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Interfacial protein engineering for spray–dried emulsions – Part I: Effects on protein distribution and physical properties

Timo Moisio [a,*], Annelie Damerau [b], Anna-Maija Lampi [b], Vieno Piironen [b], Pirkko Forssell [a], Riitta Partanen [a]

[a] VTT Technical Research Centre of Finland, P.O. Box 1000, 02044 VTT, Finland
[b] Department of Food and Environmental Sciences, University of Helsinki, P.O. Box 27, 00014 Helsinki, Finland

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ABSTRACT

Distribution of protein and oil in aqueous and spray–dried emulsions and the effect of protein cross-linking on emulsion properties and matrix–water interactions were investigated. Sodium caseinate and sunflower oil were used to make emulsions which were spray dried using maltodextrin as a wall material. 3% Na–caseinate concentration showed optimum emulsion and process stability as observed in CLSM images, droplet size data and in the amount of heptane-extractable oil from spray–dried emulsions. Transglutaminase cross-linking prior to emulsification slightly increased the amount of protein both on the oil droplet interface and on the particle surface as confirmed by analysis of continuous phase protein in the feed emulsion and by XPS measurements from the powder surface. DSC and water sorption measurements were used to study the physical state of the matrix. Glass transition occurred between RH 54% and 75% at room temperature and it was not affected by cross-linking.

1. Introduction

Spray drying of oil-in-water emulsions results in particles which are composed of a matrix, oil droplets and two types of interfaces, a particle outer surface and an interface surrounding the oil droplet within the matrix. As recently demonstrated, oxygen transfer is the rate-limiting step for oxidation in the glassy state of spray–dried emulsions, if the reaction is not suppressed by low temperature (Orlien, Risbo, Rantanen, & Skibsted, 2006). While the roles of the physical state of the matrix and morphology, as well as the particle of the surface in controlling oil stability have been elucidated over more than a decade, the oil–matrix interface has gained only little attention (Moreau & Rosenberg, 1998; Shaw, McClements, & Decker, 2007). However, as suggested already by Moreau and Rosenberg (1998) and as indicated by a more recent study performed by Klinkesorn, Sophanodora, Chinachoti, Decker, and McClements (2006), the oil-droplet interface may well be equally or even more important than the matrix in controlling oxidative stability of oil in spray–dried emulsion powders.

The recent progress in understanding plasticisation of glassy carbohydrate matrices on molecular level provides useful setup for attempts to correlate structure and structural changes with function. In order to successfully develop a matrix capable of preventing oxidation of sensitive oils, a particular challenge is to link the mass transfer-induced oxidation with the overall structure. The key issue is the effect of water on matrix mobility and the way this reflects to oxygen diffusion across the matrix. The approach of using glassy carbohydrates has been a generally accepted strategy for designing optimal encapsulation systems (Ubbink & Krüger, 2006). However, the recent observations indicate that it is not only the glass transition temperature which controls the molecular mobility of small permeants. Both water and low molar mass carbohydrates decrease glass transition temperature of amorphous carbohydrates, but water strongly increases mobility of small permeants while low molar mass carbohydrates might reduce their mobility. This is said to be due to enhanced molecular packing of the matrix caused by small carbohydrates (Townrow, Roussenova, Giardiello, Alam, & Ubbink, 2010).

Research to design the oil–water interfaces towards better oxygen barriers in aqueous o/w emulsions already indicates the real potential of this approach. Protein-interfaces have shown to slow down oxidation, especially at pHs below the protein iso-electric point (Hu, McClements, & Decker, 2003). The positive effect was suggested to be due to electrostatic repulsion of iron from the interface. Nano-size silica particles in combination with milk protein were shown to reduce oxidation which was explained to be caused by a thicker interface or by reduced surface area between the oil and the aqueous phase (Kargar, Spyropoulos, & Norton, 2011). Our recent studies (Ma et al., 2012) strongly indicate that enzymatic cross-linking of milk protein significantly reduces the rate of oxidation in an aqueous emulsion. Contradictory results

* Corresponding author. Tel.: +358 40 168 0003; fax: +358 20 722 7071.
E-mail address: Timo.Moisio@vtt.fi (T. Moisio).

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were reported in an earlier study of cross-linked milk protein (Kellerby, McClements, & Decker, 2006). Difference was explained by limited availability of oxygen in Kellerby et al. (2006). Furthermore, in Ma et al. (2012) cross-linking was performed prior to emulsification while Kellerby et al. (2006) used cross-linking after emulsification. The structure of the interfaces from these different processes is expected to be different, as it is known that interfacial elasticity is more increased by the interfacial cross-linking (Partanen et al., 2009), which in oil–water systems has been shown to reduce the thickness of the interfacial layer (Partanen, Forssell, Mackie, & Blomberg, 2013). Tikekar, Johnson, and Nitin (2011) introduced a novel method to measure real-time in situ oxygen transport across WPI and SDS based emulsion interfaces. These selected emulsions were not effective in limiting oxygen transport across the interface but the method can be used to evaluate barrier properties of other emulsion-based encapsulation systems.

In our previous work the particular area of interest has been the stabilisation of oils against non-enzymatic oxidation. The dramatic effect of water activity on the oxidative stability of oils has been shown, but the change of matrix material from carbohydrates to whey protein isolate led to a deviation from Labuza's water activity map (Labuza & Dugan, 1971; Partanen, Hakala, Sjövall, Kallio, & Forssell, 2005; Partanen et al., 2008). The major aim of the present work was to relate the properties of the interfacial protein layer and humidity-response of carbohydrate matrix to susceptibility towards oxidation. Moreover, aim was to study to which extent the interfacial modification affects not only the interface, but also the distribution of the components within the spray–dried emulsion. In this first part of the study, the effect of transglutaminase-induced cross-linking on the distributions of oil and sodium caseinate emulsifier in aqueous emulsions and spray–dried emulsion particles were investigated. Further on, the consequences of protein cross-linking on water vapour sorption and its relation with plasticisation of the powder matrix were determined. The second part was focused on the oxidation of spray–dried emulsions at various humidities (Damerau et al., in press).

2. Materials and methods

2.1. Materials

Sunflower oil was from Bunge Finland Oy (Raisio, Finland). Sodium caseinate was from Kaslink Foods (Koria, Finland). Na–caseinate contained minimum of 90% protein, maximum of 1.5% fat, 4.0% of ash and 5.5% of moisture. Maltodextrin was purchased from Grain Processing Corporation (Muscatine, Iowa, USA). The dextrose equivalence (DE) of the maltodextrin was 22.2. Sodium hydroxide (1 M) was used for pH adjustment for emulsions and sodium azide was used for better microbiological stability of emulsions. Both were of analytical grade. All the solutions were prepared in milliQ-water. Transglutaminase enzyme was used to cross-link Na–caseinate. Microbial transglutaminase Activa MP was from Ajinomoto (Japan) and it was further purified and assessed for activity using protocols described by Lantto, Puolanne, Kalkkinen, Buchert, and Autio (2005).

2.2. Preparation of the spray–dried emulsion

2.2.1. Emulsion preparation

Na–caseinate was used at 0.5–3.0% (w/w) aqueous solutions. Na–caseinate was first dissolved in boiling milliQ-water and mixed with magnetic stirrer overnight. Sodium azide (0.04% of the protein solution) was added to the solution to maintain microbiological stability. The solution was pre-homogenised with sunflower oil (20% oil of the weight of the emulsion) with a Heidolph Diax 900 (Germany) homogeniser at speed 6/6 for 2×2 min. Pre-emulsion was fed to high pressure homogeniser (Microfluidics M-110Y) and processing was performed 10 times through pressure chambers (75 μm interaction chamber and 200 μm auxiliary processing module) at 50 MPa pressure.

2.2.2. Enzymatic cross-linking

Cross-linking for spray–dried emulsions was performed in protein solution prior to emulsification. Transglutaminase activity was 100 nkat/g of protein, Na–caseinate concentration was 3% and incubation time was 4 h at room temperature. The enzyme was not inactivated but after incubation time, the solution was mixed with maltodextrin and spray dried within 2 h. Cross-linking was confirmed with SDS–PAGE.

2.2.3. Feed emulsion preparation

Maltodextrin was dissolved in milliQ-water (50%-solution). Maltodextrin solution pH was adjusted with 1 M NaOH to 6.5 to prevent precipitation of Na–caseinate. Feed emulsions were prepared by mixing the emulsion and maltodextrin solution for 3 min with magnetic stirrer. Dry matter composition of the feed emulsion was 30% of sunflower oil, 67% of maltodextrin and 3% of Na–caseinate. The dry matter content of the feed emulsion was 36%.

2.2.4. Spray drying of feed emulsion

Feed emulsions were spray dried by a Niro Mobile Minor (Denmark) laboratory spray drier with a rotary atomiser. The inlet air temperature was adjusted to 180 °C, and the outlet temperature was kept at 80 ± 2 °C by controlling the feed rate. The rotating speed of the atomiser was 22,000 rpm. Samples were collected to the collection vessels under the chamber.

2.3. Droplet and particle size distribution

Droplet size distribution of emulsions and feed emulsions and particle size distribution of spray–dried emulsions were analysed by laser diffraction using a Coulter LS 230 (Beckman Coulter, USA) equipped with polarisation intensity differential scattering (PIDS) assembly for particles smaller than 0.4 μm. Emulsion samples were diluted in water and scattering was measured for 90 s in two consecutive runs. Powders were dispersed in ethanol and measured as emulsions. The refractive indices of 1.33 for water and 1.47 for oil were used for emulsions. Refractive indices of 1.36 for ethanol and 1.5 for spray–dried particle were used for spray–dried emulsions.

2.4. Continuous phase protein determination

Emulsions (10 ml) were centrifuged at 40 °C and 30,000*g* for 90 min. The aqueous phase was withdrawn and filtered through 400, 200 and 100 μm filters. Protein concentration was determined from the filtrate using the Bio-Rad DC protein assay kit (Bio-Rad, Richmond, USA) based on reaction of protein with an alkaline copper tartrate solution and Folin reagent. Characteristic blue colour of the reaction was measured spectrophotometrically at 750 nm absorbance wavelength. Protein concentration was calculated using bovine albumin serum as a standard.

2.5. Confocal microscopy

The emulsions and feed emulsions were visualised using confocal laser scanning microscopy (CLSM) equipment consisting of a Bio-Rad Radiance Plus confocal scanning system (Bio-Rad, Hemel Hempstead, Hertfordshire, UK) attached to a Nikon Eclipse E600 microscope (Nikon Corp., Tokyo, Japan). For imaging, lipids were