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# Exposure assessment of trace elements in fresh eggs from free-range and home-grown hens analysed by inductively coupled plasma optical emission spectrometry (ICP-OES)

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A B S T R A C T

A total of 21 metals (Na, K, Ca, Mg, V, Mn, Fe, Cu, Zn, Cr, Mo, Co, B, Ba, Li, Sr, Ni, Si, Al, Pb, Cd) have been analysed, using inductively coupled plasma atomic emission spectroscopy (ICP-OES), in a total of 288 eggs from free-range and home-grown hens in order to establish differences in metallic content according to the breeding system. Method accuracy was checked by the analysis of certified reference materials (SRM 1515 Apple Leaves, SRM 1548a Typical Diet and SRM 1567a Wheat Flour from NIST) and the recovery percentages were between 97.7–103%. The results obtained were in good agreement with 95% confidence limit with the certified concentrations, relative standard deviation (RSD) values were lower than 25%. Significant differences ($p < 0.05$) in the metal content between the two breeding systems have been demonstrated. The daily consumption of eggs from free-range hens (24.3 g/person/day for children, 31.2 g/person/day for adults) contributes to the intake of metals, such as Na (2.7% adults, 2.1% children), Fe (7.4% males, 3.7% females, 4.3% children), Zn (4.4% males, 5.9% females, 4.1% children) and Cu (9.6% adults, 8.3% children). Macroelements and trace elements contribution is lower in home-grown eggs and with higher amounts of toxic metals.

## 1. Introduction

Eggs are an important food because they are an economically affordable source of high-quality protein and nutrients (Exler et al., 2013). They provide nutrients such as folic acid, riboflavin, vitamins, among others (Navligio et al., 2012). Although the caloric content is low, eggs have important concentrations of cholesterol. The nutritional composition varies from the egg yolk to the egg white, with the majority of micronutrients, fat, cholesterol and carotenoids being in the yolk (Domingo, 2014).

The breeding system of the laying hen, as well as their feeding, influences nutritional differences between the different eggs. According to Commission Regulation (EC) No. 589/2008 of 23 June 2008, eggs from free-range hens are identified by the number 1 in the egg code (EC, 2008). These hens live a poultry house and an open-air pen, as laid down in Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens (EC, 1999). On the other hand, home-grown hens, which come from small private pens, have been considered as a healthier, more economical and environmentally friendly alternative (Waegeneers et al., 2009a). However, several studies show that eggs from home-grown hens have higher levels of toxic metals from contaminated soils, home-grown plants, pollution, etc. (Van Overmeire et al., 2009; Waegeneers et al., 2009a,b).

In Spain, the average consumption of eggs according to data from the Spanish Agency for Food Safety and Nutrition (AESAN) is 24.3 g/person/day for children aged 7–12, and 31.2 g/person/day for adults over 17 years of age (AESAN, 2006).

Hens' eggs can provide macroelements, trace elements and toxic metals to the human diet. Soils or contaminated food increase the accumulation of metals in both hens and their eggs, so the consumption of eggs is a means of human exposure to these metals. (Waegeneers et al., 2009b; Grace and MacFarlane, 2016).

The macroelements (Ca, Na, K, Mg) are essential in large concentrations for the human organism. Sodium and potassium play an important role in the transmission of nerve impulses. Calcium forms part of the bone and tooth structure and is needed to activate certain enzymes. Magnesium is a necessary cofactor in different enzymes (IOM, 2001; González-Weller et al., 2015).

Regarding the trace elements, some of them such as Fe, Zn and Cu

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are essential for humans. But others such as B, Ba, Sr or V are not essential but they are needed by some plants and organisms (IOM, 2001; EFSA, 2004; WHO, 2010; SCHER, 2012; González-Weller et al., 2015).

The toxic metals (Al, Cd and Pb) do not have any function in the human organism and furthermore they are accumulative. Aluminium is known to cause neurotoxicity, this metal accumulates in the brain and this can result in diseases such as dementia or Alzheimer's disease (González-Weller et al., 2010; Al Juhaiman, 2010). Lead, absorbed in large amount by children, accumulates in bone tissue and long-term exposure effects include renal failure, motor paralysis, etc. (Rubio et al., 2004). Cadmium, characterized by a long residence time in humans, can interfere with the enzymatic system causing several adverse effects such as hypertension, renal dysfunction and damage to the nervous system (González-Weller et al., 2015).

Therefore, due to the importance of hens' eggs in the diet, as well as the growing interest in home-grown food, which is seen as a contaminant-free alternative, this study was carried out to determine the concentration of 21 metals (Na, K, Ca, Mg, V, Mn, Fe, Cu, Zn, Cr, Mo, Co, B, Ba, Li, Sr, Ni, Si, Al, Pb and Cd) in eggs of free-range and home-grown hens in order to estimate the nutritional composition depending on the breeding system, and to evaluate the toxic risk.

Inductively coupled plasma atomic emission spectroscopy (ICP-OES) has been used to perform the metal analysis, as it is a technique with favourable detection limits for metals (Schorin et al., 1998). ICP-OES is one of the best techniques to determine trace elements in food samples (Luis et al., 2015; Rubio et al., 2017). Nowadays, ICP-OES is widely used because it can determine trace elements at low levels (Hardisson et al., 2017; Alzahrani et al., 2017).

## 2. Material and methods

All the solutions used in the present study were prepared from analytical reagent grade chemicals and high-purity deionised water from a Milli-Q water purification system (Millipore, MA, USA). 65% (v/v) nitric acid ($HNO_3$) (Merck, Darmstadt, Germany) was used to the analysis and the sample preparation. The manufacturer's instructions concerning the management of the residues were followed. The acid residues were disposed of by authorized people.

### *2.1. Instrumentation*

An ICP-OES model ICAP 6300 Duo Thermo Scientific (Waltham, MA, USA) was used for the determination of the metals, under the following instrumental conditions: approximate RF power, 1150 W; Gas flow (nebulizer gas flow, auxiliary gas flow), 0.5 L/min; Injection of the sample to the pump flow, 50 rpm; stabilization time, 0 s (Luis et al., 2015). Liquid argon (99.999% purity, Air Liquide, Spain) was used in the ICP-OES metal determination.

The ICP-OES was equipped with an automatic sampler model CETAC ASX-520 (Thermo Fisher Scientific, MA, USA) and with a CID86 chip (Charge Injection Device) which allows the user to choose wavelengths from 166 to 847 nm. In addition, the model ICAP 6300 Duo Thermo Scientific (Walthman, MA, USA) is analytically versatile and environmentally safe because it reduces the consumption of the purge gas and is more stable, has less noise and a better dynamic range than previous designs and its non-destructive readout allows optimum signal-to-noise measurements at all concentration levels.

The analytical calibration curves were prepared on a daily basis before the sample measurement. The calibration curves were prepared from a multi-element stock solution Multi-Element Std. SCP28AES (SCP Science, Baie-D'Urfe, QC, Canada) of 100 mg/L of V, Mn, Fe, Cu, Zn, Cr, Mo, Co, B, Ba, Li, Sr, Ni, Si, Al, Pb and Cd (Merck, Darmstadt, Germany) and a stock solution IV-STOCK-2 of 10000 μg/mL of Ca, Na, Mg, K (Inorganic Ventures, Christianburg, VA, USA). Different batch patterns have been used for the construction of the calibration curves to those used in the addition of samples, to obtain the accuracy and precision parameters of the method. The calibration curves were prepared using 1.5% nitric acid ($HNO_3$) (Merck, Darmstadt, Germany).

**Table 1**
Origin of the studied samples.

| Type | Manufacturer | No. Packages | Origin |
|---|---|---|---|
| Home-grown eggs | M1 | 2 | La Laguna, Tenerife (Spain) |
| | M2 | 2 | |
| | M3 | 2 | La Esperanza, Tenerife (Spain) |
| Free-range eggs | M4 | 2 | Güímar, Tenerife (Spain) |
| | M5 | 2 | Arafo, Tenerife (Spain) |
| | M6 | 2 | La Orotava, Tenerife (Spain) |

Quality controls were carried out based on the recovery percentage study obtained with the reference material under reproducible conditions in order to verify the accuracy of the analytical procedure (Luis et al., 2015; Rubio et al., 2017). The certified reference materials used, which were kept in a desiccator under controlled conditions of temperature and humidity, were as follows: apple leaves (NIST SRM 1515, Gaithersburg, MD, USA), typical diet (NIST SRM 1548a, Gaithersburg, MD, USA), wheat flour (NIST SRM 1567a, Gaithersburg, MD, USA) (Dybczynski, 2002; Iyengar et al., 2002; Nardi et al., 2009).

### *2.2. Samples and sampling*

The sample set consisted of a total of 288 eggs of which 144 eggs (12 packages of a dozen eggs each) were from free-range hens and 144 eggs were from home-grown hens. The samples were collected in local shops and supermarkets on the island of Tenerife (Canary Islands, Spain) (Table 1). The samples were acquired between November 2015 to April 2016 and the packages corresponded to different laying dates.

The most popular brands sold in Tenerife were selected as the samples, as they were at the time the most widely consumed by the local population. The sampling has been performed according to the recommendations in the relevant European regulations which are Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs, Commission Regulation (EU) No 836/2011 of 19 August 2011 amending Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs, and the Commission Regulation (EU) 2016/582 of 15 April 2016 amending Regulation (EC) No 333/2007 of 28 March 2007 as regards the analysis of inorganic arsenic, lead and polycyclic aromatic hydrocarbons and certain performance criteria for analysis (EC, 2007, 2011, 2016).

Each egg was divided into yolk and egg white using a plastic egg yolk separator (Tupperware, MA, USA) and homogenized egg samples were homogenized with a plastic fork.

Free-range eggs were from ISA-Brown hybrid hens, these hens are the product of the miscegenation between the White Rhode Island and the Red Rhode Island hens, both from the USA. The home-grown eggs were from local hens on the island of Tenerife.

The free-range hens were fed on cereals (split corn, soybean wheat, barley, corn flakes and oat flakes). The home-grown hens were fed on vegetables and cereals.

### *2.3. Treatment of samples*

Three grams of each separate sample were weighed to perform the analysis using Mettler Toledo scales (Mettler Toledo, Columbus, OH, USA) in porcelain crucibles (Staalich, Berlin, Germany) and then placed in an oven (Nabertherm Inc., DE, USA) at 70 °C for 24 h for their