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RESEARCH

Effect of Lachnanthocarpone on the Structural and Functional Properties of a Protein-Starch-Based Film: A Promising Alternative for Active Packaging

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Abstract
Due to their adaptability, plastics are used in everyday products, including food, which need to be protected to retard spoilage reactions like lipid oxidation. However, today, the interest is in developing active food packaging materials with natural compounds such as α-tocopherol and lachnanthocarpone (2,6-dihydroxy-9-phenyl-1*H*-phenalen-1-one). The first is a natural antioxidant that has been successfully incorporated in different films. The second is a powerful natural antioxidant, with more than twice the activity of Trolox in vitro, which has scarcely been explored for its application in food or food packaging. The objective of the study presented here was twofold: first, it intended to evaluate, through a solvent-casting technique, the effects of these two antioxidants on some properties of films based on cassava starch and concentrated whey protein, and second, it aimed to analyze the biodegradability and functionality of lachnanthocarpone for developing active packaging using a commercial fresh-semi-hard cheese as a reference food. Results of the study showed significant differences between the films with antioxidants and the control film (without antioxidants); thus, the latter had the highest light transmittance of the films, indicating that the food was subjected to more significant oxidative damage. On the other hand, the former showed biodegradability characteristics and less quantity of hexanal production after 30 days of storage, which demonstrated its protective effect on the reference food, leading to lower lipid oxidation. These results suggest that the developed films with incorporated antioxidants are a promising alternative for reducing plastic food packaging materials, with the added value of oxidative protection.

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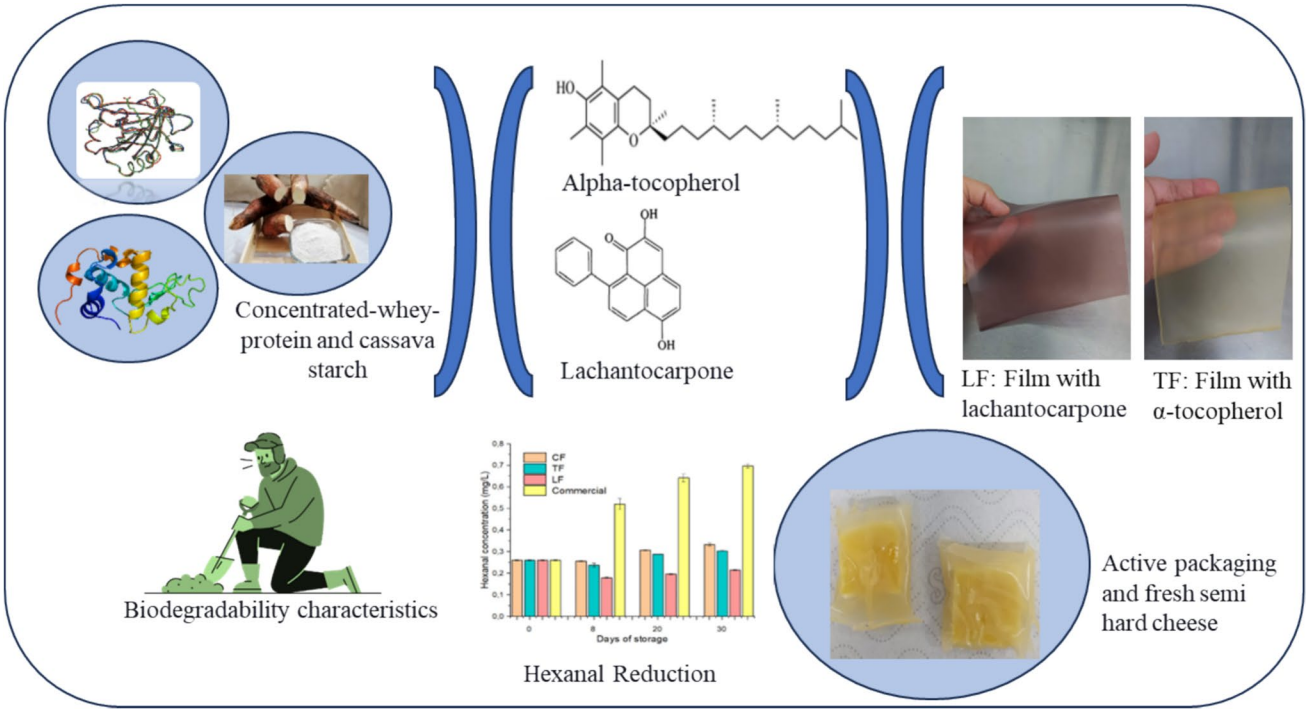
Graphical Abstract



Keywords Antioxidant food packaging · Casting · Hexanal · Films · Tocopherol

Introduction

Petroleum-derived plastics have become a daily occurrence for modern humans, forming an essential part of their basic needs in various aspects such as food packaging (Ait-Oubahou et al., 2019). The increase in food production and the volume of packaging material made from these non-biodegradable raw materials has generated more accumulation of plastics in a single decade than in the previous 40 years (UN Environment Programme, 2020). According to Tucki et al. (2022), in 2020, plastic production reached 367 million tons a year worldwide, with 40% being demanded by the packaging industry (Walker & Fequet, 2023). This production is expected to double in 20 years (Walker & Fequet, 2023), which provides an idea of the amount of waste that will accumulate in the coming years.

Today, there is greater awareness among consumers of the damage generated by the accumulation of single-use packaging materials, and many efforts are being made by governments and institutions to develop recyclable and biodegradable packaging materials. Likewise, the academy is evaluating natural raw materials or materials derived from agro-industrial waste for the development of biopolymers with eco-friendly, renewable, and biodegradable characteristics (Ramakrishnan et al., 2023; Zhang et al., 2024), which means that there is great potential for the development of food packaging materials. This development has already started. In 2020, the global production capacity of biodegradable/bio-based packaging materials was estimated to be at 2.11 million tons; in 2022, it was 2.4 million tons; and by 2025, it will be 2.87 million tons (Amin et al., 2021; Cheng et al., 2024).

There is interest in developing biodegradable food preservation materials based on safe biomolecules such as polysaccharides and proteins (Zhang et al., 2023d). The most researched biopolymers in the packaging area have been polysaccharides due to their easy access, abundance in nature, low cost, non-toxicity, and renewability (Moeini et al., 2021). In particular, cassava starch has stood out for generating rapidly biodegradable, homogeneous, flexible materials that exhibit high transparency, making it an excellent and effective option for obtaining films (Gunathilake & Somendrika, 2024). However, it is important to highlight that starches have some shortcomings, like their hydrophilic character and their poor moisture barrier properties, which generate limits in their application for the development of food packaging. To improve the

properties of these materials without compromising their biodegradability, strategies such as the incorporation of cross-linking agents, nanocomposites, and structural modification of biopolymers, among others, have been applied (Zhang et al., 2023b, c). Another option to improve these shortcomings is to combine them with other non-synthetic compounds such as proteins (Alias et al., 2022), especially those from dairy sources, because these proteins generate denaturation during heating and there is aggregation into insoluble clumps, helping to improve the moisture barrier. Such combinations will make it possible to obtain materials that can compete with the advantages that synthetic packaging materials have had for decades (Papadaki et al., 2022). Currently, whey proteins play an essential role due to their low production cost: they allow the revaluation of a by-product such as whey, positively impact the environment, and provide thermoplastic, mechanical, and oxygen barrier characteristics which are desirable in the development of new packaging materials (Alipour et al., 2023; Alvarez-Perez et al., 2022; Silva et al., 2023). These properties stem from its hydrophobic groups and the disulfide bonds of its structure and can serve as vehicles for many functional ingredients, such as antioxidants, which improve the functionality of packaging materials (Moeini et al., 2021).

In recent years, active antioxidant packaging has also emerged as a solution (Song et al., 2022) and shows great promise in improving food preservation when it is found in packaging materials developed from biopolymers (Zhang et al., 2020), hence the growing interest in incorporating natural antioxidants such as tocopherols, polyphenols, plant extracts, and essential oils into packaging materials (Yildirim et al., 2018). To select these antioxidants, their capacity to capture free radicals and the structural characteristics that allow an adequate interaction with the protein chains of the film must be considered, without inducing adverse effects. This, added to their organoleptic specifications, imposes an "intelligent selection of candidates" criterion. Previously, α-tocopherol had been successfully incorporated in whey protein–based films made through casting methods (Agudelo-Cuartas et al., 2020), showing an excellent barrier property in the UV region; thus, it could be used as a reference to evaluate the effectiveness of other natural compounds. Similarly, lachnanthocarpone (2,6-dihydroxy-9-phenyl-1*H*-phenalen-1-one), an important pigment in the fruit capsules of *Lachnanthes tinctoria*, was one of the first phenylphenalenones isolated from plants (Salazar & Otálvaro, 2024). In 2013, studies demonstrated that lachnanthocarpone is a powerful antioxidant with more than twice the activity of Trolox in vitro. Additionally, its aromatic structure simulates a hydrophobic steroid mimetic arrangement similar to that of the ABD rings of the steroidal nucleus, allowing it to interact selectively with proteins such as beta-lactoglobulin (Duque et al., 2013). To date, no study has been carried out to evaluate its application as a natural antioxidant in foods or natural food packaging.

The central objective of the present study was to evaluate the effects of antioxidant incorporation (α-tocopherol and lachnanthocarpone, separately) on some mechanical, microstructural, and functional properties of films, based on concentrated whey protein and cassava starch, and analyze the potential of lachnanthocarpone for developing biodegradable antioxidant packaging and their functionality. These films were fabricated and characterized for the first time using the solvent casting technique.

Materials and Methods

Materials

For the development of the study, concentrated whey protein (WPC; 80% m/m), supplied by the company Ingredients and Functional Products (IPF) (Itagüí, Antioquia, Colombia), and native cassava starch (with an apparent amylose content of 21.65%), supplied by the company Poltec S.A.S (La Estrella, Antioquia, Colombia), were used. The antioxidants α-tocopherol (Sigma-Aldrich, USA) and lachnanthocarpone, supplied by the SIMBIOMENA research laboratory at Universidad de Antioquia (UdeA, Medellín, Colombia), were incorporated. Finally, polysorbate 80 (Tween® 80), sorbitan monostearate (Span® 60) (Sigma-Aldrich, USA), and glycerol (J.T.Baker, Germany) were incorporated to improve the plasticity of the material.

Lachnanthocarpone Safety Testing

Considering that lachnanthocarpone has not been evaluated directly in food or in biodegradable food packaging, a safety test of the compound was initially included. It was carried out using in vitro cytotoxicity tests with cells similar to those present in the skin around the mouth, to determine its safety when ingested. The safety testing was performed following the provisions of the ISO 10993–5:2009 Biological Evaluation of Medical Devices—Part 5: Test for In vitro Cytotoxicity Standard, which is the internal procedure PGLH 043 for the biological evaluation of an extract or medical devices. The (HaCat) cell line, which is highly sensitive to human epidermal keratinocytes and derived from non-malignant human keratinocytes, was used. The in vitro cytotoxicity assay was made, employing the tetrazolium-based colorimetric method (MTT). The cells were exposed to 50 mg/l, 100 mg/l, and 150 mg/l concentrations of lachnanthocarpone for 24 h. Likewise, the cytotoxicity of the compound, once incorporated into the film, was evaluated. The results correspond to the average of three evaluations.

Lachnanthocarpone Antioxidant Activity

The antioxidant capacity of pure lachnanthocarpone and the compound incorporated in the film were measured using the oxygen radical absorbance capacity (ORAC) method. In this assay, the oxidative degradation kinetics of fluorescein are monitored at wavelengths of 485 nm and 520 nm, respectively, and the reaction is quantified based on a calibration curve of the Trolox standard (hydrophilic analogue of alpha-tocopherol). For sample preparation, 20 mg of lachnanthocarpone was taken and brought to 2.0 ml with 70% ethanol as solvent, and centrifuged for 10 min at 13,000 rpm. The supernatant was diluted 1 in 1000 using phosphate buffer pH 7.3, and this dilution was analyzed. For the film sample, cm^2 of sample films (≈1 g) were cut and weighed, then 45 ml of 70% ethanol was added and subjected to bath ultrasound for 40 min and was homogenized in an Ultraturrax®. This homogenate was centrifuged at 9000 rpm for 20 min. Finally, the supernatant was completed to 50 ml and then diluted 1 in 50 using a pH 7.3 phosphate buffer. This dilution was subjected to analysis. The results were expressed as µmol of Trolox equivalent per 100 g of the sample (µmol TE/100 g sample) as an average of three measurements.

Film Preparation

Control Film (CF)

The film-forming solution (FFS) without antioxidants was prepared with the methodology proposed by Agudelo-Cuartas et al. (2023) with some modifications: 10.0 g of WPC was dissolved in 80.0 g of distilled water under continuous stirring at 750 rpm during 3.0 min, and at 25 °C, the pH was adjusted to 7 with an aqueous solution of 0.1 M NaOH. Simultaneously, a solution of 5.0 g of starch, 5.0 g of glycerol, and enough distilled water to complete 100.0 g was prepared with constant stirring and homogenized using an Ultraturrax® IKA T25 homogenizer (Labotechnik, Germany) for 5 min at 3500 rpm. The thermal treatment of the solution was made at 67.0 ± 0.5 °C, using a water bath (Lauda Alpha, Germany) and mixing constantly for 20 min. Then, the solution was cooled down in an ice bath until it reached room temperature.

Film with α-Tocopherol Incorporation (TF)

To facilitate the incorporation of α-tocopherol into the film, an organic phase composed of 0.43 g of α-tocopherol, 0.26 g of Span 60, and 0.22 g of ethanol was prepared and mixed with a magnetic stirrer for 10 min at 35 °C. Simultaneously, the aqueous phase of 34.0 g of distilled water and 0.42 g of Tween 80, mixed with a magnetic stirrer for 10 min at 25 °C, was prepared. Subsequently, the two phases were mixed and homogenized using an Ultraturrax® IKA T25 homogenizer (Labotechnik, Germany) for 5 min at 3500 rpm, and left to rest for 3 min. These conditions for the incorporation of α-tocopherol into the film were previously optimized by Agudelo-Cuartas et al. (2023).

It has been previously stated that α-tocopherol at a concentration of 2% is sufficient to generate antioxidant activity (Granda-Restrepo et al., 2014). The film-forming solution (FFS) with α-tocopherol was prepared similarly to the control film with 10.0 g of WPC dissolved in 25 g of distilled water under continuous stirring at 750 rpm for 3.0 min at 25 °C and with pH adjusted; 32.5 g of α-tocopherol homogenized was added simultaneously with a solution of 5.0 g of starch, 5.0 g of glycerol, and enough distilled water to complete 100.0 g. The solution was prepared with constant stirring and homogenized using an Ultraturrax® IKA T25 homogenizer (Labotechnik, Germany) for 5 min at 3500 rpm. The thermal treatment of the solution was at 75.5 ± 0.5 °C, using a water bath (Lauda Alpha, Germany) and mixing constantly for 20 min. Then, the solution was cooled down in an ice bath until it reached room temperature (25 ± 2 °C).

Film with Lachnanthocarpone Incorporation (LF)

The antioxidant lachnanthocarpone is in the form of crystals; hence, to facilitate their incorporation into films, it is necessary to dissolve them with ethanol. This is why for this study, a solution of lachnanthocarpone-ethanol (SLE) at 100 mg/l was prepared and stored in refrigerated conditions (≈7 °C) until used. For the preparation of the film-forming solution (FFS) with lachnanthocarpone, the methodology proposed by Agudelo-Cuartas et al. (2023) was followed with some modifications which were similar to those made to the control film; 10.0 g of WPC was dissolved in 80.0 g of distilled water under continuous stirring at 750 rpm for 3.0 min and at 25 °C, and the pH was adjusted to 7 with an aqueous solution of 0.1 M NaOH. Then, a solution of 0.7 g of SLE was added and, simultaneously, another solution of 5.0 g of starch, 5.0 g of glycerol, and distilled water to complete 100.0 g was added. The whole process involved constant stirring and then homogenization. The thermal treatment of the solution was at 67.0 ± 0.5 °C, using a water bath and mixing constantly for 20 min. Then, it was cooled down in an ice bath until it reached room temperature. All the films (CF, TF, and LF) were made by pouring this resulting mixture into glass molds (17 cm × 17 cm) and spreading the mixture with an ink draw rod. Finally, they were dried at 45 °C for 10 h in an air oven (Binder FD 56, Germany).

Film Characterization

Thickness, Moisture Content, and Water Solubility

Thickness of films was determined by taking ten random measurements from the surface with a digital

micrometer (Mitutoyo 293–230-30 MDC-25 MX, Japan). Then, the arithmetic mean was calculated and the results were expressed in mm (Wang et al., 2022). Moisture content was settled by drying the samples at 105 °C for 24 h, and the constant weight was verified according to Wang et al. (2022), and the results were expressed as the average of three measurements. Finally, solubility in water was established by immersing the dried samples obtained in the moisture content analysis in 50 ml of distilled water for 24 h at room temperature (≈25 °C). These samples were then removed from the water and dried in an air oven (105 °C) overnight. The water solubility was calculated as the ratio of the solid mass of the supernatant to the amount of the original weight (Luchese et al., 2021). The results were expressed as an average of three measurements.

Mechanical Properties

Mechanical properties of casting film samples were measured in a universal machine (Shimadzu, Autograph AGS-X with a capacity of 5 tons) using tensile tests at room temperature, according to ASTM D882. Samples were prepared with a rectangular cell size of 150 mm × 25 mm. The travel speed of the machine was 10 mm/min. The average of the five measurements was used to express the results.

Barrier Properties

Light Barrier Properties

Light transmittance of the films was determined with a UV–vis recording spectrophotometer (Mapada Instruments, Shanghai, China). A blank cell was used as a control. The films made through casting were cut into dimensions of 10 mm × 40 mm, and the light transmission at 500 nm was measured for each sample (Zhang et al., 2023a). The results corresponded to the average of three evaluations.

Water Vapor Permeability (WVP)

The water vapor permeability tests were carried out for the samples considering the gravimetric method specified in the ASTM E-96 standard. Each sample was cut into circles with a diameter of 35 mm similar to that of the cells and an effective permeation area of 0.00096 m^2. Then, the cells were filled with water, and the amount of water lost was measured daily until a constant value (Toas, 1989). The temperature and humidity for test were 20 °C ± 0.5 and 60 ± 2% RH. The analysis was carried out under atmospheric pressure (that is, 640 mmHg (0.84 atm)). The results corresponded to the average of three evaluations.

Oxygen Permeability (OP)

Casting samples' oxygen permeability tests were carried out using a static permeability cell based on the ASTM E2945-14 standard, filling the cells with 100% nitrogen, and measuring the amount of oxygen that was entering the cell through a platinum sensor twice a day during the week. The conditions under which the tests were carried out were 20.0 ± 0.5 °C temperature and 60 ± 2% relative humidity (E35 Committee, 2021). The results corresponded to the average of three measurements.

Microstructure

Microstructure analyses were carried out using a scanning electron microscope (SEM JEOL JSM-6490 LV). Then, the film's surface section and cross-section were evaluated. To evaluate the film's cross-section, liquid nitrogen was applied, and cryofracture was performed. The samples were vacuum coated with gold before analysis, and the tungsten filament was operated at 20 kV (Zhang et al., 2023a).

Biodegradability

The biodegradability test under composting conditions was carried out using the ISO 14855–1:2018 standard "Method according to the analysis of carbon dioxide generated. Part 1: General Method" and ASTM D5338-15:2021 "Method for determining aerobic biodegradation of plastic materials under controlled composting conditions." The films' biodegradability tests were performed with lachnanthocarpone (LF) and α-tocopherol (TF), and they were carried out for 30 days at 58 ± 2 °C where the percentage of biodegradability was obtained from the accumulated carbon dioxide emission concerning the maximum CO_2 potential (D20 Committee, 2021).

Functionality

Functionality is the test that allows evaluating the capacity of the antioxidants α-tocopherol and lachnanthocarpone to delay oxidation in a cheese used as a reference food. A commercial fresh semi-hard cheese with 26% fat and packaged in polyethylene multilayer was acquired from a local supermarket. It was opened, and then 4 cm × 4 cm pieces were cut. Cheese pieces were completely wrapped with a film without antioxidants, as a CF, and with films carrying the antioxidants lachnanthocarpone (LF) and α-tocopherol (TF). Other cheese pieces were completely wrapped with their commercial package. All the cheese samples were stored for 30 days under refrigeration conditions (≈7 °C) and exposed to

continuous incandescent light using an E12 110v 7 W bulb. After 8 days, 20 days, and 30 days of storage, the control, commercial, and active packaging (with antioxidants) were removed from the surface of the cheese samples, and the hexanal concentration was evaluated as a chemical indication to monitor lipid oxidation (Agudelo-Cuartas, 2022; Granda-Restrepo et al., 2009a, b).

For the hexanal quantification, a gas chromatograph (GC) (Agilent 6890 Series, USA), equipped with a flame ionization detector (FID) and a DB-WAX (Crossbonded® Carbowax®) column of 30 m × 0.32 mm internal diameter × 0.25 µm film thickness, was used. The H_2 flow was 40 ml/min in split mode, the injector temperature was 300 °C, and the oven temperature was maintained at 50 °C for 6 min, then increased to 230 °C at a rate of 20 °C/min maintained for 10 min. The hexanal calibration curves (0.05 to 5 mg/l) were prepared, and the handling of cheese samples was carried out according to Agudelo-Cuartas (2022). The results of four treatments (commercial, CF, LF, TF) were plotted for hexanal concentration (in mg/l) against storage time (in days). The results were expressed as the average of three measurements for the day and the treatment.

Statistical Analysis

The results of each analysis were expressed as means ± standard deviation from the indicated estimations (at least in triplicate). An analysis of variance (ANOVA) with Fisher's least significant difference (LSD) test was used to determine significant differences among the means at a significance level of $p < 0.05$ using Statgraphics® Centurion XVII V17.2.00 software (StatPoint Technologies, Inc., USA).

Results and Discussion

Lachnanthocarpone Safety Testing

As seen in Fig. 1, the cell viability results for concentrations of 50 mg/l, 100 mg/l, and 150 mg/l of the lachnanthocarpone compound were 97 ± 3%, 86 ± 6%, and 68 ± 4%, respectively. Since the reduction of cell viability by more than 30% is considered a cytotoxic effect, these results suggested that direct contact with the compound or its incorporation at 50 mg/l and 100 mg/l concentrations in the polymer matrix would not have a potential cytotoxic effect. For this reason, it was decided to use a concentration of 100 mg/l for further studies.

Once the films were available, both the viability of the HaCat line cells in contact with films with incorporated lachnanthocarpone (100 mg/l) (LF) and the viability of control film without antioxidant (CF) were evaluated. Cells without contact with the sample materials were also evaluated and called untreated cells (NT). The results showed percentages of viability equal to 100 ± 1%, 100 ± 1%, and 100 ± 7%, for NT, CF, and LF, respectively, as seen in Fig. 2. These results suggest that the component exerts its activity safely in the polymer matrix, presenting a cell viability of almost 100%, similar to the cell viability obtained for the film and cells not treated with this compound (NT), which is favorable to its use as an antioxidant in polymeric materials that will be in contact with food.

Lachnanthocarpone Antioxidant Activity

When evaluating the antioxidant capacity of the lachnanthocarpone compound, values of 816,316 ± 27,120 µM

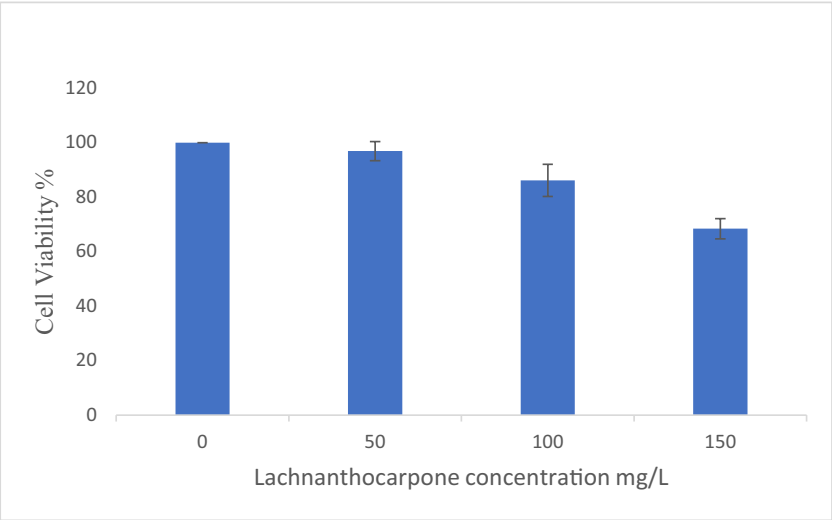


Fig. 1 Cell viability using different lachnanthocarpone concentrations

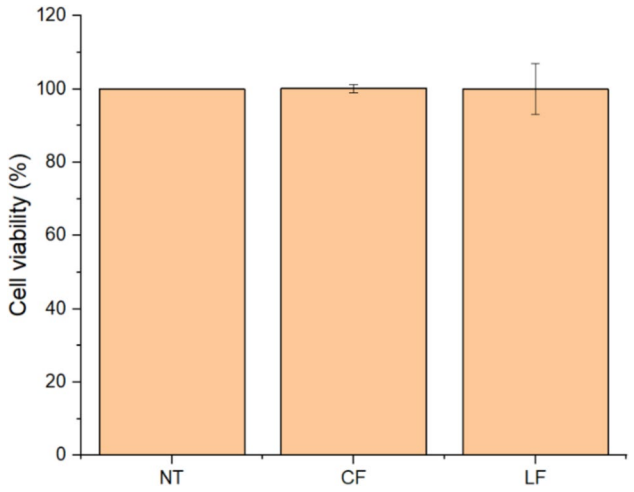


Fig. 2 Cell viability for a control film without antioxidant (CF) and with lachnanthocarpone (LF) and untreated cells (NT)

TE/100 g sample were found. These values are much higher than those reported for 65 samples, including vegetables, fruits, vegetable products, and health supplements with content ranging from 720 µM TE/100 g to 310,878 µM TE/100 g (Thuy et al., 2021). Other authors, when evaluating the total antioxidant capacity of meat and meat products consumed in a reference "Spanish standard diet," reported values from 797 to 4890 µmol TE/100 g. Specifically, the highest ORAC value was found in cured meat samples, in Iberian cured ham (4890 ± 443 µmol TE/100 g), whereas the lowest level of 797 ± 68 µmol TE/100 g was found in Frankfurt sausages. Products like mortadela with olives, sobrasada, and salami showed intermediate values ranging between 1107 ± 123 µmol TE/100 g and 1011 ± 63 µmol TE/100 g (Martínez et al., 2014). The structure of lachnanthocarpone (2,4-dihydroxy-9-phenyl-1*H*-phenalen-1-one), being a polyhydroxylated aromatic, gives it the possibility of reacting by transferring hydrogen atoms (HAT) where the phenyl rings and hydroxyl groups participate, generating a coordinated system that blocks oxidation reactions (Duque et al., 2013). On the other hand, when evaluating the antioxidant capacity in the film containing concentrated whey protein, cassava starch, glycerol, and lachnanthocarpone (100 mg/l) (LF), values of 16,273 ± 305 µmol TE/100 g sample were quantified, which indicates that the antioxidant capacity of lachnanthocarpone remains after the interaction processes with the other components of the material and the drying process to which the film was subjected. This value is even higher than the one reported for red wine (3135 ± 312 µmol TE/100 g) (Martínez et al., 2014). This indicates the potential of lachnanthocarpone to be used as an antioxidant compound in packaging materials for food preservation.

Film Characterization

Thickness, Moisture Content, and Water Solubility

Table 1 shows that the thickness of the films with antioxidant incorporation TF and LF presented statistically significant differences from the control film without antioxidants. These differences in thickness may be due to the form of incorporation of the antioxidant; that is, the α-tocopherol was incorporated through an emulsion and in a higher concentration than the lachnanthocarpone, which was incorporated as a solution with ethanol and at a lower concentration. Therefore, it is possible that the TF was thicker and the LF was thinner. This was seen in the study carried out by Tsironi et al. (2022). The authors demonstrated that since the concentration of active components such as essential oils increased in films based on whey protein isolate, a greater film thickness was also obtained. Thickness is related to some parameters that influence the conservation of packaged foods, such as light transmission, water vapor permeability, and some mechanical properties. Regarding moisture content (%), there were no significant differences between TF and LF. However, it was observed that the addition of antioxidant components decreased the moisture content compared to CF, possibly due to their hydrophobic nature.

The films' integrity under high moisture usually depends on water solubility. In this study, water solubility (%) from LF was not significantly different from CF but presented significantly lower values than TF (Table 1). This solubility index provided information about the behavior of the films in an aqueous environment and was a factor that influenced their biodegradability (Martins et al., 2012). However, the TF's solubility was greater than that of the LF and CF, which indicated that its hydrophilicity was not similar, presenting a lower integrity in water. This can be attributed to the interaction force of α-tocopherol with the polymer matrix in aqueous medium being lower than that of lachnanthocarpone in the LF. It is also important to note that, during the solubility test, the films presented good integrity and did not separate into parts, as far as it could be perceived by the naked eye, despite being in direct contact with water for an extended time.

Table 1 Thickness, moisture content, and water solubility, for developed films

Film	Thickness (mm)	MC (%)	WS (%)
CF	0.145 ± 0.008^{a}	19.1 ± 0.5^{b}	17.8 ± 0.9^{a}
LF	0.129 ± 0.008^{b}	16.6 ± 1.6^{a}	16.7 ± 0.2^{a}
TF	0.167 ± 0.007^{c}	17.2 ± 1.3^{ab}	20.4 ± 1.8^{b}

Values followed by at least a common superscript letter are not significantly different ($p > 0.05$)

CF film without antioxidant, *LF* film with lachnanthocarpone, *TF* film with α-tocopherol, *MC* moisture content, *WS* water solubility

Mechanical Properties

The mechanical deformation parameters at the break, tensile strength, and elastic modulus were evaluated and are summarized in Table 2. The mechanical properties of the polymeric material used as packaging are important to maintain its integrity during storage and handling (Ribeiro-Santos et al., 2018). The tensile strength (TS) allows us to know how resistant the material is to the applied pressures, the deformation at break (DB) indicates its resistance to deformation (Abedi-Firoozjah et al., 2023), and the elastic modulus (ME) is an indicator of film stiffness. The higher the modulus, the more rigid the material is (Ortega et al., 2017).

The films with the compounds lachnanthocarpone (LF) and α-tocopherol (TF) presented statistically significantly higher TS values compared to the CF (Table 2), indicating that CF has a weaker structure and was characterized by being more flexible (more significant deformation), as indicated by the results of DB and ME, which were significantly lower in CF than in their counterparts. That was possibly due to the influence of the water content, which, together with glycerol, acts as a plasticizer, helping to achieve greater flexibility (Ribeiro-Santos et al., 2018). Other authors have also reported greater flexibility in films developed from proteins, polysaccharides, and glycerol, due to the protein–polysaccharide interaction and the plasticizing effect of glycerol, which increased the mobility of the polymer chains (Cortés-Rodríguez et al., 2020). In a study carried out by Abedi-Firoozjah et al. (2023), the authors found that the addition of coconut shell as an active agent to films based on polyvinyl alcohol and starch led to more interactions between various components, which decreased the DB and, therefore, both films presented a lower stretching capacity. This behavior was also evident in TF and was related to the high values of ME obtained and thickness of 16.877 ± 1.937 MPa and 0.167 ± 0.007 mm, respectively. The reduction was also observed during the test, since the fractures of the specimens occurred more quickly for TF than for LF and CF, presenting greater rigidity.

Table 2 Mechanical properties for developed films

Film	DB (mm/mm)	TS (MPa)	ME (MPa)
CF	0.571 ± 0.116^{a}	0.494 ± 0.056^{a}	4.443 ± 0.781^{a}
LF	0.418 ± 0.061^{b}	0.788 ± 0.104^{b}	14.575 ± 4.222^{b}
TF	0.109 ± 0.030^{c}	0.838 ± 0.088^{b}	16.877 ± 1.937^{b}

Values followed by at least a common superscript letter are not significantly different ($p > 0.05$)

CF film without antioxidant, *LF* film with lachnanthocarpone, *TF* film with α-tocopherol, *DB* strain at break, *TS* tensile strength, *ME* elastic modulus

Barrier Properties

Table 3 shows the results obtained from WVP for the CF, LF, and TF. WVP is a factor that helps determine the quality of food packaging materials since it evaluates the vapor transfer between the medium and the food through the polymeric packaging material (Vianna et al., 2021). It was observed that the addition of lachnanthocarpone decreased the average WVP value to $15{,}872.9 \pm 4196.5$ g/mm/m^2 atm day, increasing the water vapor permeability barrier. However, no significant differences were found between the LF and CF. Similar values were reported before by Iamareerat et al. (2018) for films based on cassava starch, glycerol, cinnamon essential oil, and sodium bentonite. They all presented values of $14{,}509.7 \pm 1882.6$ g/mm/m^2 atm day for concentrations of 1.5% and 0% of cinnamon oil and sodium bentonite, respectively (P1), and $14{,}840.1 \pm 965.6$ g/mm/m^2 atm day for concentrations of 1.5 and 0.75% of cinnamon oil and sodium bentonite, respectively (P2). These results were obtained for films with thicknesses of 0.340 ± 0.01 mm (P1) and 0.320 ± 0.02 mm (P2), respectively. Such values were higher than those obtained for LF of 0.129 ± 0.008 mm. On the other hand, the addition of α-tocopherol increased the average WVP values compared to the control film. However, no significant differences were found between the LF and CF. In addition, when LF and TF were compared, the latter presented significantly higher values. This may have been because the addition of α-tocopherol in emulsion affects the cohesive forces of the network formed by starch-WPC, increasing the molecular spaces and facilitating the movement of water vapor through the polymer matrix. These results are consistent with those reported by Agudelo-Cuartas et al. (2020) who found that the WPC-based film + the

Table 3 Barrier properties for developed films

Film	Thickness (mm)	WVP (g/mm/m^2 atm day)	OP (cm^3/mm/m^2 atm day)	LT (%)
CF	0.145 ± 0.008^{a}	16851.5 ± 4959.4^{ab}	67.81 ± 7.89^{a}	23.59 ± 0.47^{a}
LF	0.129 ± 0.008^{b}	15872.9 ± 4196.5^{a}	82.32 ± 14.30^{a}	17.59 ± 0.43^{b}
TF	0.167 ± 0.007^{c}	20537.4 ± 5999.9^{b}	62.77 ± 10.66^{a}	20.89 ± 0.36^{c}

Values followed by at least a common superscript letter are not significantly different ($p > 0.05$)

CF film without antioxidant, *LF* film with lachnanthocarpone, *TF* film with α-tocopherol, *WVP* water vapor permeability, *OP* oxygen permeability, *LT* light transmittance

addition of a nanoemulsion with 2% tocopherol increased the WVP values compared to the control film. This meant that the cohesive forces of the protein network allowed the movement of water vapor through the WPC matrix.

Regarding OP, the entry of oxygen into foods can cause oxidation, affecting sensory changes and deteriorating nutrients. Furthermore, it can increase the multiplication rate of aerobic bacteria in the air, affecting food safety (Sothornvit & Pitak, 2007). As shown in Table 3, in this study, there were no significant differences between the three samples. In addition, the OP values found for CF, LF, and TF showed better protection compared to the oxygen barrier offered by some synthetic polymers, such as low-density polyethylene (190–200 $cm^3/mm/m^2/atm$ day) and polypropylene (80–95 $cm^3/mm/m^2/atm$ day). CF and TF mainly presented a barrier similar to high-density polyethylene (40–70 $cm^3/mm/m^2/atm$ day) and polylactic acid (PLA) (60–80 $cm^3/mm/m^2$ atm day). These results indicate the potential of the films developed in this study to be used as packaging that protects food from oxidation reactions (Keller & Kouzes, 2017; Sothornvit & Pitak, 2007). Concerning light transmittance, visible light comprises wavelengths between approximately 350 nm and 600 nm. According to Robertson (2013), this range is the most harmful because the food is subjected to more significant oxidative damage. As seen in Table 3, the light transmittance (%) obtained for all the films did not exceed values of 21%. However, the films with incorporated antioxidants, LF and TF, had statistically significantly lower light transmittance than that obtained for the control film CF, which indicated that these compounds provide better light protection, a favorable aspect for their use in the development of materials intended for food preservation. The formulation of the films made it possible to reach a light transmittance even lower than that achieved in other studies with films made from WPC with tocopherol and natamycin, where the light transmittance at 500 nm reached values close to 50% (Agudelo-Cuartas et al., 2020).

Film Microstructure

The microstructure of the films depends mainly on the interactions between the film components and can influence their functional, mechanical, barrier, and optical properties (Vianna et al., 2021). The cross-sectional and surface SEM images of the CF, TF, and LF are shown in Fig. 3A, C, and E and B, D, respectively. The images show good integration of the components in the polymer matrix, a smooth fracture surface, and good compatibility of all the components' parts. They also show the heterogeneity of the CF, through waves that could have been formed when this was drying in the air oven, and the compact structure of the TF, which indicates a good distribution of the emulsion droplets. However, spaces or cracks can be seen on its surface, which could also cause the higher WVP value for this film. The LF surface image, in particular, allows us to observe that the component interacts better with the polymer structure, producing a more homogeneous film, with fewer imperfections, which constitutes a better barrier to gases and water vapor. In the CF and LF, small gaps can be seen in the cross-section of the matrix, which can be attributed to tiny air bubbles that could have been incorporated in the stirring stage of the film-forming solution (Moreno et al., 2017).

Biodegradability

The biodegradability obtained for the LF was $85.40 \pm 8.74\%$, and that for the TF was $43.11 \pm 6.03\%$, which represents a statistically significantly lower degradation rate. The biodegradability values obtained for the films show that they experienced aerobic biodegradation in the compost during the 30 days of evaluation. These data also present higher values than those found by Leejarkpai et al. (2011) in their research. The authors evaluated the biodegradability of plastic materials developed from microcrystalline cellulose (MCE) and PLA, obtaining values of 94.34% and 85.75%, respectively, in 90 days. In addition, they found that polyethylene (PE) materials and PE/starch mixture degraded by only 0.56% and 11.5%, respectively, in the same period. All this allows us to conclude that the materials obtained in this study (TF and LF) present specifications that can be considered biodegradable and of great potential for packaging in the food industry.

Functionality

The functionality of the control (CF), commercial, and active packaging materials (TF and LF) was evaluated using a food matrix with high-fat content: a fresh semi-hard cheese. The upper part of Fig. 4 shows the films made with α-tocopherol (TF) and lachnanthocarpone (LF). To the naked eye, it appears that the film elaborated with lachnanthocarpone presents a fuchsia color characteristic of the antioxidant. However, on contact with cheese, the difference is less noticeable. The bottom part of Fig. 4 presents the hexanal concentration in the samples stored in refrigeration. As can be seen, the commercial cheese already had an initial concentration of hexanal equivalent to 0.26 ± 0.002 mg/l. Thus, this was used as the starting point to evaluate the behavior of the compound in the different treatments.

During storage, a variable production of hexanal was quantified between samples. The cheese in direct contact with the commercial packaging generated a statistically significant higher hexanal content compared to the other

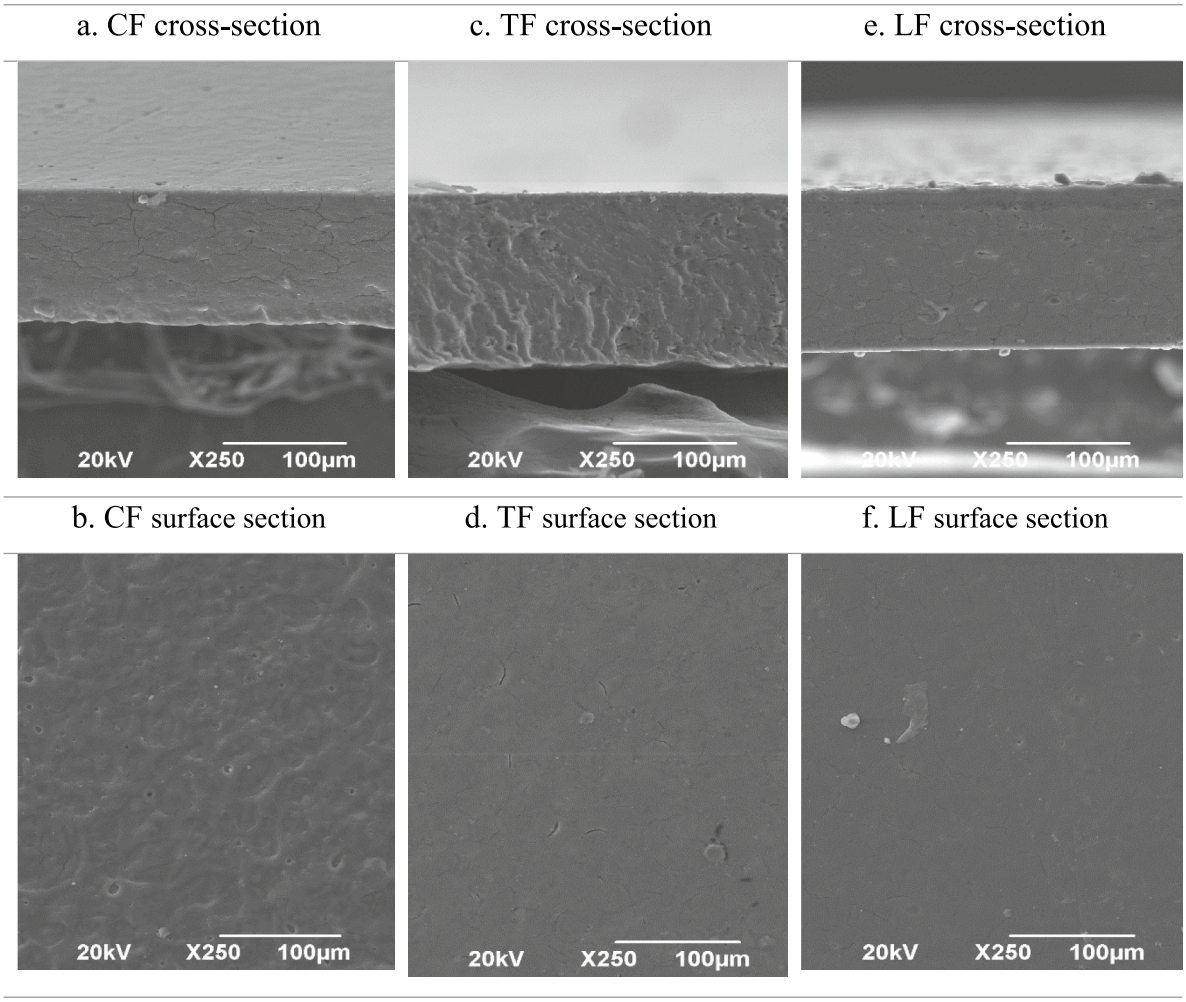


Fig. 3 SEM images of the cross-section (**A**, **C**, **E**) and surface section (**B**, **D**, **F**) of the CF, TF, and LF

treatments. That content was 0.521 ± 0.024 mg/l for 8 days, 0.643 ± 0.019 mg/l for 20 days, and 0.698 ± 0.010 mg/l for 30 days, which represented the highest concentrations of the entire study. These values were in agreement with a previous study carried out by Nzekoue et al. (2019), who evaluated hexanal production in Italian cheese packaged in polyethylene. The authors found an initial hexanal concentration of 0.012 ± 0.001 mg/kg, and a refrigerated storage value of 0.55 ± 0.06 mg/kg at 30 days. As for the cheese stored using the control film (without antioxidants), this showed an increase in hexanal production during storage equal to 0.257 ± 0.002 mg/l, 0.308 ± 0.001 mg/l, and 0.333 ± 0.007 mg/l for 8 days, 20 days, and 30 days, respectively. Since those values were significantly lower than those obtained using the commercial packaging, it was concluded that the packaging material made by casting with concentrated whey protein and starch had a protective effect on the lipid oxidation process of cheese. This could be explained by the material's high barrier to oxygen (67.81 ± 7.89 cm^3/mm/m^2 atm day). It could also be explained by the interaction phenomenon that occurred in β-lactoglobulin and hexanal, which was confirmed by Ince et al. (2024). These authors established that the β-lactoglobulin composes the greatest proportion in whey (up to 65%) and produces non-covalent interactions with hexanal, which modifies the secondary structure of the protein and affects the availability of hexanal in the medium, generating indirect protection to the food.

Regarding the hexanal concentration in cheese samples stored in films made with incorporated antioxidants TF and LF, they were significantly lower than the CF. On the one side, TF presented values of 0.238 ± 0.009 mg/l, 0.290 ± 0.000 mg/l, and 0.304 ± 0.002 mg/l for 8 days, 20 days, and 30 days, respectively. On the other hand, in all cases, LF presented values that remained below the different treatments. These values were 0.180 ± 0.002 mg/l, 0.197 ± 0.001 mg/l, and 0.215 ± 0.003 mg/l for 8 days, 20 days, and 30 days, respectively. The results show the promising effect of TF and LF as antioxidants in the

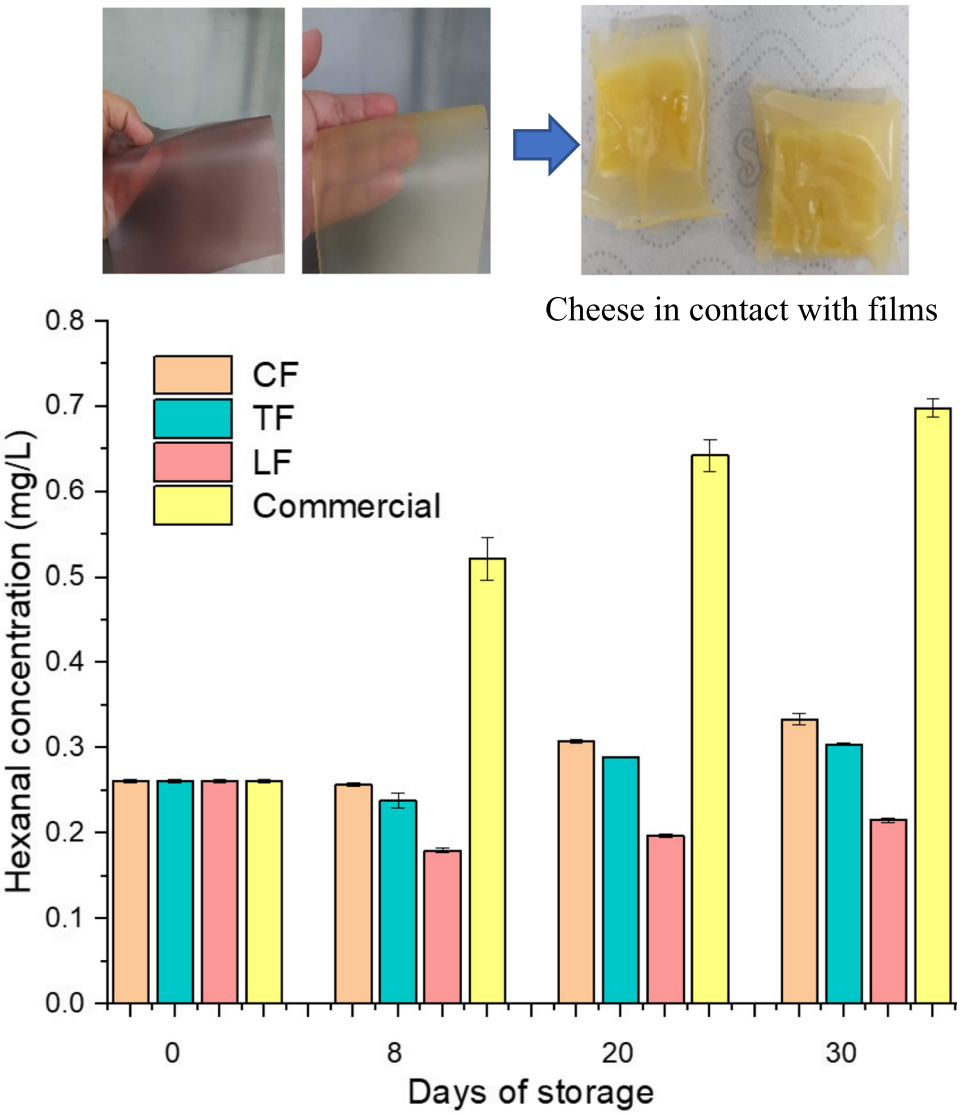


Fig. 4 Effect of active packaging prepared by casting and with antioxidants on the hexanal concentration in cheese. CF, film without antioxidant; LF, film with lachnanthocarpone; TF, film with α-tocopherol

development of food contact films. Specifically, they demonstrate that when high-fat cheese is protected only with a synthetic packaging material without antioxidants, the lipid oxidation process gradually increases until it reaches values in the hexanal concentration that consumers can easily perceive since the hexanal has a very low sensory detection threshold and is equivalent to 0.03–0.01 ppm in water and is associated with a grassy odor that is generally disliked by consumers (Ince et al., 2024).

Other authors have conducted similar studies. when analyzing double-cream cheese, for example, Agudelo-Cuartas (2022) found that, in general, the cheese stored for 8 weeks presented a lower production of hexanal (0.11 mg/kg) compared to cheese stored in packaging that was similar but lacked the antioxidant compounds. Also, when studying whole milk powder, Granda-Restrepo et al., (2009a, b) found a significant effect of the packaging with tocopherol when compared to the same packaging system without the antioxidant. In sum, the positive impact of the antioxidants can be seen in both cases, which indicates that their presence is crucial in delaying the oxidation process, as had been stated before (Kola & Carvalho, 2023).

Conclusions

The formulation developed for the films showed significant differences between the films with antioxidants and the control film (without antioxidants), achieving better oxygen barrier properties than those of synthetic materials derived from petroleum. This indicates that the materials used for the development of active packaging present good properties to maintain food quality and protect foods from more significant oxidative damage. As expected, the film

with lachnanthocarpone showed the least amount of hexanal production after 30 days of storage, which indicated its protective effect on cheese as a model/reference food, leading to lower lipid oxidation. Therefore, the developed biodegradable films have good properties, which make them a promising alternative to reducing plastic materials in food packaging. Regarding functionality, cheese is a dairy product in which whey is generated during production. Whey is also the source of the concentrated proteins used to prepare the materials in this study. It, thus, becomes an essential element to close the cycle in favor of sustainability since the waste from the cheese-making process would be used as raw material to produce active packaging materials, constituting a comprehensive strategy for developing the bioeconomy.

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Declarations

Competing Interests The authors declare no competing interests.

References

Abedi-Firoozjah, R., Chabook, N., Rostami, O., Heydari, M., Kolahdouz-Nasiri, A., Javanmardi, F., Abdolmaleki, K., & Mousavi Khaneghah, A. (2023). PVA/starch films: An updated review of their preparation, characterization, and diverse applications in the food industry. *Polymer Testing, 118*, 107903. https://doi.org/10.1016/j.polymertesting.2022.107903

Agudelo-Cuartas, C., Granda-Restrepo, D., Sobral, P. J. A., Hernandez, H., & Castro, W. (2020). Characterization of whey protein-based films incorporated with natamycin and nanoemulsion of α-tocopherol. *Heliyon, 6*(4), e03809. https://doi.org/10.1016/j.heliyon.2020.e03809

Agudelo-Cuartas, C., Granda-Restrepo, D., Sobral, P. J. A., & Hernandez, H. (2023). Nanometric modeling of migration of α-tocopherol from whey protein-based films on the cheese surface. *Journal of Food Engineering, 358*, 111661. https://doi.org/10.1016/j.jfoodeng.2023.111661

Agudelo-Cuartas, C. (2022). *Desarrollo de un envase activo biodegradable a partir de proteínas de lactosuero y nanoemulsiones encapsulando α-tocoferol como antioxidante y natamicina como antimicrobiano* [Tesis de Doctorado en Ciencias Farmacéuticas y Alimentarias]. Universidad de Antioquia. https://bibliotecadigital.udea.edu.co/dspace/handle/10495/40644

Ait-Oubahou, A., Nur Hanani, Z. A., & Jamilah, B. (2019). Packaging. In *Postharvest technology of perishable horticultural commodities* (pp. 375–399). Elsevier. https://doi.org/10.1016/B978-0-12-813276-0.00011-0

Alias, A. R., Wan, M. K., & Sarbon, N. M. (2022). Emerging materials and technologies of multi-layer film for food packaging application: A review. *Food Control, 136*, 108875. https://doi.org/10.1016/j.foodcont.2022.108875

Alipour, A., Rahaiee, S., Rajaei Litkohi, H., Jamali, S. N., & Jafari, S. M. (2023). Development and optimization of whey protein-*Lepidium perfoliatum* gum packaging films: An approach towards antimicrobial and biodegradable films. *Industrial Crops and Products, 196*, 116447. https://doi.org/10.1016/j.indcrop.2023.116447

Alvarez-Perez, O. B., Ventura-Sobrevilla, J. M., Torres-León, C., Rojas-Molina, R., Rodríguez-Herrera, R., Aguilar-González, M. A., & Aguilar, C. N. (2022). Development and characterization of whey protein films incorporated with tarbush polyphenols and candelilla wax. *Food Bioscience, 45*, 101505. https://doi.org/10.1016/j.fbio.2021.101505

Amin, U., Khan, M. U., Majeed, Y., Rebezov, M., Khayrullin, M., Bobkova, E., Shariati, M. A., Chung, I. M., & Thiruvengadam, M. (2021). Potentials of polysaccharides, lipids and proteins in biodegradable food packaging applications. *International Journal of Biological Macromolecules, 183*, 2184–2198. https://doi.org/10.1016/j.ijbiomac.2021.05.182

Cheng, J., Gao, R., Zhu, Y., & Lin, Q. (2024). Applications of biodegradable materials in food packaging: A review. *Alexandria Engineering Journal, 91*, 70–83. https://doi.org/10.1016/j.aej.2024.01.080

Cortés-Rodríguez, M., Villegas-Yépez, C., Gil González, J. H., Rodríguez, P. E., & Ortega-Toro, R. (2020). Development and evaluation of edible films based on cassava starch, whey protein, and bees wax. *Heliyon, 6*(9), e04884. https://doi.org/10.1016/j.heliyon.2020.e04884

D20 Committee. (2021). Test method for determining aerobic biodegradation of plastic materials under controlled composting conditions, incorporating thermophilic temperatures. *ASTM International*. https://doi.org/10.1520/D5338-15R21

Duque, L., Zapata, C., Rojano, B., Schneider, B., & Otálvaro, F. (2013). Radical scavenging capacity of 2,4-dihydroxy-9-phenyl-1*H*-phenalen-1-one: A functional group exclusion approach. *Organic Letters, 15*(14), 3542–3545. https://doi.org/10.1021/ol400384z

E35 Committee. (2021). Test method for film permeability determination using static permeability cells. *ASTM International*. https://doi.org/10.1520/E2945-14R21

Granda-Restrepo, D. M., Soto-Valdez, H., Peralta, E., Troncoso-Rojas, R., Vallejo-Córdoba, B., Gámez-Meza, N., & Graciano-Verdugo, A. Z. (2009a). Migration of α-tocopherol from an active multilayer film into whole milk powder. *Food Research International, 42*(10), 1396–1402. https://doi.org/10.1016/j.foodres.2009.07.007

Granda-Restrepo, D., Peralta, E., Troncoso-Rojas, R., & Soto-Valdez, H. (2009b). Release of antioxidants from co-extruded active packaging developed for whole milk powder. *International Dairy Journal, 19*(8), 481–488. https://doi.org/10.1016/j.idairyj.2009.01.002

Granda-Restrepo, D., Medina-Pineda, Y., Culebras-Rubio, M., & Gómez-Clari, C. (2014). Desarrollo y Caracterización de una Película Activa Biodegradable con Antioxidantes (Alfa-tocoferol) a partir de las Proteínas del Lactosuero. *VITAE, Revista de la Facultad de Química Farmacéutica, 21*(1), 11–19.

Gunathilake, I. A. D. S. R., & Somendrika, M. A. D. (2024). Development of a biodegradable packaging with antimicrobial properties from cassava starch by incorporating *Ocimum tenuiflorum* extract. *Food Chemistry Advances, 4*, 100658. https://doi.org/10.1016/j.focha.2024.100658

Iamareerat, B., Singh, M., Sadiq, M. B., & Anal, A. K. (2018). Reinforced cassava starch based edible film incorporated with essential oil and sodium bentonite nanoclay as food packaging material. *Journal of Food Science and Technology, 55*, 1953–1959. https://doi.org/10.1007/s13197-018-3100-7

Ince, C., Condict, L., Ashton, J., Stockmann, R., & Kasapis, S. (2024). Molecular characterisation of interactions between β-lactoglobulin and hexanal – An off flavour compound. *Food Hydrocolloids, 146*, 109260. https://doi.org/10.1016/j.foodhyd.2023.109260

Keller, P. E., & Kouzes, R. T. (2017). Water vapor permeation in plastics (No. PNNL--26070, 1411940; p. PNNL--26070, 1411940). https://doi.org/10.2172/1411940. Available at https://www.pnnl.gov/main/publications/external/technical_reports/PNNL-26070.pdf

Kola, V., & Carvalho, I. S. (2023). Plant extracts as additives in biodegradable films and coatings in active food packaging. *Food Bioscience, 54*, 102860. https://doi.org/10.1016/j.fbio.2023.102860

Leejarkpai, T., Suwanmanee, U., Rudeekit, Y., & Mungcharoen, T. (2011). Biodegradable kinetics of plastics under controlled composting conditions. *Waste Management, 31*(6), 1153–1161. https://doi.org/10.1016/j.wasman.2010.12.011

Luchese, C. L., Pavoni, J. M. F., & Tessaro, I. C. (2021). Influence of the incorporation form of waste from the production of orange juice in the properties of cassava starch-based films. *Food Hydrocolloids, 117*, 106730. https://doi.org/10.1016/j.foodhyd.2021.106730

Martínez, J., Nieto, G., & Ros, G. (2014). Total antioxidant capacity of meat and meat products consumed in a reference 'Spanish standard diet.' *International Journal of Food Science & Technology, 49*(12), 2610–2618. https://doi.org/10.1111/ijfs.12577

Martins, J. T., Cerqueira, M. A., & Vicente, A. A. (2012). Influence of α-tocopherol on physicochemical properties of chitosan-based films. *Food Hydrocolloids, 27*(1), 220–227. https://doi.org/10.1016/j.foodhyd.2011.06.011

Moeini, A., Germann, N., Malinconico, M., & Santagata, G. (2021). Formulation of secondary compounds as additives of biopolymer-based food packaging: A review. *Trends in Food Science & Technology, 114*, 342–354. https://doi.org/10.1016/j.tifs.2021.05.040

Moreno, O., Cárdenas, J., Atarés, L., & Chiralt, A. (2017). Influence of starch oxidation on the functionality of starch-gelatin based active films. *Carbohydrate Polymers, 178*, 147–158. https://doi.org/10.1016/j.carbpol.2017.08.128

Nzekoue, F. K., Caprioli, G., Fiorini, D., Torregiani, E., Vittori, S., & Sagratini, G. (2019). HS-SPME-GC-MS technique for FFA and hexanal analysis in different cheese packaging in the course of long term storage. *Food Research International, 121*, 730–737. https://doi.org/10.1016/j.foodres.2018.12.048

Ortega, F., Giannuzzi, L., Arce, V. B., & García, M. A. (2017). Active composite starch films containing green synthetized silver nanoparticles. *Food Hydrocolloids, 70*, 152–162. https://doi.org/10.1016/j.foodhyd.2017.03.036

Papadaki, A., Kachrimanidou, V., K. Lappa, I., Andriotis, H., Eriotou, E., Mandala, I., & Kopsahelis, N. (2022). Tuning the physical and functional properties of whey protein edible films: Effect of pH and inclusion of antioxidants from spent coffee grounds. *Sustainable Chemistry and Pharmacy, 27*, 100700. https://doi.org/10.1016/j.scp.2022.100700

Ramakrishnan, R., Kulandhaivelu, S. V., Roy, S., & Viswanathan, V. P. (2023). Characterisation of ternary blend film of alginate/carboxymethyl cellulose/starch for packaging applications. *Industrial Crops and Products, 193*, 116114. https://doi.org/10.1016/j.indcrop.2022.116114

Ribeiro-Santos, R., De Melo, N. R., Andrade, M., Azevedo, G., Machado, A. V., Carvalho-Costa, D., & Sanches-Silva, A. (2018). Whey protein active films incorporated with a blend of essential oils: Characterization and effectiveness. *Packaging Technology and Science, 31*(1), 27–40. https://doi.org/10.1002/pts.2352

Robertson, G. L. (2013). Food packaging: Principles and practice (3rd ed). CRC Press. https://doi.org/10.1201/b21347

Salazar, A., & Otálvaro, F. (2024). A gram-scale synthesis of lachnanthocarpone. *Results in Chemistry, 7*, 101404. https://doi.org/10.1016/j.rechem.2024.101404

Silva, K. F., Marques, C. S., De Freitas Junior, A., Dias, M. V., & Mori, F. A. (2023). Whey protein isolate and kraft lignin multifunctional films for potential food packaging application: UV block and antioxidant potential. *Food Bioscience, 53*, 102581. https://doi.org/10.1016/j.fbio.2023.102581

Song, T., Qian, S., Lan, T., Wu, Y., Liu, J., & Zhang, H. (2022). Recent advances in bio-based smart active packaging materials. *Foods, 11*(15), 2228. https://doi.org/10.3390/foods11152228

Sothornvit, R., & Pitak, N. (2007). Oxygen permeability and mechanical properties of banana films. *Food Research International, 40*(3), 365–370. https://doi.org/10.1016/j.foodres.2006.10.010

Thuy, L. T., Thuong, N. N., Bang, N. T., Quynh, D. T., Trang, V. T., & Mai, P. T. N. (2021). Determination of antioxidant capacity in some foods by oxygen radical absorbance capacity (ORAC). *Vietnam Journal of Food Control, 4*(3), 199-206. pdf. https://doi.org/10.47866/2615-9252/vjfc.3826

Toas, M. (1989). Results of the 1985 round-Robin test series using ASTM E 96–80. American Society for Testing and Materials. https://compass.astm.org/document/?contentCode=ASTM%7CSTP22959S%7Cen-US

Tsironi, M., Kosma, I. S., & Badeka, A. V. (2022). The effect of whey protein films with ginger and rosemary essential oils on microbiological quality and physicochemical properties of minced lamb meat. *Sustainability, 14*(6), 3434. https://doi.org/10.3390/su14063434

Tucki, K., Orynycz, O., Wasiak, A., Gola, A., & Mieszkalski, L. (2022). Potential routes to the sustainability of the food packaging industry. *Sustainability, 14*(7), 3924. https://doi.org/10.3390/su14073924

UN Environment Programme. (2020). *Beat plastic pollution [informative]*. UNEP. https://www.unep.org/interactives/beat-plastic-pollution/. Accessed 6 Oct 2023.

Vianna, T. C., Marinho, C. O., Marangoni Júnior, L., Ibrahim, S. A., & Vieira, R. P. (2021). Essential oils as additives in active starch-based food packaging films: A review. *International Journal of Biological Macromolecules, 182*, 1803–1819. https://doi.org/10.1016/j.ijbiomac.2021.05.170

Walker, T. R., & Fequet, L. (2023). Current trends of unsustainable plastic production and micro(nano)plastic pollution. *TrAC Trends in Analytical Chemistry, 160*, 116984. https://doi.org/10.1016/j.trac.2023.116984

Wang, Y., Wang, X., Hu, G., Al-Romaima, A., Liu, X., Bai, X., Li, J., Li, Z., & Qiu, M. (2022). Effect of green coffee oil as a natural active emulsifying agent on the properties of corn starch-based films. *LWT, 170*, 114087. https://doi.org/10.1016/j.lwt.2022.114087

Yildirim, S., Röcker, B., Pettersen, M. K., Nilsen-Nygaard, J., Ayhan, Z., Rutkaite, R., Radusin, T., Suminska, P., Marcos, B., & Coma, V. (2018). Active packaging applications for food. *Comprehensive Reviews in Food Science and Food Safety, 17*(1), 165–199. https://doi.org/10.1111/1541-4337.12322

Zhang, W., Li, X., & Jiang, W. (2020). Development of antioxidant chitosan film with banana peels extract and its application as coating in maintaining the storage quality of apple. *International Journal of Biological Macromolecules, 154*, 1205–1214. https://doi.org/10.1016/j.ijbiomac.2019.10.275

Zhang, J., Han, Y., Ben, Z., Han, T., & Yin, P. (2023a). Effect of branched polyethyleneimine and citric acid on the structural, physical and antibacterial properties of corn starch/chitosan films. *International Journal of Biological Macromolecules, 231*, 123186. https://doi.org/10.1016/j.ijbiomac.2023.123186

Zhang, W., Ahari, H., Zhang, Z., & Jafari, S. M. (2023b). Role of silica (SiO_2) nano/micro-particles in the functionality of degradable packaging films/coatings and their application in food preservation. *Trends in Food Science & Technology, 133*, 75–86. https://doi.org/10.1016/j.tifs.2023.01.009

Zhang, W., Roy, S., Ezati, P., Yang, D.-P., & Rhim, J.-W. (2023c). Tannic acid: A green crosslinker for biopolymer-based food packaging films. *Trends in Food Science & Technology, 136*, 11–23. https://doi.org/10.1016/j.tifs.2023.04.004

Zhang, W., Sani, M. A., Zhang, Z., McClements, D. J., & Jafari, S. M. (2023d). High performance biopolymeric packaging films containing zinc oxide nanoparticles for fresh food preservation: A review. *International Journal of Biological Macromolecules, 230*, 123188. https://doi.org/10.1016/j.ijbiomac.2023.123188

Zhang, W., Khan, A., Ezati, P., Priyadarshi, R., Sani, M. A., Rathod, N. B., Goksen, G., & Rhim, J.-W. (2024). Advances in sustainable food packaging applications of chitosan/polyvinyl alcohol blend films. *Food Chemistry, 443*, 138506. https://doi.org/10.1016/j.foodchem.2024.138506