Food Chemistry 194 (2016) 111–116

Contents lists available at ScienceDirect

Food Chemistry

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

Effects of storage and cooking on the antioxidant capacity of laying hen eggs

Chamila Nimalaratne [a], Andreas Schieber [a,b], Jianping Wu [a,*]

[a] Department of Agricultural, Food and Nutritional Science (AFNS), 4-10 Agriculture/Forestry Centre, University of Alberta, Edmonton, AB T6G 2P5, Canada
[b] Department of Nutritional and Food Sciences, University of Bonn, Roemerstrasse 164, D-53117 Bonn, Germany

ARTICLE INFO

Article history:
Received 28 November 2014
Received in revised form 10 July 2015
Accepted 23 July 2015
Available online 26 July 2015

Keywords:
Egg yolk
Antioxidants
Storage
Nutrient-enriched eggs
Free amino acids
Carotenoids

ABSTRACT

The aromatic amino acids and carotenoids are the major contributors to the antioxidant properties of egg yolk. This study aimed to evaluate the effect of simulated retail storage and domestic cooking on the antioxidant activity as well as on the aromatic amino acid and carotenoid contents in ordinary table eggs, omega 3/lutein (n-3/lutein) enriched eggs, and eggs from heritage chicken breeds. The oxygen radical scavenging capacity (ORAC) was the highest in n-3/lutein enriched eggs (161.4 μmol TE/g sample), while eggs from heritage white leghorns (HW) showed the lowest levels (127.6 μmol TE/g sample). Six weeks of storage at refrigerated temperature did not change the ORAC values, as well as the contents of free amino acid, carotenoid, and malondialdehyde (MDA) in egg yolk. Boiling and frying however, significantly reduced the ORAC value, and the contents of free amino acid, lutein and zeaxanthin, and increased the MDA content in eggs. Our results showed that the antioxidant activity is stable during six weeks of simulated retail storage.

1. Introduction

Chicken eggs are economically affordable nutritious food commodity containing important macronutrients such as highly digestible proteins and lipids, as well as micro-nutrients such as vitamins and minerals. Eggs are also viewed as an efficient vehicle for delivering various nutrients, for example omega-3 fatty acids, vitamin E, selenium, and lutein, which are thought to provide additional protection against certain disease conditions (Sim, 1998). Eggs enriched with these nutrients are referred to as 'designer eggs', which can be obtained through formulating hens' feed (Surai & Sparks, 2001). The concept of designer eggs fits nicely with the increasing needs of health conscious consumers, with omega-3 enriched eggs being most popular (Sim & Sunwoo, 2002). Egg constituents also impart various biological activities including antimicrobial, antioxidant, antihypertensive, immunomodulatory and antiadhesive properties, which may be beneficial beyond the basic nutrition (Kovacs-Nolan, Phillips, & Mine, 2005). Among various bioactivities, the antioxidant activity of egg-derived compounds has attracted great attention due to the possible roles of antioxidants against various chronic diseases including heart diseases (Dávalos, Miguel, Bartolomé, & López-Fandiño, 2004; Katayama, Ishikawa, Fan, & Mine, 2007). Recently, we found that two aromatic amino acids, tryptophan and tyrosine, contribute to the antioxidant properties of egg yolk (Nimalaratne, Lopes-Lutz, Schieber, & Wu, 2011). Egg proteins and derived peptides, phospholipids, vitamin E, carotenoids and phosvitin are other compounds that contribute to the total antioxidant activity of egg yolk (Hargitai et al., 2006; Katayama, Xu, Fan, & Mine, 2006; Mohiti-Asli, Shariatmadari, Lotfollahian, & Mazuji, 2008).

However, the antioxidant properties of egg yolk may vary depending on several factors, for instance egg types, processing and storage conditions. In Canada, approximately 70% of the total eggs produced is consumed as table eggs, which are stored at refrigerated temperature with an approximate shelf life of one month, while the rest is processed into liquid, frozen or dried products (Agriculture and Agri-Food Canada, 2013). It is known that industrial processing techniques such as spray-drying can result in increased lipid oxidation and loss of antioxidant capacity in eggs (Galobart, Barroeta, Baucells, & Guardiola, 2001; Lai, Gray, Buckley, & Kelly, 1995; Morgan & Armstrong, 1992). Table eggs are usually subjected to domestic cooking methods such as frying, boiling, scrambling etc., which are also known to reduce the antioxidant properties and the content of free amino acids (Nimalaratne et al., 2011), and increase lipid oxidation of eggs, especially in n-3 PUFA enriched eggs (Cortinas, Galobart, Barroeta, Baucells, & Grashorn, 2003; Ren, Perez, Zuidhof, Renema, & Wu, 2013). Lipid

* Corresponding author.
E-mail address: jwu3@ualberta.ca (J. Wu).

http://dx.doi.org/10.1016/j.foodchem.2015.07.116
0308-8146/

oxidation occurs during storage, especially at elevated temperatures (Mohiti-Asli et al., 2008), and is more pronounced in n-3 PUFA enriched eggs, due mainly to the presence of the unsaturated bonds (Hayat, Cherian, Pasha, Khattak, & Jabbar, 2010; Ren et al., 2013). Most available data on lipid oxidation or oxidative stability are focused on dried egg products and omega-3 enriched eggs, whereas information on the antioxidant activity of table eggs during retail storage and cooking is limited. Given the fact that free aromatic amino acids and carotenoids are the major contributors to the antioxidant capacity of egg yolk, the main objective of this study was to evaluate the effects of simulated retail storage conditions and cooking on the antioxidant capacity, the contents of free amino acids and carotenoids, and lipid oxidation. To compare the antioxidant capacity of different eggs, four different types of eggs were used in the study.

2. Materials and methods

2.1. Chemicals

Lutein (xanthophyll from marigold), zeaxanthin, trichloroacetic acid, 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH), 2-Thiobarbituric acid (TBA), and anhydrous monobasic sodium phosphate, amino acid standards, ethanolamine (EA), b-aminobutyric acid (BABA) and ophthaldialdehyde (OPA) were obtained from Sigma–Aldrich (Oakville, ON, Canada). Light petroleum ether, methanol, acetone, ethyl acetate, TBME (*tert*-butyl methyl ether) and HPLC-grade water were purchased from Fisher Scientific (Ottawa, ON, Canada). Fluorescein disodium and trolox were obtained from Acros Organics (Morris Plains, NJ, USA).

2.2. Types of eggs

Four types of fresh shell eggs ($n = 72$ each) were used in this study. The eggs were not sterilized or UV treated before. Normal table eggs and eggs from two heritage chicken breeds (white leghorns and brown leghorns) were obtained from the Poultry Research Centre of the University of Alberta (Edmonton, AB, Canada). Heritage chicken breeds are characterized by an original genetic line; they are not selected based on egg laying or quality performances. Eggs enriched in lutein and omega-3 fatty acids were purchased from a local supermarket (Edmonton, AB, Canada). Eggs were stored under normal retail storage conditions, i.e. at 4 °C in packages for 35 days and analyzed on a weekly basis.

2.3. Cooking of eggs

Cooking of eggs was carried out on days 1, 7, 14, 21, 28, 35 after storage as follows. A set of twelve eggs was taken from each type and divided into three groups for boiling, frying and raw egg yolks. For boiling, whole shell eggs were placed in a saucepan as a single layer, with water up to 3–5 cm above the eggs, and boiled for 10 min. The eggs were then placed under running tap water for 5 min and peeled, and yolks were separated from whites. Fried eggs were prepared using a frying pan (model SK200TY non-stick frying pan, Black & and Decker Canada Inc., Brockville, ON, Canada) preheated to 205 °C. Eggs were fried for 6 min (3 min each side) and the yolks were separated from whites. Raw egg yolks were used as a control. To prepare raw yolk samples, egg yolks were manually separated from whites and wiped with a filter paper to remove adhered albumins. Cooked and raw egg yolks were pooled and subjected to freeze drying in containers covered with aluminum foil to protect samples from light induced oxidation. Dim light conditions were used throughout the cooking period. Freeze-dried samples were ground to obtain a fine powder. The processed samples were stored in the dark at −20 °C in airtight sealed plastic containers until analysis. All analyses were conducted using freeze-dried egg yolk samples and the results were reported based on the weight of freeze-dried egg yolk sample.

2.4. Extraction and analysis of free amino acids

Free amino acids were extracted using the method described by Ohkubo, Sawaguchi, Hamatsu, and Matsubara (2006). Approximately 150 mg of freeze-dried egg yolk was extracted with 1 mL of 6% trichloroacetic acid by vortexing followed by centrifugation at 6000*g* for 20 min using a bench top centrifuge (Centrifuge 5418, Eppendorf, Hamburg, Germany). The supernatant was filtered with 0.45 μm nylon syringe filter (Mandel Scientific Corp., Guelph, ON, Canada) and used for HPLC analysis. Free amino acids were determined as described by others previously (Sedgwick, Fenton, & Thompson, 1991) using ethanolamine (EA) and β-amino-n-butyric acid (BABA) as the internal standards with minor modifications. Briefly, the sample solutions were prepared in HPLC vials by adding 25 μL of sample or standard, 100 μL of internal standard mixture (contain 25 nmol/mL of BABA and EA in Milli Q water), 200 μL of saturated $K_2B_4O_7$ and 75 μL Milli Q water (the pH of the final mixture should be around 9). Samples were derivatized using OPA immediately prior to the injection. Amino acids were separated using binary solvent system (solvent A: 0.1 M sodium acetate; solvent B: methanol) on a Supelcosil LC-18 reverse phase column (4.6 × 150 mm, 3 μm) with a Supelco LC-18 reverse phase guard column (4.6 × 50 mm) using a HPLC system equipped with a Fluorichrom detector. The gradient was 0% B to 19% B in 0.1 min, 19% B for 14.9 min, 19% B to 32% B in 10 min, 32% B to 42% B in 0.1 min, 42% B to 50% B in 7.9 min, 50% B to 70% B in 7 min, 70% B to 100% B in 2 min, l00% B for 2 min, 100% B to 0% B in 1 min with a flow rate of 1.1 mL/min. Four replicates were conducted for each sample.

2.5. Extraction and analysis of carotenoids

Yolk samples were extracted according to Schlatterer and Breithaupt (2006) with minor modifications as described previously (Nimalaratne, Lopes-Lutz, Schieber, & Wu, 2012). Approximately 0.5 g of freeze dried egg yolk powder was extracted three times (3 mL each) using a ternary solvent mixture (methanol:ethyl acetate:petroleum ether, 1:1:1, v/v/v) with 0.1% BHT at room temperature for 1 min using a vortex mixer (model 945404, Fisher Scientific, Edmonton, AB, Canada) and the combined supernatants were evaporated under nitrogen gas. The residue was dissolved in 2 mL of TBME:methanol (3:1, v/v). These samples were filtered through 0.45 μm nylon membrane filter and analyzed by UFLC. Duplicate extractions were performed and the samples were protected from light during the extraction and analysis.

Samples were analyzed using a C_{30} reversed-phase column, 100 mm × 2.0 mm I.D. and 3 μm particle size (YMC America, Allentown, PA, USA) operated at 21 °C. Analyses were performed on a Shimadzu UFLC-XR system (Shimadzu, Kyoto, Japan) equipped with a diode array detector (Model SPD-M20A). The injection volume was 3 μL. A binary solvent system consisting of mobile phase A (methanol:water, 90:10, v/v) and mobile phase B (TBME:methanol, 80:20, v/v) was used with the following gradient: 0–8 min (8–40% B), 8–13 min (40–100% B), 13–14.5 min (100% B), 14.5–14.6 min (8% B). The flow rate was 0.3 mL/min and the carotenoids were detected at 450 nm. Analyses were duplicated. Identification of carotenoids was based on their UV spectra, retention time and order of elution compared to the standard compounds while quantification was based on seven point standard calibration curves as described in Nimalaratne et al. (2012).