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# Avaliação das propriedades físico-químicas e antibacterianas de filmes à base de biopolímeros incorporando-se extrato de *Zingiber officinale*

## *Evaluation of the physicochemical and antibacterial properties of films based on biopolymers incorporating Zingiber officinale extract*

## *Evaluación de las propiedades fisicoquímicas y antibacterianas de películas basadas en biopolímeros que incorporan extracto de Zingiber officinale*

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**Resumo**

Os polissacarídeos são um material sustentável para revestimentos e filmes comestíveis, pois não são tóxicos, estão amplamente disponíveis na natureza e têm permeabilidade seletiva ao $CO_2$ e $O_2$. Neste trabalho, uma pesquisa de laboratório em uma base quali/quantitativa, foram desenvolvidos filmes de alginato de sódio, com e sem reticulação pós-formação do filme, além de filmes de quitosana incorporando-se extrato de *Z. officinale* como aditivo antimicrobiano. Foram comparadas várias propriedades como a solubilidade, o conteúdo de umidade, o grau de intumescimento, a morfologia e a atividade antimicrobiana dos filmes preparados. Os filmes de alginato com reticulação e incorporação de extrato de *Z. officinale* mostram as melhores características para serem utilizados como curativo medicamentoso, uma vez que apresenta baixa solubilidade em água, maiores graus de intumescimento e menor conteúdo de umidade. Além disso, o filme de alginato com reticulação e incorporação de média concentração de extrato de *Z. officinale* apresentou atividade antimicrobiana contra *Bacillus cereus*.

**Palavras-chave:** Atividade antimicrobiana; Filmes funcionais; Grau de intumescimento; Morfologia; Solubilidade; Umidade.

**Abstract**

Polysaccharides are a sustainable material for coatings and edible films, as they are nontoxic, widely available in nature and have selective permeability to $CO_2$ and $O_2$. In this work, a laboratory research on a quali/quantitative basis, sodium alginate films were developed, with and without post-film cross-linking, as well as chitosan films incorporating *Z. officinale* extract as an antimicrobial additive. Several properties such as solubility, moisture content, swelling, morphology and antimicrobial activity of prepared films were compared. The alginate films with crosslinking and incorporation of extract of *Z. officinale* showed the best characteristics to be used as medicated dressing, since it presents low solubility in water, higher swelling, and lower moisture content. In addition, the alginate film with crosslinking and incorporation of medium concentration of *Z. officinale* extract showed antimicrobial activity against *Bacillus cereus*.

**Keywords:** Antimicrobial activity; Functional films; Swelling degree; Morphology; Moisture; Solubility.

**Resumen**

Los polisacáridos son un material sostenible para recubrimientos y películas comestibles, ya que no son tóxicos, están ampliamente disponibles en la naturaleza y tienen una permeabilidad selectiva al $CO_2$ y al $O_2$. En este trabajo, una investigación de laboratorio sobre una base cualitativa/cuantitativa, se desarrollaron películas de alginato de sodio, con y sin reticulación posterior a la película, además de películas de quitosano que incorporan extracto de *Z. officinale* como aditivo antimicrobiano. Se compararon varias propiedades, tales como solubilidad, contenido de humedad, grado de hinchamiento, morfología y actividad antimicrobiana de las películas preparadas. Las películas de alginato con reticulación e incorporación de extracto de *Z. officinale* muestran las mejores características para usarse como apósito medicinal, ya que tiene baja solubilidad en agua, mayor grado de hinchazón y menor contenido de humedad. Además, la película de alginato con reticulación e incorporando una concentración media de extracto de *Z. officinale* mostró actividad antimicrobiana contra *Bacillus cereus*.

**Palabras clave:** Actividad antimicrobiana; Películas funcionales; Grado de hinchazón; Morfología; Solubilidad, Humedad.

## 1. Introduction

Increasingly, consumers are concerned with the consumption of more natural, high quality and safer food that has packaging that does not pollute and is made through sustainable and inexpensive processes. Food packaging has its main function isolation of food from the surrounding environment, reduction of interaction with deteriorating factors, loss of active compounds, and extension of shelf life (Mohamed, El-Sakhawy & El-Sakhawy, 2020).

When films are applied as a thin layer to food, they act as a barrier against the surrounding environment. These coatings extend the shelf life of food by acting as a barrier to moisture and gases. Edible coatings also improve functional properties when incorporating biologically active components (Sharma, Shehin, Kaur & Vyas, 2018). A definition for edible coatings and films is that they are primary packaging made from edible ingredients. An edible film can be applied directly as a thin layer, by immersion, spraying, but a previously formed film can be used as a food packaging (Mohamed, El-Sakhawy & El-Sakhawy, 2020; Sharma, Shehin, Kaur & Vyas, 2018).

Polysaccharides are a sustainable material for coatings and edible films, as they are nontoxic, widely available in nature and have selective permeability to $CO_2$ and $O_2$

(Mohamed, El-Sakhawy & El-Sakhawy, 2020; Sharma, Shehin, Kaur & Vyas, 2018). Alginates are natural polysaccharides composed of different unit ratios of α-L-guluronate (G) and R-D-mannuronate (M) in (1–4) chain sequences. Alginates produce a strong and insoluble polymeric gel when reacting with divalent cations, mainly $Ca^{2+}$. Thus, alginate-based coatings and films can increase the shelf life and food quality, forming a barrier against water, maintaining flavour and delaying fat oxidation (Varaprasad, K., Jayaramudu, T., Kanikireddy, V., Toro, C. & Sadiku, 2020; Mohamed, El-Sakhawy & El-Sakhawy, 2020).

Chitosan, produced by deacetylation of chitin, is a cationic polysaccharide with antimicrobial, antioxidant, film-forming, texturizing and binding properties. As a coating agent, this polysaccharide can, slow the growth of certain fungi, delay ripening, in addition to reducing ethylene production, among other functions. Due to its non-toxicity, biocompatibility and biodegradability, chitosan has been used in the biomedical, food and chemical industries. Chitosan films are clear, flexible, and resistant and display good resistance to fat and oil, and $O_2$, but are highly moisture sensitive (Khan, Jamil, Akhtar, Bashir & Yar, 2019; Mohamed, El-Sakhawy & El-Sakhawy, 2020).

Essential oils and extracts from plants and spices exhibit antimicrobial and antioxidant properties, making them interesting additives for use in both the food and pharmaceutical industries. In recent years, essential oils have been extensively studied as additives in films and coatings. Due to their lipidic nature, they are believed to help reduce the water vapor permeability of hydrophilic films and impact other film properties such as traction, optical and structural, in addition to providing biological effects (Atarés & Chiralt, 2015). The application of natural extracts or essential oils incorporated in edible coatings demonstrated a decrease in respiration rate, reduction of microbial deterioration, as well as, antifungal and antibacterial action of these films in fresh products (Mohamed, El-Sakhawy & El-Sakhawy, 2020).

*Zingiber officinale* (popular known as ginger) is a spice cultivated for its flavour and pungency. Ginger essential oil and extracts are valuable products because of their aromatic and medicinal properties (Kubra & Rao, 2012). Ginger is widely used in traditional oriental medicine for cold, influenza, digestive disorders, nausea, vomiting, stomach problems, diarrhoea, arthritis, rheumatic disorders, migraines, headaches, heart problems, hypertension, stimulant, aromatic, antitumor, antioxidant, anti-inflammatory and cancer prevention (Kubra & Rao, 2012).

Therefore, the aim of the present paper was to prepare and study the physical-chemical and antimicrobial properties of alginate and chitosan films incorporating *Zingiber officinale* extract.

## 2. Methodology

The present work was a laboratory research on a quali/quantitative basis (Pereira et al., 2018).

**Extraction of *Z. officinale* by Soxhlet:** The sample was extracted with ethanol, which was heated together with the sample (crushed *Zingiber officinale* root) for 2 h. After extraction, the extract was filtered, dried in a rotary evaporator, and stored in a freezer at -5°C.

**Antibacterial activity of *Z. officinale* extract:** In the bioassays, the antimicrobial activity of the *Z. officinale* extract was tested against different microorganisms (Gram-negative bacteria: *Escherichia coli* ATCC 35218, Gram-positive bacteria: *Staphylococcus aureus* ATCC 29213 and *Bacillus cereus* ATCC 11778). To assess the antibacterial behaviour of the extract, the bacterial culture was diluted to a final concentration of $10^6$ cells/mL, and 500 µL of this solution was spread on new agar plates with LB medium. The plates were then placed in the incubator at 37°C for 2 h, The 5 mm diameter sterile discs were dipped aseptically in the extract for 3 minute and placed over nutrient agar plates seeded with bacterial culture. The plates were incubated at 37°C for 24 hours and the diameter of the inhibition zones was measured in millimetres. Antimicrobial assays were performed in triplicate with each bacterial strain. (CLSI, 2018; Balouiri, Sadiki & Ibnsouda, 2016).

**GC-MS analysis of the *Z. officinale* extract:** The GC-MS analysis were performed in a Shimadzu High Resolution Gas Chromatograph, model 2010 with Mass Spectrometry Detector, using an Agilent DB-5MS (30 m x 0.25 mm - 0.25 µm) column. The operating conditions of the equipment were: Injector temperature: 220°C; Injection Mode: Splitless; Sampling time: 2 minutes; Flow control mode: Linear speed (45.0 cm.seg-1); Pressure: 15.7 psi; Total flow: 19.4 mL min-1; Column flow: 1.49 mL min-1; Column temperature: Gradient mode (80-280°C). The Parameters of the Mass Spectrometry Detector were: Ion source temperature: 200°C; Interface temperature: 280°C; Solvent cutting time: 3 minutes; Detector voltage: Relating to the result of the Tuning; Initial detection time: 3.0 minutes; Final

detection time: 17.0 minutes; Acquisition mode: SCAN; Acquisition time: 0.25 seconds; SCAN mass/charge ratio (m/z): 40 to 600; Injection volume: 1 μL.

**Preparation of sodium alginate films:** The sodium alginate films were obtained according to the casting technique in two ways, according to the modified Norajit & Ryu (2011) methodology:

- in two stages, with pre-crosslinking in the formulation and post drying of the films.
- in one step, only with pre-crosslinking.
-

To obtain the alginate films in two stages, 2g of sodium alginate and 0.01 g of $CaCl_2$ were solubilized in 100 ml of distilled water at 80ºC with mechanical stirring. Then, glycerol (1.5 g/g sodium alginate) and *Z. officinale* extract (5, 10 and 20% w/w in relation to sodium alginate) were added. The film-forming solution was deposited in silicone forms and dried in an oven at 40ºC for 24 h. After drying, the films were immersed in a 2% $CaCl_2$ solution for 30 s and dried again at 40°C until easily removed from the support.
To obtain the films in a single step, cross-linking with 2% $CaCl_2$ solution was not performed after drying the films. Control films, without the extract, were also prepared (in one and two stages) and submitted to all subsequent evaluations.

**Preparation of chitosan films:** Chitosan films were obtained according to the casting technique in accordance with the modified Siripatrawan & Harte (2010) methodology. The film-forming solution was prepared by dissolving 1% chitosan in a 1% acetic acid solution. This solution was maintained under mechanical agitation for 24 h at room temperature. Subsequently, glycerol (30% w/w in relation to chitosan) and extract of *Z. officinale* (5, 10 and 20% w/w in relation to chitosan) were added. The film-forming solution was deposited in silicone forms and dried in an oven at 40°C for 24 h. Control films, without the extract, were also prepared, and submitted to all subsequent evaluations.

**Visual aspect of the films:** Visual and tactile analyses were performed subjectively. The films were evaluated considering parameters such as homogeneity (absence of insoluble particles and uniform colour), continuity (absence of breaks or fractures after drying), flexibility, ease of detachment from the support and ease of handling (Seixas, Turbiani, Salomão, Souza & Gimenes, 2013).

**Film thickness:** The thickness control of the films was determined using a digital micrometre (MITUTOYO, model MDC-25S, resolution 0.001 mm, USA). The final thickness was calculated by the arithmetic mean of ten random measurements over a fixed area.

**Analysis of film morphology:** The surface of the films was studied using optical microscopy. The films were analysed with essential oil and without essential oil, using a Leica digital microscope (Model MZ8, Leica AG, Heerbrugg, Switzerland) attached to a computer and a camera.

**Moisture content of films (W):** The total mass of a 2.5 cm diameter film sample was quantified and subsequently taken to an oven maintained at 105ºC for 24 h (Seixas, Turbiani, Salomão, Souza & Gimenes, 2013). After this period, the final dry matter was quantified. The moisture content of the film (W) is expressed as a function of the initial dry mass of the film using the equation:

$$W = \frac{(Mi - Msf)}{Mi} \; x \; 100$$

In which:

W - is the final moisture of the film (%).

Mi - is the initial mass of the sample (g).

Msf - is the final dry mass of the sample (g).

**Water solubility of the films (S):** A 2.5 cm diameter film sample was immersed in excess distilled water and the system was kept under gentle agitation at 25 ºC for 24 h, using an orbital Shaker (Seixas, Turbiani, Salomão, Souza & Gimenes, 2013). The final dry mass was determined by submitting this sample to drying (105 ºC for 24 h). The solubility of the film (S) is expressed as a function of the initial dry mass of the film using equation:

$$S = \frac{(Msi - Msf)}{Msi} \; x \; 100 \qquad \textbf{(1)}$$

In which:

S - is the amount of soluble matter (%).

Msi - is the initial mass of the sample (g).

Msf - is the final dry mass of the sample (g).

**Swelling Degree:** The total initial mass of a film sample was quantified, and the material was immersed in distilled water for different periods of time (Seixas, Turbiani, Salomão, Souza & Gimenes, 2013). Every 10 minutes the film was removed from the water, its total mass determined, and the sample returned to the water, until the mass of the film stabilized. The excess moisture on the surface of the samples was removed, placing it between two sheets of filter paper, before each weighing. The degree of swelling (GI) can be calculated according to equation 2:

$$GI = \frac{(Mu - Mi)}{Mi} \quad (2)$$

In which:

GI - is the degree of swelling of the film.

Mu - is the mass of the sample taken from the solution (g).

Mi - is the initial mass of the sample (g).

**Antibacterial activity of the film:** In these bioassays, the antimicrobial activity of the obtained *Z. officinale* extract was tested against different microorganisms (Gram-negative bacteria: *E. coli* ATCC 35218, Gram-positive bacteria: *S. aureus* ATCC 29213 and *B. cereus* ATCC 11778). To assess the antibacterial behaviour of the extract, the bacterial culture was diluted to a final concentration of 106 cells/mL, and 500 µL of this solution was spread on new agar plates with LB medium. The plates were then placed in the incubator at 37°C for 2 h, The 2.5 mm diameter film discs were placed over nutrient agar plates seeded with bacterial culture. The plates were incubated at 37°C for 24 h and the diameter of inhibition zones was measured in millimetres. Antimicrobial assay was performed in triplicate with each bacterial strain. (CLSI, 2018; Balouiri, Sadiki & Ibnsouda, 2016).

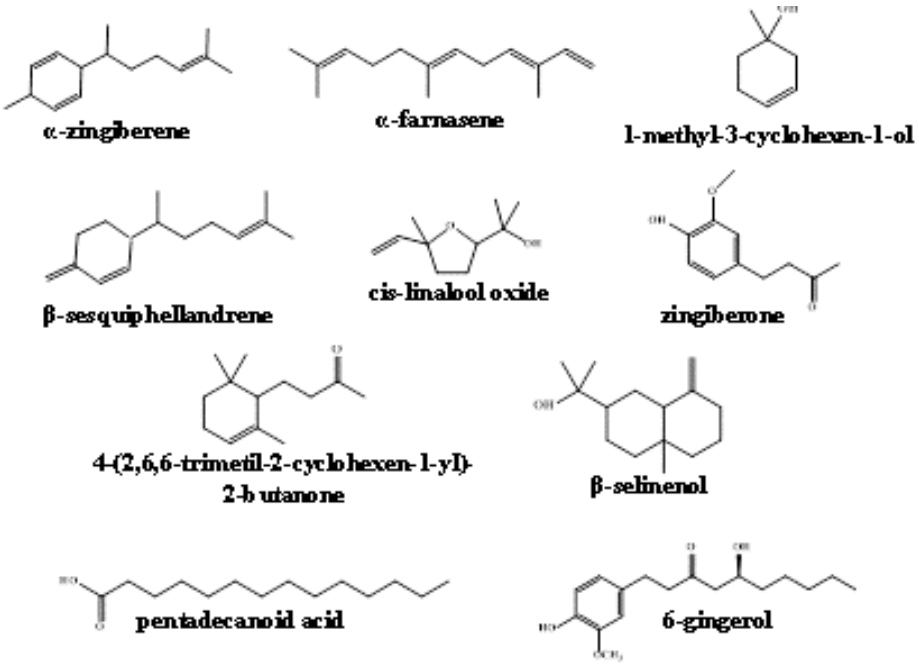
## 3. Results and Discussion

**Antibacterial activity of *Z. officinale* extract:** the antimicrobial bioassays performed with the extract of *Z. officinale* inhibited 100% of the growth of the three bacteria tested, one gram-negative and two gram-positive. According to Gull et al. (2012), ethanol and methanol extracts of *Z. officinale* were effective against eight different characterized drug resistant bacterial strains (*Salmonella typhi*, *Shigella*, *Pseudomonas aeruginosa*, *E. coli*, *B. subtilis*, *S. aureus*, *Staphylococcus epidermidis* and *Klebsiella pneumoniae*), but *E. coli* and *Shigella* showed the maximum susceptibility. Lucky, Igbinosa & Jonahan (2017), studied ethanol

extracts of *Z. officinale* against *Pseudomonas aeruginosa*, *B. subtilis*, *Aspergillus flavus* and *Candida albicans* and the extract was more effective against *B. subtilis* and *Candida albicans.* The authors also performed a phytochemical screening of the extract and found the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, phenols and steroids. Although, the literature shows high antimicrobial activity of *Z. officinale* ethanol extract (Gull et al. 2012; Lucky, Igbinosa & Jonahan, 2017), in this work the extract inhibited 100% the growth of all the studied microorganism, presenting a better antimicrobial activity than the literature.

**GC-MS analysis of the *Z. officinale* extract:** analysis by gas chromatography coupled with mass spectrometry revealed the presence of 9 peaks that were identified as: α-zingiberene, α-farnasene, 1-methyl-3-cyclohexen-1-ol, β-sesquiphellandrene, cis-linalool oxide, zingiberone, 4-(2,6,6-trimetil-2-cyclohexen-1-yl)-2-butanone, β-selinenol, pentadecanoid acid and 6-gingerol and the major compound is gingerol. The chemical structures of the identified compounds are shown in Figure 1.

**Figure 1:** Chemical structure of the compounds present in *Z. officinale* extract studied.




Source: Authors (2020).

The literature related that typical ginger essential oil and extracts presenting a high content of sesquiterpene, particularly, zingiberene, ar-curcumene, β-bisabolene, and β-sesquiphellandrene, while important monoterpenoids normally include geranial, neral, and

camphene, which are related to antimicrobial activity of ginger essential oil (Gull et al. 2012). The gingerols, (pungent principles of ginger), which are chemically relative to capsaicin, are biologically active components of ginger, specially related to anti-inflammatory, antidiabetic, antioxidant, anticancer and antimicrobial activity against both Gram-positive and Gram-negative microorganisms. The diarylheptanoids have been found to possess antioxidant, antihepatotoxic, anti-inflammatory, antiproliferative, antiemetic, chemopreventive, and antitumor activities (Gull et al. 2012; Lucky, Igbinosa & Jonahan, 2017; Oyedemi, Kotsia, Stapleton & Gibbons, 2019). By this is possible conclude that α-zingiberene, α-farnasene, β-sesquiphellandrene and, especially, 6-gingerol are responsible for the antimicrobial activity of *Z. officinale* extract studied in this work, as 6-gingerol is the major compound in the studied extract.

Even though is possible to find in the literature several articles describing the antimicrobial properties of *Z officinale* but finding articles about the mechanism of action is not quite as easy. Apparently, the antimicrobial mechanism of action of gingerols is related to the number of carbon side chains of these compounds (Lee, Kim, Choi, Ham, Park & Lee, 2018). The anti-inflammatory activity of 6-gingerol is mediated by the macrophage inhibition and neutrophils activation, negatively affecting monocyte and leukocyte migration (Ezzat, Ezzat, Okba, Menze & Abdel-Naim, 2018). The 6-gingerol also inhibit biofilm formation of *P. aeruginosa* and *C. albicans* without affecting the planktonic cell growth and showed no chemical toxicity and is effective for metabolic syndrome, cardiovascular disease, dementia, arthritis, diabetes, osteoporosis, cancers, and infectious diseases. Studies revealed that presence of the hydroxyl moiety in 6-gingerol influences proinflammatory gene activation. According to Lucky, Igbinosa & Jonahan (2017), phylogenetic analysis of *Z. officinale* samples demonstrated that samples from different geographical origins were genetically indistinguishable. Although, the authors reported that *Z. officinale* samples from different origins presented no differences in major volatile compounds, significant differences in non-volatile composition, especially in 6-, 8- and 10-gingerols, which are the most active anti-inflammatory components in this species, were observed,

**Visual aspect:** visual and tactile analyses were carried out, and both alginate films without crosslinking and chitosan proved to be homogeneous and with good continuity (without breaks) and easy detachment of the support. However, alginate films proved to be more gelatinous and, when detached, adhered to other surfaces and, sometimes, ended up breaking. Chitosan films, on the other hand, proved to be more rigid and less flexible, thus not

presenting this problem. The crosslinked alginate films had some adherence to the surface of the plate and, when removed, broke.

**Thickness:** the thickness of the prepared films was measured at five points in the prepared films and the averages are shown in Table 1.

**Table 1:** Physical properties of the studied films.

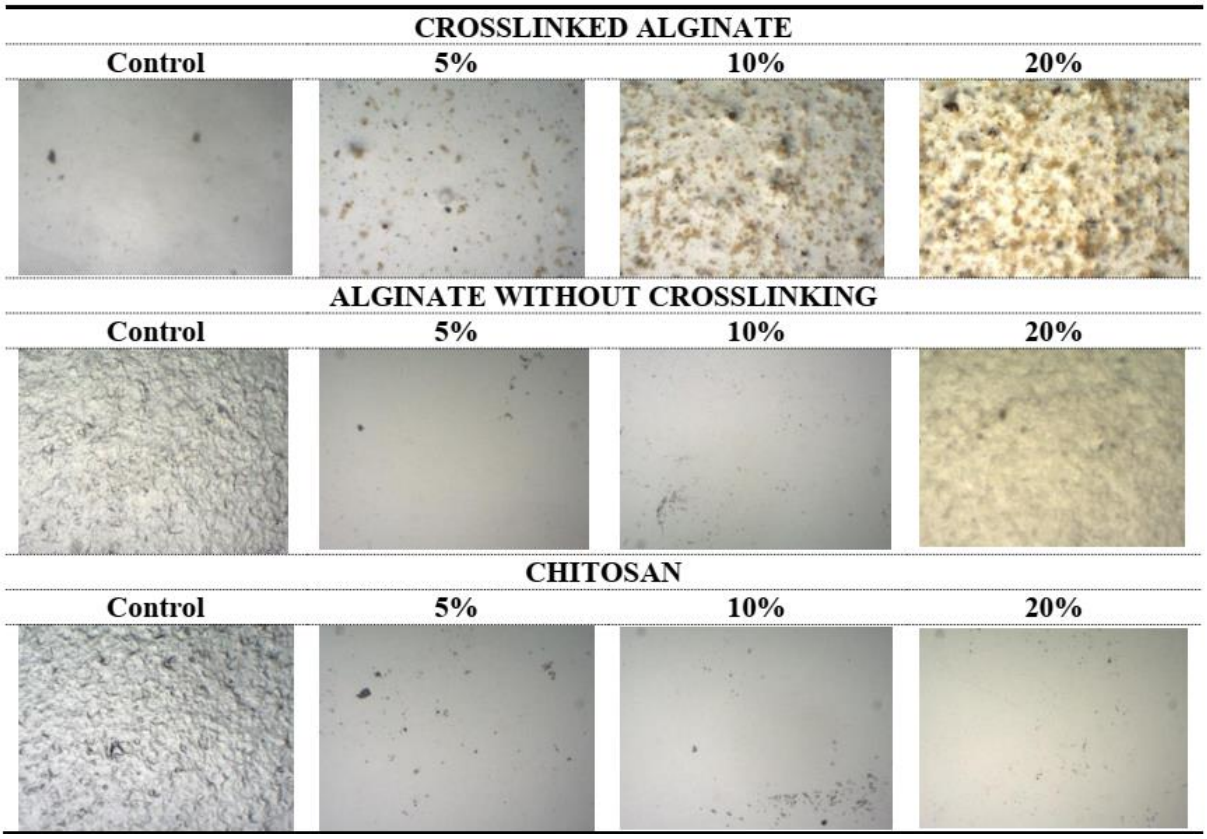
| Films | Thickness (mm) | W (%) | S (%) | SD |
|---|---|---|---|---|
| **Crosslinked alginate** | | | | |
| Control | 1.942 | 14.29 | 42.86 | 3.00 |
| 5% | 1.174 | 20.00 | 42.86 | 2.05 |
| 10% | 0.909 | 20.00 | 44.44 | 2.25 |
| 20% | 1.903 | 23.53 | 42.86 | 2.06 |
| **Alginate without crosslinking** | | | | |
| Control | 0.310 | 66.60 | 83.33 | 3.28 |
| 5% | 0.219 | 54.16 | 85.67 | 1.66 |
| 10% | 0.326 | 62.77 | 83.71 | 1.56 |
| 20% | 0.214 | 58.39 | 82.16 | 1.06 |
| **Chitosan** | | | | |
| Control | 0.112 | 11.40 | 100.00 | - |
| 5% | 0.411 | 16.06 | 100.00 | - |
| 10% | 0.185 | 11.66 | 100.00 | - |
| 20% | 0.281 | 11.20 | 100.00 | - |

Source: Authors (2020).

As can be seen from Table 1, the alginates films with crosslinking were thicker than the films without crosslinking, as was observed by Benavides, Villalobos-Carvajal & Reyes (2012). The alginate films are thinner than alginates films with crosslinking and is almost the same for alginates films without crosslinking. The concentration of extract incorporated in the films, apparently, has no relation to the thickness of the films, different from that observed by Norajit & Ryu (2011). In this study, the authors observed an increase in the thickness of the films with an increase in the concentration of green tea extract.

**Morphology analysis:** the morphology of the films prepared in this study was analysed using optical microscopy and the images are represented in Figure 2.

**Figure 2:** Optical micrograph of films produced.




Source: Authors (2020).

From Figure 2 it is observed that in alginate films without crosslinking, the control shows many irregularities on the surface, however, films with incorporation of low and medium concentration of *Z. officinale* extract present a smooth surface, without cracks and with small imperfections. The alginate film without crosslinking, with a high concentration of *Z. officinale* extract, present irregularities, that is, the high concentration of the extract impaired its dispersion in the film matrix. In chitosan films, the same is observed, the control shows many irregularities on the surface, however, all films incorporated with extracts of *Z. officinale* present a smooth and crack-free surface. However, in crosslinked alginate films, the observed pattern is the opposite, the control has a smooth and regular surface, whereas films with different extract concentrations have irregularities on their surface that increase with increasing concentration. In addition, it is observed that in these films the extract is presented in specific domains, which increase with the increase in the concentration of the extract. Apparently, the extract did not disperse in the film matrix, as observed by Liakos et al (2014). In this work, the authors prepared films of sodium alginate with surfactant Igepal CO-520 and incorporated cinnamon and mint essential oils (Liakos et al, 2014). This was not expected as

crosslinking is a technique in which the structure of the polymer matrix becomes more rigid and the essential oil or extract remains in the matrix's reticules.

**Moisture content of films (W):** the moisture analysis of the films results are shown in Table 1 and the sodium alginate films without crosslinking presented much higher humidity than those of chitosan and alginate with crosslinking, which explains the gelatinous behaviour of the films described previously in the visual aspect. Apparently, the incorporation of extract in different concentrations does not have a direct relationship between its concentration and moisture. The moisture content may be related to the polymeric structure of these films. Comparing the crosslinked alginate films and the chitosan films it is observed that the chitosan films have a slightly lower moisture content. According to Giz et al. (2020), with the increase in crosslinking, the chain entanglements also increase so, maybe, there is less space to retain water molecules.

**Water solubility of the films (S):** the analysis of the water solubility of the studied films was also performed in triplicate and the average data obtained are shown in Table 1. Considering that in edible coatings and in pharmaceutical applications low solubility is necessary, the use of chitosan films is out of the question, as prepared in this work. Although Sabbah et al. (2019), reported the addition of a plasticizer on chitosan-based films can modify the physicochemical characteristics of the film improving its applications. According to the authors, the addition of a plasticizers decreases the intermolecular forces along the polymer chains, impart flexibility and lower the glass transition temperature (Sabbah et al., 2019).

Despite the alginate without crosslinking films not being completed soluble, these films present a higher water solubility than the alginate with the crosslinking, so only the alginate crosslinking films have technological application in this case. According to Giz et al. (2020), the plasticizer and the crosslinking affects the water solubility of the films, the higher the amount of the plasticizer and the calcium chloride, lower the solubility.

In the alginate films with crosslinking, apparently, the incorporation of extract in different concentrations does not have a direct relationship between its concentration and the water solubility.

**Swelling Degree:** the degree of film swelling was also performed in triplicate for each of the film matrixes and the average of the values obtained is shown in Table 1. However, as expected due to the solubility test performed previously, the chitosan films were completely

solubilized in the first 10 min of immersion. That was also observer by Giz, et al. (2020). It is observed that alginate films with and without crosslinking and incorporated extract have a lower swelling degree than their respective control films. Thus, apparently, the addition of extract to the polymeric matrix impairs the absorption of water by the films, but without a direct relationship between the concentration of the extract and the swelling degree. Comparing the films incorporation the extract cross-linked and non-cross-linking, it is observed that the cross-linked films had a higher swelling degree than non-cross-linked films, which was not expected due to the moisture content data obtained in this study.

**Antibacterial activity of films:** antimicrobial activity of the prepared films was tested against the same microorganisms as the ginger extract. The data obtained from the bioassays performed are shown in Table 2.

**Table 2:** Antimicrobial activity of the studied films.

| **Films** | **Inhibition zones (mm)** | | |
|---|---|---|---|
| **Crosslinked alginate** | ***B. cereus*** | ***E. coli*** | ***S. aureus*** |
| Control | - | - | - |
| 5% | 12 | - | - |
| 10% | **20** | 12 | - |
| 20% | 15 | - | - |
| **Alginate without crosslinking** | | | |
| Control | - | - | - |
| 5% | - | - | - |
| 10% | - | - | - |
| 20% | - | - | - |
| **Chitosan** | | | |
| Control | - | - | - |
| 5% | - | - | - |
| 10% | - | - | - |
| 20% | - | - | - |

Source: Authors (2020).

It can be seen from Table 2 that the alginate films with crosslinking and a medium concentration of *Z. officinale* extract showed a slight antimicrobial activity, especially against *B. cereus*. The other films did not display antimicrobial activity. As in alginate films with crosslinking, there is the presence of external domains of extract, this may have contributed to its release into the culture medium, inhibiting microbial growth. Benavides, Villalobos-Carvajal & Reyes (2012) also observed in their study that films were more effective against

Gram-positive bacteria than against Gram-negative bacteria.

Biopolymeric films and coatings incorporating essential oils are promising as they are sustainable alternatives for use both as edible coatings in food, instead of conventional plastic packaging, or biomedical systems (Mohamed, El-Sakhawy & El-Sakhawy, 2020). Saxena, Sharma & Maity (2020), describe the popularity that coatings and films incorporating extracts and essential oils have been receiving in recent years, describing several studies using different coatings in combination with natural extracts or essential oils applied to different fruits and vegetables.

So, the crosslinking seems to be especially important in all the properties studied including the antimicrobial activity. The crosslinking apparently influences the matrix structure and the dispersion of the essential oils or extract incorporated in the films. By the present work, the alginate film with crosslinking and incorporating a medium concentration of *Z. officinale* extract may be an option as antimicrobial wound dressing or edible coating applied to minimally processed food.

## 4. Final Considerations

It is concluded that the ethanolic extract of *Z. officinale* affects the structure of alginate films, with and without crosslinking, as well as chitosan films. Regarding the studied properties, alginate films with crosslinking and incorporation of *Z. officinale* extract show the best characteristics to be used as a medicinal dressing as they present low water solubility, higher swelling degrees, and lower moisture content. In addition, the alginate film with crosslinking and incorporating a medium concentration of *Z. officinale* extract showed antimicrobial activity against *B. cereus* and could be used as edible coating in minimally processed fruits and as wound dressing healing.

In a future study the authors intend to apply the, the alginate film with crosslinking and incorporating a medium concentration of *Z. officinale* extract in a minimally processed fruit to explore the microbiological properties of this film, to study the shelf live and the sensorial acceptance of the of the recovered fruit.

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