COST ACTION FA1001

The application of Innovative Fundamental Food-Structure-Property Relationships to the Design of Foods for Health, Wellness and Pleasure

WG1+WG2+WG3 meeting

"THE NANO, MICRO, MACRO CONFLUENCE IN FOOD STRUCTURE FOR HEALTH, WELLNESS AND PLEASURE"

Bucharest, Romania
February 27-28, 2014

Book of Extended Abstracts

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EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

EDITURA ACADEMICA

Microencapsulation of probiotics envisaged for folate production in human intestine environment

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A balanced nutrition is a great concern in Human health and a preventive way to avoid diseases. Folate, a B-complex vitamin, is crucial in a significant group of cellular metabolic reactions. The daily intake of folate recommended for an adult varies between 200 and 400 μg; these values double in the case of pregnant women. The intake of folate is inefficient because its chemical forms can be extremely unstable in food products and after ingestion. The consumption of probiotics that produce this vitamin directly in the intestine could improve folate intake. However, the survival ratio of probiotics to gastric conditions is a problem than needs to be tackled. Several technologies can be applied aiming at probiotic encapsulation and each of them provides microcapsules with different characteristics in terms of particles' size and type of capsule. Emulsification allows the production of a wide particle size range from 0.2 to 5000 μm, whereas extrusion yields a smaller range size but does not provide particles under 300 μm *[1]*. Considering the envisaged application of probiotics into foods, this study was focused in particles smaller than 100 μm, produced by microemulsification techniques, to avoid modifying food texture.

This work describes the design and production of a model to support and protect probiotics through the gastrointestinal tract, until they reach the intestine. Considering the small size and chemical composition of the particles, the layer-by-layer technique was used to increase the resistance of the microcapsule to the gastro-intestinal conditions. In the first stage described here, the model was created and characterized without probiotics, to better understand how the different compounds interact each other.

The main structure is a microemulsion which was produced with a 1.5% sodium alginate solution, 0.05 M $CaCl_2$ solution, vegetable oil and Tween 80 *[2]*. After the production of the main structure (alginate microcapsule) the concentration of each layer (layer-by-layer) was optimised, considering the modulus of the highest value of zeta-potential as the indicator of a successful adhesion of a given layer to the previous one (**Fig. 1**). The first layer was built with the adhesion of a poly-L-lysine solution (a positively charged material) and considering different concentrations of this protein, where a concentration of 0.1% presented the best results. For the second layer an optimization of the concentration of a sodium alginate solution (a negatively charged material) was performed and the best concentration tested was 0.03%.

After the z-potential tests, confocal microscopy was used in order to confirm the consequent adhesion of the layers, and if they were in the correct order (**Fig. 2**). Concerning the resolution of the confocal microscope and the thickness of each layer, only two layers were labelled, the first and the third layer. The first layer, constituted by poly-L-lysine, was labelled with Fluorescein isothiocyanate (FITC) and the third layer, constituted by chitosan, was labelled with Rhodamine. **Fig. 2** clearly shows the existence of those

different layers, and also indirectly proving the existence of the second layer, because it is the negative charged compound, being responsible to connect to the first layer and third layer, both positively charged.

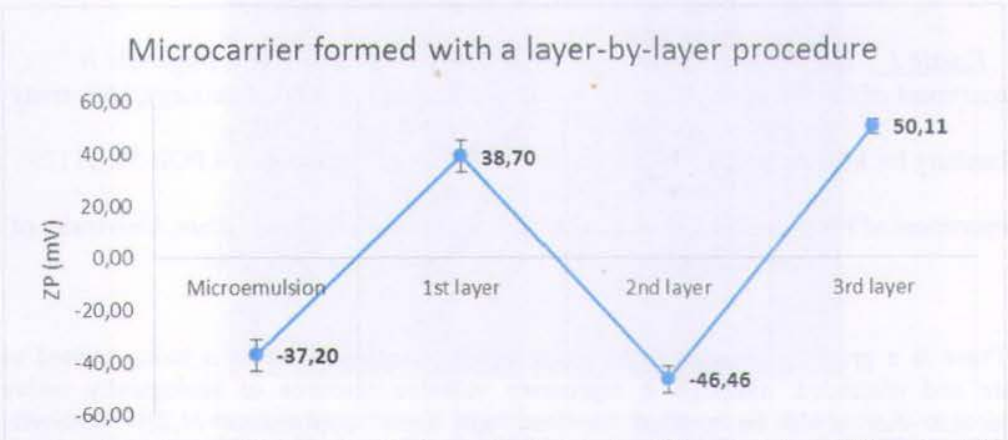

Fig. 1. Values of z-potential for each of the layers of poly-L-lysine (1st layer), alginate (2nd layer) and chitosan (3rd layer)

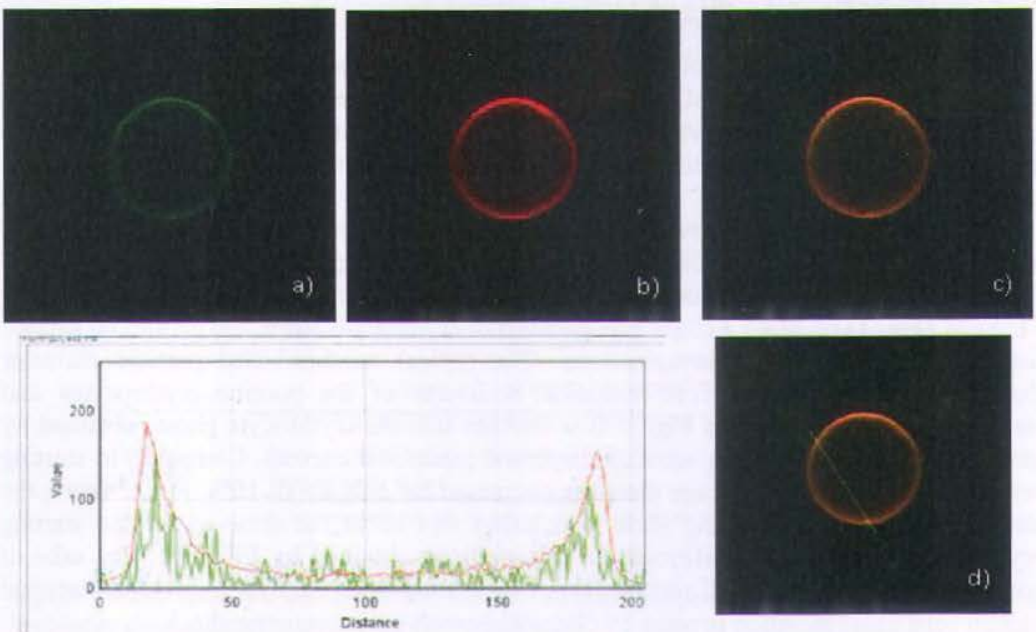

Fig. 2. Layer-by-layer confocal microscopy results, a) FITC in green representing the poly-L-lysine layer (1st layer), b) Rhodamine in red representing the chitosan layer (3rd layer), c) FITC/Poly-L-lysine and Rhodamine/Chitosan alone, d) cross section of the microcapsule where the fluorescence intensity is quantified (green – FITC; red – Rhodamine), allowing an estimate of the diameter of each layer (diameter of the particle – 8,302 μm).

References

[1]Burgain J, Gaiani C, Linder M. 2011. J Food Eng 104: 467-483.
[2]Sheu TY, Marshal, RT. 1993. J Food Sci 54: 557-561.