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Effect of selected pre-treatments on folate recovery of granule suspensions prepared from hen egg yolk

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ABSTRACT

Fractionation of hen egg yolk allowed the concentration of 5-methyl-tetrahydro-folate (5-MTHF) in granule faction of yolk. Consequently, the pre-treatment methods naming as increasing ionic strength and power ultrasound was used to improve concentration of folate in granule fraction. The composition of granules after pre-treatment was characterized. The polyacrylamide gel electrophoresis showed that ionic strength modifications induced the loss of granule structure by solubilisation of proteins. Granule contained 21 μg of 5-MTHF/g granules extract on dry basis. However, by increasing ionic strength (>0.15 M NaCl) concentration of 5-MTHF was decreased in granule fraction due to integration of granule structure and release of folate in plasma fraction. Concerning ultrasound pre-treatments, the significant changes in total protein, lipid, cholesterol, fatty acid and 5-MTHF content of granules was not observed by increasing the ultrasound time. All results were indicative of the stable structure of granule and that its modification is difficult. The application of salt addition and increasing ionic strength indicates the importance of the existence of phosphocalcic bridges between phosphate groups of HDL-phosvitin and 5-MTHF which keeps folate (5-MTHF) stable in this compact network.

1. Introduction

Hen egg yolk is a major ingredient for many food products due to its excellent functional properties such as, antioxidant and mineral binding properties of phosvitin in yolk granule (Choi, Jung, Choi, Kim, & Ha, 2005; Jiang & Mine, 2001; Lee, Han, & Decker, 2002; Nakamura, Ogawa, Nakai, Kato, & Kitts, 1998), egg yolk lecithin (Rossi, 2007), egg yolk antibodies (Schade, Zhang, & Terzolo, 2007) and etc. Hen egg yolk is a complex system of an aqueous phase (plasma) and insoluble granules. Native egg yolk can be separated into its main fractions of granule and plasma by centrifugation techniques. Over the last 40 years, different centrifugal techniques have been studied to separate egg yolk components (Laca, Paredes, & Diaz, 2010; Mc Bee & Cotterill, 1979; Strixner & Kulozik, 2013). Granules are major components of yolk, and contain 70% lipovitellins, 16% phosvitin and 12% low density lipoproteins (LDL) (Causeret, Matringe, & Lorient, 1991). Egg yolk granule is mainly composed of proteins and contains low lipid content compared to egg yolk (Laca, Paredes, Rendueles, & Díaz, 2014), moreover, higher 5-methyl-tetrahdro-folate (5-MTHF) content compared to the native egg yolk (Naderi, House, & Pouliot, 2014). 5-MTHF is the most biologically active form of B9-vitamin also generically known as folate (Seyoum & Selhub, 1998). Considering dried egg yolk containing 3–6 μg of 5-MTHF per gram sample, separated granule at lab-/pilot-scale contains 10–20 μg folate/g dry granule (Naderi et al., 2014), which corresponds to 5% of the recommended daily allowance of 400 μg folate for adults (Medicine & Medicine, 1998). Hence, the granules with high protein and low cholesterol contents (Laca et al., 2010) together with enrichment in 5-MTHF (Naderi et al., 2014) should make their utilization more interesting as health ingredient.

Granular units consist of a high density lipoprotein (HDL)-phosvitin complexes. The structure of granules closely depends on ionic strength and presence of bi-or polyvalent mineral cations (Anton & Gandemer, 1997). Granular complex structures form a compact and non-soluble network. The role of possible protein–-protein interactions between granule HDL-phosvitin and low density lipoprotein (LDL)-apoprotein constituents in forming this stable network is still unexplored (Strixner, Sterr, Kulozik, & Gebhardt, 2014). The present work aims at improving 5-MTHF recovery by removing soluble compounds from granules. Initially, the

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applied technique for disrupting the granular structure should have minimum adverse effect on 5-MTHF stability.

Previous studies showed the importance of modification of ionic strength on some physiochemical properties of granules such as viscosity and solubility (Causeret et al., 1991). It was presented that the solubility of granules was relatively increased (80%) when ionic strength fixed to 0.3 M NaCl, and remained the same even at 0.5 M NaCl (Anton & Gandemer, 1997). Chang, Powrie, and Fennema (1977) also observed complete dissociation of granules when adding 1.71 M NaCl to the yolk. Findings of another study stated that unrelated to the concentration of protein in granule this dissociation occurs when the concentration of NaCl reaches 0.58 M (Causeret et al., 1992). In accordance with these studies, in our preliminary experiments the granules were diluted in different concentration of NaCl solutions from 0 to 0.75 M. Based on our observations the dissociation of granules occurred in 0.5 M NaCl and remained the same at 0.55 and 0.75 M (data are not presented). Subsequently, we attempted to re-suspend granule in saline solution with gradient concentration (up to maximum 0.5 M NaCl) and study the effect of structural integration on composition of granule, mainly 5-MTHF.

Also, application of ultrasound assisted extraction process is well documented in food and related industries (Vilkhu, Mawson, Simons, & Bates, 2008) with the aim of increasing the yield and rate of extraction, and achieving higher processing throughput while the nutritional and bioactivity of certain constituents of food can be preserved (Soria & Villamiel, 2010). The pre-ultrasound treatment is effective in size reduction and maximizes surface area for achieving rapid and complete extraction (Balachandran, Kentish, Mawson, & Ashokkumar, 2006). In different studies, the application of ultrasound was introduced being useful in degradation of high molecular weight proteins and cholesterol in egg yolk (Sun, Yang, Zhong, Zhang, & Wang, 2011). Moreover, ultrasonication can break up whey protein aggregates and assist in breaking apart casein micelles (Chandrapala, Martin, Zisu, Kentish, & Ashokkumar, 2012).

Granule has compact and insoluble structure and we attempted to disrupt granules by applying pre-treatment techniques (increasing ionic strength and using power ultrasound) followed by fractionation through centrifugation. The ultimate goal was using non-toxic procedures to modify egg yolk granule composition and study the effect on composition of granular sub-fractions.

2. Materials and methods

2.1. Materials and chemicals

Petroleum ether, ethyl ether, chloroform, methanol, acetic acid and hydrochloric acid were purchased from Fisher Scientific (NJ, USA). Sodium chloride, carbon tetrachloride (99.9% purity), (+)-sodium L-ascorbate, and 5-MTHFdisodium salt (90% purity) were obtained from Sigma–Aldrich (MO, USA). Cholesterol (Cholest-5-en-3β-ol with purity of >99%) was purchased from Merck (Darmstadt, Germany). Acetonitrile and ethanol was of HPLC gradient grade and from Sigma–Aldrich (MO, USA); other chemicals were of analytical quality. Water was purified ($\leq$0.1 μS cm^{-1}) using a Milli-Q system of Millipore (LA, USA). The stock solutions of folate for HPLC, 50 nM, were prepared in ascorbate buffer (20 g/L sodium ascorbate; 12.1 g/L Trizma base; pH 7.8). The SRM (standard reference material) 1544 Fatty Acids and Cholesterol in a Frozen Diet Composite and SRM 1946 Lake Superior Fish Tissue were purchased from the National Institute of Standards and Technology (NIST) and they were used to support the measurement of fatty acids.

2.2. Preparation of yolk and granules

Fresh white shell eggs of weight class L (58–63 g) were purchased separately for each batch of experiments from a local supermarket. The eggs were broken manually and the yolks were separated from albumen and chalazae using blotting paper. The vitelline membranes were ruptured by using tweezers and yolk material was collected. The yolk was diluted with Milli-Q water (1:1 v/v) and fractionated based on the method described by (Naderi et al., 2014), using a tubular bowl centrifuge (CEPA Centrifuge, series LE, Germany) with a maximum centrifugal force of 40,000×g. The diluted fresh egg yolk was pumped into the centrifuge and granules were separated from plasma. The sedimented granule fraction in centrifuge cylinder was collected and divided into three parts: one part of granule was kept in their native condition (control granule: G_C), second part was used for pre-treatment analysis using saline solution, and the third part was used for ultrasound power pre-treatment analysis.

2.3. Application of pre-treatments on granule suspensions

2.3.1. Re-suspension in saline solution (NaCl) with different ionic strength

The granules were diluted (1:2 w/v) in NaCl solutions of increasing ionic strength (0, 0.1, 0.15, 0.25, 0.55 M NaCl) and homogenized by Ultra-Turrax T-25 treatment (IKA® Werke Staufen, Germany) for 30 s pulses at 6500 rpm. Then samples were centrifuged at 10,000×g for 20 min and the supernatants were separated from precipitated granule and each fraction was collected in separate tubes. Consequently, according to their ionic strength, there were two set of samples: granules (G_{H2O}, $G_{0.1}$, $G_{0.15}$, $G_{0.25}$) and plasma (P_{H2O}, $P_{0.1}$, $P_{0.15}$, $P_{0.25}$). Each set of samples were analyzed separately.

2.3.2. Ultrasound treatments

The granule fractions separated from yolk were freeze-dried at −70 °C under vacuum by using pilot lyophilizer (VirTis Ultra 50L, SP Scientific, NY, USA). The dry matter of freeze-dried granules was 99.26 ± 0.74 (g/100 g). The granules were mixed with ×2 volume of Milli-Q water. The granule solutions were accurately weighed and dispersed with an ultrasonic instrument (Virsonic 475 Cell Disrupter) in a 100 mL sealed flask. The actual power delivered into the system was 95 W at 20% amplitude. An ultrasonic probe with a tip diameter of 7 mm was fitted into the flask and the tip was inserted at the half height of the solution. The ultrasonic process time was set at 0, 5 and 10 min for 95 W at 20% amplitude. The temperature of solutions was controlled by immersion the sample flask into an ice-bath. After ultrasound treatment, all samples were centrifuged (10,000×g; 20 min; 23 °C). All the experiments were performed in triplicate. The analyses were performed on each separated fraction. Weights were registered before and after centrifugation.

2.4. Chemical analysis

Moisture and total lipid content were determined based on the official method of the AOAC (2005) method. The total protein content of egg yolk and its fractions was determined with a nitrogen gas analyzer system (Model 601-500, LECO Corporation, St Joseph, MI, USA). The instrument was previously calibrated with ethylenediaminetetraacetic acid (EDTA). Total protein determined from the nitrogen content of the yolk material using a conversion factor of 6.25 for all egg samples. Sodium was determined by ICP (ICP-OES, Optima 4300, Dual View, Perkin–Elmer, Shelton, CT, USA). The wavelength used for Na element was 589 nm. Each