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Short communication

Functional and antioxidant properties of whey protein hydrolysate/pectin complexes in emulsions and spray-dried microcapsules

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ABSTRACT

The aim of the present study was to investigate the potential of partially hydrolysed whey proteins to microencapsulate fish oil by spray-drying bilayer feed emulsions containing pectin, an oppositely charged biopolymer. Microcapsules were composed of fish oil, β-lactoglobulin (β-LG) or hydrolysates thereof produced by trypsin (DH6) and glucose syrup (DE38) as the matrix component. Pectin was attached to protein single-layer emulsions via electrostatic interactions using the layer-by-layer technique at pH 4.0. All emulsions exhibited good process stability during atomisation and drying as indicated by oil droplet size distribution and accordingly the microencapsulation efficiency was high (≥ 95.2 %) for all samples. The hydroperoxide formation in fish oil microcapsules prepared from single-layer feed emulsions was reduced in the presence of DH6 due to increased accessibility of antioxidant amino acids. The attachment of pectin to protein- or peptide-stabilised single-layer emulsions yielded bilayer microcapsules with increased stability (lower hydroperoxide content during storage). Increased thickness of the interfacial layer and the immobilisation of prooxidative ions by pectin may add to the antioxidative effect of the hydrolysate.

1. Introduction

The stabilisation of emulsions by multiple layers of oppositely charged biopolymers has received increasing attention in the last decade. The formation of strong electrostatic complexes between proteins and anionic polysaccharides results from their positive and negative charge below the isoelectric point of the protein (Rodríguez Patino & Pilosof, 2011). Bilayer emulsions prepared by the layer-by-layer technique, which involves consecutive adsorption of a second biopolymer component to oppositely charged primary emulsion droplets, mostly exhibit increased physical stability compared to single layer emulsions (Güzey & McClements, 2006; Serfert et al., 2013).

Several factors affect the stability of lipids prone to oxidation in complex food matrices and the physical and chemical composition of the interface in an o/w emulsion plays a key role in the prevention of autoxidation. β-LG has been used for the stabilisation of emulsions, due to its antioxidant activity (Elias et al., 2006). Partial enzymatic hydrolysis may enhance the antioxidant effect of β-LG, since many antioxidant amino acids are buried within its native globular structure. Besides their stabilisation of sensitive lipophilic ingredients in liquid emulsions (Elias et al., 2006), β-LG hydrolysates also retarded lipid oxidation in spray-dried emulsions (Tamm et al., 2015). Electrostatic attachment of pectin to primary emulsion droplets stabilised with proteins (e.g. β-LG) yields a thicker physical barrier to hinder metal-lipid interactions and furthermore pectin is known to chelate prooxidative metals (Chen, McClements, & Decker, 2010). Accordingly, interfacial complexes of β-LG/pectin may reduce the formation of hydroperoxides in fish oil microencapsulated by spray-drying (Serfert et al., 2013).

Aim of the present study was to investigate the potential of bilayer-stabilised emulsions prepared from partially hydrolysed β-LG and pectin for the microencapsulation of lipophilic bioactive ingredients. β-LG and its hydrolysates were characterised by their peptide distribution (SEC and SDS-PAGE). Physical properties of liquid emulsions (oil droplet size distribution and ζ-potential),

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emulsion stability during spray-drying and subsequent stability of the encapsulated fish oil were examined. The latter includes microencapsulation efficiency and hydroperoxide formation.

2. Material and methods

2.1. Materials

Refined fish oil (Omevital 18/12 TG Gold, 12 % docosahexaenoic acid, 21 % eicosapentaenoic acid) was purchased from BASF Personal Care and Nutrition GmbH, Illertissen, Germany. β-lactoglobulin (β-LG, Davisco Foods International Inc., Le Sueur, USA) was used as cationic emulsifier at pH 4.0 unmodified and enzymatically hydrolysed. Enzymatic hydrolysis was conducted with trypsin (cat#T8003, EC:3.4.21.4, 12238 BAEE units/mg protein; Sigma Aldrich, Taufkirchen, Germany). Three different types of pectin were used as anionic polysaccharides to form an additional interfacial layer around the oil droplets: A low-methoxylated pectin (LMP (Herbstreith & Fox, Neuenbürg, Germany)) and two types of high-methoxylated pectin (HMP1 ((Herbstreith & Fox) and HMP2 (CP Kelco, Lille Skensved, Denmark)) with varying degree of methoxylation (DM) and galacturonic acid content (GC), which were donated by the suppliers. LMP, HMP1 and HMP2 exhibited a DM of 33, 72 and 70 % analysed by the method of Blumenkrantz and Asboe-Hansen (1973) and the GC was 88, 76 and 82 % determined as described by Baeuerle, Otterbach, Gierschner, and Baumann (1977), respectively. Glucose syrup (C*Dry01934, DE38) was purchased from Cargill Deutschland GmbH, Krefeld, Germany.

2.2. Enzymatic hydrolysis of β-LG

Enzymatic hydrolysis of β-LG was performed as described elsewhere (Tamm et al., 2015). A solution containing 7 wt% β-LG was hydrolysed to a degree of hydrolysis (DH) of 6 % (DH6) using the pH-stat method of Adler-Nissen (1986) and an enzyme/substrate-ratio of 1:800.

2.3. Molecular weight distribution of β-LG and DH6

The characterisation of the peptide composition of β-LG and DH6 was performed by tris-tricine SDS-PAGE (Schägger, 2006) and size exclusion chromatography (SEC) as described elsewhere (Tamm et al., 2015). SDS-PAGE was performed under reducing conditions using DL-dithiothreitol (Sigma Aldrich). For comparison a standard marker (cat#MWSDS17S (Sigma Aldrich)) was used, containing seven polypeptides between 2.5 and 17.0 kDa. SEC analysis was conducted on a TSKgel G2000SW column (7.5 mm ID × 30 cm, particle size 10 μm, Tosoh Biosciences LLC, USA) in series with a TSKgel CW guard column (7.5 mm ID × 4 cm, particle size 10 μm). An isocratic elution of 30 % acetonitrile containing 0.1 % TFA (v/v) was used at a flow rate of 1.0 mL/min and the elution was monitored with UV absorbance at 214 nm.

2.4. Preparation and physical characterisation of single and bilayer emulsions

Emulsions were prepared using the layer-by-layer technique. Coarse emulsions were produced by rotor-stator homogenisation (21.500 rpm, 90 s; T25 basic, IKA, Staufen, Germany) and contained 10 wt% fish oil, 0.5 wt% β-LG or DH6 (in 0.1 M acetate buffer) and 44.5 wt% glucose syrup. After high-pressure homogenisation (500 bar, 3 passes; PandaPlus, GEA Niro Soavi, Parma, Italy), emulsions were diluted with buffered solutions containing either glucose syrup (single layer) or glucose syrup and 0.4 wt% pectin (bilayer), respectively. These emulsions were again homogenised (400 bar, 1 pass) to yield final single layer (0.25 wt% protein, 5 wt% fish oil, 44.5 wt% glucose syrup) and bilayer emulsions (additionally 0.2 wt% pectin).

The oil droplet size of emulsions before and after spray-drying was analysed by laser diffraction (LA-950, Horiba Jobin Yvon GmbH, Bensheim, Germany). Results of the volume distribution are reported as the 90th percentile of the oil droplets, since this parameter is sensitive for shifts towards larger droplets. Measurements were performed in triplicate.

The ζ-potential of emulsions was examined using a Zetasizer (Nano-ZS, Malvern Instruments GmbH, Herrenberg, Germany). Emulsions were diluted 200-fold with 0.1 M acetate buffer prior to ζ-potential-measurements and analysed in triplicate.

2.5. Spray-drying of single and bilayer emulsions and characterisation of spray-dried microcapsules

Microencapsulation of fish oil was conducted by spray-drying feed emulsions (see Section 2.4) on a pilot-scale spray-dryer (Mobile minor, Niro A/S, Copenhagen, Denmark) at 180/70 °C inlet/outlet temperature with a two-fluid nozzle (1.9 bar). For evaluation of the microencapsulation efficiency, the extractable oil content in microcapsules was analysed. After extraction of the non-encapsulated oil using petrol ether, the oil content was determined gravimetrically (Westergaard, 2004).

2.6. Lipid oxidation of microencapsulated fish oil

The lipid oxidation of microencapsulated fish oil was monitored by following the hydroperoxide formation for 12 weeks. Samples were stored in desiccators in the dark at room temperature in an atmosphere of 33 % relative humidity. Microcapsules were incubated in an aqueous solution containing 0.7 wt% pectinase (Sigma Aldrich, cat#17389) to digest the pectin layer. Subsequently the oil was extracted by a blend of 2-propanol/isooctane and the hydroperoxide content was analysed to quantify the extent of lipid oxidation (International Dairy Federation, 1991) with slight modifications as reported by Tamm et al. (2015). Each determination included by two repetitions of the oil extraction and three analytical replicates of each extraction.

3. Results and discussion

3.1. Molecular weight distribution of β-LG and DH6

Enzymatic hydrolysis of β-LG using trypsin affected the molecular weight distribution and the results are shown in Fig. 1 β-LG mainly contains one protein as shown by the most intense band around 18 kDa in the SDS-PAGE gel and the SEC results. DH6 exhibits strong bands between 6.2 and 8.2 kDa and at 2.5 kDa in the SDS-PAGE gel and the SEC results confirm the peptides in DH6 are mostly smaller than 10 kDa. Most notable is the absence of the β-LG band in the SDS-PAGE indicating that the majority of β-LG was hydrolysed by trypsin. SEC results however show that over one third of the peptides in DH6 is larger than 10 kDa. This can be explained by either newly formed disulphide bonds between peptides or the formation of peptide aggregates that cannot be dissociated by the SEC eluent (Le Maux et al., 2013).

3.2. Characterisation of single and bilayer emulsions and spray-dried microcapsules

The physicochemical properties of feed emulsions and spray-dried microcapsules are shown in Table 1. All emulsions were physically stable during atomisation and drying as indicated by a