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Method of Test for Natural Edible Colorants - Test of Heavy Metals in Plant Carbon

1. Scope

This method is applicable for the determination of arsenic, cadmium, mercury and lead in plant carbon of natural edible colorant.

2. Method

After microwave assisted acid digestion under an autoclave style or a closed vessel style microwave digester, 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. Autoclave style microwave digester: > 1000 W with temperature control and pressurized system.

2.1.3. Closed vessel style microwave digester: > 1000 W with temperature control and pressure feedback system.

2.1.4. Acid steam cleaning system.

2.2. Chemicals

Nitric acid, ultrapure grade (67-70%) and reagent grade;

Hydrogen peroxide (30%), ultrapure grade;

Deionized water, resistivity ≥ 18 MΩ • cm (at 25°C);

Arsenic, cadmium, mercury, lead and gold, 1000 μg/mL, reference standards, ICP grade;

Germanium, rhodium, iridium and bismuth, 1000 μg/mL, internal standards, ICP grade.

2.3. Apparatus

2.3.1. Microwave digestion flask(note): quartz.

2.3.2. High pressure microwave digestion flask(note): Teflon.

2.3.3. Volumetric flask(note): 50 mL.

2.3.4. Storage tube: 50 mL, PP.

2.3.5. 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. Reagent preparation

2.4.1. 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.

2.4.2. 15% (w/w) nitric acid

Add 150 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

2.5.1. Autoclave style microwave digester

Accurately transfer 0.5 mL of germanium, rhodium, iridium and bismuth internal standards and 5 mL of gold reference standard 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 1 μg/mL (containing 10 μg/mL gold reference standard) as the internal standard solution.

2.5.2. Closed vessel style microwave digester

Accurately transfer 0.5 mL of germanium, rhodium, iridium and bismuth internal standards and 5 mL of gold reference standard to each 50-mL volumetric flask, make up to volume with 15% (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 15% (w/w) nitric acid to 1 μg/mL (containing 10 μg/mL gold reference standard) as the internal standard solution.

2.6. Standard solution preparation

2.6.1. Autoclave style microwave digester

Accurately transfer 0.5 mL of arsenic, cadmium, mercury 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 from section 2.5.1, dilute with 5% (w/w) nitric acid to 0-25 ng/mL (containing 10 ng/mL internal standards and 100 ng/mL gold reference standard), and transfer to storage tubes as the standard solutions.

2.6.2. Closed vessel style microwave digester

Accurately transfer 0.5 mL of arsenic, cadmium, mercury and lead reference standards to each 50-mL volumetric flask, make up to volume with 15% (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 internal standard solution from section 2.5.2., dilute with 15% (w/w) nitric acid to 0-25 ng/mL (containing 10 ng/mL internal standards and 100 ng/mL gold reference standard), 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 arsenic, cadmium, mercury and lead by the ratios of the signal intensity of arsenic, cadmium, mercury and lead to that of the internal standard vs. the added concentrations.

ICP-MS operating condition(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	
Arsenic	75	Germanium	74
Cadmium	114、111、112	Rhodium	103
Mercury	202、200、201	Iridium	193
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

2.8.1. Autoclave style microwave digester:

Transfer about 0.2 g of the well-mixed sample accurately weighed into a microwave digestion flask, add 0.5 mL of the internal standard solution from section 2.5.1, 6 mL of nitric acid (ultrapure grade) and 1 mL of

hydrogen peroxide, stand at room temperature for at least 1 hr, 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 microwave 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 from section 2.5.1, 6 mL of nitric acid (ultrapure grade) and 1 mL of hydrogen peroxide, stand at room temperature for at least 1 hr, and perform the same procedure described above as the blank solution.

Autoclave style microwave digester operating conditions(note):

Step \ Condition	Power (W)	Heating time (min)	Duration time (min)	Temperature (°C)	Pressure (bar)
1	1200	30	15	250	160

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

2.8.2. Closed vessel style microwave digester:

Transfer about 0.2 g of the well-mixed sample accurately weighed into a high pressure microwave digestion flask, add 0.5 mL of the internal standard solution from section 2.5.2 and 10 mL of nitric acid (ultrapure grade), stand at room temperature for at least 1 hr, and then digest according to the following conditions. After cooling to room temperature, add 5 mL of nitric acid (ultrapure grade) and 3 mL of hydrogen peroxide, and digest again according to the following conditions. After cooling to room temperature, transfer to a 50-mL volumetric flask. Wash and rinse the residue in the high pressure microwave 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 high pressure microwave digestion flask, add 0.5 mL of the internal standard solution from section 2.5.2 and 10 mL of nitric acid (ultrapure grade), stand at room temperature for at least 1 hr, and

perform the same procedure described above as the blank solution.

Closed-vessel style microwave digester operating conditions(note):

Step \ Condition	Power (W)	Heating time (min)	Duration time (min)	Temperature (°C)
1	1000	5	0	100
2	1000	15	5	220
3	1800	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 appropriate rate, and operate according to the conditions in section 2.7. Calculate the amount of arsenic, cadmium, mercury or lead in the sample based on the ratio of the signal intensity of arsenic, cadmium, mercury or lead to that of the internal standard by the following formula:

The amount of arsenic, cadmium, mercury or lead in the sample (mg/kg)

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

C: the concentration of arsenic, cadmium, mercury or lead in the sample solution calculated by the standard curve (ng/mL)

C_0:.the concentration of arsenic, cadmium, mercury 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 for arsenic, cadmium and mercury are all 0.05 mg/kg and that for lead is 0.1 mg/kg.
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. Xu, Y. H., Iwashita, A., Nakajima, T., Yamashita, H., Takanashi, H. and Ohki, A. 2005. Effect of HF addition on the microwave-assisted acid-digestion for the determination of metals in coal by inductively coupled plasma-atomic emission spectrometry. Talanta 66: 58-64.
2. The Ministry of Health and Welfare, Taiwan. 2021. Method of test for heavy metals in edible fat, oil and cream (MOHWH0029.00) promulgated on August 26, 2021.