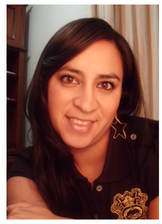
P-071 **Microencapsulation of *Bifidobacteriu breve* ATCC 15700 with amaranth starches**

Falfán, CRN[1*#], Martínez, BF[1], Gaytán[1], MM, Grosso, CRF[2], Arvizu MSM[3], Amaya LSL[3]
[1] Cinvestav-Qro. Lib. Norp. 2000, Real de Juriquilla, Qro, México. [2] Univ Campinas, Campinas-SP Brazil.[3] Unive Autónoma de Querétaro, Qro, México.
[*] Supervisor [#] pa_llely@ hotmail.com



INTRODUCTION AND OBJECTIVES

The viability of the probiotics must be maintained during manufacture, storage, and delivery to the target site in the gastrointestinal tract to exert their health benefit. The survival of probiotics is genus-, species-, and strain-dependent, and composition of the other ingredients in the preparation will also affect their viability (Del Piano 2006). Recently spray drying has gained more attention as an alternative for microencapsulation of probiotics, because the low cost of manufacture and can be easily scaled up. Various polymer systems have been used to encapsulate probiotic microorganisms. Starch is a dietary component that has an important role in colonic physiology and functions and potential protective role against colorectal cancer (Cassidy 1994), however the native starch present limitations that reduce their use at the industrial level. Chemical modification involves the introduction of functional groups into the starch molecule, resulting in markedly altered physico-chemical properties (Hermansson, 1996). Starches derivatives using reactive extrusion are materials with better characteristics of solubility and viscosity to be used in spray-drying (Murua 2009). The aim of this work was to study microencapsulate *Bifidobacterium breve* ATCC 15700 by spray-drying in modified amaranth starch.

MATERIAL AND METHODS

Preparation of modified amaranth starches. Amaranth starch was isolated in the laboratory by the alkali steeping method (Yanez 1986) from *Amaranthus* seeds variety Nutrizol. The native starch was hydrolyzed using HCl (3.4% dry basis at 50 °C for 6 h). The hydrolyzed starch was chemically modified using reactive thermoplastic extrusion process, resulting acetylated, succinated and phosphated starches. The change in chemical structure of the starch was qualitatively analyzed using FT-IR (Perkin Elmer, Spectrum GX) and the pasting properties of modified starches were measured in a Rapid Visco Analyzer (RVA).

Microencapsulation of B. breve. Culture of *Bifidobacterium breve* ATCC 15700 were obtained after activation by two successive transfers in MRS broth, under anaerobic conditions. Cultures in late-log phase were harvested by centrifugation at 4500 rpm for 15 min, (Centrifuge Hermle Z200A), washed twice in a sterile solution with 0.9% peptone. The pellet was resuspended in 10 ml of a sterile solution of reconstituted skimmed milk, which was added 25 g (d.b.) of modified starch in distilled water with 10^9 CFU/g as final bacterial concentration. Microencapsulation was developed by Spray Drying (SD-Basic Spray-Dryer, LabPlant, Huddersfield, UK). Drying conditions were: inlet air temperature 100°C ± 1°C, outlet air temperature 70 °C ± 5, nozzle diameter 0.5mm, liquid flow rate 2 ml/min. Bacteria were enumerated on MRS agar using the pour plate method. The plates were incubated at 37 °C for 48 h in anaerobic jars. Cell counts were performed in triplicate and means were reported. The external morphology of the capsules was evaluated by scanning electronic microcopy (ESEM EDDAX, GSE).

RESULTS AND DISCUSSION

The viscosity profile for modified starches (figure 1), shows a very low viscosity after the chemical modification and extrusion processing, due to the granule fragmentation resulting in a reduction in this parameter, compared to native amaranth starch which has a viscosity of 2500 ± 0.030 cP.

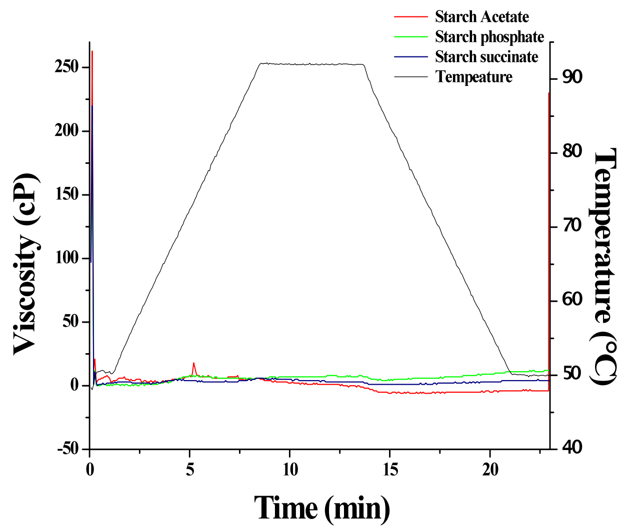


Figure 1. Viscosity profiles of amaranth starches chemically modified by extrusion process

FT-IR spectroscopy was used to verify the change in the chemical structures of starch molecules resulting from esterification. The FTIR spectra (Fig. 2) shows that amaranth native starch and modified starches have a similar profiles. However the modified starches showed less intensity. A comparison of the spectrum of native starch with acetylated starch clearly indicated the introduction of acetyl moiety through a band at 1730.8 cm^{-1} and compared with succinated starch this show a new absorption band at 1726 cm^{-1}, which suggest the formation of ester carbonyl groups. No difference between spectra of native starch and phosphated starch were observed probably due to a strong dependence on hydration in the stretching vibration of PO_2^{-2} group (Lewis and McElhaney, 1996).

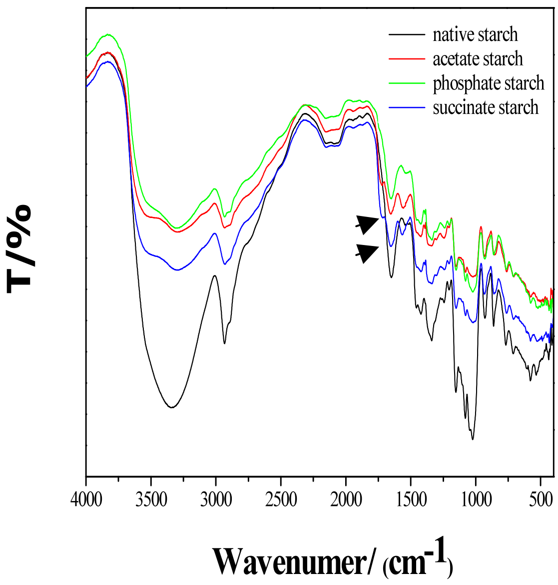


Figure 2. FTIR spectra of amaranth native starch and modified starches.

The bacteria survival was evaluated after spray-drying process with the three modified starches (Table 1). Higher survival was obtained with acetylated starch. The phosphated and succinated starch did not show significantly different. The photomicrographs showed that no bacteria were found in free form. A high efficiency of encapsulation during drying process (87.0 - 93.0%) was obtained. The shape of the microcapsules was typical for microcapsules produced by spray-drying with concavities on the surface as a result of fast water evaporation. The microcapsules size obtained in this study were less than 20 µm, which can contribute in reducing the impact on the texture when they are incorporated in a food.

Table 1. Survival of microcapsules containing *B. breve* ATCC 15700

Starch Modification	Before the process	After the process
	(Log CFU g^{-1})	
Acetate	9.8 ± 0.050	9.05 ± 0.070 a
Phosphate	9.8 ± 0.010	8.8 ± 0.000 b
Succinate	9.8 ± 0.050	8.75 ± 0.070 b

Different letters in the same column of each starch sample indicate statistical difference ($p<0.05$).

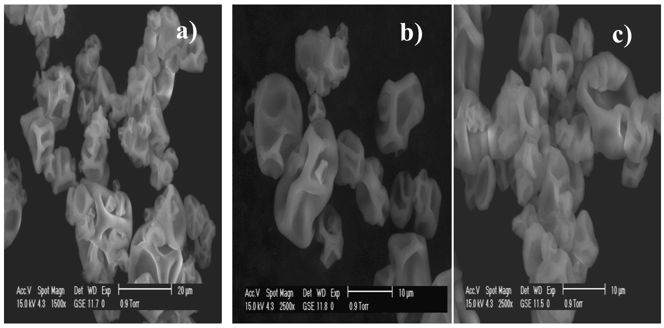


Figure 3. Morphology of encapsulated *B. breve* with (a) acetate starch, (b) phosphate starch, (c) succinate starch from amaranth.

CONCLUSIONS

The physical and chemical properties of modified starches were greatly influenced by chemical modification using reactive thermoplastic extrusion process. Microencapsulation by spray-drying using the amaranth starches (acetylated, succinated and phosphated) as wall material, was shown to be a convenient process to obtain microcapsules containing *Bifidobacterium breve* ATCC 15700.

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