Food Bioscience 18 (2017) 38–45

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Physicochemical and functional properties of livetins fraction from hen egg yolk

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ARTICLE INFO

Keywords:
Egg yolk livetins
Protein solubility
Isoelectric point
Physicochemical properties
Functional properties

ABSTRACT

This study aimed to evaluate the physicochemical (solubility, surface hydrophobicity, zeta potential, denaturation enthalpy, and protein structural properties) and functional properties (foaming and emulsifying properties) of livetins fraction extracted from hen egg yolk in order to enhance its applications in food and pharmaceutical industries. The effects of pH on protein solubility, surface hydrophobicity (S_0), surface charge, foaming properties and emulsification activities were studied. Protein solubility of livetins was above 86% over a wide pH range (2–12). The surface hydrophobicity analysis of livetins fraction showed the highest fluorescence intensity at pH 2.0. The livetin fraction exhibited a net zero charge at pH 5.70. Thermal analysis of livetins was performed by differential scanning calorimetry (DSC). Denaturation enthalpy (ΔH) and denaturation temperature were 1.29 J/g and 83.3 °C, respectively. Fourier transform infrared (FTIR) spectroscopy indicated the presence of secondary structural elements comprised of β-sheets, α-helices and turns in livetins. The livetins had foaming capacity (21–58%) and emulsification activity (7.3–9.7 m^2/g) at various pH levels (2–12). The foaming ability and emulsification properties (emulsifying activity index and emulsion stability index) were affected by the pH of the medium. The findings suggest that bioactive livetins fraction from hen egg yolk with physicochemical and functional properties could be useful in food and nutraceutical industries for various applications as a functional ingredient.

1. Introduction

Egg yolk is well known as a natural oil-in-water emulsion. Because of its multifunctional properties egg yolk is extensively used in the food, medical, pharmaceutical, and cosmetics industries (Laca, Paredes, Rendueles, & Diaz, 2015). The protein content of egg yolk represents about 15.7–16.6% of all hen egg yolk compounds. Livetins fraction with α, β, and γ-livetins is relatively heterogeneous and accounts for about 9.3% of hen egg yolk proteins. All livetins are water-soluble and the relative amount of the three livetins in the yolk is 2:5:3, respectively. The major fraction of γ-livetin is IgY (immunoglobulin Y) while albumin is the prime component of α-livetin, and α-2-glycoprotein is the major constituent of β-livetin (Kovacs-Nolan, Phillips, & Mine, 2005; Schade & Anibal Chacana, 2007).

The individual components of yolk are difficult to separate, but egg yolk can be easily fractionated by simple dilution and centrifugation into pellet (granule) and supernatant (plasma) (Laca et al., 2015). Fractionation of yolk could enlarge its field of application and increase its value. This study focuses on the IgY-rich livetins fraction, which can be extracted from egg yolk using 10× dilution and centrifugation. The 10-fold dilution method is most widely used in IgY preparation from egg yolk, which allows the isolation of IgY immunoglobulin with a high degree of purity and recovery (95%) and this process could be readily applied on a commercial scale (Ahn, Lee, Singam, Lee, & Kim, 2006).

IgY antibodies, the predominant serum immunoglobulin in birds, reptiles and amphibians, are transferred from the serum of females to the egg yolk, where they offer passive immunity to embryos and neonates. Each hen egg contains 150–200 mg of IgY. The ease of collection and production and often recognized multiple epitopes make IgY a potential source for immunological supplementation of foods (Ahn et al., 2006; Yang, Wen, Zhao, Zhang, & Zhou, 2014). IgY has been used as a food additive to prevent traveler's diarrhea and fever caused by rotavirus or prophylactically applied in baby food in order to increase their resistance (Eckert et al., 2014; Silvan & Tambourgi, 2010). But studies related to its physicochemical and functional properties are still limited.

The evaluation of physicochemical properties of food proteins is very important, particularly if they are intended for use in different food products. Protein solubility is the most basic functional property. Other functional properties such as emulsification capacity and foaming

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http://dx.doi.org/10.1016/j.fbio.2017.04.002
Received 29 November 2016; Received in revised form 4 April 2017; Accepted 15 April 2017
Available online 16 April 2017

properties are mainly dictated by protein solubility and therefore solubility is a good indicator of the protein functionality, and its potential applications (Kinsella & Melachouris, 1976). Unique functional properties of several specific proteins have been exploited for many years (Foegeding & Davis, 2011). Several studies have reported the physicochemical properties of whole egg yolk and its fractions (granule and plasma) (Laca et al., 2015; Rannou et al., 2015). Anton and Gandemer (1997) reported the functional properties (solubility and emulsifying properties) of whole egg yolk and major yolk fractions of plasma and granules obtained using 2x dilution. In recent years, numerous studies have reported the physicochemical and functional properties, especially emulsifying properties, of whole egg yolk and its granule and plasma fractions prepared under different experimental conditions (Denmat, Anton, & Beaumal, 2000; Ercelebi & Ibanoglu, 2010; Ibanoglu & Ercelebi, 2007; Monfort, Manas, Condón, Raso, & Alvarez, 2012; Navidghasemizad, Temelli, & Wu, 2014; Rannou et al., 2015; Sergey & Melnikov, 2002). Phosvitin from egg yolk has also been reported to possess good emulsifying properties at various pH levels (Castellani, David-Briand, Gue´rin-Dubiard, & Anton, 2005; Chung & Ferrier, 1992). However, the literature is lacking information on the physicochemical and functional properties of the IgY-rich livetins fraction obtained from hen egg yolk. If physicochemical and functional properties of livetins were better known that would pave way for more efficient or more specific use of this fraction (livetins) in food, nutraceutical, medical and pharmaceutical industries. Hence, the objectives of this study were to determine the physicochemical and functional properties of the livetins fraction obtained from egg yolk in a wide range of pH conditions.

2. Materials and methods

2.1. Egg yolk fractionation

Freshly laid white shell eggs were obtained from the Poultry Research Centre of the University of Alberta (Edmonton, Alberta, Canada). Egg yolks were manually separated from the white and carefully rolled on Whatman paper (grade No. 4) to remove all albumen from the vitellin membrane. The vitelline membrane was then punctured with a sharp blade and the content of the egg yolk was collected in a beaker placed in an ice bath. Egg yolk water-soluble livetins fraction was separated according to Ahn et al. (2006). Egg yolk was diluted ten times (w/w) with distilled water and pH was adjusted to 5.0 using 1 M HCl. The yolk slurry was kept for 1 h (without stirring) at 4 °C and then centrifuged at 2800 × *g* for 30 min at 4 °C. The water-soluble supernatant that contains IgY was collected, and passed through the ultrafiltration cartridge (Millipore Corporation, model CDUF002TT, Billerica, MA, USA) with a molecular weight cut-off of 30 kDa to concentrate the supernatant. The concentrated sample was freeze dried (Labconco, model 7806020, Kansas, MO, USA), and stored at −20 °C until further testing.

2.2. Yield and proximate composition analysis

Total nitrogen content of the livetins was measured by Dumas Combustion method using a TruSpec CN analyser (Leco Corp., St Joseph, MI, USA). Protein content was calculated by multiplying the total nitrogen value by the conversion factor of 6.25. Fat content was determined by extracting with petroleum ether for 8 h using a soxhlet apparatus and the fat content was expressed as g/100 g sample. The average yield was calculated by measuring the amount of freeze-dried livetins recovered as a percentage of 100 g fresh egg yolk used for the experiment.

2.3. SDS-PAGE profile of livetins

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) under non-reducing conditions was carried out using 4–20% Mini-PROTEAN TGX precast protein gels (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using Mini-PROTEAN tetra cell unit (Bio-Rad, Hercules, CA, USA) to check the status of livetins (including IgY). Five milligrams (in 750 µl water) sample was mixed with 250 µl of 20% SDS for at least 5 min to dissolve proteins. About 50 µl of protein solution was mixed with 50 µl Laemmli sample buffer (1:1) (Bio-Rad, Hercules, CA, USA) and heated at 90 °C for 5 min. About 25 µl of sample was loaded in a well and electrophoresis was performed at constant voltage (150 V). Gels were stained (4 h) in a staining solution containing 0.1% Coomassie Brilliant Blue R-250, 30% methanol and 20% acetic acid, and de-staining was carried out using a solution containing methanol, glacial acetic acid and water (3:2:5). Approximate molecular weight of the livetins was determined using broad range (10–250 kDa) pre-stained protein marker from Bio-Rad (Hercules, CA, USA). The gels were scanned using an Alpha Innotech gel scanner (Alpha Innotech Corp., San Leandro, CA, USA) with FluorChem SP software.

2.4. Protein solubility

Solubility of livetins was determined according to Klompong, Benjakul, Kantachote, and Shahidi (2007). An aliquot of the sample (200 mg) was added to 20 ml of distilled water, and the mixture was adjusted to the desired pH value (2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12) using 0.5 N HCl or 0.5 N NaOH. The mixture was stirred at room temperature (22 ± 2 °C) for 30 min, and then centrifuged at 4500 × *g* for 30 min at 4 °C. Protein content of the supernatant was determined by the Dumas Combustion method using a TruSpec CN analyser (Leco Corp., St Joseph, MI, USA). Protein solubility was calculated using the following equation.

$$\text{Protein solubility}(\%) = \frac{\text{Protein content in supernatant}}{\text{Total protein content in sample}} \times 100$$

2.5. Determination of surface hydrophobicity

The surface hydrophobicity of livetins was determined using the fluorescent probe 1-anilinonaphthalene-8-sulfonic acid (ANS), as described earlier (Wu, Hettiarachchy, & Qi, 1998). The 0.003% livetin solution was prepared in millliQ water, and pH was adjusted to desired pH value (2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12) with 0.5 N HCl or 0.5 N NaOH. Twenty microliters of ANS (8.0 mM) was added to 4 ml of the protein solution. The fluorescence intensity (FI) of livetins was measured using a Shimadzu RF-5301PC spectrofluorophotometer (Kyoto, Japan) at an excitation wavelength of 390 nm and an emission wavelength of 470 nm.

2.6. Zeta potential (ζ) measurement

Surface charge of livetins was measured using Zetasizer Nano ZSP (Malvern Instruments, Worcs, UK). Livetin samples were prepared in milli-Q water at 0.1% (w/v) and adjusted to the desired pH value (2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12) using 0.5 N NaOH or 0.5 N HCl. Then, the samples were subjected to zeta potential measurement.

2.7. Thermal analysis of livetins by a differential scanning calorimeter (DSC)

Thermal analysis of livetins in aqueous solution (30 mg/ml) was carried out using a differential scanning calorimeter (TA DSC Q2000, TA Instruments, New Castle, DE, USA) at a heating rate of 1 °C/min from 10 to 120 °C using nitrogen as purge gas. All samples were weighed accurately in aluminum pans and sealed with hermetic lids. An empty hermatically sealed aluminum pan was used as reference. The enthalpy values were calculated from the peak area of the thermogram.