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Scaling-up a process for the preparation of folate-enriched protein extracts from hen egg yolks

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

[a] Institute of Nutrition and Functional Foods (INAF), Department of Food Science and Nutrition, 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

A simple separation process was used to fractionate egg yolk into plasma and granule fractions through the use of gravitational separation with laboratory- or pilot-scale centrifuges. The granule fractions at pilot-scale presented with higher protein concentrations (66–69%) and lower lipid contents (20–26%), on a dry basis, as compared to the plasma fraction. The recovery of protein, based on the raw material (egg yolk), was calculated to be 96% and 89% for the laboratory- and pilot-scale fractionation processes, respectively. Good agreement was achieved between the laboratory- and pilot-scale centrifugation processes, in terms of chemical composition and recovery of egg yolk components. The plasma fraction preserved 66–71% of the lipid with the laboratory-scale and 75–77% with the pilot-scale fractionation process. It was confirmed by SDS–PAGE and 2D-gel electrophoresis analysis that each egg yolk fraction incorporated different amount of LDL and HDL. SDS–PAGE and 2D-gel electrophoresis analysis revealed different profile patterns for the plasma and granule fractions. Folate content in plasma and granule fractions was measured using HPLC analysis. Folate analysis revealed that the plasma fraction of egg yolk was devoid of folate (5-CH_3-H_4folate), whereas the granule fraction was concentrated by a 3-fold factor in comparison to native egg yolk.

1. Introduction

Eggs represent an important source of nutritional and bioactive ingredients usable in the food, medical, pharmaceutical, cosmetic, and biotechnological industries (Anton, 2007).

Egg proteins are highly digestible, containing essential amino acids in a profile that is comparable to the ideal balance of amino acids needed by humans. Eggs also supply various minerals and major vitamins. The hen egg has been also considered as an excellent natural source of folate (Strandler et al., 2011), contributing 10–20% of the daily requirement for folate (Song and Kerver, 2000).

The ability of the egg to be used as functional food has been established (Surai and Sparks, 2001). Enrichment of eggs with anti-oxidants and vitamins has been well documented (Sahlin and House, 2006; Surai and Sparks, 2001). With respect to the water-soluble vitamin folate, recent studies have revealed that the folate content of eggs can be enhanced due to the hen's ability to convert folic acid from fortified feed to natural forms (Hebert et al., 2005; House et al., 2002) with high bioavailability (House et al., 2003; Sugiyama et al., 2012). Folic acid, the synthetic form of folate vitamin, either from supplements or fortification, has been shown to reduce the incidence of neural tube defects in pregnancies (MRC, 1991; Ray et al., 2002). However, an excess of the synthetic form of folate for the population as a whole may not necessarily be beneficial (Smith et al., 2008). There are several studies that seem to indicate a potential risk of excess synthetic folic acid intake (Mason et al., 2007; Wien et al., 2012). One study showed an apparent increase in the incidence of colorectal cancer after the mandatory fortification of folic acid in both the USA and Canada (Mason et al., 2007). Conversely, the native dietary forms of folate in physiological amounts are known to have no adverse effects. Egg yolk has been reported to contain 87–100% of folate in the form of 5-CH_3-H_4folate as the most abundant form of folate (Seyoum and Selhub, 1993; Strandler et al., 2011; Vahteristo et al., 1997), with folate principally restricted to the yolk fraction. According to recent studies, one egg (60 g) provides 40–50 μg of folate which corresponds to 10–15% of the recommended daily allowance of 400 μg for adults or to 13–16% of the estimated average requirements of 320 μg/day (Institute of Medicine, 1998).

Egg yolk is a very complex biological system consisting of non-soluble protein aggregates (granules) in suspension in a clear

* Corresponding author. Tel.: +1 418 656 5988; fax: +1 418 656 3353.
E-mail address: Yves.Pouliot@fsaa.ulaval.ca (Y. Pouliot).

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yellow fluid (plasma) that contains low-density lipoproteins (LDLs) and soluble proteins (Anton, 2006). Classical separation methods of egg yolk have utilized solvents (Kwan et al., 1991), or conditions which in turn caused the protein fractions of egg yolk to denature (Liot, 2002). However, in order to preserve the techno-functional properties of egg yolk fractions, the native behavior of egg yolk must remain unaffected (Ternes and Werlein, 1987). Egg yolk can also be fractionated into two fractions of plasma and granules by dilution followed by centrifugation. Previous studies have relied on fractionation processes based on discontinuous lab scale methods (McBEE and Cottwrill, 1979), where centrifugation at high *g*-forces (10,000*g*) and long residence times (45 min) is utilized. In recent studies, the discontinuous fractionation methods with long residence times have been applied to determine the specific functional properties of granule/plasma-fractions where these methods provided the advantage of high separation efficiencies (Laca et al., 2010, 2011; Strixner and Kulozik, 2013) in separation of the fat and aqueous fractions of egg yolk. Studies by Laca et al. (2010, 2011) resulted in a granule fraction that contained 34% lipid and 60% protein. However those methods utilized different strategies (e.g. pH adjustment, the use of acids, bases and/or salts with extreme water dilution) which all have disadvantages such as complexity of procedures, or compatibility for ultimate use as a food or medicinal ingredient.

The use of centrifugation steps offers high clarifying potential and throughputs (Lander et al., 2005) for the separation of biological products, as the volume of the particles or the density gradient between the particles and the liquid are small (Spelter et al., 2010). The tubular bowl centrifuges are well established efficient unit operations in the field of biotechnology (Stahl et al., 2008) and they offer the highest centrifugal forces (Lander et al., 2005) and can be used to scale-up an egg-yolk fractionation procedure.

The objective of the present study was to develop and scale-up a sequential separation method using water to fractionate native hen egg yolk for the preparation of a folate-enriched protein extract. The present work was based on the hypothesis that folate would be found in higher proportion in the protein fraction (granule). The composition of fractions generated from laboratory-scale and pilot processes were characterized and mass balances were determined.

2. Materials and methods

2.1. Hen eggs

Fresh white shelled eggs of weight class M (53–56 g) or L (58–63 g) were purchased from a local supermarket and stored in a cooling chamber at 4 °C until further use.

2.2. Chemicals

All chemicals, unless specified otherwise, were obtained from VWR (Quebec, Canada). Acetonitrile was of HPLC gradient grade; other chemicals were of analytical quality. Water was purified ($\leqslant$0.1 μS cm^{-1}) using a Milli-Q system (Millipore, USA). Standard for HPLC method; 5-Methyltetrahydrofolic acid (5-CH_3-H_4folate) disodium salt was from Sigma–Aldrich (Wisconsin, USA). The standard folate was purchased in lyophilized form and was stored at −20 °C until use. 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 calibration solutions were prepared immediately before use by diluting the stock solution with extraction buffer (20 g/L sodium ascorbate; 12.1 g/L Trizma base; pH 7.8).

2.3. Fractionation of egg yolk

Each egg was weighed, and then yolks and albumen were separated manually and the weights were registered. The residual albumen and chalazae were eliminated manually from the yolk using blotting paper. The removal of the vitelline membrane was achieved using tweezers. Next, the yolk material was diluted by mixing with Milli-Q water (1:1 v/v) and gently homogenized by stirring with a glass rod in a beaker cooled in an ice bath.

2.3.1. Lab scale process

The laboratory-scale fractionation method was developed as a modification of the procedure described by Laca et al. (2010). All experiments were performed on three separate batches by choosing one dozen eggs for each batch.

The egg yolk fractionation studies were performed by using a RC-5B PLUS centrifuge (SORVALL® Superspeed®, Newtown, CT, USA) and a fixed-angle rotor (SS-34) by applying the conditions of 10,000*g* at 4 °C for 45 min. Recent findings have shown the absence of granule-particles in plasma and the complete separation of granule and plasma with centrifugation at 10,000*g* (Strixner and Kulozik, 2013). As a result, we chose to utilize a g-force of 10,000*g* for the lab-scale study. Diluted fresh egg yolk material was placed in centrifugation tubes (50-mL) and filled to capacity. Following centrifugation, the tubes were decanted slowly to separate the plasma and granule fractions. The deep orange-yellow supernatant stream was defined as the plasma and the sediment was defined as the granular fraction. Both fractions were stored over night at 4 °C for further investigations. All experiments were performed in triplicate.

2.3.2. Pilot scale process

All experiments were performed on three separate batches, with two dozen large size eggs per batch. The fractionation studies were performed on a tubular bowl centrifuge (CEPA Centrifuge, series LE, Germany) with a maximum centrifugal force of 40,000*g*. The tubular bowl centrifuge (Fig. 1) is equipped with (a) a cylinder type SK which is a serum clarifying cylinder, and the suspension is then led by (b) a feed pump from below over the (c) nozzle holder with a screwed on nozzle. The liquid (diluted egg yolk) is injected into the cylinder and cleaned continuously by the centrifugal force. The cleaned or separated liquid is collected at the outlet aperture at the cylinder head into the (d) collecting trays and is drained off via the apertures of the collecting trays.

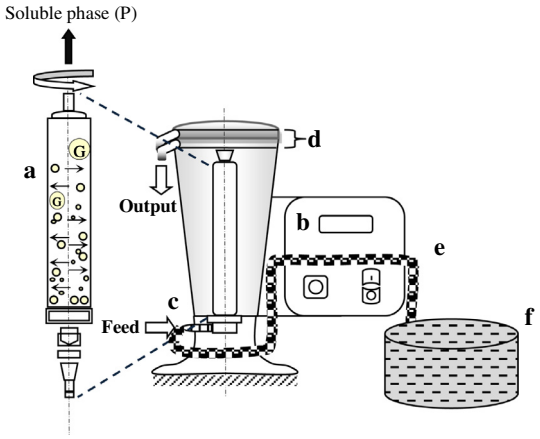

Fig. 1. Schematic of the tubular bowl vertical centrifuge (CEPA Centrifuge, series LE, Germany) used for pilot-scale experiments. Cross-sectional views: (a) cylinder type SK, (b) feed pump, (c) nozzle, (d) collecting trays, (e) tube, and (f) feeding tank. P: Plasma, G: Granule.