Microencapsulation of glucosyltransferase by ionic gelation

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Introduction

Isomaltulose or palatinose® is a non-cariogenic sugar, naturally present in very small quantities in honey, sugar cane extract and other sugar-rich fluids. This alternative sugar is commercially obtained by the enzymatic conversion of sucrose catalyzed by glucosyltransferase. The isomaltulose shows physicochemical and organoleptic properties similar to those of sucrose and has a low glycemic index. The safety of isomaltulose has been comprehensively verified, resulting in its approval as human food (Lina et al., 1997), and it is widely used commercially as a sucrose substitute in foods, soft drinks and medicines. The use of enzyme immobilization techniques is well known to produce stable and reusable biocatalysts since a hydrogel matrix could protect the enzyme from environmental conditions such as pH, temperature, organic solvents and other compounds capable of inactivating them. The immobilized catalyst could also be handled more easily and recovered from the solution without difficulty (Derkavos et al., 1991). Within this framework, the microencapsulation by ionic gelation is straightforward and inexpensive. Pectins, which gelation mechanism is mainly governed by their degree of esterification (DE), are among the most widely studied ionic polysaccharides with useful and versatile properties in this application area. The addition of hydrophobic compounds such as lipids can improve the retention of the hydrophilic compounds used as core materials in these matrices. These microcapsules present high water content and low water solubility, and they can be produced in different sizes and shapes. In this work the microencapsulation of glucosyltransferase produced by the strain *Erwinia* sp. D12 was evaluated using low methoxyl pectin with or without fat mixture addition (butter milk plus oleic acid, 1:1) wet and freeze dried. Microparticles characterizations with respect to morphology, average size and enzymatic activity after 6 cycles of production of isomaltulose from sucrose solutions were evaluated.

Material and methods

Microorganism and culture maintenance: A strain of *Erwinia* sp. D12 (glucosyltransferase producer), isolated in the laboratory of Food Biochemistry, Faculty of Food Engineering - UNICAMP, was cultivated in inclined agar tubes, composed of nutrient agar + 0.3% sucrose (m/v) for 15 hours at 30°C and then stored at 4°C.

Production of glucosyltransferase: The production of cell biomass was carried out using a medium composed by sugar cane molasses (150 g l^{-1}), corn steep liquor-Milhocina® (20 g l^{-1}), yeast extract Prodex Lac SD® (15 g l^{-1}) and adjusted to pH 7.5. A loop full of culture was inoculated into 250 ml Erlenmeyer flasks containing 50 ml of inoculum medium, incubated in an orbital shaker (New Brunswick Scientific, Edison, NJ, USA) at 200 rpm for 15 h at 30°C and then a 10% (v/v) inoculum was added to the medium. The liquid cultures were incubated at 30°C and 200 rpm in a rotator shaker. After 8 hours the culture was centrifuged (Centrifuge Beckman J2-51, Beckman-Coulter, Inc., Fullerton, CA, USA) at 10000 x g for 15 minutes. The cell mass was washed twice with distilled water and then suspended in 50 ml of distilled water. The cell suspension was cooled to

5ºC and disrupted by ultrasonic oscillation 180 W for 20 sec (Labline Instruments, Inc., Illinois, USA). After cell wall disruption, the samples were centrifuged at 10000 g for 15 min at 5 ºC. The enzyme activity, humidity and total protein (Kjeldahl) of the supernatant was determined. The supernatant obtained was considered as glucosyltransferase crude extract.

Microcapsules production: Amidated low methoxyl pectin (LMP) was used for the enzyme immobilization. A 2% (m/m) of polymer solution was heated with slowly stirring until complete dissolution of pectin, and a few drops of paprika oleoresin were added for a better visualization of the microcapsules. After the system was cooled to 30°C, glucosyltransferase crude extract, 5% (v/m) with respect to the final concentration was added. The mixture was homogenized with an Ultra Turrax T50 (10000rpm/1 min) and sprayed using a double fluid atomizer (Lab Plant, UK) with a compressed air sprinkler (P = 0.15kg_f/cm^2) on a 2% (m/v) $CaCl_2$ at 25°C with constant stirring. After a curing period of 30 minutes at 25°C in the $CaCl_2$ solution, the capsules were sieved (25μm) washed with 600 ml of distilled water and stored at 5°C (WC) The total protein concentration and enzyme activity of glucosyltransferase was determined in the microparticles and also in the residual calcium chloride. The preparations were made in triplicate. Another preparation was produced with the addition of 2% (w/w) of fat material (1:1, butter milk: oleic acid) as previously described.

Microcapsules drying: Microcapsules were frozen (-18°C) and subsequently freeze dried (72 hr). The freeze-dried capsules (FDC), were kept at -18°C. The glucosyltranferase crude extract was lyophilized in the same conditions and the enzymatic activity and humidity also determined.

Physico-chemical characterization of microcapsules: The microcapsules ((WC) and (FDC)) were characterized in relation to the amount of total protein (Kjeldahl), humidity and efficiency of encapsulation (E.E.%) of the filling material, measured by the ratio of the concentration of total protein remaining in the capsules after the cure process and the amount of protein present in the solution, prior to the formation of the capsules, expressed as a percentage. (E.E.% = [encapsulated protein (g) x 100] / [Initial protein (g)])

Microcapsules morphology: WC morphology was analyzed by optical microscopy, using objectives of 2X and 10X. The average diameter of the WC was also determined. FDC were set on stubs of aluminum using copper adhesive tape and covered by a thin layer of gold (Balzers - Sputter Coater SCD050) 40mA/180s, at 24°C and observed in the scanning electron microscope (Jeol, JSM 5800LV, Tokyo, Japan).

Evaluation of the activity of the encapsulated enzyme: Samples of 20g of WC, or 1.2g of FDC with fat, or 0.7g of FDC without fat were added to 100 ml of 10% sucrose solution. The microcapsules were kept inside an adapted container (sieve 25μm) allowing the recovery of the capsules from the reaction medium. The system was placed on agitation, at 35°C. After 30 min. aliquots were collected and glucosyltransferase activity determined. The remaining reaction media was discarded and replaced by and identical one to start another cycle of reaction. This procedure was repeated for 6 cycles. All experiments were done in triplicate.

Glucosyltransferase activity measurement: The glucosyltransferase activity was performed by the increase of the reducing power from a solution containing sucrose, described by Park et al. (1996) with modifications. A mixture of 450 μL of a 10% (w/v) sucrose solution in water (pH 6.0) and 50 μL of enzyme solution was incubated for 20 min at 35ºC. The reducing sugars were measured by Somogyi method (Somogyi, 1945) using glucose as standard. One activity unit (U) of glucosyltransferase was defined as the amount of enzyme that liberates one μmol of reducing

sugars/minute/ml of the enzyme from sucrose under standard assay conditions. The specific activity was performed dividing the enzymatic activity by the amount of total protein.

Results and Discussion

The protein encapsulation efficiency (E.E.%) was slightly higher for the capsules with fat (55%) than for the capsules without fat (51%), and the moisture content (WC) was 94% and 97% respectively, as it may be observed in table 1. The figures 1 and 2 indicate that all the capsules showed spherical shape, and multinucleated core distribution. The average size was about 196 μm (microcapsules with fat) and 173 μm (microcapsules without fat). Bourgeois et al. (2006) reported that the protein encapsulation efficiency was 75% and 45% for non-amidated and amidated pectin beads, respectively, with immobilized β-lactamase.

Table 1. Characterization of microparticles

	Humidity (%w/w)	Total Protein (%w/w)	E.E. %	Diameter (μm)
WC without fat	97	1.8	51	196
WC with fat	94	1.0	55	173

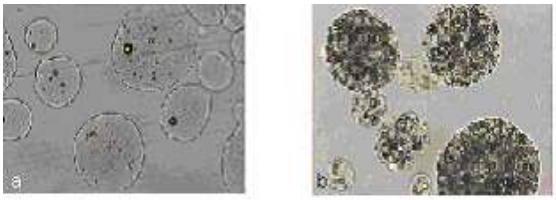

Fig 1. WC micrographs obtained by optical microscopy after production (10x). a) without fat, b) with fat.

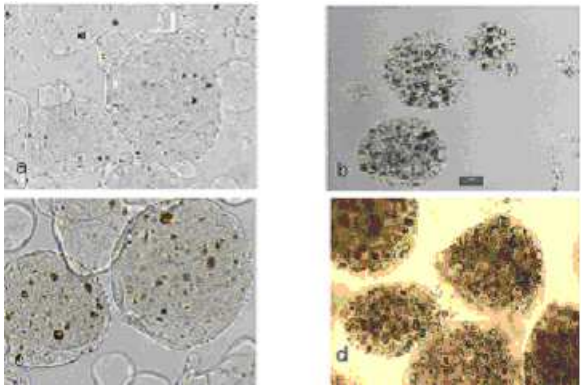

Fig 2. WC (a. without fat, b. with fat) and FDC (c. without fat, d. with fat) after the conversion from sucrose to isomaltulose assay with glucosyltransferase encapsulated.

FDC with fat maintained its size and largely spherical shape, as could be observed by Scanning Electron Microscopy (SEM) (figure 3), while FDC without fat were much more brittle and crushed.

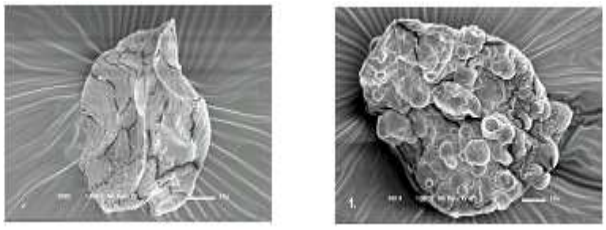

Fig 3. SEM micrographs of FDC a) capsules without fat, b) capsules with fat.

Figure 4 indicated that the higher enzyme activity was obtained for WC with fat, with retention of specified activity during the 1st 3 cycles of reaction (30 min), decreasing 60% of the activity in the 4th and 5th cycle and 80% in the 6th cycle. The activity of the enzyme with FDC was considerably lower compared with wet particle system. Also the enzyme freeze dried non- encapsulated had shown the same low enzymatic activity capacity.

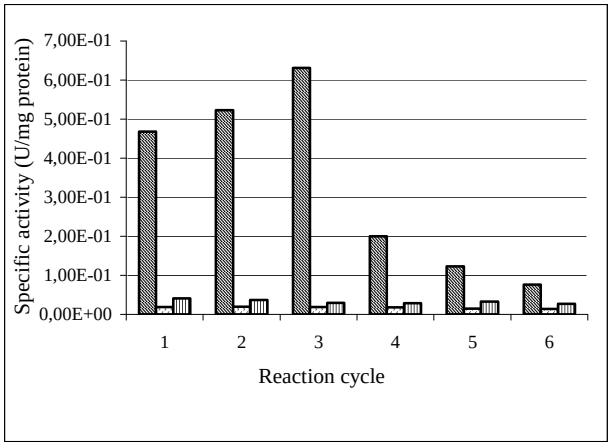

Fig 4. Enzymatic activity ▨ WC with fat, ▫ WC without fat ◫ FDC with fat

Conclusions

The results indicate that the immobilization of this enzyme by ionic gelation was feasible. However, the freeze drying process needs to be adjusted, as the handling and maintenance of lyophilized microcapsules is easier than wet particles.

References

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