Properties of propolis microencapsulated

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INTRODUCTION

Propolis (bee glue) is a strongly adhesive resinous bee hive product collected by honeybees that has been used by man since ancient times due to its pharmaceutical properties (Burdock, 1998). Its chemical composition is complex and typically consists of waxes, resins, water, inorganics, phenolics, and essential oils, the exact composition depending upon the source plant(s).
Among the products such as bee honey, royal jelly, pollen, among others, the propolis is notable both for its therapeutic properties, such as antimicrobial activity, inflammatory, healing, anesthetic and antioxidant (Bankova, 1989; Ghisalberti, 1979). However, for the use of this material on a larger scale and in different formulations, such as food additive, there are obstacles such as the strong flavor and the difficulty of solubilization, which normally requires the sale in the form of ethanolic extractive solution. In this case, the technology of microencapsulation is relevant, because it has settled limitations in the use of food additives and ingredients, since it can mask undesirable flavors, reduce volatility, reactivity and hygroscopicity, increasing the solubility, and allowing an increase in stability of these in adverse environmental conditions (Brannon-Peppas, 1993).
In this context, this study aimed to prepare microspheres of propolis using two encapsulating agents and characterizes the powder obtained by morphological characteristics, physicochemical and antioxidant properties.

MATERIAL AND METHODS

Propolis extractive solution was prepared with propolis/ethanol ratio of 30/70 (w/w). This extract was used as core. Octenyl-succinate starch (OSA starch) (Corn Products, Brazil) and gum arabic (CNI- Colloides Naturels International, Brazil) were used as the encapsulating agents.

Four dispersions were prepared to microencapsulate the extract of propolis. They differed in the proportion core/encapsulating agent and the type of agent (OSA starch or gum arabic). These dispersions were known by the treatments 1, 2, 3 and 4 described below:

- Treatment 1 = 1 part of extract of propolis/6 parts of solution 30% (w / w) of gum arabic;
- Treatment 2 = 1 part of extract of propolis/4 parts of solution 30% (w / w) of gum arabic;
- Treatment 3 = 1 part of extract of propolis /6 parts of solution 30% (m / m) of OSA starch;
- Treatment 4 = 1 part of extract of propolis/4parts of solution 30% (m/m) of OSA starch.

Ultra-Turrax homogenizer digital model T25 (IKA) was used to prepare the dispersions, with rotation of 15000 rpm for a period of approximately 2 minutes. The solutions were atomized in a laboratory spray dryer (model 1.0 MSD, from Labmaq do Brasil Ltda.), according with the following operating parameters: temperature input 120º C and feed flow rate of 1 L/h.

The morphology of the microspheres was observed with a scanning electron microscope (JEOL JSM–T300, Tokyo, Japan) at an accelerating voltage of 5 kV. Sample diameter and size distribution were measured using laser diffractometer (Mastersizer X, Malvern Instrument, UK). The average particle size was expressed as the volume mean diameter in µm.The solubility of the powder in cold water (S) was determined according to methodology described by Singh (2003). For the

hygroscopicity (H) measurements, samples (about 2 g) of each powder were placed in Petri dishes at 25°C in an airtight plastic container containing Na_2SO_4 saturated solution (81% RH). After 1 week the samples were weighed and hygroscopicity was expressed as g of water absorbed/100g of dry solids (Cai & Corke, 2000). It also determined the moisture (M) and the water activity (AW) of the powders. The coloration (parameters L, a *, b *) of the powders was measured using a Hunter Lab colorimeter according to the methodology described by Cai & Cork (2000).
The resistance of the material in the process of microencapsulation was determined by quantification of phenolic compounds in the feed and in the powders (after reconstituting) by a spectrophotometric method of Folin-Ciocateau described by Woisky & Salatino (1998). The determination of the resistance was obtained by calculating the loss of phenolic compounds, using gallic acid as standard. Spectrophotometry method was also used to evaluate the antioxidant activity of propolis microspheres. For this, the activity sequestrant of DPPH radical was quantified as the methodology described by Chen et al. (2003). To the determination of phenolic compounds and to achieve a measure of antioxidant activity, it was necessary the powder dissolution in water, and then to solubilize the core (propolis) in ethyl alcohol 80%. After this step, were done the colorimetrics reactions.

RESULTS AND DISCUSSION

With respect to morphology, it was observed that for both encapsulating agents, gum arabic and OSA starch, the microcapsules showed rounded outer surface of teeth with training or concavities and varying sizes (Figure 1 and 2). The appearance of teeth on the surface is attributed to the rapid evaporation of liquid droplets during drying in the atomizer (Rosenberg, 1985).

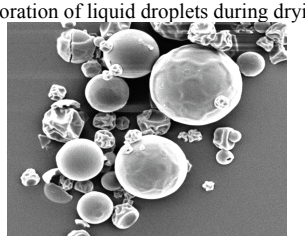

Figure 1 : SEM micrographs (5000x) of microspheres of propolis in gum arabic.

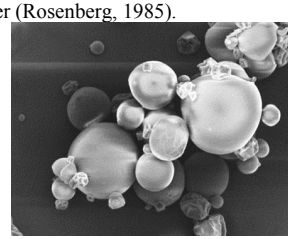

Figure 2 : SEM micrographs (5000x) of microspheres of propolis in OSA starch.

Regarding the distribution of particles, all treatments showed clearly bimodal behavior. In diameter, the statistical analysis of results found a significant difference ($P < 0.05$) among the four types of treatments. In general, the microspheres produced with gum arabic had an average diameter greater than the average diameter of produced with OSA starch. This result can be explained by the fact that gum arabic retained more water after drying (Table 1), which helps to prevent shrinkage the capsule. The average sizes of particles (Table 1) were produced within the range of size of particles produced by atomization, which varies from 5 to 150 µm, according Thies (1995). In general, the values of moisture and water activity, described in Table 1, are usually obtained for powders and are sufficient to ensure microbiological stability to the material. With respect to solubility, by analyzing the values obtained it is possible to infer that the two encapsulating agents showed high solubility in water. This result is excellent, as most certainly extends the range of applications can be given to propolis, including as an additive in food, where the presence of alcohol is totally undesirable. Among the treatments studied, the 3 shown the highest solubility, the closer to 100%. The samples were slightly hygroscopic (Table 1), which results in ease of storage, handling and applications of the powders. Samples prepared with gum arabic (treatments 1 and 2) were

significantly more hygroscopic than those prepared with OSA starch (treatments 3 and 4). This difference can be attributed to the polarity of encapsulating.

	Treatments			
	1	2	3	4
M (%)	9.3±0.4 [b]	12.6±0.4 [a]	4.9±0.3 [d]	7.2±0.6 [c]
AW	0.33±0.01 [b]	0.39±0.02 [a]	0.25±0.02 [d]	0.29±0.01 [c]
S (%)	86.0±3.0 [b]	84.0±0.3 [b]	94.0±2.0 [a]	86.0±2.0 [b]
H	29.3±0.6 [a]	27.4±0.9 [a]	15.8±0.9 [b]	13.8±0.1 [b]
D (μm)	24.0±0.6 [a]	23.3±0.4 [b]	15.0±0.3 [d]	16.0±0.0 [c]

Table 1 : Moisture, water activity, solubility and diameter size of the powders.

By Tukey test, equal letters in the same line do not differ significantly between them (p> 0.05)

Treatments	L	a*	b*
1	80.08	-3.63	20.18
2	80.28	-3.39	18.91
3	81.54	-3.01	15.43
4	80.84	-3.25	17.61

Table 2: Parameters of color – CIELAB

•Treatment 1: 1 propolis/6 gum arabic;
•Treatment 2: 1 propolis/4 gum arabic;
•Treatment 3: 1 propolis/6 OSA starch;
•Treatment 4: 1 propolis/4 OSA starch;

For color data, it is known that the parameter L, which varies between 0 and 100, is related to brightness of the sample and this way, both encapsulating values obtained were similar, which indicate that the post had dye trend light. For the parameter a*, it is known that the more negative it is more the sample tends to color green and one more time for both encapsulating the values were similar. For the parameter b *, which indicates the level of yellow, it is possible to conclude that the microcapsules of gum (Treatments 1 and 2) showed slightly higher values when compared to the microcapsules of starch (Treatments 3 and 4), this result can be attributed to the fact that the gum is more transparent that the starch, so lets get more light, which was reflected by flavonoids (pigments of propolis) in the spectrum that gives it color, with more yellowish hue to the capsules of gum therefore slightly higher values for the parameter b, for treatments 1 and 2.

The results to the antioxidant properties of propolis have shown that the process of microencapsulation was not deleterious to the compounds responsible for this activity (Table 3). In general the samples containing more propolis, treatments 2 and 4 showed higher antioxidant activity, except for the higher concentrations tested (3000 ppm, for samples encapsulated with starch and 5000 ppm for samples encapsulated with gum arabic), where results were very similar for both treatments. These results suggest that the highest concentrations were close to the saturation concentration for this activity, so you can not work with a higher concentration, with the aim to increase this activity. The concentration of treatments was not identical, because the samples concentrations were adjusted to minimize effects of turbidity caused by the reaction of the encapsulating material with the reagents used in this analysis.

The results were similar to those obtained by Marquele et al. (2006) and Souza et al. (2007), which dried extract of propolis by spray-drying and determined that the dried material showed high antioxidant activity and was able to inhibit 50% of lipid oxidation in concentrations ranging from 2.5 to 5 mg / ml.

As in the process of atomization was used a high temperature, was evaluated the effect of employment of this process of phenolic compounds in propolis. For this was done a determination of these compounds in dispersion and in powder reconstituted. According to results showed in Table 4 the operating parameters used in the spray were appropriate because the loss of phenolic compounds present in propolis can be considered small.

Among encapsulating agents tested, it was observed that with the gum arabic the loss of phenolic compounds was lower than with the OSA starch. Then, is possible to infer that gum arabic offered greater protection than OSA starch. Maybe the gum recovered more such compounds, which protected during exposure to high temperature of the process. This difference can be attributed to the higher molecular weight and stronger film-forming properties of the gum arabic.

Treatment	Concentration (ppm)	Antioxidant Activity (%)
1	500	23.82 ± 1.23
	2000	72.20 ± 4.63
	5000	88.95 ± 3.26
2	500	33.41 ± 0.73
	2000	78.70 ± 1.23
	5000	84.18 ± 3.37
3	600	34.60 ± 2.57
	1500	61.54 ± 4.01
	3000	87.59 ± 1.14
4	600	52.42 ± 1.45
	1500	80.55 ± 1.64
	3000	87.40 ± 1.45

Table 3 : Evaluation of the antioxidant properties of propolis microencapsulated.

Treatment	Loss of phenolic (%)
1	3.4 ± 0.3[a]
2	3.0 ± 2[a]
3	9.0 ± 2[b]
4	10.5 ± 1[b]

Table 4: Resistance to the process of microencapsulation

Furthermore, different concentrations of encapsulating tested did not affect the strength of the phenolic to the process of spraying, since there was no difference between treatments with the same encapsulant prepared.

CONCLUSION

The process, operating conditions of spray and encapsulating agents were adequate to obtain propolis in the form of powder, alcohol-free and with good solubility in water which expands the scope of application, especially in food. This work has proved that it is possible to encapsulate extract of propolis with gum arabic and OSA starch and this process is not harmful to the phenolic compounds present in the material, and preserves its antioxidant activity.

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