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Spray drying behaviour and functionality of emulsions with β-lactoglobulin/pectin interfacial complexes

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ABSTRACT

Aim of the present study was to investigate the impact of atomization and drying on the functionality of emulsions with a bilayered oil–water interface consisting of a globular protein (β-lactoglobulin, bLG) and anionic polysaccharides (pectins with varying degree of methoxylation). With regard to the atomization process, the emulsion spray droplet size generally decreased with increasing atomization energy. The spray droplet size distribution was narrower with rotary atomization compared to two-fluid nozzle atomization. The single droplet drying behaviour of the differently stabilized emulsions was similar as examined by acoustic levitation. With more than 95%, microencapsulation efficiency was high in all spray-dried particles. However, a shift in oil droplet size upon reconstitution indicated that oil droplet coalescence occurred within the process which was less pronounced in bilayer emulsions compared to the bLG-stabilised single layer emulsion. Data from interfacial viscoelasticity measurements showed distinct differences, which may explain oil droplet coalescence. The oxidative stability of encapsulated oil was influenced by both the physical state of the emulsions and the different constituents at the o/w-interface with bLG and low methoxylated pectin giving the best protection of the oil.

1. Introduction

The targeted use of synergistic effects resulting from the interactions of proteins and polysaccharides on the formation and stabilisation of food systems like emulsions and foams is of large interest (Dickinson, 2011; Ganzevles, Zinoviadou, van Vliet, Cohen Stuart, & de Jongh, 2006; Liu, Zhao, Liu, & Zhao, 2011; Lutz, Aserin, Wicker, & Garti, 2009; Matalanis, Jones, & McClements, 2011; McClements, 2006; Murray, Durga, Yusoff, & Stoyanov, 2011; Perez, Sánchez, Rodríguez Patino, Rubiolo, & Santiago, 2011; Rodríguez Patino & Pilosof, 2011; Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998; Stone & Nickerson, 2012; Xu, Wang, Jiang, Yuan, & Gao, 2012; Ye, 2008) as polysaccharides increase the physical stability via electrostatic and/or steric effects, increased viscosity in the aqueous phase and by modification of the interfacial rheological properties (Bouyer, Mekhloufi, Rosilio, Grossiord, & Agnely, 2012; Dickinson, 2003, 2009). Rodríguez Patino and Pilosof (2011) recently reviewed that the strength of the protein–polysaccharide (non-covalent) interactions may vary substantially by pH and/or ionic strength of the aqueous phase. At pH close to the isoelectric point (pI) or above, weak reversible complexes tend to be formed between proteins and anionic polysaccharides as the proteins carry nearly zero overall charge or a net negative charge, respectively. In contrast, strong electrostatic complexes between proteins and polysaccharides will be formed at pH below the pI. The type of interaction between proteins and polysaccharides affects the interfacial rheological characteristics of both the air–water (a/w) and the oil–water (o/w) interface of food formulations like dispersions and emulsions (Rodríguez Patino & Pilosof, 2011).

For stabilisation of o/w-emulsions by protein–polysaccharide complexes, two alternative procedures are possible. One approach is the addition of both protein and polysaccharide during

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emulsification (co-adsorption); the other technique involves the sequential adsorption of protein and polysaccharide by the so-called layer-by-layer technique (Jourdain, Schmitt, Leser, Murray, & Dickinson, 2009; Rodríguez Patino & Pilosof, 2011). In case of the latter technique, irrespective of the type of constituents at the o/w-interface, bi- or multilayer emulsions were generally shown to exert a higher physical and/or oxidative stability than single layer emulsions in the liquid state (Guzey, Kim, & McClements, 2004; Guzey & McClements, 2007; Klinkesorn, Sophanodora, Chinachoti, McClements, & Decker, 2005; Li et al., 2010; McClements, Decker, & Weiss, 2007). Among other constituents, the use of bLG and pectin for the preparation of self-assembled bilayers around oil droplets in an emulsion has also been described in the literature in terms of physical (Cho, Decker, & McClements, 2009; Guzey et al., 2004; Guzey & McClements, 2007; Moreau, Kim, Decker, & McClements, 2003) and oxidative stability (Katsuda, McClements, Miglioranza, & Decker, 2008) of the emulsions.

Spray-drying is a frequently applied technique to increase the stability of the emulsified food ingredients like flavours or nutritional oils (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007; Jafari, Assadpoor, He, & Bhandari, 2008; Taneja & Singh, 2012; de Vos, Faas, Spasojevic, & Sikkema, 2010). As reviewed by Drusch (2013) the preservation of the interfacial structure is of utmost importance to ensure a high microencapsulation efficiency and to maintain the desired functionality. Recently, a decrease in microencapsulation efficiency and a higher rate of lipid oxidation in spray-dried bilayer-emulsions based on lecithin and chitosan was reported (Serfert et al., in press). A similar observation was found by Gharsallaoui, Saurel, et al. (2010), who described a shift in oil droplet size in reconstituted bilayer emulsions prepared from pea protein and pectin. However, it needs to be mentioned that flavour retention during spray-drying was increased in the pea protein–pectin-based bilayer emulsions compared to pea protein-stabilised single layer emulsions. At present no data on the oxidative stability of spray-dried and reconstituted liquid single and bilayer emulsions containing bLG and pectin are available in the literature. Moreover, literature on characterization of the atomization and drying behaviour as well as interfacial dilatational rheology of single and bilayer emulsions is scarce.

Hence, the objective of the present study was to investigate the impact of atomization and drying on the functionality of emulsions with an o/w interface consisting of a globular protein (bLG) and sequentially adsorbed anionic polysaccharides (pectins with varying degree of methoxylation) at a pH well below the pI. A similar approach as described before for the characterisation of spray-dried dispersed systems with interfacial bilayer containing lecithin/chitosan (Serfert et al., in press) was used to characterize the emulsions investigated in the present study. The investigation included the changes in the spray droplet size during atomization, the drying behaviour by single droplet experiments with acoustic levitation, the size of free volume elements in the spray-dried matrix using positronium annihilation lifetime spectroscopy and finally oxidative stability during storage of liquid, reconstituted and spray-dried single and bilayer emulsions.

2. Materials and methods

2.1. Materials

Fish oil (Omevital 18/12 TG Gold, BASF Personal Care and Nutrition GmbH, Illertissen, Germany) was used as a model substance and contained 10.9 and 16.5% docosahexaenoic acid and eicosapentaenoic acid, respectively, as specified by the supplier. The oil was stabilized with mixed tocopherols by the manufacturer and was used without further treatment. bLG (Davisco Foods International Inc., Le Sueur, USA) was used as cationic emulsifier at pH 4.0, whereas pectins with varying degree of methoxylation (DM) were used as anionic polysaccharides to form a bilayer interface around the oil droplets: high methoxylated pectin (HMP) with DM of 71% and low methoxylated pectin (LMP) with DM of 38%. Both pectins were obtained from Herbstreith & Fox KG (Neuenbürg, Germany). The contents of galacturonic acid as specified by the manufacturer were 84 and 87% for the HMP and LMP, respectively. Before use in the present study, the DM and the content of galacturonic acid of the pectins were analysed photometrically by the chromotropic acid (Baeuerle, Otterbach, Gierschner, & Baumann, 1977) and the *m*-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973), respectively. The DM amounted to 29 and 74% and the galacturonic acid contents were 92 and 80% for the LMP and HMP, respectively. Glucose syrup (DE38; C*Dry 01934) was provided by Cargill Deutschland GmbH, Krefeld, Germany. Acetic acid and sodium acetate were of analytical grade and obtained from Carl Roth GmbH & Co. KG (Karlsruhe, Germany).

2.2. Preparation of single and bilayer emulsions

Preliminary experiments with varying amounts of bLG (0.25–1.5 wt%) and different homogenization conditions (100–500 bar, 1–3 passes) were carried out in order to produce physically stable bLG-single layer stock emulsions containing 10.0 wt% fish oil. These stock emulsions were further diluted with solutions of glucose syrup or glucose syrup/HMP or LMP to obtain stable primary (single layer) and secondary (bilayer) emulsions, respectively. For the preparation of bilayer emulsions, the amount of pectin was varied (0.05–0.35 wt%). As a result, all other experiments were performed according to the procedure as follows: First, a coarse bLG-single layer stock emulsion was prepared by emulsifying 10.0 wt% fish oil in 0.1 M acetate buffer (pH 4.0) containing 0.5 wt% bLG and 44.5 wt% glucose syrup by high shear homogenization (19,000 rpm, 1 min; Ystral GmbH, Ballrechten-Dottingen, Germany). The emulsion was further homogenized at 500 bar with three passes using a high-pressure homogeniser (Panda 2K; Niro Soavi Deutschland, Lübeck, Germany). The stock emulsion was subsequently diluted with acetate buffer based solutions containing glucose syrup or glucose syrup and 0.4 wt% pectin (HMP or LMP) to obtain a diluted bLG-single layer and bLG–pectin bilayer emulsion, respectively. The single and bilayer emulsions were further homogenized at 400 bar with one pass. Before, the glucose syrup/pectin solutions were prepared by magnetic stirring at room temperature until complete dissolution occurred. The final bLG-single layer emulsion contained 0.25 wt% bLG, 5.0 wt% fish oil and 44.5 wt% glucose syrup, whereas the bLG–pectin bilayer emulsion additionally contained 0.2 wt% HMP or LMP, respectively. As the amount of glucose syrup was kept constant (44.5 wt%), slight variations in the amount of total solids in the single layer (49.75 wt%) and bilayer emulsion (49.95 wt%) occurred. The pH of emulsions amounted to pH 4.0 in the bLG-single layer emulsion and to pH 4.0 and 3.9 in the bilayer emulsions containing HMP and LMP, respectively, and was not further adjusted. After spray drying, reconstitution of single and bilayer emulsions was done by dissolving microcapsules in acetate buffer by gentle magnetic stirring for about 15 min. In order to remove oxygen inserted by the stirring process, emulsions were purged with nitrogen for 1 min.

2.3. Characterization of liquid single and bilayer emulsions

2.3.1. Physicochemical characterization

The viscosity of emulsions was determined using a rotational viscometer (Haake Viscotester 7L, Thermo Electron Corporation,

Dreieich, Germany) with spindle L2 at 200 rpm. Values were recorded after 30 s at 20 °C. The electrical charge of emulsion droplets was determined via zeta potential by electrophoretic light scattering (Zetasizer Nano-ZS, Malvern Instruments GmbH, Herrenberg, Germany) with disposable capillary cells (DTS1061) by applying the Smoluchowski model after 200 fold dilution of emulsion samples with acetic acid buffer (pH 4.0). The oil droplet size of single and bilayer emulsions before and after spray drying, i.e. after reconstitution, was analysed by static laser diffraction (Helos; Sympatec GmbH, Clausthal-Zellerfeld, Germany) equipped with the module CUVETTE. Prior to analysis of reconstituted emulsions, an aliquot of powder was dissolved in acetate buffer (pH 4.0) and sonicated in an ultrasonic bath for 5 s. Results are reported as 10th, 50th and 90th percentile of the size of oil droplets.

The interfacial dilatational rheology, i.e. the viscoelastic response of a single layer of bLG or the mixed layer of bLG/pectin to a change in interfacial area, at the o/w-interface was analysed with an automated drop tensiometer (OCA-20, Dataphysics Instruments GmbH, Filderstadt, Germany) with an oscillating drop generator unit (ODG-20). Sinusoidal oscillations at a frequency f (0.1 Hz) with a volume amplitude of 10% were conducted, resulting in a change of surface area ($\Delta A/A$). Each measurement consisted of 10 cycles. The interfacial dilatational modulus E^*, its elastic E', and viscous E'' components and the phase angle φ were derived from the change in surface tension σ (dilatational stress) resulting from a small change in surface area (dilatational strain) (Lucassen & Van Den Tempel, 1972). For a perfect elastic material, stress and strain are in phase ($\varphi = 0$) and the imaginary term is zero. In case of a perfectly viscous material, $\varphi = 90°$ and the real part is zero (Rodríguez Patino, Rodríguez Niño, & Sánchez, 1999). For the analysis a drop of the aqueous solution was placed in a cuvette filled with MCT oil (Gustav Hees Oleochemische Erzeugnisse GmbH, Stuttgart, Germany). The latter was purified according to the procedure of Rodriguez Patino et al. (Rodríguez Patino, Navarro Garcia, & Rodriguez Niño, 2001) to ensure the absence of surface-active constituents.

2.3.2. Analysis of single and bilayer emulsions during atomization and single droplet drying

For analysis of the single and the bilayer emulsion during atomization, refined commercial rapeseed oil (obtained from the local supermarket) was used instead of fish oil. The emulsions were atomized by co-current two-fluid nozzle and rotary atomizer (SL 24-50/M, Niro A/S, Copenhagen, Denmark). The two-fluid nozzle was used in normal position with an orifice diameter of 1.0 mm. The emulsions were fed to two-fluid nozzle and rotary atomizer by a peristaltic pump at liquid flow rates of 65 ml/min, respectively. Rotary atomizer and two-fluid nozzle were driven by pressurized air at 1.5–3.0 and 1.5–4.0 bar, respectively, absolute pressure. Pressure was measured by a pressure gauge and controlled by a pressure regulation valve (Fairchild regulator). The set-up of test rigs is described elsewhere (Serfert et al., in press). Measurement of the droplet size distribution was carried out by laser diffraction (Spraytec, Malvern Instruments GmbH, Herrenberg, Germany). As light scattering model, the Mie-theory and a standard-opaque-particle-model (refraction index: $1.5 + i \cdot 1.0$) were used. To prevent errors due to beam steering effects caused by atomization gas the scattering data from the detectors 1 to 6 were not considered in case of the two-fluid nozzle. The Sauter mean diameter d_{32} was calculated, as it is commonly used to characterize droplet size distribution of sprays. The width of droplet size distribution was expressed as span: $= (d_{90,3} - d_{10,3})/d_{50,3}$, where $d_{x,3}$ is the diameter corresponding to x% of a volumetric cumulative droplet size distribution.

The drying behaviour of single and bilayer emulsions was analysed by acoustic levitation at 80 °C. The levitation technique allows a contactless positioning of samples (single droplets) in a standing ultrasonic wave between a transducer and a reflector and offers a direct means of observing a drying droplet and measuring its drying rate. The experiments were conducted in a custom made acoustic levitator as described recently (Serfert et al., in press). The shadow of the levitated particle was captured and the cross sectional area was determined by software (ProAnalyst). The decrease of the cross sectional area could be correlated directly with the mass transfer from liquid to gaseous phase via calibration. Firstly, the area was converted into droplet volume. Data were then flattened with an algorithm presented by Savitzky and Golay (1964) and by regarding the first derivation converted into a mass flow with a presumed density that equals the density of water at 80 °C. In order to achieve comparability between drying kinetics of a single droplet during the drying process as well as between drying kinetics of droplets of different sizes, volume transfer density was calculated. The latter was obtained by dividing the decrease in volume by the surface of the droplet at a given time interval.

2.4. Spray drying of single and bilayer emulsions

Microcapsules were prepared by spray drying of single layer and bilayer emulsions on a pilot plant spray dryer (Mobile Minor 2000, Niro A/S, Copenhagen, Denmark) at 180/70 °C inlet/outlet temperature and 4 bar with rotary atomization as described above. The fish oil content amounted to 10% of the dry matter.

2.5. Characterization of spray-dried carrier matrix particles and microcapsules prepared from single and bilayer emulsions

The particle size of microcapsules was determined after dispersing an aliquot of powder in inert oil (MCT oil, Gustav Hees Oleochemische Erzeugnisse GmbH, Stuttgart, Germany) by laser diffraction. Analyses were performed with three replicates. The surface morphology of spray-dried emulsions was analysed by scanning electron microscopy with a CamScan 44 REM/EDX (CamScan USA Inc, Cranberry Township PA, USA) at the Institute of Geosciences, Department of Geology, University of Kiel. Extractable oil was measured gravimetrically after extraction with petrol ether as described by Westergaard (2004). In addition, positron annihilation lifetime spectroscopy (PALS) experiments were performed in order to investigate the effect of excess pectin on the size of free volume elements in spray-dried carrier matrix without oil. Carrier matrix particles consisted of HMP or LMP and glucose syrup in different ratios ranging from 1/10 to 1/40. Samples were equilibrated at 20 °C and 33% relative humidity prior to analysis. The measurements were performed in a fast-fast coincidence setup with a custom-made temperature-controllable sample holder under high vacuum conditions as described elsewhere (Drusch et al., 2009; Kruse et al., 2005).

2.6. Lipid oxidation of fish oil in liquid single and bilayer emulsions and microcapsules

100 g of each liquid original and reconstituted single and bilayer emulsion were stored in 250 ml screw cap bottles at 20 °C in the dark for a period of 3 weeks (in duplicate). Single and bilayer microcapsules were stored at 20 °C and 33% relative humidity in the dark for a period of 8 weeks (in duplicate). Lipid oxidation was monitored through analysis of the hydroperoxide content measured via ferric thiocyanate hydroperoxide method (International Dairy Federation, 1991) with slight modifications after extraction of the oil with isooctane/isopropanol 50:50 (v/v).

3. Results and discussion

3.1. Characterization of liquid single and bilayer emulsions

3.1.1. Physicochemical characterization

Before microencapsulation, optimum concentrations of pectin were identified to obtain physically stable bilayer emulsions. Below 0.15 wt% pectin, bilayer emulsions were not physically stable which can be attributed to bridging flocculation leading to emulsion destabilization (Guzey & McClements, 2006). Regardless of the type of pectin, physically stable emulsions were obtained at pectin concentrations above 0.15 wt% within the concentration range (0.05–0.35 wt%) studied. No phase separation, which may occur during depletion flocculation, was observed during three weeks of storage. These findings are in accordance with the stability map of o/w-emulsions at a constant concentration of bLG and different oil contents and pectin concentrations by Cho et al. (2009), although pH (3.5) and DM of pectin (60%) differed. In the present study, the zeta potential of the single layer emulsion was +35.5 mV, whereas a charge reversal was induced by the addition of pectin up to a concentration of 0.35 wt%. Above pectin concentration of 0.2 wt% the zeta potential was almost constant at −17.3 and −30.0 mV for bLG–HMP-stabilized and the bLG–LMP stabilized bilayer emulsions (Fig. 1), respectively, which can be attributed to saturation of the bLG-stabilized interface with pectin. The difference in zeta potential between the two bilayer emulsions can be explained by the difference in the degree of methoxylation and thus by differences in overall charge. LMP has generally a higher overall charge compared to HMP.

Viscosity of the single layer emulsion was 39 mPa s, whereas viscosity of bilayer emulsions increased to 66 and 59 mPa s in HMP and LMP bilayer emulsions, respectively. The surface tension of the single layer emulsion was 42.7 mN/m and was not significantly affected by pectin addition: In the bilayer emulsions the surface tension was 43.6 and 48.4 mN/m when stabilized with HMP and LMP, respectively. As described by Hecht and King (2000), surface tension and viscosity are important parameters to evaluate the behaviour of solutions during spray drying with respect to the morphology of spray-dried particles and the occurrence of particle ballooning.

3.1.2. Analysis of single and bilayer emulsions during atomization and single droplet drying

Generally, droplet size distributions and spray patterns differ between two-fluid nozzle and rotary atomizer (Huang, Kumar, & Mujumdar, 2006). During atomization, the spray droplet size (d_{32}) decreased with increasing energy input, irrespective of the method of atomization (Fig. 2). Within the gas pressure range studied,

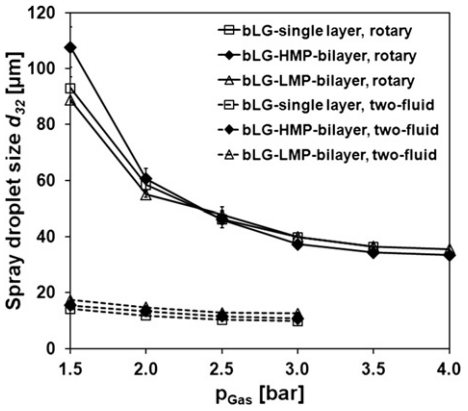

Fig. 2. Spray droplet size (d_{32}) of single and bilayer emulsions atomized at different energy inputs by two-fluid (A) and rotary atomizer (B).

emulsion spray droplets atomized by rotary atomizer were larger than droplets obtained by two-fluid atomization. Furthermore, in two-fluid nozzle atomization, moderate differences occurred between single and bilayer emulsions due to differences in viscosity (see 3.1.1). In rotary atomization, the effect of varying viscosity of differently stabilised emulsions on spray droplet size distribution was negligible above a gas pressure of 2.0 bar as at high atomizer revolution and low flow rate, liquids drip directly from the edge of the rotating disc. In case of dripping, droplet formation is quasi-static and not a function of viscosity (Walzel, 2010). Also with rotary atomization, the span of the spray droplet size distribution was narrower (0.8–1.2) than with two-fluid atomization (1.6–3.5). These observations are in good agreement with those of a previous study in which lecithin single layer and lecithin/chitosan bilayer emulsions were characterized (Serfert et al., in press). As a narrow particle size distribution induces a better overall drying performance (Huang et al., 2006; Walzel, 2011), rotary atomization was chosen for the preparation of single and bilayer microcapsules. During atomization, oil droplet sizes of the emulsions were only slightly affected by different energy inputs (data not shown). Drying behaviour of differently stabilized emulsions measured as volume transfer density during acoustic levitation of a single droplet was also similar (Fig. 3).

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

The sizes (d_{32}) of the microcapsules varied between 9.5 µm for bLG–HMP bilayer microcapsules and 10.1 µm for bLG–LMP

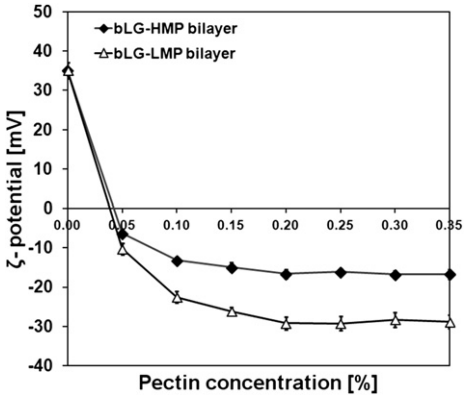

Fig. 1. Electrical charge of oil droplets in bLG based bilayer emulsions as a function of pectin type (HMP, LMP) and concentration.

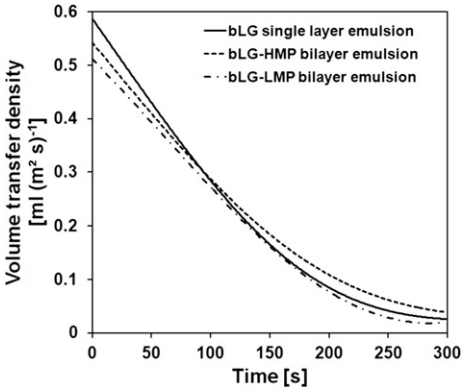

Fig. 3. Volume transfer densities of levitated single droplets of single and bilayer emulsions at 80 °C.

bilayer microcapsules. With respect to the surface morphology, no differences could be observed between single and bilayer microcapsules by SEM (Fig. 4). Extractable oil content of bLG-single layer microcapsules amounted to 5.6%. In bilayer microcapsules, amount of extractable oil was 4.1 and 1.5% when stabilized by bLG–HMP and bLG–LMP, respectively. Amount of extractable oil and thus microencapsulation efficiency depend on oil droplet size in liquid emulsions and the physical stability of the dispersed systems during drying and particle formation. Table 1 shows that distinct differences occurred in oil droplet size distribution after reconstitution of spray-dried microcapsules in single layer and bilayer emulsions prepared with HMP, which may explain differences in microencapsulation efficiency. Based on these findings, differences in interfacial dilatational modulus between the two pectin stabilised bilayers are likely to occur. However, in the present study the interfacial dilatational modulus E^* was in a similar magnitude in both binary bLG/pectin solutions (34.5 mN m^{-1}) but higher compared to the protein solution (26.0 mN m^{-1}) indicating an increasing solid-like character of the film (Table 2). These results are in agreement with data reported by Gharsallaoui, Yamauchi, Chambin, Cases, and Saurel (2010) on the interfacial dilatational rheology in pea protein–pectin stabilised emulsions. The decrease in phase angle φ when pectin is present confirms increased elasticity of bLG/pectin films (Ganzevles et al., 2006; Jourdain et al., 2009). In the present study, it was not possible to perform a sequential layering of bLG and pectin in interfacial dilatational rheology for technical reasons. According to Ganzevles, Fokkink, van Vliet, Cohen Stuart, and de Jongh (2008), a film of a simultaneous adsorbed protein–pectin complex will be thinner and will contain less material in the first protein layer than in sequentially adsorbed films and this might affect comparison of measurements performed in the present study with the behaviour of emulsions during spray-drying. A more severe point to discuss is the limited knowledge of the molecular structure of pectins and their effect on bilayer formation. Sperber, Schols, Cohen Stuart, Norde, and Voragen (2009) described the influence of overall charge and charge density of well-defined pectins on complex formation of bLG and pectin. Thus, differences in cooperativity between bLG and pectin can affect the stability of bilayer emulsions during spray drying and/or storage.

Table 1
Percentile (10, 50 and 90) of oil droplet sizes in liquid and reconstituted bLG-single layer and bLG–pectin bilayer emulsions (means ± standard deviation; bLG, β-lactoglobulin; HMP, high methoxylated pectin; LMP, low methoxylated pectin).

Sample	Percentile of oil droplet size [μm]		
	10th	50th	90th
Liquid emulsions before spray drying			
bLG	0.30 ± 0.00	0.73 ± 0.01	1.43 ± 0.03
bLG/HMP	0.34 ± 0.00	0.78 ± 0.00	1.47 ± 0.00
bLG/LMP	0.40 ± 0.00	0.95 ± 0.01	1.83 ± 0.03
Reconstituted emulsions after spray drying			
bLG	0.38 ± 0.00	0.97 ± 0.01	2.41 ± 0.00
bLG/HMP	0.38 ± 0.01	0.97 ± 0.01	3.07 ± 0.14
bLG/LMP	0.38 ± 0.01	0.90 ± 0.01	1.91 ± 0.05

3.3. Lipid oxidation of fish oil in liquid single and bilayer emulsions and microcapsules

It is often described in the literature that emulsion oil droplets with an anionic surface charge exhibit a lower oxidative stability in comparison to cationic emulsion oil droplets (Djordjevic, Cercaci, Alamed, McClements, & Decker, 2007; Gudipati, Sandra, McClements, & Decker, 2010; Klinkesorn et al., 2005; Shaw, McClements, & Decker, 2007; Taherian, Britten, Sabik, & Fustier, 2011). A possible explanation is the repulsion of prooxidative metal ions by the positive charge. However, in the present study a higher oxidative stability was observed in emulsions having a negative zeta potential (Fig. 5A). Furthermore, oxidative stability varied depending on the degree of methoxylation of pectin. After three weeks of storage, hydroperoxide content amounted to 33 mmol/kg oil in bilayer emulsions containing LMP, whereas in

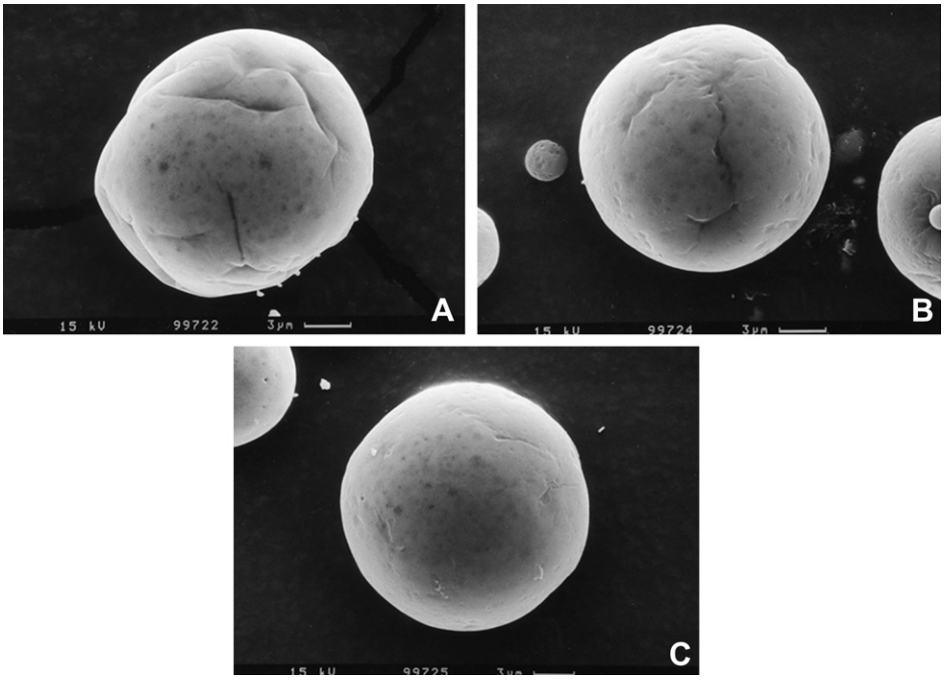

Fig. 4. SEM micrographs of bLG-single layer (A), bLG–HMP bilayer (B) and bLG–LMP bilayer (C) microcapsules.

Table 2
Mean values of viscoelastic properties at the o/w-interface of a single layer of bLG and a mixed layer of bLG and pectin at an amplitude of 0.1 mm and a frequency of 0.1 Hz (bLG, β-lactoglobulin; HMP, high methoxylated pectin; LMP, low methoxylated pectin).

Interfacial dilatational modulus E^* [mN m^{-1}]			Real part E' [mN m^{-1}]			Imaginary part E'' [mN m^{-1}]			Phase angle φ [°]		
bLG	+HMP	+LMP	bLG	+HMP	+LMP	bLG	+HMP	+LMP	bLG	+HMP	+LMP
26.0	34.7	34.5	25.6	34.3	34.3	4.4	5.1	3.4	9.9	8.5	5.7

bilayer emulsions with HMP the hydroperoxide content was 52 mmol/kg oil. In single layer emulsions a hydroperoxide content >50 mmol/kg oil was already reached after 2 weeks of storage. Regarding the surface charge of an emulsion and its oxidative stability, a similar observation was reported by Katsuda et al. (2008) for fish oil in bLG and bLG–pectin stabilized single and bilayer emulsions, respectively. They assumed that the interface in bilayer emulsions consisting of bLG and pectin was thicker than the interface of single layer emulsions consisting solely of bLG. By that means metal–lipid interactions would be inhibited. Furthermore the authors assumed that the iron binding capacity of pectin is responsible for their observation. Recently, Chen, McClements, and Decker (2010) investigated the role of continuous phase pectins in fish oil emulsions stabilized by non-ionic surfactant (Brij 35). The oxidative stability was lower in emulsions with high methoxylated pectin (DM 70%) than with low methoxylated pectin (DM 37%). They also showed that iron-binding capacity of the latter was higher than in high methoxylated pectin, whereas no differences in radical-scavenging ability between the pectins could be observed (Chen et al., 2010).

In the present study, in comparison to the liquid emulsions the oxidative stability in the reconstituted emulsions was generally lower, especially in bilayer emulsions (Fig. 5B). Zeta potential of reconstituted emulsions was similar to the zeta potential of liquid emulsions prior to spray-drying and did not change during three weeks of storage. As described by Shaw et al. (2007), the zeta potential indicates changes in the conformation of the single and bilayer formation around oil droplets after reconstitution of spray-dried powders. However, data from oil droplet size analysis after reconstitution of spray-dried emulsions showed a significant change in oil droplet size distribution in bLG-single layer and bLG–HMP bilayer emulsions (Table 1). Furthermore, flocculation occurred in the reconstituted emulsions. Depletion flocculation has been described for pea protein–pectin stabilised emulsions upon addition of maltodextrin (Gharsallaoui, Yamauchi, et al., 2010) and this phenomenon might have occurred during the drying process when total solids content of drying droplets increases. Furthermore close proximity of oil droplets as it occurs in the flocs in reconstituted emulsions facilitates interactions between droplets and facilitates lipid oxidation.

With respect to microcapsules in the present study, the course of lipid oxidation was similar in bLG-single layer and bLG–HMP bilayer microcapsules (Fig. 5C). At the end of storage, the hydroperoxide content amounted to 72 and 64 mmol/kg oil in single layer microcapsules and bLG–HMP bilayer microcapsules, respectively. In contrast, the highest oxidative stability was observed in bLG–LMP bilayer microcapsules with 37 mmol/kg oil after 8 weeks of storage. The microencapsulation efficiency was above 95% in all samples. It is generally accepted that microencapsulation efficiency is a crucial point in terms of stability of microcapsules and may partly be responsible for differences in oxidative stability in the present study. However, Drusch and Berg (2008) have shown that in case of a high microencapsulation efficiency other parameters like oil droplet distribution have to be considered. Another key parameter governing lipid oxidation in spray-dried emulsions is the size of free volume elements. It has been shown in the past that the molecular weight composition of carbohydrate sources (Drusch et al., 2009) as well as an excess of polymeric compounds (Serfert et al., in press) affect the size of free volume elements which in turn may affect oxygen diffusivity. The ortho-positronium-lifetime of 1.3 ns is well in agreement with data on glucose syrup with a dextrose equivalent of 36 in previous studies (Drusch et al., 2009). In the present study data from PALS measurements showed no clear tendencies towards an increase of free volume elements in spray-dried carrier matrix particles with a high pectin:glucose syrup ratio and thus excess pectin in the matrix (Table 3). An increase in the ortho-positronium lifetime was only observed for a spray-dried mixture of glucose syrup and LMP at a ratio of 1:10. As reviewed by Lopes da Silva and Rao (2006), degree of methoxylation, botanical source of pectins and processing conditions can significantly affect conformation of the galacturonan backbone and its flexibility. As a consequence, the radius of gyration is not proportional to the degree of methoxylation and thus also molecular packaging and average size of free volume elements. However, in spray-dried microcapsules the ratio between pectin and glucose syrup was very low (approx. 1:200) and differences in free volume elements and thus oxygen diffusivity between HMP vs. LMP cannot be expected.

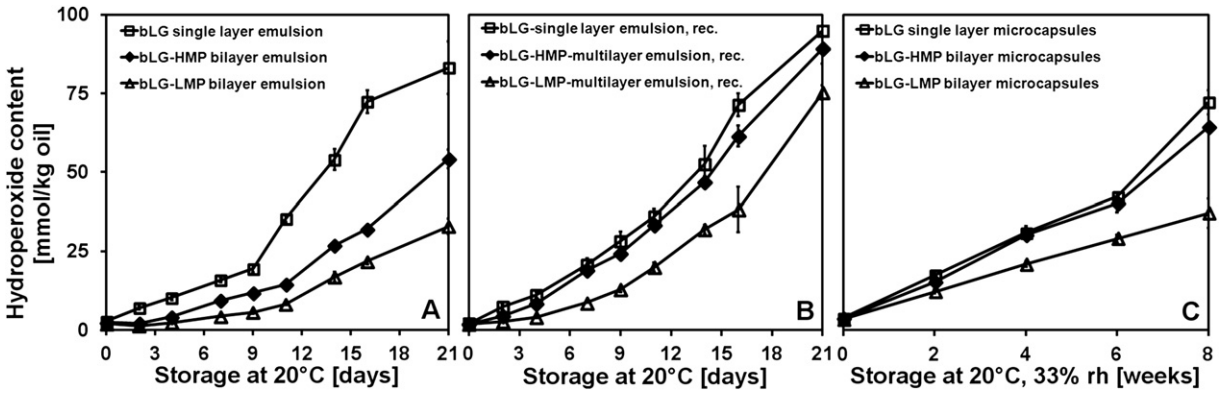

Fig. 5. Development of hydroperoxide contents in liquid (A), reconstituted liquid (B) single and bilayer emulsions and microcapsules (C) during storage at 20 °C (and 33% rh).

Table 3
Ortho-positronium lifetime (τ_3) for spray-dried carrier matrix particles containing glucose syrup and pectin in different ratios (means ± standard deviation; HMP, high methoxylated pectin; LMP, low methoxylated pectin).

Pectin:glucose syrup ratio, type of pectin	τ_3 [ns] ± SD
1:40, LMP	1.36 ± 0.02
1:20, LMP	1.28 ± 0.01
1:10, LMP	1.99 ± 0.02
1:40, HMP	1.27 ± 0.01
1:20, HMP	1.30 ± 0.00
1:10, HMP	1.28 ± 0.01

4. Conclusions

The present study provides new insights into characterization of protein based single and bilayer emulsions with regard to the spray drying process and functionality of spray-dried solid particles. Droplet sizes after atomization of single and bilayer emulsions were mainly affected by atomization conditions and only to a limited extent by emulsion composition. Volume transfer densities during levitation of single droplets were similar for all samples indicating a similar drying behaviour of single layer and bilayer emulsions. Microencapsulation efficiency was high and a shift in oil droplet size indicated that oil droplet coalescence occurred within the process, especially in bLG-single layer and bLG–HMP-bilayer systems. Data from interfacial elasticity measurements partially explain the improved stability of bilayer emulsions. However, since distinct differences between the two pectins used for bilayer formation occurred, further research should focus on a detailed investigation of the impact of molecular structure of pectins on their interaction with bLG and the resulting properties of the interfacial bilayer. Finally, the study clearly demonstrates synergistic effects of bLG and low methoxylated pectin on oxidative stability of lipophilic ingredients not only in liquid but also in a spray-dried state.

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References

Baeuerle, D., Otterbach, G., Gierschner, K., & Baumann, G. (1977). Bestimmungen des Polyuronidgehaltes und des Veresterungsgrades des Pectinanteiles in Handelspektinpräparaten, Apfelsäften und Apfelmaceraten. *Deutsche Lebensmittelrundschau, 73*, 281–286.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Chemistry, 54*, 484–489.

Bouyer, E., Mekhloufi, G., Rosilio, V., Grossiord, J.-L., & Agnely, F. (2012). Proteins, polysaccharides, and their complexes used as stabilizers for emulsions: alternatives to synthetic surfactants in the pharmaceutical field? *International Journal of Pharmaceutics, 436*(1–2), 359–378.

Chen, B., McClements, D. J., & Decker, E. A. (2010). Role of continuous phase anionic polysaccharides on the oxidative stability of Menhaden oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 58*(6), 3779–3784.

Cho, Y.-H., Decker, E. A., & McClements, D. J. (2009). Competitive adsorption of mixed anionic polysaccharides at the surfaces of protein-coated lipid droplets. *Langmuir, 25*(5), 2654–2660.

Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids, 17*(1), 25–39.

Dickinson, E. (2009). Hydrocolloids as emulsifiers and emulsion stabilizers. *Food Hydrocolloids, 23*(6), 1473–1482.

Dickinson, E. (2011). Mixed biopolymers at interfaces: competitive adsorption and multilayer structures. *Food Hydrocolloids, 25*(8), 1966–1983.

Djordjevic, D., Cercaci, L., Alamed, J., McClements, D. J., & Decker, E. A. (2007). Chemical and physical stability of citral and limonene in sodium dodecyl sulfate–chitosan and gum arabic-stabilized oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 55*(9), 3585–3591.

Drusch, S. (2013). An industry perspective on the advantages and disadvantages of different fish oil delivery systems. In N. Garti, & D. J. McClements (Eds.), *Encapsulation technologies and delivery systems for food ingredients and nutraceuticals*. Cambridge: Woodhead Publishing Ltd.

Drusch, S., & Berg, S. (2008). Extractable oil in microcapsules prepared by spray-drying: localisation, determination and impact on oxidative stability. *Food Chemistry, 109*(1), 17–24.

Drusch, S., Rätzke, K., Shaikh, M. Q., Serfert, Y., Steckel, H., Scampicchio, M., et al. (2009). Differences in free volume elements of the carrier matrix affect the stability of microencapsulated lipophilic food ingredients. *Food Biophysics, 4*, 42–48.

Ganzevles, R. A., Fokkink, R., van Vliet, T., Cohen Stuart, M. A., & de Jongh, H. H. J. (2008). Structure of mixed b-lactoglobulin/pectin adsorbed layers at air/water interfaces; a spectroscopy study. *Journal of Colloid and Interface Science, 317*(1), 137–147.

Ganzevles, R. A., Zinoviadou, K., van Vliet, T., Cohen Stuart, M. A., & de Jongh, H. H. J. (2006). Modulating surface rheology by electrostatic protein/polysaccharide interactions. *Langmuir, 22*(24), 10089–10096.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., & Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: an overview. *Food Research International, 40*(9), 1107–1121.

Gharsallaoui, A., Saurel, R., Chambin, O., Cases, E., Voilley, A., & Cayot, P. (2010). Utilisation of pectin coating to enhance spray-dry stability of pea protein-stabilised oil-in-water emulsions. *Food Chemistry, 122*(2), 447–454.

Gharsallaoui, A., Yamauchi, K., Chambin, O., Cases, E., & Saurel, R. (2010). Effect of high methoxyl pectin on pea protein in aqueous solution and at oil/water interface. *Carbohydrate Polymers, 80*(3), 817–827.

Gudipati, V., Sandra, S., McClements, D. J., & Decker, E. A. (2010). Oxidative stability and in vitro digestibility of fish oil-in-water emulsions containing multilayered membranes. *Journal of Agricultural and Food Chemistry, 58*(13), 8093–8099.

Guzey, D., Kim, H. J., & McClements, D. J. (2004). Factors influencing the production of o/w-emulsions stabilized by β-lactoglobulin -pectin membranes. *Food Hydrocolloids, 18*, 967–975.

Guzey, D., & McClements, D. J. (2006). Formation, stability and properties of multilayer emulsions for application in the food industry. *Advances in Colloid and Interface Science, 128–130*, 227–248.

Guzey, D., & McClements, D. J. (2007). Impact of electrostatic interactions on formation and stability of emulsions containing oil droplets coated by beta-lactoglobulin–pectin complexes. *Journal of Agricultural and Food Chemistry, 55*(2), 475–485.

Hecht, J. P., & King, C. J. (2000). Spray drying: influence of developing drop morphology on drying rates and retention of volatile substances. 1. Single-drop experiments. *Industrial & Engineering Chemistry Research, 39*(6), 1756–1765.

Huang, L. X., Kumar, K., & Mujumdar, A. S. (2006). A comparative study of a spray dryer with rotary disc atomizer and pressure nozzle using computational fluid dynamic simulations. *Chemical Engineering and Processing, 45*(6), 461–470.

International Dairy Federation. (1991). Anhydrous milk fat. Determination of peroxide value. In *International IDF standards 1991*, Square Vergot 41, Brussels (Belgium).

Jafari, S. M., Assadpoor, E., He, Y., & Bhandari, B. R. (2008). Encapsulation efficiency of food flavours and oils during spray drying. *Drying Technology, 26*, 816–835.

Jourdain, L. S., Schmitt, C., Leser, M. E., Murray, B. S., & Dickinson, E. (2009). Mixed layers of sodium caseinate + dextran Sulfate: Influence of order of addition to oil–water interface. *Langmuir, 25*(17), 10026–10037.

Katsuda, M. S., McClements, D. J., Miglioranza, L. H. S., & Decker, E. A. (2008). Physical and oxidative stability of fish oil-in-water emulsions stabilized with β-lactoglobulin and pectin. *Journal of Agricultural and Food Chemistry, 56*(14), 5926–5931.

Klinkesorn, U., Sophanodora, P., Chinachoti, P., McClements, D. J., & Decker, E. A. (2005). Increasing the oxidative stability of liquid and dried tuna oil-in-water emulsions with electrostatic layer-by-layer deposition technology. *Journal of Agricultural and Food Chemistry, 53*(11), 4561–4566.

Kruse, J., Kanzow, J., Rätzke, K., Faupel, F., Heuchel, M., Frahn, J., et al. (2005). Free volume in polyimides: positron annihilation experiments and molecular modeling. *Macromolecules, 38*(23), 9638–9643.

Li, Y., Hu, M., Xiao, H., Du, Y., Decker, E. A., & McClements, D. J. (2010). Controlling the functional performance of emulsion-based delivery systems using multicomponent biopolymer coatings. *European Journal of Pharmaceutics and Biopharmaceutics, 76*(1), 38–47.

Liu, L., Zhao, Q., Liu, T., & Zhao, M. (2011). Dynamic surface pressure and dilatational viscoelasticity of sodium caseinate/xanthan gum mixtures at the oil–water interface. *Food Hydrocolloids, 25*(5), 921–927.

Lopes da Silva, J. A., Rao, M. A., Stephen, A. M., Philips, G. O., & Williams, P. A. (2006). Pectins: structure, functionality, and uses. In A. M. Stephen, et al. (Eds.), *Food polysaccharides and their applications*. Boca Raton: CRC Press, Taylor & Francis Group, LLC.

Lucassen, J., & Van Den Tempel, M. (1972). Dynamic measurements of dilational properties of a liquid interface. *Chemical Engineering Science, 27*(6), 1283–1291.

Lutz, R., Aserin, A., Wicker, L., & Garti, N. (2009). Release of electrolytes from W/O/W double emulsions stabilized by a soluble complex of modified pectin and whey protein isolate. *Colloids and Surfaces B: Biointerfaces, 74*(1), 178–185.

Matalanis, A., Jones, O. G., & McClements, D. J. (2011). Structured biopolymer-based delivery systems for encapsulation, protection, and release of lipophilic compounds. *Food Hydrocolloids, 25*(8), 1865–1880.

McClements, D. J. (2006). Non-covalent interactions between proteins and polysaccharides. *Biotechnology Advances, 24*(6), 621–625.

McClements, D. J., Decker, E. A., & Weiss, J. (2007). Emulsion based delivery systems for lipophilic bioactive components. *Journal of Food Science, 72*(8), 109–124.

Moreau, L., Kim, H.-J., Decker, E. A., & McClements, D. J. (2003). Production and characterization of oil-in-water emulsions containing droplets stabilized by b-lactoglobulin–pectin membranes. *Journal of Agricultural and Food Chemistry, 51*(22), 6612–6617.

Murray, B. S., Durga, K., Yusoff, A., & Stoyanov, S. D. (2011). Stabilization of foams and emulsions by mixtures of surface active food-grade particles and proteins. *Food Hydrocolloids, 25*(4), 627–638.

Perez, A. A., Sánchez, C. C., Rodríguez Patino, J. M., Rubiolo, A. C., & Santiago, L. G. (2011). Surface adsorption behaviour of milk whey protein and pectin mixtures under conditions of air–water interface saturation. *Colloids and Surfaces B: Biointerfaces, 85*(2), 306–315.

Rodríguez Patino, J. M., Navarro Garcia, J. M., & Rodriguez Niño, M. R. (2001). Protein–lipid interactions at the oil–water interface. *Colloids and Surfaces B: Biointerfaces, 21*(1–3), 207–216.

Rodríguez Patino, J. M., & Pilosof, A. M. R. (2011). Protein–polysaccharide interactions at fluid interfaces. *Food Hydrocolloids, 25*(8), 1925–1937.

Rodríguez Patino, J. M., Rodríguez Niño, M. R., & Sánchez, C. C. (1999). Adsorption of whey protein isolate at the oil–water interface as a function of processing conditions: a Rheokinetic study. *Journal of Agricultural and Food Chemistry, 47*(6), 2241–2248.

Savitzky, A., & Golay, M. J. E. (1964). Smoothing and differentiation of data by simplified least squares procedures. *Analytical Chemistry, 36*(8), 1627–1639.

Schmitt, C., Sanchez, C., Desobry-Banon, S., & Hardy, J. (1998). Structure and technofunctional properties of protein–polysaccharide complexes: a review. *Critical Reviews in Food Science and Nutrition, 38*(8), 689–753.

Serfert, Y., Drusch, S., Schwarz, K., Schröder, J., Mescher, A., Laackmann, J., et al. Characterization of the spray drying behaviour of emulsions containing oil droplets with a structured interface. *Journal of Microencapsulation*, in press.

Shaw, L. A., McClements, D. J., & Decker, E. A. (2007). Spray-dried multilayered emulsions as a delivery method for ω-3 fatty acids into food systems. *Journal of Agricultural and Food Chemistry, 55*, 3112–3119.

Sperber, B. L. H. M., Schols, H. A., Cohen Stuart, M. A., Norde, W., & Voragen, A. G. J. (2009). Influence of the overall charge and local charge density of pectin on the complex formation between pectin and b-lactoglobulin. *Food Hydrocolloids, 23*(3), 765–772.

Stone, A. K., & Nickerson, M. T. (2012). Formation and functionality of whey protein isolate- (kappa-, iota-, and lambda-type) carrageenan electrostatic complexes. *Food Hydrocolloids, 27*(2), 271–277.

Taherian, A. R., Britten, M., Sabik, H., & Fustier, P. (2011). Ability of whey protein isolate and/or fish gelatin to inhibit physical separation and lipid oxidation in fish oil-in-water beverage emulsion. *Food Hydrocolloids, 25*(5), 868–878.

Taneja, A., & Singh, H. (2012). Challenges for the delivery of long-chain n-3 fatty acids in functional foods. In M. P. Doyle, & T. R. Klaenhammer (Eds.), *Annual review of food science and technology, Vol. 3* (pp. 105–123). Palo Alto: Annual Reviews.

de Vos, P., Faas, M. M., Spasojevic, M., & Sikkema, J. (2010). Encapsulation for preservation of functionality and targeted delivery of bioactive food components. *International Dairy Journal, 20*(4), 292–302.

Walzel, P. (2010). Spraying and atomizing of liquids. In *Ullmann's encyclopedia of industrial chemistry*. Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA.

Walzel, P. (2011). Influence of the spray method on product quality and morphology in spray drying. *Chemical Engineering & Technology, 34*(7), 1039–1048.

Westergaard, V. (2004). *Milk powder technology. Evaporation and spray-drying* (5th ed.). Kopenhagen: Niro A/S.

Xu, D., Wang, X., Jiang, J., Yuan, F., & Gao, Y. (2012). Impact of whey protein – beet pectin conjugation on the physicochemical stability of b-carotene emulsions. *Food Hydrocolloids, 28*(2), 258–266.

Ye, A. (2008). Complexation between milk proteins and polysaccharides via electrostatic interaction: principles and applications – a review. *International Journal of Food Science & Technology, 43*(3), 406–415.