Encapsulation and release kinetics of bioactives from whey protein microgels

O'Neill, G. Jacquier, J.C. O'Sullivan, M. O'Riordan, E. D.*
Food for Health Ireland, Univ College Dublin, Ireland (graham.oneill@ucd.ie)

INTRODUCTION AND OBJECTIVES

Functional foods have great potential to maintain and improve health. Many of the compounds used in these foods are sensitive to the gastrointestinal environment and require protection to ensure their bioavailability (de Vos et al., 2009). Traditional pharmaceutical methods are generally not suitable for use in foods, hence the necessity for the development of GRAS delivery systems. Food protein microgels have been shown as effective encapsulation agents for a range of bioactives (Chen & Subirade, 2006). However the site and rate of release of a bioactive within the GIT is important in order to maximise its physiological efficacy. The aim of this work was to develop a food grade encapsulation system based on whey protein microgels to provide sustained delivery of low Mw, hydrophobic compounds (a dipeptide and riboflavin). Drying the microbeads at ambient temperatures was explored as a means of further controlling the release of the compounds from these matrices.

MATERIALS AND METHODS

Materials

Whey Protein Isolate (WPI) was obtained from Davisco Food Ingredients Int. (Le Sueur, Minn., U.S.A.). Calcium chloride dihydrate was purchased from E.Merck (Damstadt, F.R. Germany). Riboflavin was purchased from Sigma Aldrich (St. Louis, MO, USA). The dipeptide (phe-trp) was purchased from Bachem (Switzerland).

Manufacture of microgels

A 10%w/w WPI solution was heat denatured at 80°C for 30mins and cooled to room temperature. The solution was systematically dropped into a 100mM calcium solution to form microgel beads, which were left for 24 hours to crosslink before washing in deionised water.

Dried Microgels

Washed microgel beads were placed on a petri dish in a convection oven at 30˚C for 24hours prior to performing the release experiments.

Microstructure of beads

Microgels were dipped in liquid nitrogen and subsequently freeze dried before SEM analysis using a FEI Philips XL30 SFEG scanning electron microscope.

Encapsulation

Preformed beads (200, 300, 400, and 600) were placed in stirred dipeptide solutions (0.2g/L) and 100µl samples were withdrawn periodically. Analysis was conducted on an Agilent 1200 HPLC system. (Agilent Technologies, Santa Clara, CA) using an Agilent Eclipse XDB-C_{18} (150 mm × 4.6 mm i.d.; 5 µm particle size) column with a C_{18} guard column (Phenomenex, Macclesfield, UK). % Encapsulation was calculated using the following equation,

$$\text{\% Encapsulation} = \frac{\text{Quantity of compound in the bead (mg)}}{\text{Quantity of compound in original solution (mg)}} \times 100$$

Release

400 beads were placed in a solution (10ml) of either dipeptide (0.5g/L) or riboflavin (0.05g/L) for 5 hours to load the microgel beads. Following this the beads were placed in deionised water to measure release. Samples (100µl) were taken periodically and analysed by HPLC.

RESULTS AND DISCUSSION

Microgel morphology

A scanning electron microscope (SEM) image of the microgels is shown in Fig 1. A uniform protein structure can be seen throughout the bead. The microgels were 1mm in diameter and white in colour.

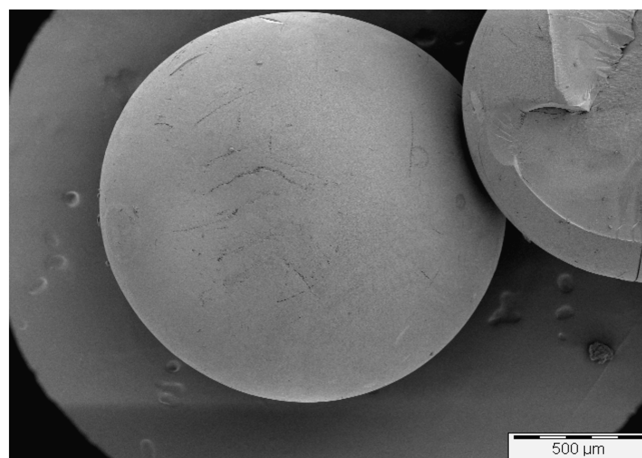

Fig. 1. SEM of cross section of a microgel (x50 mag).

Encapsulation

The level and rate of encapsulation of the dipeptide by the microgels at a range of bead:solution ratios are shown in Fig 2. As the bead:solution ratio increased, the % encapsulation increased with a maximum of

59% encapsulation achieved. In all experiments the rate of encapsulation was first order.

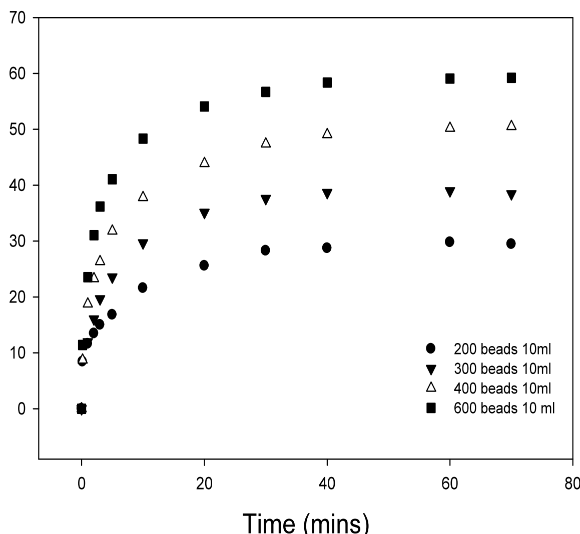

Fig. 2. Kinetics of dipeptide encapsulation at a range of bead: solution ratios.

Release

The release of dipeptide from wet or dry beads is shown in Fig. 3. The wet beads provide a rapid release that plateaus quickly compared to that of the dried bead which gives a sustained release. The release of dipeptide at 60 minutes was faster from wet beads (48%) than from the dry beads (16%) representing a 'burst' and 'controlled' release system respectively.

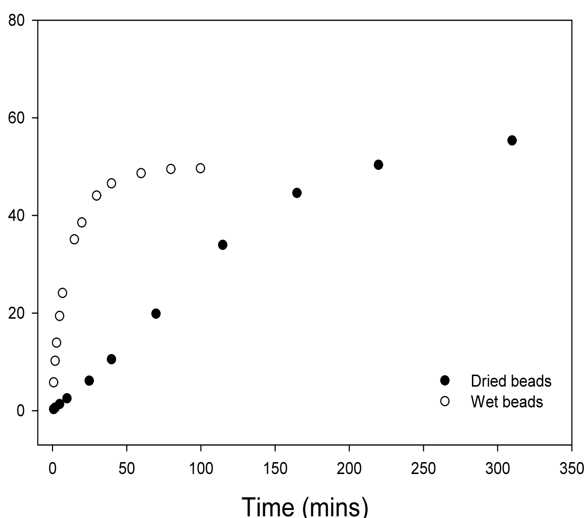

Fig. 3. Release kinetics of a dipeptide from 400 microgel beads, wet (○) and dry (●).

The release profile of riboflavin is very similar to that of the dipeptide showing a sustained release profile from the dried beads releasing 18% in the first hour. The release is continuous over a 5 hour period. This is desirable as it would prevent rapid unloading at a localised site in the intestine, instead providing a continuous supply of the vitamin. For more potent actives this is essential to stop dangerous peaks in concentration of the active that could result in adverse health effects.

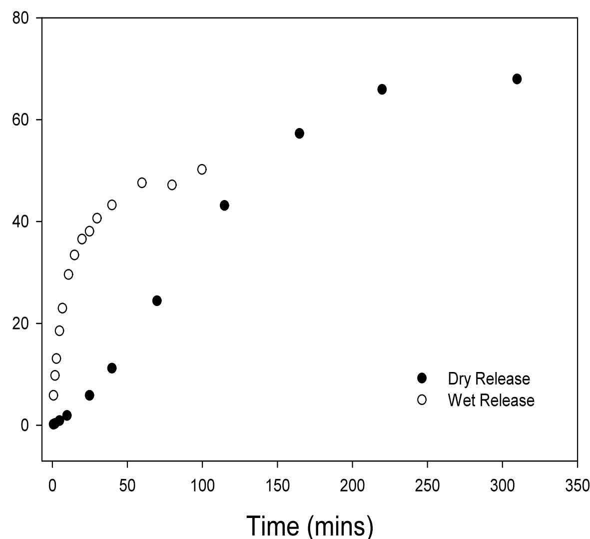

Fig. 4. Release kinetics of riboflavin from microgels, wet (○) and dry (●).

CONCLUSION

Cold set whey protein microgels were used to successfully encapsulate low Mw, hydrophobic molecules. Optimisation of the bead:solution ratio resulted in an increase in the % encapsulation of the dipeptide. Drying of the beads resulted in many benefits, including the controlled release of low Mw, hydrophobic bioactives. The reduction in size of the beads by drying (<200µm) and the low water activity would facilitate incorporation into foods and long term storage. In conclusion dried whey protein microgels show good potential as a food grade encapsulation system.

REFERENCES

- de Vos, P., Faas, M. M., Spasojevic, M. & Sikkema, J. (2009) *Encapsulation for preservation of functionality and targeted delivery of bioactive food components.* International Dairy Journal, 20, 292-302.
- Chen, L. & Subirade, M. (2006) *Alginate-whey protein granular microspheres as oral delivery vehicles for bioactive compounds.* Biomaterials, 27,(2006) 4646-4654.

ACKNOWLEDGMENTS

This research was part funded bygrant aid under the Food Institutional Research Measure, which is administered by the department of Agriculture and in Ireland, and Food for Health Ireland, Food Science Building, University College Cork (Enterprise Ireland Grant No. CC20080001).