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Production and Characterization of Biodegradable Films Incorporated with Clove Essential Oil/β-cyclodextrin Microcapsules

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Biodegradable films can be designed to contain natural antioxidants, vitamins and antimicrobials in order to extend shelf life of the product keeping the natural sensorial properties. The objective of this study was production and characterization of biodegradables films incorporated with clove essential oil (CEO) microcapsules. The CEO was encapsulated in β-cyclodextrin (β-CD) by freeze drying and spray drying methods. The encapsulation efficiencies were 277.17 and 575.49 mg CEO/ g of sample, for spray drying and freeze drying methods, respectively. Alginate films incorporated with CEO/β-CD inclusion complexes were prepared by casting method, and investigated for mechanical and barrier properties. Generally, the films prepared with CEO encapsulated, compared with the control films, were less transparent, and less flexible. The CEO/β-CD inclusion complex increased water vapor permeability (WVP) of films and decreased their elasticity, contributing to greater hardness. The films were evaluated by moisture sorption isotherms and it was observed that the encapsulation decreased the hydrophilicity of cyclodextrin, and consequently, the films. The films developed could be a possible future application for shelf life extension of fresh food.

1. Introduction

Essential oils are lipophilic volatile compounds extracted from plants. Many oils have showed strong antimicrobial properties when tested in *in vitro* experiments. As essential oils are natural ingredients, their use in food products is highly desirable, since the consumer has avoided synthetic additives in their food. Commercial applications of these oils require a suitable formulation consisting of biodegradables compounds that protect them from degradation and evaporation, while allowing their controlled release (Soliman, et al., 2013).

There are several essential oils that have an inhibitory effect on pathogenic microorganisms, such as cinnamon oil, clove extract, oregano and trans-cinnamaldehyde. Clove oil is an essential oil used as a fragrant and flavoring agent in a variety of food and cosmetic products. However, pungent taste, volatility, light sensitivity and poor water solubility make it unsuitable to use as such (Hernández-Sánchez et al., 2012).

Cyclodextrins are oligosaccharides, in the form of a truncated cone, having a hydrophobic cavity and a hydrophilic exterior. This characteristic of CDs allows molecules to be trapped in their cavity, improving their stability under various environmental conditions, such as exposure to oxygen, light and heat. Another characteristic of cyclodextrins is to convert a liquid, insoluble compound in water, into a powder with good aqueous solubility, facilitating the handling of the encapsulated material (Szente et al., 1998).

Encapsulation in cyclodextrins can mask the flavor of the essential oils and protect them against oxidation or thermal degradation, allowing the oils to effectively remain as antimicrobial agents over a wide variety of environmental conditions and over a long period of time (Hill et al., 2013).

The inclusion of clove essential oil in cyclodextrins has been investigated for several researchers, with good results of encapsulation (Hill et al., 2013; Hernández-Sánchez et al., 2012; Wang et al., 2011). However, in these studies the authors just produce and characterize the inclusion complex. The incorporation of clove

essential oil/β-CD inclusion complex in the formulation of biodegradables films could supplement the application in food packages.

Sun et al. (2014) developed β-cyclodextrin inclusion with essential oils in chitosan edible films and investigated their antimicrobial, mechanical and physical properties. The authors observed that incorporation of low amounts of essential oil/β-CD in the chitosan films improved its antimicrobial properties, and the mechanical properties of films incorporated with essential oil/β-CD in chitosan were as effective for active food packaging as films without the additional incorporation of essential oil/β-CD oil in chitosan.

The present study aims to produce and characterize biodegradables films incorporated of clove essential oil encapsulated with β-cyclodextrin.

2. Materials and methods

2.1. Materials

β-cyclodextrin was kindly provided by Roquette Fréres (France). Clove essential oil (eugenol content of 85 %) was acquired from Ferquima (São Paulo, Brazil). Sodium alginate was donated from FMC (São Paulo, Brazil). All the other chemicals used were of analytical grade.

2.2. Preparation of essential oil/β-cyclodextrin inclusion complex

An aqueous solution of β-cyclodextrin (15 mM) was prepared at room temperature. Then clove essential oil was added in a molar ratio of 1:1 (based on eugenol content). The mixture was homogenized in a sealed container for 24 hours, at room temperature, to allow the formation of inclusion complex and prevent the loss of volatiles compounds to the atmosphere. Then, solution was dried in a spray dryer and freeze dryer equipment (Hill et al., 2013).

The freeze drying process the solution was frozen and freeze drying at -50 °C and 1.09 Pa, for 48 h. The spray drying process was performed with a scale spray dryer (Labmaq Brazil, Model MDS 1.0), at constant air inlet temperature of 150 ºC and outlet temperature of 86 °C, in a feed flow rate of 6.67 mL/min, and pressure of 1.05 mbar. The dried formulations were stored in a closed container, under refrigeration, for further characterization.

2.3. Characterization of essential oil/β-cyclodextrin inclusion complex

Encapsulation efficiency

The powder dried by spray and freeze drying was dissolved in ethanol (3.12 g/L). The dispersion was sonicated for 20 min, and then centrifuged for 10 min, at 2000 rpm. The absorbance of supernatant was measured in a spectrophotometer at 280 nm. The essential oil content into microcapsules was obtained from a calibration curve. The experiment was performed in triplicate.

Powders solubility

Water solubility of powders obtained by spray drying and freeze drying methods was performed according to the Cortés-Rojas et al. (2014). Briefly, 100 mg of powders were dissolved in 10 mL of distilled water and stirred magnetically for 10 min, at room ambient. The solution was centrifuged at 3500 rpm, for 5 minutes. An aliquot of approximately 2 g of the supernatant was transferred to a previously weighted Petri dish, and dried in an oven at 105 °C, for 5 h. The results were expressed as g of soluble powder per 100 g of water. The experiment was performed in triplicate.

2.4. Preparation of biodegradables films

Biodegradables films were prepared by casting method, from aqueous solution of alginate (2 % w/v), encapsulated clove essential oil (1 % w/v), and glycerin (25 % of solid content in solution). The solutions were placed in acrylic plate, and dried at 50 °C, for 20 h. The films were removed from the plate and stored for further characterization. Two control samples were prepared to comparing to the films elaborated with encapsulated essential oil produced by spray and freeze drying (0.26 % and 0.55 % v/v, respectively).

The films were named: F1 for film containing 0.26 % of free essential oil, F2 for films containing essential oil encapsulated by spray drying, F3 for film containing 0.55 % of free essential oil and F4 for film containing essential oil encapsulated by freeze drying.

2.5. Characterization of biodegradables films

Color parameters: the color parameters were determined by a Konica Minolta CR-410 portable colorimeter, using CIEL*a*b* system. The experiment was performed in triplicate.

Films thickness: a manual micrometer (Mitutoyo, Brazil) was used to measure the films thickness, with an accuracy of ± 0.01 mm, in ten random locations for each film.

Water solubility: water solubility of biodegradables films was determined according to Gontard et al.(1992) methodology.

Water vapor permeability (WVP): The WVP of the films was determined according to the standard testing method ASTM (1995). Each sample was fixed onto an aluminium capsule, with an internal diameter of 60 mm, using silicone wax, so as to guarantee that the migration of moisture only occurred via the film. Calcium chloride was inserted into each capsule, and the capsules were stored in desiccators at 25 ± 2 °C, with a saturated $MgNO_3$ solution (53 % RH), for 72 hours, to maintain the relative humidity gradient.
Periodic weighing was carried out until the rate of mass gain was constant. The permeability rate to water vapor was determined according to Equation 1.

$$WVP = \frac{WVPR.\delta}{P_s.\left(\frac{RH_1 - RH_2}{100}\right)} \quad (1)$$

where WVP is the permeability to water vapor (g/m.Pa.s.), WVPR is the water vapor permeation rate (g / m^2 .s), δ the average film thickness (mean of 6 measurements) (m), Ps is the vapor saturation pressure at the test temperature (Pa), RH_1 the relative humidity outside the capsule (%) and RH_2 the relative humidity inside the capsule (%).
Mechanical properties: tensile strength (TS) and elongation at break (EB) tests were performed at room temperature using a texturometer (model TA.TX2i, Stable Micro Systems, England), according to the standard testing method ASTM D–882–91 (1995). Sample films were stored under 53 % relative humidity for seven days. The samples were cut into strips measuring 80 mm in length by 6 mm in width and were tied to the pneumatic grips of the texturometer. Initially, the grips were spaced 30 mm apart and the traction velocity was 500 mm/min. The properties investigated were maximum tensile strength (MPa), elongation at break (%), and elastic modulus (MPa). Ten measurements were collected for each sample.
Sorption isotherms: films were stored in a desiccator containing calcium chloride for 20 days. The sorption isotherms of the films were determined in a desiccator containing saturated saline solutions, at eight different RH settings (11.3, 33, 43, 53, 75, 84, 90 %), at 25 °C. The samples were weighed until equilibrium moisture content. All tests were performed in triplicate. The Guggenheim-Anderson-de Boer (GAB) model (Eq. 2) was used to fit the data.

$$X_w = \frac{m_0.C.K.a_w}{[(1 - K.a_w).(1 - K.a_w + C.K.a_w)]} \quad (2)$$

where X_w (g water/g dried mass) is the equilibrium moisture content; m_0 the monolayer value; a_w water activity; C and K are constants of Guggenheim, which represents the heat of sorption in the first layer and the heat of sorption of the multilayers, respectively.

2.6. Statistical analysis

Analysis of variance (ANOVA) was conducted using a STATISTICA 7.0 software. Significantly different means ($p < 0.05$) were separated by Tukey's test.

3. Results and discussion

The process yield for drying of microcapsules was 23.64 and 85.53 % for spray and freeze drying, respectively. The low process yield of spray drying technique was due to the adhesiveness and cohesiveness of the particles while interacting with the drying chamber (Behboudi-Jobbehdar et al., 2013).
Characterization of powders dried by spray and freeze drying is presented in Table 1.

Table 1. Characterization of powders dried by spray and freeze drying.

Drying method	Encapsulation efficiency (mg oil/g powder)	Solubility (g of soluble powder/ 100 g of water)
Spray drying	$277.17^b \pm 5.69$	$70.00^a \pm 0.00$
Freeze drying	$575.49^a \pm 3.68$	$66.67^a \pm 5.77$

Equal letters in the same column did not differ among themselves statistically ($p < 0.05$)

Spray dried powders has low encapsulation efficiency when compared to freeze dried powders. In spray drying technique, the exposition time of material to high temperatures may have caused partial loss of essential oil, while the freeze drying technique it works with low temperatures, preserving its quality.
In relation to water solubility, a statistical analysis demonstrated that there were no significant differences between the samples, at level of 5%.

Characterization of biodegradables films is showed in Table 2.
Color parameters of samples demonstrated that films prepared with encapsulated essential oil (F2 and F4) were more opaque and less yellowish than films prepared with free essential oil (F1 and F3). This result is due to the presence of cyclodextrin in the product. In relation to water solubility, it is observed that all films have good water solubility and there was no significant difference between them ($p < 0.05$).
Clove essential oil is a hydrophobic molecule, and it presence in formulations decrease the water vapor permeability of films. In this study the WVP was not affected significantly by concentration of essential oil in the formulations (F1 and F3), probably because the low concentrations of oil used in the formulations. However, films containing inclusion complexes showed higher WVP value than films containing free essential oil. β-cyclodextrin is a compound derived from starch, and it is known that films elaborated with starch shows lost on water vapor permeability properties (Brandelero et al., 2013).
Tensile strength was not influenced by the presence of essential oil/β-CD inclusion complex prepared by spray drying (Table 2). However, film containing inclusion complex prepared by freeze drying (F4) showed lower tensile strength than film containing inclusion complex prepared by spray drying (F2) and film containing free essential oil (F3).

Table 2. Characterization of biodegradables films.

		F1	F2	F3	F4
Color	L*	$88.25^{a} \pm 0.13$	$87.47^{a,b} \pm 0.37$	$88.20^{a} \pm 0.70$	$86.87^{b} \pm 0.21$
parameters	a*	$-4.32^{a,b} \pm 0.05$	$-4.63^{a} \pm 0.20$	$-4.06^{b} \pm 0.02$	$-4.42^{a} \pm 0.13$
	b*	$6.13^{a} \pm 0.40$	$8.36^{b} \pm 1.49$	$5.16^{a} \pm 0.16$	$8.95^{b} \pm 0.37$
Water solubility (%)		$99.95^{a} \pm 0.01$	$99.96^{a} \pm 0.01$	$99.93^{a} \pm 0.02$	$99.96^{a} \pm 0.01$
Thickness (μm)		$98.40^{a,b} \pm 0.01$	$74.50^{b} \pm 0.00$	$37.90^{c} \pm 0.00$	$91.42^{a} \pm 0.00$
WVP ($x10^{11}$) (g/m.Pa.s)		$1.63^{a} \pm 0.08$	$3.95^{b} \pm 0.10$	$1.38^{a} \pm 0.42$	$2.74^{b} \pm 1.30$
Tensile strength (Mpa)		$47.66^{a} \pm 6.52$	$46.91^{a} \pm 3.80$	$50.11^{a} \pm 7.73$	$30.65^{b} \pm 4.10$
Elongation (%)		$56.18^{a,b} \pm 7.67$	$43.74^{a} \pm 13.59$	$64.95^{b} \pm 6.75$	$47.09^{a} \pm 5.56$

Equal letters in the same line did not differ among themselves statistically ($p < 0.05$)

The incorporation of essential oil/β-CD inclusion complex in films formulations decreased their elasticity, contributing to greater hardness, as showed in elongation test of films (Table 2).
Sorption isotherms of films samples are showed in Figure 1. Films showed greater tendency to absorb water from the relative humidity of 60%.

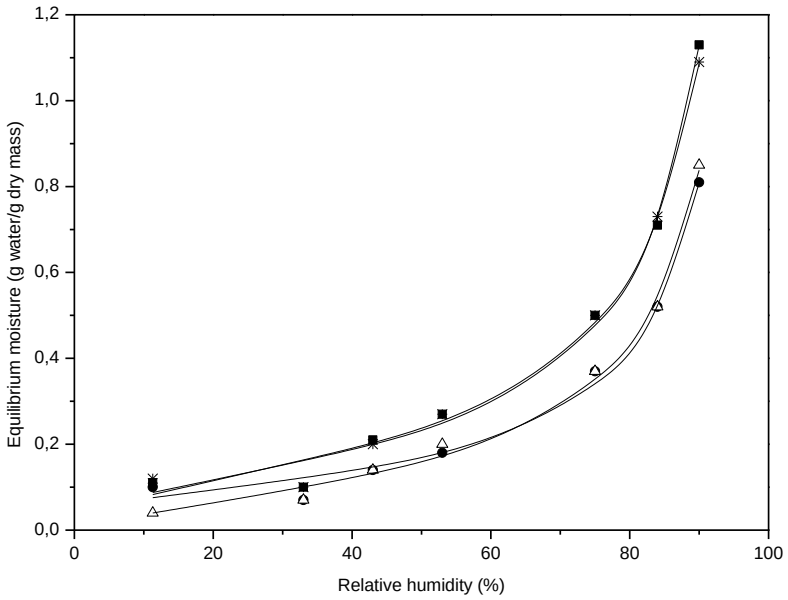

Figure 1. Sorption isotherms of biodegradables films. F1 (filled square), F2 (filled circle), F3 (asterisk), F4 (open triangle) formulation. The lines are derived from GAB model.

The sorption isotherms established for the films were modeled using GAB model, and the parameters obtained are summarized in Table 3.

The isotherm modelling was considered to be satisfactory. Out of the constants showed in Table 3, the monolayer value (m_0) is of particular interest because it indicates the amount of water that is strongly adsorbed to specific sites of the film surface (Melo et al., 2011). Monolayer values of films containing free essential oil are very close, and similar result was observed to monolayer value of films containing encapsulated essential oil. However, it is observe difference on monolayer values between films containing free oil and encapsulated oil. The films containing encapsulated essential oil shows lower monolayer value than others films. It is known that the films produced with empty β-CD, i.e. pure β-CD shows an increase of monolayer value, due to the hydrophilic nature of empty β-CD (Motta et al., 2011). Therefore, the encapsulation decreased the hydrophilicity of β-CD. This result corroborate with the results of water solubility of inclusion complexes (Table 1), that it were lower when compared to water solubility of β-cyclodextrin, in the conditions studied.

Table 3. GAB model parameters for biodegradables films.

Film samples	m_0	C	K	R^2
F1	0.1273	12.564	0.0098	0.9965
F2	0.0893	24.097	0.0098	0.9952
F3	0.1350	9.684	0.0097	0.9963
F4	0.1003	4.377	0.0098	0.9971

All the samples showed K value very close and less than unit. The C parameter is related to the monolayer heat sorption and in this work it was observed a significant difference between the films samples. The C and K parameters presented value less than unit and more than two, respectively, characteristic of isotherm type II, according to Blahovec (2004).

4. Conclusions

Spray and freeze drying techniques showed good results in encapsulation of clove essential oil in β-CD, with higher yield process and encapsulation efficiency obtained by freeze drying technique. Biodegradables films prepared with encapsulated essential oil were more opaque and less yellowish than the films prepared with free essential oil. All film samples showed high water solubility, with no significant difference between them ($p < 0.05$). Films prepared with encapsulated essential oil showed an increase on the water vapor permeability and lower elasticity. The sorption isotherms showed that the encapsulation decreased the hydrophilicity of cyclodextrin and, consequently, hydrophilicity of films. The use of encapsulated essential oil in biodegradables films is a promising alternative for the application of this antimicrobial compound in food.

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References

ASTM**,** American Society for Testing and Materials**,**1996, Test methods for tensile properties of thin plastic sheeting – D 882-91.Annual book of ASTM standards. Philadelphia, ASTM.

Behboudi-Jobbehdar, S., Soukoulis, C., Yonekura, L. and Fisk, I., 2013, Optimization of spray-drying process conditions for the production of maximally viable microencapsulated L. acidophilus NCIMB 701748. Drying Technology, 31(11), pp.1274-1283. DOI: 10.1080/07373937.2013.788509.

Blahovec, J., 2004, Sorption isotherms in materials of biological origin mathematical and physical approach. Journal of Food Engineering, 65(4), pp.489-495. DOI: 10.1016/j.jfoodeng.2004.02.012.

Brandelero, R.P.H., Grossmann, M.V. and Yamashita, F., 2013. Hidrofilicidade de Filmes de Amido/Poli (Butileno Adipato Co-Tereftalato)(Pbat) Adicionados de Tween 80 e Óleo de Soja. *Polímeros*, *23*(2), pp.270-275.

Cortés-Rojas, D.F., Souza, C.R. and Oliveira, W.P., 2014, Encapsulation of eugenol rich clove extract in solid lipid carriers. Journal of Food Engineering, 127, pp.34-42.

Gontard N., Guilbert S. and CUQ J.L., 1992, Edible wheat gluten films: influence of the main process variables on film properties using response surface methodology. Journal of food science, 57(1), pp.190-195, DOI: 10.1111/j.1365-2621.1992.tb05453.x

Hernández-Sánchez, P., López-Miranda, S., Lucas-Abellán, C. and Núñez-Delicado, E., 2012. Complexation of Eugenol (EG), as Main Component of Clove Oil and as Pure Compound, with [Beta]-and HP-[Beta]-CDs. Food and Nutrition Sciences, 3(6), p.716.

Hill L.E., Gomes C., and Taylor T.M., 2013, Characterization of beta-cyclodextrin inclusion complexes containing essential oils (trans-cinnamaldehyde, eugenol, cinnamon bark, and clove bud extracts) for antimicrobial delivery applications. LWT-Food Science and Technology, 51(1), pp.86-93.

Melo, C.P., Grossmann, M.V., Yamashita, F., Youssef, E.Y., Dall'Antônia, L.H. and Mali, S., 2011. Effect of manufacturing process and xanthan gum addition on the properties of cassava starch films. Journal of Polymers and the Environment, 19(3), pp.739-749. DOI: 10.1007/s10924-011-0325-1.

Motta, C., Martelli, S.M., Soldi, V. and Barreto, P.L., 2011, Biodegradable films containing α-tocopherol/β-cyclodextrin complex. In: 11° Congresso Brasileiro de Polímeros, Campos do Jordão, São Paulo, Brazil.

Soliman, E.A., El-Moghazy, A.Y., El-Din, M.M. and Massoud, M.A., 2013. Microencapsulation of essential oils within alginate: formulation and in vitro evaluation of antifungal activity.

Sun, X., Sui, S., Ference, C., Zhang, Y., Sun, S., Zhou, N., Zhu, W. and Zhou, K., 2014. Antimicrobial and mechanical properties of β-cyclodextrin inclusion with essential oils in chitosan films. Journal of agricultural and food chemistry, 62(35), pp.8914-8918.

Szente L., Harangi J., Szejtli J. 1998, Long-term storage stability studies on flavor-b-cyclodextrin complexes. Proceedings of the 4th International Symposium on Cyclodextrin. The Netherlands: Kluwer. pp 545–549.

Wang, T., Li, B., Si, H., Lin, L. and Chen, L., 2011. Release characteristics and antibacterial activity of solid state eugenol/β-cyclodextrin inclusion complex. Journal of Inclusion Phenomena and Macrocyclic Chemistry, 71(1-2), pp.207-213. DOI: 10.1007/s10847-011-9928-3.