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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