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EFFECT OF ADDITION OF DIFFERENT TYPES OF MODIFIED CLAYS IN THE PROPERTIES OF OPACITY AND SOLUBILITY OF ZEIN BIOFILM

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ABSTRACT Zein is an alcohol-soluble protein contained in the endosperm tissue of *zea mays*. The degree of polymerization of zein allows producing “useful high molecular weight” polyamide or polyester linear polymers. It can be used to produce films and coatings. The zein biofilms, even with the addition of plasticizers still show brittleness of the films caused by high intermolecular force between the protein chains of these materials. Thus, compared with polymers derived from oil, this polymer has different characteristics and properties. In other to improve some of these characteristics, two types of modified clays (hydrophilic and hydrophobic) were added at 1, 2 and 4% (w/w) in the films produced by “casting” methodology. Biofilms (zein-Cloisite® 15A and zein-Cloisite® 30B) were analyzed by water solubility and opacity and results were compared with zein-oleic acid film. Related to opacity, clay’s and control’s biofilms are more yellow than polyethylene, because of carotenoids pigments, but, the presence of Cloisite® 15A (1 and 2%) and Cloisite® 30B (4%) did not differ statistically among them. However, the higher opacity was obtained for Cloisite® 15A (4%) due to particles of clay dispersed in the film. The results of solubility showed that the sample with Cloisite® 15A (2%) showed lower solubility, thus, it supposes that there was great interaction between the clay and filmogenic matrix; the others samples, control and Cloisite® 30B (1, 2 and 4%) and 15A (1%), did not differ statistically. The films with 4% of Cloisite® 15A had the higher solubility among them suggesting that there was the intercalation of the clay into the matrix.

Keywords: biofilms, zein, organoclays.

INTRODUCTION Because of the broad amount of materials used in packaging industries are produced from fossil fuels which are practically un-degradable, nowadays, there is a considerable interest on development of biodegradable materials as a contribution to minimize environmental problems (SORRENTINO, GORASSI and VITTORIA, 2007). In recent years, many studies have been developed to create and characterize biofilms with elevated potential for application in agriculture and food products.

Unfortunately, so far, the use of biodegradable films for food packaging has been strongly limited because of the poor barrier properties and weak mechanical properties shown by these natural polymers. Polymers of nanocomposite often show high properties compared to the conventional. They are more resistant, thicker, have thermal and oxidative stabilities and good barrier properties besides flame retardance behavior. These properties are enhanced by the nanoparticules that fill spots on the matrix. Biodegradability of a packaging material can also be enhanced with the introduction of inorganic particles, such as clay, into the biopolymeric matrix (SOZER, KOKINI, 2009; PAVLIDOU, PAPASPYRIDES, 2008). However, some functional properties of these biofilms, like opacity and solubility, can be altered and need to be analyzed.

The transparency of the material shows the intensity of light passing through it. Lower transparency characterizes a limitation of the light passing through the material. To elaborate biofilms that will be used as package or coating for foods, a higher transparency is desirable when original characteristics of the product like color should be visible (YANG and PAULSON, 2000). The opacity grade depends on the contents and size of lipids particles. The lipids addition change the appearance of the films making them more opaque (BERTAN, 2005). It believes that nanoparticules addition in films solution can modify the transparency of films.

Solubility in water is another important property of the films, because of some applications need insolubility in water to maintain integrity of the product, like chemist pills and seeds. According to Veiga-Santos (2004), the determination of solubility in water for biodegradable films is important, mainly in films elaborated with carbohydrate and proteins, which have elevated water affinity.

The purpose of this study was to elaborate zein biomaterial added with nanoparticules to obtain nanocomposites and to determine their functional properties.

MATERIAL AND METHODS

Materials Corn zein was acquired from Freeman Industries Inc., Tuckahoe, NY; ethanol 75% (Synth, Brazil) was used as solvent; oleic acid (VETEC, Brazil) used as plasticizers; emulsifier (EMUSTAB- Duas Rodas Industrial Ltda, Brazil) as stabilizer; and glycerol (Merck, Brazil) as assistant agent of plasticizer. Modified nanoclays were purchased from Southern Clay Products Inc. (USA).

Preparation of zein biofilms The control film were prepared by dissolving granular zein in 75% ethanol solution to a concentration of 20% (w/v) at room temperature (~25ºC). Oleic acid was added at a ratio of 70 g/100 g zein (w/w), glycerol at a ratio of 30 g/100 g zein (w/w) and emulsifier at a ratio of 5 g/100 g zein (w/w). The mixture was stirred in a water bath at 60 to 65°C for 10 min. The samples with clays added followed the same sequences except with addition of Cloisite®15A and 30B (1%, 2% and 4% related to zein amount). Clay was mixed apart of the filmogenic solution and added only after homogenization of the others materials. Filmogenic solutions were casted on rectangular supports of polystyrene and maintained at room temperature (25°C) for 48 hours to dry (KLEEN et al., 2002 with adaptations). After drying, films were peeled out and stored inside desiccator at 58% relative humidity (RH) before analyses. Thicknesses of the films were determined by arithmetic mean of six values measured in six randomized points of each sample using a digital micrometer with 0.001 mm resolution (Digimess, Brazil).

Biofilms characterization and visual aspect. For all seven samples, visual and tactile analyses were carried out to choose only homogenous samples. Samples with absence of insoluble particles, uniform color, without rupture or brittle zones and easy to remove from the support were considered. Film with 4% of Cloisite® 15A did not show those characteristics; in this sample it was observed insoluble particles after drying. So, it concludes that there was not enough incorporation of the clay into zein matrix on these samples. Tactile analyses shown that films with Cloisite®15A and 30B were, most of the time, thicker than the control film. The great thickness of Cloisite®15A and 30B indicates some interaction of the clay with the matrix.

Opacity was determined with a UV/VIS spectrophotometer (Quimis, Brazil), as proposed by Gounga et al. (2007). Samples of rectangular shape were applied to the internal wall of the cuvette. Three replications were done for each film at 600 nm. Film transparency was calculated by dividing the absorbance at 600 nm with the film thickness.

Water Solubility was determined in triplicate, according to the method proposed by Gontard et al. (1994). Samples (2.0 cm in diameter) were immersed in 50 mL distilled water and the system slowly stirred mechanically (72rpm) at 25 ± 1°C in a shaker (MA-410, Marconi, Brazil) for 24 h. After this period, the samples were removed from the solution and dried in an air oven (105 °C, 24h), the difference in weight was used to calculate the water soluble matter as a percentage of initial weight.

Statistical analysis Analyses of Variance (ANOVA) were performed considering a randomized experimental design and Tukey's tests applied to compare data means at 5% probability using a computational program ESTAT, version 2.0, according to Banzatto and Kronka (2006).

RESULTS AND DISCUSSION

Opacity Table 1 presents opacity values for films produced with Cloisite 15A and Cloisite 30B in the proportion of 1, 2 and 4% related to zein mass, as well the results for control film (only zein and plasticizers) and polyethylene.

Table 1. Opacity of zein biofilms added with nanoclays

Samples	Thickness	Absorbance 600nm	Opacity
Polyethylene	0,05 ± 0,00	0,01 ± 0,00	2,12 ± 0,09[e]
Control (Zein)	0,19 ± 0,01	1,13 ± 0,04	5,81 ± 0,20[bc]
Zein + Cloisite® 15A (1%)	0,23 ± 0,02	1,31 ± 0,02	5,69 ± 0,21[c]
Zein + Cloisite® 15A (2%)	0,22 ± 0,02	1,32 ± 0,03	6,05 ± 0,13[ab]
Zein + Cloisite® 15A (4%)	0,24 ± 0,02	1,41 ± 0,03	6,08 ± 0,16[a]
Zein+ Cloisite® 30B (1%)	0,26 ± 0,02	1,37 ± 0,02	5,18 ± 0,07[d]
Zein + Cloisite® 30B (2%)	0,25 ± 0,03	1,24 ± 0,03	5,05 ± 0,14[d]
Zein+ Cloisite® 30B (4%)	0,23 ± 0,02	1,28 ± 0,02	5,64 ± 0,10[c]

[a,b,c,d,e] - Means followed by the same letter in each column are not different according to Tukey´s test ($p<0.05$)

From the results of opacity, it was observed that polyethylene is translucent and show a decreased opacity compared with zein biofilms. These results were expected, according to Sessa et al. (2003) because of the yellow color zein due to the carotenoids: b-carotene, zeaxanthin and lutein. In addition, the control film, most of the time, had lower thickness and high flow of the filmogenic solution in relation to others films made with clay. The first insight suggests that these characteristics could reduce the opacity of the films. However, statistics tests shown that the values were not different from the others samples that had higher thickness (0.22 to 0.23mm): Cloisite® 15A (1 and 2%) and Cloisite® 30B (4%). So, it concludes that little differences on thickness among the samples were not relevance to change the opacity.

There were no statistics differences between the results of samples with Cloisite® 15A (1%) and Cloisite® 30B (4%). Although they have different hydrophobicity, the amount of Cloisite 15A (1%) added might be insufficient for a complete interaction with protein matrix. As Cloisite® 30B (4%) is more hydrophilic the interaction with zein might be limited and pores allowed the passage of the light through the material.

Besides of the color of polymer matrix the presence of fatty acids also contributes to the higher opacity of these samples. Bertan et al. (2005) found in their study that the high amount of fatty acids added to hydrophobic film on the basis of gelatin increased the opacity of the film. Yang and Paulson (2000) also observed an increase in opacity of films based on gellan, due to the concentration of the mixture of stearic acid/palmitic acid.

The presence of clay´s particles dispersed in the film with Cloisite® 15A (4%) provided higher opacity, suggesting that this concentration was very high to ensure the intercalation in the film, or that the “casting” technique was inadequate to ensure an efficient dispersion of these nanocomposite.

Although the sample Cloisite® 15A (2%) had homogeneous characteristics and also particles did not were observed, the result was not different from those of the control and with Cloisite® 15A (4%). The first insight suggests that these clays interacted with contents of film, but due to similar means with Cloisite® 15A (4%) it is believed that the presence of little particles, only detected by spectrophotometer, were responsible for the result.

The lower opacity was observed for Cloisite® 30B (1 and 2%). Probably, the hydrophilic nature of clay was unable to promote an effective incorporation of the protein matrix, allowing the formation of pores, which permitted the passage of polarized light.

This lack of interaction between clay and zein was evident by its opacity that was lower than the control film.

There are no published data relating addition of clay with increasing or decreasing opacity in polymers. Analyses of film microstructure might help to better understanding the arrangements of films components.

Water solubility Table 2 shows solubility of films prepared with zein and clays.

Table 2. Solubility of zein biofilms added with nanoclays

Sample	Thickness	Solubility (%)
Control (Zein)	0,14 ± 0,00	6,81 ± 0,79[ab]
Zein + Cloisite® 15A (1%)	0,26 ± 0,01	5,02 ± 0,06[bc]
Zein + Cloisite® 15A (2%)	0,27 ± 0,01	4,62 ± 0,62[c]
Zein+ Cloisite® 15A (4%)	0,26 ± 0,01	7,92 ± 0,57[a]
Zein+ Cloisite® 30B (1%)	0,28 ± 0,02	5,76 ± 0,90[bc]
Zein+ Cloisite® 30B (2%)	0,27 ± 0,02	5,26 ± 0,98[bc]
Zein+ Cloisite® 30B (4%)	0,24 ± 0,03	5,11 ± 0,23[bc]

[a,b,c] - Means followed by the same letter in each column are not different according to Tukey´s test ($p<0.05$)

The results of solubility in water for control and Cloisite® 30B (1, 2 and 4%) and 15A (1%) were not statistically different. The additions of Cloisite® 30B, in all concentrations, were not enough to decrease the solubility because of the hydrophilic characteristics. The similarity with control indicates intercalation between clay and matrix. However, the sample Cloisite® 15A (1%) should show differences between Cloisite® 30B and control, because of hidrophobicity, but it is believed that the amount of clay were not enough to make the incorporation with all film contents.

As mentioned earlier, the sample with Cloisite® 15A (4%) had features visually non-homogeneous which resulted in higher solubility. It indicates that the clay particles did not incorporate into the matrix and the water passed through the pores.

Lack of incorporation of matrix components increased the solubility of the samples. Batista et al. (2005) observed that the addition of fatty acids in films with pectin tended to decrease their solubility. However, failure to incorporate the fatty acids in the filmogenic matrix contributed to the greater exposure of the hydrocolloid matrix, promoting the solubility in water.

The sample with Cloisite® 15A (2%), which had lower solubility, possessed proper conditions (hydrophobic nature) and concentration to ensure a better interaction with the matrix.

There is no enough information in the literature correlating the addition of clay to increase or decrease the solubility in polymers. An analysis of the microstructure of the film could help to better understand the phenomenon.

CONCLUSION

The results of opacity did not differ statistically among control, Cloisite® 15A (1 and 2%) and Cloisite® 30B (4%). The lower opacity among samples of this study was observed for Cloisite® 30B (1 and 2%). It is suggested that the hydrophilic nature of clay was unable to promote an effective incorporation of the protein matrix. Other representative results of opacity were obtained from Cloisite® 15A (4%), due to the presence of particles in the film, higher opacity were obtained. In relation to solubility, the samples control and Cloisite® 30B (1, 2 and 4%) and 15A (1%) were not statistically different and reduction in solubility was observed only in the sample Cloisite® 15A (2%), which possessed appropriated conditions (hydrophobic nature) and concentration to ensure a better interaction with the matrix.

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