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Optical and Structural Properties of Zein-Xanthan Gum Biofilms

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Summary: Structural and optical characteristics of zein-based films produced with different xanthan gum concentrations have been studied in this work. Scanning electronic microscopy (SEM) and optical microscopy (OM) were performed to identify if the incorporation of the material into the matrix film, formed a homogeneous structure, as well as to characterize its constituents as the colour and shape. SEM showed a homogeneous matrix for the control (0% xanthan) with good lipid distribution. However, when the samples were investigated by OM, lipids globules in the control biofilm appeared larger and more dispersed in the matrix than the others samples. Transparency/opacity test measurements by UV-VIS analysis indicated that the addition of xanthan to the film matrix lowered significantly its transparency properties Overall, the addition of xanthan gum favoured lipid dispersion in the matrix, making biomaterials more homogeneous, although with less transparency.

Keywords: biopolymer; composites; xanthan gum; zein

Introduction

Zein, the main corn protein and alcohol soluble, is commercially produced from corn gluten, a coproduct of the corn wet milling process. This protein has low biological value due to the amino acid imbalance: high contents of leucin and glutamine and low content of lysine and tryptophan.[1–3] A major advantage of zein, as compared to the other corn protein, is its polymerization characteristics. It has twice more potential than necessary to produce linear polyamide/polyester polymers.

The xanthan or xanthan gum is an important food additive due to its functional properties as well as the improvement in several food characteristics.[4] Xanthan is a natural polymer, hydrophilic, produced by microorganisms of the gender *Xanthomonas campestris*.[5] Because of the excellent rheological properties it has been used as thickener, stabilizer, emulsifier, and suspension agent in several products and process by chemical, cosmetics and food industries, among others.[6]

According to Lai and Padua,[7] zein biofilms show good transparency. When plasticizers are added to the matrix, the material becomes more flexible, although some properties can be modified as, for example, increasing opacity.[8–9] In order to improve the material characteristics, copolymerizations and different polymeric blends have been produced and characterized.[10]

During polymeric materials development, a physical mixture of two or more polymers forming a polymeric blend attracted, most of the time, more attention than polymer synthesis. This happened mainly because the combination of polymer properties resulted in materials with different properties that are often better than the individual polymer properties. This proceeding is easier and less expensive than the

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investigation of new synthetic route. The properties of final polymeric blend depend on the miscibility of the constituents or the morphological structure of each phase in the case of heterogeneous blends.[11]

Scanning electronic microscopy (SEM), among other techniques, has been used for understanding polymer-polymer miscibility.[12] Material incorporation in the matrix can form a homogeneous or heterogeneous structure depending on their interactions. Optical microscopy (OM) and SEM allow the identification of the material incorporated in the matrix and permit its characterization through the observation of colour and shape.

The transparency/opacity of the material shows its capacity to block the light. Low transparency indicates that the material is a good light blocker. Biofilms used as packing or food covering should have high transparency when the original characteristics of the packed product have to be visible.[13] However the material can become more susceptible to the heat.[14]

The objective of this research is to produce composites biofilms with zein-xanthan gum at different xanthan concentrations, and to determine their optical and structural characteristics. SEM, OM and transparency/opacity determinations were used to characterize the biofilms.

Materials and Methods

For each treatment, zein-based materials were prepared by dissolving granular zein (F4000, Freeman Industries, Tuckahoe, NY) in a 75% aqueous ethanol, to a concentration of 16% (w/v) at room temperature. Oleic acid was added at a ratio of 70 g/100 g zein (w/w), while stirring the solution on a water bath at $62 \pm 2\,^{\circ}C$. Glycerol was added at a ratio of 30 g/100 g zein (w/w) and 0.01% of sorbitan/emulsifier or four concentrations of xanthan added. Xanthan concentrations (w/v) were 0.01, 0.02, 0.03 and 0.04% related to the total material. was stirred mechanically 10 min and submitted to 20 mHz of ultrasonic frequency (Fisher Scientific®) for another 10 min, after which they were cast on rectangular acrylic plates and maintained at room temperature (25 °C) for 48 hours to dry. After drying, the formed films were peeled off and stored inside the desiccator at 58% relative humidity until analyses.

The thickness of films was determined by the arithmetic mean of six values measured in six randomized points of each sample using a digital micrometer with 0.001 mm resolution (Digimess model).

For SEM analysis, film samples of 12 mm diameter, taken in duplicate, were fixed on stubs with double-sided tape with conductive copper and covered with 35 nm of gold (Emitech K550, England). Samples were observed under an electronic microscope (LEO 435 VP, England) at 15 kV in a climatized room.

OM was used to identify the formed compounds in films Xylidine ponceau (pH = 3.5), which permits the detection of cationic protein radicals.[15] The coloration technique of Periodic acid- Schiff (PAS) was used to identify neutral polysaccharides and glycoproteins.[16] The samples, taken in duplicate, were strained directly without previous fixation and dehydration because of the zein solubility in alcohol solutions, which are used to fix the material. Instead of the fixation by the ethanol-based solution, the glass slides were dried in an oven (Odontobras ECB 1.2 Digital, Brazil) at 37 °C for 24 h and mounted with Canadian balsam. After 24 h, the samples were analyzed at room temperature in an optical microscope (Olympus BX 60) with an image capture system (Olympus DP 71). Different points in the sample were observed with 10X magnification.

Films apparent transparency was determined with a UV-Vis spectrophotometer (Quimis, Brazil) as proposed by Gounga *et al.*.[17] 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.

Analyses of variance (ANOVA) was performed considering a randomized

experimental design and Tukey's test applied to compare data means at 5% probability using a computational program ESTAT, version 2.0, according to Banzatto and Kronka.[18]

Results and Discussion

Thickness average of the films was 0.12 ± 0.03 mm. The homogeneity of the samples observed by electromicrographs can be shown in Figure 1, where it observed that an increase in xanthan concentration promoted changes in the surface of the material, interfering with the compounds distribution. The control (Figure 1a) presented the best distribution of fat globules, which is identified by the black points in the picture. Apparently, there was no formation of a continuous layer of the matrix in the other films with xanthan concentrations. Similar observations of several points on the material surface were found for zein biofilms in the work done by Ghanbarzadeh *et al.*[19] and with some domains, which already appear to indicate phase separation.[20] The first insight suggests that these points are microbubbles entrapped inside the matrix or spaces occupied by glycerol before the drying process.[21] However, there is a possibility of phase separation between zein and glycerol due to a low interaction between these two compounds.

During biofilms formation, difficulties to homogenize the solution were observed as xanthan concentrations increased, manly 0.03 or 0.04%, when some lumps appeared as spots in the film. In order to better characterize these lumps as well as the points found on the surface, OM was performed to observe the homogeneity through distribution of protein and fat globules.

Figure 2 and 3 show the images magnified ten times (500 μm) obtained for each xanthan concentration using two kinds of sample preparation: Xylidine ponceau (pH = 2.5) and PAS staining. The gray colour in Figure 2 represents protein fraction and the white points the fat globules.

As xanthan concentration increases the size of fat globules decreases, enhancing

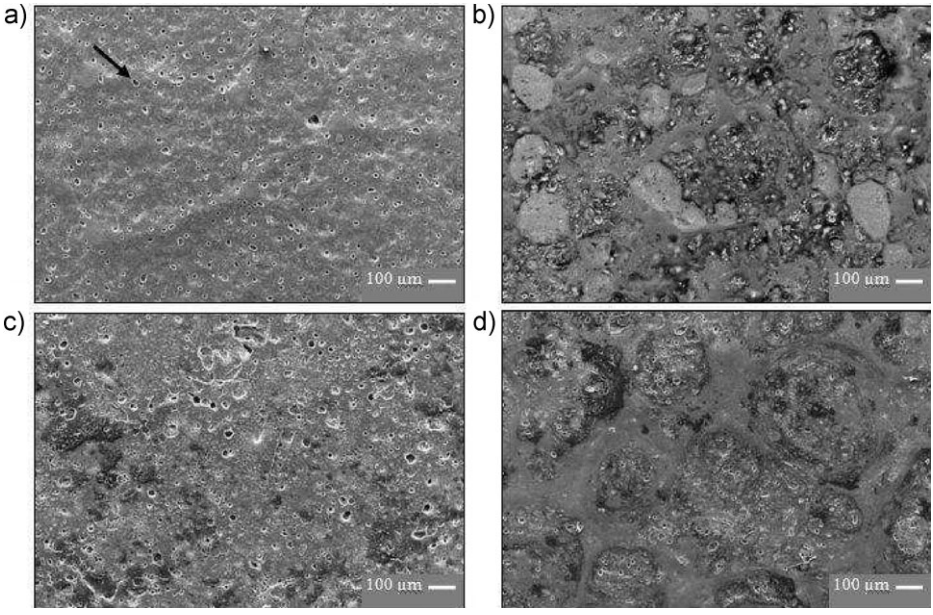

Figure 1.
SEM of zein-xanthan biofilms: a) 0% xanthan (control); b) 0.01% xanthan; c) 0.02% xanthan; d) 0.04% xanthan.

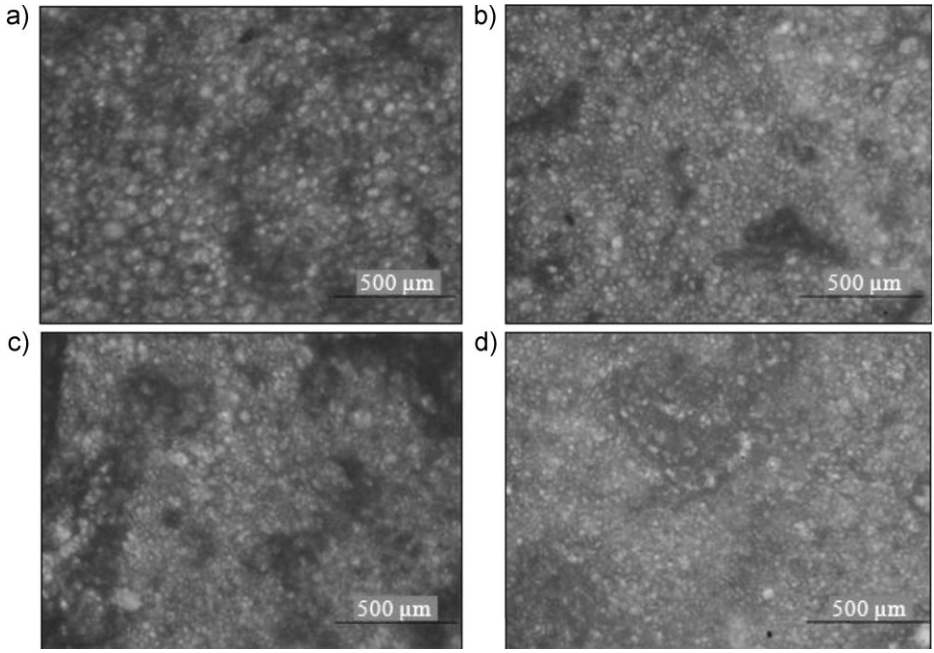

Figure 2.
OM with Xylidine ponceau staining for zein-xanthan biofilms: a) 0% xanthan (control); b) 0.01% xanthan; c) 0.03% xanthan; d) 0.04% xanthan.

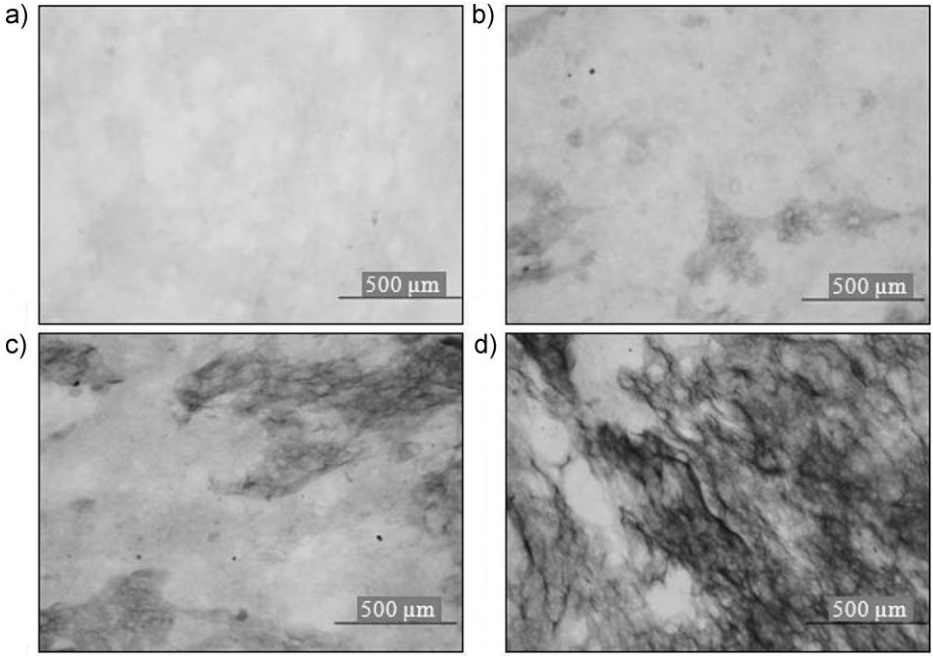

Figure 3.
OM with PAS staining for zein-xanthan biofilms: a) 0% xanthan (control); b) 0.01% xanthan; c) 0.03% xanthan; d) 0.04% xanthan.

Table 1.
Transparency of the zein-xanthan biofilms obtained by UV-Vis spectrometry.

Material	Absorbance $_{600\ nm}$	Transparency
Zein + Sorbitan	0.963 ± 0.04	7.570 ± 0.29^{b}
Zein + Xanthan 0.01%	0.822 ± 0.08	9.605 ± 0.95^{a}
Zein + Xanthan 0.02%	0.811 ± 0.03	7.277 ± 0.26^{b}
Zein + Xanthan 0.03%	0.623 ± 0.09	7.624 ± 1.13^{b}
Zein + Xanthan 0.04%	0.869 ± 0.04	5.565 ± 0.30^{c}

^{a,b,c} – Means followed by the same letters in each column are not different by Tukeýs test ($p < 0.05$)

better homogenization of the material into the film matrix. From Figure 3 it can be observed that increasing xanthan concentration resulted in samples with grayish more coloured. Figure 3d (0.04% xanthan) shows lower amount of fat dispersed, confirming the observations shown in Figure 2 (Xylidine ponceau staining).

The control sample also presents a slight grayish colour, which indicates that the PAS reagent stained the hydroxyl radicals of the matrix structure.[22]

After optical microscopy analyses, it can be concluded that the observations previously considered as porous on SEM image, are indeed fat globules dispersed into the film matrix. Thus it is confirmed that xanthan addition improves homogenization of the compounds mainly with respect to the size and distribution of the fat globules.

Transparency tests done by UV-Vis spectrometer confirmed the observations of PAS analyses (Table 1).

Films with 0.01% xanthan demonstrated better transparency and 0.04% xanthan demonstrated lower transparency as shown by the peaks with less intensity in the PAS results. This can be explained by better homogenization of the material with 0.04% xanthan, resulting in less space between the fat globules, making difficult to the passage of light and consequently decreasing the absorbance.

Conclusion

It was possible to produce biofilms composed of zein-xanthan gum. The film prepared with 0.04% of xanthan was visually less homogeneous due to the presence of lumps across surface. However, OM analyses has concluded that the observations previously considered as pores on SEM images, are indeed fat globules dispersed into the film matrix. Thus, it is confirmed that xanthan addition improves homogenization of the compounds, mainly with respect to the size and disposition of the fat globules. The addition of xanthan gum favoured lipid dispersion in the matrix, making the biofilms more homogeneous but decreases the transparency of the material.

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