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Effect of Different Essential Oils on the Properties of Edible Coatings Based on Yam (*Dioscorea rotundata* L.) Starch and Its Application in Strawberry (*Fragaria vesca* L.) Preservation

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Abstract: Every year the world loses about 50% of fruits and vegetables post-harvest and in the supply chain. The use of biodegradable coatings and films with antioxidant properties has been considered an excellent alternative to extend the shelf life of food. Therefore, the objective of this work was to develop a coating based on yam (*Dioscorea rotundata* L.) starch-containing lime, fennel, and lavender essential oils to extend the shelf life of strawberries (*Fragaria vesca l.*). The tensile properties, barrier properties (water vapour permeability (WVP) and oxygen permeability (OP)), moisture content, water-solubility, absorption capacity, water contact angle, optical properties, the antioxidant activity of the resultant starch-based coatings were evaluated. After that, the active properties of the coatings were assessed on strawberries inoculated with *Aspergillus niger* during 14 days of storage at 25 °C. The results showed that the incorporation of essential oils improved the elongation and WVP and provided antioxidant capacity and antimicrobial activity in the films. In particular, the essential oil of lime showed higher antioxidant activity. This fact caused the unwanted modification of other properties, such as the decrease in tensile strength, elastic modulus and increase in OP. The present study revealed the potential use of lime, fennel, and lavender essential oils incorporated into a polymeric yam starch matrix to produce biodegradable active films (antioxidant and antimicrobial). Obtained films showed to be a viable alternative to increase the shelf life of strawberries and protect them against Aspergillus niger.

Keywords: physicochemical properties; shelf life; lime essential oil; fennel essential oil; lavender essential oil

1. Introduction

The consumption of fruits and vegetables has increased worldwide, considering the increase in the potential consumers interested in the nutritional properties of these products. However, they are perishable products that, in a short time, lose their physical attributes and nutritional value. In this way, it is essential to maintain the quality of fruits and vegetables after harvest to reduce agro-industrial waste since it has been estimated that approximately one-third of the food produced for human consumption is lost or wasted on the planet, corresponding to 50% of food losses in fruits and vegetables [1]. Strawberry

(*Fragaria vesca* L.) is a widely accepted crop worldwide, and specifically in South America, Colombia has 36.5 T/ha of strawberry production per year [2]. Currently, there is an interest in reducing post-harvest residues of fruits and vegetables caused during food handling, storage, and transport. Therefore, methods have been employed to extend shelf life by using high-water vapour barrier packaging, edible coatings, heat treatment, UV-C plasma treatment, controlled and modified atmosphere systems. These treatments could extend shelf life and reduce post-harvest losses while maintaining fruit quality [3].

Edible coatings improve the appearance of the fruit, increase shelf life, and maintain fruit quality during storage. It is a technology that contributes to the environment by controlling moisture loss, gas exchange, or oxidation processes. In addition, they provide an additional protective layer to produce and create a modified atmosphere around the product [4]. Edible coatings can be prepared from proteins, polysaccharides, and lipids and by their combination. Different polysaccharides such as starch, chitosan, pectin, alginate, gums, and gels have been used to coat fresh fruits and vegetables. Edible polysaccharide-based coatings maintain the post-harvest quality of fruits and vegetables and are environmentally friendly [5,6].

Starch is a polysaccharide with wide application in the food industry due to its easy availability, low extraction cost, biocompatibility, biodegradability, edibility, and good film-forming capacity [7]. In particular, yam (*Dioscorea rotundata* L.) has called attention to being the third most crucial tuber globally with an estimated world production of 7258 million tons and an average yield of 8351.5 kg ha^{-1} registered for the year 2018 [8]. Moreover, due to the yam's excellent nutritional and energy quality, its starch has interesting functional and industrial properties comparable to cereals. Studies based on yam starch showed higher Young's modulus and strength values than cassava, indicating its high applicability [9].

The incorporation of essential oils into the polymeric matrix of fruit films and coatings adds bioactive compounds that provide antioxidant and antimicrobial properties. This process allows the controlled release of the active substances on the surface of the fruit for a longer time, which is a great advantage over the direct application of the essential oil, which can modify the flavour and quality of the product [10]. An example is the essential oil of lime, which is used in the food industry due to its chemical and sensory characteristics. This oil is composed of a mixture of terpenes (75%), oxygenated complexes (12%) and sesquiterpenes (3%), being limonene, γ-terpene, and citral its main active compounds, which have shown high antibacterial activity against different gram-positive and gram-negative bacteria, such as *P. aeruginosa, E. coli, S. typhimurium,* and *S. aureus* [11,12]. On the other hand, some studies have reported the high antioxidant, antifungal, and antibacterial properties of fennel essential oil, attributed to its main active compounds, ketones, olefins, and anethole, present in the oil in a proportion of 48.86%, 33.07%, and 9.78%, respectively [13,14]. Other studies have considered lavender essential oil as a bioactive component to enhance the biological properties of biodegradable films due to its antibacterial, anti-inflammatory, antifungal, and especially analgesic and antioxidant properties [15,16].

Therefore, the objective of this work is to develop a coating based on yam (*Dioscorea rotundata* L.) starch-containing lime, fennel, and lavender essential oils to study their physicochemical properties antioxidant capacity and conduct assays of antimicrobial activity using strawberries inoculated with *Aspergillus niger*.

2. Materials and Methods

2.1. Raw Materials

Yam starch was obtained from tubers provided by the Association of Tuber Producers (San Juan Nepomuceno, Bolívar, Colombia). The starch had 28.3% amylose and 71.7% amylopectin content. Glycerol, soy lecithin, and other reagents were provided by Sigma-Aldrich and Panreac (Bogotá–Colombia). Essential oils were obtained from Now Foods (Bloomingdale, IL, USA) with 100% purity.

2.2. Preparation and Characterization of Starch-Based Films

The films were made by casting. For this, yam starch was used as a polymer, glycerol as a plasticizer, soy lecithin as an interface agent, and three essential oils (EO) (lime (LiEO), fennel (FeEO), and lavender (LaEO)) as active agents. The MIC of Aspergillus Niger was not considered to calculate the essential oil added to the films. An amount high enough to have active effects was calculated but not so high that there would be oil bleeding effects. For this, some preliminary studies have been used (Gómez-Contreras et al., 2021 and Ortega-Toro et al., 2014). The starch was gelatinized in distilled water at 90 °C for 20 min. In another beaker, 2 g soy lecithin and 2 g of essential oil were dispersed in 38 g of distilled water. They were sonicated at 40 kHz and 40% power for 300 s (1 s on and 1 s off) according to the methodology reported by Jiménez et al. [17]. After that, the gelatinized starch, glycerol, and lecithin-EO emulsions were mixed, keeping the proportions shown in Table 1. Finally, film-forming dispersions (FFD) were mixed with a rotor-stator homogenizer (Ultraturrax T25, Janke and Kunkel, Germany) for 2 min at 13,500 rpm followed by 5 min at 20,500 rpm. The film-forming solutions were placed on 400 cm^2 plates and dried at room temperature for 36 h. The films were removed and conditioned in desiccators with Mg $(NO_3)_2$ saturated solutions and conditioned one week before their characterization.

Table 1. Studied formulations expressed in mass fraction.

Formulations	Starch	Glycerol	Soy Lecithin	LiEO	FeEO	LaEO
F1	0.8000	0.2000				
$F2_{li}$	0.7407	0.1852	0.0370	0.0370		
$F3_{fe}$	0.7407	0.1852	0.0370		0.0370	
$F4_{la}$	0.7407	0.1852	0.0370			0.0370
$F5_{lifela}$	0.7407	0.1852	0.0370	0.0123	0.0123	0.0123

2.3. Thickness

Before testing, the thickness of yam starch films was measured using a digital micrometre (TL268, Shanghai, China) with ±0.001 mm accuracy. Measurements were performed and averaged in five different points, two in each end and one in the middle.

2.4. Tensile Properties

The mechanical properties were carried out with samples previously conditioned for 1 or 5 weeks under 25 °C and 53% RH conditions. About seven replicates of each sample were used. The tensile strength (TS), the elastic modulus (EM), and the elongation (E) of the films were determined, according to the standard method ASTM D882 39. EM using the universal test equipment (model TA.XTplus, Stable Micro Systems, Haslemere, England). TS and E were determined from the stress-strain curves, estimated from the force-distance data obtained for the films (2.5 cm wide and 10 cm long). Balanced samples were used in the film spreading jaws of the testing machine and stretched at 50 mm min $^{-1}$ until they broke. The relative humidity (R.H.) of the environment was maintained at approximately 53% during the tests at 25 °C.

2.5. Barrier Properties

2.5.1. Water Vapour Permeability (WVP)

The WVP of the films was determined using the ASTM E96-95 40 gravimetric method, using samples conditioned one week in hermetic desiccators at 25 °C and 53% RH considering the modification proposed by McHugh, Avena-Bustillos, and Krochta [18]. The films were chosen based on the absence of physical defects. First, distilled water was incorporated into the Payne permeation cups (3.5 cm diameter, Elcometer 5100/1, Argenteau, Belgium) to expose the film to 100% RH on one side. Then, each cup was placed in a cabinet of balanced relative humidity at 25 °C, with a fan placed in the upper part of the cup to reduce the resistance to the transport of water vapour, avoiding the stagnant layer effect in this exposure. It should be noted that the RH of the cabinets (53%) was kept constant using supersaturated solutions of magnesium nitrate-6-hydrate. The free surface of the film during film formation was exposed to the lowest relative humidity to simulate the actual application of films in high water activity products when stored at intermediate relative humidity. Subsequently, the cups were periodically weighed (0.0001 g). The water vapour transmission (*WVTR*) was determined from the slope obtained from the regression analysis of the weight loss data about time, once the state stationary, divided by the area of the film. Considering the *WVTR* data, the vapour pressure on the inner surface of the film (p 2) was obtained with Equation (1), proposed by McHugh et al. [18], to correct the effect of the concentration gradients established in the stagnant air space inside the bowl.

$$WVTR = \frac{PDL_n \left[\frac{P - P_2}{P - P_1}\right]}{RT\,\Delta z} \quad (1)$$

where P, total pressure (atm); D, the diffusivity of water through the air at 25 °C (m^2/s); R, gas law constant (82.057×10^{-3} m^3 atm $kmol^{-1}$ K^{-1}); T, absolute temperature (K); Δz, mean height of the stagnant air gap (m), considering the initial and final z value; P_1, water vapour pressure on the surface of the solution (atm); and P_2, corrected water vapour pressure at the inner surface of the film (atm). The water vapour permeability was calculated with Equation (2) as a function of P_2 and P_3 (pressure on the outer surface of the film in the cabinet). Permeability was achieved by multiplying the permeability by the average film thickness.

$$Permeance = \frac{WVTR}{P_2 - P_3} \quad (2)$$

2.5.2. Oxygen Permeability (OP)

The evaluation of oxygen permeability was carried out in triplicate with the Mocon OX-TRAN Model 2/23 ML equipment (Lippke, Neuwied, Germany) at 53% RH and 25 °C. The yam starch film samples were conditioned previously for one week at 25 °C and 53% RH using saturated solutions of magnesium nitrate-6-hydrate. Two samples were used in the equipment for the analysis, and they were conditioned in the cells for six hours. Then the transmission values were determined every 20 min until equilibrium was reached. The exposure area during the tests was 50 cm^2 for each formulation. It should be noted that the thickness of the film was considered in all cases to obtain oxygen permeability.

2.6. Moisture Content

The moisture content was made with average values of the samples of the films in triplicate and previously conditioned at 53% RH. They were dried for 24 h at 60 °C in a natural convection oven (J.P. Selecta, S.A., Barcelona, Spain) and subsequently incorporated in a desiccator for two weeks in the presence of P_2O_5 at 25 °C.

2.7. Solubility in Water

The solubility test of the films was carried out in triplicate for each formulation, taking into account the initial and final weights. First, they were immersed in containers with distilled water in a ratio of 1:10, film: water, respectively, for 48 h. Next, the samples were taken to a natural convection oven (J.P. Selecta, S.A., Barcelona, Spain) for 24 h with a temperature of 60 °C to remove the free water. Then they were taken to a P_2O_5 desiccator at 25 °C for two weeks, which was performed to remove tightly bound water.

2.8. Water Absorption Capacity

The absorption capacity measurement of the films was based on the parameters of ASTM-D570, so that the dried films were immersed in approximately 20 mL of distilled water for 30 min and then weighed again to determine the amount of water absorbed. Finally, the percentage of absorbed water is calculated according to Equation (3).

$$\% \text{ Absorption} = \frac{Humid\ film\ weight - Dry\ film\ weight}{Dry\ film\ weight} \quad (3)$$

2.9. Water Contact Angle

The contact angle (θ) on the surface of the films was calculated with 10 replicates per formulation. The shape of a sessile drop (0.01 mL) was studied after 10 s with a video-based contact angle meter model OCA 20 (DataPhysics Instruments GmbH, Filderstadt, Germany). Image analyzes were mirrored using SCA20 software.

2.10. Optical Properties: Gloss and Internal Transmittance

The Kubelka-Munk theory was used to establish the properties of spectral reflectance. The transparency of the film was established by applying the Kubelka-Munk theory for multiple scattering to the reflection spectra [19]. The surface reflectance spectra of the films were determined from 400 to 700 nm with a CM-3600d Spectro-colorimeter (Minolta Co., Tokyo, Japan) on a black and white background. Considering that as light passes through the film, it is absorbed and partially scattered, the absorption (K) and scattering (S) coefficients are quantified. The internal transmittance (Ti) of the films was quantified using Equation (4). Thus, R_0 is the reflectance of the film on an ideal black background. Parameters a and b were calculated using the Equations (5) and (6), where R is the reflectance of the sample layer supported by a known reflectance R_g. Measurements were taken in triplicate for each sample on the free film surface. A wavelength of 450 nm was considered for the analysis.

$$Ti = \sqrt{(a - R_0)^2 - b^2} \quad (4)$$

$$a = \frac{1}{2}\left(R + \frac{R_0 - R + R_g}{R_O R_g}\right) \quad (5)$$

$$b = \sqrt{a^2 - 1} \quad (6)$$

The gloss was determined according to the ASTM D523 [20] standard method, on the surface of the free film, with an angle of incidence of 60°, using a flat surface gloss meter (model 3 nh, Shenzhen Threenh Technology Co., Shenzhen, China). For this, measurements were taken in triplicate for each sample, and three films of each formulation were considered. All the results were expressed as gloss units, about a highly polished surface of black glass standard with an approximate value of 100. The transparency and gloss measurements were carried out with films conditioned one week in hermetic desiccators at 25 °C and 53% RH.

2.11. Antioxidant Capacity

The antioxidant capacity of the films was determined using the 2,2-diphenyl-1-pikryl-hydroxyl (DPPH) reduction method [21]. 30 µL of samples diluted in water (1:10 for powder films) were mixed with 1 mL of 0.1 mM DPPH in methanol. The mixture was vortexed and allowed to stand at room temperature in the dark (40 min) before measuring absorbance at 517 nm. In the same way, 30 µL of samples were diluted in water (1:10 for the powder films) and 1 mL of ammonium solution of 2.2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS+) from Sigma Aldrich®, the solutions were diluted in mixed methanol [22]. After six minutes of reaction in the dark, the absorbance at 734 nm was monitored using a spectrophotometer (UV Visible Thermo Scientific Genesys 10S, Dreieich, Germany). The results were expressed to the IC50 parameter, which allows measuring the DPPH and ABTS+ radical scavenging capacity of the AE to an antioxidant standard. The lower the IC_{50} value, the greater the antioxidant power it will have in the analyzed sample.

2.12. Coating Effect on Strawberry Preservation: Coating Application on Strawberries, Development of Strawberries Inoculated with Aspergillus niger

The coating application was applied on strawberries (*Fragaria vesca* L.) and compared with the application without coating. The fruits were selected according to their degree of maturity and the absence of defects, washed with 1% sodium hypochlorite for 2 min, and then washed twice in sterile water. The fungus (*Aspergillus niger*) was inoculated both before (curative treatment) and after (preventive treatment) of the coating of the fruit with the film-forming dispersion. Inoculation was performed using a sterilized needle to transfer 5 µL of a 10^6 spore suspension/mL. The coating-forming dispersion was sprayed onto the surface of the fruit and dried under ambient conditions. The strawberry samples were stored in cabinets at 25 °C and 85% RH (using a supersaturated KCl solution) for two weeks when the coating was dry. Finally, visual observation was made every five days to determine the appearance of the fruit and the incidence of fungi during storage. In the same way, weight loss was controlled in uninoculated fruits (coated and uncoated). For the above, 20 strawberries were considered in each series (coated and uncoated fruits).

2.13. Statistical Analysis

Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, MD, USA) was used to carry out statistical analyses of data through analysis of variance (ANOVA). Fisher's least significant difference (LSD) was used at the 95% confidence level. All tests were carried out in triplicate.

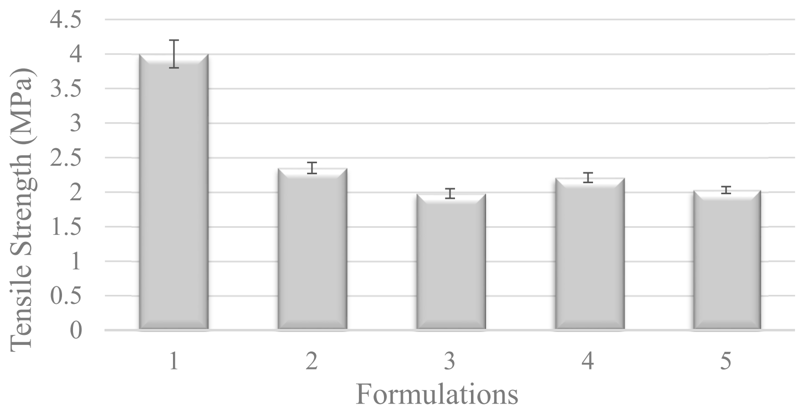
3. Results

3.1. Tensile Properties

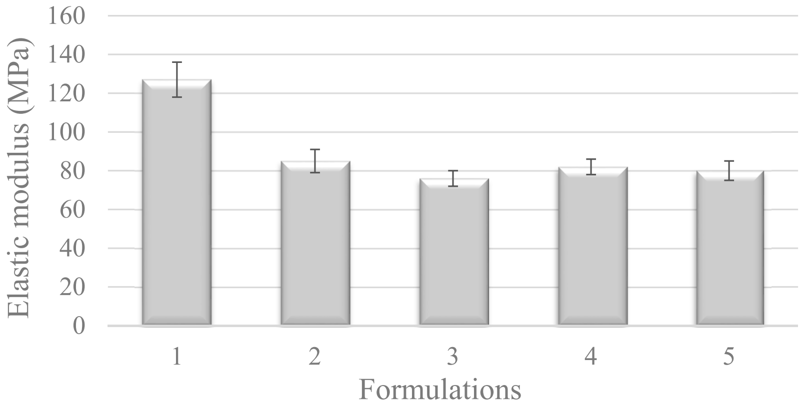
Figure 1 shows the tensile strength, modulus of elasticity, and elongation at the breaking point of the studied films stored for one week at 53% RH and 25 °C. The tensile strength is between 1.98 and 4.0 MPa, the elastic modulus was found between approximately 76 and 127 MPa, and the elongation varied between 16 and 150% for the formulations with essential oil.

In Figure 1 shows that an increase in the concentration of starch and glycerol influences the films' mechanical properties. Consequently, F1 showed a value significantly higher ($p < 0.05$) in tensile strength since the other formulations contained oils; these substances could function as a plasticizer of the system, increasing the molecular mobility. Therefore, the TS and EM decrease, and the E increases significantly ($p < 0.05$) with the oil content. Different authors have reported that the addition of essential oils significantly changes tensile strength (TS) and elongation at break (EB) of films based on starch. For instance, Amaral do Evangelho et al., 2019 reported that adding orange essential oil into corn starch films decreased the tensile strength and elongation of the films because the film structure featured discontinuities in the presence of essential oil [23]. On the other hand, Souza et al., 2013 developed cassava starch composite films incorporated with cinnamon essential oil, concluding that cinnamon essential oil significantly decreased TS and increased EB, reducing the interaction of the intermolecular forces between polymer chains [24].

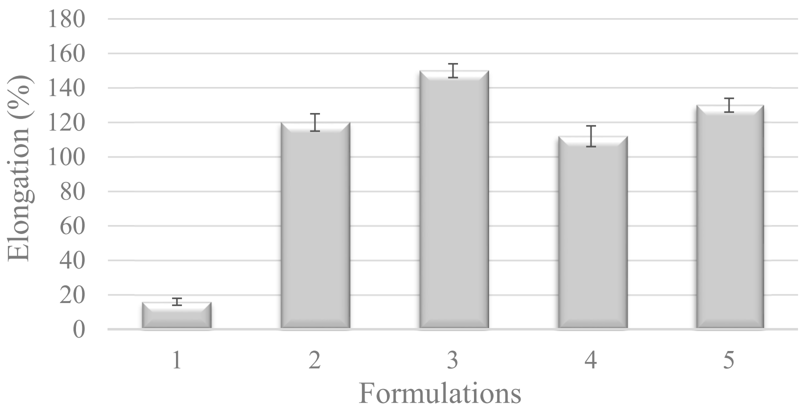
It is essential to mention that the higher the value of the modulus, the stiffer the material. In this way, the films with essential oil showed similar values, while the control film showed the maximum value in elastic modulus. These values are similar to those reported for films based on corn and wheat starch incorporated with lemon essential oil (lo). The reported results showed that the incorporation of LO caused a decrease in water content, transparency, whiteness index (WI), water vapour permeability (WVP), solubility, and tensile strength properties. In this way, they evaluated that the tensile strength decreased as the essential oil concentration increased and, conversely, the elongation at break increased compared to the control film [25]. This phenomenon could be due, as mentioned above, to an increase in molecular mobility promoted by the oils. This effect is seen in the significantly increase in elongation. Other authors suggest that the decrease in tensile strength can be attributed to the change in polymer-polymer interactions in polymer-oil interactions caused by the addition of essential oils and the mixture of both polymers. These less rigid and more deformable materials can be applied to manufacturing food packaging materials [26]. In general, similar studies highlight that the tensile strength of yam starch films is better than wheat and corn starch films, which produce weak strength [27]. Possibly it is because the amylose content of yam starch is higher than in cassava or wheat starch. This fact would be related to a higher crystallinity, increasing the tensile strength and rigidity of the material [28].



(**a**)



(**b**)



(**c**)

Figure 1. Mean values and standard deviation of the tensile strength (**a**), elastic modulus (**b**), and elongation at a breakpoint (**c**) for the different films stored for one week at 53% RH and 25 °C. The formulations: 1 (without essential oil), 2 (essential oil of lime), 3 (essential oil of fennel), 4 (essential oil of lavender), 5 (essential oil of lime, fennel and lavender).

3.2. Thickness and Barrier Properties

Table 2 shows the values of thickness (µm), water vapour, and oxygen permeability after one week of storage at 53% RH and 25 °C. Thickness is an essential characteristic of coatings because it affects barrier properties such as WVP and other gases. It is observed that when adding essential oils to the starch-based matrix, the thickness decreased significantly ($p < 0.05$). The values of the thicknesses of the formulations containing essential oils were statistically similar.

Table 2. Mean values and standard deviation of thickness (µm), water vapour (g· mm kPa^{-1} h^{-1} m^{-2}) and oxygen ($\times 10^{15}$ cm^3 m^{-1} s^{-1} Pa^{-1}) permeabilities of the different films after one week of storage at 53% relative humidity and 25 °C.

Formulations	Thickness	WVP	OP
F1	244 ± 4 ^{a}	19.2 ± 1.2 ^{a}	2.4 ± 0.3 ^{a}
$F2_{li}$	175 ± 4 ^{b}	11.8 ± 0.5 ^{b}	3.2 ± 0.5 ^{b}
$F3_{fe}$	172 ± 5 ^{b}	11.4 ± 0.6 ^{b}	2.7 ± 0.4 ^{ab}
$F4_{la}$	180 ± 8 ^{b}	12.1 ± 0.9 ^{b}	3.1 ± 0.2 ^{b}
$F5_{lifela}$	175 ± 6 ^{b}	11.7 ± 0.8 ^{b}	3.0 ± 0.2 ^{b}

Different superscript letters within the same column indicate significant differences among formulations ($p < 0.05$).

The highest value of WVP was for the F1 film with 19.2 (g mm kPa^{-1} h^{-1} m^{-2}), while the film $F3_{fe}$ obtained the lowest value. The behaviour of WVP is significantly influenced ($p < 0.05$) with the addition of essential oils to the films. The oxygen permeability data ranged between 2.4 and 3.2 ($\times 10^{15}$ cm^3 m^{-1} s^{-1} Pa^{-1}). Regarding WVP, adding a hydrophobic substance causes a decrease in this parameter. This phenomenon is because higher tortuosity is created inside the polymeric matrix as the water vapour molecules pass, slowing down the permeation [29]. An improvement in WVP of active biodegradable films based on cassava starch and pumpkin residue extract and oregano oil was reported by Dos Santos Caetano et al., 2018 The incorporation of natural compounds decreased WVP, reducing the water absorption by the films since these substances are complex and highly hydrophobic mixtures [30]. In the case of OP, an opposite effect is created; the presence of hydrophobic substances slightly accelerates the permeation of oxygen molecules. As is well-known, the oxygen permeability of starch films depends highly on plasticizer content and relative humidity. In this regard, the essential oil, acting as a plasticizer, could increase oxygen permeability [31]. Ghasemlou et al., 2013 reported similar results in corn starch-based films containing Zataria multiflora Boiss or Mentha pulegium [32]. Adding 3% (*v*/*v*) of either essential oil to the films significantly increased the OP values. This behaviour was attributed to the swelling of the starch polymer matrix because of volatile oils. In this way, oxygen may penetrate through oil/starch interfaces, providing oxygen-penetration channels when higher essential oil concentrations are added to the films.

3.3. Interaction with Water

Table 3 shows the moisture content, water-solubility, absorption capacity, and contact angle values for the studied films. As expected, the addition of hydrophobic molecules to the material significantly modified the properties related to the interaction with water.

Regarding the moisture content, no significant differences ($p < 0.05$) were observed between the yam flour films in addition to essential oil. However, a higher moisture content (0.0067) was observed for F1 compared to the other formulations. This fact suggests that the amylose interaction favoured the hydrophilicity of the material. Finely dispersed oil molecules between the starch chains could occupy intermolecular spaces, preventing the water molecules from easily interacting with the starch hydroxyls [33]. This fact would favour a decrease in the moisture content of the material, given that the incorporation of hydrophobic substances throughout the polysaccharide matrix tends to reduce possible interactions between water and functional groups of the polymer [34].

Table 3. Mean values and standard deviation of moisture content (Xw, (g water/g dried film)), solubility in water (g solubilized film/g initial dried film), water absorption capacity (g water/ g dried film), and contact angle with water (°) of the different films after one week of storage at 53% relative humidity and 25 °C.

Formulations	Xw	Solubility in Water	Water Absorption Capacity	Water Contact Angle (°)
F1	0.067 ± 0.005 [a]	0.182 ± 0.005 [a]	0.81 ± 0.02 [a]	56 ± 2 [c]
$F2_{li}$	0.042 ± 0.003 [b]	0.142 ± 0.004 [b]	0.95 ± 0.03 [b]	62 ± 2 [b]
$F3_{fe}$	0.045 ± 0.005 [b]	0.141 ± 0.002 [b]	0.98 ± 0.04 [b]	67 ± 2 [a]
$F4_{la}$	0.043 ± 0.005 [b]	0.148 ± 0.005 [b]	0.94 ± 0.03 [b]	63 ± 2 [b]
$F5_{lifela}$	0.042 ± 0.004 [b]	0.145 ± 0.003 [b]	0.96 ± 0.02 [b]	64 ± 2 [ab]

Different superscript letters within the same column indicate significant differences among formulations ($p < 0.05$).

The results of the solubility of the films are related to the moisture content, so the higher value of solubility (F1) indicates a lower water resistance. Possibly the presence of oil molecules between the starch chains can weaken the intermolecular forces between the amylose and amylopectin chains, promoting the system's instability in an aqueous medium [31]. As have been reported, the addition of essential oil significantly changes the water solubility of starch films. In some cases, essential oils increase this property, and in other cases, a reduction is observed, suggesting that hydrophilic compounds increase the film solubility while hydrophobic compounds decrease it [35]. Cai et al., 2020 found that the solubility of starch films increased with increasing thyme essential oil microcapsule content due to the hydrophilicity of the microcapsules [36]. The high-water solubility of biomaterials can be advantageous for the biodegradation process of the films [37].

Water absorption is a critical film factor, especially for packaging applications, demonstrating the film's sensitivity to liquid water contact [38]. Films in the presence of essential oil did not show significant differences between them ($p < 0.05$), with a maximum value for $F3_{fe}$ of 0.98, while F1 showed the minimum value of 0.81. Therefore, all films achieved medium absorption. The decrease in the intermolecular forces of the starch chains could make it easier for the liquid water molecules to penetrate the matrix more easily. Interestingly, the opposite case would be given for water vapour molecules (moisture content and permeability to water vapour) [36].

Table 3 shows that the yarn films presented values between 56 and 62° of contact angle with water. The contact angles were higher in the films containing essential oils, due to the presence of oily molecules that increase the surface tension of the water droplets on the surface of the film. Other authors also observed similar water contact angle values for yam starch-based films ranging from 44.0° to 63.3°, indicating that the higher the contact angle value, the more hydrophobic the material [39].

3.4. Optical Properties

The optical properties of food packaging materials play an important role in the appearance and acceptance of packaging by consumers [40]. Table 4 presents the optical properties (gloss at an angle of incidence of 85° and internal transmittance at 650 nm) of yam starch films with and without essential oils. The gloss values obtained were between 32.1 and 38.7°, so they can be considered low gloss. In F3, the gloss decreased significantly ($p > 0.05$) compared to the other formulations. This property is related to the surface roughness of the films. The more homogeneous the surface, the brighter it will be. Therefore, the analyzed films had surface roughness, possibly due to the drying process and the retrogradation of the starch chains on the surface of the films. In addition, essential oils have been reported to reduce the transparency and increase the opacity of starch films due to a modification in the crystalline structure and increased light scattering generated by the oil droplets in the polymer matrix [41]. For instance, Cai et al., 2020 reported a reduction in corn starch films' optical properties (e.g., transparency) due to the presence of thyme essential oil microcapsules [36]. All the films here developed mean internal transmittance values associated with greater internal homogeneity of the film. As expected, the dispersed phase of essential oil causes the internal homogeneity of the material to decrease as a consequence of higher diffraction of the light 33. From a food preservation point of view, reduced transparency in food packaging materials can also be a positive feature because light scattering can help prevent photo-oxidation and degradation of organic compounds in food products [42].

Table 4. Mean and standard deviation of brightness (°), internal transmittance (nm), of the different films after one week at 53% relative humidity and 25 °C.

Formulations	Gloss (85°)	Internal Transmittance (650 nm)
F1	38.7 ± 0.4^{a}	65.0 ± 1.5^{a}
$F2_{li}$	35.4 ± 0.4^{b}	61.2 ± 1.9^{b}
$F3_{fe}$	32.1 ± 0.5^{c}	60.1 ± 2.1^{b}
$F4_{la}$	36.2 ± 0.3^{b}	59.8 ± 2.3^{b}
$F5_{lifela}$	35.1 ± 0.2^{b}	60.3 ± 2.5^{b}

Different superscript letters within the same column indicate significant differences among formulations ($p < 0.05$).

3.5. Coating Effect on Strawberry Preservation

3.5.1. Antioxidant Properties

Table 5 shows the DPPH and ABTS antioxidant activity (IC_{50} values) of lime, fennel, and lavender essential oils. In the same way, the antioxidant activity of films with essential oils incorporation is shown. The lower the IC_{50} parameter, the substance or material has the greater antioxidant activity [Guidelines for accurate EC_{50}/IC_{50} estimation].

Table 5. Mean values and standard deviation of DPPH IC_{50} and ABTS IC_{50} of the films and, lime (li), fennel (fe), and lavender (la) essential oils.

Sample	DPPH IC_{50} (mL EO/ mg DPPH)	ABTS IC_{50} (mL EO/ mg DPPH)
EO_{li}	1.9 ± 0.2^{a}	0.26 ± 0.03^{a}
EO_{fe}	0.042 ± 0.003^{b}	0.0081 ± 0.0003^{b}
EO_{la}	0.029 ± 0.002^{c}	0.0052 ± 0.0002^{c}
	DPPH IC_{50} (mg film/mg DPPH)	ABTS IC_{50} (mg film/mg DPPH)
F1	0	0
$F2_{li}$	38 ± 0.4^{a}	8.06 ± 0.03^{a}
$F3_{fe}$	10.05 ± 0.02^{c}	0.26 ± 0.02^{c}
$F4_{la}$	6.67 ± 0.03^{d}	0.18 ± 0.02^{c}
$F5_{lifela}$	14.9 ± 0.3^{b}	2.6 ± 0.03^{b}

Different superscript letters within the same column indicate significant differences among formulations ($p < 0.05$). The samples represent: EO_{li} (essential oil of lime), EO_{fe} (essential oil of fennel) and EO_{la} (essential oil of lavender).

Lavender essential oil (EOla) showed higher antioxidant activity compared to the other essential oils. Consequently, the DPPH radical scavenging activity of the F4la film was significantly higher ($p < 0.05$). The high antioxidant activity of EOla is attributed to their main constituents, such as 1,5-Dimethyl-1-vinyl-4-hexenylbutyrate, 1,3,7-Octatriene, 3,7-dimethyl-, Eucalyptol, and Camphor 43. Hui et al., 2010 reported a strong antioxidant activity of EOla against lipid peroxidation in a linoleic acid model system assessed by the β-carotene bleaching test. Lime essential oil (EOli) and derived films showed the lowest antioxidant activity compared to the other essential oils [43]. The bioactive properties of EOli have been attributed to the volatile components, e.g., terpenes, oxygenated compounds, and sesquiterpenes, specifically, the terpene compound limonene [1-methyl-4-(prop-1-en-

2-yl)cyclohex-1-ene) [44,45]. The antioxidant activity of films containing EOli was also observed in previous studies using 5 to 50 µg/mL concentrations. It was found that the oil has an antioxidant activity comparable (27.9 to 87.54%) to the reference compound, i.e., ascorbic acid (42.18 to 93.83%). The free radical scavenging activity increased as the concentrations increased; however, ascorbic acid (IC50: 13.68) was approximately 1.5 times more potent than essential oil of lime-based on the IC50 value [46]. In general, the addition of essential oils into the polymer matrix improves the antioxidant capacity of the final material. All the films presented antioxidant activity, and they can be applied in active packaging systems to extend the shelf life of food products [47].

3.5.2. Coating Application on Strawberries

Figure 2 shows the weight loss experienced by coated and uncoated strawberries stored at 25 °C and 85% RH for two weeks. From the first days of storage, it was observed that the coated strawberries lost weight in a less accelerated way than the coated strawberries. As of day 8, uncoated strawberries show an acceleration in weight loss. This weight loss is directly related to the moisture request of the fruits. As seen in the water vapour permeability test, the films with essential oils had lower WVP than the control. Thus, the graph shows that the weight loss in uncoated strawberries was 26.2 g of water/100 g of fruit, and in coated strawberries, 3.3 g of water/100 g of fruit. It was possible to reduce 22.8 g of water/100 g of fruit from the weight loss using coatings. Similar studies have indicated that the weight loss of fresh strawberries is due to water loss [48]. Furthermore, coating thickness and porosity have been shown to affect gas and water exchange [49]. Cai et al., 2020 found that mangoes treated with a starch film containing thyme essential oil microcapsules after 10 days of storage had a lower weight loss than those without film, extending the weight loss of mangoes within a specific range. These results suggested that films and coatings play an essential role as a water barrier between the fruit and the environment throughout the storage period [36].

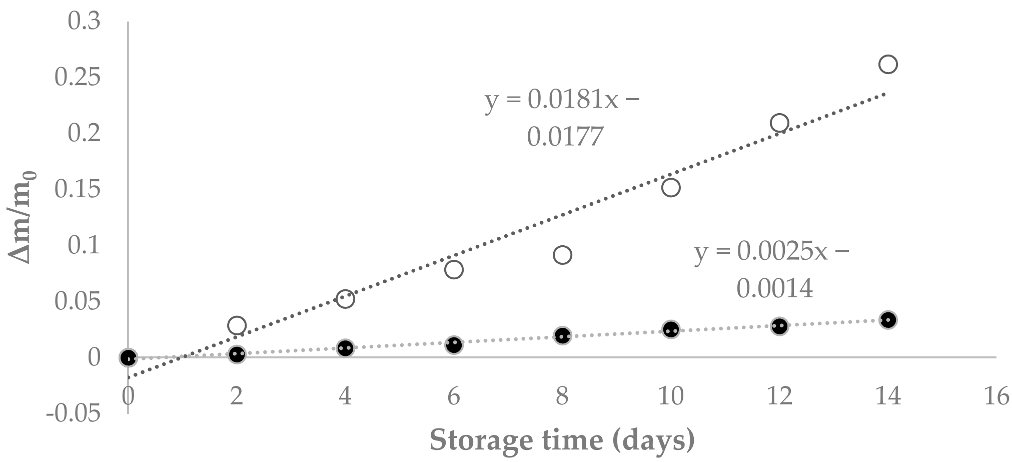


Figure 2. Weight loss of strawberries with (•) and without (○) coating (F5 formulation) stored under 85% RH at 25 °C for two weeks.

The changes of strawberry inoculated with *Aspergillus niger* stored at 85% RH and 25 °C for two weeks are shown in Table 6. According to the present study, the growth of *Aspergillus niger* increased with storage time on uncoated strawberries inoculated with the fungus (C_{with_i}). The study showed that the addition of essential oils inhibited the growth of *Aspergillus niger* until day 8. After this day, the presence of *Aspergillus niger* is observed in the RF5with_i, although to a lesser degree than in the C_{with_i}. After 10 days of storage, the physical appearance of inoculated coated strawberries decreased remarkably due to the fungus and softening phenomena. Therefore, the coating effectively reduced the *Aspergillus niger* population in strawberries during storage at 25 °C. Previous studies have also reported that films or coatings with antimicrobial properties incorporated with different essential oils and extracts can successfully inhibit the growth of *Aspergillus* in different foodstuffs. Moreover, functional coatings can inhibit a wide range of bacteria. In this way, Bezerra de Aquino et al., 2015 studied the effect of edible chitosan–cassava starch coatings containing a mixture of Lippia gracilis Schauer genotypes on the shelf life of guavas during storage at room temperature for 10 days. They concluded that the total number of mesophilic aerobic bacteria and the counts of fungi and yeasts were statistically lower in the coated fruits [50]. Similar results were obtained by Issa et al., 2017 where the use of thyme essential oil in sweet potato starch-based nanocomposite films reduced the population of Salmonella Typhimurium and Escherichia coli in fresh spinach leaves. These results demonstrate the advantages of using edible active films to reduce food spoilage [51].

Table 6. Strawberry inoculated with *Aspergillus niger*: samples without coating ($C_{without\_i}$: Uninoculated and uncoated; C_{with_i}: Inoculated without coating) and samples with coating ($RF5_{without\_i}$: without inoculum and with coating; $RF5_{with\_i}$: inoculated and coated) stored at 85% RH and 25 °C for two weeks.

Day	Treatment	Good Appearance (%)	Softening (%)	Aspergillus Presence (%)
0	$C_{without\_i}$	100	0	0
	C_{with_i}	100	0	0
	$RF5_{without\_i}$	100	0	0
	$RF5_{with\_i}$	100	0	0
2	$C_{without\_i}$	100	0	0
	C_{with_i}	90	0	10
	$RF5_{without\_i}$	100	0	0
	$RF5_{with\_i}$	100	0	0
4	$C_{without\_i}$	100	0	0
	C_{with_i}	80	10	20
	$RF5_{without\_i}$	100	0	0
	$RF5_{with\_i}$	100	0	0
6	$C_{without\_i}$	90	10	0
	C_{with_i}	60	30	40
	$RF5_{without\_i}$	100	0	0
	$RF5_{with\_i}$	100	0	0
8	$C_{without\_i}$	85	15	0
	C_{with_i}	30	50	70
	$RF5_{without\_i}$	100	0	0
	$RF5_{with\_i}$	100	0	0
10	$C_{without\_i}$	70	30	0
	C_{with_i}	0	70	100
	$RF5_{without\_i}$	100	0	0
	$RF5_{with\_i}$	95	0	5
12	$C_{without\_i}$	65	35	0
	C_{with_i}	0	100	100
	$RF5_{without\_i}$	95	5	0
	$RF5_{with\_i}$	90	5	10
14	$C_{without\_i}$	50	50	0
	C_{with_i}	0	100	100
	$RF5_{without\_i}$	90	10	0
	$RF5_{with\_i}$	85	10	15

4. Conclusions

It was possible to incorporate essential oils of lime, fennel, and lavender into yam starch-based films. The effect of the addition of essential oils on the tensile properties, the barrier properties, the moisture content, the solubility in water, the absorption capacity, the angle of contact with water and the optical properties of the films were studied. The mechanical properties indicated that the tensile strength varied between 1.98 and 4 MPa. The modulus of elasticity was found between 76 and 127 MPa, and the elongation was between 16 and 130% for the formulations with essential oils. A plasticizing effect of essential oils was observed. As expected, WVP decreased, and OP increased due to the hydrophobic nature of the oil. In the same way, the addition of oil affected the properties of interaction with water. The films with oils showed lower moisture content, less solubility in water, and a slightly higher water absorption capacity. About antioxidant properties, lavender oil and derived films had the highest antioxidant capacity. Regarding the coating application on strawberries, it was observed that the strawberries coated with F5 lost 22.8 g of water/100 g strawberry less than those not coated during the 14 days of storage. In addition, the total inhibition of *Aspergillus niger* was observed until day 8 of storage. From day 10, it was observed in a much lower proportion than on the control samples. This study has shown the potential application of yam starch-based coatings with the content of essential oils lavender, lime, and fennel on the preservation of strawberry, both to avoid weight loss and protect it against *Aspergillus niger*.

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