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Physicochemical properties of leftover egg yolk after livetins removal

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ABSTRACT

Isolation of immunoglobulin Y (IgY) from egg yolk using water-dilution method generates large quantities of leftover pellet as the co-product. Although egg yolk is well known for its great emulsion property, there is a lack of understanding on how livetins removal would affect the emulsion and rheological properties of the pellet. Therefore, the objective of this study was to determine the effect of soluble protein removal on emulsifying and rheological characteristics of the leftover egg yolk. Egg yolk pellet exhibited distinct structural and physicochemical properties after soluble protein removal. Emulsions prepared from pellet were more vulnerable to coalescence instability than that of egg yolk, although both had a similar oil droplet size. Egg yolk displayed a Newtonian behaviour, compared to a shear-thinning behaviour of pellet. Pellet showed a higher apparent viscosity as well as higher viscoelastic moduli than those of egg yolk, probably due to increased hydrogen bond and hydrophobic interactions in the pellet. Therefore, the changes on the emulsion stability and the rheological properties of egg yolk after soluble proteins removal should be considered in food formulation and processing. Further study is needed to improve the emulsion property of pellet for uses in the food industry.

1. Introduction

Egg yolk is well known for its excellent emulsifying property and has been applied extensively in different food products as an emulsifier. It is also an abundant and inexpensive source of many bioactives (Buxmann, Bindrich, Heinz, Knorr, & Franke, 2010; Kovacs-Nolan, Phillips, & Mine, 2005). Livetins are a class of water-soluble proteins, accounting for 10 g/100 g of fresh egg yolk proteins (Bernardi & Cook, 1960; Kovacs-Nolan & Mine, 2004). Livetins along with low density lipoproteins (LDL), high density lipoproteins (HDL) and phosvitin are the major components found in egg yolk (Kovacs-Nolan et al., 2005). Egg yolk immunoglobulin Y (IgY), a member of livetins, is analogous to mammalian immunoglobulin E. The presence of antibody in egg yolk represents its potential as an immunotherapeutic agent for applications in oral vaccination and passive immunization against bacterial and viral pathogens (Schade et al., 2005; Schade, Zhang, & Terzolo, 2007).

Egg yolk can be separated into two distinct fractions: granules and plasma using high speed centrifugation at 20,000 × g for 12 h or at 78,000 × g for 6 h (Saari, Fennema, & Powrie, 1964; Schmidt, Bessman, Hickey, & Thannhauser, 1956) or through aqueous dilution (Anton & Gandemer, 1997; McBee & Cotterill, 1979). Plasma prepared from 2-fold dilution contains 77–81 g/100 g of egg yolk dry matter and is composed of 73 g/100 g lipids, 25 g/100 g proteins and 2 g/100 g ash. The major constituents of plasma are LDL (85 g/100 g) and livetins (15 g/100 g). Granules make up 19–23 g/100 g of egg yolk dry matter and are composed of 70 g/100 g HDL, 16 g/100 g phosvitin and 12 g/100 g LDL (LDLg) (McBee & Cotterill, 1979). Granules have low solubility and emulsifying activity in distilled water or at neutral pH, but similar emulsifying activity to plasma at ionic strength above 0.1 mol/L at neutral pH, due to the disruption of phosphocalcic bridges and solubilization of HDL and phosvitin (Anton & Gandemer, 1997; Dyerhurdon & Nnanna, 1993). Granules showed better emulsifying stability in their solubilized form, but at acidic pH 3, plasma exhibited better emulsifying properties while granules were prone to creaming (Le Denmat, Anton, & Beaumal, 2000). Kwan, Li-Chan, Helbig, & Nakai (1991) showed that by increasing dilution factor from 2 to 10 using distilled water at egg yolk natural pH 6, over 90 g/100 g of egg yolk lipids precipitated in the granules, whereas the aqueous phase contained mostly soluble livetins with minor amount of lipids in it. The 10-fold dilution method is most widely used in IgY preparation from egg yolk. Kwan et al. (1991) reported that emulsions prepared from the pellet had slightly higher emulsifying activity index and the mayonnaise prepared from the leftover egg yolk showed acceptable properties.

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Although there are extensive studies regarding the emulsifying properties of egg yolk and its fractions i.e. granules and plasma under different thermal, enzymatic, ionic strength and pH conditions (Anton et al., 2003; Anton & Gandemer, 1997; Buxmann et al., 2010; Campbell, Raikos, & Euston, 2005; Davey, Zabik, & Dawson, 1969; Kiosseoglou, 2004; Kiosseoglou & Sherman, 1983; Le Denmat, Anton, & Gandemer, 1999; Nilsson, Osmark, Fernandez, & Bergenstahl, 2007; Sirvente et al., 2007), the effect of livetins removal on the emulsion and rheological properties of the pellet has not been extensively studied.

Livetins account for less than 2 g/100 g of egg yolk dry matter; the pellet after IgY isolation is an abundant source of phospholipids and proteins which can be applied as an emulsifier or can be further processed for value added phospholipids extraction. Therefore, the objective of this study was to determine the effect of soluble protein removal on emulsifying and rheological characteristics of the leftover egg yolk.

2. Materials and methods

2.1. Egg yolk fractionation

White shell eggs (grade A), produced by Sparks egg farmers of Lucerne Inc. (Calgary, Alberta, Canada), were obtained from Safeway stores (Edmonton, Alberta, Canada). Egg yolks were separated from the albumen manually and rolled on grade No. 5 Whatman paper to remove albumen from yolk. The vitelline membrane was punctured with a sharp blade and egg yolk content was collected in a beaker placed in an ice bath. Egg yolk pellet was prepared according to Kwan et al. (1991) with some modifications. Egg yolk was diluted 10-fold with MilliQ water and pH was adjusted to 6 using 1 mol/L HCl. The yolk slurry was mixed for 1 h at 4 °C and was centrifuged at 10,000 × g, at 4 °C for 15 min to separate water-soluble proteins. Then, the supernatant was separated from the pellet. The pellet was centrifuged once again under the same conditions and liquid was separated from the pellet. The egg yolk, pellet and supernatant were freeze dried (Labconco, model 7806020, Kansas, MO, USA) for proximate and protein profile analyses.

2.2. Preparation of reconstituted egg yolk

Supernatant, obtained from 10-fold dilution of egg yolk was divided into small containers and freeze dried. About 1.5–2 g of the freeze-dried supernatant was added back to about 20 g fresh pellet, containing about 43 g/100 g dry matter, in order to replicate the composition of the original intact egg yolk (about 52 g/100 g dry matter), which is referred to as reconstituted egg yolk. Emulsion properties of the reconstituted egg yolk were compared to those of the original yolk and pellet.

2.3. Proximate analysis

Total lipid content was determined according to Hara and Radin (1978) using hexane and isopropanol as solvents and the lipid content was expressed as g/100 g on dry weight basis. Total nitrogen content was determined by Dumas Combustion method using a TruSpec CN analyser (Leco Corp., St Joseph, MI, USA). Total protein concentration was then calculated by multiplying the total nitrogen content by the conversion factor of 6.25.

2.4. Protein profile

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of yolk and its fractions (pellet and supernatant) were carried out in 10–20% Tris–HCl ready gels in a Mini-PROTEAN tetra cell (Bio-Rad Laboratories, Inc., Hercules, CA, USA) attached to a PowerPac Basic electrophoresis apparatus (Bio-Rad Laboratories Inc., Hercules, CA, USA). A high molecular weight protein marker was also obtained from Bio-Rad Laboratories, Inc. (Hercules, CA, USA). Before running the gels, lipids were removed from the freeze dried samples by hexane/isopropanol (3/2, v/v) and the lipid phase was separated by centrifugation at 10,000 × g for 15 min and samples were then dried under a gentle stream of nitrogen. Samples were dissolved in 1X SDS-PAGE running buffer (Tris base 300 g/L, Glycine 140 g/L and sodium dodecyl sulphate 10 g/L) at a concentration of 2.5 mg/mL and shaken in a thermomixer (Eppendorf, Hamburg, Germany) for at least 1 h at 20 °C to dissolve proteins. About 50 μL of protein solution was mixed with 45 μL Laemmli sample buffer and 5 μL of 2-mercaptoethanol (Sigma–Aldrich, St. Louis, MO, USA) and heated at 90 °C for 5 min. About 20 μL of sample was loaded in a well and 1X SDS-PAGE running buffer was used. Gels were stained in a bath of staining solution, shaking gently on an orbital shaker (Lab-Line Max Rotator, Model 4631, Dobunq, IA, USA) for 1 h. The staining solution composition was 90 mL distilled water, 250 mL methanol, 50 mL acetic acid and 100 mL of 10 g/L Coomassie blue. After staining, samples were destained using destaining solution, composed of 500 mL methanol, 400 mL distilled water and 100 mL acetic acid. The gels were scanned using an Alpha Innotech gel scanner (Alpha Innotech Corp., San Leandro, CA, USA) with FluorChem SP software.

2.5. Oil droplet size, flocculation index (FI) and coalescence index (CI)

Freeze-dried egg yolk and pellet were re-constituted with distilled water into final 30 mg/mL protein concentration under stirring using a magnetic stirrer in a cold room (5 °C), at 500 rpm for 1 h to obtain a homogenous suspension. The pH of the suspension was adjusted to pH 6, which is the natural pH of egg yolk using 0.1 mol eq/L HCl or NaOH. Oil-in-water emulsions were prepared by adding 20% (mL/100 mL) sunflower oil. Sunflower oil and protein sample were homogenized for 1.5 min at 20,000 rpm using a high-speed disperser (Ultra-Turrax T25; Janke & Kunkel IKA® Labortechnik, Staufen, Germany). Oil droplet size of emulsions, expressed as volume weighted mean diameter ($d_{4,3}$), was measured by laser light diffraction (Mastersizer 2000S, Malvern Instruments Ltd., Chicago, IL, USA). Emulsion was diluted 10-fold in 10 (g/L) SDS solution. The refractive index for water as dispersant was 1.33 and for oil was 1.46 at 20 °C. At least eight individual egg yolk, pellet and reconstituted yolk emulsions were prepared for the characterization of emulsions. Flocculation and coalescence indices were calculated according to Sirvente et al. (2007). For flocculation determination, emulsions were kept for 4 h at ambient temperature (20 °C). After 4 h, emulsion was diluted 10-fold using water (pH 6) and its volume weighted mean diameter ($d_{4,3}$) was compared to that of the fresh ones, already measured in the presence of 1 (g/L) SDS. Flocculation index was calculated as follows:

$$\text{Flocculation index} = (d_{4,3} \text{ of flocs}/d_{4,3} \text{ of droplets}) \times 10$$

For coalescence index determination, prepared emulsions were stored stationary at 5 °C for 7 days. After 7 days, coalescence index was calculated by estimating the specific surface area (m^2/mL) as $SS = 6/d_{3,2}$, where $d_{3,2}$ is the surface weighted mean diameter of the oil droplet. Prior to coalescence index measurement, samples were diluted 10-fold in 10 (g/L) SDS. The coalescence index was calculated as follows:

Coalescence index$= (SS_0 - SS_7/SS_0) \times 100$, where SS_0 and SS_7 are the specific surface areas of oil droplets of the emulsions stored for 0 and 7 days, respectively.