Research Article

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Evaluation of physicochemical properties of film-based alginate for food packing applications

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Abstract: The indiscriminate use of films as synthetic primary packaging, for the conservation and transport of fruit and vegetable products in postharvest, causes disposal problems. In the present work, films based on sodium alginate were synthesized and characterized, with alginate as a biopolymer matrix, glycerol (plasticizer), oleic acid (control of hydrophobicity), and calcium chloride (cross-linking agent). The dynamic mechanical, thermal, structural, and hydrophobicity properties were studied. In the case of dynamic mechanical properties, they were analyzed at a temperature of −50°C, because food packaging goes through storage during its cold chain, showing biofilm stability under these conditions. On the other hand, infrared spectroscopy analysis showed that the carboxylate and carboxy functional groups serve as a link for all the components, and oleic acid is also serving as a plasticizer and, to a lesser degree, as a hydrophilicity controller.

Keywords: biofilms, primary packaging, cold storage, postharvest, cross-linking

1 Introduction

There is a growing interest in the scientific community for food quality assurance. Worldwide, 1,300 million tons of food that are produced are considered as food loss and waste. In Latin America, an average of 127 million tons of food waste is generated per year, with fruits and vegetables being one of the most affected products, which is equivalent to 55%, due to the handling and storage conditions during postharvest as well as deterioration factors. All these postharvest handling are derived from the respiration rates, since some of them are considered as climacteric products (1,2). In this sense, the main function of packaging is to maintain nutritional and safety quality in food during storage and transport as well as to increase the shelf life (3). Thus, to date, the production of petroleum-derived packaging materials per year has increased alarmingly. Furthermore, the degradation of petroleum-derived packaging materials is a slow and laborious process – due to the presence of nondegradable components used for its synthesis – which has led to the generation of serious environmental problems, due to their ultimate disposal in ecosystems (4,5).

On the other hand, the growing demand by consumers for natural foods has led researchers in the areas of food and engineering to improve packaging materials – for example, seeking to develop new materials based on biopolymers, including those from renewable sources. This is the case with agroindustrial waste, which has resulted in the generation of green processes, which include a synergy...
between food and packaging (3,6). In addition to this, new trends involving modern practices and lifestyles are laying the foundations for the evolution of innovative preservation techniques, without compromising the quality and safety characteristics of food (7). According to the European Bioplastics Association, biopolymers are defined as polymers that can be manufactured from renewable sources and that have to be biodegradable and – specifically – compostable, in order to serve as fertilizers and/or soil conditioners. Biodegradability is directly related to the bonds of the chemical structure of the materials, rather than the source from which they come. In particular, the chemical bond type plays an important role in the time in which it can degrade the polymer (4). Among the polymers that can be used for the development of primary food packaging materials, especially for the film and coating types, proteins, polysaccharides, lipids, and polyesters are taken, purely or in combination (4,5). One of the advantages that proteins and polysaccharides present for the synthesis of the packaging films is their oxygen barrier properties at low or high relative humidity values, together with their acceptable characteristics of mechanical stress, low humidity, and water vapor barrier properties, due to the hydrophilic character of the chemical structure of the network strongly, which is linked by its hydrogen bonds. However, when it comes to mechanical and water vapor barrier properties, biopolymers still cannot compete with petroleum-based materials, which is a challenge for new research (2,3,8).

Among the strategies that have been examined to improve the properties of biodegradable packaging materials to compete with synthetic polymers is the development of cross-linking polymers. These structures form an integrated network through a process in which the chains are connected inter- and intra-molecularly, through covalent and noncovalent bonds. The cross-linked structures in biopolymers occur to a greater extent in proteins and polysaccharides, which have the characteristic of having numerous functional groups, acting as a link in reactions of this type. In addition to cross-linking processes, other alternatives to improve the properties of this type of material include chemical modifications of polymers, the addition of plasticizers, and the addition of nanoparticles as reinforcement or polymeric compatibilizers (3). The addition of plasticizers modifies the mechanical properties of polymers by reducing cohesion in the film structure and weakening intermolecular forces, causing an improvement in the mechanical properties of polymers, and also serving as part of a hydrophilic polymer matrix that changes the barrier properties, especially of water vapor. Polysaccharides have the ability to form films and coatings, among which are cellulose, chitosan, pectin, and alginate, to mention just a few (9,10). In addition to being effective in the transfer of gases such as O₂ and CO₂ and retarding the loss of volatile components, its hydrophilicity results in low water vapor barrier properties. Carra-geenans and alginites are highly hygroscopic; and when these are used as packaging films, they provide temporary protection against the loss of water in food. These characteristics of permeability are of vital importance, especially in fruits and vegetables, since they prevent the deterioration of these foods, delaying their respiration and aging as a consequence of the metabolic processes in the postharvest stage (4,11,12).

Alginate is a biopolymer with a block structure that can form resistant materials, with a variety of applications in textiles, manufacturing, medicine, and food. It is a linear anionic polysaccharide soluble in water and considered as a complex mixture of oligosaccharides composed of blocks of poly-mannuronic acid and poly-guluronic acid derived from monomers with bonds of \( \beta(1\rightarrow4) \) mannuronic acid and \( \alpha(1\rightarrow4) \) guluronic acid, respectively. While the \( \beta(1\rightarrow4) \) link is considered to form a flexible, linear-type structure, the \( \alpha(1\rightarrow4) \) link gives rise to a rigid axial chain structure, causing steric rejection (near the carboxylic group, COOH) of the polymer chain by conformational folding of the structure (13). The composition of the monomer blocks and the sequence affects the final properties of the alginate gels and the materials developed, as a consequence of the selectivity of the biopolymer bonds with the cross-linking agent when used to stabilize the resulting structure. For example, in the case of films synthesized from this polymer, the mechanical properties are related to the number of active sites (functional groups), for the formation of “eggbox” structures, improving the barrier and mechanical properties as well as their cohesiveness and stiffness (6). Thus, the focus of this work is on the study and analysis of the interactions and influence of the plasticizer, a second compound to impart a hydrophilic/hydrophobic character (oleic acid also provides plasticizing characteristics) and a cross-linking agent, over the properties of an alginate matrix, for its subsequent application in the development of a primary packaging film for fruits and vegetables in postharvest storage, in a modified atmosphere in cold temperatures.

2 Materials and methods

For the synthesis of the biofilms, sodium alginate from Sigma-Aldrich was used as the polymer matrix. Glycerol
was purchased from Meyer’s Company, oleic acid from Drugstore Cosmopolita Company, and calcium chloride from Sigma-Aldrich.

2.1 Synthesis of biofilms

The biofilms were synthesized in three blocks. The components were an alginate matrix, glycerol (as a plasticizer), oleic acid (as a hydrophilicity/hydrophobicity controller), and calcium chloride (CaCl₂) as a cross-linking agent, as shown in Table 1. In all cases, each component was added at different concentrations, and the Teflon molds used were 15 cm in diameter. Once the biofilms were obtained, they were stored in sealable bags at room temperature.

2.1.1 Biofilms (alginate–glycerol) added with oleic acid without cross-linking (block 1)

The solution for the polymer matrix was prepared by casting, dissolving the sodium alginate in distilled water at a concentration of 1% (w/v), heated at a temperature of 70°C for 20 min, and kept under constant stirring. Next, glycerol was added at the concentrations of 0.25%, 0.5%, and 1% (v/v), and oleic acid was added at 0.25% (v/v), with stirring at 600 rpm for an additional 20 min. Once this time had elapsed, the mixture was poured into the molds to form the films. Finally, the films were placed on a drying stove at a temperature of between 40°C and 45°C for 12 h.

2.1.2 Biofilms (alginate–glycerol) without addition of oleic acid and cross-linking (block 2)

In this case, the films were cross-linking with CaCl₂. Once the film was formed and dried – with the previously used conditions and without the addition of oleic acid – the films were kept in the molds. Subsequently, they were cross-linked with a CaCl₂ solution at concentrations of 0.75% and 1% (v/v), with a contact time of 2 min. After the time had elapsed, they were removed from the mold and dried for 5 min at 40°C in a drying oven.

2.1.3 Biofilms (alginate–glycerol) added with oleic acid and cross-linking (block 3)

For the synthesis of these films, oleic acid was added to the solution of sodium alginate, and glycerol was added during the stirring step at a temperature of 50°C and kept under constant stirring for 4 h. After 4 h had elapsed, the container with the obtained gel was placed in a desiccator, and a vacuum pressure of 51 kPa was applied for 15 min for the elimination of the bubbles obtained during the mixing of the components. Subsequently, it was emptied into the circular molds, which were dried at 38°C for 12 h. Once the films were dry, the CaCl₂ solution was added, maintaining contact between the cross-linking agent and the films for 1 min more. The films were dried at 40°C for 5 min and were finally removed from the mold.

Table 1: Biofilm formulation

| 1% (% w/v) sodium alginate (A1) matrix |
|---------------------------------------|
| None cross-linked and added with glycerol | Biofilms without oleic acid | Biofilms with oleic acid |
| A1 | A1Ao |
| AG25 | AG25Ao |
| AG5 | AG5Ao |
| AG1 | AG1Ao |
| Cross-linked and added with glycerol | Biofilms with addition of glycerol, cross-linked at 0.75% | Biofilms with addition of glycerol, cross-linked at 1% |
| ACC75 | ACC1 |
| AG25Cc75 | AG25Cc1 |
| AG5Cc75 | AG5Cc1 |
| AG1Cc75 | AG1Cc1 |
| Cross-linked and added with glycerol and oleic acid | Biofilms cross-linked at 0.75% | Biofilms cross-linked at 1% |
| ACC75Ao | ACC1Ao |
| AG25Cc75Ao | AG25Cc1Ao |
| AG5Cc75Ao | AG5Cc1Ao |
| AG1Cc75Ao | AG1Cc1Ao |

2.2 Characterization

The synthesized films were analyzed by dynamic mechanical analysis (DMA) as well as thermal, structural, and hydrophobicity examination. DMA was carried out on a Q800 device (TA Instruments, Inc.), with a temperature range from −50°C to 200°C at a heating rate of 10°C every 5 min. The thermal stability was evaluated by means of TA Instruments simultaneous with DSC/TGA equipment model SDTQ600 (TA Instruments, Inc.) in a temperature range from 30°C to 800°C, with a speed heating of 10°C every 5 min. To study the changes in the chemical structure of the sodium alginate matrix and the different components of the formulation, the analysis was carried out by
infrared spectrophotometry (FTIR), using a Spectrum One device (Perkin Elmer), in an attenuated total reflectance mode (ATR-FTIR; ATR Universal Pike-MIRacle) accessory, with a 12-scan at a resolution of 4 cm⁻¹, in a wavenumber range of 4,000–400 cm⁻¹. The hydrophilic property was evaluated by measuring the drop formation with a Canon DS126621 camera (Macro 0.25 m/0.8 ft, lens ft/s 18–55 mm, diameter 58 mm) and a portable photo studio with backdrops (black, white, orange, blue, red, and green). The images were digitized in Drop Snake LBADSA software to determine the contact angle, and the water vapor permeability (WVP) was analyzed by the ASTM E96/96M-16 (Water method). Samples were tested by triplicate, and the values were averaged.

3 Results and discussion

3.1 DMA

DMA analysis was performed to determine the effect of the added components – glycerol, oleic acid, and calcium chloride (cross-linking agent) – on the polymeric matrix of sodium alginate as well as to determine the influence of these on the storage module ($E'$) (Table 2) and tan $\delta$ (Table 3), with respect to temperature (see Figure 1). The storage module measures the absorbed energy and the ability of a material to withstand loads or stress, while the tan $\delta$ provides information related to changes associated with the movement of the polymer chains and also determines the glass transition temperature ($T_g$) and its viscoelastic behavior. The start of the analysis was carried out at low temperatures (from −50°C), since these biofilms are intended to be used as primary packaging in climacteric foods such as fruits and vegetables. Mechanical behavior is of vital importance in these materials to guarantee food safety and to control gas permeability through films (14,15).

Table 2 and Figure 1a–c show the storage modulus ($E'$). Here it can be seen that at temperatures of −50°C in alginate/glycerol biofilms without the addition of oleic acid, there is a complex and tangled molecular structures with maximum values of 11,430 MPa (AG25). However, when oleic acid is added to this formulation (AG25Ao) – for the control of hydrophilicity in the polymeric matrix – the $E'$ value falls to 1,776 MPa (Figure 1a). This behavior shows that the chemical structure of this component (with a carboxyl group and a double bond) interacts with the polymer chain of alginate and glycerol, and intermolecular electrostatic attractions are established in complex manners. The resulting structure hinders the movement of the polymer chains, and the storage modulus $E'$ is decreased by nearly 100% specifically at low temperatures and when the concentration of glycerol is increased, but with the concentration of oleic acid remaining constant (AG1Ao). In Figure 1b, we can see...
that when only the cross-linking agent (CaCl₂) is added to the alginate matrix, the value of \( E' \) again decreases by 7% \((E' = 10,410 \text{ MPa} \) at a CaCl₂ concentration of 0.75%; sample ACc75). Moreover, when the concentration of the cross-linking agent is increased to 1% (ACc1), the storage modulus decreases further – up to 25% \((E' = 8,367 \text{ MPa})\) – presenting a behavior that is inversely proportional to the concentration of the cross-linking agent.

These phenomena are observed at a temperature of \(-50^\circ\text{C}\), which indicates that – under these conditions – the plasticity of the biofilm is a function of the concentration of the cross-linking agent, with the understanding that the purpose of this component is the union of the polysaccharide (rigid morphology and better mechanical properties of the biopolymer) from semicrystalline to amorphous zones. On the other hand, the width of the signals and areas not defined in the thermograms means that in these areas, there is a dispersion of the molecules caused by a heterogeneous arrangement in the structure of the polymer matrix.

Table 3 and Figure 1 show the \( \tan \delta \) values range from \(-50^\circ\text{C}\) to \(250^\circ\text{C}\). The analysis temperatures were taken as a function of the phases, where the changes in the thermograms are being presented, so they were divided into three stages for their examination. The temperature ranges from \(-50^\circ\text{C}\) were chosen depending on the application that these materials are intended to have, that is, whether they are to be used as packaging films for storing climacteric foods in a cold chain, whose average

### Table 3: Damping properties at the transition temperature of the alginate/glycerol/oleic acid/CaCl₂ biofilm formulation

| Biofilms     | Phase 1         | Phase 2         | Phase 3         |
|--------------|-----------------|-----------------|-----------------|
|              | Thermal transition \( ^\circ\text{C} \) | \( \tan \delta \) | Thermal transition \( ^\circ\text{C} \) | \( \tan \delta \) | Thermal transition \( ^\circ\text{C} \) | \( \tan \delta \) |
| A1           | -15.71          | 0.055           | 30.77           | 0.084           | 204.64          | 0.630           |
| AG25         | -37.68          | 0.134           | -10.31          | 0.101           | 197.11          | 0.702           |
| AG5          | -27.77          | 0.535           | 9.94            | 0.374           | 194.47          | 0.545           |
| AG1          | 33.16           | 0.415           | -4.03           | 0.490           | 159.58          | 0.316           |
| A1Ao         | -26.08          | 0.240           | 9.48            | 0.325           | 216.49          | 0.748           |
| AG25Ao       | -23.03          | 0.184           | 11.74           | 0.251           | 104.80          | 0.199           |
| AG5Ao        | -23.22          | 0.169           | 1.86            | 0.184           | 121.00          | 0.112           |
| AG1Ao        | -40.02          | 0.076           | 105.44          | 0.081           | 179.94          | 0.210           |
| AG25Cc75Ao   | 23.03           | 0.184           | 11.74           | 0.251           | 174.41          | 0.224           |
| AG5Cc75Ao    | -23.03          | 0.184           | 11.74           | 0.251           | 174.41          | 0.224           |
| A1Cc75Ao     | 23.03           | 0.184           | 11.74           | 0.251           | 174.41          | 0.224           |
| AG1Cc75Ao    | 30.97           | 0.141           | 5.17            | 0.093           | 196.27          | 0.481           |
| A1Cc1Ao      | 19.82           | 0.078           | 17.8            | 0.077           | 192.35          | 0.656           |
| AG25Cc1Ao    | 34.47           | 0.128           | 7.46            | 0.212           | 73.65           | 0.121           |
| AG5Cc1Ao     | 39.97           | 0.170           | -3.76           | 0.118           | 60.64           | 0.128           |
| AG1Cc75Ao    | 41.08           | 0.118           | 12.18           | 0.239           | 57.58           | 0.338           |
| A1Ac1Ao      | 42.00           | 0.118           | 79.63           | 0.110           | 114.81          | 0.208           |
| AG25Cc1Ac1Ao | 36.76           | 0.153           | 40.73           | 0.144           | 225.65          | 0.893           |
| AG1Cc1Ac1Ao  | 29.67           | 0.156           | 17.71           | 0.203           | 47.58           | 0.226           |

glycerol and oleic acid in the formulation – in combination with the cross-linking agent – provides viscoelastic properties in the biofilm, allowing the chains to relax. This phenomenon is presenting while an increase is reflected until to reach the \( E_{\text{max}} \) values at their yield temperature of 3,117 and 4,883 MPa, for the samples of ACc75Ao and ACC1Ao, respectively. As the temperature increases, the storage modules generally show a decrease in their values, which means that the films go through transition states, from rigid to soft, so the biopolymer is considered to pass from semicrystalline to amorphous zones. On the other hand, the width of the signals and areas not defined in the thermograms means that in these areas, there is a dispersion of the molecules caused by a heterogeneous arrangement in the structure of the polymer matrix.
temperature of storage is approximately 4°C. In addition, the behavior of the material at these temperatures was studied in order to observe the changes in rigidity, flexibility, and stability of the structure of the polymer chains when these materials are subjected to these temperature changes.

In Figure 1d–e, three thermal transition phases can be observed, showing in general that the sodium alginate matrix shows high capacity to store energy, where the number of binding sites for the formation of the “eggbox” structures is related to the mechanical properties of this type of material (6). In phase 1, shown in Table 3, we can see that in a temperature range of approximately −50°C to 30°C (related to \( T_g \)), with the addition of glycerol (together with oleic acid, with the latter also being considered as plasticizer (17) but of the hydrophobic...
On the other hand, when calcium chloride concentration at which it is present plays an important role, the energy; however, when combined with oleic acid, the compatibility limit between glycerol/oleic acid/CaCl₂ and the polysaccharide – in this case, the alginate. In the particular case of synthesized biofilms, there are limit concentrations of glycerol of 0.5%, when oleic acid is present at a concentration of 0.25% and in the case of the cross-linking agent between 0.75% and 1%. These interactions are being carried out primarily by the affinity of the functional groups present (18).

### 3.2 Differential scanning calorimetry analysis

Figure 2 and Table 4 presents the thermograms by differential scanning calorimetry (DSC) of the biofilms synthesized via casting. In the first two phases, a narrow and defined endothermic peak can be observed, followed by a combination of exothermic and endothermic peaks, equally defined (triplet of peaks α, β, and γ). In the case of endothermic peaks between 51°C and 66°C (first phase), they are related to the water from the sodium alginate solution, the bound water and the hydrophilic groups (COO⁻ and OH groups) of the polymer chain (20), in addition to the fact that interactions are taking place between glycerol and oleic acid as the first reaction. However, when adding calcium chloride, the thermal transition decreases by 13%, due to the formation of stable structures, by binding via hydrogen bonds with glycerol/oleic acid and subsequently with alginate. Despite these interactions, the presence of glycerol, oleic acid, and calcium chloride does not cause a noticeable change in the thermal transitions of this first phase.

When the combination of peaks α, β, and γ in the second phase appears (Figure 2d), there is a shift and an increase in transition temperatures. The exothermic peak α (135–212°C) occurs as a result of depolymerization reactions in the decarboxylation of protonated carboxylic groups and oxidation reactions of the polymeric matrix, as well as the breakdown of glycosidic bonds, loss of hydroxy groups and the evolution in the formation of CO₂. This second phase is also closely related to the breakdown of the interactions of the polymeric matrix with glycerol, oleic acid and calcium chloride (endothermic transition β, 170–237°C) and with the evaporation and degradation of the glycerol present (exothermic transition γ, 204–266°C), as shown in Table 4 and Figure 2d (20–22). Here, it is
evident that the presence of the combination of glycerol/oleic acid increases thermal stability by 10%, but when the synergy between glycerol/oleic acid/CaCl₂ is established, this property is improved by up to 20% compared to the pure polymeric matrix. This is attributed to the cross-linking networks between glycerol and alginate. The presence of glycerol causes an increase in the binding sites, to form stable structures of the “eggbox” type, showing great affinity with these two components, via hydroxyl groups and the calcium ion of the salt.

As for the third phase of degradation, temperatures basically obey exothermic processes and will depend on the system of components present, so they can start from values closer to 350°C for alginate (glycerol/oleic acid/CaCl₂), 650°C for alginate–glycerol, and 660°C for alginate–(glycerol/CaCl₂) (23). The behavior is attributed to the beginning of the decomposition of the carbonaceous material of the alginate matrix, and the difference in the temperature ranges is attributed to the interactions that are manifesting in the components of the formulation (23). Ultimately, the decomposition of biofilms occurs in the range of 720–780°C.

3.3 Thermogravimetric analysis

Thermogravimetric analysis (TGA) and the study of stability and thermal reactions are important, especially when films are going to be used as food packaging. Figure 3 shows three weight loss degradation phases for
synthesized biofilms. The first step occurred between 135°C and 203°C and is related to the water removal to the alginate films by 26% on average. The second stage appears at 242–264°C derived from the degradation of polysaccharides, the bond breakdown and the loss of the hydroxyl groups, as well as start the loss of plasticizer (22). While increasing the concentration of hydroxyl groups, the loss mass also tends to rise by up to 80% (AG1) and

Table 4: Combined bands α, β, and γ. Endo- and exothermal transitions of biofilms depending on the components of glycerol/oleic acid/calcium chloride

| Sample | α   | β   | γ   | Sample | α   | β   | γ   | Sample | α   | β   | γ   |
|--------|-----|-----|-----|--------|-----|-----|-----|--------|-----|-----|-----|
| A1     | 208.0 | 212.6 | 232.6 | ACc75  | 203.1 | 210.2 | 242.7 | ACc75Ao | 203.8 | 220.6 | 266.5 |
| AG25   | 204.3 | 220.0 | 233.4 | AG25Cc75 | — | 211.7 | 244.2 | AG25Cc75Ao | 207.9 | 237.7 | 227.9 |
| AG5    | 135.3 | 170.7 | 203.6 | AG5Cc75 | — | 213.1 | —     | AG5Cc75Ao | 171.1 | 205.9 | 227.9 |
| AG1    | 135.3 | 195.4 | 205.1 | AG1Cc75 | 184.5 a | 206.4 b | 241.5 c | AG1Cc75Ao | 183.6 | 205.1 | 222.7 |
| A1Ao   | 212.3 | —     | 232.8 | ACc1   | 201.0 | 208.1 | 248.9 | ACc1Ao | 205.9 | 223.1 | 261.8 |
| AG25Ao | 204.3 | 216.5 | 229.0 | AG25Cc1 | — | 217.3 | 249.2 | AG25Cc1Ao | 208.5 | 230.4 | —     |
| AG5Ao  | 144.5 | 181.7 | 206.3 | AG5Cc1 | 208.4 | 232.1 | 253.6 | AG5Cc1Ao | 179.3 | 209.4 | 229.1 |
| AG1Ao  | 135.8 | 204.1 | —     | AG1Cc1 | — | 208.4 | 224.7 | AG1Cc1Ao | 177.9 | 210.2 | 235.9 |

I In samples with superscripts a, b, and c, the process is reversed; endo, exo, and endo.
II The values that are not reported are due to the fact that the peaks are not occurring in the described areas.

Figure 3: TGA plots of (a) none cross-linked and added with glycerol, without and with oleic acid; (b) cross-linked and added with glycerol; and (c) cross-linked and added with glycerol and oleic acid.
70% (AG1Ao), compared to pure alginate. Nonetheless, the weight loss in this phase is around 39%. This phenomenon is observed just when glycerol and oleic acid are part of the formulation in the alginate matrix. The third step begins in the temperature range of 467–484°C, which is associated with the degradation of the organic structure of alginate (12%). Conformation modifications and the release of volatile compounds take place as the temperature increases, indicating the bulk of the matrix. Beyond this temperature, the remaining weight loss indicates the char process of the carbonaceous material (Figure 3a) (21,24). However, when the CaCl₂ cross-linking agent is added to the matrix and interacts with the alginate and glycerol (Figure 3b), the first phase of degradation suffers a thermal slight decrease. At the same time, weight loss also drops to 23% at a lower temperature (131–200°C). The diminishing of active groups from glycerol (OH) as a result of the bonding process with calcium chloride solution, also gives rise to a reduction in the concentration of water. In the second phase, however, the temperature remains unchanged compared to the biofilms previously described (see Figure 3a), which is attributed to the interaction between the polymer, chain of alginate and the CaCl₂ through plasticizer. The same does not hold true for the lost weight, which suffers an increase of 33%, coming from the cross-linking salt agent. On the other hand, at the third wave, the rate of the chemical structure changes shifted to higher-range temperatures (492–510°C). Due to the presence of the polyvalent calcium ion, the thermic behavior improved – before decomposition began – and the char reactions appear from the oxide compounds (24). Finally, in the third group of formulated biofilms (Figure 3c), a similar behavior to those registered previously is again being presented. This suggests that the plasticizer (mainly glycerol) decreases the temperatures at which structural chemical changes are taking place, while the cross-linking agent (CaCl₂) increases the temperature of these reactions in each of the three phases presented in the biofilms.

### 3.4 FTIR analysis of biofilms

FTIR analysis was performed to determine the main functional groups and interactions of compounds of the different formulations for the synthesis of biofilms, in addition to the formation of new functional groups as a consequence of the resulting bonds. Figure 4 shows the FTIR spectrum of the biofilms synthesized in the alginate matrix and in the different synthesized formulations. We can observe the characteristic bands of alginate, A1 (Figure 4a) at 3,275 cm⁻¹; the stretch of the hydroxyl groups v(OH), v(CH₂), and v(CH₃) at 2,923 cm⁻¹; and the alkyl groups at 2,857 cm⁻¹ (23). The bands at 1,595 and 1,406 cm⁻¹ correspond to the vibrations of the carboxylate groups v(COO⁻) in the symmetric and asymmetric modes, respectively, of the pyranoside ring of the alginate matrix (25). Small peaks at 1,088 and 947 cm⁻¹ have been related to the group v(CO) with the stretching vibrations of the pyranoside ring and stretching of the CO group (deformation of the vibrations of C–C–H and C–O–H), respectively (23). An intense band at 1,027 cm⁻¹ is related to either a v(C–O) bond or the glycosidic bond (15). In Figure 4a, the alginate matrix spectra with the addition of glycerol are presented (AG25, AG5, AG1). Here, we can see an increase in hydroxyl groups due to the contribution of the glycerol molecule at 3,275 cm⁻¹, as well as the alkyl groups observed at 2,923 and 2,857 cm⁻¹; coming from the groups v(CH₃) asymmetric and symmetric vibrations, respectively. In the case of the carboxylate groups at 1,595 and 1,406 cm⁻¹, the interaction with the pyranoside ring only occurs when glycerol is in a concentration of 1%. This is not the case for the v(C–O) groups of the pyranoside ring at 1,088 and 947 cm⁻¹, where it is observed that at all concentrations of glycerol, there are interactions between both molecules, showing a high binding affinity. In the case of the v(C–O) group, the peak is at 1,027 cm⁻¹. However, coming from the glycosidic bond, the binding is only obtained when the glycerol concentration is at 1%. On the other hand, when there is an interaction with oleic acid (Figure 4b, samples A1 and A1Ao), there is no contribution from the hydroxyl and alkyl groups; they are not compatible with the alginate matrix at this specific formulation. However, for the rest of the alginate bands – that is, the carboxylate (COO⁻) and carboxy (CO) groups – there is a significant capacity for interaction with oleic acid and alginate. In Figure 4c, the infrared spectrum is shown when the CaCl₂ cross-linking agent is added to the alginate/oleic acid formulation (A1, ACc75Ao, ACc75, ACc1Ao, ACc1), where we can observe a manifestation of the bidentate cross-linking typical of the alginate matrix (26). The hydroxy and alkyl groups only interact when there is the combination of 0.25% oleic acid and CaCl₂ in both concentrations (0.75% and 1%), displaying the affinity of the bond in the polar zone of the oleic acid molecule (COOH). In the absence of oleic acid, there is no bond between the alginate and the cross-linking. Once again, it presents the carboxylate and carboxy groups – with the bands at 1,595, 1,406, 1,088, 1,027, and 947 cm⁻¹ – which participate in the links of the three components of this formulation, with alginate/oleic acid/CaCl₂, being more related to the formation of an “eggbox”-type structure.
When the glycerol is added – instead of oleic acid – and interacts with the cross-linking agent in the formulation (A1, AG25Cc75, AG5Cc75, AG1Cc75), as seen in Figure 4d, neither the hydroxy nor the alkyl groups of this molecule participate in the bonds with the alginate matrix. Additionally, there is a higher degree of binding, and in the area of the carboxyl groups and their derived functional groups – such as COO⁻ and CO – of the

Figure 4: FTIR spectra of alginate films of (a) Alginate/glycerol, (b) Alginate/oleic acid, (c) Alginate/oleic acid/crosslinked, (d) Alginate/glycerol/crosslinked and (e) Alginate/glycerol/oleic acid/crosslinked.
pyranoside ring and of the glycosidic bond. In the band at 1,027 cm\(^{-1}\), which corresponds to the glycosidic bond, it is observed that at a concentration of 1% glycerol, in combination with alginate and CaCl\(_2\), there is a limit of miscibility where all components added can interact without affecting the properties. Finally, when all the components of the formulation are interacting; samples A1, AG25Cc75Ao, AG5Cc75Ao, and AG1Cc75Ao (alginate/glycerol/oleic acid/CaCl\(_2\) in Figure 4e), the dominant functional groups for the formation of bonds are being presented with the carboxylate and carboxy groups of the pyranoside ring, but not the hydroxy and alkyl groups, which do not participate in the linking of the components of the formulation. In summary, the main active sites for the binding of all compounds are occurring in the pyranoside ring of the alginate polymer. The one with the highest binding affinity is oleic acid, due to the group in the polar zone of this molecule (COOH). On the contrary, when the glycerol and oleic acid interact in the formulation, competition is created, which is closely related to the concentration of glycerol.

### 3.5 Contact angle analysis

In Table 5, the analysis of the contact angle is presented, which is related to the degree of hydrophilicity and hydrophobicity of the biofilms. In all cases, it has a hydrophilic behavior. However, in particular, the presence of only oleic acid in the formulation – with its contribution of nonpolar groups, coming from the aliphatic chain with a terminal methyl group – causes an increase in hydrophobicity of 12% compared to the alginate matrix (A1 and A1Ao) (27). In the same way, we can observe that the glycerol alone contributes to the hydroxyl groups (OH), resulting in a decrease in hydrophobicity of close to 16% (A1 and AG1). If – together – glycerol and oleic acid are added to the formulation (which has a nonpolar zone as well as a polar zone in its chemical structure through its carboxyl group [COOH]), this brings about a synergic behavior by increasing the hydrophobicity up to 40%. This is reflected in the biofilms of the pure alginate matrix, with a contact angle of 50°; however, when oleic acid is added – and glycerol is also present AG25Ao – the value decreases to 30°. This effect is also exhibited when the CaCl\(_2\) cross-linking agent is incorporated (AG25Cc1), and the combination of the three components – glycerol, oleic acid, and calcium chloride (AG25Cc1Ao) – affects the degree of hydrophobicity of the films. This is due to the contribution of the functional groups, which are working as binding and hydrophilic sites at the same time. In general, the trend is a saturation of the composition, in which all the components can interact, and it accepts at most 0.25% of glycerol, before a contrary phenomenon occurs in the hydrophobicity properties.

### 3.6 WVP

WVP is an essential property for food packing, indicating water transfer between food, such as fruits and vegetables, and the atmosphere. WVP should be as low as possible, to decrease the dehydration process of food in order to keep it fresh (21,28). Table 5 shows the WVP as well as thickness data of biofilms. The tendency of biofilms is

| Table 5: Biofilms properties, water vapor permeability, thickness, and contact angle |
|-------------------------------------------|
| **Biofilms** | **Water vapor permeability (WVP)** (g m\(^{-1}\) h\(^{-1}\) Pa\(^{-1}\)) \(\times 10^{-6}\) | **Thickness** (mm) | **Contact angle (°)** |
|-------------------------------------------|
| **Biofilms added with glycerol, oleic acid without cross-linking agent** |
| A1 | 1.733\(^a\) | 0.0184\(^ab\) | 50 |
| AG25 | 1.302\(^a\) | 0.0055\(^ab\) | 50 |
| AG5 | 2.954\(^a\) | 0.0126\(^ab\) | 44 |
| AG1 | 2.947\(^a\) | 0.0126\(^ab\) | 42 |
| A1Ao | 2.952\(^a\) | 0.0566\(^ab\) | 56 |
| AG5Ao | 3.13\(^a\) | 0.0055\(^ab\) | 40 |
| AG1Ao | 9.22\(^a\) | 0.0372\(^ab\) | 33 |
| **Biofilms with addition of glycerol, cross-linking agent, and without oleic acid** |
| A1Cc75 | 5.075\(^a\) | 0.0056\(^ab\) | 49 |
| AG25Cc75 | 3.489\(^a\) | 0.0243\(^ab\) | 35 |
| AG5Cc75 | 4.405\(^a\) | 0.0452\(^ab\) | 49 |
| AG1Cc75 | 4.434\(^a\) | 0.0851\(^ab\) | 43 |
| A1Cc1 | 3.588\(^a\) | 0.0199\(^ab\) | 50 |
| AG25Cc1 | 4.662\(^a\) | 0.0098\(^ab\) | 33 |
| AG5Cc1 | 6.054\(^a\) | 0.0633\(^ab\) | 41 |
| AG1Cc1 | 3.65\(^a\) | 0.0553\(^ab\) | 50 |
| **Biofilms added with glycerol, oleic acid, and cross-linking agent** |
| A1Cc75Ao | 1.254\(^a\) | 0.1184\(^ab\) | 49 |
| AG25Cc75Ao | 6.624\(^a\) | 0.516\(^a\) | 35 |
| AG5Cc75Ao | 4.070\(^a\) | 0.0540\(^ab\) | 49 |
| AG1Cc75Ao | 6.883\(^a\) | 0.0617\(^ab\) | 43 |
| A1Cc1Ao | 3.616\(^a\) | 0.0404\(^b\) | 50 |
| AG25Cc1Ao | 3.213\(^a\) | 0.0892\(^ab\) | 33 |
| AG5Cc1Ao | 2.122\(^a\) | 0.0056\(^ab\) | 41 |
| AG1Cc1Ao | 7.183\(^a\) | 0.0426\(^ab\) | 50 |

Mean of three repetitions ± standard deviation. Comparisons between means were made using a Tukey test. Different letters indicate significant differences between formulations (\(p \leq 0.05\)).
that the WVP values are generally higher than the pure alginate matrix. This phenomenon might be caused by the sorption and transport of water molecules throughout the polar groups, where the WVP increase is more evident when the glycerol and oleic acid are added to the matrix, so it is also related to the concentration of these components. On the other hand, hydrophilic and hydrophobic ratio is closely related to the WVP properties (28,29), which is in accordance with the behavior observed in this study. The analysis of variance did not show a significant difference in WVP in each of the groups. But if the values of first group of films are compared with the third group, here it can be seen that the permeability values are higher, due to the increase in the hydrophilic and polar groups present in the structure. Regarding the thickness of the biofilms, the analysis of variance showed a significant difference in group 3. Also, in Table 5, it can be observed that the thickness of the film rises between the formulations; when the cross-linking agent is present, the thicknesses is highest in biofilms.

4 Conclusions

In the present work, biofilms were formulated based on alginate, glycerol (as a plasticizer), oleic acid (for the control of hydrophilicity/hydrophobicity), and calcium chloride (as a cross-linking agent). Biofilms have high storage modulus ($E'$) at the start of analysis and at a temperature of −50°C, with 11,140 MPa for the pure alginate matrix, which is characteristic of a polymer that fits into a rigid structure; beside each added component and concentration play an important role in the ability to absorb energy. At the end of the analysis, the maximum values of $E'$ show a stability of the biofilm when it goes from cold to hot temperatures. In the case of tan δ, three phases of transition and rearrangement of the polymeric structure take place. Also, it was found that oleic acid behaves as a second plasticizer. In thermal analysis, different degradation stages were observed, throughout the synergy of glycerol/oleic acid and CaCl$_2$ by the affinity of functional groups, leading to an improvement in thermal stability. Structural analysis (FTIR) of the biofilms showed that specific active sites are observed for the binding of all the compounds, with the carboxylate and carboxy groups (COO$^-$ and CO). Both of them present in the pyranoside ring of the alginate polymer have the highest affinity for the interactions of the formulation compounds. Oleic acid has an important characteristic in its chemical structure, such that the polar zone through the carboxyl group (COOH) actively participates to function as a bridge for glycerol and the cross-linking agent. On the contrary, when the glycerol and oleic acid are present in the formulation, a competition will appear to the linking process, which is closely related to the polar properties and the concentrations of both components. As for the WVP and contact angle, all the films have to show a hydrophilic behavior; although by adding only oleic acid to the alginate matrix, this property slightly decreases. Finally, it is important to note that these biofilms are intended to be applied as films in primary packaging for the storage of postharvest fruit and vegetable products, during storage in the cold chain to guarantee food safety and nutritional quality. This is closely related to the thermomechanical properties at low temperatures. The polymers are able to recover without presenting breakage of the polymeric structures caused by temperature, at the same time allowing the permeability of breathing gases ($O_2$, $CO_2$, and water vapor) for the aforementioned types of foods.

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