O6-4 **Coating of protein-based micro-beads for improved protection of sensitive ingredients**

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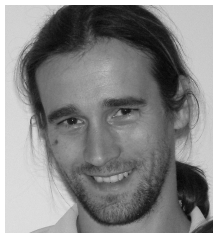

INTRODUCTION AND OBJECTIVES

There is considerable interest in the development of dietary supplements with physiologically active components that benefit the composition and bio-activity of health-promoting gut microflora. Probiotic bacteria, such as lactic acid bacteria and bifidobacteria are the most widely studied bacteria in the probiotic field and are permanent residence of the intestinal microbiota (Siro 2008). However, from a processing point of view, integration of probiotic bacteria into food systems represents a difficult challenge to a food manufacturer (Ross 2005). Thus, probiotics should be technologically suitable for integration into different food systems so that they retain viability and efficacy throughout storage and following consumption. The wide use of dairy proteins, in a variety of foods, opens interesting opportunities for milk proteins as cost-effective delivery systems for bioactive compounds such as probiotic bacteria. In a previous study (Doherty 2009) it was shown that entrapment of probiotic bacteria, such as Lactobacillus rhamnosus GG (LGG®), in dairy protein-based micro-beads provided excellent storage viability of cells in fruit juice. In addition, it was shown that entrapped probiotic bacteria survived in high numbers during simulated *in vitro*/*ex vivo* and *in vivo* porcine gastric transit.
Coating of microbeads with polysaccharides by electrostatic deposition was also shown to positively influence the stability of probiotic bacteria during gastric transit and could notably delay release in the small intestine (Doherty 2009). However, analytical methods for monitoring effective coating of micro-beads are limited and can be unreliable. In this paper we present a combination of several method used for the characterisation microbeads before and after single/double coating.

MATERIAL AND METHODS

Sample preparation and encapsulation: A milk protein formulation with and without polysaccharides, was rehydrated in distilled water for 16 hours at 4ºC under slight agitation (150rpm). The solution was treated and subsequently stored at 4ºC following neutral pH adjustment using 10 mM HCl (Brodkorb 2010). The bacterial concentrate and protein suspension were in some cases blended, yielding a probiotic population corresponding to the stationary phase concentration (10^9cfu/mL). Monodisperse protein micro-beads were prepared aseptically using an encapsulation device (Inotech Encapsulator®, Dottikon, Switzerland) with a 150µm nozzle size. The beads were agitated gently for a pre-determined time period, subsequently recovered and used immediately for (i) single or double coating or (ii) simulated gastric transit.

Bacterial strain and culture conditions: Some microbeads contained probiotic bacteria, in which case the cell suspension was mixed with the protein solution prior micro-bead production. The probiotic strain Lactobacillus rhamnosus GG (ATCC 53103, Valio Ltd., Finland), was procured from University College Cork, under a restricted materials transfer agreement. Harvested cells were stored as stock solutions in MRS broth (Oxoid Ltd., Hampshire, U.K.) containing 50% (v/v) aqueous glycerol at -20°C. The frozen culture was grown in MRS broth at 37°C under anaerobic conditions; achieved using activated Anaerocult A gas packs (Merck, Darmstadt, Germany). Stationary phase cells destined for encapsulation were propagated from 1% (v/v) inoculums for 19 hours at 37°C. Cells were harvested by centrifugation, washed and re-suspended to obtain a concentrated cell suspension, which was used for the micro-bead production as described above.

Micro-bead Coating: Six different polysaccharide coating materials were kindly donated by Cybercolloids Ltd. (Cork, Ireland) and assays were developed for testing the adsorption efficiency of each coating biopolymer to the protein micro-bead surface. Stock solutions of each biopolymer were autoclaved at 121ºC for 15 minutes. The optimum addition ratio of micro-beads to coating solution was established for each biopolymer solution to facilitate electrostatic deposition of the coating material onto the micro-bead surface (Brodkorb 2010). Coated micro-beads were subsequently recovered from the respective suspension and assayed during ex vivo gastro-intestinal (GI) incubation.

Zeta (ξ) Potential Measurements: Uncoated and coated micro-beads, formulated at various pH's, were homogenized and their zeta potential was in 10 mM KNO_3 using a Zetasizer (Malvern, Worchester, UK).
Microscopy: Characterisation of micro-beads and their coating was visually examined under a Leica TCS SP5 confocal scanning laser microscope (CSLM) (Leica Microsystems, Wetzler, Germany). Samples structures were stained using a method involving LIVE/DEAD BacLight® (Invitrogen Ireland) cell viability stain.

Infrared analysis: Attenuated Total Reflection- Fourier Transform Infrared (ATR-FTIR) measurements of freshly prepared uncoated and coated micro-beads were performed using a Bruker Tensor 27 spectrometer (Bruker Optik, Gmbh, Ettlingen, Germany) fitted with a thermally controlled BioATR Cell II, which was specifically designed for measuring proteins in aqueous solution. The design of the ATR crystal (ZnSe) allowed the evanescent wave to penetrate samples approximately 6µm in depth. Spectra were acquired at 25°C and averaged over 128 scans at a resolution of 4 cm^{-1} using Bruker Opus 5.5 software. After atmospheric compensation for absorbance of CO_2(g) and H_2O(g, l), the entire spectra were vector-normalised and compared.

RESULTS AND DISCUSSION

High resolution, confocal microscopy revealed a coarse surface morphology of the protein micro-beads.

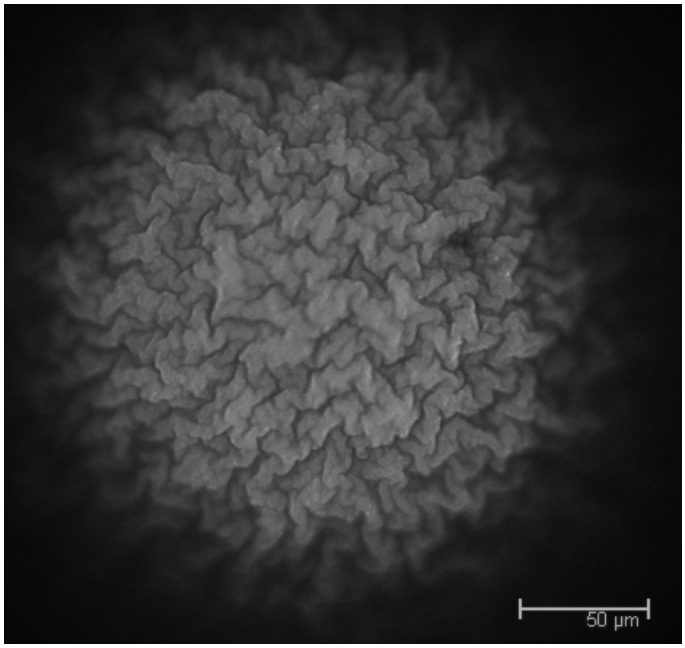

Figure 1: Confocal image of stained protein microbead.

Exposure of protein-based micro-beads to polysaccharides in solution induced a uniform coating as shown by confocal microscopy. Coating layers could be visualised by negative staining of the surrounding. Their thickness strongly depended on the nature of the polymers, presumably influenced by the charge difference between core and coating material. These electrostatic differences could be monitored by measuring the zeta potential of the homogenised micro-beads. The zeta-potential increased to positive values when microbeads were coated with positively charged polysaccharides. In addition, a clear decrease in the zeta-potential was observed if a double coating, i.e. protein (core) / polysaccharides (1^{st} coating) / protein (2^{nd} coating), was applied.

FTIR measurement of the neat micro-beads gave unsatisfactory results due to the low contact area of the spherical beads with the ATR crystal. However, physical treatment such as homogenisation and/or pressure against the crystal gave satisfactory spectra with clear differences between neat protein beads and microbeads with additional polysaccharides coating.

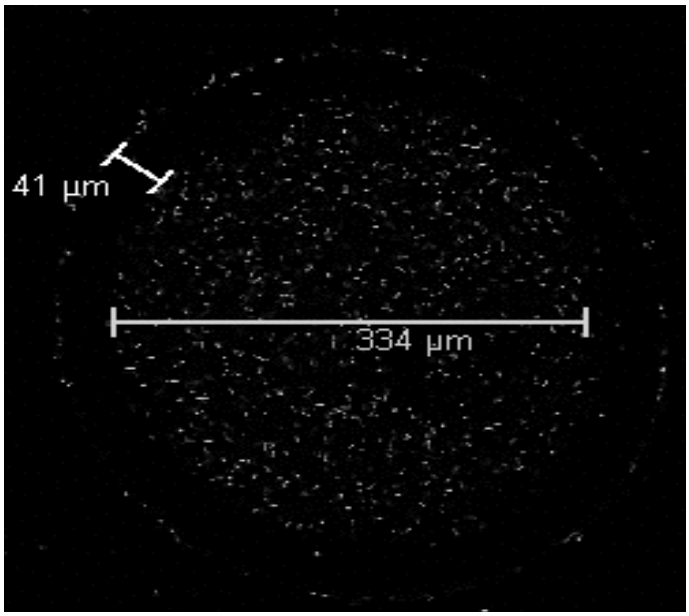

Figure 2: Confocal image of coated micro-bead, loaded with live probiotic bacteria (white roods). Negative staining of cells outside the microbeads revealed the un-stained polysaccharide coating layer.

CONCLUSIONS

Coating of microbeads can be a useful tool to control targeted release of microbeads. By combining analytical methods such as confocal microscopy, zeta-potential measurement and FTIR, sequential coating of microbeads can be monitored and characterised in detail.

REFERENCES

• Brodkorb, A. *et al. Method of producing micro-beads* (2010), Patent Application (PCT/EP2010/054846)

• Doherty *et al.* (2008) *Evaluation of protein matrices as structural safeguards for controlled delivery of probiotic bacteria.* in XVIIth International Conference on Bioencapsulation, Groningen, Netherlands: September 24-26, 2009

• Ross, R. P. et al. (2005) *Overcoming the technological hurdles in the development of probiotic foods.* Journal of Applied Microbiology 98(6) 1410-7.

• Siro, I., et al. (2008) *Functional Food. Product development, marketing and consumer acceptance – A review.* Appetite 51 (3) 456-467