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Full Length Research Paper

Microencapsulation of the natural urucum pigment with chitosan by spray drying in different solvents

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The food industry expects increasingly complex properties from food ingredients and such complex properties can often only be provided by microencapsulation. A number of methods are reported for microencapsulation but the most popular technique employed in industry is spray drying. Urucum has many applications in the food industry. In this study, we report the process of urucum microencapsulation into chitosan by spray drying. Characterization by scanning electron microscope, infrared spectroscopy, thermogravimetric analysis, differential scanning calorimetry and color were used to analyze solid materials obtained in different carboxylic acids.

Key words: Chitosan, urucum, spray-drying, natural dyes, microencapsulation.

INTRODUCTION

There has recently arisen great interest in natural pigments, mainly due to the demand for healthier food products, environmental concerns and the opportunities for creating new markets. The use of natural pigments requires a chemical knowledge of their molecules and stability in order to adapt them to the conditions of use during processing, packaging and distribution (Ferrari, 2004; Aggarwal and Shishodia, 2006; Downham and Collins, 2000).

The industry requires technologies which protect the natural pigments in the environment, due to their instability in the presence of light, air, humidity and high temperatures (Downham and Collins, 2000; Mapari et al., 2005; Francis, 2000). Currently, in order to provide this protection one alternative is microencapsulation technology (Dziezak, 1988; Benita, 1996; Ciapara et al., 2004; Lyng et al., 2005).

Microencapsulation by spray drying is the most common method of encapsulation of food ingredients because of it being more economical and of extensive use in the food industry. It is used to produce dry powders, granules or agglomerates (Chawla et al., 1994; Barbosa et al., 2005; Schrooyen et al., 2001). The spray drying process involves three stages: preparation of the dispersion or emulsion, homogenization and atomization (Ré, 2006; Desai and Park, 2005; Shahidi and Han, 1993; Stephane et al., 1997). In this method the active compound is surrounded by a protective matrix, normally a polymer (Paradkar et al., 2004). Typical materials include gum acacia, maltodextrins, hydrophobic starch, carboxymethylcellulose and mixtures thereof (Barbosa et al., 2005; Gouin, 2004).

The biopolymer chitosan is a copolymer formed of units of 2-deoxy-N-acetyl-D-glucosamine and 2-deoxy-D-glucosamine joined by β-1,4-glycoside bonds, obtained from the alkaline deacetylation of chitin present in the exoskeleton of crustaceans. Due to the nature of its chemical configuration and its biodegradability properties, low toxicity and biocompatibility, chitosan has been employed in the preparation of films, gels and microspheres (Lorenzo-Lamosa et al., 1998; Liu et al., 2001; Krajewska, 2004). Chitosan is insoluble in water, but becomes a cationic polyelectrolyte when dissolved in acid solutions such as acetic, citric, lactic, formic or chloridric acid.

Urucum pigment is extracted from the external layer of seeds of the species *Bixa orellana* and normally shows

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two forms: liposoluble bixin and norbixin, which is hydrosoluble. These compounds belong to the class of carotenoids which have conjugated double bonds and act as antioxidants (Stahl and Sies, 2005; Kiokias and Gordon, 2003).

Due to the importance of chitosan, which is already used in different areas of industry such as pharmaceutical, biomedical, cosmetics and food products, the objective of this study is to evaluate the process of urucum pigment microencapsulation by spray drying in different solvents, using the biopolymer chitosan as the encapsulating agent.

MATERIALS AND METHODS

Materials

Chitosan, degree of deacetylation 90%, was purchased from Purifarma, Brazil. The urucum pigment (AM-200-WS-P) was kindly provided by Christen – Hansen Ind. and Com. Ltda, with hydrosoluble power containing 1% of norbixin. All other reagents employed were of analytical grade.

Spray drying

Dispersions containing 500 mg of urucum and 3 g of the polymer were prepared in the following solutions: acetic acid 5%, lactic acid 5% and citric acid 5%; for the lactic acid solution a sample containing 1% Tween-80 was also prepared. The dispersions were homogenized and the spray drying was carried out using a Buchi 191 mini spray drier, containing a 0.5 mm atomizer inside a chamber of 44 cm height and 10.5 cm diameter. The samples were atomized standardizing the air inlet temperature at 180 ± 5°C and the air outlet temperature at 100 ± 5°C, with a positive manometric pressure of 5 bars. Under these conditions the device can dry up to 600 mL of the resulting solution per hour. The drying time varied between 15-20 min. The size of the particles obtained in the drying process by spray drying is influenced by the spray flow, thus a flow of 400 NL/h was used. After drying, the product was colleted in the form of a powder.

Infrared spectroscopy

The products obtained after drying were analyzed through infrared spectroscopy using a Perkin Elmer infrared spectrometer, Model FT-PC-16. The samples were prepared as KBr pellets.

Morphology of the microspheres

The external morphology, porosity and average size of the samples were analyzed with a scanning electron microscope (Philips, Model XL 30). The samples were placed over stabs, being treated with a gold film and then micrographed.

Thermogravimetric analysis

The samples were analyzed with a Shimadzu thermogravimetric analyzer (TGA50, Kyoto, Japan) in a nitrogen atmosphere. The heating rate of the experiments was $10^{o}C\ min^{-1}$. The nitrogen flow was maintained at 50 mL min^{-1} and samples of ca. 12 mg were used for all experiments.

Differential scanning calorimetry

Thermograms of the pigment, original chitosan and pigment-loaded microspheres were obtained with a Shimadzu differential scanning calorimeter (DSC50, Kyoto, Japan) interfaced to a computer. Samples (12 mg) were sealed in aluminum pans. The scanning rate throughout the study was $10^{o}C\ min^{-1}$. All tests were carried out in duplicate.

Color qualification

For the qualification of the color a Lab Hunter system was used, which consists of a rectangular coordinates system for the definition of color in terms of luminosity (L*), red versus green (a*) and yellow versus blue (b*). The qualification of the color of the samples was carried out by the direct reading of the reflectance of the coordinates L*, a* and b*, using a Jobin-Yvon U1000 double monochromator coupled to a GaAs photomultiplicator and to a conventional photon counter. As a standard, the standard illuminant A, incandescent light, was used.

RESULTS AND DISCUSSION

Since the viscosity of the final solution is one of the limitations to the use of spray drying, a wall material is always used which results in a solution which can be easily taken to the atomizer and then dried (Dziezak, 1988; Gouin, 2004). Thus, all of the solutions used in this study were easily atomized.

Scanning electron microscopy

The atomized samples were morphologically characterized, including in this study, the particle size distribution. The micrographs of the samples atomized in different solvents are shown in Figures 1 a-d. It can be observed that the samples had very similar external morphologies, being spherical, without fissures or apparent porosity, which is very important to guarantee the effective protection of the encapsulated material. For a mixed population of microspheres, it was observed that the particle size was not homogeneous for the different samples, an average particle size in the range of 2 – 20 μm being found. Small particles around bigger particles were observed for all samples, Figure 1.

Visually, the encapsulated samples appeared in the form of extremely fine, colored and water soluble powers. It can be seen in Figure 1 that the samples prepared in citric acid and lactic acid were agglutinated. This may be attributed to an increase in the humidification of the sample, which causes agglutination of the particles (Muzzarelli et al., 2004). Of the acids employed in the sample preparation, acetic acid is the acid with the lowest molecular mass (60 g/mol) and the lowest ebullition point (117.9°C), it being the most volatile. The addition of the emulsifier Tween-80 to the chitosan/lactic acid/pigment sample, decreased the agglutination of the particles, Figure 1d.

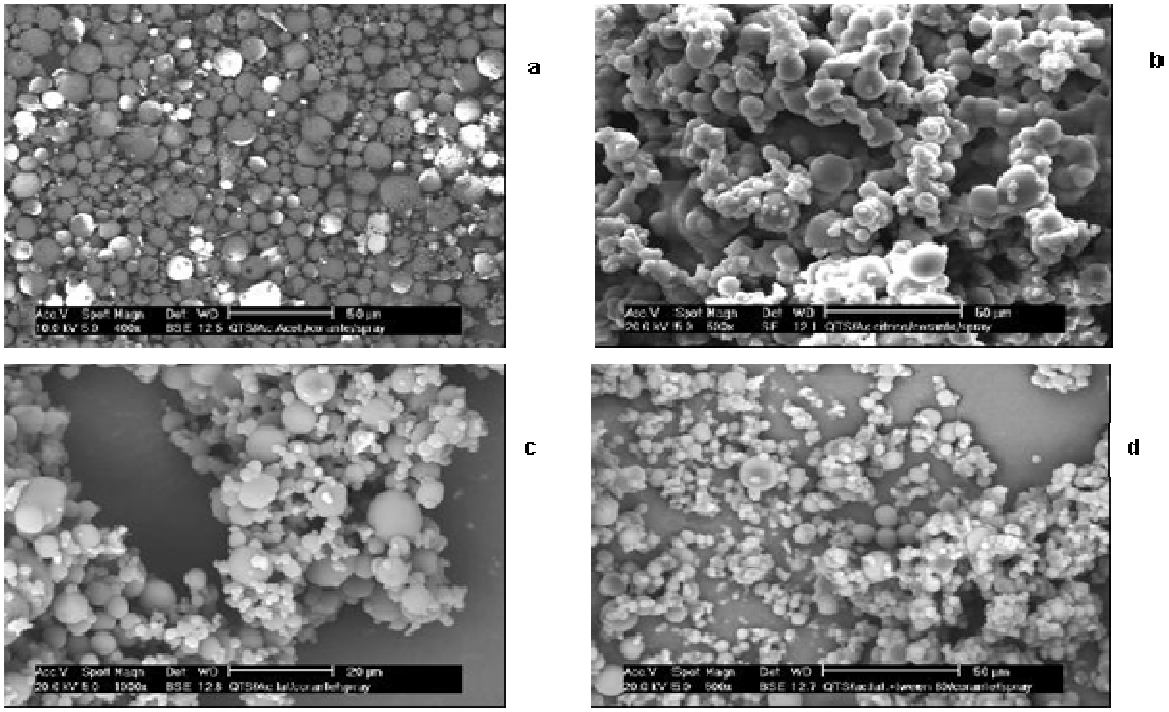

Figure 1. Scanning electron microscopy of urucum/chitosan samples atomized in spray drying in different solvents: a) acetic acid 5%; b) citric acid 5%; c) lactic acid 5% and d) lactic acid 5%/Tween-80.

Infrared spectroscopy (IR)

Figure 2 shows the FT-IR spectra of the samples under study. The FT-IR spectrum of chitosan powder shows characteristic absorption bands at 3443, 2923, and 2867 cm^{-1}, which represent the presence of an OH group, and CH_2 and CH_3 groups, respectively. The amino group has a characteristic absorption band in the region of 3400-3500 cm^{-1}, which must have been masked by the absorption band due to the OH group (Silverstein and Webster, 1998; Shanmugasundaram et al., 2001). The >C=O stretching (amide I) peak at 1659 cm^{-1} representing the structure of N-acetylglucosamine, as well as the NH_2 stretching (amide II) peak at 1567 cm^{-1} representing the glucosamine functional group, appeared in the spectrum of chitosan powder (Muzarelli, 1977; Nunthanid et al., 2004). The spectra of the spray-dried chitosan-acetate, chitosan-lactate and chitosan-citrate show strong peaks at 1555, 1586 and 1592 cm^{-1}, respectively. In addition, the strong peak at 1500-1600 cm^{-1} and the weak peak near the 1400 cm^{-1} region, was attributed to an asymmetric and a symmetric carboxylate anion stretching, respectively (Nunthanid et al., 2004; Orienti et al., 2002).

The carbonyl stretching peak at 1659 cm^{-1} (amide I peak) disappeared and a new peak near 1630 cm^{-1} assigned to an asymmetric NH_3^+ bending was observed (Silverstein and Webster, 1998).

Moreover, a new peak appeared at 1730 and 1721 cm^{-1} in the spectra of chitosan-lactate and chitosan-citrate, which could be assigned to the carbonyl groups of lactic acid and citric acid, respectively.

This indicated that the spray-dried chitosan powder, using acetic acid, lactic acid and citric acid as the dissolving vehicle could yield spray-dried powders of chitosan acetate, chitosan-lactate and chitosan-citrate, respectively, indicating the formation of a salt between the $–COO^-$ groups of the acids and the $–NH_3^+$ groups of chitosan (Lorenzo-Lamosa et al., 1998; Orienti et al., 2002).

Similar IR spectra were observed in the urucum-loaded chitosan using several acids as the dissolving vehicle. It was therefore confirmed that urucum, specifically norbixin, might interact with chitosan at the position of an amino group to form a salt.

Thermogravimetric analysis (TGA)

Figure 3 shows the isothermal profile obtained as a function of time in a thermogravimetric analyzer, for the chitosan/urucum sample produced in acetic acid, at a temperature of 180°C. It was observed that during this period in which the sample was exposed for three hours to a temperature of 180°C, there was no significant degradation or mass loss, only the loss of a small quantity of humidity from the sample (3.78%) being observed. This result indicates that the temperature employed in the urucum microencapsulation process using spray drying, with chitosan as the encapsulating agent, did not alter the

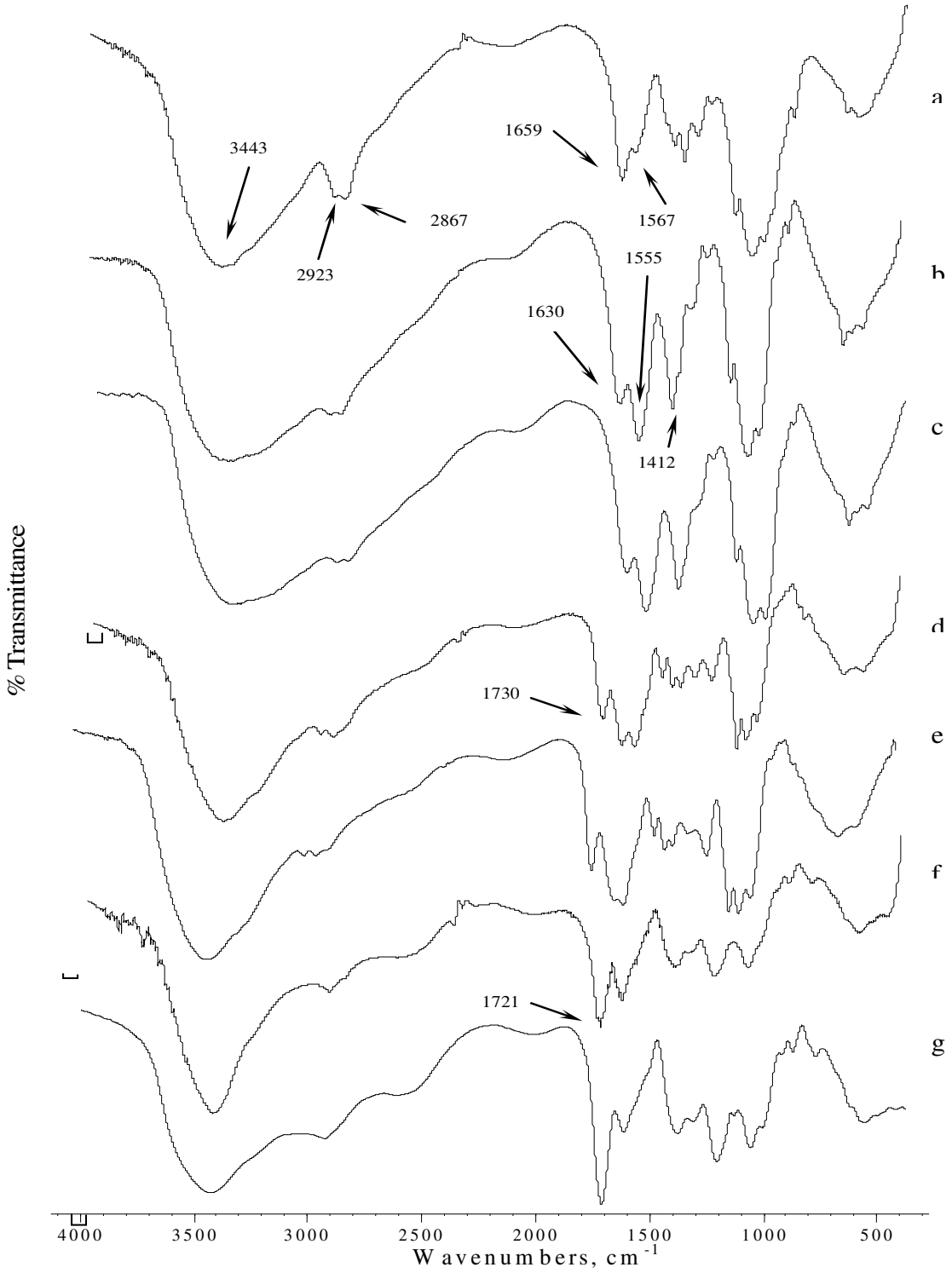

Figure 2. FT-IR spectrum of spray-dried chitosan powder (a), chitosan acetate (b), chitosan acetate/urucum (c), chitosan lactate (d), chitosan lactate/urucum (e), chitosan citrate (f), chitosan citrate/urucum (g).

pigment characteristics and the process was carried out effectively, this temperature being appropriate for use in the sample drying process. The optimum inlet temperature range for the preparation of chitosan microspheres using the spray drying method from aqueous solution of chitosan was found to be 160-180°C. When the inlet temperature is set to below 140°C, the solvent in the droplets can not be fully evaporated (He et al., 1999).

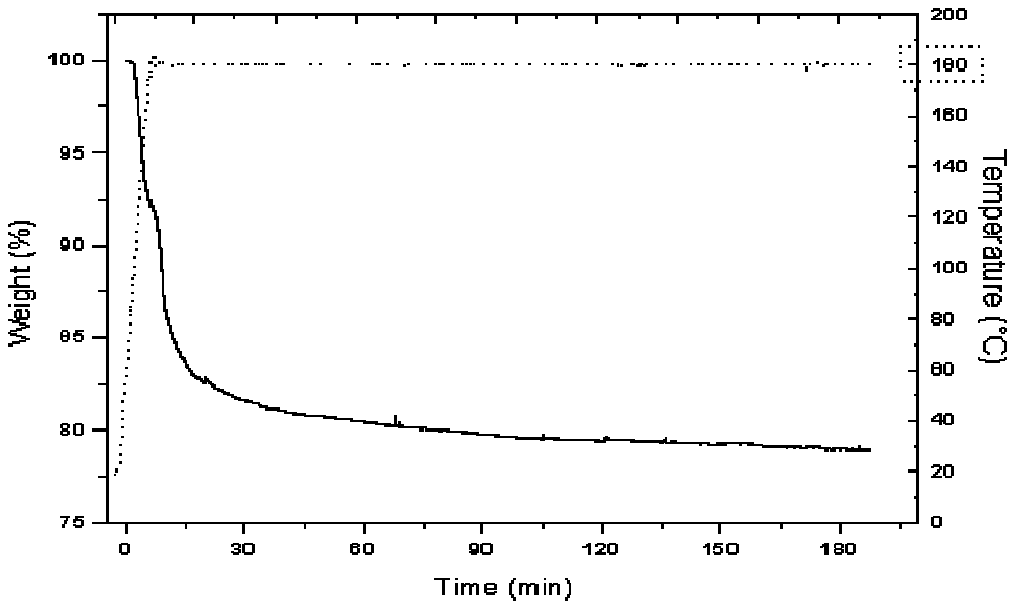

Figure 3. Isotherm obtained through thermogravimetric analysis of the chitosan/urucum/acetic acid sample as a function of time, at a temperature of 180°C

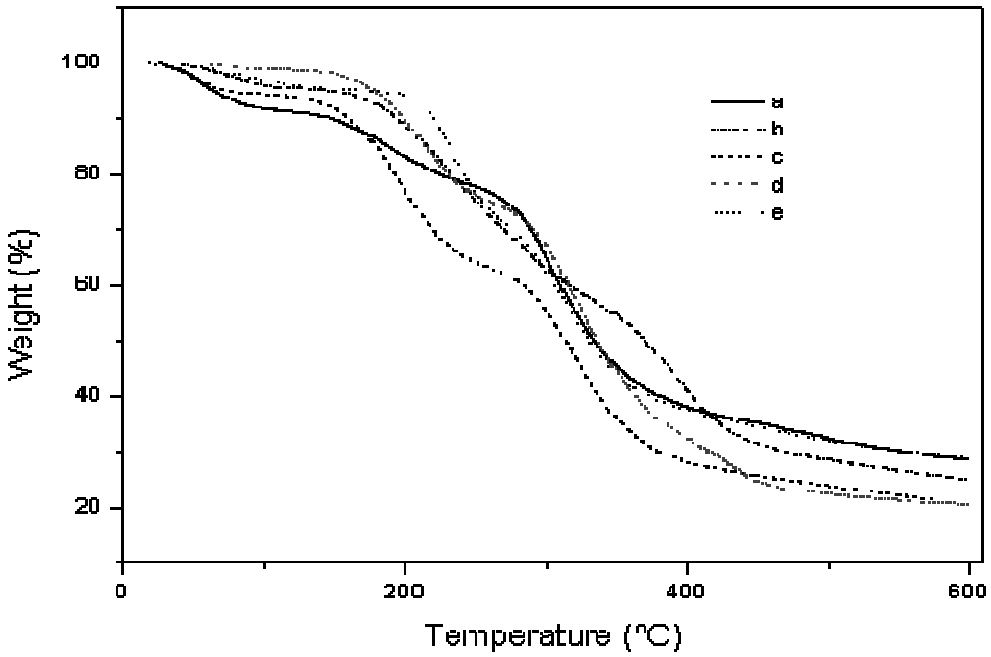

Figure 4. Thermogravimetric analysis of atomized chitosan/urucum samples: a) acetic acid; b) citric acid; c) lactic acid; d) lactic acid/Tween-80 and e) urucum.

Figure 4 shows the mass loss profile for the atomized samples and for the urucum pigment. It can be observed that all of the samples have a quantity of free humidity, this being due to the solvent used in the sample preparation process which was not totally volatilized during the drying (Figure 4 a-d).

Each atomized sample has a distinct decomposition peak: chitosan/acetic acid/urucum 302.40°C; chitosan/ citric acid/uucum 392.48°C; chitosan/lactic acid/urucum 318.09°C and chitosan/lactic acid/urucum/Tween-80 317.39°C (Figure 4 a-d). The pure pigment gave a de-composition peak at 233.13 and 317.3°C (Figure 4 e).

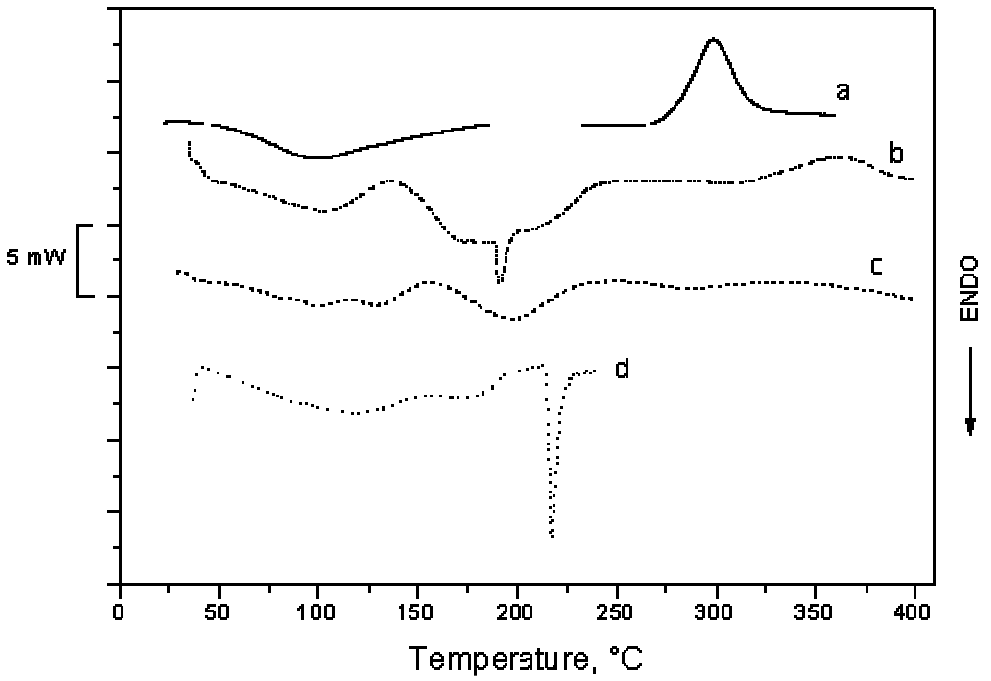

Figure 5. DSC analysis for atomized chitosan/urucum samples and urucum pigment: a) acetic acid; b) citric acid; c) lactic acid and d) urucum.

The results suggest a greater thermal stability of the chitosan sample loaded with urucum prepared in a citric acid solution (Figure 4 b). This finding can be attributed to the higher number of carboxylic groups of the citric acid, in relation to the other acids employed in the preparation of the samples, thus allowing a greater interaction between the NH_3^+ of the chitosan and the $-COO^-$ of the citric acid (Demarger-Andre and Domard, 1994; Park et al., 2001; Yamaguchi et al., 2003). Furthermore, of the acids employed, citric acid is the one which can carry out a greater ionic interaction with chitosan and a possible interaction by hydrogen bonding with the pigment, and may thus be acting in this way as a reticulant agent, increasing, consequently, the thermal stability of the sample (Jain et al., 2004).

It can be seen in Figures 4 b-d that for the samples produced in citric acid and lactic acid a mass loss at around 200°C occurs, which may be attributed to a water molecule chemical bound to the product, this being recrystallized in the drying chamber. Adamiec and Modrzejewska characterized microgranules of chitosan, prepared by the spray drying technique, by DSC and interpreted the presence of endothermic peaks above 100°C in the spectrum, as a possible indication of different mechanisms of water binding in the samples (Adamiec and Modrzejewska, 2005).

Differential scanning calorimetry (DSC)

Figure 5 shows the results of the differential scanning calorimetry analysis for atomized samples and the urucum pigment. It can be observed for all samples that there is an endothermic peak in the range 50-120°C, relating to the humidity loss of the sample. For the samples prepared in citric acid and lactic acid another endothermic peak was found at around 200°C, which is attributed to a loss of crystallization water (Figure 5 b-c). This peak confirms the mass loss at around 200°C, observed in the TGA of the samples obtained in citric acid and lactic acid (Figure 4 b-d). For the pigment, an endothermic peak is observed at around 220°C (Figure 5 d).

The exothermic peak at 274.83°C (Figure 5 a), relates to the polymer decomposition, which is shown to be displaced when compared to the DSC of pure chitosan (296°C). This same peak is also displaced at higher temperatures, in the DSC of the chitosan/urucum samples prepared in citric acid and lactic acid found at 360 and 330°C, respectively (Figures 5 b-c). These displacements are attributed to possible interactions between the polymer and the pigment. The DSC data confirm a greater thermal stability of the sample prepared in citric acid also observed in the TGA of the atomized samples (Figure 4 b).

Color

The Lab Hunter system is a rectangular coordinates system which defines the color in terms of luminosity (L), red versus green (a) and yellow versus blue (b) (Francis

Table 1. Color parameters of the Lab Hunter system for the powder samples of chitosan with urucum obtained by spray drying.

Sample	L*	a*	b*
Chitosan/acetic acid/urucum	69.65	21.03	45.80
Chitosan/citric acid/urucum	71.14	18.23	31.82
Chitosan/lactic acid/ urucum	67.37	15.86	34.38
Chitosan /lactic acid/urucum/Tween-80	65.28	13.28	33.91
Urucum pigment	46.19	30.21	31.34

and Clydesdale, 1975). The color variables observed for the atomized samples were basically, an increase in luminosity, a decrease in red and an increase in yellow contents. Table 1 shows the values for L*, a* and b* obtained for the atomized samples.

It can be observed in Table 1 that of the atomized samples, the chitosan/urucum sample prepared in citric acid gave the highest value for L*, it being visually the lightest. The a* Hunter coordinate which quantifies the red intensity, showed a similar behavior for all samples, it being slightly higher for the chitosan/urucum sample prepared in acetic acid. It can be seen that all samples have a positive a* value. The b* Hunter coordinate which quantifies the yellow intensity, also shows a higher value for the sample prepared in acetic acid. All samples had positive b* values.

On comparing the color parameters found for the atomized samples with those determined for the pure pigment, it can be seen that the atomized samples were lighter, Table 1. On the other hand, the differences in the color parameters found for the atomized samples and the pure pigment may be attributed to the effects of the heat to which the samples were submitted in the spray drying process. The heat effects may initially cause, in principle, a process of degradation of the red components which make up the urucum, which would explain a more accentuated decrease for the a* parameter. However, on observing the spacing of L*, a* and b*, it can be seen that the samples are found in the quadrant where the red color mixes with the yellow color, resulting in a reddish chestnut color, characteristic of the urucum pigment. Ferreira et al. (1999). submitted commercial hydrosoluble solutions of urucum to different time/temperature treatments in order to investigate the color stability, an increase in luminosity being observed, with an increase in the yellow and a decrease in the red contents (Ferreira et al., 1999).

Conclusion

This study demonstrates that urucum pigment can be successfully incorporated into chitosan by means of a spray-drying process, resulting in dry and colorful powders which are water soluble. Urucum pigment is widely used in the food industry and interest in its application in the pharmaceutical and cosmetics industry is growing.

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