P-107 **Encapsulation of *Bacillus Thuringensis* with waxy corn starch**

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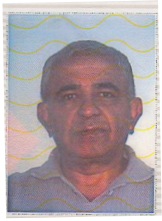

INTRODUCTION AND OBJECTIVES

One of the most widely used bioinsecticide against pest insects control is a protein extract obtained from *Bacillus thuringiensis* (*Bt*). Proper encapsulating materials are especially important for the best efficiency and benefit of Bt encapsulation (Imre et al. 2005). Native starches and starch hydrolysis products can be changed by phosphorylating them with hydrophobic groups or negatively charged groups using thermoplastic extrusion. The aim of this work was to modify waxy corn starch using thermoplastic extrusion and after encapsulate dried spores of *Bacillus thuringiensis* serovariety Kurstaki HD-1 by spray drying.

MATERIAL AND METHODS

Modified waxy corn starch (CPI San Juan del Rio, Qro), sodium tripolyphosphate (Sigma–Aldrich) and lyophilized spores from *Bt* were used.

Starches were hydrolyzed with HCl (3.4% dry basis, at 50°C for 6 h) sodium tripolyphosphate was added to the dried powder of hydrolyzed starch and mixed, pH was adjusted at 5.0–5.2 and moisture content adjusted to 15–16%.

The samples were modified using a laboratory single screw extruder. Barrel temperatures were 70–80, 150 and 180°C at the feeding, transition and high pressure extrusion zones, respectively. The change in chemical structure of the starch was qualitatively analyzed using FT-IR (Perkin Elmer, Spectrum GX).

Microencapsulation was developed by spray drying (LabPlant, UK) using emulsions containing 30 g (d.b.) of shell material/100 mL of water and 2 g/100 g of dried spores from *Bt* and 4% of orange peel oil as phagostimulant with respect to the solids. Drying conditions were: inlet air temperature of 180 ± 1 °C, outlet air temperature of 100 ± 5 °C, nozzle diameter of 0.5 mm, liquid flow rate 7.5 mL/min.and 70 m^3/h air flow.

The pasting properties of modified starches were measured in a 3C Rapid Visco Analyzer (RVA).

The samples were observed with a polarized light microscopy (Olympus Optical Co., LTD, Japan).The external morphology of the capsules was evaluated by scanning electronic microscopy (ESEM EDDAX, GSE).. Water activity was measured using the Aqua Lab equipment at 25ºC, mean particle size was measured using a Zetasizer nano equipment.

The presence of *Bt* was quantified by plating aliquots of the encapsulated lyophilized spores using physiological serial dilutions incubated at 30±2 °C and the number of microorganisms calculated at 24, 48 and 72 h. Colonies of *Bt* derived from the microcapsules samples were examined by light microscopy after Gram staining of the bacterial colonies that grew after 24 and 72 h using lugol, ethylic alcohol and safranine.

RESULTS AND DISCUSSION

The modified starch, with hydrophobic characteristics, showed low viscosity values (4.52 RVA units) and high water solubility (77 %) (Figure 1) compared to raw waxy corn starch. The extrusion process enhanced the fragmentation of starch producing shell materials with better characteristics of solubility and viscosity.

The spray-dried microcapsules exhibited low water activity (0.42) with a small mean particle size (7.55μm).

In Figure 2 no difference between spectra of waxy maize starch and phosphate starch were observed probably due to a strong dependence on hydration in stretching vibration of PO_2^{-2} group (Lewis and McElhaney 1996).

Microcapsules with encapsulated *Bt* (Figure 3) showed a smooth external morphology, in the form of irregular spheres with deformations, although free of pores and cracks, which is important in the liberation of the bioinsecticide since it could depend of the porosity and integrity of the surface of the microcapsules. The morphology of the interior of the particles showed a void center and *Bt* was dispersed between the internal walls in small drops.

The *Bt* colonies showed fast growth after 24h. The plates of the dilutions from 10^{-1} to 10^{-3} of phosphorylated waxy corn starch had a saturation of colonies, which could not be counted, and in the rest of the dilutions the count only it was possible after 48 h (5.7 x 10^6 UFC/g, 10^{-4} dilution and 3.8 x 10^7 UFC/g x 10^{-5} dilution for phosphorylated waxy starch. Whereas in raw corn starch the count was low 4.7 X 10^6 UFC/g UFC/g, 10^{-4} dilution and 3.0 X 10^7 UFC/g UFC/g x 10^{-5} dilution).
At 24h of incubation was observed the *Bt* by morphology and Gram stains, colonies with a violet-pink background, and these colonies were recognized as *Bt*. The *Bt* showed extended and cane form being a bacillus gram (+). In the

Gram staining done after 72 h was observed that already spore had formed, thus were not tinned and presented a spherical form (Figure 4).

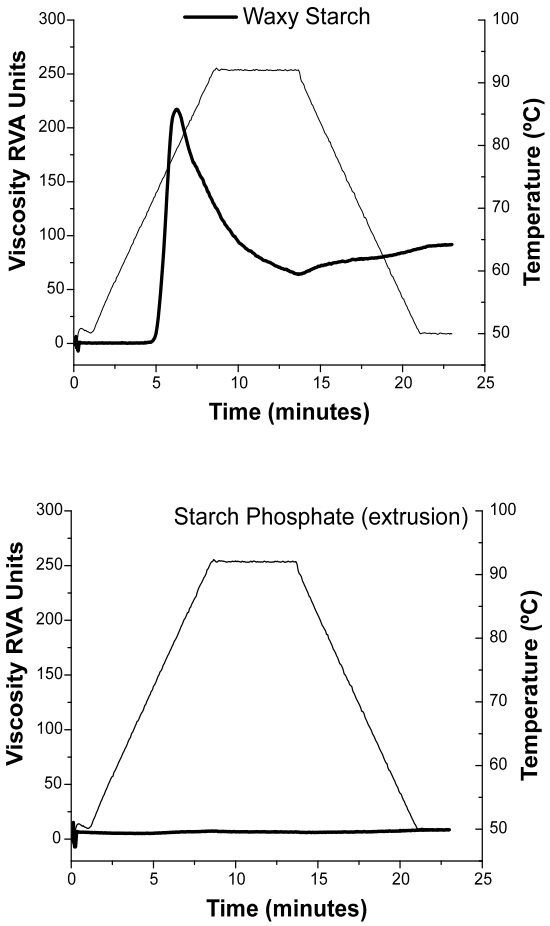

Figure 1. Viscosity profiles of raw and modified starch phosphates.

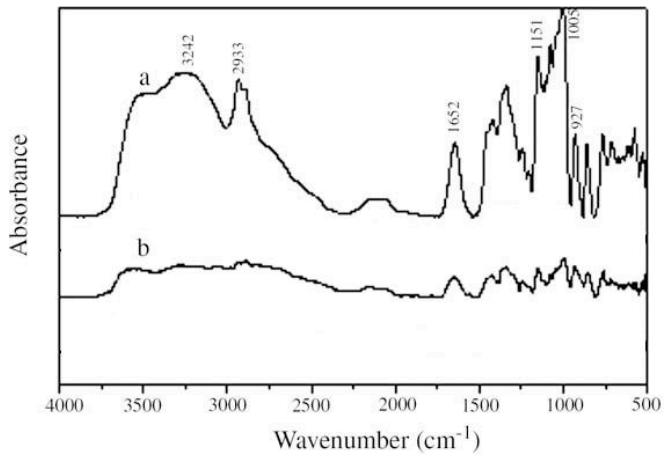

Figure 2. FTIR spectra of (a) waxy corn starch and (b) waxy corn starch phosphate.

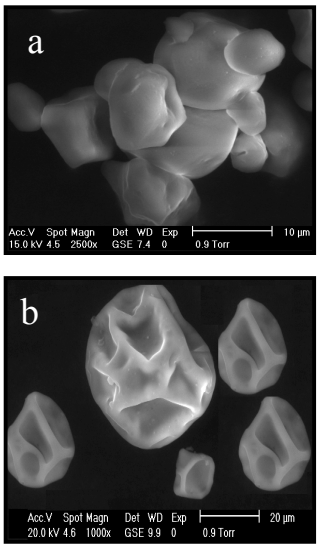

Figure 3. Microparticles morphology of encapsulated Bt with raw waxy corn (a) and phosphorylated waxy corn (b).

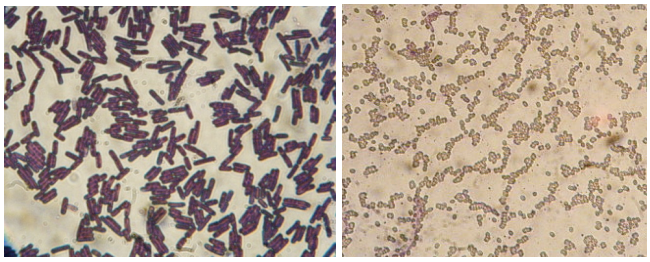

Figure 4. Gram staining of *Bt* 10^{-5} dilution at 24h (left) and at 72h (right).

CONCLUSIONS

The extrusion process enhanced the solubility and viscosity of the modified starch. The negatively charged groups introduced to starch phosphate improved the capabilities of the starch derivative making them good alternative for the encapsulation of bioinsecticide *Bt*.

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