Nano-encapsulations liberated from plant protein microparticles for oral delivery of bioactive compounds

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INTRODUCTION

Oral administration is by far the most convenient way for delivery of bioactive compounds, especially when repeated or routine administration is necessary. However, this route is restricted for many bioactive compounds that have poor solubility, poor permeability, and/or poor stability in the gastro-intestinal environment (Sahana et al., 2008). Polymeric nanoparticles are promising candidates for oral delivery of bioactive compounds. In order to preserve functionality, nanoparticles must survive the harsh gastric conditions of low pH and pepsin digestive enzymes. A major drawback of these dispersions is their tendency to decrease their interfacial surface area and then aggregate (Li and Kaner, 2006). Our objective was to develop plant protein-based emulsion microparticles for oral administration of lipophilic bioactive compounds. This may provide a new approach for targeted and controlled delivery of nano-encapsulations in the human gut by avoiding nanoparticle aggregation and degradation during storage or in stomach conditions. This research paper describes the preparation, characterization, and evaluation of emulsion microparticles based on barley proteins. Microparticle degradation and bioactive compound release behaviours were studied using in vitro systems.

MATERIALS AND METHODS

Regular barley grains (Falcon) were kindly provided by Alberta Agricultural and Rural Development, Lacombe, Alberta. Barley protein content was 13.2% (w/w, dry status).

The microcapsules were prepared by pre-emulsifying processing followed by a high pressure homogenization treatment. The size of the microparticles in wet status was measured at room temperature by dynamic light scattering using a Zetasizer NanoS instrument (model ZEN1600, Malvern Instruments Ltd, UK). The morphology of the spray-dried microparticles was observed with a scanning electron microscope (SEM, S-2500, Hitachi, Tokyo, Japan) operating at 15KV.

Extraction of oil from barley protein microparticles was based on the method described by Beaulieu et al. (Beaulieu et al., 2002). The encapsulation efficiency (*EE*) were calculated by the following equations:

$EE\ (\%) = W_{encapsulated\ oil} / W_{total\ oil} \times 100$; where $W_{encapsulated\ oil}$ represents the weight of oil encapsulated in the microparticles and and $W_{total\ oil}$ represents the oil added initially in the particle formation mixture.

Release profiles of the microparticles were studied by incubating them in four different release media: HCl-saline solution (pH 2.0); phosphate-buffered saline (pH 7.4) or PBS; simulated gastric fluid (SGF) (pH 2.0) with 0.1% pepsin (w/v): and simulated intestinal fluid (SIF) (pH 7.4) with 1.0% pancreatin (w/v). Changes in microparticle morphology after incubating in SGF and SIF were observed using the TEM. The samples were prepared by coating a copper grid with a thin layer of digestive suspension and then staining with 1% (w/v) phosphotungstic acid. Excess liquid was blotted from the grid, and then samples were air dried and examined using the TEM at an accelerating voltage of 120 kV.

RESULTS AND DISCUSSIONS

Microcapsule preparation

SEM photographs of the spray-dried microparticles are shown in Fig. 1. These particles demonstrated diameters ranging from 3-5 μm with a spherical shape.

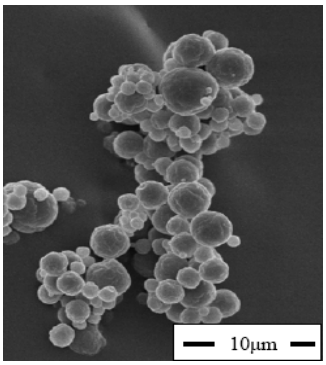

Fig. 1. Surface morphology of spray-dried barley protein microparticles by TEM.

Beta-carotene was selected as the model bioactive compound, since this precursor of vitamin A is well recognized as a disease-preventing antioxidant. Barley protein microparticles demonstrated very high encapsulation value (92.9-97.0%), indicating most of the added β-carotene was encapsulated in the barley protein microparticles.

In vitro release

The release properties of the microparticles were investigated in the simulated gastric and intestinal fluids with and without digestive enzymes. A control experiment verified that β-carotene cannot be released from microparticles without digestive enzymes in pH 2.0 and 7.4 buffers, indicating that the integrity of the microparticles was well maintained. Thus only β-carotene release profiles in SGF with pepsin and SIF with pancreatin were described in Fig. 2. In SGF with pepsin, β-carotene was slowly released from microparticles and less than 5% β-carotene was detected in the release medium after 2 h of the test. Interestingly, in SIF with pancreatin, β-carotene was steadily released from the microparticles at almost zero-order release kinetics (r^2 = 0.97) in the first 2 h. Over time the release curve levelled off gradually, until after 6 h when 91.6% of the β-carotene had been released.

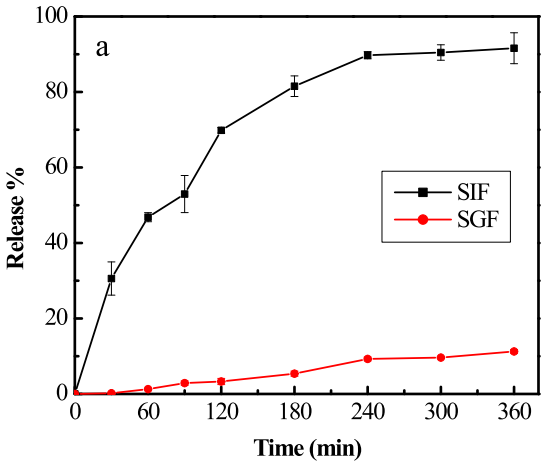

Fig. 2. Release profile of β-carotene in the simulated gastric (SGF) and intestinal (SIF) fluids with digestive enzymes from barley protein

In vitro degradation

Fig. 3 shows the morphology changes of BGH microparticles in SGF and SIF. Nanoparticles with average sizes between 20-30 nm predominated as a result of microparticle bulk matrix degradation when incubated in SGF for 30 min (Fig. 3a). After 1 h of incubation, bulk matrices disappeared with mono-dispersed nanoparticles remaining in the release medium (Fig. 3b). Interestingly, in SIF with pancreatin, both liberated nanoparticles and the original BGH microparticles were degraded within 6 h of incubation, leaving well dispersed nano-emulsions in the SIF medium. Fig. 3d shows emulsions released from nanoparticles. These released nano-emulsions were probably stabilized by the soluble protein hydrolysates in the release media, which can improve the absorption of the incorporated β-carotene in the small intestine.

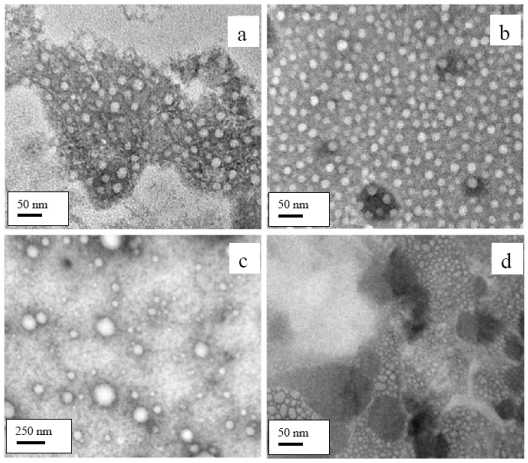

Fig. 3. Morphology changes of microparticles in the SGF and SIF by TEM: nanoparticles observed after incubating microparticles in SGF with pepsin (a) for 30 min and (b) for 1 h, and nanoparticles incubated in SIF (c) without pancreatin for 2 h, and (d) with pancreatin for 6 h.

CONCLUSION

This research is the first to report that nano-encapsulations were formed as a result of enzymatic degradation of barley protein microparticle bulk matrix in a simulated gastric tract. These nano-encapsulations delivered β-carotene to a simulated human intestinal tract intact, where they were degraded by pancreatic enzymes and steadily released the β-carotene. This *in vitro* system shows potential to facilitate lipophilic bioactive compound absorption in the human digestive tract, which needs to be proven in future *in vivo* experiments.

ACKOWLEDGEMENT

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