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Foodstuffs — Determination of hydride-reactive arsenic compounds in rice by atomic absorption spectrometry (Hydride-AAS) following acid extraction

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Foodstuffs - Determination of hydride-reactive arsenic compounds in rice by atomic absorption spectrometry (Hydride-AAS) following acid extraction

Détermination de composés arséniés réactifs aux hydrures dans le riz par spectrométrie d'absorption atomique (SAA-Génération d'Hydrures) après extraction acide

Lebensmittel - Bestimmung von Hydrid-bildenden Arsen-Verbindungen in Reis nach Säureextraktion mit Atomabsorptionsspektrometrie (Hydrid-AAS)

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Foreword

This document (CEN/TS 16731:2014) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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1 Scope

This Technical Specification describes a screening procedure for the determination of nitric-acid extractable inorganic arsenic in rice with hydride generation-AAS.

The method has been developed and validated for the application of analysis in rice. It has been validated in an interlaboratory study according to ISO 5725 [2] on parboiled rice and brown rice having an inorganic arsenic content of 0,092 mg/kg and 0,191 mg/kg.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 13804, *Foodstuffs — Determination of elements and their chemical species — General considerations and specific requirements*

3 Principle

Organic and inorganic arsenic compounds are extracted from the rice using diluted nitric acid. When determining the arsenic by hydride generation technique, only reducible forms of arsenic react. Of the organic arsenic compounds only a low proportion of dimethylarsinic acid reacts to form a hydride and methylarsonic acid is typically not present in rice. The gaseous hydride is transferred into a heated measuring cell (quartz cuvettes or coated graphite tube), by a stream of carrier gas, and decomposed. The absorption line of arsenic at 193,7 nm serves as a measure of the arsenic concentration [3], [4].

The procedure is exclusively applicable to rice [5].

The determination of the inorganic fraction of arsenic requires a determination of arsenic with hydride generation AAS; direct measurement of the extract by graphite furnace AAS, using ICP-MS or ICP-OES provides incorrect results.

4 Reagents

Unless stated otherwise, chemicals of analytical grade shall be used and “solution” means aqueous solution.

The water shall be of the corresponding purity.

The arsenic concentration of reagents and water shall be so low that it does not influence the result of the determination.

4.1 Hydrochloric acid, w = 30 % [1], ρ = 1,15 g/ml [2].

4.2 Nitric acid, concentrated, w = 65 %.

4.3 Diluted nitric acid, c = 0,28 mol/l[3].

Dilute 3,7 ml of nitric acid (4.2) to 200 ml with water.

4.4 Sodium borohydride, w ≥ 96 %.

1) w = mass fraction.

2) ρ = mass concentration.

3) c = substance concentration.

4.5 Sodium hydroxide, $w \geq 98$ %.

4.6 Sodium borohydride solution, e.g. $\rho = 3$ g/l.

Dissolve 1 g of sodium hydroxide pellets (4.5) in water, add 3 g of sodium borohydride (4.4) and dilute to 1 000 ml with water.

Prepare the solution freshly every day of analysis. If the solution contains undissolved fractions, filter before use.

The mass concentration ρ of the sodium borohydride solution can vary depending on the system being used. Therefore, follow the manufacturer's instructions.

4.7 Carrier solution, diluted hydrochloric acid, e.g. $w = 1{,}5$ %.

Dilute 50 ml of hydrochloric acid (4.1) to 1 000 ml with water.

The mass concentration ρ of the carrier solution can vary slightly depending on the system being used. Therefore, follow the manufacturer's instructions.

4.8 L-ascorbic acid, $w \geq 99{,}7$ %.

4.9 Potassium iodide, $w \geq 99{,}5$ %.

4.10 Solution of potassium iodide and ascorbic acid:

Dilute 5 g of potassium iodide (4.9) and 5 g of ascorbic acid (4.8) in water and make up to 100 ml. Prepare the solution freshly daily. The mass concentrations of the potassium iodide and the ascorbic acid can slightly vary depending on the system being used. Therefore, follow the manufacturer's instructions.

4.11 Arsenic stock solution, $\rho = 1\,000$ mg/l.

The use of a commercial, certified stock solution is recommended.

4.12 Arsenic standard solutions:

Prepare arsenic standard solutions by diluting the arsenic stock solution (4.11) in several steps.

The arsenic standard solutions shall contain sufficient amounts of hydrochloric acid (at least 3 ml of hydrochloric acid (4.1) per 100 ml, $w = 0{,}9$ %).

Example of a dilution series:

1 000 mg/l → 10 mg/l → 0,1 mg/l

An arsenic standard solution with a mass concentration of $\rho = 10$ mg/l of arsenic in hydrochloric acid ($w = 6$ %) is stable for at least one month.

4.13 Antifoaming agent, based on polydimethylsiloxane or silicone oil.

5 Apparatus

In order to minimize any contaminations, pre-treat carefully all apparatus and auxiliary equipment coming into direct contact with the sample and the solutions being used, in accordance with EN 13804. If the extraction vessels are used for several times, they should be cleaned by heating to 95 °C for 1 h, using nitric acid (w = 13 %).

When using glassware (e.g. Erlenmeyer flasks, beakers, graduated flasks, pipettes), ensure that it does not release any arsenic to the solutions which come into contact with the glassware.

5.1 Atomic absorption spectrometer, comprising a measurement data acquisition system and the required accessories for the hydride generation technique.

5.2 Element-specific lamp for arsenic, (hollow-cathode lamp or electrode-less discharge lamp).

5.3 Centrifuge, with an acceleration of at least 1 000 g[4)].

5.4 Syringe filter (unit), pore size of 0,45 µm, diameter of 25 mm, compatible for use with diluted nitric acid (4.3).

5.5 Extraction vessels, e.g. 30 ml or 50 ml tubes of polypropylene, with gas-tight screw closures and sufficient pressure stability.

Centrifuge tubes with screw closures are suitable.

5.6 Temperature controlled heating apparatus, for an extraction temperature of 95 °C, e.g. heating block or water bath; the heating block should be provided with an accurately fitting insert for the vessels used. The vessels should have contact with the wall in order to ensure good heat transfer.

In order to achieve an extraction temperature of 95 °C, the heating block shall be adjustable to a temperature of at least 120 °C.

5.7 Temperature measuring device, for controlling the temperature in the extraction vessel.

6 Procedure

6.1 Sample preparation

6.1.1 General

Prior to the extraction, the rice shall be finely ground, while avoiding the generation of excess heat. The particle size should be less than 500 µm.

The measurement should be performed as soon as possible after extraction. If this is not possible, store extracts in a refrigerator but for not longer than 2 d.

6.1.2 Extraction

Weigh 1 g ± 0,01 g of rice flour into a closable extraction vessel (5.5) and add 10 ml of diluted nitric acid (4.3). The ratio of 1:10 (test portion/extracting agent) shall be adhered to. Close the extraction vessels tightly and mix the content intensely using a test tube shaker. There shall be no remaining lumps. Afterwards, place the vessels in a pre-heated heating block and extract for 90 min at 95 °C (±4 °C). Alternatively, a boiling water bath can be used for the extraction. The extraction time starts as soon as the temperature in the vessel reaches 95 °C. Shake the vessel occasionally (one to two times during extraction) without opening. A constant stirring with a magnetic stirrer can be helpful.

Additionally, prepare a blank value with 10 ml of diluted nitric acid (4.3) and treat in the same manner as the samples.

The extraction temperature of 95 °C (±4 °C) shall be reached in the extraction vessels; the measurement time starts when this temperature has been reached. It is recommended to fill a reference vessel with the same

4) g = 9,81 m/s^2.

amount of water and to measure the temperature in the vessel by a temperature sensor (hole in the fitted lid). Experience has shown that there are differences in the temperature measured in the heating block and that measured in the solution.

Alternatively, the extraction can also be performed in a microwave-heated apparatus, using gas-tight vessels. In this case, ensure that the temperature is measured inside a reference vessel and that the sensor is calibrated for a temperature of 95 °C. Measuring the temperature with infrared sensors is not suitable for this type of extraction.

Seal the vessels gas-tight in order to avoid evaporation losses and to keep the extraction volume constant. Therefore, it is no longer necessary to make the volume up to a final volume after cooling down. It shall be kept in mind that the vessels are pressurized and that only plastics vessels shall be used which are correspondent to temperature and pressure stability. In general, polyethylene or polypropylene vessels are suitable.

After the extraction, cool down the vessel and open only afterwards, if applicable. Separate the solids from the solution as soon as possible. Firstly, centrifuge the sample for 10 min, and filter through a syringe filter (5.4). The application of an ultracentrifuge (20 000 g to 25 000 g) is recommended as, in that case, filtration is no longer necessary, or considerably facilitated. It is recommended to filter just an aliquot, as filtration can be very difficult due to proteins and carbohydrates in the sample. The extract shall be free of particles. The solution should be measured immediately by hydride generation AAS. If this is not possible, store the extraction solution in a suitable vessel in the refrigerator. Since new particles can be regenerated after only one day, check the extract before starting the measurement, and filter if necessary.

When stored in the refrigerator (5 °C to 8 °C), the extract is stable for 2 d.

6.1.3 Pre-reduction of reference, blank and sample solution

Depending on the hydride system being used, it can become necessary to use larger or smaller volumes than described as follows. The ratios stated, however, shall be adhered to.

For the purpose of preparing a 1 µg As/l reference solution, pipette the following solutions into the hydride generation system's analysis vessel:

— 200 µl of the standard solution with 0,1 mg of As/l (4.12);

— 13,8 ml of diluted nitric acid (4.3);

— 2,0 ml of the potassium iodide/ascorbic acid solution (4.10);

— 4,0 ml of hydrochloric acid (4.1).

Mix the solution after each addition. Finally, leave the vessel open or loosely covered with lintless paper, at room temperature. When preparing a 3 µg As/l reference solution, take 600 µl of the standard solution with 0,1 mg of As/l (4.12) and 13,4 ml of diluted nitric acid (4.3); the quantities of the other reagents are not changed.

It is recommended to prepare 5 reference solutions with e.g. 1 µg/l, 3 µg/l, 5 µg/l, 8 µg/l and 10 µg/l each, following the described scheme.

For the purposes of preparing a blank solution, pipette the following solutions into the analysis vessel:

— 14 ml of diluted nitric acid (4.3);

— 2,0 ml of the potassium iodide/ascorbic acid solution (4.10);

— 4,0 ml of hydrochloric acid (4.1).

For the preparation of the pre-reduced sample solution, pipette the following volumes into the analysis vessel. For the stated composition, dilute the sample solution at a ratio of 1:10, this factor shall be taken into consideration when calculating the content.

— 0,5 ml of extracted sample solution (6.1.2);

— 3 ml of diluted nitric acid (4.3);

— 0,5 ml of the potassium iodide/ascorbic acid solution (4.10);

— 1,0 ml of hydrochloric acid (4.1).

After the addition of KI/Ascorbic acid pre-reductant, the solutions shall be left for 2 h at room temperature before measurement. This is to ensure that the pre-reduction of As(V) to As(III) is complete.

For the pre-reduction, up to 1,0 ml of nitric acid extract in accordance with 6.1.2 can be used. A lower amount of the sample solution is regulated by addition of diluted nitric acid (4.3). The concentrations of the acid and of the reducing agent shall be identical in all measurement solutions.

After extraction, the solutions are coloured yellow. Interferences with the pre-reduction caused by this yellowing have not been observed.

NOTE 1 Undiluted extracts may foam in the measurement of hydride generation system. This can be avoided due to a lower use of the sample solution for the pre-reduction and according volume compensation with diluted nitric acid (4.3). Foam formation can also be reduced by adding some drops of a suitable antifoaming agent (4.13) to the carrier solution (4.7).

NOTE 2 Comparable results were obtained from the pre-reduction in accordance with EN 14627 [1].

6.2 Atomic absorption spectrometry (Hydride generation AAS)

6.2.1 Operating conditions for the hydride generation AAS

The following two procedures are recommended for hydride generation technique:

a) Continuous flow system where sample and reagents are continuously caused to react until a signal is created which is independent of the time.

b) Flow-injection procedure where the sample is dosed into the hydrochloric-acid carrier solution via a selectable-volume sample loop, and caused to react with the reducing agent in the dosing unit. The resulting signal is time-dependant.

Since, for the two procedures, different sample volumes are used, different detection limits for the procedure variants are obtained.

The concentration of reagents used for hydride generation (e.g. reducing agent sodium borohydride-solution (4.6) and carrier solution (4.7)) may vary depending on the procedure and apparatus used.

For the purposes of developing a measurement programme, set the apparatus in accordance with the manufacturer's operating instructions. Afterwards, the settings shall be optimized by the user.

The hydride formation is delayed by the co-extracted organic accompanying substances. Thus, the measurement times shall be chosen long enough to cover the full hydride signal.

Occasionally, test the reactivity of the hydride generation system to dimethylarsenic acid (DMA) by adding a known amount of DMA to a sample extract before the pre-reduction. The DMA hydride generation efficiency should be less than 10 %.

Apparatus systems where the hydride is concentrated in a graphite tube (e.g. HydrEA coupling[5)], FIAS-Furnace coupling[6)]) offer better detection capabilities and an improved signal evaluation as a result of the concentration step and the subsequent atomization.

6.2.2 AAS measurement

For all measurements, take only the pre-treated solutions (6.1.3).

Zeroing of the apparatus is performed using the blank solution (6.1.3).

For the purposes of establishing the reference function, measure the extinctions of the reference solutions using different element concentrations. Determine the reference function by applying pairs of measured values. Ensure that the reference function is linear. Measure the sample solution directly or, if the absorbance is outside the calibration range, following the appropriate dilution (as described in 6.1.3).

In the case of longer measurement series, check zero point and reference function repeatedly.

7 Evaluation

Calculate the mass fraction w of inorganic arsenic in the sample in mg/kg in accordance with Formula (1).

$$w = \frac{a \cdot V \cdot F}{m \cdot 1\,000} \tag{1}$$

where

- a is the mass concentration of arsenic determined in the measurement solution when applying hydride generation-AAS, in µg/l;
- V is the volume of the nitric acid solution used for the extraction, in ml;
- F is the dilution factor, taking into consideration the pre-reduction and, if applicable in the case of high arsenic concentrations, further dilutions;
- m is the mass of the test portion used for the extraction, in g.

Express the result with 3 significant figures.

8 Precision

8.1 General

Details of the interlaboratory test of the precision of the method are summarized in Annex A. The values derived from this test may not be applicable to analyte concentration ranges and matrices other than given in Annex A.

8.2 Repeatability

The absolute difference between two single test results found on identical test material by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability limit r in not more than 5 % of the cases.

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5) HydrEA coupling is the trade name of a product supplied by Analytik Jena. This information is given for the convenience of users of this Technical Specification and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead the same results.

6) FIAS-Furnace coupling is the trade name of a product supplied by Perkin Elmer. This information is given for the convenience of users of this Technical Specification and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead the same results.

The values are:

Parboiled rice	$\overline{x}$ = 0,092 mg/kg	r = 0,018 mg/kg
Brown rice	$\overline{x}$ = 0,191 mg/kg	r = 0,043 mg/kg

8.3 Reproducibility

The absolute difference between two single test results found on identical test material reported by two laboratories will exceed the reproducibility limit R in not more than 5 % of the cases.

The values are:

Parboiled rice	$\overline{x}$ = 0,092 mg/kg	R = 0,039 mg/kg
Brown rice	$\overline{x}$ = 0,191 mg/kg	R = 0,076 mg/kg

9 Test report

The test report shall contain at least the following data:

a) all information necessary for the identification of the sample;

b) a reference to this Technical Specification or to the method used;

c) the date and time of sampling procedure (if known);

d) the date of receipt;

e) the date of test;

f) the results and the units in which the results have been expressed;

g) any particular points observed in the course of the test;

h) any operations not specified in the method or regarded as optional which might have affected the results.

Annex A
(informative)

Precision data

This Technical Specification has been elaborated by Working Group “Elementanalytik” of the Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (Federal Office of Consumer Protection and Food Safety, BVL) according to Article 64 of the German Foods Act and validated in an interlaboratory study. A total of 11 of all participants have worked according to the provisions of this Technical Specification. In addition, one participant has achieved similar results by applying hydride generation ICP-OES. However, this laboratory was assessed as an outlier. The interlaboratory study lead to the results as laid down in Table A.1 on mass concentrations of inorganic arsenic.

Table A.1 — Precision data

Parameter	Sample 1 Parboiled rice	Sample 2 Brown rice
Number of participating laboratories	12	12
Number of laboratories following elimination of outliers	11	11
Number of outliers	1	1
Mean value (mg/kg)	0,092	0,191
Reproducibility standard deviation s_R (mg/kg)	0,014	0,027
Relative reproducibility standard deviation $s_{R,rel}$	15,0 %	14,3 %
Reproducibility limit R, (mg/kg)	0,039	0,076
Relative reproducibility limit R_{rel}	42,1 %	40,0 %
Repeatability standard deviation s_r (mg/kg)	0,006	0,016
Relative repeatability standard deviation $s_{r,rel}$	6,85 %	8,12 %
Repeatability limit r (mg/kg)	0,018	0,043
Relative repeatability limit r_{rel}	19,2 %	22,7 %
Relative Horwitz-standard deviation	22,9 %	20,5 %
Horrat-Value	0,66	0,70

Bibliography

[1] EN 14627, *Foodstuffs — Determination of trace elements — Determination of total arsenic and selenium by hydride generation atomic absorption spectrometry (HGAAS) after pressure digestion*

[2] ISO 5725 (all parts), *Accuracy (trueness and precision) of measurement methods and results*

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