Cold set whey protein microgels as immobilization matrices for food bioactives

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INTRODUCTION AND OBJECTIVE

There is a drive to produce innovative foods containing bioactive ingredients (De Vos et al., 2010). Food bioactives from a wide variety of sources have demonstrated many health benefits including antioxidant and antimicrobial activity (McClements et al., 2009). Despite promising biological activity, many food bioactives cannot be simply added to foods and require encapsulation/immobilization within protective matrices to enable their successful incorporation into novel food systems (Augustin & Hemar, 2009).
This study aimed to assess the potential of cold-set whey protein microgels for their suitability to remain stable within typical food system environments, enable the immobilization of bioactive ingredients and contribute to the protection and sustained release of those bioactives.

MATERIALS AND METHODS

Preparation of microgels
Whey protein microgels were prepared by two cold-set calcium induced gelation mechanisms - an external and an internal gelation technique. Pre heat-treated whey protein isolate (WPI) emulsions (with up to 20 % w/w soyoil) were either added; dropwise to calcium solution (external gelation) or into a double emulsion (internal gelation) containing a calcium source to form sub-millimetre microgels at ambient temperature.
Microgel stability
Microgels were characterized by scanning electron microscopy, and their integrity was assessed over a range of food relevant pH values (2.5, 4.5 and 7), temperatures (4, 25, 37 °C) and in the presence of chelating agents (EDTA, citric acid) by determining soluble protein leached from microgels incubated within those solutions over time (up to 14 days).
Bioactive immobilization
Immobilization of bioactives within these matrices was examined by either an entrapment process (bioactive added to the microgel-forming solution) or by sorption onto the pre-formed WPI matrices. A prediction model was generated based on the pH ionization profile of the WPI and suitably charged bioactive ingredients (cationic and anionic amino acids and peptides) to interpret the binding of these bioactives by the sorption process to the microgels.
Bioactive release
Microgels (0, 10 or 20 % w/w lipid filled) were incubated in simulated gastric and intestinal fluids (at 37 °C, U.S. Pharmacopeia, 2004) in the presence or the absence of enzymes. Both the digestion of the protein matrix and the release of the vitamin riboflavin were monitored with time.

RESULTS AND DISCUSSION

Microgels were successfully prepared by the external (1 mm diameter microgels) and the internal (100 µm diameter microgels) gelation methods. The smaller size (100 µm or less, Fig. 1) of internal gelation microgels combined with their soft gelatinous nature could be particularly favorable for incorporation into functional foods.

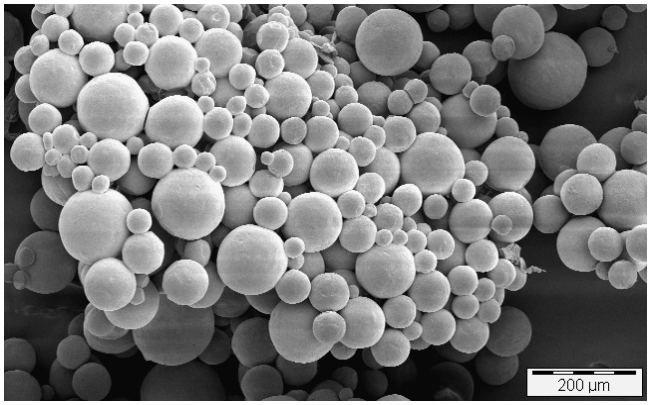

Figure 1: Internal gelation microgels. Mag x 100

Microgels were sufficiently water-insoluble, maintaining their integrity (> 90 % retention of the protein-based matrix) despite changes in pH, temperatures (Fig. 2) and the presence of chelating agents.

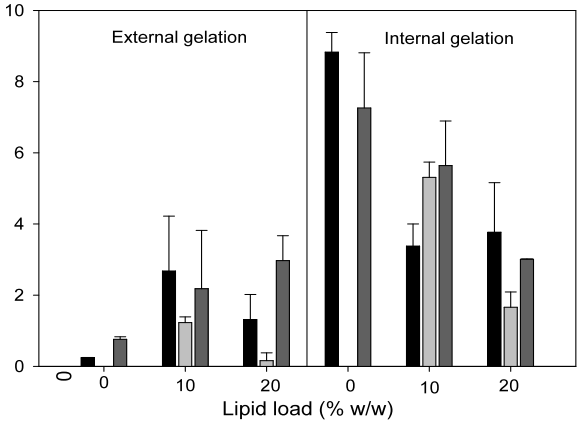

Figure 2 Soluble protein (% of total) in microgels after incubation for 14 days at 37°C. At pH 2.5 (■), 4.5 (■) or 7 (■).

At a chilled environment (4 °C), the integrity of the microgels was most ensured, indicating good potential for incorporation into chilled food products. Microgels were successful at entrapping food bioactives (> 82 % immobilization of the vitamin riboflavin). The incorporation of lipids (10 or 20 % w/w) into these matrices enabled a significant ($p \leq 0.05$) delay in matrix digestion, and release of bioactive under simulated gastro-intestinal conditions (Fig. 3).

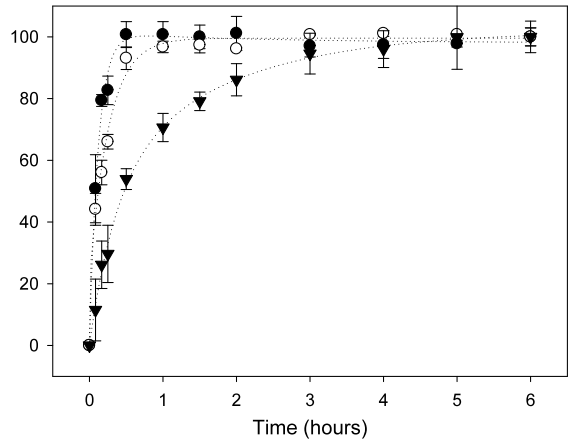

Figure 3: Effect of lipid load (●) 0 %, (○) 10 % or (▼) 20 % w/w, within externally gelled microgels on the release of riboflavin with time in simulated gastric fluid.

The incorporation of lipids into the microgels negated the burst release of riboflavin (microgels with no lipid), and instead, a composite matrix (20 % w/w lipid) capable of a sustained release of bioactive ingredient was possible.

In an opposite approach to entrapment, the immobilization of bioactives by a sorption process onto pre-formed WPI matrices was investigated.

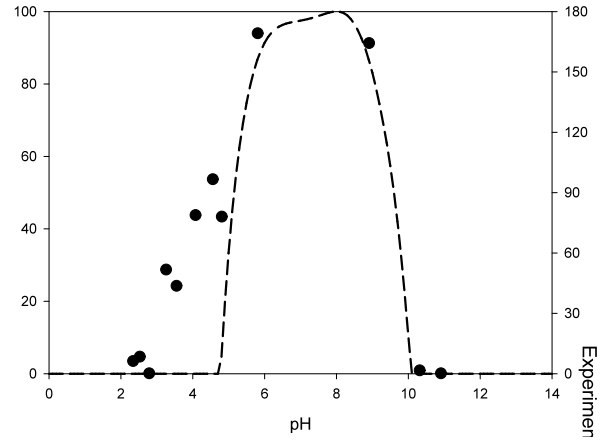

Figure 4: The binding of KHIQK peptide by the WPI microbeads over a pH range. Experimental data (●) Predicted data (-).

The immobilization of charged bioactives (amino acids, peptides, vitamins) by a sorption process onto pre-formed matrices was shown to be pH dependent.

Additionally salt affected this pH binding, indicating an electrostatic interaction between suitably charged bioactives and the microgels. A prediction model based on the ionization profile of the WPI and individual bioactives enabled interpretation of this binding, with experimental results correlating to that of the prediction model very well (Fig. 4), in particular the pH at which maximum binding occurs.

CONCLUSIONS

Cold set whey protein microgels can be manipulated to achieve high immobilization of a range of food bioactives and to affect the release of those bioactives. Internal gelation microgels have feasible potential for incorporation into foods due to their relatively discrete size (100 μm or less), and their stability is most ensured at 4 °C and pH 4.5 – indicating a chilled fermented food could be an ideal environment. This study also highlights the ability of the microgels as sorbents for the immobilization of a variety of ionisable bioactives. The prediction model could benefit from greater input to allow the interpretation of the binding of more complex bioactives, but shows good potential for a novel reverse engineering approach to maximising immobilization of bioactives via a charge interaction.

REFERENCES

- Augustin, M. A. & Hemar, Y. (2009) *Nano- and micro-structured assemblies for encapsulation of food ingredients.* Chemical Society Reviews, 38 (4) 902-912.
- De Vos, P. et al. (2010) *Encapsulation for preservation of functionality and targeted delivery of bioactive food components.* International Dairy Journal, 20 (4) 292-302.
- McClements, D. J. et al. (2009) *Structural Design Principles for Delivery of Bioactive Components in Nutraceuticals and Functional Foods.* Critical Reviews in Food Science and Nutrition, 49 (6) 577 - 606.
- U.S. Pharmacopeia (2004) *Simulated gastric and intestinal fluids,* T.S. In: Board of Trustees, Editor, The U.S. Pharmacopeia 27, The National Formulary Vol. 22, Rockville, M.D., p. 272.

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