Method of Test for Heavy Metals in Eggs

1. Scope

This method is applicable for the determination of copper and lead in eggs.

2. Method

After microwave assisted acid digestion, heavy metals are determined by inductively coupled plasma mass spectrometry (ICP-MS).

2.1. Equipment

2.1.1. Inductively coupled plasma mass spectrometer.

2.1.2. Microwave digester: > 1000 W with temperature control and pressure feedback system.

2.1.3. Homogenizer.

2.1.4. Acid steam cleaning system.

2.2. Chemicals

Nitric acid, ultrapure grade (67-70%) and reagent grade;
Deionized water, resistivity ≥ 18 MΩ • cm (at 25°C);
Copper and lead, 1000 μg/mL, reference standards, ICP grade;
Rhodium and bismuth, 1000 μg/mL, internal standard, ICP grade.

2.3. Apparatus

2.3.1. Microwave digestion flask(Note): quartz or Teflon.

2.3.2. Volumetric flask(Note): 50 mL.

2.3.3. Storage tube: 50 mL, PP.

2.3.4. Membrane filter: 0.45 μm, PTFE.

Note: After cleaning, use an acid steam cleaning system to clean the apparatus with nitric acid (reagent grade) vapor for 2 hours, or soak the apparatus in nitric acid (reagent grade) : water (1:1, v/v) overnight. Take the apparatus out, wash away the residual nitric acid with deionized water and dry.

2.4. 5% (w/w) nitric acid

Add 50 mL of nitric acid (ultrapure grade) slowly into 500 mL of deionized water, and dilute to 1000 mL with deionized water.

2.5. Internal standard solution preparation

Accurately transfer 0.5 mL of rhodium and bismuth internal

standards to each 50-mL volumetric flask, make up to volume with 5% (w/w) nitric acid, and transfer to storage tubes as the internal standard stock solutions. When to use, mix appropriate amount of the internal standard stock solutions, and dilute with 5% (w/w) nitric acid to 1000 ng/mL as the internal standard solution.

2.6. Standard solution preparation

Accurately transfer 0.5 mL of copper and lead reference standards to each 50-mL volumetric flask, make up to volume with 5% (w/w) nitric acid, and transfer to storage tubes as the standard stock solutions. When to use, mix appropriate amount of the standard stock solutions and the internal standard solution, dilute with 5% (w/w) nitric acid to 0-50 ng/mL (containing 10 ng/mL internal standards), and transfer to storage tubes as the standard solutions.

2.7. Standard curve preparation

Inject the standard solutions into the ICP-MS at the appropriate rate, and operate according to the following conditions. Establish the standard curves of copper and lead by the ratios of the signal intensity of copper or lead to that of the internal standard vs. the added concentrations.

ICP-MS operating conditions(Note):

Radiofrequency power: 1500 W.

Plasma argon flow rate: 15 L/min.

Auxiliary argon flow rate: 0.9 L/min.

Nebulizer argon flow rate: 1.0 L/min.

Atomic mass (*m/z*):

Analyte		Internal standard	
Copper	63、65	Rhodium	103
Lead	208、206、207	Bismuth	209

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2.8. Sample solution preparation

Homogenize the egg white and egg yolk after removing the shell from the egg sample, transfer about 0.5 g of the homogenized sample accurately weighed into a microwave digestion flask, add

0.5 mL of the internal standard solution and 6 mL of nitric acid (ultrapure grade), and then digest according to the following conditions. After cooling to room temperature, transfer to a 50-mL volumetric flask. Wash and rinse the residue in the digestion flask with 5 mL of deionized water several times. Add the washings to the same volumetric flask, and make up to volume with deionized water. Transfer to a storage tube, filter with a membrane filter, and take the filtrate as the sample solution. Take an empty microwave digestion flask, add 0.5 mL of the internal standard solution and 6 mL of nitric acid (ultrapure grade), and perform the same procedure described above as the blank solution.

Microwave digester operating conditions(Note):

Condition / Step	Power (W)	Heating time (min)	Duration time (min)	Temperature (°C)
1	1200	5	-	70
2	1200	15	-	140
3	1200	10	10	240

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2.9. Quantification

Inject the sample solution, the blank solution and the standard solutions into the ICP-MS separately at the appropriate rate, and operate according to the conditions in section 2.7. Calculate the amount of copper or lead in the sample based on the ratio of the signal intensity of copper or lead to that of the internal standard by the following formula:

The amount of copper or lead in the sample (mg/kg)

$$= \frac{(C - C_0) \times V}{M \times 1000}$$

Where,

C: the concentration of copper or lead in the sample solution calculated by the standard curve (ng/mL)

C_0: the concentration of copper or lead in the blank solution calculated by the standard curve (ng/mL)

V: the final make-up volume of the sample (mL)

M: the weight of the sample (g)

Remark: 1. The limits of quantification (LOQs) are 0.2 mg/kg for copper, and 0.02 mg/kg for lead.

2. Further validation should be performed when interference compounds appear in samples.

3..When the test is conducted by other instruments, verification by the certified reference material (CRM) or the standard reference material (SRM), or validation of the method should be performed.

Reference

1. USFDA. 2015. Elemental analysis manual for food and related products. 4.7. Inductively coupled plasma - mass spectrometric determination of arsenic, cadmium, chromium, lead, mercury, and other elements in food using microwave assisted digestion.
2. Lai, I. C., Tsai, M. C., Lin, Y. W., Dai, H. Y., Chu, C. M., Liao, C. D., Kao, Y. M., Zhou, X. G., Cheng, H. F. 2016. The Development of the Method for Lead and Copper in Eggs. 2016 Annual Research Report of the Taiwan Food and Drug Administration.