From Cheese Whey Permeate To An Anti-*Listeria* Food Packaging Device: Bacterial Cellulose Nanocrystals/Sakacin-A Conjugates (Nanosak)

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Abstract

In the present project cheese whey permeate (CWP), the residual by-product obtained by extraction of whey proteins from cheese whey, was used as substrate for the growth of bacterial species that produce two appealing molecules: the anti-listerial bacteriocin sakacin-A and bacterial cellulose (BC). BC is then turned into nanocrystals (BCNCs) that are finally conjugated with sakacin-A to obtain an innovative antimicrobial device for food which could support *Listeria monocytogenes* growth.

Sakacin-A was produced by *Lactobacillus sakei* DSMZ 6333 in liquid cultures. The highest bacteriocin production (around 300 AU/mL) was achieved after 9 h at 26°C; a food-grade, salt-free enriched sakacin-A extract was obtained by using a gravity reverse phase chromatography. BC was produced by *Komagataeibacter xylinus* DSMZ 2325 by static fermentation of CWP in presence of 0.5 U/mL of β-galactosidase at 30°C; after 7 days, BC yield was around 7 g/L. BCNCs were then obtained by acid hydrolysis mediated by sulfuric acid, with the goal of removing the amorphous regions of BC and introduce a net negative charge by esterification on the hydroxyl group on C6.

BCNCs/sakacin-A conjugates were prepared by exploiting their opposite charge: enriched sakacin-A extract was mixed with BCNCs and, after incubation, conjugates collected by centrifugation have a specific activity of 100 AU/mg BCNCs. Among all peptides present in the enriched sample, sakacin-A appears to preferentially absorb onto BCNCs, thus allowing its further purification.

Sakacin-A as well its BCNCs conjugates were then included in a hydroxypropil-cellulose coating spread onto paper sheets at a concentration of 5 and 25 AU/cm^2. The addition of the coating did not bring any significant change in the oxygen barrier properties of the cellulosic substrate. In a similar way, the static contact angle of both uncoated and coated substrate was of approximately 130°. However, the presence of BCNCs seemed to increase the swelling phenomenon of the coating.

Sakacin A was also included in whey, caseine and cellulose derived matrices to prepare films and coatings with diverse results. The kinetics of Sakacin-A released from active films to aqueous food was analyzed by immersion of samples in water (as simulant) and measuring the anti-*Listeria* activity of the simulant after increasing times of exposure.

In vitro and *in vivo* antimicrobial trials were carried out on real food products demonstrated their anti-listerial effectiveness, proving that the developed devices can contribute to increase shelf life, quality and safety of perishable foods.

Keywords: *sakacin-A, bacterial cellulose, cellulose nanocrystals, active packaging, Listeria*

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