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Biofilms Composed of Alginate and Pectin: Effect of Concentration of Crosslinker and Plasticizer Agents

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Biofilms are defined as flexible films prepared from biological materials such as proteins and polysaccharides that act as barriers to outside elements. These materials have potential application in medical, pharmaceutical and food area. Their use depends on various parameters such as mechanical properties (strength and flexibility), barrier properties (permeability to water vapor), water solubility, among others. This work aims to characterize biofilms made from pectin and alginate. The films were prepared by casting. This technique consists in preparing a film solution (2 % w/v) followed by the application of it in a holder for solvent evaporation. The manufacturing process of the films consisted of two stages. First the alginate and pectin (1:1) were dissolved in water containing 0.04 g of CaCl2.2H2O/g macromolecule and 0.6 g glycerol/g macromolecule. The second stage (crosslinking complementary) consisted of immersing the films in 50 mL of a solution CaCl2.2H2O and glycerol, both with different concentrations, for 30 minutes. Was evaluated the effects of the plasticizer (glycerol 5, 7 and 10 % v/v) and crosslinking agent (Ca++ 3, 5 and 7 % w/v) on mechanical properties, water solubility, degree of swelling and permeability to water vapor in the biofilms. The films, with an average thickness of 0.07mm, were attractive appearance, acceptable mechanical properties, moisture content around 20 % and water solubility in the range of 32 to 55 %. These films have a degree of swelling around 1 to 3.5 %. The water vapor permeability is moderate and the values are typical of biofilms hydrophilic.

1. Introduction

Biofilms are formed from natural polymers, of animal or vegetable origin, such as polysaccharides, lipids and proteins. These materials, when released into the environment, are converted into simple compounds that do not harm the biosystem (Chandra and Rustgi, 1998). Pectin, a polysaccharide that can be extracted from citrus peels, and alginate, a carbohydrate extracted from various species of algae *Phaeophyceae*, for being natural polymers, have low cost, high stability, good gelling properties, biocompatibility, atoxicity and easy modification chemistry and biochemistry (Bunhak et al., 2007). Thus, these materials have great potential for use in the preparation of biofilms, covering of food, drug coating, among others.

The materials used in the preparation of the films present advantages and disadvantages, and their combination can promote improvement in the desired characteristics. Films made from pure pectin show up completely soluble in water, besides having poor mechanical properties. On the other hand biofilms made from alginate, demonstrate high rigidity and low deformability. In this sense, the two polysaccharides were used in the preparation of the biofilms, seeking with this, the improvement of their properties.

For the development of a filmogenic solution are required basic constituents such as: high molecular mass polymers, solvents, plasticizers and crosslinking agents. Each of these materials is used aiming to provide certain characteristics to the biofilm. Thus, this study examined the influence of concentration of calcium chloride (crosslinking agent) and glycerol (plasticizing agent) on the properties of the films.

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2. Experimental

2.1 Materials

The biofilms were produced from a blend of citric pectin (Vetec PA, Brazil, HM) and sodium alginate (PA Synth, Brazil). Furthermore were used Calcium chloride dihydrate (Merck ACS, USA) as crosslinking agent and glycerol (Synth PA, Brazil) as plasticizer.

2.2 Preparation of biofilms

The biofilms made of pectin and sodium alginate were obtained according Turbiani (2007), using the technique of casting. A polymer solution with a mass fraction of 2 % (g/mL) was prepared by dissolving pectin and alginate (1:1) in distilled water containing glycerol (0.6 g glycerol/g macromolecule) at 30 °C. The solution was mechanically stirred at 800 rpm for 1 h until complete dissolution. Subsequently, it increased the solution temperature to 70 °C and 30 mL of a solution of calcium chloride dihydrate ($CaCl_2.2H_2O$ 0.04 g/g macromolecule) was added to the system at a flow rate of 1 mL/min. This filmogenic solution was then transferred into polypropylene plates (diameter = 14 cm). The plates were brought to a circulating air oven at 40 °C for about 20 h. In this step the film formed is water soluble and should undergo a supplementary crosslinking. The crosslinking complementary consists in immersing the film for 30 min in a room temperature bath consisting of 50 mL of a solution containing $CaCl_2.2H_2O$ (3, 5, and 7 %) (g/mL) and glycerol (5, 7 and 10 %) (v/v). Then, the films were removed from the bath and maintained on a support for 20 h, at ambient temperature, for drying. The films were stored in a desiccator at a relative humidity of 52 %, for a period of three days to reach the equilibrium moisture in this environment before its characterization.

2.3 Film Characterization

The films were characterized with respect to its thickness, surface characteristics, moisture content, water solubility, degree of swelling, mechanical properties (stress and elongation at break) and permeability to water vapor.

2.3.1 Solubility (S)

The water solubility was determined According to Irissin-Mangata et al. (2001). The Samples of biofilm were weighted and the value of its mass was named (*idm*), subsequently the samples were immersed in 100 mL of distilled water and the system maintained under mild agitation at 25 ºC for 24 h using a shaker Tecnical TE-421. The end dry mass (*edm*) was determined by subjecting this sample to oven drying (105 °C for 24 h). The solubility of the biofilm (*S*) was expressed in terms of the initial dry mass (*idm*) of the biofilm through the Eq (1).

$$S = \frac{(idm - edm)}{idm}.100 \quad (1)$$

2.3.2 Thickness

The thickness of the films was determined using a digital micrometer (MITUTOYO, Model MDC-25S, resolution 0.001 mm, USA).

2.3.3 Degree of swelling (DS)

The degree of swelling of the films was determined according Turbiani (2007). The total initial mass (mi) of a film sample of 2.5 cm diameter was determined and the material was immersed in distilled water for different time periods. At certain periods of time, film was removed from the water and its total mass (mt) was determined, then the sample returned to water, this process was repeated until the weight of the sample film was kept constant. Excess moisture on the sample surface was removed by placing the film between two sheets of filter paper, before each weighing. The degree of swelling (DS) was calculated according to Eq (2).

$$DS = \frac{(mt - mi)}{mi} \quad (2)$$

2.3.4 Permeability to water vapor (K_{pvw})

The permeability to water vapor (K_{pvw}) was determined gravimetrically according to Method E95-96 (ASTM, 1995b), using small capsules made of plastic and aluminum. Granular calcium chloride was used to fill the bottom of the cell, keeping the moisture inside the capsule in the amount of 2 %. Biofilm samples were fixed on the cells. This system has remained within a desiccator with a relative humidity of 68 % contained, conferred by a saturated solution of sodium chloride. Thus, there was permeation of water vapor through the film between the two atmospheres with different humidities.

2.3.5 Mechanical Properties

The Stress at break (T) and the elongation at break (E) of the biofilms were obtained using the method D882 (ASTM, 1995a), using a texturometer TA.XT2 (Stable Microsystems, UK). The resistance to traction was expressed as the maximum tensile strength divided by the initial cross-sectional area of the film strip and the elongation at break, as a percentage of the original length.

2.3.6 Surface characteristic

For visualization of the surface characteristics of the films, it was used a scanning electron microscope Shimadzu SS - 550, Superscan, software Superscan SS-550. The samples were adhered to a support with the use of a double-sided tape of conductive carbon and subsequently metallized with gold to ensure electrical conductivity of the surface observation.

3. Results and Discussion

The films made from pectin and alginate showed up with a slightly yellowish color, translucent and homogeneous surface.

Through the microscopic images of composite films for different concentrations of crosslinker solution, shown in Figure 1, we can observe that the films presented a matrix filmogenic continuous and compact. This behavior demonstrates the chemical compatibility of the two polysaccharides.

The films formulated from a crosslinker solution containing 3 % $CaCl_2$ (w/v) and 5 % glycerol (v/v) originated the surface more uniform among all the treatments. Having, thus, the total incorporation of crosslinking agents and plasticizers to filmogenic matrix.

For films prepared with 7 % $CaCl_2$ (w/v) is possible to observe the initiation of the formation of small granules of calcium on the surface of the film, evidence that for crosslinking concentrations higher than this may be a saturation limit of the absorption of ions in the film.

When we analyzed the images under varying concentration of glycerol is possible to note that the increase of this factor produces films rougher surface. With this, they become more flexible and consequently less resistant to tension, in addition to having greater permeability to water vapor since its display area becomes relatively greater.

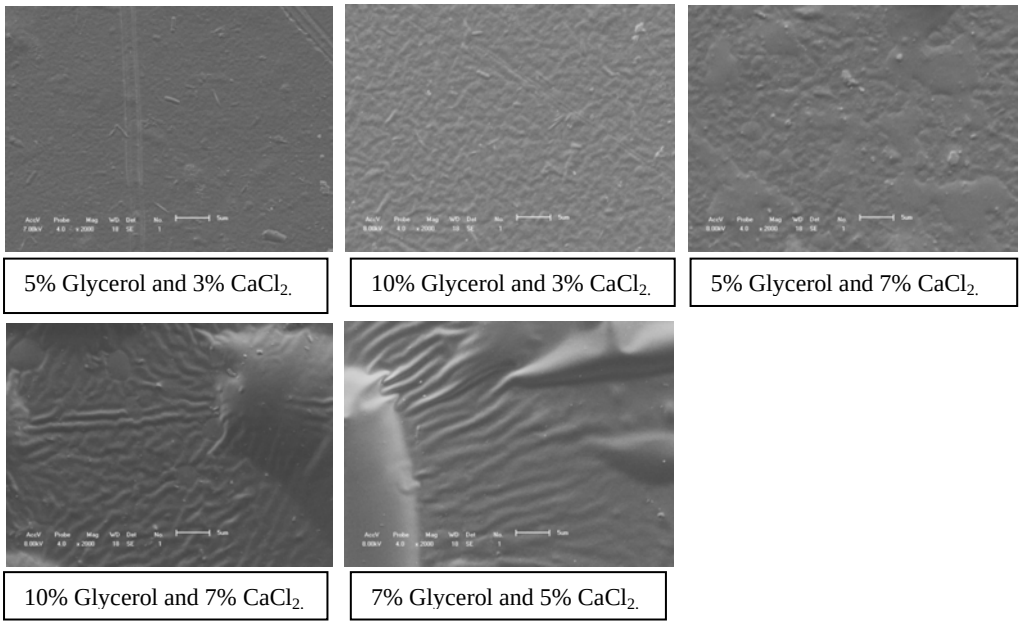

Figure 1: Scanning electron microscopy at the surface of the biofilms composed of pectin and alginate, to different concentrations of crosslinker solution for the 2nd stage (2000 X mag).

Although in some applications high solubility of the films may be desirable low solubility edible films is one of the most important requirement for food and pharmaceutical applications. The water solubility displayed by the biofilms treated with different concentrations of crosslinker solution is shown in Table 1. The films

presented values of solubility between 32.88 and 51.98 %. Silva et al. (2009) found values between 8.8 and 37.2 % of mass solubilized for composite biofilms of pectin and alginate. For biofilms of pure pectin added fatty acid, Batista (2004) obtained materials 100 % soluble in water. Showing, with that, the highly hygroscopic characteristics of pectins. Turbiani (2007) prepared biofilms from sodium alginate crosslinked with 0.8 % $CaCl_2$ in the 1st stage and crosslinked in the 2nd stage with a solution containing 5 % glycerol and different contents of $CaCl_2$. The solubility found by the author was maintained around 13 %. Comparing the values for water solubility obtained in this work with biofilms made from pure polysaccharides, one realizes that the biofilm originated from the blend of the two, showed intermediate greatness.

It can be seen that increasing the concentration of crosslinker results in a significant decrease the solubility of the biofilms, indicating that the ions Ca^{++} actually promoted crosslinking of the polymer chains, thereby increasing the concentration of Ca^{++} makes the intermolecular bonds more cohesive and arranged, making, thus, the solubilization of the biofilms. Moreover, increasing the concentration of glycerol promotes a slight increase of the solubilization of the films. This may be an indication that the plasticizer used can peel off on the polymer matrix, causing it to gaps in the material, making it more accessible to water molecules. Thus, the use of glycerol as the plasticizer is not suitable for preparation of materials that need to present low solubility. Another important factor to be considered is the hygroscopic character of glycerol, contributing to the increase in the moisture films, as can be seen in Table 2.

The examination of the data presented in Table 1 shows that the increase in concentration of plasticizer promotes an increase in the permeability of the films. It is also observed that the permeability of the films decreases as the concentration of Ca^{++} increases.

The classification of films in relation to their barrier properties to water vapor can be established by comparing some material already on the market. According to the classification given by Krochta and Mulder-Johnston (1997). Films of alginate and pectin present as moderate barriers to water vapor. The values of K_{pvw} (7.7 to 9.82 g.mm/m^2.dia.kPa), shown in Table 1, are slightly larger than those found in films obtained from other formulations. Films based on fatty acid and pectin (0.04 g pectin/mL) showed K_{pvw} equal to 6.80 g.mm/m^2.dia.kPa (Batista, 2004). Films of alginate crosslinked with sodium (3 % $CaCl_2$) presented water vapor permeability around 4.5 g.mm/m^2.dia.kPa (Turbiani, 2007). Higher values of permeability to water vapor found may be justified by the use of plasticizing agent, the inclusion of glycerol molecules between the polymer chains causes the spacing therebetween increases, facilitating with this diffusion of water vapour through the film (Yang and Paulson, 2000).

Table 1: Values of stress at break (T), percent elongation at break (E), solubilized in water mass (S) and water vapour permeability (K_{pvw}) to variations in the concentration of crosslinker solution.

Glycerol (%) (mL/mL)	$CaCl_2$ (%) (g/mL)	T (MPa)	E (%)	S (%)	K_{pvw} (g.mm)/(m^2.dia.kPa)
10	3	25.49 (± 6.58)	13.99 (± 3.55)	51.98 (± 0.96)	9.82 (± 0.027)
10	7	28.82 (± 9.07)	9.35 (± 4.65)	36.45 (± 0.49)	8.29 (± 0.039)
5	7	37.22 (± 4.55)	2.18 (± 2.58)	32.88 (± 0.57)	7.72 (± 0.014)
5	3	27.47 (± 6.98)	3.64 (± 5.25)	48.50 (± 1.25)	8.77 (± 0.025)
7	5	30.31(± 8.97)	2.90 (±5.67)	36.78 (± 0.42)	8.16 (± 0.028)

The tensile strength is for the film a mechanical resistance, which can be attributed to the cohesion between the matrix of the filmogenic polymer chains, whereas the elongation is a measure of the plasticity of the film, namely its capacity to stretch to occur before their rupture (Cuq et al., 1995). We can see from the data presented in Table 1 that the stress at break (T) decreases with increasing glycerol concentration, this is due to the fact that the plasticizer makes the film more flexible and consequently less rigid. It is also possible to note that increasing the concentration of Ca^{++} provides an increase in tensile strength of the films, this increase can be explained by the development of crosslinking between the carboxyl groups present in the molecules of alginate and pectin and Ca^{++} (Pavlath et. al., 1999). The films presented measures of tension around 25.49 to 37.22 MPa, these values are lower than those found by Silva et al. (2009) to formulate films of pectin and alginate. This may be due to the the fragility of the films as the

preparation for the analysis, since small cracks on the sides of samples facilitate the breakup of the film (Turbiani, 2007).

Regarding the property of stretching the plasticizer contribute significantly to increase the elasticity of the films. The crosslinking agent in turn causes decrease in elongation of the film, however, produces a less pronounced effect than the former. The elongation values remained between 2.18 and 13.99 %. Turbiani (2007) obtained pure alginate films with low elongation properties, around 0.71 to 2.66 %, demonstrating with this, the feature of alginate to form rigid films and little elastic. For films of fatty acid and pectin, Batista (2004) found values ranging from 1.77 to 5.99 %. As a result, the composite films presented a sharp improvement in their elastic properties.

Table 2 shows the values of the degree of swelling of the films submitted to different concentrations of crosslinker solution. In this experiment the films remained intact after 36 min of immersion in water and showed similar appearance to the original film. The swelling degree of equilibrium reached after 36 minutes. The values found remained between 1.09 and 2.18, which is consistent with those obtained by Silva et al. (2009) for composite films of pectin and alginate.

Table 2: Swelling degree, average thickness and moisture content for the films of pectin and alginate to variations in the concentration of crosslinker solution for the 2nd stage.

Glycerol (%) (mL/mL)	$CaCl_2$ (%) (g/mL)	SD	Thickness (mm)	Moisture (%)
10	3	2.18	0.076 (± 0.001)	0.33 (± 0.015)
10	7	1.64	0.065 (± 0.004)	0.28 (± 0.014)
5	7	1.09	0.051 (± 0.005)	0.20 (± 0.056)
5	3	1.99	0.076 (± 0.003)	0.27 (± 0.10)
7	5	1.94	0.063 (± 0.004)	0.16 (± 0.006)

4. Conclusions

Biofilms prepared by the blend of pectin and alginate presented improved properties when compared to the films prepared from the pure polymers. The composite films presented up translucent and homogeneous. When analyzing the effect of the crosslinker agent on the characteristics of the films can be concluded that this contributes to the decreased solubility and permeability to water vapor, addition to providing increased resistance of the material. The plasticizer used provided obtain biofilms more malleable in the sense that increased elasticity thereof. The films were considered moderate barrier to water vapor. The synthesized materials presented better qualities when obtained from using a crosslinker solution composed of 5 % (mL/mL) of glycerol and 7 % (g/mL) $CaCl_2$.

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