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Food-based carriers for the delivery of pancreatic enzymes to elderly for restoring digestion and tackling malnutrition

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INTRODUCTION

European population is ageing. It is estimated that the proportion of old population (individuals aged >65 years) will grow from 16% in 2010 to 29% by 2060.¹ Malnutrition is correlated with morbidity and mortality of elderly people. Malabsorption of nutrients due to pancreatic insufficiency is amongst the reasons that cause weight loss in the elderly² and several human trials have clearly established that the pancreatic exocrine secretion does decline in advanced age.^{3,4}

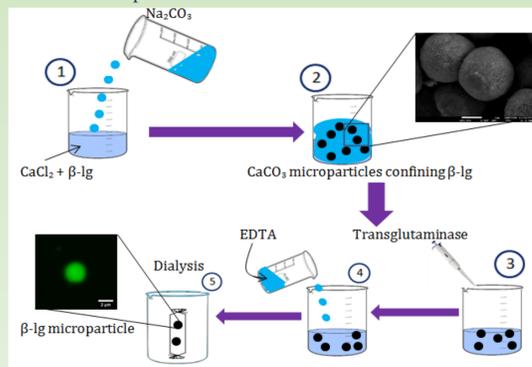
This project aims at developing beverages and gels specifically dedicated to deliver pancreatic secretions and highly nutritive proteins to tackle elderly malnutrition. The beverages and gels are based on aqueous two-phase system emulsion. Aqueous two-phase system or water-in-water emulsions consist of droplets of a macromolecular crowded aqueous solution, phase separated within a coexisting immiscible aqueous phase.⁵ These emulsions are more biocompatible than oil-containing emulsions and are increasingly used for diverse objectives including bioactive delivery and synthesis of hydrogel particles.⁶

OBJECTIVES

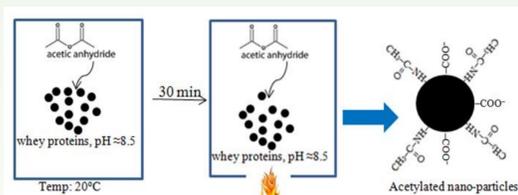
The objectives of the project are to (1) develop a high-protein emulsion for elderly nutrition, (2) design a physiologically-relevant dynamic digestion model of elderly by taking into account the changes observed in the gastrointestinal tract with aging (salivary flow, gastric acidification, decrease in enzyme secretions...), and (3) assess the ability of the carriers developed to deliver pancreatic enzymes in the small intestine during dynamic *in vitro* digestion. Since it will be made of whey proteins, the emulsion will also deliver branched amino acids that act as signals in the elderly and allow to restore protein muscle synthesis in subjects suffering from sarcopenia (loss of muscle mass during aging).

METHODS AND FINDINGS

The project is performed in successive steps.



Initially, we developed a method by which the major whey protein, β -lactoglobulin (β -lg) is at first encapsulated through a facile procedure within CaCO_3 micro-templates, followed by enzymatic crosslinking of the protein and succeeding removal of the template to form protein microparticles. Protein encapsulation increased the colloidal stability and influenced the micro-morphology of CaCO_3 microparticles. Dynamic light scattering and ζ -potential measurements, supported by gel electrophoresis indicated that β -lg was entrapped in monomeric form. In subsequent to enzymatic crosslinking and template dissolution, confocal laser scanning microscopy illustrated that the resulting protein particles were spherical, micron sized ($\approx 3\mu$) and structurally homogenous. It was found based on Fourier transform infra-red spectroscopy that enzymatic crosslinking twisted the β -sheet structures of β -lg. Gel electrophoresis and intrinsic fluorescence measurement showed that in addition to the enzymatic polymerization, thiol-disulfide exchange reactions contributed in protein particles formation. It was also concluded based on intrinsic fluorescence measurement that tryptophan microenvironment was not influenced by the enzymatic crosslinking. The fabricated β -lg particles did not undergo significant denaturation by subsequent heating. This may open a route to deliver near-native whey protein microparticles to final consumers.



Later, we hydrophobized whey proteins. For this purpose, whey protein isolate (WPI) powder was dissolved in distilled water (65 mg/mL) and stirred for 2 h at 20°C, sodium azide was added (100 ppm) as antimicrobial agent. After storage for 18 h at 5°C to warrant complete hydration, the WPI solution (9.5 mL) was titrated successively with acetic anhydride (two injections, each 50 μ L) and 7 M NaOH (five 50- μ L injections and three 10- μ L injections). The ratio of acetic anhydride to whey proteins was ≈ 0.18 g/g. This was followed by titration of the WPI solution with 1 M NaOH (2-3 injections immediately, and 3-4 injections over time, each 10 μ L) during the next 30 min to keep pH above 8.0. Immediately after the last alkaline titration, the WPI solution (pH ≈ 8.70) was heat-treated at 80°C for 20 min while being stirred at 800 RPM, after which pH dropped to 7.41 ± 0.03 . Subsequently, the pH of the acetylated heat-treated WPI solution was increased to 8.25 ± 0.1 by adding 1 M NaOH (35 μ L). The pH value did not change during further storage. The procedure yielded whey protein nano-particles (75 nm) with a high degree ($\geq 90\%$) of acetylation of free amino groups and a significantly low isoelectric point (3.70). The surface hydrophobicity of acetylated protein and β -sheet structure content correlated. Acetylation decreased inter-molecular crosslinking of whey proteins due to heating. It was also found that mobility of α -lactalbumin in non-reducing electrophoresis gel was higher after acetylation.

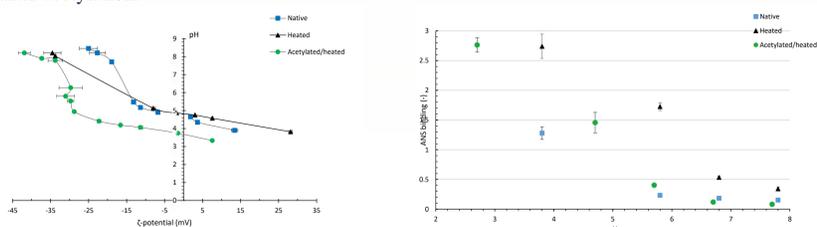


Figure 1. Zeta-potential and ANS-binding of WPI samples at different pH values.

Subsequently, the hydrophobized whey protein nano-particles were used for the fabrication of aqueous two-phase system emulsion. Emulsion were prepared as follows: WPI samples (native, heated only and acetylated/heat-treated) were supplemented with different concentrations (0, 5.2, 10.5 and 15.8 w/w%) of erythritol. Then, sodium alginate solution (60 mg/mL) as disperse phase (0.5 g) was titrated (during 15 sec) into WPI-erythritol mixed solution (9.5 g) as continuous phase while being stirred at 30,000 RPM (T 10 basic Ultra-Turrax®, IKA, Staufen, Germany). The solution was stirred for additional 2 min. As a model cargo, which has a distinct λ_{max} from proteins (i.e. 590 nm vs. 240 nm), crystal violet dye was added into alginate solution at a ratio of 1:99. The dye-loaded alginate was emulsified by the same method described above. Resulting two aqueous phase emulsions had a fixed disperse phase-to-continuous phase weight ratio of 5:95 but consisted of different WPI solution and erythritol contents, i.e. 95 vs. 0, 90 vs. 5, 85 vs. 10, and 80 vs. 15 w/w%.

The surface tension, contact angle, and apparent viscosity of WPI solutions, as well as, the microstructure of the thin layer of air-dried WPI samples were examined. Emulsion samples were imaged by light microscopy and confocal laser scanning microscopy.

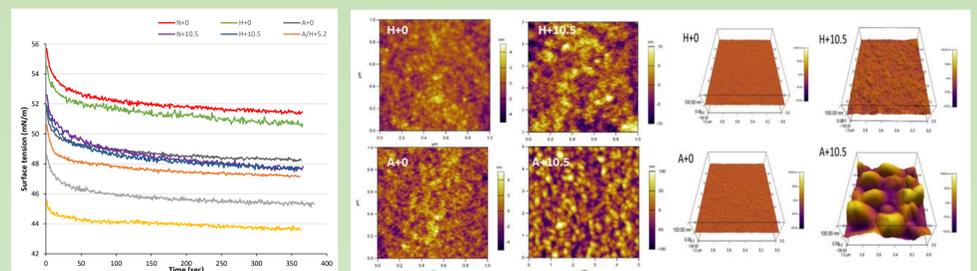


Fig. 2. Surface tension and the AFM images of different WPI samples with added erythritol. N, H and A abbreviate native, heat-treated and hydrophobized WPI samples, respectively. The digits indicate erythritol concentration (%w/w) in WPI solution.

Heat-treatment, hydrophobization and erythritol addition decreased the surface tension of whey protein solutions. It was hypothesized based on viscosity measurement and AFM imaging that erythritol addition at a sufficiently high concentration (i.e. ≥ 10.5 %w/w) into WPI solution induced interaction of hydrophobized WPI nano-particles through their acetyl moieties, resulting in development of a fine network.

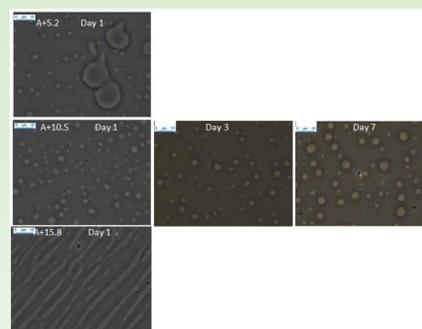


Fig. 3. Light microscopy images of water-in-water alginate in erythritol-added hydrophobized WPI solution. N, H and A abbreviate native, heat-treated and hydrophobized WPI samples, respectively. The digits indicate erythritol concentration (%w/w) in WPI solution.

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