SciVerse ScienceDirect

Procedia Food Science 1 (2011) 1374 – 1380

Procedia Food Science

11th International Congress on Engineering and Foods (ICEF11)

Preliminary study on microbeads production by co-extrusion technology

Laura Piazza*, Tommaso Roversi

Department of Food Science and Microbiology, University of Milan, Via Mangiagalli 25, Milan 20133, Italy

Abstract

The present paper describes preliminary results on the development of a batch lab-scale co-extrusion device for the production of alginate beads as carrier for hydrophilic compounds. A dual jet of liquid core (aqueous solution of glucose or vitamin B_{12} or olive oil) and liquid shell material (aqueous solution of alginates differing for guluronic to mannuronic ratio) was pumped through concentric extrusion nozzles and droplets were formed by means of a rotating fluid jet cutter. The shell of the bead was then hardened in the hardening bath containing a calcium chloride solution. Optimization of the co-extrusion plant accounts for the management of the operative conditions within the hardening unit (incubation time, molarity of the gelling agent), and for the control of the surface-tension-driven breakup of laminar jets which was managed by varying the jet cutter - hardening unit distance. Beads enriched in vitamin B_{12}, that were formed according to the best plant set-up, were tested for the bioactive release when stressed under thermal conditions simulating typical dairy products processes. Cyanocobalamin was retained at all.

Selection and/or peer-review under responsibility of 11th International Congress on Engineering and Food (ICEF 11) Executive Committee.

Keywords: microbeads; co-extrusion process; alginate; material properties; vitamin B12.

1. Introduction

Microencapsulation technology is receiving increasing commercial attention for protection and delivery of bioactives in food applications. Many bioactives are prone to degradation, they are unstable and incorporation in foods can alter flavour, odour and texture. They may be directly added to food if they are in a comparable format with the food matrix and their direct addition does not impact negatively with the bioavailability of the bioactive. More often, bioactivity needs to be maintained in order to have a physiological function when delivered to its particular target site within the body.

* Corresponding author. Tel.: 0039-0250319222; fax: 0039-0250319191.
E-mail address: laura.piazza@unimi.it

Selection and/or peer-review under responsibility of 11th International Congress on Engineering and Food (ICEF 11) Executive Committee.

doi:10.1016/j.profoo.2011.09.204

In addition to the selection of appropriate shell formulation materials, the challenges in developing commercially viable microcapsules rely on the selection of the most appropriate process to provide the desired morphology, stability, and release mechanism, and on the economic feasibility of large-scale production [1].

The present paper describes preliminary data on the development of a co-extrusion device for the production of alginate beads as carrier for hydrophilic compounds. In particular, the optimization of water-in-water- hydrogel microcapsules focuses on the interactions between the liquid dispersed and the continuous gelled phases because complex fluodynamic regimes take place in multiphase systems when beads are formed which could compromise the release/retention performance. The application of alginate beads as carriers for an hydrophilic bioactive requires that conditions used during the extrusion process should be defined as to ensure high the bioactive retention under different environments during technological treatment of the real food that will include the beads in their formulation.

2. Materials and Methods

2.1. Chemicals

Algogel 6020 (Cargill Inc., France) and Sodium alginate RPH (Carlo Erba, Italy) were used as wall materials in beads production. Sodium azide was used to prevent microbiological growth. Commercial olive oil and an aqueous solution of glucose syrup (Cargill Inc., France) (40:60 v/v) were used as filler materials. Calcium chloride and cyanocobalamin were purchased from Sigma Aldrich.

2.2. Alginate characterization

^{1}H NMR spectra of alginate solutions were acquired on a Bruker Avance 500 spectrometer (Bruker, Karlsruhe, Germany). Calculations of M/G ratio was performed as described by Grasdalen et al [2].

The average molecular weight (M_w) of alginates and the polidispersity index (P.I.) were determined by size exclusion chromatography with multiangle light scattering (SEC-MALS) using two columns (TSK gel GMPWXL, Tosoh, 7.8mm ID x 30cm, Viscotek) in series as well as a triple detector array (Viscotek mod. 302 TDA).

Flow curves were obtained with a control stress rheometer (SR-5000, Rheometrics) using a double couette geometry over a shear stress range of 0.01–100 Pa at temperature of 20°C.

The surface tension of the alginate solutions and of fillers was determined at room temperature by means of the Wilhelmy balance technique using a contact angle tensiometer (DCA-100, FTA Europe Ltd, Cambridge, UK).

2.3. Co-extrusion device

Spherical alginate gel beads (0.6 mm diameter) entrapping alginate solution, water solution of glucose or vitamin B_{12} or filled with olive oil were produced by a co-extrusion process. A batch coaxial encapsulation unit was realized on lab scale. Fig. 1 shows the experimental set up. The alginate water dispersion (2) and the filler phase (1) are simultaneously pushed by peristaltic pumps (3a-3b) to the coaxial nozzle (4) which consists of two sub-nozzles allocated in a coaxial way (Nisco Engineering AG, Zurich). The core material is pumped into the inner nozzle, while the alginate dispersion is pumped through the annulus, allowing true "core-shell" morphology of the beads. As the liquid stream (about 19 ml/min) exits the nozzle, a rotating fluid jet cutter (400 rpm; 5a-5b) processes the fluid to defined droplets and ensures that the drops fall vertically downwards in the gelling bath (6) where the beads shell is hardened. Once liquid alginate dispersion is contacted with polycation (Ca^{++}), it immediately transforms into gel by a quick ionotropic reaction. All studies were conducted at 25°C.

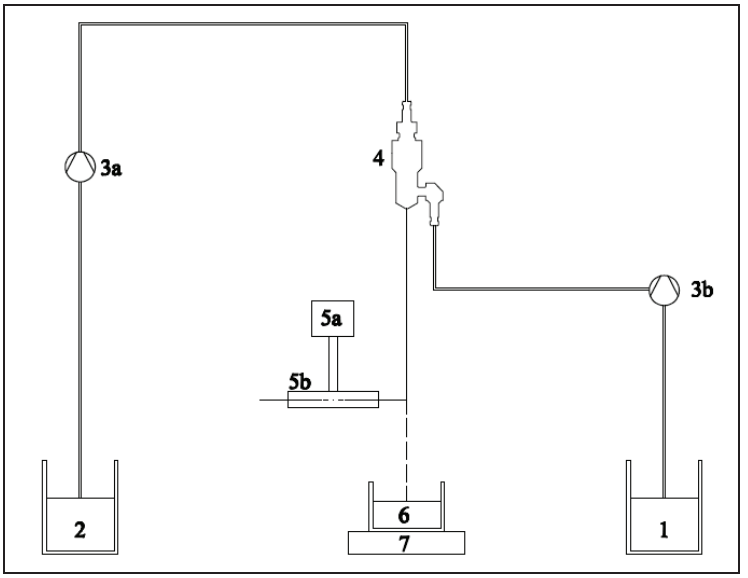

Fig. 1. Schematic representation of the co-extrusion device. 1) filler solution supply 2) wall material solution supply 3) peristaltic pump 4) nozzle 5) jet cutter (a:engine b: rotating disc) 6) gelation bath 7) magnetic stirrer

2.4. Beads size measurements

The size of the polymerized alginate beads was determined by analyzing, through the Image Pro Plus 6.2 software (Media Cybernetics, USA), digital images of beads that were acquired connecting a digital camera (Cyber-shot DSC-W70, Sony, China) to a microscope (D Laborlux Leitz, Germany). Results are given as average size of 10 measured beads. The spherical dimension is expressed by the apparent radius index (AR) as described by Chan et al. [3]. AR = average diameter / smaller diameter, normal to the major. Beads were considered spherical if the apparent radius was less than 1.1.

2.5. Mechanical properties of the beads

The mechanical properties of the beads were measured using a Texture Analyser (TA.XT Plus, Stable Micro Systems, UK) equipped with a parallel plate geometry. The test consisted in the compression (7.5 mm/min) of five capsules placed on the lower plate. An index of rigidity was taken in the linear region of the force/distance curve $\Delta F/\Delta d$ ($N \cdot mm^{-1}$), together with compression work at 50% deformation (J). In order to compare the mechanical data obtained from different batches, all the parameters above described were normalized by the weights of the beads. Results are therefore expressed as $N \cdot mm^{-1} \cdot g^{-1}$ and $J \cdot g^{-1}$ for the rigidity index and the compression work, respectively.

2.6. Released of the carried bioactive

Release from microbeads of cyanocobalamin was evaluated by quantifying the amount of vitamin that was residual in the beads after two heat treatments were performed on water dispersions containing the microspheres. Operative conditions were 40°C for 300 min and 80°C for 5 min respectively. The

concentration of the diffusing bioactive was determined by means of a Cary 100 BIO UV–Visible spectrophotometer at 361nm.

3. Results and Discussion

Once the basic assembly of the co-extrusion encapsulation device was outlined, investigations were carried out to reveal a clear operating region and the wall and core materials limits within which spherical Ca -alginate beads could be formed.

Alginate is a linear co-polymer composed of two monomeric units, D-mannuronic acid and L-guluronic acid. These monomers occur in the alginate molecule as regions made up exclusively of one unit or the other, referred to as M-blocks or G-blocks, or as regions in which the monomers approximate an alternating sequence. The calcium reactivity of alginates is a consequence of the particular molecular geometries of each of these regions. It has been established that alginates with a narrow molecular weight distribution (low polidispersity index PI) show a more pronounced Newtonian rheology than alginates with a high PI [4]. Flow behavior could therefore interfere with the shear stress over the nozzle surface and consequently over the choice of the range of the extrusion conditions. The commercial alginates that were tested for the beads production have similar polydispersity index (M_W: 328.312 and 391.719 kDa, PI: 2.966 and 2.313 for Algogel 6020 and Alginate RPH, respectively), but they differ for Guluronic/Mannuronic ratio, being this chemical index higher for Algogel 6020 (56/44 and 42/58 for Algogel 6020 and Alginate RPH, respectively). Results of preliminary investigations which are omitted in this paper, showed that the difference in chemical composition of alginates has noteworthy impact on jets flow properties, notwithstanding similarity in polidispersity, which dramatically influences homogeneity in the size and the mechanical properties of the hardened beads: solutions of alginates (2% w/v) differing for guluronic to mannuronic ratio exhibit different processability, mainly governed by difference in viscosity (9.59 -23.92 Pa·s for high guluronic and high mannuronic polysaccharides, respectively) and surface tension (58.6 – 49.0 ($N \cdot m^{-1}$) $\cdot 10^{-3}$ for high guluronic and high mannuronic polysaccharides, respectively). Within the limits of the liquid properties, the Algogel 6020 was finally selected as wall material to be used in the production of bioactive carriers of desired shape and consistency.

Alginate concentration, which strongly affects the fluid viscosity, was adjusted in such a way that bead integrity was not disrupted by contact with the stirred $CaCl_2$ solution. A good compromise was achieved with alginate concentrations between 1 and 2%. Gelation time (here assumed as equal to the incubation time in the $CaCl_2$ 1M solution) for alginate in alginate beads was 20 min and the nozzle - jet cutter distance, that allows reproducible droplets to form, was kept equal to 15 cm. Morphological and mechanical tests showed that a 2% (w/v) concentration of alginate solution allowed the smallest and the most deformable beads to form (figure 2).

In the recent years, it has become a common trend to produce perfectly spherical Ca-alginate particles. The reasons could be to develop highly reproducible controlled release rates, which may be critical in some biomedical, pharmaceutical applications and physiological adsorption. Most of the co-extrusion plants for the production of alginate beads are currently optimized for beads filled with a hydrophobic dispersed phase. When using miscible wall and core materials, the control of fluodynamics is more complicated and production of two-phases beads is compromised. In this work, experiments were undertaken to produce beads with constant bead size and mechanical resistance to be used as carriers of olive oil, of an hydrophilic phase (glucose : water 60:40 v/v) and finally of the glucose : water 60:40 v/v phase including a water soluble bioactive compound: the cyanocobalamin.

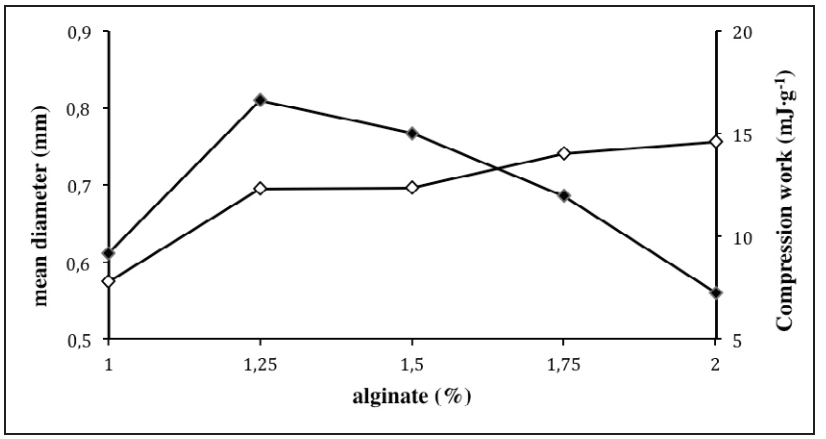

Fig. 2. Mean bead diameter (filled squares) and compression work at 50% strain (empty squares) as a function of alginate concentration for alginate/alginate coextruded beads

In view of the optimization of the co-extrusion plant, the incubation time in the gelling bath, the molarity of the gelling agent and the "nozzle-jet cutter" distance were judged to be critical.

The influence of gelation time of 2% alginate solutions was investigated up to 60 min reaction time in a $CaCl_2$ 0.1M gelling bath, with a path equal to 5 cm for the formed drops before reaching the hardening vessel. The co-extruded phases consisted in olive oil and the glucose solution, respectively.

While the mean diameter of beads was not affected by the compatibility/incompatibility of the outer and inner phases, mechanical properties of the beds were. When formed in the presence of an immiscible filler phase, beads show higher mechanical resistance (63.80, 94.14, 134.54 and 86.29, 93.31, 98.33 $N \cdot mm^{-1} \cdot g^{-1}$ at 20 min, 30 min, 60 min for alginate/ oil beads and alginate /glucose solution, respectively). Micro-fracture events of the gelled wall were clearly visible in compression traces for the both products and were more consistent for alginate/glucose solution beads due to differences in the water/oil, water/water interface behavior.

With an incubation time equal to 20 minutes in the $CaCl_2$ solution, the previous coextruded beads were formed under different molarity of the gelling agent: 0.1-0.2-0.5-1-2M. The higher the counter ion up to 1M, the higher the mechanical properties of the beads, with alginate/oils beads showing higher performances over all the gelling conditions (e.g. rigidity index equal to 281.52 and 105.02 $N \cdot mm^{-1} \cdot g^{-1}$; $W_{50\%}$ = 11.18 and 5.19 $(J \cdot g^{-1}) \cdot 10^{-3}$, for alginate/oil beads and alginate/glucose solution, respectively in 1M $CaCl_2$ solution). Beyond this molarity, the decrease in mechanical properties was assigned to an excess of calcium, which might have resulted in structural heterogeneity, leading to lower average strength [5].

Besides materials variables, a further critical point should be addressed, that is the surface-tension-driven breakup of laminar jets.

The stability analysis of liquid jets includes the effects of viscosity and aerodynamic interaction with the surrounding gas in order to determine the breakup process and the prediction of drop sizes. The liquid jet out of the co-extrusion nozzle is naturally unstable and breaks up into droplets. For a straight, free falling jet under gravity, the size of the primary drops and jet break-up length are a function of the jet velocity [6] where the breakup length is the height of the continuous column of liquid at the nozzle exit. Jet velocities, aerodynamic forces and internal flows within the jet may have influence, with a different extent, on the jet break up into drops. The breakup of the jet can additionally be dependent on the nozzle geometry. The main target of getting an uniform distribution of beads sizes, implies the jet cutter working with continuous fluid streams, i.e. below the break up length. In addition, after shaping is operated by the jet cutter, the drops shape deformation should finally be caused by impaction, where the viscous and the

surface tension forces of the liquid drop could not overcome the impact force exerted when it hit the gelling bath.

Since the co-extrusion unit under study was designed to keep constant flow rates of downstreams of both wall and core materials and since the nozzle geometry was kept constant too, the liquid jet stability was controlled by controlling the collection distance (nozzle-gelation bath distance). Velocity profiles along the jet axis are supposed to be different in jets of the co-extruded alginate/olive oil and alginate/glucose due to the differences in viscosity and in surface tension at the interface of the composite jets. Consequently, the break up length is expected to differ for the systems under study. Beads were therefore produced by varying the nozzle-jet cutter distance. Results of the experimental assessment of mechanical properties of beads (results are not shown for seek of brevity) showed that a critical length exists, and in the case it is exceeded beads loose in mechanical performances: their rigidity decreases and the homogeneity of the structure is lost, as it can be concluded on the basis of the increase in the number of the fracture events taking place during the mechanical compression tests. The break-up length resulted to be higher for the oil in water jet. In conclusion, the nozzle-jet cutter distance was therefore set at 10 cm for the water in water jet, and at 15 cm for the oil in water one.

After successful conditions for co-extruded beads production were defined, a preliminary application consisted in the production of microspheres filled with a bioactive compound: the cyanocobalamin. An aqueous solution of glucose syrup (40:60 v/v) and cyanocobalamin (0.03 mM) was used as filler phase in alginate beads. Aim of investigation was the evaluation of the role of the network density of the alginate gel on vitamin B12 retention/release when beads were let to rest in water under mild and severe thermal treatments. Two concentration of the gelling agent in the hardening unit of the plant were experimented: 0.1 and 1M solution of $CaCl_2$, respectively. As an example, figure 3 shows values of the amount of vitamin that was residual after heat treatments in the beads that were hardened in a 0.1 M solution of $CaCl_2$. The bioactive concentration was determined by means of UV–Visible spectrophotometric measurement (A_{361nm}). The microspheres were dispersed in water and heated at 40°C up to 300 minutes or 80°C up to 5 min minutes, respectively, in order to simulate the operative conditions currently used in yoghurt fermentation and in dairy creams HTST pasteurization. Analytical data showed a complete bioactive retention under both thermal treatments, due to the proved thermal resistance of the alginate gel wall of the microspheres, which also express efficient barrier properties to cyanocobalamin diffusion and release. Similar results were obtained when using the highest counter ion concentration in the gelling bath.

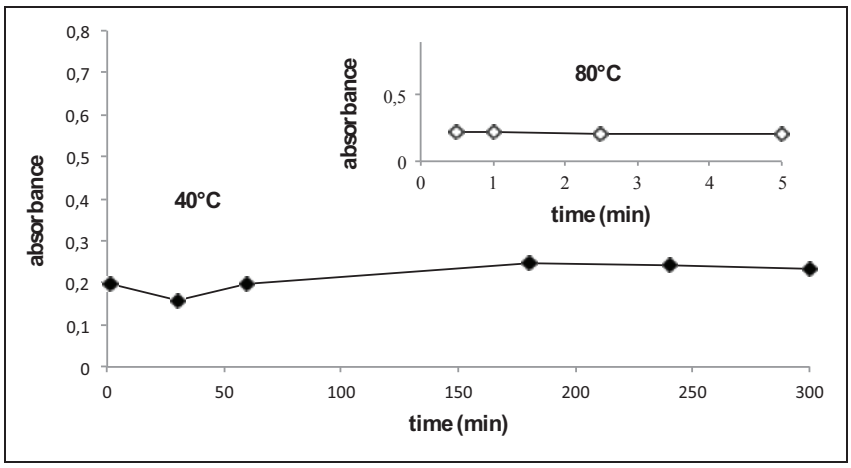

Fig. 3. Amount of vitamin B_{12} that was residual in the alginate beads ($CaCl_2$ 0.1M as gelling agent) after heat treatments (40°C and 80°C) performed on water dispersions containing the microspheres

4. Conclusion

Although co-extrusion has been used for many years, formation of beads with desired size and spherical-shaped still requires some trial and error works on the liquid formulation and experimental set-up (e.g. solution viscosity or surface tension, tip size, collecting distance etc.). If the conditions are not optimum, deformed beads or beads with unsuitable structure properties could be produced.

An empirical approach was used in this work to determine the relationship between the process variables (i.e. material properties and experimental set-up) on the shape, the size of alginate co-extruded microspheres and bioactive release. Until now, there are limited efforts to develop models that comprehensively correlate the material properties and experimental conditions on the shape and size of the beads formed and release of the carried diffusants. Results could be used for prediction models aimed to process optimization and to evaluate process limitations.

References

[1] Gouin, S., 2004. Microencapsulation: industrial appraisal of existing technologies and trends. Trends in Food Science & Technology, Volume 15, Issue 7-8, Pages 330-347

[2] Grasdalen, H., 1983. Hig-field ^{1}H-N.M.R. spectroscopy of alginate: sequential structure and linkage conformations. Carboidrate Research, 118: 255-260.

[3] Chan, E. S., Lee, B. B., 2007. Dimensionless number analysis for production of spherical Ca-alginate beads. XVIth International Conference on Bioencapsulation, Ireland, (2008), P54.

[4] Serp, D., Cantana, E., Heinzen, C., Von Stockar, U. and Marison, I. W., 2000. Characterization of an encapsulation device for the production of monodisperse alginate beads for cell immobilization. Biotechnology and Bioengineering, 70: 41–53.

[5] Kuo C.K. and Ma X.P., 2001. Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: Part 1. Structure, gelation rate and mechanical properties. Biomaterial Volume 22, Issue 6, Pages 511-521

[6] Blaisot, J.B., Adeline, S., 2003. Instabilities on a free falling jet under an internal flow breakup mode regime. Int. J. Multiphase Flow 29, 629–653.

Presented at ICEF11 (May 22-26, 2011 – Athens, Greece) as paper FPE1259.