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THE DETERMINATION OF COPPER IN FOODSTUFFS

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THE DETERMINATION OF COPPER IN FOODSTUFFS

1. SCOPE:

This method specifies a reference method for the determination of copper in foodstuffs.

2. FIELD OF APPLICATION:

The method described is applicable to all foodstuffs and biological material to determine copper down to 0.05 mg/kg.

3. DEFINITION:

Copper content of foodstuffs: the copper content determined according to the procedure described in this recommendation and expressed in milligrammes per kilogramme.

4. PRINCIPLE:

Wet combustion by means of nitric and sulphuric acids of the test portion in an all glass apparatus according to Thielepape. An oxidizing medium is maintained throughout the digestion. The destruction is finished with hydrogen peroxide.

The digest is diluted with water and copper is chelated with ammonium-pyrrolidine-dithiocarbamate in methylisobutylketone at pH 3.

The copper content of the organic phase is determined by atomic absorption measurement at 324·7 nm.

5. REAGENTS:

All reagents should be of analytical reagent quality. Water must be redistilled from an all-glass apparatus of Pyrex* or other resistent glass. Water should be re-distilled water.

5.1 Sulphuric acid, 98% ($^m/_m$) d = 1.84
Should be checked for the absence of heavy metals.

5.2 Nitric acid, 65% ($^m/_m$) d = 1.40
Should be checked for the absence of heavy metals.

5.3 Hydrogen peroxide, 30% ($^m/_m$).
Should be checked for the absence of heavy metals.

5.4 Ammonium pyrrolidine dithiocarbamate solution (1% $^m/_v$):
Dissolve 1 g ammonium pyrrolidine dithiocarbamate in 100 ml of water.

This solution must be prepared just before use.

5.5 Methylisobutylketone.

* Mention of a company 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 that may be also suitable.

5.6 Concentrated copper stock-standard solution (100 µg Cu/ml):
Dissolve 100.0 mg copper turnings (p.a.) in 20 ml of nitric acid (65%) in a beaker of 100 ml. Transfer the content of the beaker after the reaction has ceased quantitatively into a graduated flask of 1 litre and make up to the mark.
(1 ml = 100 ug Cu).
NOTE: Some glass surfaces absorb copper ions. From experience it is noticed that shaking of the copper standards just before preparing the standards solutions will overcome this effect.

5.7 Copper stock-standard solution (5 µg Cu/ml):
Dilute concentrated copper stock-standard solution (5.6.) $^{1}/_{20}$ by pipetting 25 ml of 5.6. into a graduated flask of 500 ml. Add 10 ml of concentrated nitric acid and make up to the mark with water.

5.8 Working copper standard solutions:
Prepared working copper standard solutions by serially diluting the copper stock-standard solution (5.7.) pending the concentration expected.

WARNING:

Fresh working copper standard solutions must be prepared just before use from the copper stock-standard solution (5.7.).

5.9 Sodium sulphate anhydrous.

5.10 Ammonium hydroxide 25% ($^{m}/v$).

6. APPARATUS AND GLASSWARE:

Glassware, including reagent bottles, must be of chemically resistent glass, preferable Pyrex or equivalent. It should be reserved for the estimation of copper and before its use the glassware should be cleaned first with a commercial available detergent solution consisting principally of a mixture of anionic-cationic and nonionic detergents together with a proteinase. The glassware should be stored overnight in the acidified (0.5 N H_2SO_4) detergent solution. Next the glassware should be rinsed 7 times with water and stored in 0.1 hydrochloric acid. Before use the glassware should be rinsed three times with double distilled water. Before each use the digestion apparatus is cleaned by boiling with nitric acid (4N), without water circulating through the condenser.

6.1 Digestion apparatus:

Consisting of a 100 ml or 250 ml two-necks round-bottom equipped with a Thielepape extractor with a double bore stopcock for withdrawing samples (Normschliff Glassgerate K.G. Wertheim (cat. no. 6.224) with a receiver capacity of 70 ml) a Dimroth condensor length of jacket 40 cm and a thermometer up to 200°C (fig. 1).

6.2 Separation funnels.
50 ml with ground joint NS 14/23 and glass stopper.

6.3 Graduated pipettes.
For delivering quantities of 0.5 ml.

6.4 One-mark pipettes with a nominal capacity of 1 ml and 5 ml.

6.5 Stop-watch.

6.6 Atomic absorption spectrophotometer. With scale expander and connected with a digital read out and/or 1mV-50 mV Stripchart recorder.

6.7 Hollow cathode lamp for copper.

6.8 Oxidant. Air, cleaned and dried through a suitable filter to remove oil, water and other foreign substances.

6.9 Fuel, acetylene.

6.10 pH-meter.

7. SAMPLE:
Proceed from a representative primary sample of at least.....grammes.

8. PROCEDURE:

8.1 Preparation of the sample:
Make the sample homogeneous, avoid contact with metals by using porcelain where ever possible (spoons etc.). If metal food grinders are used, check them for possible copper contamination.

8.2 Test portion:
Weigh into the digestion flask to the nearest 10 mg about 5 g of the homogenized sample.

8.3 Digestion:
Add 15 ml concentrated nitric acid, 5 ml concentrated sulphuric acid and some glassbeads. In case of dry material such as cereals etc. 10 ml of water is first added to the sample before adding nitric acid. Mix well and connect the Thielepape extractor and water condenser. Allow mixture to stand at room temperature overnight in order to prevent foaming during first stage of digestion. Heat, first by means of a soft flame, such as that of an Argand burner, remove flame from digestion apparatus as necessary to minimize escape of nitrogen oxides from the top of condenser. Maintaining full heat, turn the tap through 90° so that liquid distils into the receiver B. Temperature of the vapour in the digestion flask at this stage will not exceed 120°C. Turn the tap through a further 90° so that the

distillate (mainly water) drain off through C. Turn the tap in such a way that liquid distils into the receiver B.

Intensify heating in such a way that nitric acid distills into the receiver. If the solution begins to darken add a few millilitres of nitric acid from the receiver with the help of the double bore stopcock.

NOTE:
In some cases addition of a new portion of 10 ml of nitric acid is necessary. Drain off the nitric acid already distilled in the receiver.

Repeat this proceeding till the solution remains yellow when heating up to white fumes of sulphuric acid appear. Digestion has been now completed.

Disconnect the roundbottom flask. To remove nitrosylsulphuric acid, add 0.5-1.0 ml hydrogen peroxide (30%) and boil off. Repeat this operation with another 0.5-1.0 ml portion of hydrogen peroxide added to the hot solution. The final solution should be colourless at most light straw in colour. Evaporate the sulphuric acid until the volume is a half to one ml. Allow to cool.

8.4 Determination:
Transfer the digest quantitatively with the help of redistilled water into a beaker of 100 ml. Adjust the pH of the solution with ammoniumhydroxide (25% $^{m}/_{v}$) to approximately 3. Transfer quantitatively the solution obtained into a separation funnel of 50 ml with the help of redistilled water. The solution should not exceed 30 ml. Pipette 1 ml of ammonium pyrrolidine dithiocarbamate and 5 ml of methylisobutylketone. Shake for 1 minute. Allow the layer to separate. Drain off the aqueous phase. Dry the organic phase by shaking with some anhydrous sodium sulphate (50-100 mg.).

Switch on the atomic absorption spectrophotometer and allow the copper-lamp to warm up for at least 30 minutes. Adjust the monochromator to give maximum signal near the recommended wavelength 324.7 nm. Slitwidth 100 micron. Adjust flame stoichiometry with gas mixture air - acetylene in such a way that maximum absorbance is obtained.

Measure the absorption of the obtained solution.

NOTE:
It is recommended to carry out a blank along with the unknown through all the subsequent steps using the same amounts of reagents throughout.

8.5 Calibration curve:
Repeat the determination procedure (8.4.) with working copper standard solutions instead of the sample solution.

Plot the absorption values or peakheights measured against the concentration of the working copper standard solutions and construct the best fitting straightline through the plotted points and the origin.

9. EXPRESSION OF RESULTS AND CALIBRATION:

9.1 Calculate the copper content (Cu) of the sample in mg/kg from the formula

$$Cu = \frac{C \times V}{g}$$

where

C = the copper content in the sample solution expressed in microgrammes per millilitre.

V = total volume of the sample solution (8.4.).

g = mass of the test portion in grammes (8.2.).

FIGURE 1

APPARATUS FOR CONTROLLED DECOMPOSITION OF ORGANIC MATERIAL