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**DETERMINATION OF TIN IN
FOODSTUFFS AND BIOLOGICAL
MATERIAL**

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DETERMINATION OF TIN IN FOODSTUFFS AND BIOLOGICAL MATERIAL

The method recommended is principally a combination of the procedure published by the Analytical Methods Committee (Analyst, 92 (1967), p.320 et seq) and the selective extraction step of tin as described by Tanaka et al. (Anal.Chim.Acta, 48 (1969), p.357 et seq). The described determinative step is preceded by a detailed description of the wet digestion of the test portion. Five laboratories have cooperated in a study of the method using macerated canned petfoods as a substrate containing tin (at two levels) as a result of contamination from the can on storage. The results were as follows:

	<u>Tin content in mg/kg</u>	
	<u>Sample A</u>	<u>Sample B</u>
Lab. A	28.8	60.2
Lab. B	24.1	60.9
Lab. C	28.6	61.7
Lab. D	26.5	57.9
Lab. E	26.1	60.4
Mean	26.8	60.2

In addition the method has been applied by one of us to kale powder of Bowen; J.Radioanal.Chem., 19 (1974), p.1215 et seq. The result was 0.23 mg/kg; literature: 0.16-0.28 mg/kg. Recovery experiments were carried out for organs of animals and duplicate 24-hour diets and proved to be satisfactory: 87% (N=10; range 78-91%) with spiking levels of 0.2 and 0.4 mg/kg and 93% (N=12; range 80-104%) with spiking levels of 1.0 and 2.0 mg/kg respectively (Vaessen et al.; Ware(n)-Chemicus, 9 (1979), p.40 et seq).

1. SCOPE AND FIELD OF APPLICATION

This method is designed for the determination of tin in foodstuffs and biological material, and is applicable to determine tin contents down to 2 µg in the test portion.

2. DEFINITION

Tin content of foodstuffs and biological material: the tin content determined according to the procedure described and expressed in milligrams per kilogram.

3. PRINCIPLE

The test portion is wet digested with a mixture of nitric and sulphuric acids followed by subsequent treatment with perchloric acid and hydrogen peroxide. The sulphuric acid residue is diluted with water to give an approximately 4.5 M concentration of the acid. Potassium iodide is then added to this solution and tin (IV) iodide is selectively extracted into cyclohexane. By shaking the organic layer with sodium hydroxide solution tin (IV) is back-extracted into aqueous solution which subsequently is acidified. Free iodine is removed by reduction with ascorbic acid, the chromophore catechol violet is added, the solution is buffered at pH 3.8

and set aside for 45 minutes at room temperature. The optical density of the resulting coloured complex with catechol violet is determined spectrophotometrically at 555 nm*.

4. REAGENTS

If not explicitly stated otherwise all reagents should be of analytical reagent quality. Water must be of the quality double distilled (or equivalent) from an all glass apparatus of Pyrex or other resistant glass.

- 4.1. Sulphuric acid; 98% (m/m), density 1.84 g/ml.
- 4.2. Nitric acid; 65% (m/m), density 1.40 g/ml; Merck** nitric acid tested by dithizone for heavy metals is very suitable.
- 4.3. Perchloric acid; 70% (m/m), density 1.67 g/ml.
- 4.4. Hydrogen peroxide; 30% (m/m).
- 4.5. Hydrochloric acid; 36% (m/m), density 1.18 g/ml.
- 4.6. Ammonia solution; 25% (m/m) as NH_3 , density 0.91 g/ml.
- 4.7. Cyclohexane.
- 4.8. Ethanol, absolute.
- 4.9. Potassium iodide.
- 4.10. Sodium hydroxide.
- 4.11. Ascorbic acid.
- 4.12. Sodium acetate trihydrate.
- 4.13. Catechol violet; Merck** or equivalent.
- 4.14. Tin, granular 30 mesh; J.T. Baker** or equivalent.
- 4.15. Sulphuric acid solution, approximately 4.5 M. Mix carefully 250 ml of sulphuric acid (4.1.) and 500 ml of water, cool down and dilute to 1000 ml with water.
- 4.16. Potassium iodide solution, approximately 5 M. Dissolve 83 gram of potassium iodide in 100 ml of water. Prepare fresh daily.
- 4.17. Sodium hydroxide solution, approximately 5 M. Dissolve 100 gram of sodiumhydroxide (4.10.) in water, dilute to 500 ml and mix. Prepare fresh each fortnight.
- 4.18. Sodium hydroxide solution, approximately 0.1 M. Dissolve 2 gram of sodium hydroxide (4.10.) in water, dilute to 500 ml and mix. Prepare fresh each fortnight.
- 4.19. Hydrochloric acid solution, approximately 5 M. Dilute 107 ml of hydrochloric acid (4.5.) with water and dilute to 250 ml.
- 4.20. Ascorbic acid solution 5% (m/v). Dissolve 1 gram of ascorbic acid (4.11.) in 20 ml of water. Prepare fresh daily.
- 4.21. Catechol violet solution 0.025% (m/v). Dissolve 25 mg of catechol violet (4.13.) in a mixture of equal parts of ethanol (4.8.) and water to produce 100 ml and mix. Prepare fresh weekly.
- 4.22. Sodium acetate solution 20% (m/v). Dissolve 50 gram of sodium acetate (4.12.) in water, dilute to 250 ml and mix.
- 4.23. Ammonia solution, approximately 5 M. Dilute 184 ml of ammonia (4.6.) with water to 500 ml.
- 4.24. Tin standard solutions.

* Each operator should check the wavelength of maximum absorption of the complex formed for his particular instrument.

** Reference to a company and/or branch name does not constitute a guarantee or warranty of the company's product and does not imply its approval to the exclusion of other products which may be equally suitable.

4.24.1. Stock solution; 200 µg/ml. Dissolve 0.1000 g tin (4.14.) in 20 ml of sulphuric acid (4.1.) by heating until fumes appear.
Cool down, dilute cautiously with 150 ml of water and cool again. Add 65 ml of sulphuric acid (4.1.) and transfer the solution to a 500 ml volumetric flask, dilute to volume with water and mix.

4.24.2. Working solution; 5 µg/ml. Pipette, immediately before use 5.0 ml stock solution (4.24.1.) into a 200 ml volumetric flask, dilute to volume with water and mix.

5. APPARATUS AND GLASSWARE

Glassware, including reagent bottles, should be soaked during 24 hours in 4 M nitric acid and before use rinsed twice with distilled water and subsequently twice with double distilled water and dried.

- 5.1. Kjeldahl flasks; long neck, 500 ml.
- 5.2. Glass beads; nitric acid treated and washed with water as indicated above.
- 5.3. Argand-burners.
- 5.4. Measuring pipettes with a glass piston; 1 and 10 ml, Fortuna^{**} (see Note^{**} on p. 1739)
- 5.5. One mark pipettes; 1, 2, 3, 4 and 5 ml.
- 5.6. Separating funnels; 100 ml, stem shortened to 1 cm.
- 5.7. Beakers; 50 ml.
- 5.8. Volumetric flasks; 25 ml.
- 5.9. pH meter.
- 5.10. Spectrophotometer.
- 5.11. Cells with 10 mm optical pathlength.

6. TEST SAMPLE

Proceed from a representative sample of preferably 200 grams and homogenize. Food grinders or other homogenisation aids used, must be checked for possible tin contamination.

7. PROCEDURE

7.1. Digestion

During the digestion nitric oxide vapours are generated and consequently all manipulations must be performed in a well ventilated fume hood.

- 7.1.1. Weigh, to the nearest 0.01 g, M_0 g. homogenized sample into a Kjeldahl flask (5.1.).
The sample portion taken for analysis should contain no more than 25 µg of tin.

NOTE

From a practical point of view the test portion should be no smaller than 0.05 gram. Whenever the tin concentration of the sample presumably is greater than 50 mg/kg, the sulphuric acid residue resulting after digestion (7.1.10.) should be properly diluted and an aliquot of the sample solution containing between 2 and 25 µg of tin should be taken for the determination. All dilutions are carried out such that the final solution is approximately 4.5 M in sulphuric acid.

- 7.1.2. Successively add 50 ml of nitric acid (4.2.), 12.5 ml of sulphuric acid (4.1.) and three glass beads (5.2.) and mix thoroughly.
- 7.1.3. Heat the flask contents to boiling using an Argand-burner (5.3.) and keep the contents boiling during the digestion. Rotate the flask occasionally to prevent caking of sample upon glass exposed to the flame

NOTE

During steps 7.1.2. and 7.1.3. excessive foaming may occur. This is easily controlled by cooling with tapwater. Foaming can be avoided when after addition of nitric acid (7.1.2.), the Kjeldahl flask contents is left overnight in a fume hood.

- 7.1.4. Maintain oxidising conditions during digestion by adding small amounts of nitric acid (4.2.) whenever the contents turn brown or darkens.
- 7.1.5. Continue digestion (7.1.4.) until all organic matter is destroyed and sulphuric acid fumes are copiously evolved. Destruction is complete when 5 minutes after fuming has started the solution remains colourless or at most, light straw coloured. If during this period a colour change is observed step 7.1.4. should be repeated.
- 7.1.6. Cool down to room temperature, add 1 ml of perchloric acid (4.3.) and reheat until copious white fumes appear. Continue heating for 5 minutes.
- 7.1.7. Remove the Argand-burner (5.3.) cool and add 1 ml of hydrogen peroxide (4.4.). Heat to fuming which is continued for 5 minutes.
- 7.1.8. Repeat the hydrogen peroxide treatment (7.1.7.) twice, each time reheating to fuming which is continued for 5 minutes.
- 7.1.9. Cool, rinse the neck of the flask with approximately 5 ml of water and reheat to fuming.
- 7.1.10. Cool down to room temperature once more and determine the tin content of the solution according to 7.2.

7.2. Determination

- 7.2.1. Quantitatively transfer the digest (7.1.10.) into a 100 ml separating funnel (5.6.) with 37.5 ml of water and mix by rotating the funnel by hand. The test solution thus obtained in approximately 4.5 M sulphuric acid.

NOTE

If an aliquot of the diluted sulphuric acid residue (7.1.1. and 7.1.10.) is taken for the determination the total volume in the separating funnel is brought to 50 ml with 4.5 M sulphuric acid.

- 7.2.2. Add 5 ml of potassium iodide (4.16.), mix and add 10 ml of cyclohexane (4.7.).
- 7.2.3. Insert the stopper, shake the funnel vigorously for 2 minutes, allow the layers to separate. Transfer the aqueous layer into a second separating funnel (5.6.), retain the cyclohexane layer. The cyclohexane layer will be coloured pink by extracted iodine.
- 7.2.4. Add 10 ml of cyclohexane (4.7.) to the contents of the second separating funnel, shake vigorously for 1 minute, allow the layers to separate and discard the aqueous phase.
- 7.2.5. Combine the cyclohexane extracts and discard any water present after combination of the extracts.
- 7.2.6. To the combined extracts successively add 5 ml of water and 1.5 ml of sodium hydroxide solution (4.17.). Insert the stopper and shake vigorously for 2 minutes. Allow to separate and transfer the aqueous layer into a beaker (5.7.) containing 2.5 ml hydrochloric acid (4.19.).
- 7.2.7. Repeat the extraction (7.2.6.) by shaking during 1 minute with 3 ml of sodium hydroxide (4.18.) and add the aqueous layer to the contents of the beaker (7.2.6.).
- 7.2.8. Wash the cyclohexane layer retained in the separating funnel with 5 ml of sodium acetate solution (4.22.) by cautiously tilting the funnel and forcing the contents to flow forth and back six times.

- 7.2.9. Decolorise the iodine present in the acidified aqueous solution (7.2.7.) by dropwise addition of ascorbic acid solution (4.20.). Add 2.0 ml of catechol violet solution (4.21.) and mix.
- 7.2.10. Add the sodium acetate washing solution (7.2.8.) to the contents of the beaker (7.2.9.) and adjust the pH of the solution to 3.8 ± 0.1 pH units with ammonia (4.23.) and/or hydrochloric acid (4.19.) using a pH meter (5.9.).
- 7.2.11. Quantitatively transfer the solution (7.2.10.) into a volumetric flask (5.8.), add 2.0 ml of ethanol (4.8.), make up to the mark with water and mix. Set aside the flask for at least 45 minutes at room temperature.
- 7.2.12. Measure the optical density (extinction) of the solution in a 10 mm cell (5.11.) using water as a reference.
- 7.2.13. Carry out a blank determination starting with 50 ml of sulphuric acid (4.15.) and carrying out steps 7.2.2. up to and including 7.2.12. The optical density of the blank should not exceed 0.070 extinction units; see note 7.3.2.
- 7.2.14. Correlate the extinction (7.2.12.) with the tin amount in μg (M_1) present in the test solution by reference to the calibration graph (7.3.) and calculate the tin concentration of the sample as indicated under 8.
- 7.3. Calibration graph
- 7.3.1. Transfer by pipette 0, 1, 2, 3, 4 and 5 ml of the tin working solution (4.24.2.) into six separating funnels (5.6.) and add sufficient sulphuric acid (4.15.) to bring the volume to 50 ml.
- 7.3.2. Determine the tin content of the solutions thus obtained as indicated under 7.2.2. up to and including 7.2.12.

NOTE

Whenever the extinction measured for the blank standard zero - exceeds 0.070 extinction units all reagent solutions should be renewed.

- 7.3.3. Calculate the linear regression ($y = a + bx$) relating the tin content of the standard in μg (y) to the measured extinction (x). Plot the calibration graph corresponding to the calculated linear regression.

8. EXPRESSION OF RESULTS

Calculate the tin content of the sample in milligrams per kilogram using the formula:

$$\text{Sn in mg/kg} = \frac{M_1}{M_0} \cdot F$$

where

M_0 is the mass in grams of the test portion (7.1.1.)

M_1 is the tin amount in micrograms present in the test solution (7.2.14.)

F is the dilution factor (7.1.1. and 7.2.1.).