Food Research International 94 (2017) 13–19

Contents lists available at ScienceDirect

Food Research International

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

Study on external factors affecting egg yolk plasma by asymmetrical flow field-flow fractionation

Haiyang Dou [a,b,*], Xiaoyue Zhang [b], Aixia Zhang [c], Jaeyeong Choi [d], Jing Wang [a], Shigang Shen [a,**], Wenhui Zhang [b], Yueqiu Li [b,**], Liang Ding [b], Seungho Lee [d,**]

[a] Key Laboratory of Analytical Science and Technology of Hebei Province, College of Chemistry and Environmental Science, Hebei University, Baoding 071002, China
[b] School of Medicine, Hebei University, Baoding 071002, China
[c] National Millet Improvement Center, Key Laboratory of Minor Cereal Crops of Hebei Province, Institute of Millet Crops, Hebei Academy of Agriculture and Forestry Science, Shijiazhuang 050035, China
[d] Department of Chemistry, Hannam University, Daejeon 34054, Republic of Korea

ARTICLE INFO

Article history:
Received 8 October 2016
Received in revised form 24 January 2017
Accepted 27 January 2017
Available online 30 January 2017

Keywords:
Asymmetrical flow field-flow fractionation
Light scattering
Egg yolk plasma
Low density lipoprotein
Aggregation

ABSTRACT

In this work, the ability of asymmetrical flow field-flow fractionation (AF4) coupled with UV–vis, multiangle light scattering (MALS), and quasi-elastic light scattering (QELS) (AF4-UV-MALS-QELS) for monitoring aggregation of components of egg yolk plasma was evaluated. The effect of external factors (i.e., pH, storage conditions, freezing and heat treatments) on the egg yolk plasma was studied. The results reveal that the aggregation mechanism of components of egg yolk plasma during heat and freezing treatment is different. The results suggest that the low density lipoproteins (LDLs) in egg yolk plasma undergo a 'clusters-fusion-gel' process under heat treatment at pH 10. The alkaline conditions promote the formation of LDL aggregates. Also, storage conditions play a role in the formation of LDL aggregates. It was found that the hen eggs stored for 7 days at room temperature contain less aggregate than those stored at 4 °C for the same period. The combination of AF4 with online MALS-QELS provided conformational information in terms of the shape and size distribution of LDL aggregates. AF4-UV-MALS-QELS was proved to be a rapid and gentle method for the separation and characterization of egg yolk plasma aggregates.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Hen egg yolk, which consists of plasma and particulate granules, is extensively used as an essential ingredient in a variety of food emulsions such as salad dressings and mayonnaise (Aluko & Mine, 1997; Kiosseoglou & Sherman, 1983). Plasma is composed of ~15% livetin and ~85% low density lipoprotein (LDL), while the granule fraction contains ~70% high density lipoprotein (HDL), ~16% phosvitin linked by phosphocalcic bridges (Anton, 2013). Recently, it has been reported that LDL fraction of egg yolk plasma is the most important contributor to egg yolk emulsifying properties (Jolivet, Boulard, Beaumal, Chardot, & Anton, 2006).

LDLs are spherical particles (17–60 nm in diameter), consisting of a core of triglycerides, cholesterol, and cholesteryl esters, surrounded by a monofilm of phospholipids in which apoproteins are embedded (Martin, Augustyniak, & Cook, 1964). LDL is a colloidal particle with a density of 0.98 g mL^{-1}. Thus, LDL is available to participate in the formation of interfacial film between oil and water. The solubility and hydrophobicity of proteins are derived from physicochemical characteristics (such as the molecular size, conformation and net charge), which could influence the emulsifying property of proteins (Anton et al., 2003). LDL aggregation can lead to a decrease in solubility and an increase in the apparent viscosity of egg yolk plasma solution due to the formation of cohesive gels (Speroni et al., 2005). LDL aggregates also play an important role in the textural properties of final food products. Many industrial food products are prepared with egg yolk under different pH and ionic strengths, which is dependent on the formulation of the products. Moreover, industrial egg yolk has to be pasteurized to ensure microbiological safety (Guilmineau & Kulozik, 2006a, 2006b). These extrinsic factors (i.e., pH, ionic strength, and freezing and heat treatments), may result in a change in the structure of LDL and/or a formation of LDL aggregates, which could affect the emulsifying properties of proteins (such as flocculation) (Speroni et al., 2005). It was reported that heating of the egg yolk prior to emulsification slightly increases the oil droplet size and drastically decreases the level of flocculation

* Correspondence to: H. Dou, Key Laboratory of Analytical Science and Technology of Hebei Province, College of Chemistry and Environmental Science, Hebei University, Baoding 071002, China.
** Corresponding authors.
E-mail addresses: douhaiyang-1984@163.com (H. Dou), shensg@hbu.edu.cn (S. Shen), lyueq@163.com (Y. Li), slee@hau.kr (S. Lee).

http://dx.doi.org/10.1016/j.foodres.2017.01.025
0963-9969/© 2017 Elsevier Ltd. All rights reserved.

(Guilmineau & Kulozik, 2006a). Harrison and Cunningham (1986) reported that freezing of egg yolk could induce irreversible viscosity increase and solubility reduction. In order to fully understand why the properties of an emulsion change, it is important to understand how the emulsifier is affected by environmental and processing conditions. Thus, detailed characterization of egg yolk treated with these external factors (pH, ionic strength, and, freezing and heat treatments) is demanded for better understanding of its functionality in emulsion.

Up to now, several analytical techniques have been applied to characterize the egg yolk protein aggregates. They include sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE), transmission electron microscopy (TEM) and quasi-elastic light scattering (QELS) (Anton et al., 2003; Speroni et al., 2005). Most egg yolk livetins can be separated by SDS-PAGE. However, delipidation of egg yolk lipoproteins prior to SDS-PAGE analysis is required to improve the electrophoretic mobility (Guilmineau, Krause, & Kulozik, 2005). TEM provides the information on both the morphology and the size distribution of the sample at the same time. QELS is easy to use and allows fast analysis. However, the size distributions obtained by both techniques may not be highly reliable or reproducible for the samples of high polydispersity, especially for those containing aggregates (Dou et al., 2013; Hagendorfer et al., 2012). Due to the small imaged area to obtain the size distribution, the reliability of the TEM analysis is decreased especially with samples of high polydispersity. And TEM is time-consuming and generally requires a relatively complicated preparation procedure for biosamples. In QELS analysis of polydisperse samples, the signals from smaller particles may be masked by those from larger ones, because the scattered light intensity is governed by the sixth power of the particle diameter. Consequently, it is essential to provide a convenient method that allows direct, rapid and cost-effective evaluation of the effect of external factors on the egg yolk plasma aggregation.

Asymmetrical flow field-flow fractionation (AF4), an alternative tool for the separation and characterization of biosamples, has attracted increasing interest in recent years owing to its broad dynamic size range (approximately from 1 nm up to about 1 μm for the AF4 normal mode) and the utilization of an "open channel" void of stationary phase or packing materials (Giddings, 1993; Wahlund & Giddings, 1987). Separation of analytes in AF4 is achieved solely through the interaction of the sample with the external perpendicular physical field, rather than through the interaction with the stationary phase. The absence of stationary phase makes AF4 gentle with no or limited shear and mechanical stress applied on the sample components, particularly suited for the analysis of delicate analytes (such as proteins) with full preservation of their native properties. Compared to microscopic technologies such as TEM, the size distribution of sample determined by AF4 is more representative as considerably larger sample amount is analyzed in single AF4 run (Dou, Zhou, Jang, & Lee, 2014; Kim, Lee, Lim, & Moon, 2013; Williams, Runyon, & Ashames, 2010). Another main advantage of AF4 is that it allows analysis of biosamples in the formulation buffer, which guarantees the maintenance of their native properties.

In our previous work (Dou et al., 2016), AF4 coupled with multiangle light scattering (MALS) and fluorescence detectors has shown potential capability for the analysis of egg yolk plasma. In the present work, the ability of AF4 coupled online with UV, MALS and QELS (AF4-UV-MALS-QELS) for monitoring of the external factor-induced changes in egg yolk plasma was evaluated. The aim of the present work is to study the effect of external factors (such as pH, storage conditions, and heat and freezing treatments) on hen egg yolk plasma by AF4-UV-MALS-QELS.

2. Materials and methods

2.1. Materials

Imidazole, sodium chloride (NaCl), potassium chloride (KCl) and calcium chloride ($CaCl_2$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrochloric acid (35–37%) and sodium hydroxide (NaOH) were purchased from Samchun Pure Chemical Co., Ltd. (Pyeongtaek, Korea) and used to adjust the pH of carrier liquid. Deionized water was obtained from a Milli-Q Plus Ultra-Pure Water system (Millipore, MA, USA). All chemicals were of analytical grade and used without further purification.

2.2. Preparation of egg yolk plasma

Fresh hen eggs were purchased from a local supermarket. The eggs were manually broken, and the albumen was discarded. The yolks were carefully rolled on a filter paper to remove albumen adhering to the vitellin membrane (Anton et al., 2003). The membrane was then punctured to flow out unspoiled egg yolk. Eight egg yolks were pooled and gently mixed at 4 °C. The egg yolk plasma was prepared by the method described by Mc Bee and Cotterill (1979) with some modification in order to study the effect of external factors on the egg yolk plasma. The salts in egg yolk mainly include 40 mM NaCl, 53 mM KCl, and 5 mM Ca^{2+} at pH = 6.2 (Dou et al., 2016). 4 mL of pooled egg yolk was diluted with an equal volume of solution with various salt compositions (i.e., NaCl, KCl, and $CaCl_2$) and pH (i.e., 3.0, 6.2 and 10.0). In some cases, 10 mM imidazole was added into carrier liquid as a buffer. Then the mixture was stirred with a magnetic stirrer for 1 h at 4 °C. The suspension was centrifuged at 10000*g* for 30 min at 4 °C, and the supernatant (plasma) was separated from the sediment (granule). The supernatant was centrifuged twice under the same conditions for complete separation of plasma and granule fractions. In order to investigate the effect of freezing and heat treatments, the egg yolk plasma samples prepared with carrier liquids containing 10 mM imidazole, 40 mM NaCl, 53 mM KCl and 5 mM Ca^{2+}, and various pHs (i.e., pH = 3.0, 6.2, or 10.0) were heated at 60 °C for 2 h. The egg yolk plasma samples prepared with carrier liquids containing the same salt compositions at pH = 6.2 were frozen at −18 °C for 4 h, 8 h, and 48 h, respectively. In order to investigate the effect of storage conditions, the whole hen eggs were stored at various temperature and time, and then egg yolk plasma samples were prepared with carrier liquid containing 10 mM imidazole, 40 mM NaCl, 53 mM KCl, and 5 mM Ca^{2+} at pH = 6.2. The prepared egg yolk plasma solution was used immediately (the same day).

2.3. AF4 analysis of egg yolk plasma

The AF4 system used in this work was an Eclipse 2 Separations System (Wyatt Technology Europe, Dernbach, Germany). It was connected to a Model 500 UV–vis detector (Chrom Tech Inc., MN, USA) operating at the wavelength of 280 nm, and a DAWN EOS MALS detector (Wyatt technology, Santa Barbara, CA, USA) operating at the wavelength of 690 nm. In addition, MALS detector was modified at an angle of 108°, where a quasi-elastic light scattering (QELS) (DynaPro NanoStar, Wyatt Technology Corporation, CA, USA) apparatus was connected via a glass fiber cord. An Agilent 1100 pump (Agilent Technologies, Waldbronn, Germany) with an in-line vacuum degasser delivered the carrier liquid into the AF4 channel. Between the pump and the AF4 channel was placed a 0.1 μm YYLP membrane filter (Millipore Corp. MA, USA) to ensure that the carrier liquid entering AF4 channel is particle-free. The channel was assembled with a 350 μm-thick Mylar spacer and a regenerated cellulose membrane with the cut-off molecular weight of 10 kDa. The actual channel thickness was measured to be 285 μm from the elution time of ferritin based on the method reported by Litzen (1993). The channel geometry was trapezoidal with the tip-to-tip length of 26.5 cm and breadths at the inlet and the outlet of 2.2 and 0.6 cm, respectively. Prior to injection into AF4 channel, egg yolk plasma solution was further diluted with the carrier liquid (5:95 v/v). The resulting sample concentration was about 3 mg mL^{-1}. Then 20 μL of egg yolk plasma solution was injected into channel at the flow rate of 0.2 mL min^{-1} for 2 min. The cross flow rate of 2.0 mL min^{-1} was

Download English Version:

https://daneshyari.com/en/article/5768280

Download Persian Version:

https://daneshyari.com/article/5768280

Daneshyari.com