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The impact of whey protein preheating on the properties of emulsion gel bead

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ABSTRACT

Thermal treatment effect (70 or 80 °C for 5 or 30 min) was evaluated on functional properties of whey protein isolate (WPI) dispersions used for the development of novel vitamin A delivery systems based on emulsion gel beads. This process combines an (O/W) emulsion diluted by a polysaccharide solution and a cold-set gelation induced by salt addition. Pre-heated WPI had a significant impact on the denaturation degree and on the surface hydrophobicity, respectively studied by differential scanning calorimetry and fluorescence. Stronger heating conditions (i.e. duration or temperature) induced complete denaturation, an increase of surface hydrophobicity and of viscosity. Under these conditions, the final emulsion showed a decrease particle size and an enhancement of stability. The resulting beads offered better vitamin A yield and stability during storage. These delivery systems bring a good protection of vitamin A to pH changes and control the release of this lipophilic component.

1. Introduction

Whey proteins are widely used as natural functional ingredients in the food industries and their use extends to various pharmaceutical applications as well as in biological systems. These proteins have a high nutritional value and are able to stabilise oil-in-water (O/W) emulsions by diffusion to (O/W) interfaces in the emulsification step, reducing surface tension (Dybowska, 2011; Millqvist-Fureby, Elofsson, & Bergenstal, 2001). Subsequently, they form thick layers which prevent droplets from coalescence or flocculation, mainly by hydrophophic interactions at the (O/W) interface (Dickinson, 2012; Dissanayake & Vasiljevic, 2009; Raikos, 2010) and also by disulfide bonds between the protein adsorbed onto oil droplets (Dickinson, 2012).

In recent decades, their ability to form a gel has been studied more and has allowed development of whey protein-stabilised emulsion gels. Indeed, whey protein-stabilised emulsions can be transformed into emulsion gels by heat treatment or acidification with glucono-δ-lactone (GDL) or addition of divalent salts ($CaCl_2$, $ZnCl_2$ or $MgCl_2$), and even enzymatic cross-linking with glutaminase for example (Beaulieu, Savoie, Paquin, & Subirade, 2002; Dickinson, 2012; Liu & Tang, 2011; Raikos, 2010). Compared to emulsions, whey protein-stabilised emulsion gels promote a better oxidative stability of lipids, a controlled release and a good potential carrier for active substances. Moreover, the cold-set gelation method induced by salt addition is interesting, because it is suitable for active substances sensitive to heating or acid, such as probiotics, vitamins, flavours, nutraceuticals and pharmaceuticals. However, in this case, protein must be heating before the emulsification step to improve the emulsifying properties of the whey proteins (Dissanayake & Vasiljevic, 2009; Kim, Cornec, & Narsimhan, 2005) and to promote the formation of soluble protein aggregates (Beaulieu et al., 2002; Liu & Tang, 2011).

Pectin is a polysaccharide commonly used in the food industry as a thickening, gelling and stabilising agent in yoghurt and acid milk drinks (Dickinson, 2012). These properties provide the possibility to encapsulate some (O/W) or (W/O) emulsions (Elmowafy, Awad, Mansour, El-Hamid, & El-Shamy, 2009). Compared to high methoxylated pectin (HMP), the use of low methoxylated pectin (LMP) promotes a cold-set gelation method by salt-induced addition without any change of the milk protein solubility (Neirynck et al., 2007). Pectin is already used to obtain simple (O/W) or multiple emulsions (O/W/W) and several studies have shown the complexation of β-lactoglobulin (β-LG) or whey protein isolate (WPI) with pectin (Guzey, Kim, & McClements, 2004; Neirynck et al., 2007; Wang & Qvist, 2000). The work of Wang and Qvist (2000) showed that pectin could be adsorbed on the surface of β-LG droplets and that molecular cooperation can be established between β-LG and LMP, when the ionic strength is moderate and creaming is prevented with the use of a low protein:pectin ratio.

Most of the work involving the use of milk proteins (i.e. β-LG) or concentrates of whey proteins (WPC) with a high proportion of proteins (i.e. 80%) was to stabilise emulsions. Some studies

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investigated the effect of thermal treatment on protein properties using different techniques (Beaulieu et al., 2002; Dissanayake & Vasiljevic, 2009; Kim et al., 2005). The ability of whey protein–polysaccharide to form complexes and the interactions between these two edible bio-polymers were studied in aqueous solution or emulsion state and correlated with the effect of pH, ionic strength, bio-polymers ratio and pectin type (Chen & Subirade, 2005; Guzey et al., 2004; Jones, Lesmes, Dubin, & McClements, 2010; Jones & McClements, 2011; Livney, 2008). The nature of complexes formed between the two bio-polymers depended on the linear charge density and hydrophobicity of the pectin molecule as well as on the pH and ionic strength of the solution (Jones & McClements, 2011). Strong electrostatic complexes could be formed between protein and anionic polysaccharide at pH below the isoelectric point (pI). On the contrary, at pH close to the pI or above, weak reversible complexes tend to be formed between proteins and polysaccharides (Guzey et al., 2004).

However, little information is available on the concept of (O/W) emulsion diluted with a polysaccharide and on the gelation of diluted emulsion to obtain beads (Li, Hu, Du, Xiao, & McClements, 2011).

This work aimed to characterise the functional properties of 12% (w/v) WPI dispersions by differential scanning calorimetry (DSC), fluorescence, rheology and surface tension measurements as a function of preheating conditions. Then, the particle size distribution (PSD) and the stability of emulsions obtained from these dispersions were evaluated. Finally, the impact of heat treatment (i.e. duration or temperature) was directly studied on the encapsulation yield, stability and *in vitro* dissolution of vitamin A (VA) entrapped into emulsion gel beads produced using an encapsulation process based on the combination of an (O/W) emulsion dilution with a cold-gelation method.

2. Materials and methods

2.1. Materials

Two bio-polymers were used in this study. BIPRO® whey protein isolate (WPI), as emulsifier, was purchased from Davisco Foods International (Le Sueur, MN). This WPI contained 92.4% protein (72.0% β-LG, 14.4% α-LA and 4.1% BSA, defined by our laboratory), 1% fat, 3% ash, 1% lactose and 4.8% moisture, as measured by the supplier's standard analysis procedures. The pH of a 12% (w/v) WPI native dispersion was 7.1. Non-amidated low methoxylated pectin (LMP, Unipectin OF 300C®) with an esterification degree ranging from 27% to 33%, extracted from lemon juice, was a gift from Cargill (Saint-Germain-en-Laye, France). This kind of pectin contains more than 80% (w/w) of galacturonic acid and the pH of a 4% (w/v) LMP dispersion was 2.6.

An oily solution of vitamin A (VA) was given by Adisseo (Commentry, France) and made of a hydrophobic antioxidant agent and a high proportion of vitamin (i.e. 80% in weight). In this study, the VA is used as a poorly water-soluble active pharmaceutical ingredient (API).

Calcium chloride dihydrate ($CaCl_2$, $2H_2O$) was supplied by VWR International (Lutterworth, UK). Octoxynol 9 (Triton X100) and (+) sodium L-ascorbate were obtained from Sigma–Aldrich (Saint Louis, MO). Sodium dihydrogen phosphate (NaH_2PO_4) and sodium chloride (NaCl) were purchased from Cooper Industrie (Melun, France). All other chemicals (e.g. HCl and NaOH) were of analytical reagent grade and used as received.

2.2. Preparation of WPI dispersions and thermal treatment conditions

Each sample containing 12% (w/v) of WPI was prepared by dispersing powdered whey protein isolate (WPI) into distilled water (pH 6.6). This WPI dispersion was then stirred for at least 2 h under gentle agitation (300 rpm). The dispersion was left to stand overnight at room temperature (i.e. 20 ± 2 °C) to ensure complete protein hydration and to avoid the formation of protein aggregates (Lefèvre & Subirade, 2000).

The native WPI dispersion can be then treated according to four different pre-heating conditions. Samples were heated at either 70 or 80 °C for 5 or 30 min, in a water bath (IKA, Staufen, Germany). They were mildly stirred by magnetic stirring to avoid particle sedimentation and to promote heat transfer. Finally, the protein dispersions were left to stand at room temperature until complete cool. As the pH of WPI denatured dispersions was close to neutrality ($pH_{70\ °C/5\ min} = 6.6$; $pH_{70\ °C/30\ min} = 6.9$; $pH_{80\ °C/5\ min} = 6.8$ and $pH_{80\ °C/30\ min} = 6.9$, neutralisation of WPI dispersions was not necessary after thermal treatment.

For this study, WPI concentration was set at 12% (w/v) in order to ensure a better emulsion stability. Indeed, a rapid evaluation of gelation ability according to concentration (up 5–15% w/v) was performed after heating at 80 °C for 30 min. The results showed that WPI dispersions had spontaneously gelled at concentrations above 12% (w/v) during cooling at room temperature. Therefore they could not be used for emulsification. However, protein dispersions were maintained in liquid state at concentrations equal to or below 12% (w/v).

2.3. Calorimetric measurements

Characterisation of 12% (w/w) WPI dispersion was achieved by a micro-calorimeter (DSC III SETARAM Instrumentation, Caluire, France). Samples (500 mg) were accurately weighed and placed into sealed aluminium pans. Each sample was measured within one day of preparation. Scanning was performed at a heating rate of 0.5 °C/min and followed for 5 h with a heating ramp from 25 up to 100 °C, using nitrogen as sweeping gas. An aluminium pan containing distilled water was used as reference. Calibration was performed using indium.

The enthalpy (ΔH), onset, peak of denaturation temperature (*Td*) and thermograms were achieved by SETSOFT software and recorded for each sample. Each DSC measurement was repeated three times and allowed to estimate the denaturation degree (*Dd*) from the following equation (Nicorescu et al., 2009):

$$\text{Denaturation degree or } Dd\,(\%) = (1 - (E_{\text{denatured}}/E_{\text{native}})) \times 100 \quad (1)$$

where $E_{\text{denatured}}$ is the energy associated with the denaturation peak of denatured protein and E_{native} is the energy associated with the denaturation peak of the native protein.

2.4. Fluorescence analysis

Fluorescence measurements were performed on a Spectrofluorometer LS50B® (Perkin–Elmer, Courtaboeuf, France). This technique allowed the study of the WPI surface hydrophobicity and the exposure of hidden hydrophobic aromatic amino acid (AAA) residues (e.g. tryptophan (Trp)).

Protein samples were diluted 4000 times (0.003% w/v) with distilled water (pH 6.6) before the fluorescence intensity was measured, to reduce the signal:noise ratio (Das & Kinsella, 1990). The WPI dispersion's intrinsic Trp fluorescence, using an excitation wavelength of 295 nm, was monitored over emission wavelengths of 300–400 nm and measured at room temperature, with a slit for excitation of 2.0 nm and a scanning speed of 60 nm/min.