Food Chemistry 136 (2013) 1551–1556

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Isotopic analysis of eggs: Evaluating sample collection and preparation

Luc Rock [a,*], Sylwia Rowe [a], Agnieszka Czerwiec [a,b], Harold Richmond [c]

[a] EERC - SPACE & ASSET Queen's University Belfast David Keir Building, Stranmillis Road Belfast, BT9-5AG Northern Ireland, United Kingdom
[b] CII - School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast Health Sciences Building, Lisburn Road Belfast, BT9-7BL Northern Ireland, United Kingdom
[c] SKEA Eggs Ltd., 146 Pomeroy Road, Pomeroy Dungannon, BT70-2TY Northern Ireland, United Kingdom

ARTICLE INFO

Article history:
Available online 16 March 2012

Keywords:
Egg
Traceability
Authenticity
Stable isotope techniques
Collection time
Sample preparation
Pasteurisation

ABSTRACT

Egg traceability/authenticity is a worldwide concern. Stable isotope techniques have been suggested as a tool to address this issue. To further validate the use of these techniques, a research project was undertaken to evaluate what effect sample collection and preparation have on the measured isotopic composition of egg components. The timing of egg collection, the timing of egg preparation after collection, and the use of pasteurisation were investigated. The C, N, O, and S isotopic compositions of egg components from 7 different production systems were measured. Two sets of eggs were collected (4 months apart). It was found that the 'isotopic fingerprint' of a particular production system was maintained over time, and that it may be possible to trace liquid egg products based on isotopic data from fresh eggs. The findings from this study support the integration of stable isotope techniques in egg traceability/authenticity systems.

1. Introduction

The safety and quality of food are major concerns in today's society. Consumers, producers, and governments want to ensure that the food being produced, traded and consumed is of the highest quality, and free from fraud. Food mis-labeling leads to mistrust in the food supply chain. In turn, tools are needed to verify the origin of food commodities to maintain consumer confidence, and to protect the industry. Stable isotope techniques are a promising tool to address food traceability and authenticity issues (e.g. Kelly, Heaton, & Hoogewerff, 2005; Reid, O'Donnell, & Downey, 2006; Luykx, & van Ruth, 2008). A range of food commodities have been analysed using these techniques, such as beef by Boner and Förstel (2004). Further examples can be found in Kelly et al. (2005).

This study focuses on the food commodity 'egg', which is an important source of protein. Global egg production is on the rise, it increased by 220% between 1970 and 2007 (IEC, 2009), and egg fraud has occurred. A 2009 newspaper headline in the UK stated that "UK authorities are seeking the extradition of an All-Ireland senior hurling winner for his alleged involvement in a multi-million fraud where eggs were falsely passed off to British consumers as being free range or organic." (O'Faolain, 2009). Another concern within the egg industry is the traceability of liquid egg products. Large amounts of eggs are transported in liquid form across Europe for which it is currently very difficult to trace the origin (BFREPA, 2009). In turn, a robust and reliable traceability system is needed to prevent fraud within the egg industry.

Stable isotope techniques have been evaluated with regards to their use in egg traceability/authenticity investigations (e.g. Rossman, 2001; Boner, 2003; Rogers, 2009). Rossman (2001) discussed the use of $\delta^{15}N$ and $\delta^{13}C$ values to establish what types of feed (plant versus animal protein; C3 versus C4 plants) were given to hens from commercial and local farms. Boner (2003) noted that $\delta^{15}N$ of egg white may enable differentiation between conventional and organic egg production. A similar observation was made by Rogers (2009) who determined the $\delta^{15}N$ and $\delta^{13}C$ values of different egg components (yolk, white, membrane) from caged, barn, free range, and organic egg production systems. Rogers (2009) concluded that $\delta^{15}N$ may a useful tracer in differentiating caged or barn from free range hens. Besides the N and C isotopic compositions of eggs, it has been suggested that the integration of the S, O, and H isotopic compositions may aid in identifying eggs from a particular region or production system (Boner, 2003). The latter study also assessed whether 5-months or 1-month composite data can be used to differentiate between different producers of organic eggs. It was noted that 1-month composite data should be used, due to smaller 'intra-producer' variability. As well, Boner (2003) looked into the impact of storage on the $\delta^{18}O$ and $\delta^{2}H$ of extracted water from egg white. Hence, previous studies have indicated that stable isotope techniques may provide a promising tool to be incorporated into egg traceability/authenticity systems; however, changes with time may complicate the 'isotopic fingerprint' of a production system.

To further validate the use of stable isotope techniques in egg traceability/authenticity studies, additional insight is needed in terms of the impact of egg collection practices or egg preparation approaches on the measured isotopic composition of egg components. Hence, a study was undertaken with a focused design

* Corresponding author. Tel.: +44 (0) 28 9097 5574; fax: +44 (0) 28 9097 4278.
E-mail addresses: l.rock@qub.ac.uk, lucrck@gmail.com (L. Rock).

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.03.041

(known origin of eggs, age of laying hens) to eliminate these other potential sources of variability. This allowed the investigation of the following objectives: (a) the timing of egg collection, (b) the timing of egg preparation, and (c) pasteurisation. A key aspect in addressing these objectives is also to assess the inter- and intra-system variability of the isotopic composition of egg components.

2. Materials and methods

2.1. Egg samples

Two sets of egg samples were collected for this study, one in December 2009 and one in April 2010 (Table 1). On each sampling occasion, eggs were randomly collected from six or seven different production systems, which included: Barn (**Barn**), Free Range Standard (**FR std**), Free Range Enriched 1 (**FR-MNS**), Free Range Enriched 2 (**FR-S O**), Organic (**Org**), Regular Cage (**Reg Cag**), and Enriched Cage (**En Cag**) (Table 1). All production systems sampled are part of SKEA Eggs Ltd. **En Cag** was not sampled in December 2009, and the main differences with **Reg Cag** are lower 'hen' stocking density and addition of nesting/scratching areas. Three types of free range eggs were sampled based on differences in dietary supplements. **FR std** are produced to BEIC (British Egg Industry Council) and Freedom Food standards using non-GM Soya and natural pigmentation. Stocking density of the birds inside the house is a maximum of 9 birds per sq metre. **FR-MNS** are produced to the same basic standards, but with the addition of marine algae rich in long chain Omega 3 being added to the hens' diet. **FR-S O** are produced to the same basic standards as the standard free range, but have their diet enriched with Omega 3 PUFAs using locally sourced salmon oil.

An attempt was made to collect eggs from hens of similar age (~50 weeks old) for all the production systems (Table 1). This was done to eliminate a potential source of variability. At present, it is not known whether the age of a laying hen, may have an impact upon the isotopic composition of the egg. This needs to be further investigated. For the December 2009 set, 3 eggs per production system were collected. Eggs from a specific production system were prepared on the same day. For the April 2010 set, 6 eggs per production system were collected, and these were split into 3 sets. 3 of the 6 eggs were prepared on the same day, one of the remaining eggs was prepared 1 week after the first 3 were prepared, another egg was prepared 2 weeks after the first 3, and the last egg was used to assess the effect of pasteurisation. Prior to sample preparation, the eggs were stored in a fridge.

2.2. Egg preparation

2.2.1. Fresh eggs

The following procedure was used to prepare an egg for isotopic analysis. At first the egg was rinsed in distiled water to remove any adhered material or company stamp on the outer shell. The egg was left to dry and then separated into egg membrane (EM), egg white (EW), egg yolk (EY), and egg shell (ESh), and each component was put into a porcelain basin. Care was taken not to break the vitelline membrane. The chalazas and any remaining EW were removed from EY by using a pipette. EY, EM, and EW were then frozen and freeze-dried for 36 h. ESh (without EM) was rinsed with acetone and left to dry in a fume cupboard overnight. Homogenisation of EW and EY was done by crushing recovered material from freeze-dryer in a plastic bag, of EM by using a freezer mill, and of ESh by using mortar and pestle. Samples were then stored in 1.5 mL vials in a desiccator. Note that lipids were removed from EY prior to isotopic analysis using a modified method of Bligh and Dryer (1959). 3.5 g of dry homogenised EY were put into a 50 ml centrifuge tube, and $CHCl_3$:MeOH in a ratio of 1:2 (v/v) was added. After centrifugation, the protein phase was recovered, dried, homogenised, and stored in 1.5 mL vials in a desiccator. The delipidation step was repeated twice.

2.2.2. Pasteurised eggs

Pasteurised EW and EY were produced by using an automatic steriliser-cooler Barriquand Steriflow system at the Ashtown Food Research Centre (AFRC) in Dublin. Prior to the pasteurisation of the EW and EY from one of the 6 eggs of each production system collected in April 2010, the system was tested and adjusted by using EW and EY from commercially bought eggs. The following procedure was used to prepare pasteurised EW and EY for isotopic analysis. Each egg was rinsed in distiled water to remove any adhered material or company stamp on the outer shell, and then separated into EM, EW, EY, and ESh into porcelain basins as described before. EW and EY were then transferred into pre-sterilised polyethylene bags with double zipper, and kept overnight in a fridge and in a cool box during transportation to AFRC. The parameters used for pasteurisation were based upon information taken from SANOVO Technology (STC, 2009). The temperature of the sample holding vessel was raised to 65 °C and maintained for 5 min for EY, and to 56 °C and maintained for 2 min for EW. After the heating process was finished, the polyethylene bags containing EY or EW were transferred to ice cold water for 15 min. Sample bags were then transferred to a cool box during transport and a fridge overnight. The following day, sample bags were frozen and the steps described in section 2.2.1. followed to complete preparation of pasteurised EY and EW for isotopic analysis.

2.3. Isotopic analyses

For EY, EW, and EM, the bulk nitrogen, carbon, and sulphur isotopic compositions were determined. For ESh, the carbon and oxygen isotopic composition of carbonate were determined. $\delta^{34}S$ of EY, EW, and EM and $\delta^{13}C$ and $\delta^{18}O$ of ESh carbonate were determined at the Stable Isotope Facility at Queen's University Belfast (QUB); whereas, $\delta^{15}N$ and $\delta^{13}C$ measurements for EY, EW, and EM were

Table 1
Description of egg sample sets.

Production system name	Abbrev.[a]	December 2009		April 2010	
		Sampled	Age of hens (weeks)	Sampled	Age of hens (weeks)
Barn	Barn	Yes	49	Yes	54
Free range standard	FR std	Yes	47	Yes	54
Free range enriched 1	FR-MNS	Yes	46	Yes	52
Free range enriched 2	FR-S O	Yes	45	Yes	33
Organic	Org	Yes	52	Yes	47
Regular cage	Reg Cag	Yes	57	Yes	41
Enriched cage	En Cag			Yes	54

[a] Abbrev.: Abbreviation used for production system within manuscript.