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Analytical Methods

A fast and feasible method for Br and I determination in whole egg powder and its fractions by ICP-MS

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ABSTRACT

A method for Br and I determination in whole egg powder and its fractions (egg white and yolk) was developed by combining microwave-induced combustion (MIC) and inductively coupled plasma mass spectrometry (ICP-MS). Using the MIC method, 350 mg of whole egg powder and its fractions were efficiently digested using 50 mmol L^{-1} NH_4OH as an absorbing solution. The limits of detection for Br and I using the MIC method followed by ICP-MS determination were 0.039 and 0.015 μg g^{-1}, respectively. Using the proposed method, agreements with the reference values between 97 and 104% for Br and I were obtained by analysis of reference material NIST 8435. Finally, it was possible to observe that Br concentration (4.59–5.29 μg g^{-1}) was higher than I (0.150–2.28 μg g^{-1}) for all the evaluated samples.
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1. Introduction

The egg is a relatively low-cost food that is consumed worldwide and presents high-quality proteins, lipids (triacylglycerol, phospholipids and cholesterol), minerals and vitamins (Miranda et al., 2015). In some countries, such as China, Japan and Mexico, the annual consumption is about 350 eggs per person (Bertechini & Mazzuco, 2013), while in Brazil this consumption is lower, around 180 eggs per person (Brazilian Poultry Association. Annual Report., 2016). Recently, typical factors of modern life have played an important role in the increased consumption of pre-cooked and processed foods. In this sense, the consumption of egg and its fractions in a powder form has increased due to the stability of these products, their ease of use, as well as their important role as a complement in human diets (Miranda et al., 2015).

Variations in egg composition can be observed depending on the type of feed, rearing and breed of laying hens (Ayadi, Khemakhem, Belgith, & Attia, 2008; Caboni et al., 2005). Although these variations can occur, limited information regarding the concentration of minerals can be found in the literature (Dahl, Johansson, Julshamn, & Meltzer, 2003; Dusová, Trávnícek, Peska, Falta, & Pálka, 2012; Haldimann, Alt, Blanc, & Blondeau, 2005; Rose et al., 2001; Travnicek, Kroupova, Herzig, & Kursa, 2006; Zikovsky & Soliman, 2002). Considering the benefits and risks of health problems due to the deficiency or excess of minerals in the diet, recently, special atention has been given to Br and I. Iodine in hormone form has several important physiological functions in the body, mainly related to the development, growth and metabolic processes. Therefore, the deficiency or excess of this element may result in human health disorders (WHO, 2007; Zimmermann, Jooste, & Pandav, 2008). In this context, the World Health Organization (WHO) has recommended an I intake of 150 μg per day as suitable for adults to maintain normal thyroid function (WHO, 2007). On the other hand, bromine still presents an unknown mechanism of action; therefore, it does not have a well-established physiological function (Pavelka, 2004; Romarís-Hortas, Bermejo-Barrera, & Moreda-Pineiro, 2012). However, the WHO recommends that adults should ingest 0.4 mg kg^{-1} of body weight of Br daily (WHO, 2009).

In this way, Br and I determination in eggs and their fractions is important to ensure the ingestion of safe levels of these elements, especially because this food is widely consumed. However, only a few studies in the literature have involved Br and/or I determination in eggs (Dahl et al., 2003; Dusová et al., 2012; Haldimann

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et al., 2005; Rose et al., 2001; Travnicek et al., 2006; Zikovsky & Soliman, 2002). Moreover, there is scarce information related to distribution of these elements in egg white and yolk (Rose et al., 2001).

Analytical techniques such as ion chromatography (IC), inductively coupled plasma optical emission spectrometry (ICP OES) and inductively coupled plasma mass spectrometry (ICP-MS) have been used to determine Br and I in foods (Knapp, Maichin, Fecher, Hasse, & Schramel, 1998; Mesko, Toralles, et al., 2016). Although ICP-MS presents high selectivity and suitable limits of detection (LODs) for Br and I determination at low concentrations (Knapp et al., 1998; Mesko, Hartwig, et al., 2011), an effective sample preparation step must be performed to obtain final solutions compatible with sample introduction systems.

In this sense, sample preparation based on alkaline extraction has usually been performed using tetramethylammonium hydroxide (TMAH) for further Br and I determination by ICP-MS. However, high blank values have been reported because this reagent is difficult to purify (Ghisi et al., 2011; Romarís-Hortas et al., 2012). Furthermore, it is important to highlight that TMAH does not allow the digestion of organic samples (basically a solubilization or extraction process), thus the use of this alkaline solution usually provides solutions with high carbon content. It results in a further dilution step prior to analysis by ICP-MS and, consequently, higher LODs (Nóbrega et al., 2006).

Thus, considering the complexity of whole egg matrix (high content of lipids and protein) (Ayadi et al., 2008; Caboni et al., 2005), the drawbacks associated with alkaline extraction, and the unsuitability of wet digestion for subsequent determination of halogens (Knapp et al., 1998), combustion methods can be considered a good alternative for sample preparation of eggs and subsequent Br and I determination by ICP-MS. Combustion methods allow the conversion of organic matrix to the respective oxidation products. These methods can reduce risks of contamination of samples and losses of analytes because they can be performed in closed vessels, such as by using a combustion bomb, Schöniger flask or microwave-induced combustion (MIC) (Barin, Flores, & Knapp, 2006). In the case of MIC system, samples are positioned on a quartz holder that is placed inside of a quartz vessel. The combustion is performed inside of this vessel pressurized with oxygen, using microwave radiation and ammonium nitrate solution for the ignition step (Barin, Flores, Mesko, Mello, & Pereira, 2014; Mesko, Costa, Picoloto, Bizzi, & Mello, 2016; Muller et al., 2013).

In this sense, MIC is a convenient alternative for extraction methods, in view that it presents high throughput, low reagent consumption and allows the use of diluted alkaline solutions for analyte absorption, as well as the achievement of digests with very low residual carbon content (RCC), which is an important parameter for analysis by various determination techniques (Flores, Barin, Paniz, Medeiros, & Knapp, 2004). Although the MIC system has been successfully applied for the digestion of organic samples for subsequent determination of halogens (Costa et al., 2015; Crizel et al., 2015; Hartwig et al., 2014; Mesko et al., 2014; Mesko, Costa, et al., 2016; Mesko, Toralles, et al., 2016; Silva et al., 2016), this method has not been applied to digestion of whole egg powder and its fractions.

The present study proposes a method using MIC to digest whole egg powder and its fractions (egg white and yolk powders) for further determination of Br and I by ICP-MS. In addition, for comparison of results, microwave-assisted extraction (MAE) using alkaline solutions and subsequent Br and I determination by ICP-MS was also performed.

2. Materials and methods

2.1. Samples

Samples of whole egg powder as well as egg white and yolk powders produced in Rio Grande do Sul and São Paulo states, Brazil, were purchased in a local market. Three brands of each type of sample (whole egg, egg white and egg yolk) were obtained, totalizing nine samples, and the brands were labeled as A to C. The optimization of the proposed method was carried out using whole egg powder (brand "A"), which was arbitrarily selected for this initial study.

The other samples were further analyzed using optimized conditions. Prior to analysis, the samples were mixed and dried at 65 °C for 8 h. The accuracy of the proposed method was evaluated by the analysis of biological reference material (RM) NIST 8435 (whole milk powder), which was submitted to the same treatment of egg samples.

2.2. Instrumentation

A food processor (model All In One 2, Philco, Brazil) and a conventional oven (model 400/2ND, DeLeo, Brazil) were used to mix and dry the samples, respectively. Samples were weighed using an analytical balance (model AY220, Marte, Brazil), with resolution of 0.0001 g and maximum load of 220 g.

A muffle furnace (model C.318.24, Quimis, Brazil) was used for ash determination, and protein determination was performed using a digestion block (model KB 40S, C Gerhardt, Brazil) and a nitrogen distiller (model MA 036 PLUS, Marconi, Brazil). A heating bath (model 18 BU B. Braun Biotech International, Thermomix, Germany) and a centrifuge (model 2–3, Sigma, Germany) were used for fat determination procedure.

A pHmeter (model MPA-210, Tecnopon, Brazil) equipped with a glass combined electrode was used to determine the pH of the solutions that were obtained after digestion by MIC.

MIC and MAE methods were performed in a microwave oven (model Multiwave 3000, Anton Paar, Austria). This system was equipped with 8 high-pressure quartz vessels when MIC was performed (volume of 80 mL, maximum temperature and pressure of 280 °C and 80 bar, respectively) (Anton Paar GmbH, 2006). A quartz device was used as a sample holder for sample preparation by MIC (Mesko, Mello, et al., 2010). On the other hand, when MAE was used for sample preparation, 8 polytetrafluoroethylene (PTFE) vessels (volume of 60 mL, maximum temperature and pressure of 260 °C and 60 bar, respectively) were used (Anton Paar GmbH, 2006).

An inductively coupled plasma optical emission spectrometer (model Spectro Ciros CCD, Spectro Analytical Instruments, Germany) with axial view configuration and optics sealed that was equipped with a quartz torch, a quartz injector tube, a cross flow nebulizer coupled to a double pass-Scott type spray chamber was used for the evaluation of carbon content in the digests obtained by MIC. Before carbon determination, volatile carbon compounds in digests were removed using argon bubbled through the solutions for 2 min (Bizzi et al., 2014). Yttrium (SpexCertiPrep, USA) was added to the samples and calibration solutions as an internal standard for carbon determination (final concentration was 1 mg L^{-1}). The operational conditions used for C determination by ICP OES have been described elsewhere (Bizzi et al., 2014).

Bromine and I determination was carried out using an inductively coupled plasma mass spectrometer (model Elan DRC II, PerkinElmer-SCIEX, Canada) equipped with a concentric nebulizer (Meinhard Associates), a cyclonic spray chamber (Glass Expansion,

Inc.) and a quartz torch with a quartz injector tube (2 mm i.d.). Instrumental performance was optimized following the instructions of the manufacturer (PerkinElmer-SCIEX, Software Guide, & Thornhill, 2003). The operational conditions have been previously described (Costa et al., 2015).

2.3. Reagents

All solutions were prepared using ultrapure water (18.3 MΩ cm) obtained from a purification system (model MegaUp, Megapurity, South Korea), and all reagents used in this study were of analytical grade. The solutions of ammonium hydroxide and TMAH that were used as absorbing and extracting solutions, respectively, were prepared from 27% NH_4OH solution (Synth, Brazil) and 25% TMAH in methanol solution (Sigma-Aldrich, USA). Ammonium nitrate solution (6 mol L^{-1}), which was used as the igniter of combustion for MIC, was prepared by dissolution of the respective solid reagent (Merck, Germany) in water.

The quartz vessels and holders were decontaminated using 6 mL of 65% HNO_3 (Vetec, Brazil) with a microwave heating program set at 1400 W for 10 min (heating) and 0 W for 20 min (cooling), followed by decontamination with ultrapure water using the same conditions (described by Costa et al. (2015)). Small discs (12 mg, 15 mm of diameter) of filter paper (0.5% ash content, Qualy, J Prolab, Brazil) were used as a combustion aid. Polyethylene (PE) films were used to wrap the samples. Before combustion, the discs of filter paper and PE films were cleaned by immersion in 20% (v v^{-1}) ethanol solution for 20 min. This step was performed using an ultrasonic bath (model USC-1800 A, Unique, 40 kHz, 155 W, Brazil), and the paper discs and PE films were further rinsed with ultrapure water and dried in a class 100 laminar flow hood (model CSLH-12, Veco, Brazil).

For the determination of Br and I by ICP-MS, standard solutions (1–10 μg L^{-1} for Br and 0.1–1 μg L^{-1} for I) were prepared by dilution of stock solutions in 10 mmol L^{-1} NH_4OH. Stock solutions (1000 mg L^{-1}) were prepared by dissolution of potassium bromide and potassium iodide salts (purity ⩾99.5%, Merck, Germany) in water.

Argon with purity of 99.996% (White Martins, Brazil) was used for plasma generation, nebulization and as the auxiliary gas for Br and I determination by ICP-MS and for C determination by ICP OES.

Petroleum ether (Proquimios, Brazil), ethyl ether (Synth, Brazil) and hydrochloric acid (Synth, Brazil) diluted in ultrapure water (1:11) were used for fat determination using the Mojonnier method (Horwitz & Latimer, 2011, chap. 34).

The following reagents were used for crude protein determination by Kjeldahl method: *i) digestion step*: 98% sulfuric acid (Synth, Brazil) and catalytic mixture of anhydrous sodium sulfate and copper sulfate pentahydrate (Synth); *ii) distillation step*: 12.5 mol L^{-1} sodium hydroxide (Isofar, Brazil), 4% (w v^{-1}) boric acid (Vetec, Brazil), methyl red (Synth, Brazil), all dissolved in water, and 0.066% (w v^{-1}) bromocresol green (Synth, Brazil) dissolved in 0.033% (w v^{-1}) ethanol (Vetec, Brazil); and *iii) titration step*: 0.01 mol L^{-1} HCl.

2.4. Physical-chemical characterization of whole egg powder and its fractions

The composition of whole egg powder and its fractions was evaluated by the determination of moisture, ash, crude protein and fat content. These procedures were preformed using whole egg powder and its fractions from brand "A", which was arbitrarily selected.

The moisture content was determined by drying the samples in an oven at 105 °C until they reached a constant weight, and ash content was determined by dry-ashing in a furnace at 550 °C (Horwitz & Latimer, 2011, chap. 34). The determination of crude protein content was performed using the Kjeldahl method, while fat determination was carried out using the Mojonnier method (Horwitz & Latimer, 2011, chap. 34).

2.5. Sample preparation

For the MIC method, a whole egg powder sample (100–350 mg, brand A) or RM NIST 8435 (350 mg) was weighed and wrapped with a PE film (8 × 8 cm). The PE film was then sealed by heating, and the excess was removed (final PE film mass used was approximately 30 mg). Before closing the PE film as a small wrap, 0, 50 or 100 μL of NH_4NO_3 solution (6 mol L^{-1}) was added directly to the sample. Then, the PE wrap containing the sample or the RM was positioned in the quartz holder with a small filter paper moistened with 50 μL of 6 mol L^{-1} NH_4NO_3 solution (Pereira et al., 2015). The quartz holder was placed inside the quartz vessel previously charged with 6 mL of absorbing solution (10–75 mmol L^{-1} NH_4OH or water). After closing, the vessel was positioned in the rotor and pressurized with 20 bar of oxygen (Praxair, Brazil), and the rotor containing the vessels was placed inside the microwave cavity. The microwave heating program used for sample preparation by MIC was: *i*) 1400 W for 5 min, and *ii*) 0 W for 20 min (cooling step). After combustion, the pressure of each vessel was released and the digests were transferred to volumetric flasks, diluted with ultrapure water up to 25 mL and subjected to Br and I determination using ICP-MS.

The accuracy was also evaluated by analyte recovery tests. In this case, a solution containing 28 mg L^{-1} Br and 4.2 mg L^{-1} I (50 μL) was added to the whole egg powder before sample preparation by MIC, using the optimized conditions. Moreover, a similar recovery test was carried out by mixing 50 mg of RM NIST 8435 with the whole egg powder sample (300 mg). Additionally, the proposed method was applied for Br and I determination in other whole eggs (brands B and C), as well as in egg white (brands A to C) and egg yolk (brands A to C).

For the MAE method, 350 mg of whole egg powder and 6 mL of 0.11 mol L^{-1} TMAH solution were transferred to PTFE vessels. Then, vessels were closed and positioned in the microwave rotor. The rotor was placed inside the oven, and the microwave heating program was started, using: *i*) 1000 W for 50 min (ramp of 10 min), and *ii*) 0 W for 20 min (cooling step). The maximum temperature used during the MAE was limited to 90 °C due to the possibility of TMAH degradation (Knapp et al., 1998; Tagami, Uchida, Hirai, Tsukada, & Takeda, 2006). After cooling, the obtained extracts were filtered, transferred to volumetric flasks and diluted with ultrapure water to 25 mL before analysis.

All statistical calculations were performed using Graph Pad InStat (Graph Pad InStat Software Inc, Version 3.00, 1997) software, and a confidence level of 95% was used.

3. Results and discussion

3.1. Physical-chemical characterization

Moisture, ash, crude protein and fat content were previously determined in whole egg powder and its fractions in order to obtain information regarding to the composition of the samples. This was done taking into account that complex matrices with high protein and lipid content can be difficult to decompose, even when the MIC method is used for sample digestion. The results obtained for physical-chemical characterization are presented in Table 1.

The values for moisture, ash content, crude protein and fat in whole egg powder and its fractions ranged from 1.4 to 4.3%, 4.0 to 7.0%, 30 to 68% and <1 to 31%, respectively. A low percentage of moisture was expected because the samples had been subjected

Table 1
Physical-chemical characterization of whole egg powder and its fractions (from brand A). Results are in % (mean ± standard deviation, n = 4).

Content	Whole egg powder	Egg white powder	Egg yolk powder
Moisture	2.9 ± 0.2	4.3 ± 0.3	1.4 ± 0.1
Ash	4.0 ± 0.4	7.0 ± 1.0	5.0 ± 0.6
Crude protein	39 ± 4.0	68 ± 5.0	30 ± 3.0
Fat	31 ± 3.0	<1.0	31 ± 4.0

to an industrial dehydration process. On the other hand, the ash content could have been related to the type of feed consumed by laying hens. It is important to mention that the egg samples used in this study were obtained from animals with a diet rich in many types of salts (sodium chloride, calcium iodate, iron sulfate, cobalt sulfate, copper sulfate, manganese sulfate, sodium selenite), according to information provided by the manufacturers.

As can be seen in Table 1, egg white powder has almost double of the crude protein content when compared to egg yolk powder. In contrast, egg yolk powder presented a fat content up to 30 times higher than egg white powder. The influence of physical-chemical characteristics in the proposed MIC method is discussed in detail in Section 3.2. In addition, it should be emphasized that part of the value not quantified in the total egg composition in the evaluated samples may be related to the presence of some additives used during the production process.

3.2. Digestion of whole egg powder and its fractions by MIC – preliminary studies

A study was initially performed to evaluate a suitable way to insert whole egg powder and its fractions into the combustion system. During the experiments in which the samples were just pressed with a hydraulic press (a common way to prepare solid samples for the MIC method) (Barin et al., 2014), it was observed that part of the fat was lost. Thus, although it has not been evaluated whether fat losses influence in Br and I concentration, a PE film was used to wrap the samples instead of pressing them, according to the procedure proposed by Costa et al. (2015).

Experiments using the samples wrapped in PE film allowed digestion of up to 300 mg of whole egg powder by MIC using an oxygen pressure of 20 bar and 6 mL of water as absorbing solution. It is important to mention that water was used in this case because the objective of this initial study was only to evaluate the higher sample mass that could be decomposed by MIC. Under these conditions, the maximum pressure reached was about 37 bar, which is lower than 50% of the maximum operating pressure of the microwave system (80 bar). Moreover, there was no statistical difference in the maximum pressure that was reached in the system during the combustion of 100 to 300 mg of whole egg powder (analysis of variance, ANOVA, confidence level of 95%). On the other hand, after the combustion of 350 mg of whole egg powder, the maximum pressure was lower than 30 bar. This behavior was due to the incomplete digestion of the sample (whole egg powder), once the presence of deposits of carbon in the walls of the quartz vessels and in the quartz holders was verified, as shown in Fig. 1A and B.

Therefore, in order to increase the sample mass that could be digested by MIC, the addition of 6 mol L^{-1} NH_4NO_3, the same solution used as the combustion igniter, into the polyethylene bag containing the sample was evaluated. Thus, with 100 µL of this solution added to the sample, it was possible to obtain a more reproducible and complete combustion that allowed the digestion of 350 mg of whole egg powder by MIC (Fig. 1C and D). However, even using 100 µL of NH_4NO_3 (6 mol L^{-1}) added to the sample, when the sample mass was increased to 400 mg the digestion was incomplete. Based on these results, the whole egg powder

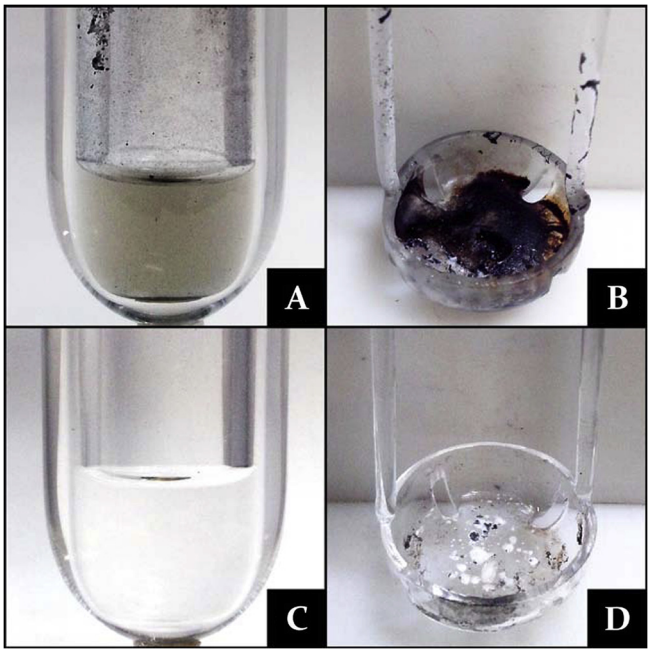


Fig. 1. Aspect of absorbing solution and the quartz holder, respectively, after decomposition of 350 mg of whole egg powder without (A, B) and with (C, D) addition of 100 µL of 6 mol L^{-1} NH_4NO_3, with O_2 pressure of 20 bar.

mass digested by MIC was different than the masses reported for other types of biological samples (500–1000 mg) (Barin et al., 2014; Costa et al., 2015). This behavior could be related to the composition of the sample, which makes difficult the decomposition of high sample masses even when MIC is used.

Egg white and yolk powders were also digested under the same conditions used for whole egg powder. It was possible to decompose egg white powder up to 750 mg, while the maximum mass of egg yolk powder that was decomposed by MIC was 350 mg, using the combustion igniter (6 mol L^{-1} NH_4NO_3) inside the wrap containing the sample. As mentioned before, this behavior should be related to the variation in the composition of the samples, as shown in Table 1. Although the maximum sample mass that could be digested by MIC was different for each type of sample, in order to keep the same conditions for all digestions, further studies were carried out using 350 mg of sample and 100 µL of NH_4NO_3 (6 mol L^{-1}) solution.

The microwave heating program was based on the methods of previous studies (Crizel et al., 2015; Hartwig et al., 2014; Mesko, Mello, et al., 2010). It was observed that the ignition for all kinds of samples (whole egg, egg white and egg yolk powders) occurred in 6 ± 2 s. Moreover, it was observed that 350 mg of samples burned in 29 ± 5 s. Thus, the heating program considered 1 min for the combustion step, 4 min for the reflux of the absorbing solution and 20 min for cooling.

3.3. Influence of absorbing solution on Br and I recoveries

Taking into account that the choice of absorbing solution in combustion methods is important to achieve suitable analyte recoveries and that alkaline solutions are recommended for halogen absorption after decomposition by MIC (Hartwig et al., 2014), in this study, NH_4OH solutions (10, 25, 50 and 75 mmol L^{-1}) and water were evaluated for Br and I absorption. The evaluation of the absorbing solution was carried out by adding the equivalent of 1.4 and 0.21 µg of Br and I, respectively, to the samples of whole egg powder (350 mg). As shown in Fig. 2, recoveries were

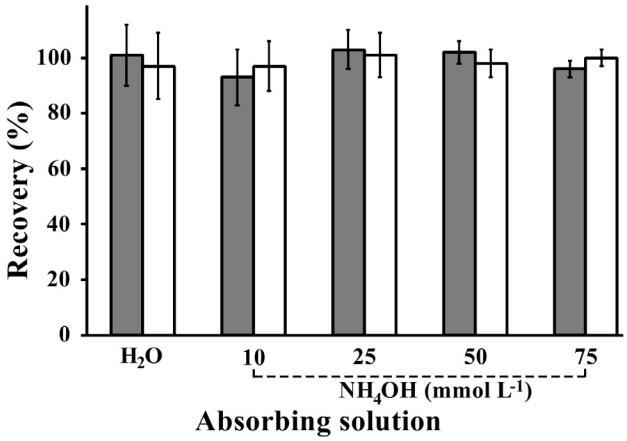


Fig. 2. Recoveries of Br (gray bars) and I (white bars) by ICP-MS in whole egg powder digested by MIC with 5 min of reflux step, using NH_4OH (10–75 mmol L^{-1}) or water as absorbing solution (0.35 g of sample, n = 3).

quantitative (96–103%) for both analytes for all evaluated solutions. However, relative standard deviations (RSDs) lower than 6% were obtained only when 50 or 75 mmol L^{-1} NH_4OH solution was used. For the other NH_4OH solutions (10 and 25 mmol L^{-1}) and for water, the RSDs obtained were about 12%.

In addition, it is important to mention that although the recoveries obtained using NH_4OH solutions or water did not present significant differences (analysis of variance, ANOVA, confidence level of 95%), the pH values of final solutions obtained after digestion by MIC were different. The pH values of final solutions were between 2.0 and 3.0 when 10 or 25 mmol L^{-1} NH_4OH solution or water was used as absorbing solution, while the pH values were between 7.0 and 8.0 with the use of NH_4OH solutions with a concentration equal to or higher than 50 mmol L^{-1}. Therefore, considering the possibility of formation of volatile species with halogens in acid medium, the lower RSD and the use of diluted solutions, which minimize the necessity of dilutions before analysis by ICP-MS, 50 mmol L^{-1} NH_4OH was chosen for further studies.

The accuracy of the MIC method using 50 mmol L^{-1} NH_4OH as the absorbing solution was evaluated by the digestion of RM NIST 8435 (whole milk powder) under the same conditions applied to the whole egg powder samples. Therefore, by the proposed method, an agreement between 97% and 104% for both analytes was obtained when the results (Br: 19.5 ± 0.9 μg g^{-1} and I: 2.39 ± 0.07 μg g^{-1}) were compared with the reference values (Br: 20.0 ± 10.0 μg g^{-1} and I: 2.30 ± 0.40 μg g^{-1}). In addition, the RSDs were lower than 6%. However, taking into account that this RM does not present a similar matrix to whole egg powder, an additional study was carried out by digestion of a mixture containing whole egg powder (300 mg) and RM NIST 8435 (50 mg). Recoveries were around 95 ± 2% for both elements, indicating the suitability of the proposed method for Br and I determination in whole egg powder and its fractions.

3.4. Determination of Br and I in whole egg powder and its fractions

After the optimization of the suitable conditions for digestion of whole egg powder and its fractions (egg white and yolk) by MIC for subsequent Br and I determination by ICP-MS, the proposed method was applied for other samples, and results are shown in Table 2.

As can be observed in Table 2, the concentration of Br (between 4.59 and 5.29 μg g^{-1}) was higher than the concentration of I (between 0.150 and 2.28 μg g^{-1}) in whole egg powder samples. Moreover, Br concentrations were higher in egg white powder, while I concentrations were higher in egg yolk powder. As previously mentioned, the laying hens were fed with many salts, including calcium iodate; thus, part of the I concentration in the evaluated samples can probably be attributed to the addition of these compounds to feed.

Table 2
Concentration of Br and I in whole egg powder and its fractions after digestion by MIC using 50 mmol L^{-1} NH_4OH as absorbing solution and determination by ICP-MS (mean expressed on a dry basis ± standard deviation, n = 4).

Brand	Sample	Concentration (μg g^{-1})	
		Br	I
A	Whole egg powder	5.29 ± 0.21	0.660 ± 0.033
	Egg white powder	16.0 ± 0.60	0.150 ± 0.007
	Egg yolk powder	2.53 ± 0.10	1.47 ± 0.07
B	Whole egg powder	4.59 ± 0.18	1.60 ± 0.08
	Egg white powder	7.10 ± 0.28	0.260 ± 0.013
	Egg yolk powder	2.50 ± 0.10	1.30 ± 0.06
C	Whole egg powder	5.19 ± 0.20	1.37 ± 0.07
	Egg white powder	6.11 ± 0.24	0.190 ± 0.009
	Egg yolk powder	2.86 ± 0.11	2.28 ± 0.11

The only information found in the literature with relation to the Br content in whole egg was that it is lower than 2.6 μg g^{-1} (Rose et al., 2001). On the other hand, it was described by some authors that whole egg presented 0.450–1.63 μg g^{-1} of I, while yolk and egg white presented 0.900–1.41 μg g^{-1} and 0.150–0.260 μg g^{-1}, respectively (Dahl et al., 2003; Dusová et al., 2012; Haldimann et al., 2005; Rose et al., 2001; Travnicek et al., 2006; Zikovsky & Soliman, 2002). However, considering that in the most of these studies the samples were not dehydrated, it is difficult to perform a comparison with the results obtained in the present work (Dusová et al., 2012; Rose et al., 2001; Travnicek et al., 2006; Zikovsky & Soliman, 2002). In spite of this, the determination of Br and I in whole egg powder and its fractions is essential, in view of the wide range of concentrations of these elements in evaluated samples, as well as the risk of health problems due to the deficiency or excess of Br and I in the human organism.

3.5. Comparison of proposed method with microwave assisted extraction using alkaline solution

Whole egg powder was also analyzed by ICP-MS after MAE using 0.11 mol L^{-1} TMAH as the extracting solution. The choice of TMAH solution was based on previous works that showed the feasibility of this alkaline solution for extraction of halogens from whole eggs (Knapp et al., 1998; Rose et al., 2001). The Br and I concentration obtained by ICP-MS after the MAE method using 0.11 mol L^{-1} TMAH solution was lower than the LODs (Br: 22.7 μg g^{-1}; I: 5.57 μg g^{-1}) obtained for this condition.

On the other hand, the LODs using the MIC method were 0.039 μg g^{-1} for Br and 0.015 μg g^{-1} for I. Regarding the LODs, it is important to mention that they were calculated from blank values and the standard deviation for 10 replicates, taking into account the sample mass (350 mg), the final volume of digests (25 mL), and, in the case of the MAE method, the dilution factor (20 times). Therefore, the main reason that the MIC and MAE LOD values were considerably different was due to the presence of high carbon content in solutions obtained by the MAE method, which required a dilution step (20 times) prior to the determination by ICP-MS. This dilution was necessary because the presence of carbon in the solution can result in carbon deposits in the plasma interface, resulting in changes of sensitivity during ICP-MS analysis. Moreover, the presence of high carbon content in the digests during ICP-MS analysis can also interfere in Br and I ionization in the plasma (Flores et al., 2004; Grindlay, Mora,

Table 3
Comparison of the main characteristics of the proposed MIC and MAE methods.

Parameter	MIC	MAE
Sample mass (g)	0.35	0.35
Reagents	6 mL of 50 mmol L^{-1} NH_4OH as absorbing solution	6 mL of 0.11 mol L^{-1} TMAH as extraction solution
Time for sample preparation (min)*	25	80
RSD (%)	1–6	–
Blank values, Br (μg L^{-1})	<0.42	14.4
Blank values, I (μg L^{-1})	<0.25	3.54
LOD, Br (μg g^{-1})	0.039	22.7
LOD, I (μg g^{-1})	0.015	5.57

* Including cooling time.

Loos-Vollebregt, & Vanhaecke, 2013). In the case of I measurements, it is possible to observe an increase in the signal intensity due to occur of charge transfer reactions between I and ionized carbon species (C^{2+}, CO^{+}, or CO_2^{+}) formed in the plasma, resulting in higher ionization of the analyte (Grindlay et al., 2013). Moreover, blank values for both analytes using MAE (Br: 14.4 μg L^{-1}; I: 3.54 μg L^{-1}) were higher than those obtained by MIC (Br: < 0.42 μg L^{-1}; I: < 0.25 μg L^{-1}).

A comparison of the main characteristics of the MIC and MAE methods is summarized in Table 3. As can be seen, the main advantages of MIC are related to the time for sample preparation, which was up to 3 times lower. The lower values of LOD obtained for the MIC method are associated to lower blank values, as well as the high efficiency of matrix oxidation, and it allows the determination of Br and I in whole egg powder and its fractions.

4. Conclusion

Considering that Br and I were quantified in trace levels in all evaluated samples, MIC was a suitable sample preparation method for subsequent analysis by ICP-MS, given that a high digestion efficiency is important to ensure reliable results by this technique. The use of a reflux step after combustion combined with the use of 50 mmol L^{-1} NH_4OH as the absorbing solution allowed the achievement of quantitative recoveries of Br and I. On the other hand, using the MAE method, the results obtained for Br and I were lower than the LODs, probably due to high blank values for both analytes and the necessity of sample dilution previous to ICP-MS analysis. Therefore, the use of MIC associated to determination by ICP-MS could be an alternative to determine Br and I in whole egg powder and its fractions, presenting advantages such as the relatively low time for digestion (25 min), which enables high throughput of sample preparation (eight samples per run), low LODs for Br and I and low generation of residues.

Regarding the concentrations of Br and I in egg fractions, it should be noted that egg white powder had higher Br concentrations, while egg yolk powder had higher I concentrations. In addition, it is essential to highlight the determination of these elements considering the range of concentration in whole egg powder and its fractions, which are widely consumed and used in many types of processed foods. Moreover, the deficiency or excess of these elements in the organism can cause problems of health. So, although there is a need for a more in-depth study to understand the behavior of Br and I concentrations in egg, these results can be considered a preliminary step in learning more about the mineral composition of this food and its byproducts. Also, it is important to mention that the proposed method can be employed in research that examines the daily intake of Br and I and the bioavailability of these elements in eggs.

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