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Phase separation behavior of egg yolk suspensions after anionic polysaccharides addition

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ABSTRACT

The objectives of this study were to understand the interactions between three anionic polysaccharides (gum arabic, xanthan gum and ι-carrageenan) and egg yolk at pH 3, 5, 6, 8, 10 and possible phase separation behavior. Zeta potential of egg yolk was not affected by gum arabic addition while it became more negative at pH 5 after xanthan gum and ι-carrageenan addition. The particle size of ι-carrageenan yolk suspension was considerably higher than the other polysaccharide yolk suspensions at pH below 6 but was dramatically decreased at alkaline pH. Most polysaccharide yolk suspensions formed either a biphasic or a monophasic system, whereas three distinct phases were observed for xanthan gum yolk suspension at pH 6. Protein profile analysis of the lipid-rich cream phase obtained from xanthan gum added yolk showed similarities to apoproteins from low density lipoproteins (LDL) of egg yolk. Microscopy analysis indicated the co-presence of xanthan gum and LDL in the creamy phase, within a network formed by xanthan gum. It was suggested that electrostatic and hydrophobic interactions between the egg yolk and xanthan gum as well as xanthan gum's rheological properties could be responsible for the unique phase separation observed in the study. The findings of this study can form the basis for future studies to develop a new method to separate LDL from egg yolk.

1. Introduction

Low density lipoproteins (LDL), accounting for about 70% of egg yolk dry matter and containing about 90% of egg yolk lipids, are small spherical particles of 16–70 nm diameter (Martinet, Saulnier, Beaumal, Courthaudon, & Anton, 2003). Due to their high content of phospholipids (PL) and unique nanostructure, LDL from egg yolk have great potential as a source for the extraction of PL or as delivery systems. A tedious ultracentrifugation method was reported for LDL separation, but the LDL yield is low (Moussa, Martinet, Trimeche, Tainturier, & Anton, 2002). Another method, consisting of egg yolk fractionation by dilution, ammonium sulfate precipitation and dialysis was also developed to isolate LDL by Moussa et al. (2002). Although the LDL yield was improved in this method up to 67%, a dialysis step was required, which may not be suitable for large scale preparation of LDL.

Polysaccharides, abundantly available natural macromolecules, are used commonly to improve emulsion stability. In addition, polysaccharides have shown potential for selective separation of proteins and lipids from complex systems such as dairy waste and egg yolk to obtain high value bioactive components and improve nutritional properties (Casal, Montilla, Moreno, Olano, & Corzo, 2006; Hatta, Kim, & Yamamoto, 1990; Hatta, Sim, & Nakai, 1988; Shah & Singh, 1992). The use of slightly anionic polysaccharides for the removal of cholesterol and PL from egg yolk has been reported previously. Hatta et al. (1988) used sodium alginate to precipitate lipoproteins from 6-fold diluted egg yolk to obtain a lipid-free supernatant fraction for further immunoglublin Y (IgY) purification. Rojas, dos Reis Coimbra, Minim, & Freitas (2007) examined the impact of several factors such as dilution and ionic strength on cholesterol removal from egg yolk using high-methoxyl pectin. They reported 6-fold egg yolk dilution at lower ionic strengths and pH of 9.2 to be the optimum condition for the precipitation of cholesterol from egg yolk. Hatta et al. (1990) studied the effects of λ-carrageenan and xanthan gum on the purification of IgY from 6-fold diluted egg yolk. Both polysaccharides could precipitate LDL; however, λ-carrageenan gave better results regarding the removal of LDL and improvement of overall IgY extraction yield compared to those of xanthan gum. These studies focused on the removal of lipids from egg yolk in order to separate IgY, where LDL were co-precipitated along with other proteins such as high density lipoproteins (HDL) and phosvitin. Despite all the above developments,

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the complex interactions between egg yolk and polysaccharides are not well understood.

In this study, three anionic polysaccharides, xanthan gum, ι-carrageenan and gum arabic, with distinctive chemical backbone and rheological properties, were used to better understand the possible interactions between yolk and these polysaccharides, which may result in the separation of LDL as a distinct fraction. Xanthan gum is an exo-polysaccharide produced by the microorganism *Xanthamonas campestris*. Xanthan gum can be dissolved in cold water and its solution exhibits pseudoplastic behavior (Phillips & Williams, 2009). Its structure consists of a linear (β 1 → 4) linked D-glucose backbone with trisaccharide side chains of glucuronic acid and mannose attached to the C-3 position of every other glucose unit. About 50% of all terminal mannose residues are pyruvated (Phillips & Williams, 2009). Gum arabic, originating from plant seeds, is a complex polysaccharide consisting of sugars such as rhamnose, glucuronic acid and arabinose as well as some proportion of nitrogenous compounds, including amino acids. Gum arabic is easily soluble in water, and its solution shows Newtonian behavior (Phillips & Williams, 2009). Carrageenans are comprised of a galactose backbone with different proportions and locations of ester sulfate groups. Carrageenans are classified as κ-, λ- and ι-carrageenans, based on their structural differences in terms of anhydrogalactose and ester sulfate contents (Phillips & Williams, 2009).

The objectives of this study were to evaluate possible interactions and resultant phase separation behavior after mixing egg yolk components with the polysaccharides. Treatments that showed potential for LDL isolation were selected for further structural and chemical analyses.

2. Experimental

2.1. Sample preparation

White shell eggs (Grade A), produced by Sparks egg farmers of Lucerne Inc. (Calgary, AB, Canada) were obtained from a local supermarket (Safeway Inc., Edmonton, AB, Canada). Xanthan gum and ι-carrageenan (Commercial grade, Type II) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gum arabic was obtained from Fluka Analytical (Saint Quentin-Fallavier, France). Egg yolks were separated manually from white and gently rolled on Whatman paper to remove albumen. The vitelline membrane was punctured with a sharp blade and egg yolk content was collected in a beaker placed in an ice bath. Polysaccharide solutions were prepared by adding 80 mg of each polysaccharide into 20 g of Milli-Q water in beakers covered with paraffin film, while stirring at ambient temperature (20 °C) until the polysaccharides became completely solvated and homogenous. Yolk/polysaccharide suspensions were prepared by mixing 20 g of fresh yolk with the polysaccharide solutions to achieve final polysaccharide concentrations of 0.4% (w/w, weight of polysaccharide/weight of fresh egg yolk). To study the effect of pH on egg yolk/polysaccharide interactions, pH values were adjusted to 3, 5, 6, 8 and 10 by adding 0.5 or 2 M NaOH or HCl while stirring the sample. After pH adjustment, egg yolk/polysaccharide suspensions were mixed for 1 h at room temperature using a magnetic stirrer at 500 rpm.

2.2. Phase separation behavior

Polysaccharide/yolk and yolk suspensions with no polysaccharide addition were centrifuged at 6000 × *g* for 30 min to observe the phase separation behavior of polysaccharide yolk suspensions. According to McBee and Cotterill (1979), egg yolk can be fractionated into two distinct phases, a soluble phase called plasma and an insoluble fraction referred to as granules by 2-fold dilution of yolk with water at pH 6, which is the natural pH of egg yolk. About 40 mL of suspension was transferred to 50 mL tubes and centrifuged to observe the phase separation. To study plasma/xanthan gum phase separation behavior, 40 mL of plasma from 2-fold diluted egg yolk was mixed with 80 mg of xanthan gum for 1 h and then centrifuged. All fractions obtained after centrifugation were carefully separated and freeze dried (Labconco, model 7806020, Kansas, MO, USA) for further analyses. All experiments were performed in triplicates.

2.3. Lipid determination

To determine the total lipid content, samples were first freeze dried (Labconco, USA). About 1 g of freeze-dried sample was added into 10 mL hexane/isopropanol (3/2, v/v) and 8 mL of 0.73% NaCl, mixed vigorously, and centrifuged at 2000 × *g* for 10 min according to Hara and Radin (1978). Supernatant was collected and dried under a gentle stream of nitrogen. Results were expressed as g/100 g of dry weight. Lipid determination was performed in duplicate using samples obtained from two different experiments.

2.4. Zeta potential (ζ)

Zeta potential of egg yolk/polysaccharide suspensions at different pH levels was measured using Zetasizer 2000 (Malvern Instruments, Worc, UK) at 20 °C. About 0.2 g of sample was diluted to a final volume of 40 mL using Milli-Q water and the pH was re-adjusted to values of 3, 5, 6, 8 or 10 using 0.1 M HCl or NaOH solution while stirring the samples. Measurements were performed on three individually prepared samples and each measurement was an average of six readings.

2.5. Particle size distribution

Particle size distribution was measured using a Mastersizer 2000S (Malvern Instruments, Ltd., Chicago, IL, USA). Water was used as dispersant and refractive index was adjusted to 1.33. Samples were dispersed in Milli-Q water at 1200 rpm until the obscuration value reached 20–30%. Particle size was measured using three individually prepared samples. Each measurement was an average of three readings for 15 s. Particle size was reported as the volume weighted mean diameter (d4,3).

2.6. Confocal laser scanning electron microscopy (CLSM)

Microstructure of cream fractions obtained from yolk/xanthan gum suspensions was studied using Leica TCS SP5 II confocal system (Leica, Mannheim, Germany). Molecular probes Alexa Fluor® 488 Concanavalin A and Alexa Fluor® 555 C2 maleimide (Molecular probes, Invitrogen, Eugen, OR, USA) were used for dying xanthan gum and proteins, respectively. Alexa Fluor 488 was prepared at a final concentration of 200 μg/mL in sodium bicarbonate buffer at pH 8.3, while Alexa Fluor 555 was prepared in 100 μM in phosphate buffer at pH 7. About 10 mg of cream fraction obtained from yolk/xanthan gum suspension was mixed directly with 100 μL of each solution and incubated overnight at 5 °C. The next day, samples were smeared on microscopy slides, covered with a cover slip (no. 2), and examined using immersion oil × 100 objectives. The CLSM was operated at two channels using the fluorescence mode at the excitation wavelength of 495 nm and the emission wavelength of 524 nm for xanthan gum visualization in green; and 555 and 565 nm excitation and emission wavelengths, respectively, for protein visualization in red, while lipids fluoresced in bright yellow to yellow–orange at this range. All images were taken at a

resolution of 512 × 512 pixel. Microscopy analysis was conducted in duplicates using two separately prepared samples.

2.7. Scanning electron microscopy (SEM)

Egg yolk, cream fractions from yolk/xanthan gum suspensions and LDL, all prepared in duplicate were picked up with a wooden stick, released into 2.5% glutaraldehyde in cacodylate buffer at pH 7.5 and left standing for 2 h at ambient temperature (20 °C). Samples were rinsed with the same buffer for 15 min each for three times, and then post fixed in 1% osmium in the same buffer as above for 2 h at room temperature. After this, samples were dehydrated in a series of ethanol baths of increasing concentration from 50%, 70%, 90% to 100%, each for 10 min. Then, they were dried with a critical point dryer, sputter coated with gold and then mounted onto a stub. The samples were examined using a scanning electron microscope (Hitachi S-2500, Tokyo, Japan).

2.8. Protein profile

Protein profiles of selected samples were analyzed at least in duplicates using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in 4–12% Criterion™ XT Bis-Tris precast polyacrylamide gels in a Criterion Cell attached to PowerPac Basic Power (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Protein markers of high range molecular weight were obtained from Bio-Rad Laboratories, Inc. (Hercules, CA, USA). The details of the protocol were similar to those reported previously (Navidghasemizad, Temelli, & Wu, 2014), except that 1× MOP buffer (Biorad) was applied as a running buffer for this study.

2.9. LDL preparation

LDL, used as a control in this study, was prepared according to Moussa et al. (2002). Briefly, ammonium sulfate was added to plasma obtained from 2-fold diluted egg yolk and stirred for 1 h at 4 °C. Then, it was centrifuged at 10,000 × g, the supernatant was collected and dialyzed against distilled water at least for 6 h. Aggregated LDL were collected for the experiments.

3. Results and discussion

3.1. Effect of pH on zeta potential (ζ) of egg yolk polysaccharide suspensions

The nature of interactions between two biopolymers such as proteins and polysaccharides can be electrostatic, hydrogen bonding, hydrophobic attractions or steric repulsion. The types of interaction are dependent on structure but can be modified drastically by environmental factors such as pH and ionic strength (McClements, 2006). Other interactions are van der Waals interactions, which are weaker forces compared to the interactions listed above but can be important for self-association and therefore aggregation of biopolymers such as proteins (Hokins, Rob, & Williams, 1998). Electrostatic interaction has been suggested as the most common force for the complex formation or phase formation of biopolymer mixtures (Samant, Singhal, Kulkarni, & Rege, 1993).

The charge of a biopolymer such as protein is affected by pH. Therefore, the effect of pH on particle charge of LDL, yolk and yolk/polysaccharide suspensions was examined. First, zeta potential of LDL was examined at different pH values because LDL makes up over 70% of egg yolk dry matter. LDL extracted from egg yolk showed a slightly positive charge at pH 6 and its isoelectric point ($\zeta = 0$) was between pH 6 and 7 (Fig. 1a). Zeta potential of egg yolk was positive at pH values of 3 and 5 (35.5 and 10.4 mV, respectively), while it was negative at pH 6 (−9.58 mV) and above (Fig. 1b). Thus,

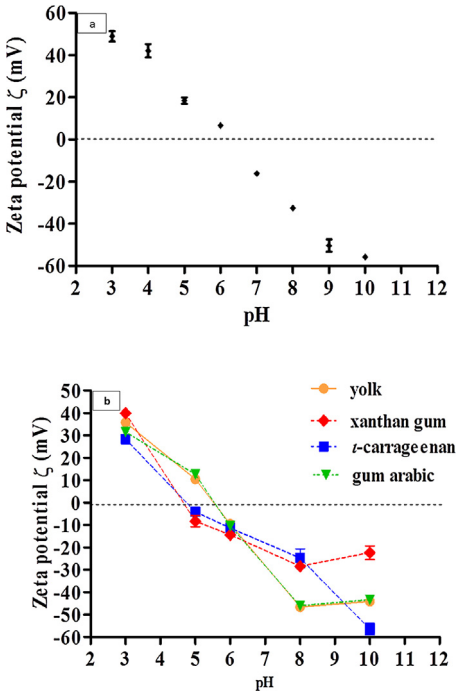

Fig. 1. Average zeta potential (ζ) of (a) control LDL and (b) suspensions prepared from egg yolk: (-●-) egg yolk; (-■-) ι-carrageenan/yolk; (-◆-) xanthan gum/yolk; and (-▼-) gum arabic/yolk as a function of pH ($n = 3$), at a concentration of 0.4% of polysaccharides.

isoelectric point of the yolk was between pH 5–6. The presence of other components such as soluble proteins and highly negatively charged proteins like phosvitin with a broad range of isoelectric points reduced the isoelectric point of the intact yolk compared to that of LDL.

Zeta potential of yolk dispersion was not affected by gum arabic addition at all applied pH conditions while it started to become more negative at pH 5 after ι-carrageenan or xanthan gum addition (Fig. 1b). At pH 8, zeta potential of these dispersions was less negative than that of yolk and it increased further for the xanthan gum addition at pH 10. The yolk/ι-carrageenan suspension was the most negatively charged suspension (−60 mV) at pH 10. It was reported that sulfated polysaccharides such as carrageenan, but not carboxylated polysaccharides such as xanthan gum and pectin, may have some interactions with proteins above their isoelectric points (Samant et al., 1993). Sulfated polysaccharides such as carrageenan interact more strongly with proteins than carboxylated polysaccharides like xanthan gum or pectin, because the electrostatic interactions between amino group ($-NH_3^+$) of proteins and sulfate group ($-OSO_3^-$) of carrageenan are stronger than those with carboxylic group ($-COO^-$) in xanthan gum and pectin (Doublier, Garnier, Renard, & Sanchez, 2000).

3.2. Effect of pH on particle size of egg yolk polysaccharide suspensions

The pH changes may also prompt electrostatic interactions, which can cause aggregation or complex formation between similar or different biopolymers. The average particle size of ι-carrageenan yolk suspension was considerably higher than those of the other polysaccharide yolk suspensions at pH below 6 (Fig. 2); it was the highest at pH 3, with an average particle size of about 540 μm, but decreased at increasing pH and reached its minimum size of −35 μm at pH 8. The occurrence of autohydrolysis of carrageenan in solution at acidic conditions may promote its electrostatic

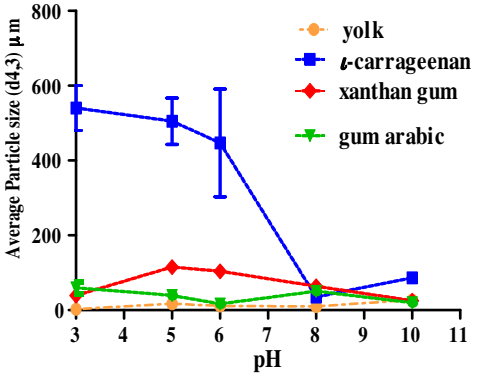

Fig. 2. The average particle size of egg yolk suspension in the presence of 0.4% concentration of gum arabic, ι-carrageenan and xanthan gum at different pH levels ($n = 3$).

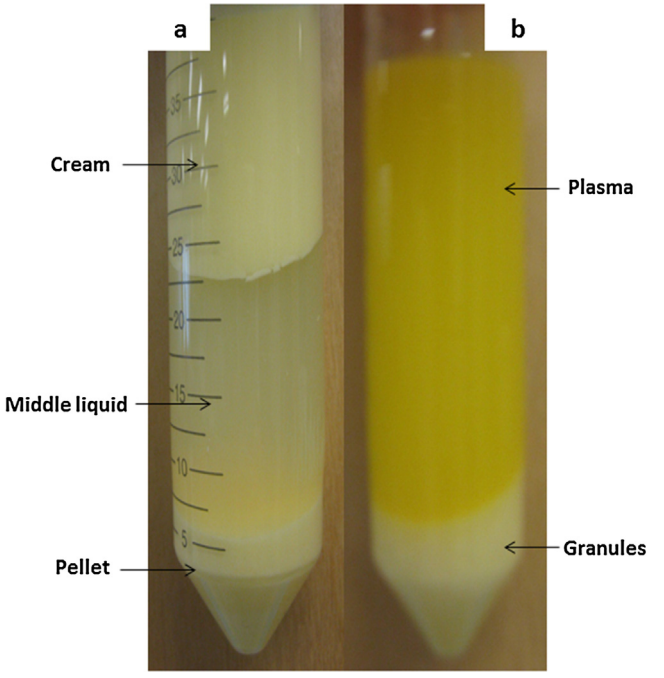

Fig. 3. Phase separation for egg yolk dilution at 0.4% polysaccharide concentration and pH 6, (a) triphasic separation for xanthan gum/yolk suspension, (b) biphasic separation for gum arabic/yolk or ι-carrageenan/yolk.

interactions with proteins due to the release of active groups, leading to the formation of aggregates (Phillips & Williams, 2009). Particle size of xanthan gum/yolk suspension increased from 38 μm at pH 3 to 115 μm at pH 5 but decreased at higher pH levels. While at pH 8, the particle size of yolk/carrageenan suspensions reached to its least (50 μm), a slight re-increase in average particle size (86 μm) was observed at pH 10. The effect of pH on the particle size of gum arabic/yolk suspensions was negligible; but the particle size was somewhat smaller at pH 6 (16 μm), a pH close to egg yolk and LDL isoelectric points, where electrostatic interactions were minimal. The decreased particle size at alkali pHs was due mainly to disassociation of HDL and phosvitin complexes (Causeret, Matringe, & Lorient, 1991), as well as possible changes in polysaccharide–egg yolk interactions.

3.3. Phase separation behavior

Two-fold diluted egg yolk as expected formed two distinct phases: an insoluble phase called granules and a liquid phase referred to as plasma. After centrifugation of polysaccharide/yolk suspensions prepared at different pH levels, most formed either a biphasic or a monophasic system, with the exception of xanthan gum yolk suspension at pH 6 where three distinct phases were formed (a cream on top, aqueous phase in the middle and a pellet at the bottom; Fig. 3). The cream phase contained approximately 72% lipids on dry basis. Shah and Singh (1992) also obtained a cream fraction rich in lipids after mixing carboxymethyl cellulose (CMC) with plasma fraction obtained from 2-fold diluted egg yolk. However, mixing CMC directly with yolk suspension at its natural pH co-precipitated LDL particles along with other proteins into a pellet fraction but no semi-solid cream was formed. Phase separation behavior was affected by the type of polysaccharide used and the pH applied during preparation.

3.4. Characterization of cream phase of xanthan gum/yolk suspension

Protein profile of the cream phase formed by the addition of xanthan gum was examined by SDS-PAGE and compared to those of LDL isolated from yolk, as well as the plasma fraction of egg yolk (Fig. 4). As expected, SDS-PAGE results showed a high level of similarity among the protein profiles of the cream from xanthan gum, the plasma and the LDL. This similarity may arise from the fact that over 85% of plasma dry matter is composed of LDL. LDL apoproteins were reported to have a molecular weight distribution ranging from 11 to 240 kDa (Aluko, Keeratiurai, & Mine, 1998; Guilmineau, Krause, & Kulozik, 2005). A major band over 250 kDa, an apoprotein from LDL, was observed in all three samples. In addition to LDL, some livetins, most probably γ-livetin, were also present in the cream phase. A comparison between the cream and the LDL showed that three more bands with molecular weights of 110, 78 and 29 kDa were present for the cream phase obtained from xanthan gum/yolk suspension (Fig. 4, Lane C1). Livetins are soluble proteins present in the plasma fraction along with LDL. Livetins in plasma are comprised of α-, β- and γ-livetins. γ-Livetin subunits have been reported to have molecular weights of 130, 104, 78 and 29 kDa based on SDS-PAGE analyses, while α- and β-livetins have molecular weights of 78, 40 and 38 kDa (Guilmineau et al., 2005). Therefore, the proteins in the cream are composed of LDL apoproteins and some livetins.

In further investigation, cream and subnatant liquid fractions obtained from plasma xanthan gum were carefully separated for further protein profile analysis as shown in Fig. 4, lanes C2 (cream) and S (liquid subnatant). Protein profiles showed that 10 major bands were present in the subnatant, which could not be absorbed into the cream. These bands had molecular weights of 78, 65, 43, 38, 33, 26, 24, 21, 13 and 10 kDa. Bands with molecular weights of 78, 40 and 38 kDa could be from α- and β-livetins, while the bands of 21 kDa as well as 78 kDa were from γ-livetin (Guilmineau et al., 2005; Jolivet, Boulard, Chardot, & Anton, 2008). Protein bands with molecular weights of 104 and 24 kDa may be related to γ-livetin, which remained in the cream fraction (Aluko et al., 1998). Two bands with molecular weights of 26 and13 kDa present in the subnatant fraction might come from LDL. Interestingly, three bands of 110, 78 and 29 kDa, observed in the cream fraction from xanthan gum/yolk suspension, were absent in the cream formed by xanthan gum/plasma treatment, meaning the purity of LDL obtained from the plasma fraction was higher than that from whole egg yolk. Overall, the results of the SDS-PAGE analysis indicated that LDL was separated into the cream phase; however, some livetins such as γ-livetin may also be present in the cream phase.

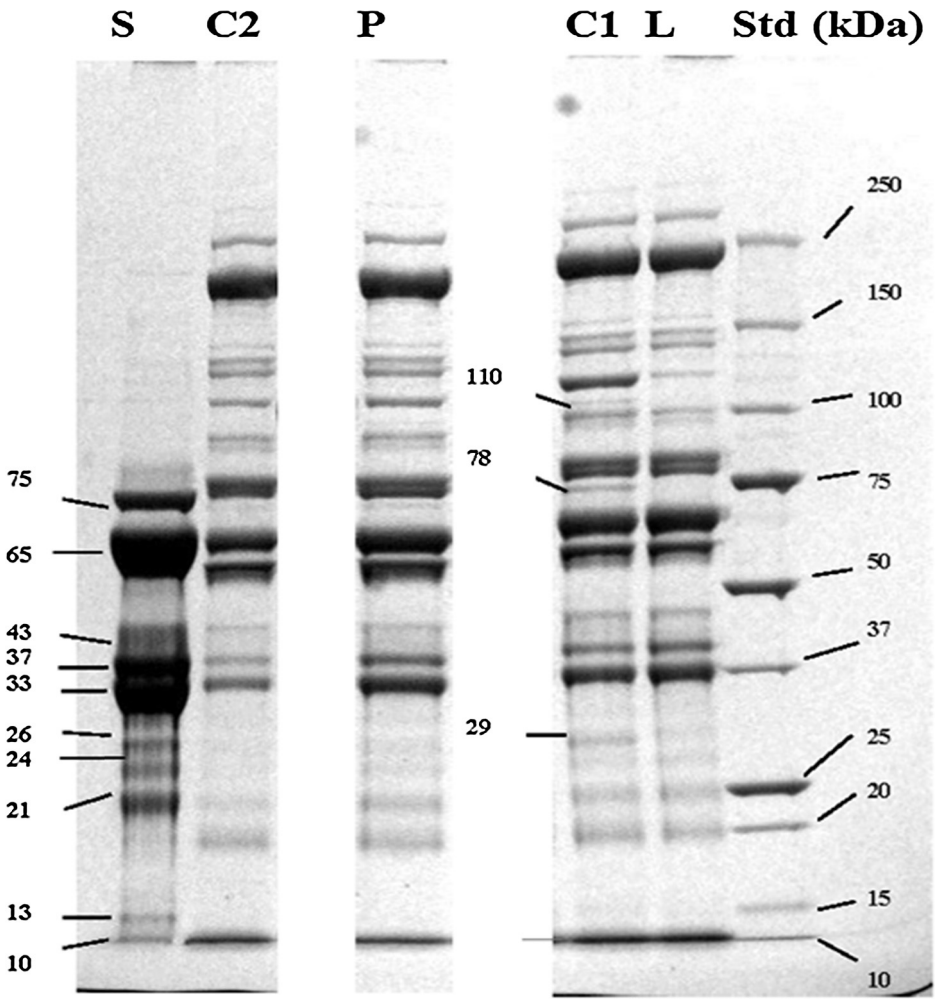

Fig. 4. Protein profile of different egg yolk fractions. (L), LDL; (C1), cream from yolk and xanthan gum suspension; (P), plasma from egg yolk 2-fold dilution; (C2), cream from plasma and xanthan gum suspension; (S), subnatant-liquid from plasma and xanthan gum suspension. Std, standard protein markers.

3.5. Microscopic analyses of cream derived from yolk–xanthan gum

Further microscopy analysis was performed to localize and confirm the co-presence of xanthan and LDL in the cream as well as to obtain additional information about the structural arrangement in the cream. As shown in Fig. 5, egg yolk shows a hive like structure dispersed with several large clumps (Fig. 5a), while LDL showed more homogenous and larger clumps (Fig. 5b). However, the cream phase (Fig. 5c) obtained after xanthan gum addition clearly showed strands of a network-like structure, with aggregated particles attached. SEM results demonstrated that egg yolk components were trapped in the network formed by xanthan.

The presence of proteins and polysaccharide and their arrangement in the cream were further examined by CLSM (Fig. 5d). In the presence of dyes, xanthan gum was visualized in green while proteins and lipids were in red and bright yellow, respectively; the presence of bright orange spots corresponded to the co-localized protein-lipid complexes (Fig. 5e). CLSM micrographs confirmed the co-presence of xanthan gum and LDL particles in the cream fraction.

3.6. Proposed interactions between egg yolk and xanthan gum

When two biopolymers are mixed, they may form monophasic or biphasic systems. Monophasic systems can be formed for two reasons: (a) both are co-soluble in the solution but do not form complexes; the biopolymers do not have any attractive interactions; (b) the interactions between biopolymers result in the formation of new complexes, which are still soluble in the solution. Phase separation and biphasic system formation in a mixture of biopolymers can happen due to associative or segregative interactions (Polyakov, Grinberg, & Tolstoguzov, 1997). If two biopolymers are not thermodynamically compatible, after mixing they form two different phases in the solution. In this case, entropic contribution due to incompatibility between two polymers is more than the enthalpic one. This may happen if two biopolymers contain the same charge and repel each other. Incompatibility may promote self-association among biopolymers of the same type and cause depletion flocculation and phase separation (Polyakov et al., 1997). In the second case, if two biopolymers contain opposite charges, then attractive interactions result in the formation of complexes, which are not soluble, and then phase separation will occur. Possible phase separation after mixing two biopolymers is the outcome of the several factors such as biopolymer interactions, pH, ionic strength and dilution factor (McClements, 2006).

As shown in Fig. 2, the net charge of egg yolk suspensions after xanthan gum or carrageenan addition became slightly negative at pH 6. This can be due to the interaction of the slightly positively charged LDL at pH 6 with the anionic polysaccharide. However, other interactions, such as hydrophobic and hydrogen bonding can also be responsible for protein-polysaccharide interactions. Previously, Goodman and Shafrir (1959) reported that the presence

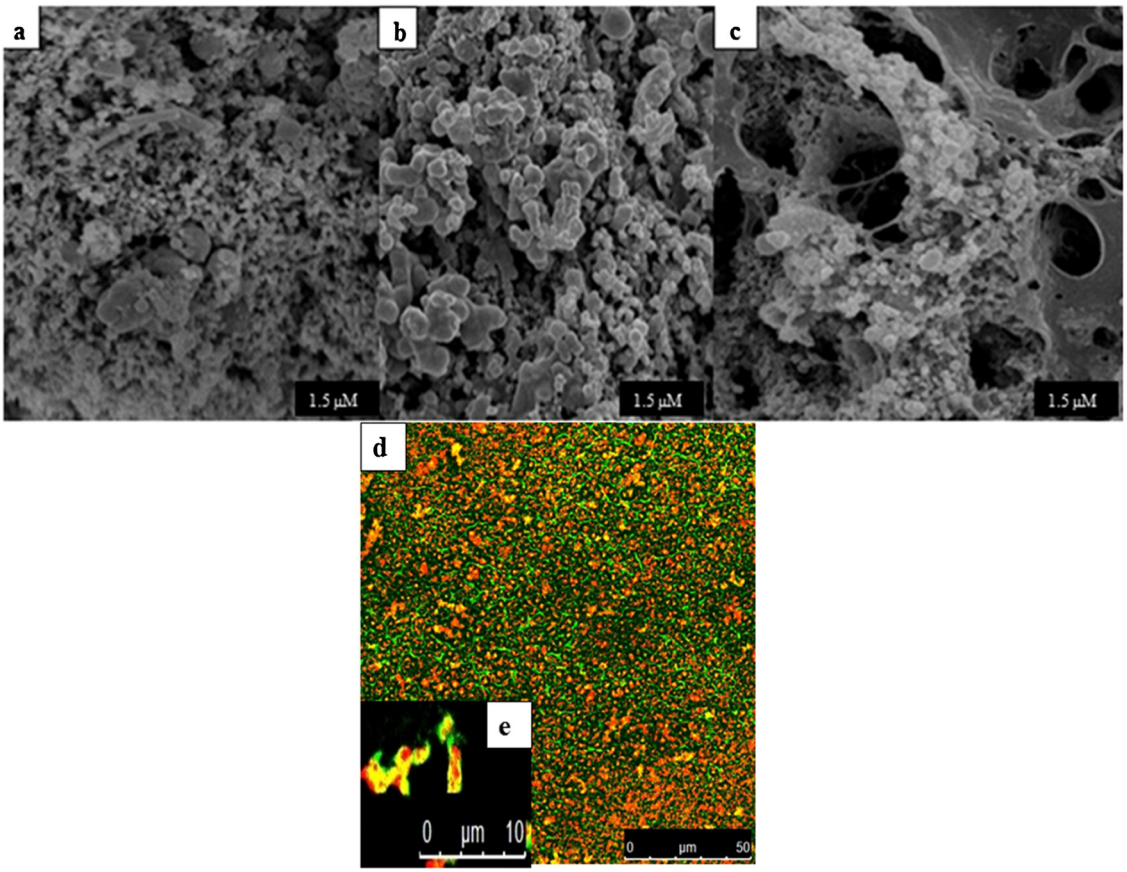

Fig. 5. SEM micrographs of different egg yolk fractions: (a) intact fresh egg yolk, (b) control LDL, (c) cream fraction from xanthan gum yolk, bar = 1.5 μm and confocal micrographs of cream fractions obtained from egg yolk/xanthan gum suspension at pH 6. Bar lengths (d) 50 μm and (e) 10 μm.

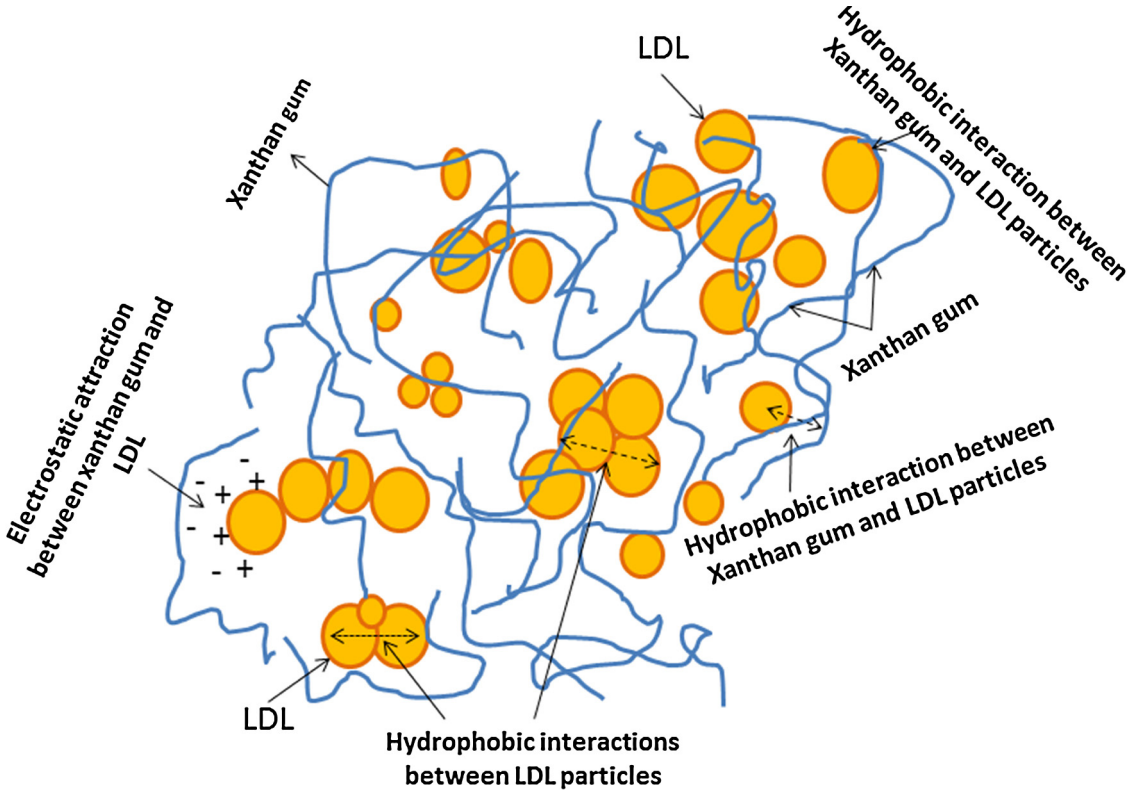

Fig. 6. Suggested mechanism for LDL separation from egg yolk after xanthan gum addition to egg yolk.

of weak hydrophobic groups in human plasma lipoproteins could promote hydrophobic interactions with polysaccharides. In egg yolk, over 40% of amino acids of LDL apoproteins are hydrophobic and some lipids on LDL surface may also show hydrophobic properties (Anton et al., 2003; Tsutsui & Obara, 1982), which may interact with the hydrophobic cavities formed by the hydrophobic pyruvate chains of xanthan gum on its ordered double helix conformation (Jouquand, Aguni, Malhiac, & Grisel, 2008). Since PL are present on the surface of LDL particles, there is always the possibility of interactions between these amphipathic compounds and hydrophilic sites of xanthan gum, which may lead to the formation of insoluble complexes (Kozarac, Cosovic, Mobius, & Dobric, 2000).

Electrostatic interactions between negatively charged xanthan gum and positively charged patches of LDL at pH 6 can be the initial force promoting the interaction between xanthan gum and LDL. As LDL itself contains a high percentage of hydrophobic apoproteins, self-aggregation among LDL particles is also possible. In fact, CLSM of cream (Fig. 5d) showed some lipid-rich aggregates, attaching to the xanthan gum strands. It has been reported that while electrostatic interactions can be the initial factor for interaction between polysaccharides and biopolymers, hydrogen or hydrophobic interactions may play a greater role in the next steps as the related functional groups get more chance for anchoring (Doublier et al., 2000).

Xanthan gum solutions have considerably higher viscosity at similar concentrations compared to the other polysaccharides tested in this study. Below transitional temperature, its helix-like structure can be considered as a rigid rod, which also forms intermolecular bonds. The intermolecular associations form a complex network of weakly bound molecules, which makes xanthan solutions to become very viscous at rest. Xanthan gum solutions also exhibit shear-thinning behavior, indicating that viscosity decreases with shear rate (Phillips & Williams, 2009). Upon mixing and centrifugation, xanthan gum viscosity decreases, facilitating the possible interactions between yolk and xanthan gum. After the removal of external force, xanthan gum solution becomes viscous again and re-forms a gel-like network. LDL trapped in the xanthan gum network will form a cream on top due to the density difference between cream and the other fractions, as major components of LDL are lipids and LDL are lighter than water. Therefore, LDL entrapped in the xanthan gum network is separated due to the density difference between cream and the rest of the suspension. A schematic summary of the suggested mechanism for LDL separation as described above has been shown in Fig. 6.

4. Conclusions

The nature of polysaccharide and pH play great roles in dictating the types of interactions between egg yolk and polysaccharides and therefore the resulting phase separation behavior. Inthis study, among the three polysaccharides investigated, i.e. gum arabic, ι-carrageenan and xanthan gum, only xanthan gum showed distinct phase separation behavior when added to egg yolk. The protein profile of the semi-solid cream separated from 2-fold diluted egg yolk using xanthan gum at egg yolk natural pH 6 was very similar to apoproteins of egg yolk LDL. A combination of electrostatic and hydrophobic forces as well as the rheological properties of xanthan gum was suggested as possible reasons for the unique separation of LDL with xanthan gum, but not with other two polysaccharides. However, further research is required to understand the exact nature of interactions between LDL and xanthan gum as well as to develop possible approaches to separate intact LDL from the polysaccharides for further potential applications.

Acknowledgments

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References

Aluko, R. E., Keeratiurai, M., & Mine, Y. (1998). Competitive adsorption between egg yolk lipoproteins and whey proteins on oil-in-water interfaces. *Colloids and Surfaces B—Biointerfaces*, *10*, 385–393.

Anton, M., Martinet, V., Dalgalarrondo, M., Beaurnal, V., David-Briand, E., & Rabesona, H. (2003). Chemical and structural characterisation of low-density lipoproteins purified from hen egg yolk. *Food Chemistry*, *83*, 175–183.

Casal, E., Montilla, A., Moreno, F. J., Olano, A., & Corzo, N. (2006). Use of chitosan for selective removal of beta-lactoglobulin from whey. *Journal of Dairy Science*, *89*, 1384–1389.

Causeret, D., Matringe, E., & Lorient, D. (1991). Ionic-strength and pH effects on compoistion and microstructure of yolk granules. *Journal of Food Science*, *56*, 1532–1536.

Doublier, J. L., Garnier, C., Renard, D., & Sanchez, C. (2000). Protein-polysaccharide interactions. *Current Opinion in Colloid and Interface Science*, *5*, 202–214.

Goodman, D. S., & Shafrir, E. (1959). The interaction of human low density lipoproteins with long-chain fatty acid anions. *Journal of the American Chemical Society*, *81*, 364–370.

Guilmineau, F., Krause, I., & Kulozik, U. (2005). Efficient analysis of egg yolk proteins and their thermal sensitivity using sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing and nonreducing conditions. *Journal of Agricultural and Food Chemistry*, *53*, 9329–9336.

Hara, A., & Radin, N. S. (1978). Lipid extraction of tissues with a low-toxicity solvent. *Analytical Biochemistry*, *90*(1), 420–426.

Hatta, H., Kim, M., & Yamamoto, T. (1990). A novel isolation method for hen egg yolk antibody-IgY. *Agricultural and Biological Chemistry*, *54*, 2531–2535.

Hatta, H., Sim, J. S., & Nakai, S. (1988). Separation of phospholipids from egg yolk and recovery of water soluble proteins. *Journal of Food Science*, *53*, 425–431.

Hokins, A. R., Rob, I. D., & Williams, P. (1998). Selective separation of proteins from mixtures using polysaccharides. *Biopolymers*, *45*, 97–104.

Jolivet, P., Boulard, C., Chardot, T., & Anton, M. (2008). New insights into the structure of apolipoprotein B from low-density lipoproteins and identification of a novel YGP-like protein in hen egg yolk. *Journal of Agricultural and Food Chemistry*, *56*, 5871–5879.

Jouquand, C., Aguni, Y., Malhiac, C., & Grisel, M. (2008). Influence of chemical composition of polysaccharides on aroma retention. *Food Hydrocolloids*, *22*, 1097–1104.

Kozarac, Z., Cosovic, B., Mobius, D., & Dobric, M. (2000). Interaction of polysaccharides with lipid monolayers. *Journal of Colloid and Interface Science*, *226*, 210–217.

Martinet, V., Saulnier, P., Beaumal, V., Courthaudon, J. L., & Anton, M. (2003). Surface properties of hen egg yolk low-density lipoproteins spread at the air–water interface. *Colloids and Surfaces B—Biointerfaces*, *31*, 185–194.

McBee, L. E., & Cotterill, O. J. (1979). Ion-exchange chromatography and electrophoresis of egg-yolk proteins. *Journal of Food Science*, *44*, 656–660.

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

Moussa, M., Martinet, V., Trimeche, A., Tainturier, D., & Anton, M. (2002). Low density lipoproteins extracted from hen egg yolk by an easy method: Cryoprotective effect on frozen–thawed bull semen. *Theriogenology*, *57*, 1695–1706.

Navidghasemizad, S., Temelli, F., & Wu, J. (2014). Physicochemical properties of leftover egg yolk after livetins removal. *LWT—Food Science and Technology*, *55*, 170–175.

Phillips, G. O., & Williams, P. A. (Eds.). (2009). *Handbook of hydrocolloids*. Boca Raton, FL: CRC Press, Cambridge. Woodhead Pub.

Polyakov, V. I., Grinberg, V. Y., & Tolstoguzov, V. B. (1997). Thermodynamic incompatibility of proteins. *Food Hydrocolloids*, *11*, 171–180.

Rojas, G. E. E., dos Reis Coimbra, J. S., Minim, L. A., & Freitas, J. S. (2007). Cholesterol removal in liquid egg yolk using high methoxyl pectins. *Carbohydrate Polymers*, *69*, 72–78.

Samant, S. K., Singhal, R. S., Kulkarni, P. R., & Rege, D. V. (1993). Protein–polysaccahride interactions: A new approach in food formulations. *International Journal of Food Science and Technology*, *28*, 547–562.

Shah, B. B., & Singh, R. K. (1992). Seperation of egg-yolk proteins and lipids with carboxylmethyl cellulose. *Journal of Food Processing and Preservation*, *16*, 275–288.

Tsutsui, T., & Obara, T. (1982). Hydrophobic components in delipidated granule of egg-yolk. *Agricultural and Biological Chemistry*, *46*, 2587–2589.