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Biodegradable pitomba starch film applied to stored coalho cheese

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ABSTRACT

Non-conventional starches are promising for application in biodegradable food packaging, contributing to the minimization of the impacts caused by conventional plastics. This study aimed to develop biodegradable films based on pitomba (Talisia esculenta) seed starch incorporated with *Cinnamomum cassia* L. essential oil and to evaluate their influence on the refrigerated storage of queijo coalho. The starch was extracted from pitomba seeds, and the films were produced using the casting technique through starch gelatinization with glycerol, containing 0% (F1), 1% (F2), 1.5% (F3), and 2% (F4) cinnamon essential oil (CEO). The films were characterized in terms of their physical and mechanical properties and applied as packaging for fresh coalho cheese stored under refrigeration for 14 days. The incorporation of cinnamon essential oil (CEO) significantly influenced the behavior of the films, resulting in increased thickness, opacity, and water vapor permeability, as well as intensified yellow coloration, without causing relevant changes in solubility. Among the evaluated formulations, F2 stood out by presenting higher tensile strength, while increasing the CEO concentration to 1.5% (F3) led to a reduction in this property. When applied to coalho cheese, the films containing CEO contributed to reducing mass loss and dehydration, as well as decreasing total and thermotolerant coliform counts. Formulations F2 and F3 were the most effective in preserving the physicochemical and microbiological characteristics during refrigerated storage of coalho cheese.

Contribution/Originality: This study contributes to the literature on the development of active biodegradable films from unconventional polymeric sources, demonstrating their effectiveness in preserving the physicochemical and microbiological quality of queijo coalho and highlighting their potential as a sustainable alternative to conventional plastics for food packaging.

1. INTRODUCTION

Food packaging plays an essential role in the protection, storage, preservation, and safe distribution of products. Despite its importance, the materials used in its production have become one of the main sources of environmental pollution, as they are predominantly composed of non-degradable synthetic polymers, such as plastics (Karmakar et al., 2024; Shaikh, Yaqoob, & Aggarwal, 2021).

The development of environmentally friendly packaging has become a promising alternative to reduce the disposal of toxic and non-biodegradable plastics. The use of natural ingredients, such as polysaccharides, as substitutes for petroleum-derived materials favors the production of biodegradable and environmentally sustainable packaging. Among these polysaccharides, starch stands out as an emerging material due to its low cost and wide availability in nature, in addition to exhibiting properties such as tensile strength, biodegradability, and ease of processing in the production of biofilms (Marichelvam et al., 2022; Tien, Nguyen, Le, Khoi, & Richel, 2023).

Since conventional starches, mainly extracted from corn, cassava, rice, and potato, often require chemical or physical modifications to meet industrial requirements (Nogueira, Carvalho, Velasco, & Matta Fakhouri, 2025), alternative starch sources have shown promise, contributing to the reduction of industrial exploitation of conventionally used species (Wilfer et al., 2021). Pitomba (*Talisia esculenta*), a fruit native to the Western Amazon and cultivated in a non-systematic manner in the North and Northeast regions of Brazil, faces challenges in technological utilization due to difficulties in pulp removal and low yield. However, the pitomba endocarp, which accounts for approximately 50% of the total fruit weight, presents a starch extraction yield of 44%, making it a promising source of non-conventional starch (Castro et al., 2021; Neri-Numa et al., 2014).

The use of essential oils associated with biodegradable packaging has attracted interest as a natural food preservative alternative, since they contain functional and bioactive compounds. Cinnamon essential oil (*Cinnamomum cassia* L.) exhibits high biological activity, including antibacterial and antioxidant properties, which can favor the reduction of microbial growth in foods during storage, thereby contributing to the preservation of perishable products (Perdana, Ruamcharoen, Panphon, & Leelakriangsak, 2021; Zhou, Wu, Chen, & He, 2021).

Cheese is a perishable product due to its high-water activity, which favors microbiological and biochemical changes from production to consumption (Ceylan, Polat, & Atasoy, 2025; Pal & Agarwal, 2024). Among the various types of cheese, *queijo coalho*, characterized as a medium- to high-moisture cheese, is obtained by milk coagulation using rennet or other appropriate coagulant enzymes and is usually marketed within up to 10 (ten) days after manufacture, a period during which it still exhibits fresh cheese characteristics (Costa et al., 2020). Therefore, the use of biodegradable packaging with active ingredients capable of extending storage time while maintaining the fresh characteristics of *queijo coalho* represents a challenge for food packaging technology.

Despite the recent interest in developing biodegradable food packaging with active properties, few studies have explored the use of non-conventional starch sources, such as pitomba (*Talisia esculenta*), combined with natural bioactive compounds for the preservation of fresh and perishable foods. Thus, this study aimed to develop biodegradable films based on pitomba (*Talisia esculenta*) seed starch incorporated with *Cinnamomum cassia* L. essential oil and to evaluate their influence on the refrigerated storage of *queijo coalho*.

2. MATERIALS AND METHODS

Pitomba fruits (*Talisia esculenta* (A. St.-Hil.) Radlk.) were collected from a rural property located in the municipality of Lagoa Seca, Paraíba, Brazil (7°08'48.9"S, 35°51'13.8"W). After harvest, the fruits were selected based on their brownish-yellow coloration and transported to the agro-industrial complex of the Escola Agrícola Assis Chateaubriand (UEPB, Campus II), where they were washed with running water and sanitized in an active chlorine solution (10 ppm for 15 min).

The peel was removed by manual pressure, and depulping for seed separation was performed according to Castro et al. (2021), consisting of immersion in 8% NaOH for 30 min, followed by rinsing and neutralization in 4% citric acid for 30 s. Cinnamon essential oil (*Cinnamomum cassia* L.; CEO) was purchased from the company Via Aroma.

2.1. Starch Extraction

Starch extraction was performed according to the method described by Loos, Hood, and Graham (1981). The seeds were immersed in a 0.2% sodium metabisulfite solution (1:2 m/v) and maintained at 4 °C for 24 h, after which they were ground and filtered through muslin cloth. The filtrate was allowed to stand for 1 h to allow starch paste decantation, followed by two washes with 0.2% sodium metabisulfite solution and one wash with absolute ethanol (95%). Subsequently, the material was dried in an oven at 50 °C for 24 h and then ground to obtain the starch powder.

2.2. Film Preparation

The films were prepared using the *casting* technique by dissolving 4 g of pitomba starch in 100 mL of distilled water containing 2% glycerol. The solution was subjected to gelatinization (95 °C for 10 min), followed by cooling to 50 °C. The CEO was added according to the proportions shown in Table 1, previously diluted in 0.1% Tween 80. The formulations were carefully spread onto acrylic plates (140 × 212 mm) and dried in an oven at 40 °C for 20 h.

Table 1. Composition of pitomba starch films and CEO.

Formulation	Composition
F1	Starch 4% + glycerol 2%
F2	Starch 4% + glycerol 2% + CEO 1% (homogenized with 0.1% Tween 80)
F3	Starch 4% + glycerol 2% + CEO 1.5% (homogenized with 0.1% Tween 80)
F4	Starch 4% + glycerol 2% + CEO 2% (homogenized with 0.1% Tween 80)

2.3. Physical Properties of the Films

2.3.1. Thickness

Film thickness was determined by measuring 10 different points of each sample using a digital micrometer (Zaas model) with a precision of 0.001 mm.

2.3.2. Opacity

Film opacity was analyzed according to a standard method, with modifications, from the British Standards Institution (BSI), as described by Mali, Grossmann, García, Martino, and Zaritzky (2004). The films were cut into 2 × 2 cm pieces and placed in the spectrophotometer cuvette. Measurements were performed at 600 nm, using air as the reference. Each sample was analyzed in triplicate. Opacity was calculated using Equation 1.

$$Opacity = \frac{Abs_{600}}{x} \qquad (1)$$

Where:

$Abs_{600} =$ Absorbance value of the film at 600 nm.

$x =$ Film thickness.

2.3.3. Solubility

Water solubility was determined according to the method described by Hosseini, Razavi, and Mousavi (2009). Film samples (10 mm × 30 mm) were dried in a forced-air circulation oven at 110 °C for 24 h to determine the initial dry mass. Subsequently, the samples were immersed in 50 mL of distilled water at 25 °C and subjected to agitation for 24 h, after which they were dried again at 110 °C for 24 h to obtain the final dry mass (msf). Solubility (%) was calculated according to Equation 2.

$$S\ (\%) = \left(\frac{msi - msf}{msi}\right) * 100 \qquad (2)$$

2.3.4. Water Vapor Permeability

Water vapor permeability (WVP) was determined according to the ASTM E96 procedure, as described by Wu et al. (2021). The films were placed over acrylic cups containing silica gel and maintained for 24 h at 25 ± 2 °C in the presence of a saturated NaCl solution, in order to establish a relative humidity gradient between the cell and the environment of 0%/75%. Measurements were performed in quintuplicate. Initially, the water vapor transmission rate (WVTR) was determined using Equation 3, followed by the calculation of WVP according to Equation 4.

$$\text{WVTR}\ (g.m^{-2}.dia^{-1}) = \frac{w}{t*A} \qquad (3)$$

$$\text{WVP}\ (g.mm.m^{-2}.dia^{-1}.kPa^{-1}) = \text{WVTR} * \frac{e}{Ps} * (UR_1 - UR_2) \qquad (4)$$

Where:

PS = Water vapor saturation pressure (kPa).

UR_1 = Relative humidity of the external environment (desiccator).

UR_2 = Relative humidity inside the cell.

e = Film thickness (mm).

2.3.5. Color Parameters

Color parameters were analyzed in triplicate using a portable WR 10 QC colorimeter, previously calibrated with a white standard plate (X = 80.5; Y = 85.3; Z = 90.0). The CIELab parameters (L*, a*, and b*) were obtained, where L* represents lightness (L* = 0, black; L* = 100, white), a* indicates the axis from green (–) to red (+), and b* represents the axis from blue (–) to yellow (+).

2.4. Mechanical Properties of the Films

The mechanical properties of the films were analyzed using a texture analyzer (TA.XT model, Stable Micro Systems), following ASTM D882-02 (ASTM International, 2001). Tests were performed at 25 °C, with an initial grip distance of 20 mm and a crosshead speed of 50 mm·min^{-1}. Samples were cut into strips (50 × 20 mm) and analyzed in quintuplicate for each formulation. Tensile strength (TS) and elongation at break (EB) were calculated using Equations 5 and 6.

$$TS = \frac{Fm}{A} \qquad (5)$$

$$EB = \frac{(\text{df})}{dg}.\ 100 \qquad (6)$$

Where:

TS = Tensile strength (MPa).

Fm = Maximum force at the moment of film break (N).

A = Cross-sectional area of the film (mm^2).

EB = Elongation at break.

df = Final extension length of the film (mm).

dg = Initial grip distance (mm).

2.5. Application of the Films

The films were applied to fresh coalho cheese, produced from cow's milk subjected to pasteurization (65 °C for 30 min), followed by cooling to 35 °C, when calcium chloride (40%) and liquid coagulant (chymosin) were added. The milk was incubated at 35 °C until coagulation was complete. The resulting curd was cut into cubes (2 cm), drained, salted, molded, and pressed, yielding a cheese with an average moisture content of 47%.

Cheese samples (20 g each) were divided into four treatments: F1 (Starch 4% + glycerol 2%), F2 (Starch 4% + glycerol 2% + CEO 1% + 0.1% Tween 80), F3 (Starch 4% + glycerol 2% + CEO 1.5% + 0.1% Tween 80), and F4 (Starch 4% + glycerol 2% + cinnamon essential oil 2% + 0.1% Tween 80). Each treatment had four replicates, which were individually packaged and sealed with the respective films and stored at 8 °C in a B.O.D. incubator for 14 days. Evaluations were performed at time zero and every 7 days, considering the parameters of pH, mass loss, moisture content, water activity, acidity, ash, total coliforms, and thermotolerant coliforms.

Mass loss (%) of the cheese was determined according to Equation 7.

$$ML(\%) = \frac{W_0 - W_t}{W_0} x100 \qquad (7)$$

Where:

ML = Mass loss (g).

W_0 = Initial sample mass (g).

W_t = Final sample mass at the evaluation time (g).

The pH was determined by direct reading using a pH meter (DL-PH model). Moisture content was evaluated by drying the samples in an oven at 105 ± 3 °C until constant weight, according to the methodology described by IAL – Adolfo Lutz Institute (2008). Water activity was measured by direct reading of the samples at an approximate temperature of 25 °C using a Labmaster aw NEO hygrometer (Novasina). Titratable acidity was determined by the titrimetric method, based on the neutralization of H^+ ions with 0.1 N sodium hydroxide (NaOH) solution, standardized with potassium bitartrate as the titrant (IAL – Adolfo Lutz Institute, 2008). Ash content was obtained by incinerating the samples in a muffle furnace at 550 °C until a residue free of carbon, with a white-gray coloration, was obtained, according to the methodology described by IAL – Adolfo Lutz Institute (2008).

The determination of total and thermotolerant coliforms was carried out using the Most Probable Number (MPN) technique with multiple tubes. This analysis was based on the performance of presumptive and confirmatory tests, according to the methodology described by Silva et al. (2017).

2.6. Statistical Analysis

For the physical properties of the films, a completely randomized design was used, with 4 treatments and 3 replicates (n = 12).

The data from the storage of cheese coated with starch films were subjected to analysis of variance (ANOVA). Based on the significance of the F-test, Tukey's test at 5% probability was applied using the Statistica software, version 5.0® (Silva & de Azevedo, 2016).

The free software MetaboAnalyst v.6.0 (Xia Lab, McGill University, Montreal, Canada) was used to generate the heat maps.

3. RESULTS AND DISCUSSION

3.1. Physical Properties of the Films

The results of the physical properties of the starch films are presented in Table 2. A variation in film thickness (0.146 to 0.196 mm) was observed due to the addition of Cinnamomum cassia L. essential oil (CEO). However, the incorporation of 1% oil (F2) did not result in a statistically significant difference in thickness compared to films with 0% (F1) and 1.5% (F3) essential oil. On the other hand, increasing the oil concentration from 1.5% to 2% (F3 to F4) significantly increased thickness, possibly due to the higher volume in the formulation. Additionally, the oil concentration may affect the gel viscosity due to the formation of hydrophobic microdroplets during the homogenization of the film-forming solution, reducing its spreadability on the plate.

Table 2. Physical properties of starch and CEO Films.

Films	Thickness (mm)	Opacity	Solubility (%)	WVP	Color		
					L*	a*	b*
F1	0.146 c	5.130 b	32.542 a	0.963 c	69.292 a	4.730 c	21.463 d
F2	0.151 bc	9.614 a	33.345 a	0.993 bc	58.733 a	20.425 b	60.092 a
F3	0.167 b	9.774 a	35.223 a	1.142 ab	57.737 a	21.340 b	55.002 b
F4	0.196 a	9.713 a	36.458 a	1.265 a	51.130 a	23.737 a	46.535 c

Note: L*, a* and b*: coordinates of the CIELAB color system.

Sharma et al. (2023), when studying TiO_2 bionanocomposite films, reported that the progressive increase in the amount of cinnamon essential oil causes a slight change in film thickness. Similar thickness values were reported by Criollo-Feijoo, Salas-Gomez, Cornejo, Auras, and Salazar (2024) for cassava bagasse starch films incorporated with oregano essential oil, which showed thickness values ranging from 0.066 mm to 0.164 mm in formulations containing 0 to 2% essential oil, with a strong positive correlation observed between starch film thickness and oil percentage. De Sousa Costa et al. (2025) also reported similar results, in which yam starch films incorporated with cinnamon cassia essential oil exhibited thickness values between 0.10 and 0.18 mm, with a progressive increase as oil concentration increased.

The opacity of pitomba starch films was significantly affected by the incorporation of CEO; however, no statistically significant difference was observed among concentrations ranging from 1 to 2%, despite a tendency toward increased values with higher concentrations. This characteristic may be associated with the refractive index of the oil, which influences light absorption by the film. Such a property favors the use of these films in food preservation by protecting against oxidation, deterioration, and changes caused by light exposure. Mouhoub et al. (2023) also reported an increase in opacity following the incorporation of cinnamon and clove essential oils into chitosan-based films.

The solubility of the films was not significantly affected by the incorporation of CEO, although a clear tendency toward increased solubility was observed as a function of CEO addition. This behavior can probably be attributed to the formation of a heterogeneous starch film structure, capable of forming pores that facilitate water penetration into the film. However, CEO concentrations of 1%, 1.5%, and 2% did not significantly affect the structure of the pitomba starch film. Shen et al. (2022) also reported an increase in solubility from 17.04% to 22.71% in *Dioscorea zingiberensis* starch films when the amount of oregano essential oil was increased from 1% to 3%, associating the inclusion of essential oils with the formation of starch films with heterogeneous structures.

Water vapor permeability (WVP) was significantly influenced by the addition of CEO (Table 2), showing a proportional increase with oil concentration. According to Al-Hashimi, Ammar, Lakshmanan, Cacciola, and Lakhssassi (2020), water vapor permeability is one of the most important factors affecting the quality of food packaging materials, since the applied film should be able to prevent or reduce water transfer between the environment and the food. These authors reported an increase in the WVP of edible millet starch films as the concentration of clove essential oil increased from 1% to 3%, which is consistent with the results obtained in the present study.

The influence of CEO on the color of pitomba starch films is presented in Table 2, in which it can be observed that lightness (L*) was not significantly affected, showing values ranging from 69.262 to 51.130. These values indicate high film lightness, which favors their use in the storage of foods sensitive to light-induced oxidation, such as products rich in fats, oils, or bioactive compounds. High lightness contributes to greater light reflection, reducing food exposure to direct radiation and, consequently, delaying photo-oxidative reactions. Li et al. (2024), when analyzing the lightness of films produced from potato starch, reported values ranging from 61.2 to 65.4, which are close to those found in this study.

The chromaticity parameter a* was significantly influenced by the addition of CEO, indicating an increase in red color intensity (a*+) as the oil concentration increased. The color parameter b* was also significantly affected by the incorporation of CEO, promoting an intensification of the yellow hue in the films. The predominance of yellow coloration in films containing CEO suggests their potential application as packaging materials for light-sensitive foods, which is further supported by the high luminosity values previously reported. Similar results were reported by Abedi, Sayadi, and Oliyaei (2024) for edible films based on emulsions containing cinnamon essential oil and chia seed mucilage, an effect attributed to the increased concentration of cinnamon essential oil.

3.2. Mechanical Properties of the Films

The results of the mechanical properties of the films indicate a significant influence of CEO addition on tensile strength (Figure 1A). The formulation containing 1% CEO (F2) exhibited the highest tensile strength value. However, increasing the CEO concentration to 1.5% and 2% resulted in a reduction of this property, with no statistical difference compared to the control film without CEO. This behavior may be attributed to the ability of the oil to penetrate the polymeric network, thereby reducing intra- and intermolecular interactions. These results suggest that higher CEO concentrations do not promote effective interactions capable of contributing to the mechanical reinforcement of pitomba starch films. Similar tensile strength values were reported by Da Costa et al. (2023) in studies on biodegradable films produced from peach palm (*Bactris gasipaes*) starch.

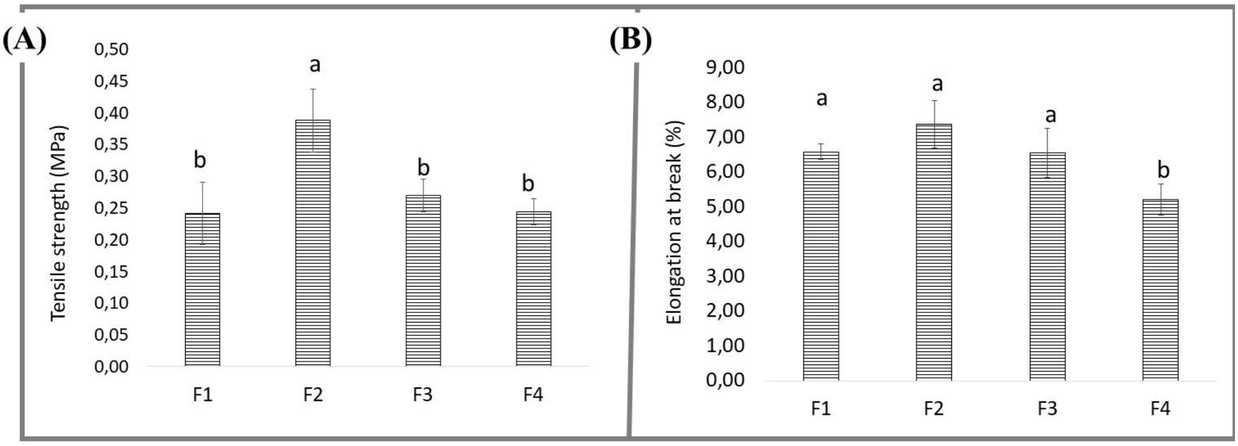


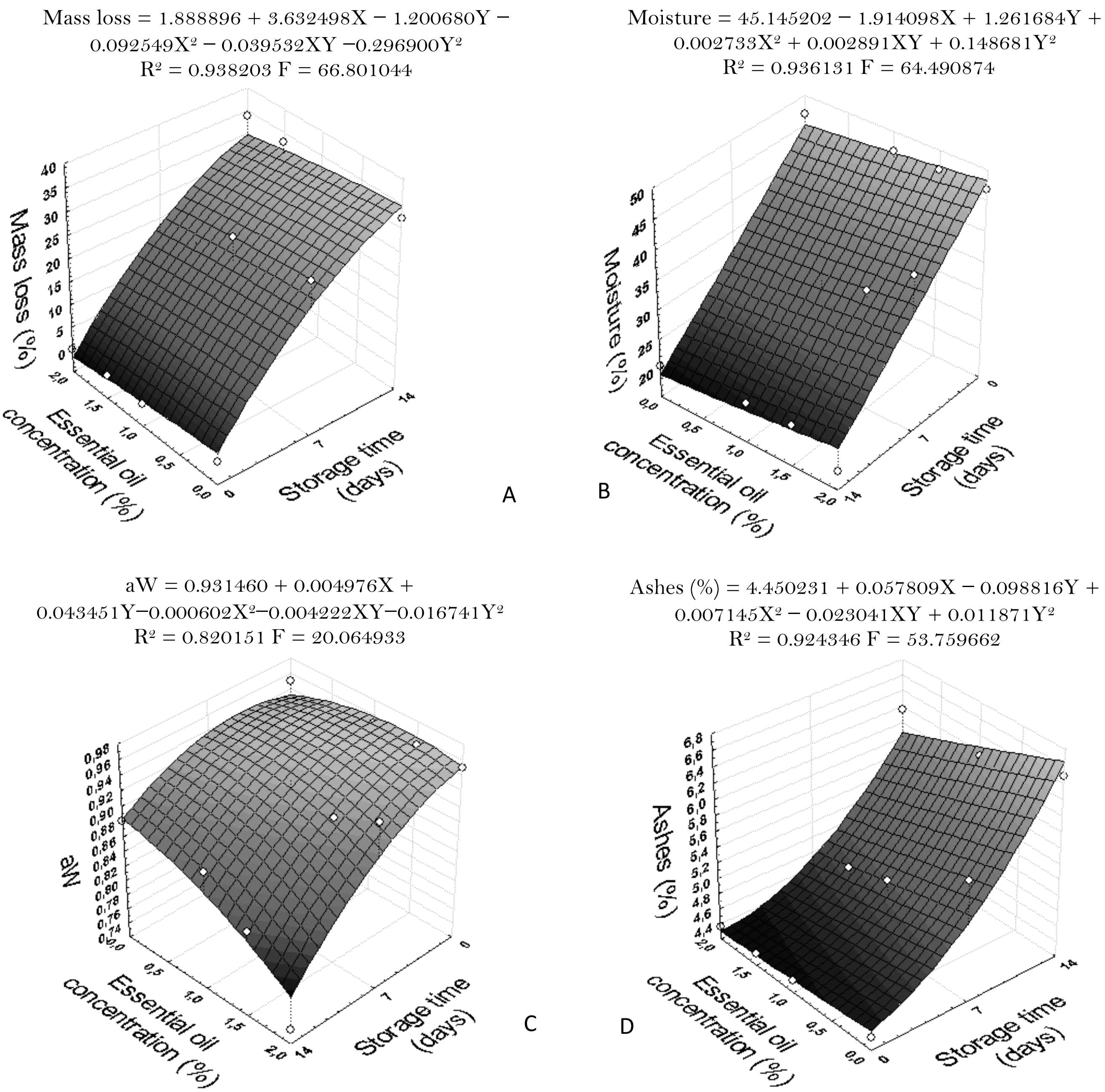
Figure 1. Tensile strength (A) and elongation at break (B) of starch films. Different letters (a–b) indicate significant differences among samples according to Tukey's test ($p < 0.05$).

The elongation at break of pitomba starch films (Figure 1B) was significantly influenced by the increase in CEO concentration from 2% (F4), whereas concentrations of 1% and 1.5% (F2 and F3) did not differ from the control formulation (F1). The low elongation at break values indicate limited elasticity, suggesting the need to increase the glycerol concentration or to add another plasticizer in future formulations to improve this property, since a matrix composed solely of starch exhibits a high-water absorption capacity, which tends to produce more brittle films. Similar results were reported by Syafiq, Sapuan, and Zuhri (2021), who observed a reduction in elongation at break with increasing CEO concentration in sugar palm starch/nanocellulose biocomposite films, corroborating the findings of this study.

3.3. Physicochemical Characterization of Coalho Cheese

The experimental results of the physicochemical evaluation of coalho cheese packaged with starch films and stored at 8 °C for 14 days are presented in Figure 2, while Table 3 shows the statistical comparison among the means. A progressive increase in mass loss was observed over the storage period for all treatments. After seven days, the lowest mass loss values were recorded for cheeses packaged with films containing CEO, which is associated with the ability of the essential oil to reduce the water vapor permeability (WVP) of the film. The lowest loss at this time was observed in cheese packaged with pitomba starch film containing 2% CEO (F4).

After 14 days of storage, the lowest mass loss (29.37%) was observed in cheese packaged with starch film containing 1% CEO (F2), differing statistically from the mass losses obtained for the other formulations (F1, F3, and F4). Similar results were reported by Silva et al. (2023), who observed better performance regarding mass loss in mozzarella cheese coated with a film-forming solution containing loquat leaf extract compared with the control cheese, attributing this behavior to the lower water vapor permeability of the film containing the plant extract.



Acidity: $= 0.055535 + 0.014168X + 0.004904Y - 0.000614X^2 + 0.001178XY - 0.001696Y^2$
$R^2 = 0.812084$ $F = 19.014772$

$pH = 6.866719 - 0.067497x + 0.065577y + 0.004611x^2 - 0.003230xy - 0.018663y^2$
$R^2 = 0.680377$ $F = 64.490874$

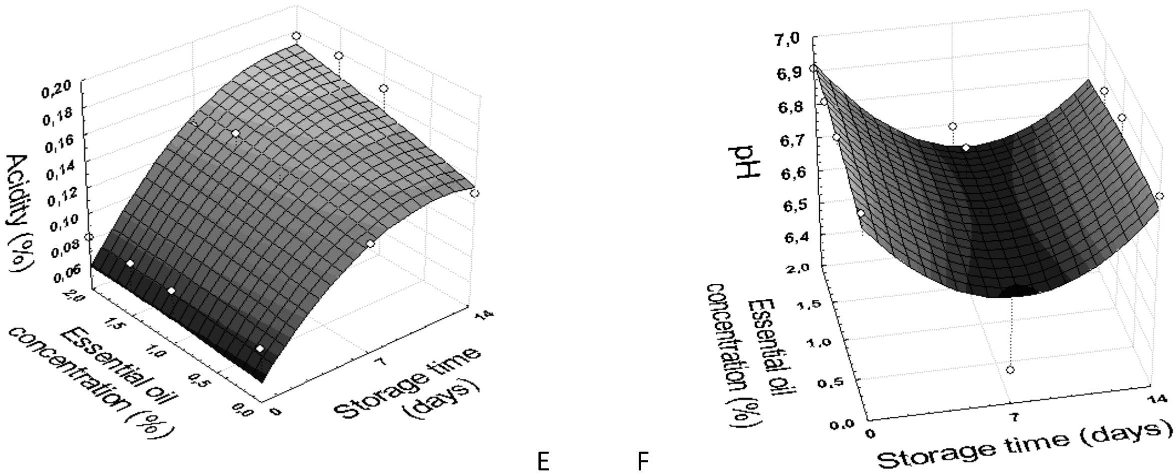


Figure 2. Quadratic multivariate regression of the characterization of mass loss (A), moisture (B), water activity (C), ash (D), acidity (E), and pH (F) of coalho cheeses packaged in pitomba starch and CEO films and stored for 14 days under refrigeration.

The multivariate modeling (Figure 2) showed satisfactory results for all evaluated parameters, with R^2 values higher than 0.68, indicating a high predictive capacity of the fitted models. These results indicate that pitomba starch films incorporated with CEO influenced the kinetic behavior of the physicochemical parameters, with moisture content and mass loss being the most strongly affected by the time × formulation interaction, reinforcing the potential use of these films as active barriers to protect against dehydration.

Table 3. Physicochemical characterization of coalho cheese packaged in pitomba starch films supplemented with CEO at concentrations of 0, 1, 1.5, and 2% and stored for 14 days.

Parameter	Days	Treatments			
		F1	F2	F3	F4
Mass loss	0	0.000 ^{cA}	0.000 ^{cA}	0.000 ^{cA}	0.000 ^{cA}
	7	28.1900 ^{bA}	26.407 ^{aA}	17.683 ^{bB}	12.427 ^{bC}
	14	32.1633 ^{aA}	29.370 ^{aB}	33.123 ^{aA}	33.820 ^{aA}
Moisture	0	46.834 ^{aA}	46.834 ^{aA}	46.834 ^{aA}	46.834 ^{aA}
	7	24.802 ^{bD}	29.383 ^{bC}	35.321 ^{bB}	41.342 ^{bA}
	14	20.299 ^{cA}	21.744 ^{cA}	21.832 ^{cA}	18.113 ^{cB}
Water activity	0	0.950 ^{aA}	0.950 ^{aA}	0.950 ^{aA}	0.950 ^{aA}
	7	0.880 ^{bC}	0.917 ^{bB}	0.917 ^{bB}	0.943 ^{aA}
	14	0.881 ^{bA}	0.878 ^{cA}	0.835 ^{cB}	0.741 ^{bC}
Ashes	0	4.361 ^{cA}	4.361 ^{cA}	4.361 ^{cA}	4.361 ^{bA}
	7	5.738 ^{bA}	5.080 ^{bB}	4.924 ^{bB}	4.505 ^{bC}
	14	6.486 ^{aA}	6.144 ^{aA}	5.471 ^{aB}	6.163 ^{aA}
Acidity	0	0.084	0.083	0.083	0.083
	7	0.174	0.153	0.210	0.193
	14	0.181	0.240	0.250	0.247
pH	0	6.907	6.907	6.907	6.907
	7	6.403	6.533	6.730	6.690
	14	6.863	6.877	6.857	6.737

Note: Lowercase letters indicate differences among columns, and uppercase letters indicate differences among rows.

Fresh coalho cheese presented an initial moisture content of 46.83%, characterizing it as a high-moisture cheese according to the classification established by the Brazil Ministry of Agriculture (1996). Throughout storage, a progressive reduction in moisture content was observed, in agreement with the recorded mass loss, which was influenced by the different concentrations of CEO incorporated into the pitomba starch films. After seven days of storage, the smallest reduction in moisture content was observed in coalho cheese packaged with films containing 2% CEO (F4). However, after 14 days, an acceleration of moisture loss was observed with increasing CEO concentration, which may be associated with the migration of vegetable oil from the film to the cheese surface. This phenomenon reduces molecular interactions within the polymeric matrix of the film, increasing its permeability and, consequently, favoring product dehydration, which may result in sensory changes such as alterations in cheese color and texture. No statistically significant differences were observed in the final moisture content of coalho cheeses packaged in pitomba starch films incorporated with 0%, 1%, and 1.5% CEO at the end of storage. Similar results were reported by Silva, Ribeiro, Teixeira, and Silva (2022), who evaluated the characterization of cheeses coated with alginate-based edible films containing *Lactococcus* strains and stored under refrigeration. The authors observed a progressive reduction in moisture content throughout the storage period; however, the presence of the coating contributed to delaying moisture loss until the eighth day. After this period, an acceleration of moisture loss was observed, resulting, at the end of storage, in values with no statistically significant difference between coated and uncoated cheeses.

Water activity showed a decreasing trend as a function of storage time and increasing CEO concentration in the films (Figure 2C). Among the evaluated formulations, films F1 and F2 demonstrated greater efficiency in preserving cheese water activity throughout the experimental period, suggesting that lower CEO proportions in the films provide a better barrier to moisture migration. The ash content of the cheeses showed a tendency to increase as a function of CEO concentration and storage time, which is probably associated with moisture loss in the samples, resulting in a relative concentration of the minerals present. This phenomenon was also reported by Lee, Jeewanthi, Park, and Paik (2016), who observed an increase in the ash content of Cheddar-type cheeses due to the addition of *Inula britannica* flower extract, which affected the cheese's water-holding capacity. However, no behavior was observed that directly relates to the influence of pitomba starch film formulations and CEO incorporation on the ash content of coalho cheese under the experimental conditions evaluated.

The results for acidity and pH parameters of the stored *queijo* coalho did not show significant differences ($p > 0.05$) among the pitomba starch film formulations or among the evaluated storage periods. Nevertheless, the graphical representation (Figure 2E) indicates a tendency toward an increase in acidity over the storage time, a behavior also reported by Leandro et al. (2021), who observed a progressive increase in titratable acidity in *queijo* coalho, with and without coating based on the residue of *Ziziphus joazeiro* Mart. fruit pulp. However, no significant differences ($p > 0.05$) were identified between the titratable acidity values of coated and uncoated cheeses during 21 days of storage.

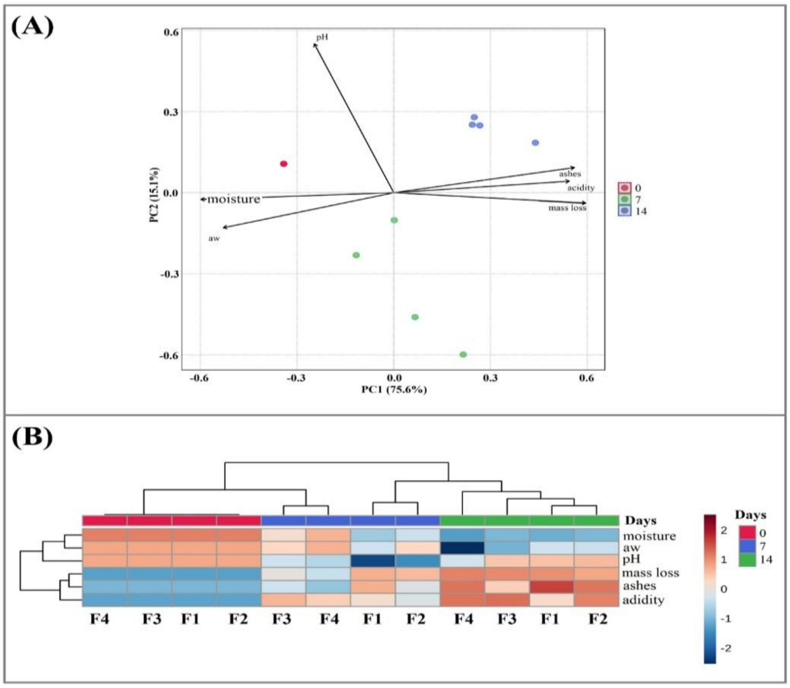


Figure 3. Multivariate analysis of the physicochemical characterization of queijo coalho packaged in pitomba starch films containing CEO at concentrations of 0%, 1%, 1.5%, and 2%, stored for 14 days: (A) Principal Component Analysis (PCA) biplot; (B) Heatmap with hierarchical clustering using Euclidean distance and the Ward method.

The pH values of the cheeses ranged from 6.907 to 6.403, indicating a low probability of microbial activity, likely favored by the storage temperature (4 °C). These results are consistent with the pH values of *queijo* coalho previously reported in the literature (Costa, Maciel, Teixeira, Vicente, & Cerqueira, 2018; Leandro et al., 2021). Silva et al. (2023) also reported stability in pH values in mozzarella cheese samples coated with films based on ripe banana peel and starch, enriched with loquat leaf extract. To complement the analysis of the physicochemical parameters obtained during the storage of *queijo* coalho, principal component analysis (PCA) and Pearson correlation were performed, and the results are presented in Figure 3.

It is observed, in the PCA analysis (Figure 3A), that the samples were grouped according to the storage time. Principal Component 1 (PC1) explained 75.6% of the total data variance, while Principal Component 2 (PC2) explained 15.1%, totaling more than 90% of the variability explained by the first two axes. PC1 was mainly influenced by the variables acidity, mass loss, and ash content, whereas PC2 reflected variations associated with pH and moisture.

Figure 3B, corresponding to the heat map with hierarchical analysis, reinforces the PCA observations by highlighting the proximity among samples belonging to the same storage time, indicating similar physicochemical profiles. Pearson's correlation analysis revealed relevant inverse associations between some variables. Moisture content and water activity showed negative correlations with acidity and mass loss, suggesting that the reduction in water content of the food is accompanied by an increase in acidity and product dehydration during storage.

These results demonstrate the effectiveness of pitomba starch films incorporated with cinnamon essential oil in reducing alterations, especially in formulations F2 and F3, which best preserved the physicochemical characteristics of coalho cheese throughout storage. The results regarding total and thermotolerant coliform counts are presented in Figure 4. A reduction in MPN/g values for total coliforms (Figure 4A) was observed in all samples at the end of the 14 days of storage; however, the coalho cheese packaged in pitomba starch film without CEO addition (F1) showed an increase in values after 7 days of storage, a behavior attributed to the absence of CEO, which may have favored the growth of the initial thermotolerant coliform microbiota during this period. The subsequent reduction in MPN observed at 14 days is possibly related to the depletion of substrates available for microbial metabolism, since new contaminations were likely prevented by the physical barrier provided by the starch film, which limited the direct contact of the cheese with the external environment.

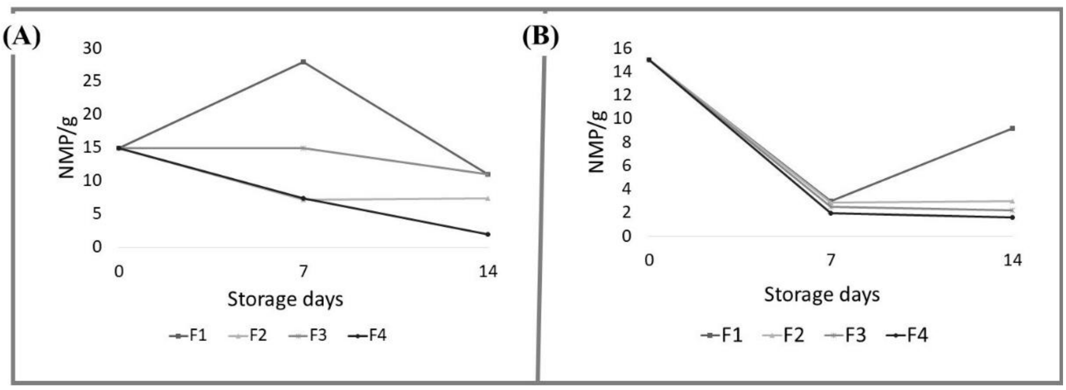


Figure 4. Results of the analyses of total coliforms (A) and thermotolerant coliforms (B) in queijo coalho packaged in pitomba starch films with different CEO concentrations, stored under refrigeration for 14 days.

The incorporation of cinnamon essential oil (CEO) into biodegradable pitomba starch films resulted in a gradual reduction in total coliform counts (MPN/g) in coalho cheese throughout the storage period, with the lowest counts observed in cheeses packaged with films containing 2% CEO (F4). A similar trend was observed for thermotolerant coliforms (Figure 4B), as cheeses coated with films without CEO addition exhibited higher microbial counts after 14 days of refrigerated storage, whereas those packaged with films containing 1%, 1.5%, and 2% CEO (F2, F3, and F4) showed significantly lower values. This antimicrobial effect is mainly attributed to cinnamaldehyde, the major active compound of CEO, whose mechanism of action involves the destabilization of bacterial cell membranes, inhibition of essential enzymes, and denaturation of intracellular proteins, ultimately leading to cell death (Fahrullah, Kisworo, & Noersidiq, 2025; Khazaei, Nateghi, Zand, Oromiehie, & Garavand, 2021).

The results for total and thermotolerant coliforms found in this study were lower than those reported by Leandro et al. (2021) for coalho cheese. This difference can be attributed to the fact that the cheese used in this research was made from pasteurized milk, which significantly reduces the initial microbial load. In contrast, studies using cheeses produced from raw milk, often sourced from informal producers, tend to show higher levels of microbial contamination, as inadequate hygiene practices during milking, processing, and handling can compromise the microbiological quality of the product (Hammad et al., 2022).

4. CONCLUSION

Pitomba seed starch represents an alternative source of polysaccharide for the development of biodegradable films. The incorporation of *Cinnamomum cassia* L. essential oil (CEO) into pitomba seed starch films demonstrated potential for application as an active packaging material for the preservation of coalho cheese stored under refrigeration. The addition of CEO induced changes in the physical and mechanical properties of the films, resulting in increased thickness, opacity, and yellowish coloration, without significantly compromising solubility or luminosity. Although higher concentrations of CEO (2%) reduced the tensile strength and elasticity of the films, the formulation containing 1% CEO showed a balance between mechanical performance and structural stability.

During refrigerated storage of coalho cheese, pitomba starch films incorporated with CEO had a positive effect on reducing mass loss and maintaining moisture and water activity, particularly in formulations F2 and F3 (1% and 1.5% CEO). These effects indicate that moderate CEO content contributes to improving the water vapor barrier, delaying product dehydration. Additionally, a reduction in total and thermotolerant coliform counts was observed in cheeses packaged with films containing CEO.

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