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Starch-based films for Red Torch ginger inflorescences postharvest conservation

Filmes à base de amido para conservação pós-colheita de inflorescências de bastão-do-imperador

Marina Romano Nogueira[1], Patrícia Duarte de Oliveira Paiva[1*], Antonio Rodrigues da Cunha Neto[1], Michele Valquíria dos Reis[1], Ângela Maria Pereira Nascimento[2], Caroline de Oliveira Timoteo[3]

[1]Universidade Federal de Lavras/UFLA, Escola de Ciências Agrárias, Departamento de Agricultura, Lavras, MG, Brasil
[2]Empresa de Pesquisa Agropecuária de Minas Gerais/EPAMIG, Prudente de Morais, MG, Brasil
[3]Universidade Federal de Lavras, Instituto de Ciências Naturais, Departamento de Biologia, Lavras, MG, Brasil
[*]Corresponding author: patriciapaiva@ufla.br
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ABSTRACT

Different products have been tested to increase the vase life of cut flowers after harvest such as the biodegradable films. These products have been used in fruits since the application on the surface may provide the modification of the atmosphere around it, although, there is no information for flowers. The objective was to evaluate the effectiveness of cassava starch films as a postharvest product for Red Torch Ginger inflorescences. On the first experiment, different starch concentrations in the solution were tested and in the second experiment starch, concentrations with plasticizers and adjuvants were evaluated. The application of the biodegradable film in Red Torch stems acted as a barrier to the gases, as observed by their water absorption rate and the maintenance of fresh weight, maintaining postharvest quality up to Day 6. The visual quality was better in the inflorescences treated with 6% starch and in the films without additives. The use of 6% starch films led to lower contents of hydrogen peroxide and lipid peroxidation and higher levels of primary-metabolism macromolecules up to Day 6, delaying senescence and increasing postharvest durability. The addition of glycerol plasticizer to the different film concentrations improved the film resistance characteristics. It is recommended the use of films based on cassava starch at a concentration of 6%. The use of the adjuvant is not essential since it changed the film's characteristics, leaving it less transparent and more viscous, hindering drying.

Index terms: Bio-based material; biodegradable films; glycerol; surfactants; vase life.

RESUMO

Diferentes produtos foram testados para aumentar a durabilidade de flores de corte após a colheita, como filmes biodegradáveis. Estes produtos têm sido utilizados em frutas desde que a aplicação na superfície proporciona a modificação da atmosfera ao seu redor. Embora, não haja informações sobre o uso desses produtos para flores. O objetivo foi avaliar a eficácia de filmes de amido de mandioca como produto pós-colheita para inflorescências de Red Torch Ginger. No primeiro experimento foram testadas diferentes concentrações de amido na solução e no segundo experimento foram avaliadas concentrações de amido com plastificantes e adjuvantes. A aplicação do filme biodegradável em hastes de bastão-do-imperador atuou como uma barreira aos gases, como observado pela sua taxa de absorção de água e a manutenção do peso fresco, mantendo a qualidade pós-colheita até o Dia 6. A qualidade visual foi melhor nas inflorescências tratadas com 6% de amido e nos filmes sem aditivos. O uso de filmes com 6% de amido levou a menores teores de peróxido de hidrogênio e peroxidação lipídica e maiores teores de macromoléculas de metabolismo primário até o Dia 6, retardando a senescência e aumentando a durabilidade pós-colheita. A adição do plastificante glicerol às diferentes concentrações de filme melhorou as características de resistência do filme. Assim, recomenda-se o uso de filmes à base de amido de mandioca na concentração de 6%. O uso do adjuvante não é essencial, pois alterou as características do filme, deixando-o menos transparente e mais viscoso, dificultando a secagem.

Termos para indexação: Material de base biológica; filmes biodegradáveis; glicerol; sulfactantes; durabilidade.

INTRODUCTION

Inflorescences of the Red Torch (*Etlingera elatior*) has a postharvest durability period of 5-10 days (Carneiro et al., 2014), shorter than other tropical plants such as *Anthurium* (Favero et al., 2020), *Heliconia psittacorum* (Malakar; Acharyya; Biswas, 2019) and *Strelitzia reginae* (Paula et al., 2021). This short durability of the Red Torch causes management problems and significant product losses after harvesting, and during the transport and commercialization processes (Carneiro et al., 2014).

Postharvest quality is related to the maintenance of plant metabolism and delayed senescence, so its improvement requires an analysis of physiological and biochemical aspects (Costa et al., 2021; Favero et al., 2020). To increase the durability of cut flowers, postharvest technologies such as films on the surface of the product and modification of the atmosphere around it have been used. These coatings have properties that act as barriers to respiratory exchange, reducing the evapotranspiration of water vapour and other gases in the plant, resulting in quality maintenance and prolonging the shelf life (Romanazzi; Moumni, 2022).

Several formulations of coatings have been developed based on starch that may be obtained from various plant sources, such as cereals, roots and tubers, fruits, and vegetables. Cassava starch has been applied to fruits, vegetables, some flowers, and roots due to its ability to form films without the addition of additives (Sanches et al., 2016). Coatings have had satisfactory effects in cherries, inhibiting peroxidase activities, preventing fruit browning, and delaying lipid peroxidation while maintaining membrane integrity (Pasquariello et al., 2015). The use of cassava starch-based films (4%) in ornamental ginger inflorescences maintains a better appearance of the flowering stems (Sanches et al., 2016).

Cassava starch, when compared to other film compositions such as chitosan, polyvinyl chloride (PVC) in addition with polymer, carnauba wax and other compounds, has a low-cost and desirable characteristic for biofilms preparation, such as good transparency, good resistance to gas exchange, resistance to mechanical damage, maintenance, and integrity of the cell wall, barrier to the incorporation of solutes and association with other compounds with fungicidal properties (Mattos et al., 2017; Guerra et al., 2019; Romanazzi; Moumni, 2022). The coatings performed using cassava starch are isotropic, odourless, tasteless, colourless, nontoxic, and biodegradable, turn in a sustainable product (Oluwasina et al., 2019). The films made with starch are brittle and not very flexible, so plasticizers are added to improve their composition and physicochemical characteristics. One example of plasticizer is the glycerol, which causes changes in the intermolecular force of the polymer chains, resulting in a less dense filmogenic matrix, enabling elongation, besides decreasing tensile strength (Tarique; Sapuan; Khalina, 2021). Lipids or surfactants are added to help fix the coating on the product whether flowers or fruits, as may facilitated adsorption and adherence (Caixeta et al., 2020). The application of cassava starch films incorporated with *Mauritia flexuosa* oil as a surfactant has provided some barrier properties against gases and water losses (Souto et al., 2021).

There is scant information regarding tropical flower coating films in the literature. As Red Torch has low durability, the use of cassava starch film is justified, since its efficiency has already been proven for other products. Additionally, it is essential to invest in sustainable technologies, since the coating practice tends to generate waste, as these products will be discarded after use. The objective was to evaluate the effectiveness of cassava starch-based films added by glycerol and adjuvants in postharvest durability of Red Torch ginger inflorescences. This study aimed to answer these questions: Can the use of starch-based films be a valuable technique to boost postharvest quality? Can the product be used alone or does the combination with adjuvants increase its viability? Does the use of starch-based film affect the aesthetic characteristics or durability of flowering stems? The contents of hydrogen peroxide, lipid peroxidation, enzymes of the antioxidant system and macromolecules of the flowering stems can be changed in consequence of the use of starch film?

MATERIAL AND METHODS

The Red Torch ginger (*Etlingera elatior*) stems were cultivated at 21º13’25”S 44º58’17”W; elevation of 920 m. The stems were harvested at 7 am and selected with the same age and opening point in clumps that had the same development conditions.

After harvesting the flowering stems of Red Torch ginger in the open stage (first ring of true flowers open), cleaned was proceeded by immersing them in water. The stems were standardized to 0.6 m and submitted to a pulsing in 20 % sucrose solution during 24 hours (Carneiro et al., 2014).

Two experiments were performed, evaluating different concentrations of starch in the solution and different concentrations of plasticizers and adjuvants added to the solution. The biofilms were prepared by diluting cassava starch powder in water, using the concentrations of 2%, 4% and 6% in tap water and keeping it at 70 °C for 1200 s until the complete gelatinization. Stems immersed in water without the presence of cassava starch were used as control. In the second experiment, after gelatinization, the solutions were cooled to 40 °C, and then plasticizer (glycerol, 1% w/w filmogenic solution) or commercial adjuvant Assist® (1% w/w filmogenic solution) were added.

For treatments, the inflorescences were submerged for approximately 20 s in the solutions, and then the stems were kept inverted during 24-h to drain the excess of the biofilm. After drying, the stems bases were placed in containers with 1 L of water for hydration and maintenance (Figure 1).

The containers were kept in a room at 21 °C and 85% ± 5% relative humidity during 12 days. The inflorescences were evaluated every 3 days by three trained evaluators based on the criteria of Carneiro et al. (2014) (Table 1).

Absorption rate

The volume of water consumed was determined, in mL/stem/day, to evaluate the hydration capacity of each flowering stem. After determining the volume consumed, water replacement was performed (Sales et al., 2021).

Colorimetry

Three fully expanded involucral bracts of each inflorescence, arranged in the second-outermost row, were measured every 3 days, positioning the Konica Minolta colorimeter model Chroma Metre CR 300 in the middle of the bract with the CIEL*a*b method, which allowed us to obtain the parameters L*, a*, hue, and chroma (Lago et al., 2020).

Figure 1: Draining and drying of the films in the inflorescences after immersion (A) and conditioning of the stems during the experiment (B).

Table 1: Criteria and scores for evaluating the postharvest visual quality of Red Torch ginger inflorescences.

SCORE	CLASS	DESCRIPTION
4	EXCELLENT	Turgid stem and inflorescences, bracts with brightness and characteristic colour.
3	GOOD	Beginning of turgor loss (only sensitive to tact); with or without the beginning of fading and/or wilting in the edges of the bracts and stems.
2	REGULAR	Decline of bracts due to visible loss of turgor and brightness of the inflorescence and stem. Edges of bracts have soggy appearance
1	BAD	Loss of pronounced turgor of the bracts and/or stems, edges of the translucent bracts, central part of the inflorescence softened
0	NO QUALITY	Waste: dry bracts and/or waterlogged appearance, with rotting of the central part of the inflorescence, and abscission of the bracts.

Source: Carneiro et al. (2014).

Fresh weight

The fresh weight was determined by weighing the flowering stems daily, and the relative weight loss was determined by the difference in weight between the evaluations.

Enzymatic activity

The enzymatic activity of peroxidase (POX), superoxide dismutase (SOD), and catalase (CAT) was determined by electrophoresis (Silva Neta et al., 2020). 1 g of fresh matter was extracted into 0.2 M Tris-HCl buffer pH 8.0 + 0.1% beta-mercaptoethanol for SOD and CAT, and for the POX enzyme, potassium phosphate buffer was used at a ratio of 300 µL per 100 mg of fresh weight. The material was vortexed and maintained for 12 h in a refrigerator, followed by centrifugation at 20,817 *g* for 1800 s at 4 °C. The electrophoresis was run in a discontinuous system of 7.5% polyacrylamide gels (separator gel) and 4.5% (concentrator gel). The running buffer used was Tris-glycine pH 8.9. A total of 0.00006 L of the sample supernatant was applied to the gel, and the electrophoretic run was performed at 150 V for 18000 s. At the end of malondialdehyde the run, the gels were revealed for the enzymes POX, SOD, and CAT.

Hydrogen peroxide and lipid peroxidation

Samples of inflorescences were collected at 3, 6, 9, and 12 days after application of the films at different concentrations. For the quantification of hydrogen peroxide (H_2O_2) and lipid peroxidation, fresh samples of 0.0002 kg of the bracts of the inflorescences were kept in the freezer at -80 °C and macerated in liquid nitrogen, homogenized in 0.0015 L of trichloroacetic acid, and centrifuged at 12,000 × *g* for 900 s at 4 °C. H_2O_2 was measured by measuring the absorbance at 390 nm in a reaction medium containing 100 mM potassium phosphate buffer pH 7.0, 0.0005 L of the extract, and 0.001 L potassium iodide (Velikova; Yordanov; Edreva, 2000), and the results are expressed in mol H_2O_2 kg^{-1} fresh weight. Lipid peroxidation was determined by quantifying thiobarbituric acid–reactive species (TBA), according to Buege and Aust (1978), and the readings were performed in a spectrophotometer at 535 and 600 nm. The results are expressed in mol malondialdehyde kg^{-1} fresh weight.

Analysis of macromolecules

For the evaluation of macromolecules of primary metabolism, the bracts were put in a forced-air oven at 60 °C to obtain their dry weight. A total of 0.2 g of dry matter was extracted together with 10 mL of 0.1 M potassium phosphate buffer (pH 7.0), put in a water bath at 40 °C for 30 min, and the mixture was centrifuged at 10,000 × *g* for 1200 s. The supernatant was collected, and the centrifugation process was repeated after adding more 10 mL of buffer. Immediately after this supernatant was collected, it was stored at -80 °C. For the quantification of the biomolecules, total soluble sugar, reducing sugars, and total soluble proteins, the anthrone, dinitrosalicylic acid, and Bradford spectrophotometric methods were used as described by Yemm and Willis (1954), Miller (1959), and Bradford (1976), all with modifications. The analyses were performed by reading in a spectrophotometer at a wavelength of 620 nm for total soluble sugars (mol glucose kg^{-1} dry weight), 540 nm for reducing sugars (mol glucose kg^{-1} dry weight), and 595 nm for total proteins (g protein kg^{-1} dry weight).

Experimental design and statistical analysis

The first experiment was conducted in a completely randomized design, with four treatments (film dose concentrations), four evaluation periods (3, 6, 9, and 12 days after insertion of the films in the rods) and 4 replicates composed of one inflorescence each. The second experiment was conducted in a completely randomized design with seven treatments (concentrations of biofilms with addition of glycerol, wit, or without adjuvant), four evaluation periods (3, 6, 9, and 12 days after insertion of the films in the rods) and 4 replicates composed of one inflorescence each. The experiments were repeated twice. The data were subjected to analysis of variance (Ferreira, 2019) and polynomial regression analysis for the parameters analysed at 5% significance or comparison of the means by the Tukey test at 5% significance for the POX enzyme activity.

RESULTS AND DISCUSSION

Quality analysis

The quality of inflorescences based on visual quality was influenced by the conditioning time and the cassava starch film concentration. Inflorescences coated with the highest concentration (6% starch) showed an average score of 3 until 9 days after harvest, while the others lost quality from day 6 (Figure 2A and 3). After day 9, all the inflorescences presented a lower visual quality that is not acceptable for commercialization.

In the experiment with additives, the inflorescences coated with the highest concentration of starch (6%) plus the plasticizer glycerol and the adjuvant Assist® showed an average score of 3 until 6 days after harvest, and the other concentrations showed loss of quality at the day 6, indicating that the use of this product affects the quality of the inflorescences (Figure 2B and Figure 4). At 9 days of evaluation, all the inflorescences, regardless of the concentrations of cassava starch film, when additive was added, showed visual quality with lower grade than acceptable for marketing.

The coating with cassava film was also efficient for ornamental ginger, however in the concentration of 4% associated with the cutting of 10 cm of the stem (Sanches et al., 2016).

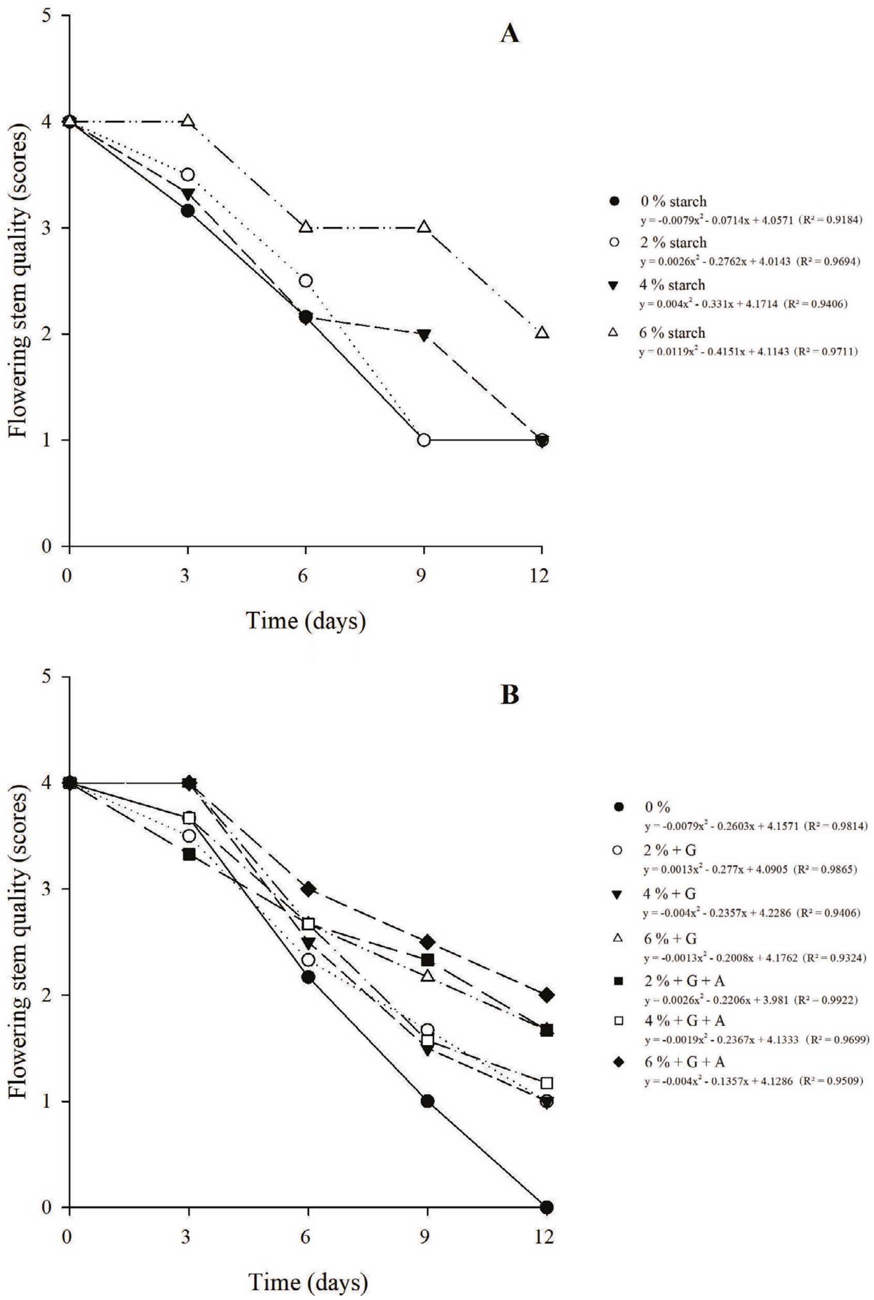

Figure 2: Visual quality of Red Torch inflorescences with films with different concentrations of cassava starch (A) and with or without additives (G = glycerol and G+A = glycerol and adjuvant) to the filmogenic solutions (B) over time.

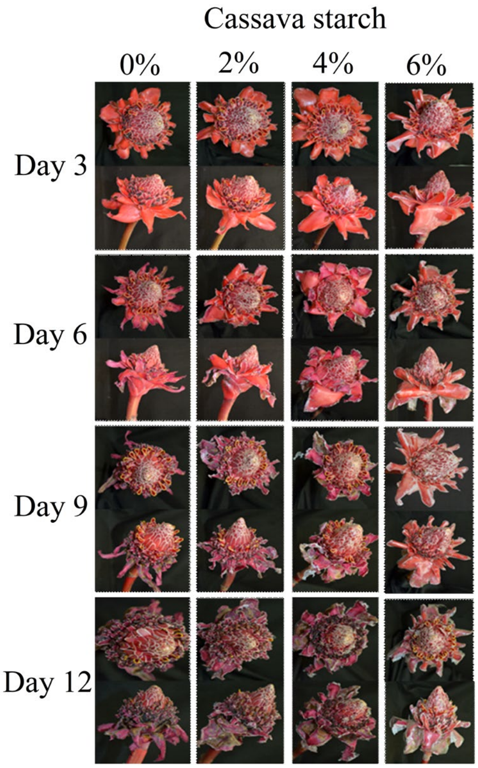

Figure 3: Red Torch inflorescences with different concentrations of cassava starch film (0, 2%, 4% and 6 %) at 3, 6, 9, and 12 days after application.

At day 9, inflorescences treated with the film presented fully damaged (Figure 5B). The inflorescences presented no commercial quality.

Starch films have low oxygen permeability; consequently, will have a lower rate of water loss of the products. Film coating creates a modified atmosphere due to permeability and change in respiratory rates (Romanazzi; Moumni, 2022). The films help to maintain the quality of coated products, delaying maturation and senescence, besides reducing dehydration, microbial growth, and losses after harvest (Pasquariello et al., 2015).

As described for other flowers and fruits, the cassava starch film was effective for Red Torch that maintained the quality of the product for longer, being indicated the concentration of 6%.

Absorption rate

The absorption rate, regardless of the treatment applied, started with a greater volume of water absorbed after harvest, decreasing over time. In the first evaluations, the absorption rate was higher in either the control group or group treated with solution 2% starch, presenting averages of 0.0329 L and 0.018 L at 3 and 6 days. The inflorescences treated with higher concentrations of cassava starch showed a mean intake of 0.0279 L and 0.016 L. On day 9, the absorption rate was similar between treatments and remained constant through day 14 (Figure 6).

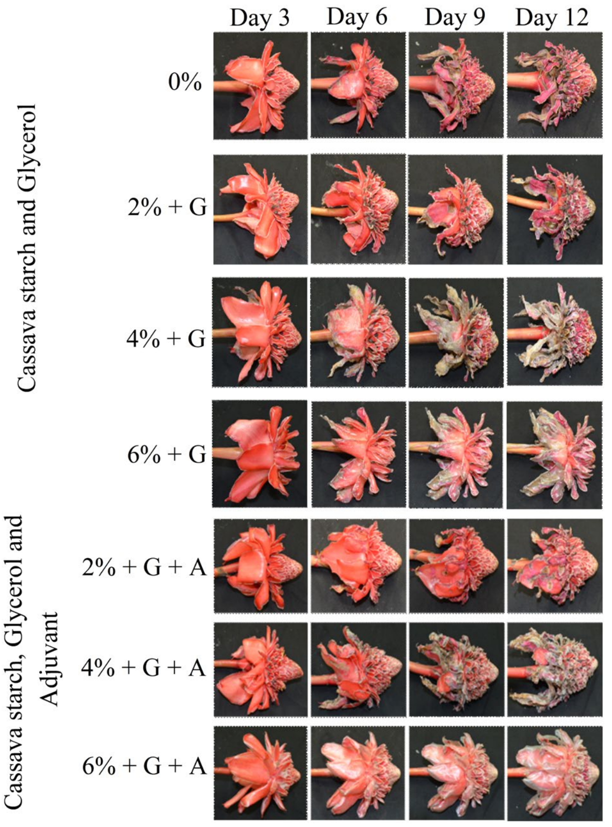

Figure 4: Red Torch inflorescences with different concentrations (0, 2%, 4%, 6%) of cassava starch film plus glycerol (G) or glycerol and adjuvant (G +A).

At 3 days after application, the film adhered to the inflorescences, forming a more efficient barrier to prevent excessive water loss, thus explaining the lower water consumption in groups with higher concentrations. In this context, the use of films is recommended as a way to create a semipermeable barrier to water and gases, as it they slow down the respiration and the senescence of the product (Guerra et al., 2019).

The modified atmosphere created in the inflorescences is characterized by the presence of an artificial barrier to the diffusion of gases around, resulting in a lower level of O_2, a higher level of CO_2, and higher water vapour content. These changes vary mainly due to the composition and thickness of the barrier and respiration rate (Guerra et al., 2019; Romanazzi; Moumni, 2022). Cassava starch formed this barrier on Red Torch stems and maintained visual quality and turgor pressure for longer time.

After day 3, the film turned brittle in all treatments, which allowed a greater loss of water and gases and, consequently, a similar water absorption rate. At days 3 and 6 after film applications, the absorption rate was highest in the control group, differing from all other coating groups, regardless of the concentration of starch and the additive used. At day 9, the absorption rates stabilized, with no difference among the inflorescences (Figure 6).

The addition of glycerol plasticizer to films with different starch concentrations provided less brittle and more flexible coatings, since this compound increases the flexibility and processing capacity, as well as the oxygen permeability (Tarique et al., 2021). On the other side, the addition of lipids, such as vegetable oil-based adjuvants, makes the filmogenic solutions denser, reducing films transparency. In addition, a low mechanical resistance and high oxygen permeability, provides good water vapor barrier properties (Souto et al., 2021).

The incorporation of adjuvants and additives aims to modify the characteristics of the films produced from cassava starch, maintaining the quality of the coated products, delaying maturation and senescence, reducing dehydration, decreasing the microbial growth rate and post-harvest losses. These occur due to the ability to create a modified atmosphere formed by the permeability of the coating and respiratory rate of the inflorescences (Romanazzi; Moumni, 2022). The use of additives in cassava starch film improved characteristics such as flexibility and adhesion, but affected and modified visual characteristics of Red Torch and was not efficient to maintain the quality of inflorescences.

Colorimetry

The luminosity scale, where the high L values represent lighter color and lower values darker color, ranges from 0 (black) to 100 (white). The luminosity data (Figure 7) indicated, on all days of evaluation, that the cassava starch film at the concentration of 6% provided higher luminosity than the other concentrations and the control. This can be explained by the fact that films in high concentration present a higher viscosity, forming a thicker film on the bracts, which was reflected in the colorimeter as higher luminosity values. On the days 6 and 9, control and coating treatments at concentrations 2% and 4% provided lower values of L*. The highest mean L* was observed in the inflorescences treated with 6% cassava starch, showing that the films formed in the bracts influenced the luminosity.

Similar to the observed in the Red Torch ginger, the use of coating with starch-based films in “Paluma” guava has resulted in higher L* values, which indicated more accentuated brightness due to the coating uses (Rocha et al., 2020).

The chromaticity (Figure 8) is defined as saturation, that is, the radial distance from the centre of the space to the color point. Saturation is directly linked to the concentration of the color element and represents the quantitative attribute of intensity. The higher value of chroma, the greater colors saturation perceptible to human eyes. Neutral colours have low saturation, while pure colors have high saturation and, therefore, are brighter to human eyes perception.

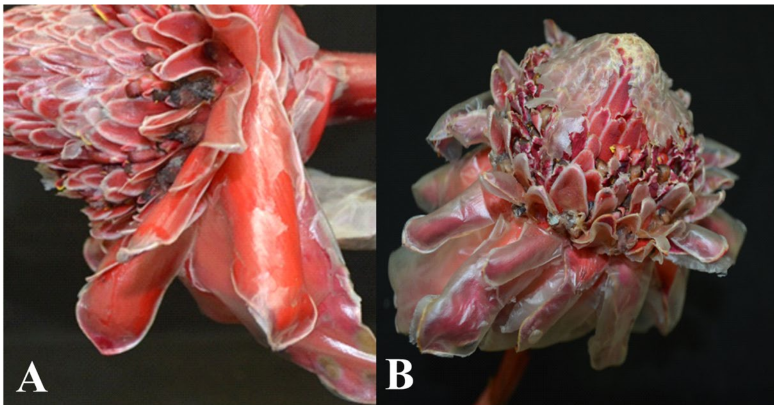

Figure 5: Detail of the inflorescences treated with film of 6% starch (A) and 6% starch + glycerol + adjuvant (B) at day 6.

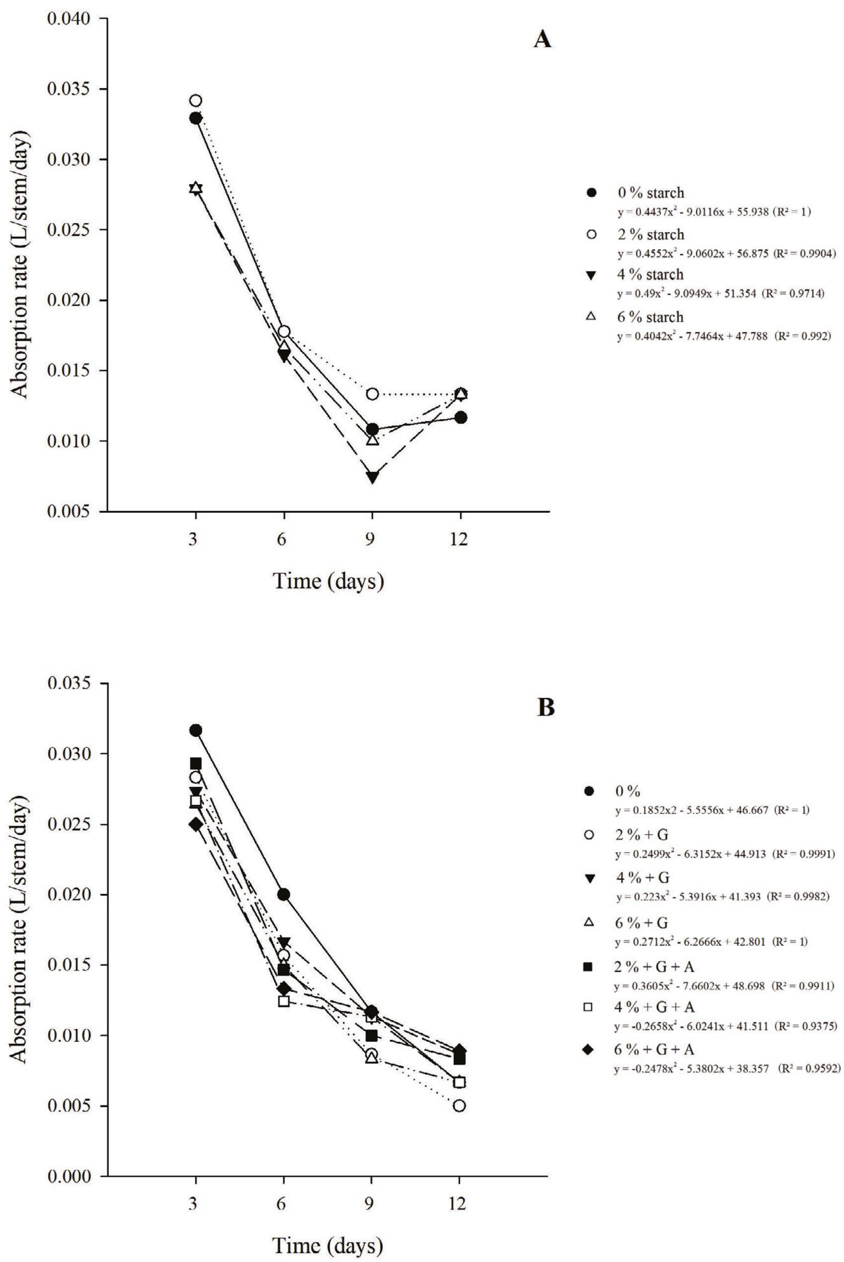

Figure 6: Absorption rate of Red Torch ginger inflorescences treated with films of different concentrations of cassava starch (A) with or without additives (G = glycerol and G+A = glycerol and adjuvant) in the filmogenic solutions (B) over time.

The chromaticity data showed that 6 days after film applications a difference was observed when comparing control and the other groups. Inflorescences that did not receive the film coating showed high saturation, with a purer color. Better colour definitions have higher chroma values, relatively lower values represent impure or grey colors and the starch film probably influenced this trait. On day 12, there was no difference between the stems contained in the control treatment and those coated with 2% cassava starch. These treatments presented higher averages compared to the inflorescences coated with 4% and 6% starch (Figure 8).

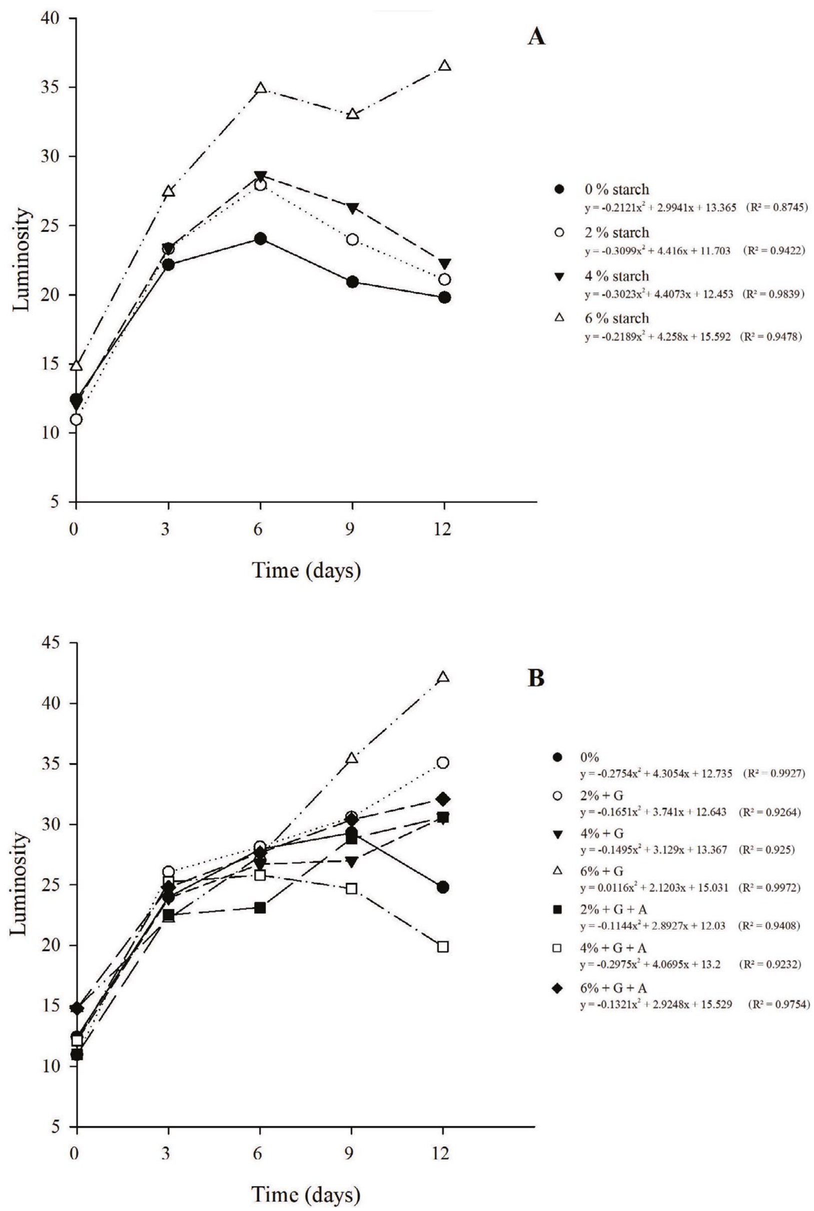

Figure 7: Luminosity values read by the colorimeter in inflorescences of Red Torch ginger with films of different concentrations of cassava starch (A) with or without additives (G = glycerol and G+A = glycerol and adjuvant) in filmogenic solutions (B) over time.

A qualitative color attribute, the hue angle, is obtained based on colors traditionally defined as reddish, greenish, etc. Graphically, the 0° angle was considered red, 90° yellow, 180° green, and 270° blue (Mcguire, 1992). The overall mean hue angle observed in this study in the bracts of Red Torch ginger treated with films at different concentrations of cassava starch was 37.48°. This result indicates a red colour of all evaluated bracts, regardless of the treatment.

Fresh weight

On the third day after the application of the film, the stems with 6% cassava starch coating presented higher fresh masses than the others. In the next evaluation, no difference was observed comparing the coated inflorescences at a concentration of 6% or 4%, being these superior to the control (no coating) or 2% cassava starch coating (Figure 9A, B). The fresh weight showed stability between treatments from day 9 after the application of the films, which is justified by the fact that the film has become fragile and released gradually from day 6 after application, allowing greater gas exchange and transpiration of inflorescences.

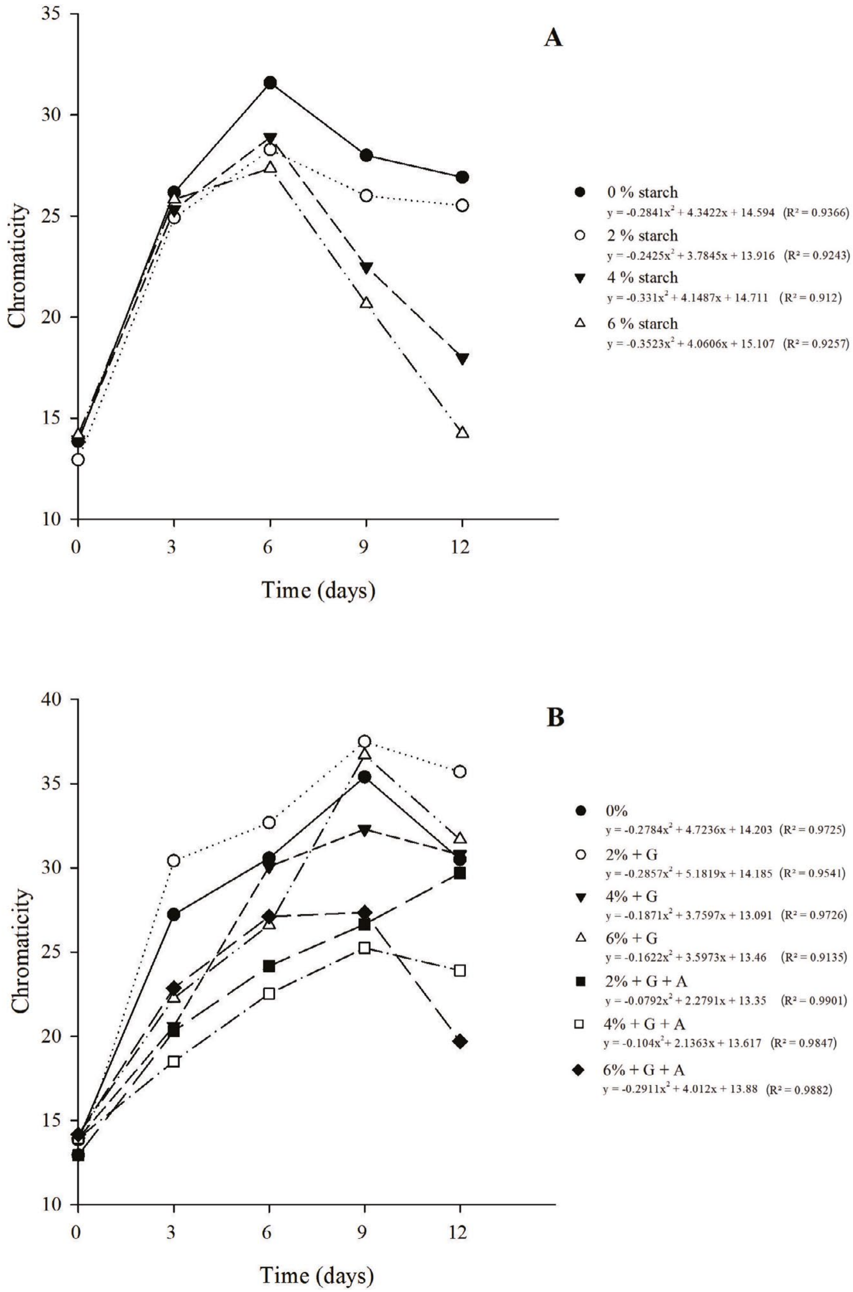

Figure 8: Chromaticity values obtained through the colorimeter in Red Torch ginger inflorescences treated with cassava starch films in different concentrations (A) solution with (G = glycerol and G+A = glycerol and adjuvant) or without additives in filmogenic solutions (B) over time.

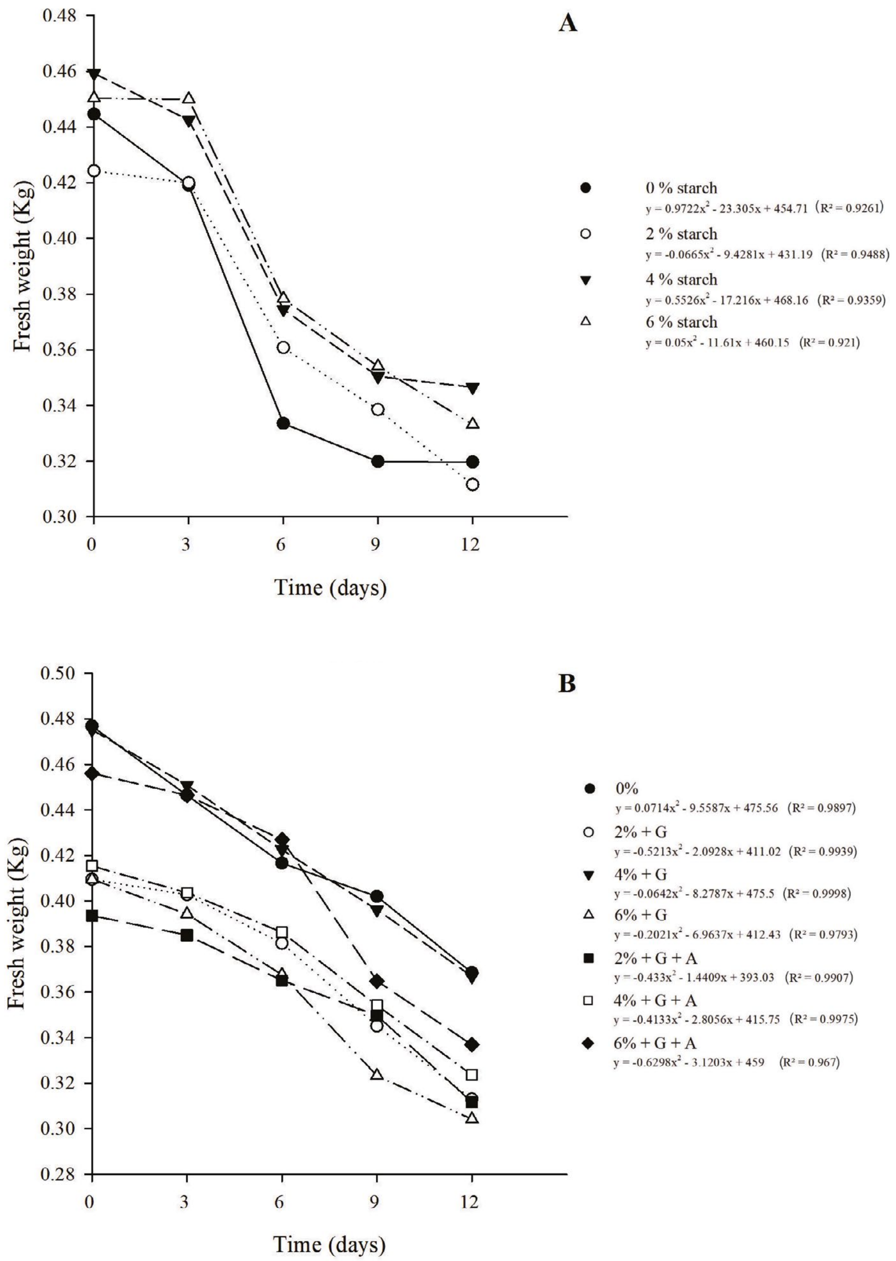

Figure 9: Fresh weight of Red Torch ginger inflorescences treated with cassava starch films at different concentrations (A) with (G = glycerol and G+A = glycerol and adjuvant) or without additives in filmogenic solutions (B) over time.

Fresh weight is an important physical characteristic that determines the longevity and postharvest quality of inflorescences, and its loss is to the transpiration process and to the reduction in water conductivity during the senescence process (Carneiro et al., 2014). The low absorption rate in stems may induce reduction in fresh weight, resulting in reduction of vase life from the day 6, since there was stability between treatments with respect to water absorption and flower stems fresh weight. Cassava starch films sprayed on ornamental ginger inflorescences at intermediate concentrations, such as 4% and 6% provided the greatest maintenance of fresh weight (Sanches et al., 2016).

On the first evaluation day, the flowering stems from the control treatment (no film application) showed higher weight loss than those from the other treatments, in both experiments (Figure 9B). The observed loss was on average 5% higher than that the other treatments. On the day 6 after the treatments application, the stems that did not receive film application still showed higher weight loss, but in a was less pronounced way (Figure 9B). On the day 9, there was a greater loss of weight inflorescences treated with 6% + G + A since after 6 days, the film detached from the bracts, allowing a greater exchange of gases and transpiration, leading to a greater loss of fresh weight.

In addition to being semipermeable barriers to gases, the coatings also perform the function of minimizing the loss of water vapour, which were measured by the loss of weight during storage. Water loss in plant products may be a reflection of high metabolic rates or may influence quality characteristics such as color, firmness, soluble solid content, and intercellular components. In the present study, similar results were obtained for fresh weight, since the stems of the control group showed higher levels of loss over time than the groups with coatings, and the 6% + G + A treatment was more efficient for maintaining the weight (Figure 10).

In the study evaluating barrier properties of cassava starch films and additives for coating blackberries, some concentrations preserved the integrity of the cuticle of the fruit and maintained its hardness in relation to the control (Peréz-Gallardo et al., 2015).

Although the use of the coating efficiently reduced the loss of fresh weight, the film interfered on the visual quality of the flowering stems. At this stage, tests were performed to remove the coating films, which were not successful due to the low solubility of the product.

Enzymatic activity

POX activity was detected by the electrophoresis technique (Figure 11). The results showed a high activity on the flowering stems, which may be due to the stress caused by cutting. On days 3 and 6 after application of the cassava starch films, low activity of POX was observed regardless of the concentration. This similarity may be a result the natural production of POX to ensure the maintenance of respiration and cell functions. On the day 9, POX had high activity in the control inflorescences, showing effectiveness of the coatings in reducing the oxidative damage caused by the senescence process in the flowering stems, especially in the stems with the highest starch concentration (6%). On the day 12, the uncoated inflorescences had one of the lowest POX activities since senescence was observed, besides an observation of higher enzyme activity in the coated plants. It is worth noting the efficiency of the application of the highest-concentration films (6%) in maintaining a low activity of the enzymes, which provided greater control of the damage caused by the oxidative process and better maintenance of the quality of the inflorescences.

The application of cassava starch-based film to lychee fruits (*Litchi chinensis*) reduces the activity of POX compared to the control treatment (Nor; Ding, 2020), as well as it was observed for the Red Torch ginger. Senescence increases the production of H_2O_2 in post-harvest plants inducing the activation of POX at the membrane level, and this enzyme can act by increasing or decreasing its activity depending on the type of coating used and the physiological stage of the tissue (Piechowiak et al., 2022).

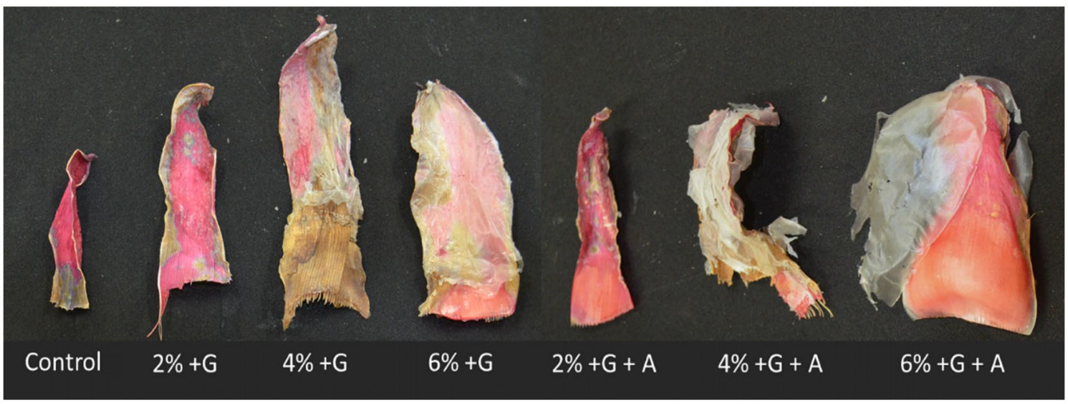

Figure 10: Details of the external bracts 12 d after application of the films at different concentrations and with or without additives (G = glycerol and G+A = glycerol and adjuvant).

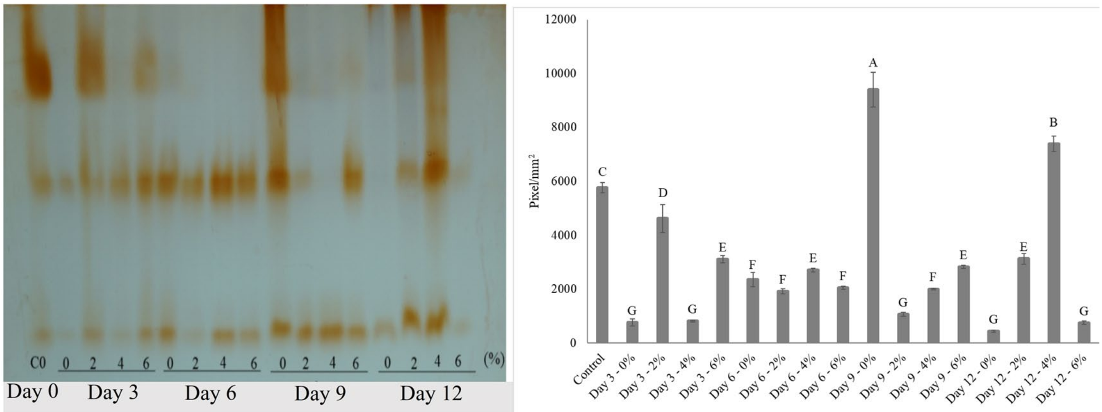

Figure 11: Acrylamide gel after electrophoresis, representing the activity of peroxidases present in the inflorescence samples with different concentrations of cassava starch over 12 days of evaluation. In the graph, the same letters over bars indicate nonsignificant differences according to the Tukey test at 5 % significance.

Through analyses performed by the electrophoresis technique, which characterizes the currently active expression of enzymes, no activity of the antioxidant system enzymes (SOD and CAT) was detected. Antioxidant enzymes such as superoxide SOD, CAT, and POX prevent oxidation under normal conditions by eliminating free radicals, but the activity of these enzymes decreases during senescence.

Under stress, in this case due to the cutting of the stems associated with the senescence process, there is an increase in reactive oxygen species that are responsible for oxidizing and modifying the cellular components (Carrera-Alvarado et al., 2021). The rapid loss of quality and senescence of Red Torch ginger stems is directly correlated with the absence of enzymes SOD and CAT that would be responsible for the antioxidative process

Hydrogen peroxide and lipid peroxidation

H_2O_2 (Figure 12 A) and lipid peroxidation (Figure 12 B) showed similar responses. On the day 3 after the application of the films, H_2O_2 increased in the groups containing the film, probably due to the stress caused by the application of the coating, damaging the lipid membrane. After this period, there was a reduction and stabilization of the values in all groups until the day 9 of evaluation, followed by an increase in the amount of peroxide and peroxidation because of the senescence process of the inflorescences and the oxidative process, except in the group with the highest concentration of cassava starch film.

The lower amounts of hydrogen peroxide and lipid peroxidation shows the efficiency of the film produced with 6% cassava starch, which acted as a barrier to gases, reducing transpiration and reduced the production of reactive oxygen species, thus promoting the maintenance of quality and durability of Red Torch rods due to lower oxidative stress. The lower peroxidase activity (Figure 11) confirms this efficiency since these parameters are directly related.

The results indicate that the use of a coating with 6% cassava starch improved the quality of the inflorescences reducing the respiratory frequency due to the barrier formed by the cassava starch film. Consequently, the film delays oxidative stress and senescence due to preservation of cell membranes increasing quality and life vase (Oluwasina et al., 2019; Romanazzi; Moumni, 2022; Souto et al., 2021).

Senescence is considered an oxidative phenomenon and involves H_2O_2 as one of the reactive species related to oxidative damage (Piechowiak et al., 2022). As observed in this study, Ferraz and Cereda (2009) indicated that the use of starch films at low concentrations (less than 3%) did not influence the biochemical characteristics related to the H_2O_2 content or lipid peroxidation on the Grand Galla roses stems. The results indicate that the use of a coating with concentration of 6% cassava starch in the stems of Red Torch improved the quality of the inflorescences, reduced oxidative stress and senescence due to preservation of cell membranes increasing quality and durability. Other studies that evaluated the production of H_2O_2 in fruits coated with films of different compositions showed results similar to ours. For example, minimally processed melons coated with 2% chitosan-based film and 0.05% trans-cinnamaldehyde also showed a reduction in H_2O_2 production, explaining the lower degree of lipid peroxidation and antioxidant enzymatic activity compared to control fruits after 20 days of storage at 4 °C (Carvalho et al., 2016).

Total protein

Total protein was higher in inflorescences coated with films at 6% on the day 3 after application but was equal to the quantity in other groups from the day 6. The lowest mean was observed in the control group on the day 1 of evaluation, i.e., on the day 3 after application of the film (Figure 13). The percentage of proteins is another way to evaluate the postharvest conditions since at this stage; there was an increase in protease activity and a decrease in protein content in senescent tissues (Zhou et al., 2022).

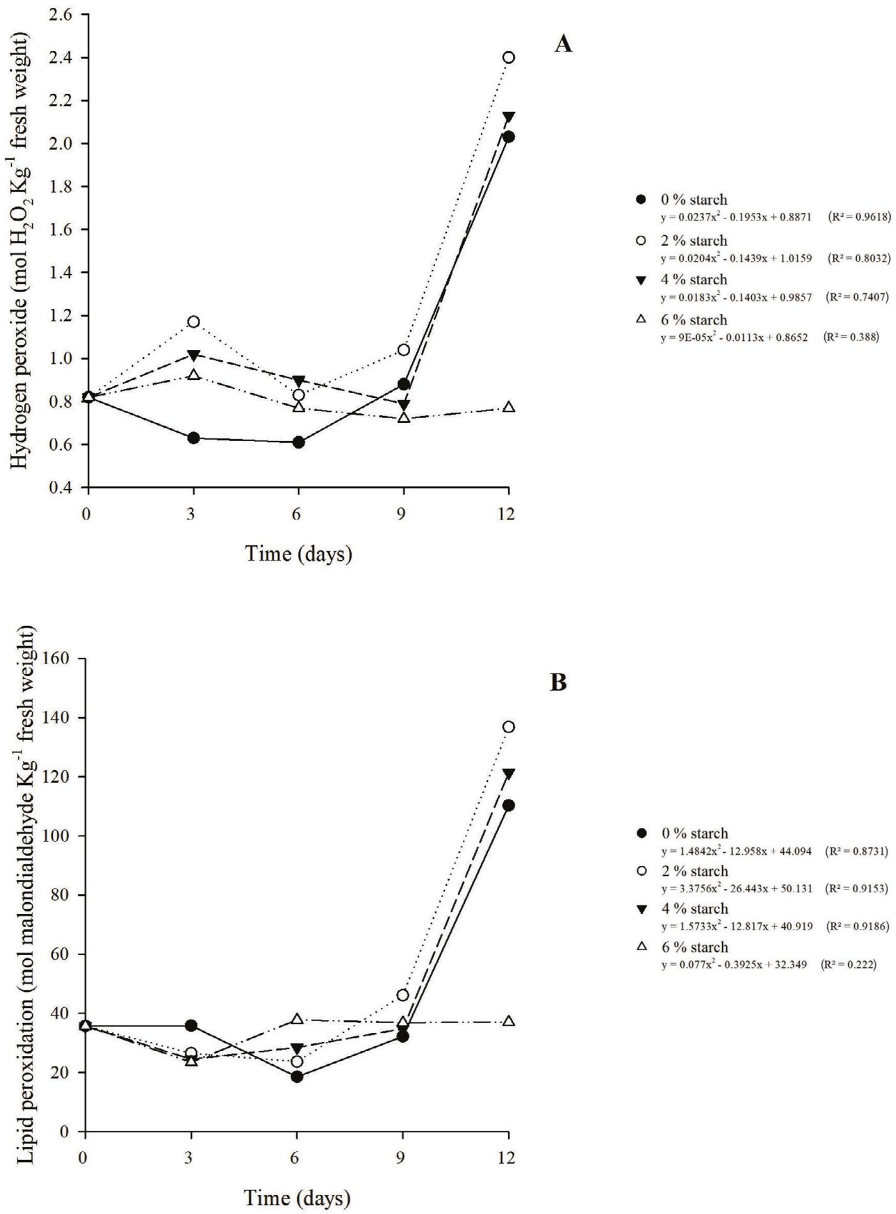

Figure 12: Quantification of hydrogen peroxide (A) and lipid peroxidation (B) over time in Red Torch ginger inflorescences coated with cassava starch films at different concentrations.

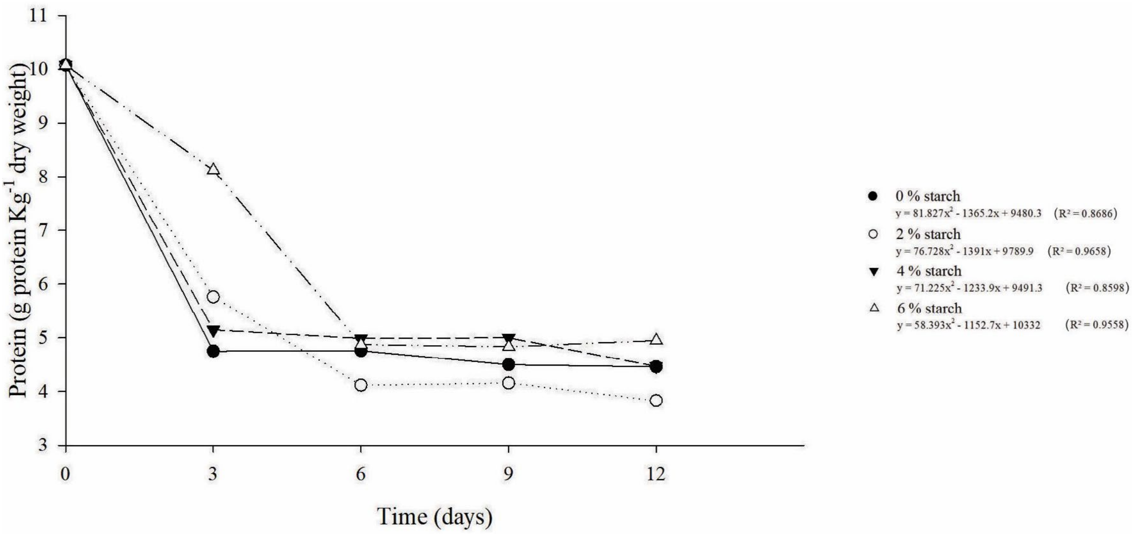

Figure 13: Total protein over time in Red Torch ginger inflorescences coated with cassava starch films at different concentrations.

Suggested that the reduction in protein content during the senescence of chrysanthemum was a result from proteins inhibition synthesis and/or an increase in the proteolytic degradation (Elanchezhian; Srivastava, 2001). In consequence, occur loss of the membranes functional capacityand an increase in ion output, leading to senescence and tissue death. Reduced protein during the senescence of cut flowers has been reported in some species, such as *Sandersonia* (Eason et al., 2002), Dendrobium (Lerslerwong; Ketsa; Van Doorn, 2009). This fact was also evidenced for Red Torch ginger, independent of the film concentration, which reduced the protein content by the senescence process.

The postharvest reduction in weight is most likely due to transpiration, in addition to the consumption of organic material, such as carbohydrates, proteins, and lipids, that is metabolized and not replaced (Oliveira Filho et al., 2022; Yin et al., 2019), confirming the protein data described in that after 6 days, 50% had been consumed, regardless of the starch concentration. The use of coatings at a concentration of 6% favoured higher protein content during the first day of postharvest conditioning than the control and the other treatments. The inflorescences treated with 2% and 4% starch film showed no differences in protein content.

Reducing and total sugars

The total sugars were low on the day 1 just after harvest excepting in the 6% starch group. Sugars in flowering stems were highest in the 6% starch group on day 3, which then decreased until the end of conditioning (Figure 14 B). Levels of starch and total soluble sugars in postharvest Red Torch ginger were higher during storage (Mattos et al., 2018). This occurred due to the mobilization and consumption of reserves by respiration during senescence. The same situation was observed for reducing sugars (Figure 14 A) in inflorescences treated with 4% and 6% starch films, which showed an increase until the day 6 after film application. It is possible that this occurred due to accumulation and mobilization. With the senescence process, after 9 days, a decrease was observed.

Sugars are the primary source of respiratory substrate, and after harvest, the content tends to decrease due to respiration (Oliveira Filho et al., 2022). Sugars may act to reduce transpiration by flowers and leaves by controlling stomatal closure and osmotic regulation of tissues, thus influencing water absorption by the stems. In cut flowers, the carbohydrate reserves may increase the longevity potential of the flowers (Mattos et al., 2018). For some species, the carbohydrates present in the flower can support an increase in postharvest durability of the flower, while in other species; they are not enough to supply the metabolism of the flowering stems after cutting (Costa et al., 2021). Thus, the maintenance of the sugar content in the Red Torch ginger inflorescences coated with 6% starch compared to the other treatments (0, 2%, 4%) shows the efficiency of this coating in delaying the consumption of reserves and increasing the postharvest durability.

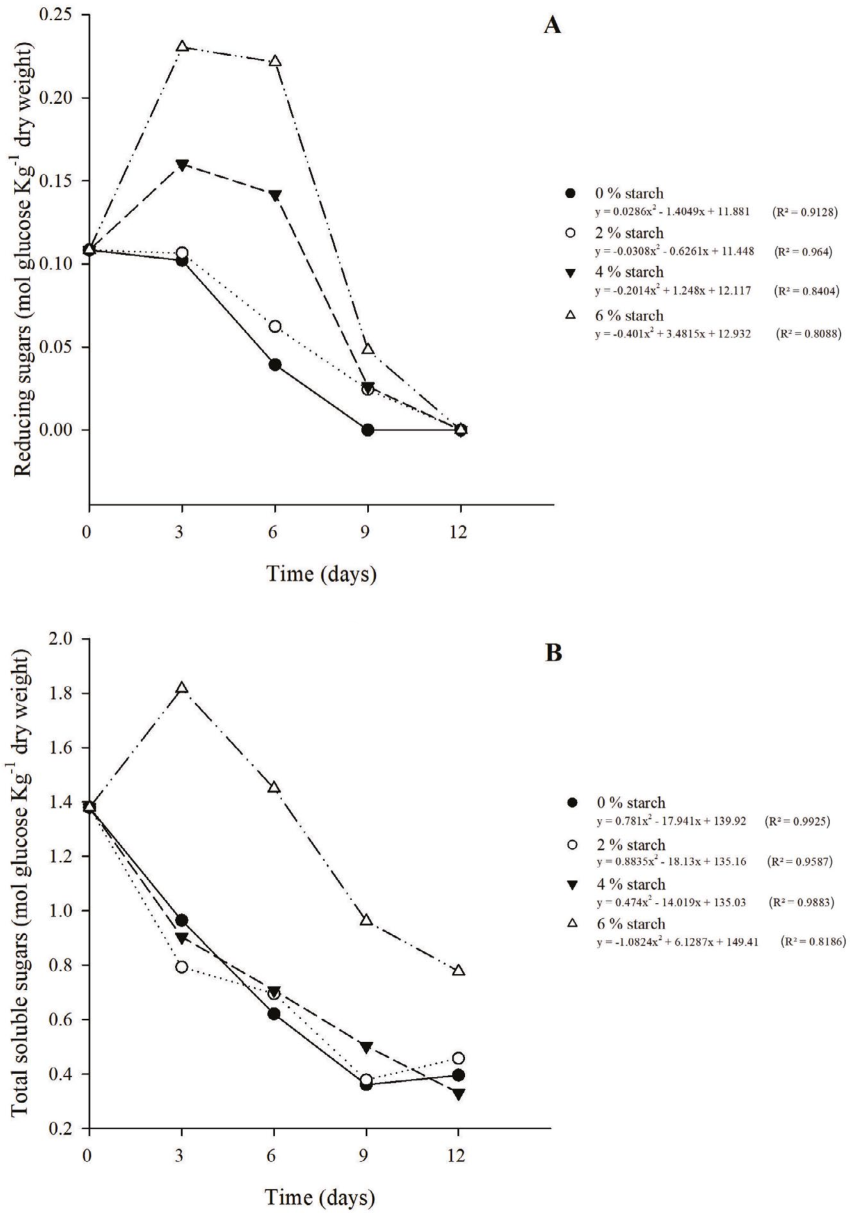

Figure 14: Reducing (A) and total sugars (B) over time in Red Torch ginger inflorescences coated with films of different starch concentrations.

As a consequence of the respiration process, senescence accelerates, so the flower loses visual qualities. With the progression of senescence, there was a loss of tissue turgidity related to the consumption of proteins responsible for maintaining the integrity and cellular structure. In consequence, the defence system was weakened, due to phenolic compounds and antioxidants, making the inflorescences more susceptible to the attack of pathogens and losses (Costa et al., 2021).

The use of cassava starch-based coatings (6%) efficiently reduced gas exchange and thereby reduced respiration, maintaining lower levels of H_2O_2 and lipid peroxidation, slowing the oxidative process and causing less damage to the cell membrane during conditioning. In addition, it was efficient at maintaining higher levels of proteins and sugars until the day 6 after application of the films, favouring quality and durability maintenance.

CONCLUSIONS

The use of cassava films is a promising technique to maintain the post-harvest quality of Red Torch ginger, being recommended 6% cassava starch, acting as a barrier to gas exchange and water loss by transpiration. The addition of glycerol is recommended since improves visual characteristics and resistance to deterioration. The use of the adjuvant is not recommended. Lower levels of hydrogen peroxide and lipid peroxidation and higher levels of macromolecules up to 6th day after application were observed.

AUTHOR CONTRIBUTION

Conceptual idea: Nogueira, M.R; Paiva, P.D.O.; Reis, M.V. Methodology design: Nogueira, M.R.; Paiva, P.D.O.; Cunha Neto, A.R.; Reis, M.V. Data collection: Nogueira, M.R.; Cunha Neto, A.R.; Nascimento, A.M.P.; Timoteo, C.O. Interpretation: Nogueira, M.R.; Paiva, P.D.O.; Cunha Neto, A.R.; Reis, M.V.; Nascimento, A.M.P.; Timoteo, C.O. Writing and editing: Nogueira, M.R.; Paiva, P.D.O.; Cunha Neto, A.R.; Reis, M.V.; Nascimento, A.M.P.; Timoteo, C.O.

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