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The use of high hydrostatic pressure to generate folate-enriched extracts from the granule fraction of hen's egg yolk

Nassim Naderi [a], Alain Doyen [a], James D. House [a,b], Yves Pouliot [a,*]

[a] Institute of Nutrition and Functional Foods (INAF), Department of Food Science, Université Laval, Québec, QC G1V 0A6, Canada
[b] Department of Human Nutritional Sciences, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

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ABSTRACT

The present work characterized the effects of pre-treatments on the protein profile and microstructure of granule fractions from egg yolk. The granule microstructure was studied using confocal laser scanning microscopy (CLSM). The protein profiles of the pre-treated granule and the corresponding plasma fraction were studied using 2D gel electrophoresis techniques. Further, we explored the potential for using high hydrostatic pressure (HHP) to promote disintegration of the granule structure. The CLSM micrographs provided evidence of the substantial disintegration of granules due to HHP (600 MPa/5 min). Results from high performance liquid chromatography (HPLC) analysis indicated high concentrations of folate in the plasma fractions (230 μg/g dry matter) separated from the HHP-treated granule. Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis revealed the localization of phosvitin in the plasma fraction, which correlated with higher folate concentrations. The results demonstrate that phosvitin and folate were stable under the HHP conditions applied in this study. These findings provide evidence of a putative interaction between phosvitin and folate, and offer an improved model for the structure of granule.

1. Introduction

The granule fraction of egg yolk, derived via centrifugation processes, can yield different compositional properties when compared to native yolk, thus creating opportunities for new applications as ingredients in the food and/or nutraceutical industries. Numerous studies have been conducted in order to understand the contribution of the native structure of yolk granule to its composition and functionality (Anton, Beaumal, & Gandemer, 2000; Castellani, Martinet, David-Briand, Guerin-Dubiard, & Anton, 2003; Guilmineau, Krause, & Kulozik, 2005; Laca, Paredes, & Diaz, 2011; Strixner & Kulozik, 2013). Granules account for 22% of yolk dry matter that composed of almost 47% of yolk proteins and 7% of yolk lipids (Anton, 2013; Anton & Gandemer, 1997). Furthermore, granules contain high density lipoproteins (HDL), phosvitin and low density lipoproteins (LDL) (Anton, Mark, & Anton, 2007).

Granules consist of numerous spherical complexes and, at low ionic strength, they form non-soluble HDL-phosvitin complexes linked by phosphocalcic bridges (Causeret, Matringe, & Lorient, 1991). The presence of numerous phosphocalcic bridges makes the microstructure of the granules very compact, poorly hydrated and accessible to enzymes. This leads to an efficient protection against such processes as thermal denaturation and heat gelation (Sirvente et al., 2007). Previous studies have indicated the importance of ionic strength on some physiochemical properties, including solubility, of egg yolk granules (Causeret, Matringe, & Lorient, 1992; Causeret et al., 1991). The high concentrations of bivalent (Ca^{+2}, Mg^{+2}) and trivalent (Fe^{+3}) cations within the granule matrix and the presence of phosphoprotein (phosvitin) suggest the existence of ionic bridges between the phosphate groups of the phosphoseryl residues within phosphoproteins (Causeret et al., 1992). Previous research has shown that the granular structure is dissociated under high saline conditions (0.3 M NaCl) (Causeret et al., 1991) due to modification of ionic bridges. Knowledge about the molecular structure of egg yolk granules and associated compositional changes due to differences in milieu conditions is limited. Previous investigations on granule structure focused on the impact

* Corresponding author.
E-mail address: Yves.Pouliot@fsaa.ulaval.ca (Y. Pouliot).

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of pH at either 4.0 or 6.5, and the results provided evidence that the sedimentation of granules at pH 4.0 resulted in a more compact conformation of the HDL components and a higher amount of bound lipid in the granule. Furthermore, LDL was observed to be incorporated largely into HDL granules at pH 4.0, due to the isoelectric conditions and steric interactions (Strixner, Sterr, Kulozik, & Gebhardt, 2014). In our previous study, we observed the importance of modifying ionic strength on selected chemical properties of egg yolk granules, including the folate and protein content (Naderi, House, & Pouliot, 2016). Ultrasound as a promising technology has been used in food industry for extraction of bioactive compounds (Chen & Zhu, 2011; Rodrigues, Pinto, & Fernandes, 2008). Compared with conventional extraction methods, application of ultrasound has several advantages, for instance higher extraction rate, shorter extraction time and minimal or no solvent requirement (Chen et al., 2010). Ultrasound has been used for increasing the rate of extraction of folate (water soluble B vitamin) from egg yolk granule and folate appeared to be stable in granule network structure (Naderi et al., 2016).

Recently, the use of high hydrostatic pressure (HHP) for food processing is finding application in the food industry. Equipment for large-scale production of HHP processed products is now commercially available (San Martin, Barbosa-Cánovas, & Swanson, 2002). The use of HHP reflects a novel, non-thermal process in which foods (liquid or solid) are subjected to pressures above 100 and up to 900 MPa (between 400 and 700 MPa in commercial systems) (Gonzalez & Barrett, 2010). High pressure can disrupt the interactions between food components (San Martin et al., 2002). The application of HHP and its effect on the retention of the water soluble vitamins B_1, B_6, and C in a multivitamin model system consisting of egg yolk or a strawberry "coulis" were previously studied (Sancho, Lambert, Demazeau, Largeteau, Bouvier, & Narbonne, 1999). Data from the latter study revealed that B_1 or B_6 vitamins were stable under HHP as retention was greater than 99%. The use of pressure energy typically disrupts weak bonds, such as hydrophobic and electrostatic ones, leaving covalent bonds unaffected (Balny, Mozhaev, & Lange, 1997; Lullien-Pellerin & Balny, 2002). It has been hypothesized that because HHP does not affect covalent bonds, small molecules such as vitamins, color, and flavor compounds will remain unaffected after treatment (San Martin et al., 2002). The high pressure method application was applied to study the iron binding capacity of phosvitin from egg yolk (Castellani, Guérin-Dubiard, David-Briand, & Anton, 2004). Castellani et al. (2004) stated that phosvitin treated by high pressure method (300, 600 MPa/10 min) did not result in aggregation and it kept its high iron binding capacity after treatments. In the current study, we characterize the effects of technological treatment methods on the protein profile and microstructure of granules separated from egg yolk. The folate content and protein profile of granules under strong treatment conditions (HHP at 600 MPa/5 min) were also studied.

2. Materials and methods

2.1. Materials and chemicals

Acetonitrile, methanol and ethanol were of HPLC gradient grade and purchased from Sigma-Aldrich (Kansas City, USA), and other chemicals were of analytical quality. Chloroform, acetic acid and hydrochloric acid were purchased from Fisher Scientific (Bridgewater, USA). Sodium chloride, (+)-sodium l-ascorbate, and 5-MTHF disodium salt (90% purity) were obtained from Sigma-Aldrich (Kansas City, USA). Water was purified ($\leq$-0.1 $\mu S\ cm^{-1}$) using a Milli-Q system of Millipore (Jaffrey, USA). Molecular probe Nile Blue A was from Sigma (Steinheim, Germany). 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 granules

Granules from fresh egg yolks were separated according to method described earlier (Naderi, House, & Pouliot, 2014). Briefly, fresh hen eggs were purchased from a local market. The eggs were broken manually, and the albumen was separated from vitelline. The yolk material was diluted with Milli-Q water (1:1 w/w). The granules were separated from diluted yolk by using a tubular bowl centrifuge (CEPA Centrifuge, series LE, Germany) with centrifugal force of 40,000*g* and flow rate of 100 mL/min.

2.3. Ionic strength modification

The granule fraction was subsequently diluted (1:2 w/w) in NaCl solutions of increasing ionic strength (0, 0.1, 0.15, 0.25, 0.55 M NaCl). The granule suspensions were mixed using Ultra-Turrax T-25 (©IKA® Werke Staufen, Germany) for 30 s at 6500 rpm. The granule suspensions were separated from the supernatant (plasma) by centrifugation at 10,000*g* for 20 min. At an ionic strength of 0.55 M NaCl, the entire granule particles dispersed in the saline solution and transformed into a yellow-translucent suspension. Further centrifugal separation and comparisons of granules were not possible, limiting comparative analysis between granules in solid state and the disintegrated granule in 0.55 M NaCl.

2.4. Mechanical treatments

Ultrasound power and HHP treatment were investigated as mechanical treatments on resuspended native granule. Portions of native granule were hydrated with 2 vol of Milli-Q water. The granule solutions were completely dissipated with an ultrasonic instrument (Virsonic 475 Cell Disrupter) in a sealed flask (100 mL), while the temperature of sample was controlled by keeping it on ice. 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 (PULSAR™ pulsing for 15 s and relaxing for 10 s) with a frequency of 20 kHz and amplitude of 95%. Thereafter, all granule solutions were centrifuged (10,000*g*; 20 min). The precipitated granules were separated from the supernatant and collected in separate vials for further analysis. The supernatants were collected and expressed as plasma for further investigations.

For HHP treatments, granule solution (1%) was prepared by resuspending the granule fraction in Milli-Q water, and hydrating the samples for 18 h at 4 °C on a rotating plate. The hydrated granule samples were transferred to 1 L polyethylene terephthalate (PET) bottles leaving no headspace. The HHP treatments were performed at 600 MPa during 5 min at room temperature in a discontinuous hydrostatic pressurization unit Hiperbaric 135 (Hiperbaric, Burgos, Spain) with water as the pressure transmission medium. The stainless-steel pressure vessel measured 0.30 m in diameter and 2.20 m in length with a working volume of 135 L. The HHP treated granule samples were centrifuged (10,000*g*; 45 min; 4 °C) to separate the granule from supernatant. All the experiments were performed in triplicate in four different batches of fractionated granule.