BIO-MICROCAPSULES PRODUCTION FOR A CONTROLLED RELEASE OF NATURAL ANTIMICROBIALS FROM PACKAGING MATERIALS

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ABSTRACT

In this work, bio-microcapsules of chitosan have been developed by a membrane process using both polymeric and ceramic membranes. This technique permitted the formation of monodispersed bio-polymer droplets which were then cross linked with a natural additive adapted for this polymer structure that enhanced the water resistance of chitosan. Furthermore, two different types of natural antimicrobial were included in the capsules making the loading both during their production and after the droplet formation. The most effective entrapping method was then selected considering the amount of natural antimicrobial incorporated. The antimicrobial activity of the microcapsules was assayed by turbidimetric methods against bacteria and yeasts selected as dangerous and common microorganisms, which may be present in fresh food. Results are reported about the relationship between the amount of natural active substances released by microcapsules and the different degree of crosslinking, as well as the effects of the technological variables investigated on the kinetics of antimicrobial release.

Key words: Bio-capsules formation, membrane technique, antimicrobial release.

INTRODUCTION

The water resistance modulation of biodegradable packaging materials is of primary importance because it allows the use of the packaging material both in contact with fresh foods and as active packaging system. In fact, the release of active compounds at rates suitable for specific sensitive foods very often implies the moisturization and the modification of the matrix where the active substances are incorporated into.

In this perspective, micro-encapsulation is a promising technology for protecting the natural active substances from the stresses and damages that can occur during the package manufacturing, for improving the capsules distribution in film, for preventing or minimising the loss of efficacy and for modulating the water resistance of the biodegradable materials. Thanks to these effects and according to their structure, the microcapsules could better control the release of the active substances and promote the interaction of the film with the active substances carrier (Rosca *et al.*, 2007). In this work, bio-microcapsules of chitosan (Srinivasa *et al.*, 2007) have been developed by a membrane process using a polymeric film. This technique permitted the formation of monodispersed bio-polymer droplets which were then cross linked with a natural additive adapted for this polymer structure that enhanced the water resistance of chitosan. Antimicrobial activity of microcapsules was assayed either in solid and liquid cultures of microrganisms selected as dangerous and which may be present in fresh food.

MATERIALS AND METHODS

Capsules preparation

The polymer, chitosan (medium MW, Sigma Aldrich), with (up to 1 wt.%) and without active agents was dissolved in a solution water/acetic acid at room temperature. The solution was magnetically stirred for at least 1 day to allow the polymer to be completely dissolved. Microcapsules have been prepared following two different procedures: a) "syringe method" b) "membrane method":

a) Used to prove the feasibility of making capsules. Capsules have been prepared by adding the polymer solution in a syringe and pressing it until the droplets were formed. The droplet enters in contact with a liquid mixture made of water/NaOH/crosslinker and the active compound, purified and semi-purified (phase 3).

b) The polymer solution (phase 1) was add to the feed tank and pressed through the mono-pore film of polyethylene (PE) which has a pore diameter of 300 mm. The droplets formed (phase 2) went in contact with the (phase 3) (Figoli *et al.*, 2007). Capsules were then left in this mixture for 24 and 48 h and then recovered using a filter paper. Capsules were left to evaporate overnight at room temperature and set in an oven under vacuum for 24h to completely remove the solvent.

Microrganisms and culture conditions

Several microbial strains were tested, in particular: Aspergillus niger, B. cereus, B. subilis, C. utilis, Enterococcus faecalis, E. coli, L. monocytogenes, Penicillium chrysogenum, Pseudomonas putida, Rhizopus oryzae, Rhodotorula rubra, Staphilococcus aureus, S. cerevisiae. They all belong to the internal collection of the Section of Industrial Microbiology of University of Milan (MIM). Yeast and filamentous fungi were grown on MEA (Malt Extract Agar) medium, of the following composition (g l-1): malt extract 20, soybean peptone 2, agar 15, glucose 20, pH 5.8, sterilisation at 118 °C for 20 min. Bacterial strains were grown on TSA (Tryptic Soy Agar, Difco) medium. Cultures were maintained as frozen stocks at - 20 °C in appropriate liquid medium in presence of 10 % glycerol (w/v), and propagated twice before use in experiments.

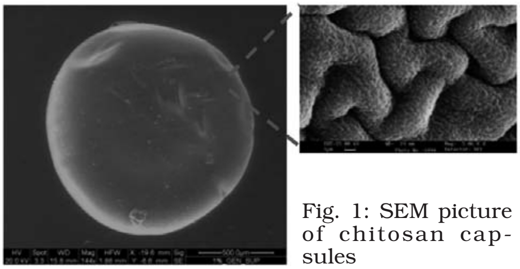

Fig. 1: SEM picture of chitosan capsules

Antimicrobial activity assay

Antimicrobial activity against indicator strains was tested either in solid and liquid cultures. As regards the first condition, 0.1 ml of a microbial suspension was added in 5 ml warm culture medium present in a Petri dish. After solidification, 20 mg of the natural antimicrobial was added, and then covered with other 5 ml culture medium. Samples were incubated at 30°C for 24 h. Antimicrobial activity was evidenced as halo of growth inhibition around the added antimicrobial. As regards assay carried out in liquid cultures, 100 ml Erlenmeyer flasks containing 10 ml TSB or MEB (media without agar) sterile medium, were inoculated with 0.1 ml of the microbial suspension, and 20 mg of the antimicrobial compound was suddenly added. A negative control sample was also set-up, without the antimicrobial. Flasks were incubated at 30 °C on an alternative shaker (200 rpm) and samples taken at appropriate intervals. Cultures were analysed for absorbance (OD 600 nm), while cell count (cells/ml) was performed employing a haemocytometer Bürker apparatus (Atanassova *et al.*, 2003).

RESULTS AND DISCUSSION

Chitosan microcapsules containing different concentration of active compounds have been prepared following the procedure above reported. A Scanning Electron Microscopy of the surface chitosan capsule, without active compound, is shown in Figure 1. The capsules present a spherical shape and a roughness surface. The capsules have an average diameter of 900 mm. Capsules containing active compound in the concentration up to 1% have been also prepared. Chitosan capsules without any active compound are usually white/transparent while the ones with the active compound resulted brown in colour as shown in figure 2. This was a clear evidence that the active compound was loaded in the microcapsules.

Antimicrobial activity against indicator strains was tested either in solid and liquid cultures. The results are summarised in table 1. In particular, the different types of active agents (purified and semi-purified) were compared as well as the effect of the cross-linker glutaraldehyde and the "additive" crosslinker .

The semipurified natural antimicrobial evidenced higher activity than the corresponding purified sample. Chitosan

Table 1. Antimicrobial activity of the different types of chitosan microcapsules prepared

	BIOPOLYMER			CHITOSAN, 2%	CHITOSAN, 2%	CHITOSAN, 2%	CHITOSAN, 2%
	ANTIMICROBIAL	SEMI-PURIFIED	PURIFIED			SEMI-PURIFIED, 1%	SEMI-PURIFIED, 1
	CROSS-LINKER			ADDITIVE	GLUTARALDEHYDE, 1%	ADDITIVE	ADDITIVE
	CROSS-LINKING TIME					24 h	48 h
M	*Saccaromyces cerevisiae*	++	-	-	-	-	+
I	*Candida utilis*	-	-	-	-	-	-
C	*Rhodotorula rubra*	++	-	-	-	-	+
R	*Enterococcus faecalis*	+	-	-	-	-	-
O	*Staphylococcus aureus*	++	+	-	-	-	+
R	*Bacillus subtilis*	++	+	-	-	-	+
G	*Bacillus cereus*	++	-	-	+/-	-	+
A	*Listeria monocytogenes*	++	+/-	-	-	-	+
N	*Pseudomonas putida*	-	-	-	-	-	-
I	*Escherichia coli*	-	-	-	-	-	-
S	*Rhizopus oryzae*	++	-	-	-	+/-	+
M	*Aspegillus niger*	++	-	-	+/-	+/-	+
S	*Penicillium chrysogenum*	++	+/-	-	-	-	+/-

crosslinked bio-microcapsules were more active after 48 h of reticulation than after 24 h. In particular, Bacillus, Listeria and the yeast strains were well inhibited by the contact active biocapsules.

CONCLUSIONS

The formation of mono-dispersed chitosan capsules, with and without active compounds, has been successfully carried out both using the “syringe” and the membrane technique.

Even if in some cases a certain degree of activity reduction was evidenced, bio-microcapsules proved their efficacy against bacteria and yeast. Furthermore, the antimicrobial activity tested in solid or liquid cultures proved an efficient procedure to evaluate bio- macrocapsule antimicrobial activity.

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