BONE PHOSPHATE

Prepared at the 33rd JECFA (1988), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003)
A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 29th JECFA (1985)

SYNONYMS Edible bone phosphate, INS No. 542

DEFINITION A heterogeneous residual mixture of calcium phosphates, principally $3Ca_3(PO_4)_2 \cdot Ca(OH)_2$, obtained by the grinding of bones which have been treated with hot water and steam under pressure; may contain unextracted fat and proteins.

Assay Not less than 30% and not more than 40% of Ca, and not less than 32% of P_2O_5.

DESCRIPTION White to pale cream coloured, odourless powder

FUNCTIONAL USES Emulsifier, moisture retaining agent, sequestrant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in ethanol and water

Test for phosphate (Vol. 4) Passes test
Use a solution obtained by dissolving 1 g of the sample by warming in 50 ml diluted hydrochloric acid.

Test for calcium (Vol. 4) Passes test
Use a solution obtained by dissolving 1 g of the sample by warming in 50 ml diluted hydrochloric acid.

PURITY

Loss on drying (Vol. 4) Not more than 2%

Loss on ignition (Vol. 4) Not more than 20%

Fluoride (Vol. 4) Total not more than 1000 mg/kg
Dissolve an amount of ash (obtained from the test for Loss on ignition) equal to 0.1 g of the sample, and proceed as described under the Limit Test, Method IV using buffer solution C.

Copper Not more than 25 mg/kg
See description under TESTS

Zinc Not more than 250 mg/kg
See description under TESTS

Arsenic (Vol. 4)	Not more than 3 mg/kg
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
Fat residue	Not more than 2% Accurately weigh 5-10 g sample. Without previous drying, extract in soxhlet or other suitable container with petroleum ether (40-60^{o}) for about 6 h. Filter extract through small hardened paper into weighed vessel, washing paper into weighed vessel, washing paper finally with small portion of hot fresh solvent. Distil or evaporate solvent at temperature ca 100^{o} and dry vessel containing residue in air oven for 1h at 100-105^{o}C. Weigh the dried residue and calculate percentage of the sample.
Protein residue (Vol. 4)	Not more than 10% (N x 6.25) Proceed as directed under *Nitrogen Determination (Kjeldahl Method)* Method II
Microbiological criteria (Vol. 4)	Total aerobic microbial count: Max 1000 in 1 g *Salmonella*: Absent in 50 g *E. coli*: Absent in 10 g

TESTS

PURITY TESTS

Copper and zinc

General precautions

Because of the min amounts of metals involved special care must be taken to reduce the reagent blanks to as low a value as possible and to avoid contamination during the test. All apparaturs should be thoroughly cleaned with a mixture of hot dilute acids (1 part hydrochloric acid, 1 part concentrated nitric acid, and 3 parts water) followed by thorough washing with water immediately before use. The methods of preparation described should be followed exactly.

Apparatus

Atomic absorption spectrophotometer equipped with air/acethylene flame and lamps for copper and zinc determination.

Reagents

Reagents shall be of an order of purity higher than accepted analytical reagent grade quality. Metal-free water (see below) shall be used throughout.

- Sulfuric acid, 98% H_2SO_4
- Nitric acid, sp.gr. 1.42
- Hydrochloric acid, sp. gr. 1.16-1.18 (conc.)
- Hydrochloric acid 5 M solution prepared by dilution of hydrochloric acid (conc.) with water
- Hydrochloric acid 0.5 M solution prepared by dilution of hydrochloric acid 5 M with water
- Water, metal free. Distilled water may be re-distilled from an all glass

apparatus or may be passed down a column of cation exchange resin, e.g., Amberlite IR 120 (H).

Standards

Standard copper solution: Dissolve 3.928 g of pure copper sulfate $CuSO_4$. $5H_20$ in distilled water, dilute to 1000 ml at 20^o with distilled water in a one-mark graduated flask. Dilute 10 ml to 100 ml with water in a one-mark graduated flask as required. 1 ml = 100 µg Cu.

Standard zinc solution: Dissolve 1.000 g of pure zinc powder in a mixture of 10 ml distilled water and 5 ml hydrochloric acid special reagent (d) and dilute to 1000 ml at 20^o with distilled water, in a one-mark graduated flask. Dilute 10 ml to 100 ml with water in a one-mark graduated flask as required. 1 ml = 100 µg Zn.

Sample preparation

Place about 2.5 g of the sample, accurately weighed, in a suitable crucible, add sufficient sulfuric acid to wet the sample, and carefully ignite at a low temperature until thoroughly charred, covering the crucible loosely with a suitable lid during the ignition. After the substance is thoroughly carbonized, add 2 ml of nitric acid and 5 drops of sulfuric acid, and cautiously heat until white fumes are evolved, then ignite, preferably in a muffle furnace, at 500^o to 600^o until all the carbon is burned off. Cool, add 4 ml of hydrochloric acid 5 M, cover, and digest on a steam bath for 10 to 15 min. Uncover, and slowly evaporate on a steam bath to dryness. Finally cool, add 10 ml 5 M hydrochloric acid and boil gently for a few min. Cool and transfer the solution to a 50-ml one-mark graduated flask washing out the Kjeldahl flask with small portions of water. Add the washings to the graduated flask and dilute to the mark with water (Solution A). To a 100 ml one mark volumetric flask pipet 10 ml of solution A and dilute to the mark with hydrochloric acid 0.5 M (Solution B). Prepare a reagent blanks using the same quantities of reagents as used in the sample preparation for obtaining solutions A and B (Blank A and Blank B).

Preparation of standard curve solutions

To a series of 100-ml one-mark volumetric flasks pipet 0, 1, 2, 3, 4 and 5 ml of each of the two standard solutions to (e) and dilute to about 50 ml. Add 20 ml of hydrochloric acid 5 M and dilute to the mark with metal-free water. These solutions then contain 0, 1.0, 2.0, 3.0, 4.0 and 5.0 µg per ml of copper and zinc.

Instrumental Conditions

Select the wavelength to be used for the particular element under consideration 324.7 nm for copper; 213.9 for zinc. The recommended settings for the various instrumental parameters vary from model to model, and certain parameters require optimization at the time of use to obtain the best results. Instruments should therefore be adjusted as described in the manufacturer's instructions using wavelength settings specified above.

Set the atomic absorption spectrophotometer to the appropriate conditions. Aspirate the strongest standard containing the element to be determined and optimize the instrument settings to give full-scale or maximum deflection on the chart recorder. Measure the absorbances of the other standards and plot a graph showing the net absorbance against the concentration of the element in

the standard solutions. Aspirate Solution A and the corresponding Blank A for determination of copper or Solution B and the corresponding Blank B for determination of zinc and determine the net absorbance. Using the graph prepared above, determine the concentration of the element in the sample solution.

Calculate the content of copper and zinc, respectively from:

$$Copper\ (mg / kg) = \frac{c \times 50}{w}$$

$$Zinc\ (mg / kg) = \frac{c \times 50}{w}$$

where
c = concentration of element (µg/ml) in the sample solution
w = the weight (g) of sample taken

METHOD OF ASSAY

Calcium:
Weigh accurately about 0.150 g of the sample. Dissolve, with the aid of gentle heat if necessary, in a mixture of 5 ml of hydrochloric acid and 3 ml of water contained in a 250 ml beaker equipped with a magnetic stirrer, and cautiously add 125 ml of water. With constant stirring, add, in the following order, 0.5 ml of triethanolamine, 300 mg of hydroxynaphthol blue indicator, and, from a 50 ml buret, about 23 ml of 0.05 M disodium ethylenediamine tetraacetate. Add sodium hydroxide solution (45 in 100) until the initial red colour changes to clear blue, then continue to add it drop wise until the colour changes to violet, then add an additional 0.5 ml. The pH is between 12.3 and 12.5. Continue the titration drop wise with the 0.05 M disodium ethylenediamine tetraacetate until the appearance of a clear blue endpoint that persists for not less than 60 sec. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 2.004 mg of Ca.

P_2O_5: Proceed as directed in the *Phosphate Determination as P_2O_5,* Method II (see Volume 4).