactus mucilage has the potential for use as an edible biocoating, as also observed in whole strawberry [13] and in kiwi slices [12], because it does not interfere with the organoleptic characteristics of the product.

In this way, formulations based on cactus mucilage can be used as edible coatings. However, the formulation containing starch and glycerol did not show a positive interaction with the plant tissue in terms of flavor. A more in-depth investigation of this formulation is suggested, since glycerol is often desirable, as it confers plasticizing properties to the biofilm [16]. These results also suggest that other plasticizers can be used with the mucilage.

Phenolic compounds and enzymatic responses

During cold storage, the concentrations of total soluble phenolic compound rose significantly in the coated and uncoated slices. This increase was more intense in the samples coated with mucilage only, followed by control treatment, and, lastly the formulation containing mucilage + starch + glycerol (Fig. 3).

The increasing amount of phenolic compounds at 4 and 10 days after minimal processing is due to handling procedures such as cutting, peeling, and others, which may induce stress in the yam root tissues. After being cut, carrot and sweet potato showed increasing phenolic compound contents and antioxidant capacity [26]. In the current study, the injury caused by minimal processing associated with cold storage after 4 and 10 days elevated the phenolic compound content, irrespective of the use of coating (Fig. 3). These phenolic

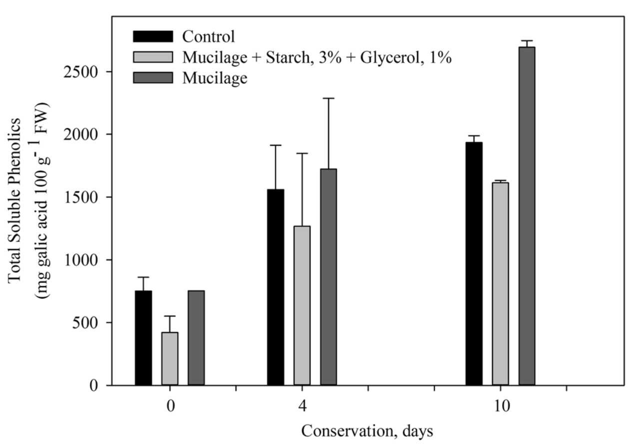

Fig. 3 Gallic acid content in minimally processed yam coated with the following formulations: control (water); cactus mucilage + cassava starch (3%) + glycerol (1%); or cactus mucilage. The slices were maintained at 5 ± 2 °C for 10 days. Bars represent the standard error of the mean

compounds can also increase the tissue's antioxidant capacity, although this parameter was not evaluated. Plants or its parts, under extreme conditions, can trigger the synthesis of phenolic compounds [27]. Despite being a defense or adaptation mechanism, in vegetables, the browning of the tissues is a consequence [28]. However, new insights on researches about secondary metabolites suggest positive effects, due phenolic compounds have health promoters nutraceutic properties [29]. Furthermore, in the case of isolated mucilage have promoted a greater accumulation of phenolic compounds (Fig. 3), which not caused browning (Fig. 2b) the compounds may be precursors of lignin, suberin, among other compounds that not cause browning. Furthermore, it is known that cactus plants have a large number of bioactive compounds [30]. Thus, its application in yam can to enrich, even more, beneficial effects on human health [31].

Polyphenol oxidase activity decreased during cold storage in the slices coated with cactus mucilage + cassava starch (3%) + glycerol (1%) and with cactus mucilage alone. Furthermore, the coated slices showed higher absolute values than control slices at all times; this trend was observed for both studied enzymes (Table 2).

Peroxidase activity in the yam slices increased in all studied treatments throughout the preservation period (Table 2). Increasing PPO and POD activities during the preservation period is a commonly observed phenomenon that precedes tissue browning [32]. In the present experiment, PPO activity only declined between 0 and 10 days of preservation in the slices coated with the formulations containing cactus mucilage + cassava starch (3%) + glycerol (1%) or cactus mucilage alone (Table 2), which coincided with the lower scores for visual appearance (Fig. 2b). This may indicate that the mucilage-containing coating partially minimized the PPO activity and consequently slowed the advance of browning. Although the coating with mucilage alone stimulated increased accumulation of phenolic compounds (Fig. 3), these accumulated phenolic compounds are possibly not involved in browning [27] (Fig. 4).

The reason why the mucilage slowed browning is still under investigation (Fig. 4). It is believed that the mucilage with and without the use of glycerol hampered the penetration of environmental O_2, which is one of the substrates of the PPO enzyme [33]. This resulted partially in decreased enzymatic activity (Table 2). Moreover, the pH of the mucilage was around 5.5, and this low value reduces the PPO activity [34].

On the other hand, the increases in POD activity observed at 4 and 10 days were significant for the slices with edible coating as compared with their uncoated counterparts (Table 2). Peroxidase is involved in browning mechanisms [35] as well as in ROS elimination mechanisms [36]. It is possible that, in the present study, the mucilage deposited as coating stimulated enzymatic

Table 2 Polyphenol oxidase and peroxidase activities in minimally processed yam coated with the following formulations: control (water); cactus mucilage + cassava starch (3%) + glycerol (1%); or cactus mucilage

Enzymes	Formulations	Days at 5 ± 2 °C		
		0	4	10
PPO (μmol Catechol min^{-1} gMF^{-1})	Control	1.9357aB	2.4949aB	2.5235aA
	Mucilage + Starch, 3% + glycerol, 1%	6.1511aA	3.3838bAB	3.0110bA
	Mucilage	5.1474aA	3.8713bA	2.5952cA
	MSD for columns = 1.0779	MSD for lines = 1.0779		
POD (μmol Guaiacol min^{-1} gMF^{-1})	Control	0.0007bA	0.0059aC	0.0041abB
	Mucilage + starch, 3% + glycerol, 1%	0.0038bA	0.0123aB	0.0116aA
	Mucilage	0.0012cA	0.0183aA	0.0086bAB
	MSD for columns = 0.0050	MSD for lines = 0.0050		

The slices were maintained at 5 ± 2 °C for 10 days

Averages followed by equal letters in the column did not differ statistically from each other by the Tukey test at 5% probability

MSD minimal significant difference

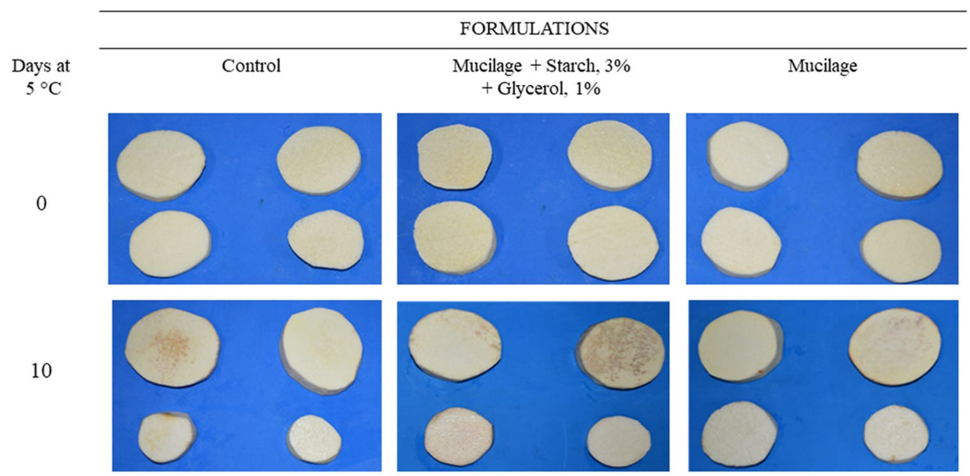

Fig. 4 Appearance of minimally processed yam coated with the following formulations: control; mucilage + starch, 3% + glycerol, 1% and mucilage. The slides were maintained at 5 ± 2 °C for 10 days

oxidative protection via POD and non-enzymatic oxidative protection through an accumulation of phenolic compounds [27] to protect the cells from oxidative damage [37]. In doing so, the mucilage worked as an elicitor [38], as well as in chitosan-containing coating on carrots [3].

The results indicate that the yield of the extraction process used here was similar than others previously works. Also, the use of citric acid solution in our extraction process is friendlier to the environment than other processes that use more toxic solvents.

Overall, hydrocolloid studied is believed to have the potential to be the base of an effective edible coating for minimally processed yam. This was one of the first scientific evidence of the use of mucilage in the yam coating composition. However, investigations are necessary to better elucidate the physiological mechanisms involving oxidative damage as well as enhance the formulation to be used to extend the quality of minimally processed yam.

The evaluated edible coating supports the proposal of value-addition to spineless cactus by indicating another use for a by-product of this plant, which is still greatly undervalued in semiarid regions of Brazil.

Conclusions

Cactus-containing coating reduced the fresh mass loss and maintained visual and sensory quality. The presence of this hydrocolloid in the coating composition increased phenolic compounds and elicited different responses from PPO and POD enzymes. Thus, the pieces of yam containing mucilage of spineless cactus as edible coating maintained the quality for 10 days at 5 °C.

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