



Lipid, fatty acid and carotenoid content of edible egg yolks from avian species: A comparative study

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ARTICLE INFO

Article history:

Received 30 March 2010

Received in revised form 19 May 2010

Accepted 13 July 2010

Keywords:

Egg yolks

Lipids

Fatty acids

Carotenoids

Ostrich

Turkey

Quail

Duck

Goose

ABSTRACT

A comparative study has been conducted of the major lipid classes composition, as well as the fatty acid and carotenoid content in the yolk of conventional eggs from five avian species (ostrich, turkey, quail, duck and goose); the nutritional indices were calculated. The neutral lipids were the major yolk fractions but their proportions varied among species. All yolks and especially ostrich's yolk were found to be an excellent source of dietary lecithin. Quail yolk displayed the lowest fat and cholesterol content and the lowest values for the cholesterol index (CI) and cholesterol-saturated fat index (CSI). It is therefore more appropriate for a healthier diet. Turkey and goose yolks contained significantly ($P < 0.05$) higher ω -3 fatty acid proportion and ω -6/ ω -3 ratio. The turkey yolk was characterised by the lowest AI and TI values, which are recommended for a healthy diet. Quail yolk lipids contained a favourable PUFA/SFA ratio. All the examined yolks contained highly bioavailable functional nutrients, such as lutein and zeaxanthin.

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1. Introduction

Diet plays an important role in the maintenance of health. Among the different products delivering essential nutrients to the body, eggs have a special place, being a rich and balanced source of essential amino and fatty acids, as well as of some minerals and vitamins (McNamara & Thesmar, 2005). Eggs are of particular interest because they are relatively rich in fatty acids, and the associated fat soluble compounds and the type and ratios of fatty acids are an important determinant of human health (Surai & Sparks, 2001). Egg yolk is an important source of glycerophospholipids (McNamara & Thesmar, 2005), which make the eggs ideal for industrial and biomedical applications, as well as for basic physicochemical studies. Eggs are also one of the least expensive single-food sources of complete protein (Kaewmanee, Benjakul, & Visessanguan, 2009). Furthermore, the egg yolk's triacylglycerols and phospholipids constitute the main source of energy and structural lipids for the developing embryo. While the majority of eggs consumed nowadays are chicken eggs, a variety of eggs from different species of birds are commercially available in differ-

ent parts of the world, from the petite quail egg to the very large ostrich egg. There are approximately 9000 species of birds adapted to a wide range of lifestyles and habitats throughout the world, consuming a very diverse range of diets (Speake, Surai, Noble, Beer, & Wood, 1999). Duck, goose and smaller eggs, such as quail eggs, as well as the largest bird eggs, from ostriches, are occasionally used as a gourmet ingredient. The eggs originating from other species, if consumed, tend to be a specialist product sold in exclusive restaurants or stores. While there is a very wide knowledge concerning the composition of yolk lipids, obtained from studies on the hen eggs, as such eggs are easily available, data for other bird yolk lipids are rare.

The aim of this research was to comparatively examine the lipid content, fatty acid composition and carotenoid content of ostrich, turkey, quail, duck and goose egg yolks. The research data may lead to a better comparison of the relative differentiation of the lipid components of the various egg yolks, and be helpful in evaluating their nutritional significance.

2. Materials and methods

2.1. Reagents and standards

The lipid standards used (purity >98%) for lipid class determination were cardiolipine, phosphatidylcholine, phosphatidylethanol-

Abbreviations: AI, atherogenic index; CI, cholesterol index; CSI, cholesterol-saturated fat index; PUFA, polyunsaturated fatty acids

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amine, phosphatidylserine, phosphatidylinositol, lyso-phosphatidylcholine, lysophosphatidylethanolamine, sphingomyelin, cholesteryl oleate, cholesterol, tristearoyl-glycerol, lauric acid, oleic acid, linoleic acid, 1,2-distearoyl-glycerol, 1-monostearoyl-rac-glycerol, all-*trans* lutein, zeaxanthin, canthaxanthin and all-*trans* β -carotene; they were all purchased from Sigma Chemical Co. (Sigma–Aldrich Company, St. Louis, MO).

The fatty acid methyl esters used as GC standard were: lauric acid M-E, L7272, *cis*-5,8,11,14,17-eicosapentaenoic acid M-E, E2012 and *cis*-4,7,10,13,16,19-docosahexaenoic acid M-E, D2659 (purity $\geq 98\%$); they were purchased from Sigma Chemical Co.; Matreya Bacterial Acid Methyl Esters CP™ Mix, Catalogue No.: 1114; Supelco™ 37 Component FAME Mix, Catalogue No.: 47885-U.

All the solvents used for sample preparation were of analytical grade, and the solvents used for Iatroscan (TLC-FID), HPLC and GC analyses were of HPLC grade. They were obtained from Merck (Darmstadt, Germany).

2.2. Sampling and sample preparation

Ostrich (African Black *Struthio camelus camelus*), turkey (*Meleagris gallopavo*), quail (*Coturnix coturnix japonica*), duck (*Anas platyrhynchos*) and goose (*Anser anser*) conventional eggs, less than 3 days after laying, were obtained from a local producer in Lakonia, Greece (3 sampling repetitions). All eggs were laid in the same period, from early September till mid November. The parent birds were maintained outdoors under free-range conditions with free access to grazing on grasses and were also provided with a commercial feed (corn 60.47%, soybean meal 12.92%, cottonseed meal 9.58%, bran 0.74%, sawdust 1.0%, limestone 6.73%, dicalcium phosphate 1.69%, salt 0.28%, mineral premix 0.20%, vitamin premix 0.20% and methionine 0.08%). As the laying parents were able to select a wide range of food items from natural sources, the relative intakes of natural versus commercial feed were not measured. Egg samples [12 ostrich eggs obtained from four different females (average weights of 1.3–1.4 kg), 27 turkey eggs obtained from nine different females (average weights of 80–88 g), 60 quail eggs obtained from six different females (average weights of 8.5–9.5 g), 15 duck eggs obtained from five different females (average weights of 65–75 g), and 24 goose eggs obtained from eight different females (average weights of 135–145 g)] were randomly selected. Egg yolks, divided in groups according to the parent bird, were separated manually from their respective whites, then homogenised at a slow speed, vacuum-packed in plastic bags, and stored at -20°C until analysed at room temperature. The analytical parameters were determined at least in triplicate for each group.

2.3. Proximate analysis

The moisture content was determined by oven drying at $105 \pm 2^{\circ}\text{C}$ to constant weight. Ash was determined by heating in a muffle furnace at 550°C to constant weight.

The total lipids were extracted according to the Bligh and Dyer method (Bligh & Dyer, 1959). After phase equilibration, the lower chloroform layer (total lipids, TL) was removed and dried in a rotary vacuum evaporator at 32°C . The extracted lipids were redissolved in chloroform/methanol (9:1, v/v). *t*-Butyl-hydroquinone was added in the prepared solutions to prevent oxidation, and the solutions were stored at 0°C until use. Afterwards, representative aliquots of all of the above mentioned samples were evaporated in pre-weighed vials to constant weight, to determine their lipid content.

2.4. Iatroscan analysis of neutral and polar lipids

The lipid classes were separated on silicic acid-coated quartz rods, chromarods (Type SIII) (5 mm silica gel-coated quartz rod, Iatron Labs, Tokyo, Japan), and were quantified using a thin-layer chromatography–flame ionisation detection system. TLC–FID analysis was performed by an Iatroscan thin-layer chromatograph (Model MK-6 TLC/FID – FPD Analyser Iatron Laboratories, Tokyo, Japan), equipped with a flame ionisation detector. The operation conditions for the Iatroscan were 160 ml/min hydrogen flow, 2 l/min air flow and 30 s/chromarod scan speed. The chromarods were activated by passing them through the FID scanner immediately before sample spotting; 1 μl of the sample solution was applied on each rod. A 1 μl Hamilton syringe (Hamilton Co., Reno, USA) was used to spot standards and samples. The rods were developed 10 cm from the origin in a lined chromatography tank with a one- or two-solvent system. To obtain a good vapour saturation of the tank, a filter paper was erected along one side of the bath, and was wetted with the solvent. Each bath was prepared 30 min before development. The temperature of the room was maintained at 22°C . After developing, the rods were dried in desiccators for a few minutes and then immediately scanned by the Iatroscan. The neutral solvent system consisted of *n*-hexane:diethylether:formic acid (*n*-H:DE:FA) (60:15:1.5 by vol.), while the polar solvent system consisted of chloroform:methanol:water (C:M:W) (50:20:2, by vol.). The determination of the weight percentages of the individual lipid classes was achieved using the standard curves obtained for each authentic standard (0.05–0.25 mg/ml per lipid), under the same chromatogram conditions and development temperatures. The correlation coefficients for the calibration curves of the thirteen lipid classes were in all cases higher than 0.998.

2.5. Gas chromatography analysis of fatty acid methyl esters

The fatty acid methyl esters (FAME) of the total lipids were prepared according to the procedure described by Sinanoglou and Miniadis-Meimaroglou (1998). Both quantitative and qualitative analysis were performed on an Agilent 6890 Series Gas Chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a flame ionisation detector. DB-23 capillary column (60 m $\times$ 0.25 mm i.d. 0.15 μm film) [50%-(cyanopropyl)-methylpolysiloxane] (Agilent Technologies, Catalogue No.: 122-2361) was used. The analysis was split injection, and the split ratio was 1:2. Helium was used as a carrier gas. The injector and detector temperature were 250°C and 260°C , respectively. The temperature was programmed at 100°C for 0 min, raised from 100 to 150°C by a rate of $10^{\circ}\text{C min}^{-1}$, and held constant at 150°C for 0 min. Then it was raised from 150 to 195°C with a rate of $2^{\circ}\text{C min}^{-1}$, and was held constant at 195°C for 5 min. It was then raised from 195 to 210°C with a rate of $1^{\circ}\text{C min}^{-1}$, it was held constant at 210°C for 0 min; it was finally raised from 210 to 240°C with a rate of $10^{\circ}\text{C min}^{-1}$, and was held constant at 240°C for 5 min. The duration of the analysis was 55.5 min (Loukas, Dimizas, Sinanoglou, & Miniadis-Meimaroglou, 2010). The injection volume was 1.0 μl . The Hewlett–Packard Chem Station Software was used to calculate the peak areas and retention times. The FAMES were identified by comparing their retention times with those of the authentic standard mixtures. The relative content of the fatty acids in the sample was determined according to Loukas et al. (2010).

2.6. Carotenoid analysis

For the identification of the different carotenoids, the egg yolk lipids were further analysed by high performance liquid chromatography (HPLC). The lipid samples were dried under nitrogen

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