Protein based materials as delivery systems of active compounds.

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INTRODUCTION

Proteins, essential components of the daily diet, are widely used in formulated foods, partly because of their nutritional value, but especially for their functional properties, which include gelling, foaming and emulsification and underlie many food sensory attributes. Among these functional properties, the network forming ability of protein and the ability to adsorb spontaneously to interfaces to stabilize polyphasic systems offer the possibility of developing GRAS biocompatible carriers for oral administration of sensitive nutraceuticals in a wide variety of foods. Moreover, under digestive conditions, food proteins break down to peptides, some of which may be bioactive and exert physiological effects in vivo.
This presentation focuses on relationships between proteins properties and their applications in protein-mediated transport and controlled release of active molecules. Since the diversity of the systems and production processes under investigation is already vast, this presentation focuses on recent progress in the design and evaluation of food-protein-based delivery systems based on gel properties of proteins.

MATERIAL AND METHODS

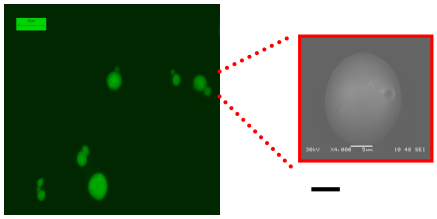
Sodium Alginate was purchased from Fluka AG, Buchs, Switzerland. Whey protein isolate (WPI) soy protein isolate (SPI) and Zein were obtained from Davisco Foods International Inc. (Le Sueur, MN, USA), Protient Inc. (St. Paul, MN, USA) and Sigma Chemical Co (St. Louis, MO, USA).

Microspheres preparation were elaborated according to Chen *et al*. (2006, 2009)

RESULTS AND DISCUSSION

Formation and characterization of gelled globular protein particles with controlled sizes Gelation of globular proteins is traditionally achieved through heat treatment limiting their use as carriers to thermally stable compounds. However, cold gelation provides an alternative gel development method for globular proteins such as whey and soy proteins. This method requires a heating step during which food proteins are denatured and polymerized into soluble aggregates, followed by a cooling step and subsequent addition of salt, which results in the formation of a gel network. The ability to form cold set gels opens interesting opportunities for food proteins as carriers of heat-sensitive bioactive compounds.

As an example, emulsification/internal cold gelation is a safe method that may be feasible to prepare food protein microparticles on large scale for food applications (Chen *et al*., 2006, 2009). Previously developed with alginate (Poncelet, 2001), the method is based on the release of calcium ions from an acid-soluble calcium salt in emulsified pre-denatured protein solution. This is achieved by an acidification with an oil-soluble acid, which partitions to the dispersed aqueous phase, releasing soluble calcium and initiating gelation. Using this technique, globular protein microspheres such as whey or soya have been successfully developed at ambient temperature (Figure 1). By modulating emulsification conditions, the particle size can be controlled from 30 to 500 µm in diameter. This method may open interesting opportunities for the use of food proteins as carriers of sensitive nutraceutical compounds in functional foods.



(a) (b)

Figure 1 - Fluorescence photomicrographs of soy protein micro-spheres (a) and scanning electron microscopic image of micro-spheres (b).

Combination of biopolymers to control release properties Another interesting property of proteins is their ability to be mixed with other biopolymers such as polysaccharide or protein allowing obtaining particles with desired release properties. The two following examples are based on mixed particles elaborated by the emulsification/internal gelation. Riboflavin (vitamin B_2), a water soluble micronutrient that plays a key role in energy metabolism, was used as a model nutrient.

In the first example, whey protein (WPI) and alginate (AL) based microspheres were elaborated according to Chen et al. (2006). A special matrix / granular structure was obtained for WPI/AL(ratio 8:2) with WPI granules 3-10 µm in diameter homogeneously distributed within an AL spherical matrix with 100 µm diameter. Release experiments, presented in Figure 2, demonstrated useful properties for these microspheres, featuring delayed riboflavin release under gastric conditions, matrix swelling in intend erosion leading to granule liberation under intestinal conditions followed by complete release of the

nutrient by pancreatic degradation of the granules. These profiles are different from the ones obtained from whey proteins particles which release riboflavin immediately in gastric step or from the ones obtained from alginate particles which release riboflavin immediately in intestinal step. These results show that mixed microspheres appear to be potentially useful as oral delivery vehicles for bioactive compounds in the food and nutraceuticals industry.

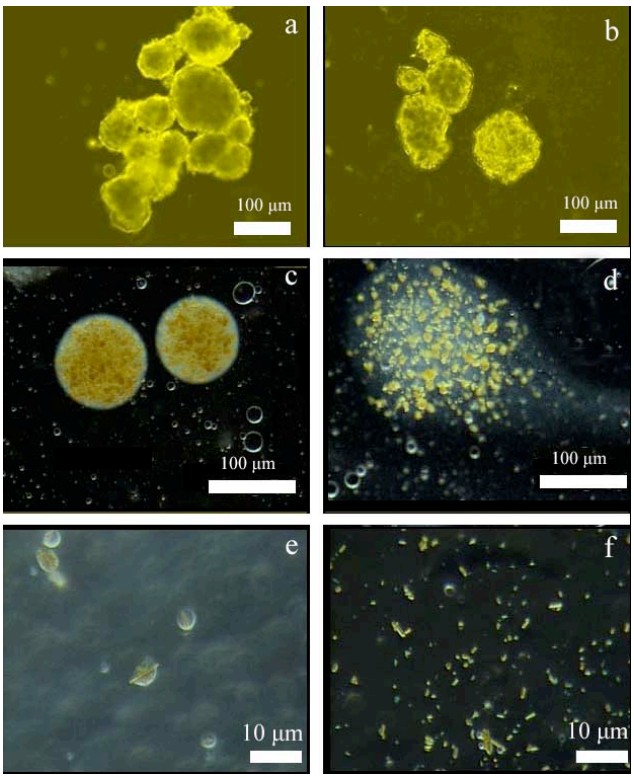


Figure 2- Micrographs of AL-WPI microspheres from degradation assays in simulated gastric and intestinal fluids. (a) pH 1.2; (b) pH 1.2 + pepsin; (c, d, e) pH 7.4; (f) pH 7.4 + pancreatin

In a recent work, we prepared soy protein isolate (SPI), zein and SPI-zein complex microspheres (15-25 µm) using a cold gelation method (Chen et al. 2009). Their payload release properties were studied with a dissolution apparatus in simulated gastric and intestinal buffers without digestive enzymes. The results showed that soy protein microparticles shed their riboflavin load, use within 15 minutes, due to the inherent hydrophilic nature of soy protein. We therefore turned to blending food proteins in order to improve the nutrient release profile of the particles and zein was chosen for this purpose, since it is hydrophobic and obtained from a very abundant source. Blending SPI and zein thus provides a convenient method of controlling the diffusion coefficient, swelling behavior and nutrient release profile of food-protein-based microspheres in simulated gastro-intestinal buffer. It is interesting to find that nutrient release rates can be controlled by adjusting the SPI/zein ratio as shown in Figure 3. The riboflavin release rate decreased progressively with increasing zein content, which may be attributed to the resulting increased hydrophobicity and crystallinity and hence decreased rate of hydration of the microspheres. Furthermore, release profiles of microspheres obtained with SPI/zein ratios of 5:5 and 3:7 displayed near-zero-order release kinetics in the simulated gastro-intestinal environment, which is a desirable characteristic for nutraceutical delivery to create novel functional foods that can have physiological benefits and/or reduce the risk of chronic disease.

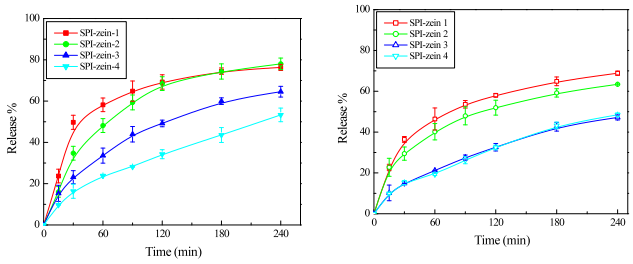


Figure 3- Release of riboflavin from SPI/zein microspheres in simulated gastric (left) and intestinal (right) fluids. Ratio: SPI/Zein-1: 8:2; SPI/Zein-2 :6 :4 SPI/Zein-3:5:5 SPI/Zein-4: 3:7

CONCLUSION

In conclusion, food proteins show great promise for developing and engineering a range of new GRAS matrices with the potential to incorporate nutraceutical compounds and provide controlled release via the oral route. Clear advantages of food protein matrices include their high nutritional value, the abundance and wide variety of their sources and their acceptability as naturally occurring food components degradable by digestive enzymes.

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