SLOVENSKI STANDARD
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Meso in mesni proizvodi - Določanje vsebnosti hidroksiprolina

Meat and meat products -- Determination of hydroxyproline content

Viandes et produits à base de viande -- Détermination de la teneur en hydroxyproline

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INTERNATIONAL STANDARD

ISO 3496

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Meat and meat products — Determination of hydroxyproline content

Viandes et produits à base de viande — Détermination de la teneur en hydroxyproline

Reference number
ISO 3496:1994(E)

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 3496 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 6, *Meat and meat products*.

This second edition cancels and replaces the first edition (ISO 3496:1978), which has been technically revised.

Annex A of this International Standard is for information only.

International Organization for Standardization
Case Postale 56 • CH-1211 Genève 20 • Switzerland

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Meat and meat products — Determination of hydroxyproline content

1 Scope

This International Standard specifies a method for the determination of the hydroxyproline content of all kinds of meat and meat products, including poultry.

It is applicable to meat and meat products containing less than 0,5 % (*m/m*) hydroxyproline.

2 Definition

For the purposes of this International Standard, the following definition applies.

2.1 hydroxyproline content of meat and meat products: Content of hydroxyproline determined according to the procedure specified in this International Standard.

The content is expressed as a percentage by mass.

3 Principle

Hydrolysis of a test portion in sulfuric acid at 105 °C. Filtration and dilution of the hydrolysate. Oxidation of the hydroxyproline by chloramine-T, followed by the formation of a red compound with *p*-dimethylaminobenzaldehyde. Photometric measurement at a wavelength of 558 nm.

4 Reagents

Use only reagents of recognized analytical quality and distilled or demineralized water or water of equivalent purity.

4.1 Sulfuric acid solution, $c(H_2SO_4) \approx 3$ mol/l.

Add 750 ml of water to a 2 litre one-mark volumetric flask. Add slowly, with agitation, 320 ml of concentrated sulfuric acid ($\rho_{20} = 1,84$ g/ml). Cool to room temperature and make up to the mark with water.

4.2 Buffer solution, pH = 6,8, consisting of:

26,0 g of citric acid monohydrate ($C_6H_8O_7.H_2O$);

14,0 g of sodium hydroxide;

78,0 g of anhydrous sodium acetate $[Na(CH_3CO_2)]$.

Dissolve the reagents in 500 ml of water and transfer quantitatively into a 1 litre one-mark volumetric flask. Add 250 ml of propan-1-ol and make up to the mark with water.

When stored at 4 °C in the dark, this solution is stable for several weeks.

4.3 Chloramine-T reagent

Dissolve 1,41 g of sodium *N*-chloro-*p*-toluenesulfonamide trihydrate (chloramine-T) in 100 ml of the buffer solution (4.2).

Prepare this solution immediately before use.

4.4 Colour reagent

Dissolve 10,0 g of *p*-dimethylaminobenzaldehyde in 35 ml of perchloric acid solution [60 % (*m/m*)] and then slowly add 65 ml of propan-2-ol.

Prepare this solution on the day of use.

If purification of *p*-dimethylaminobenzaldehyde is necessary (see note 3 in 8.4), proceed as follows. Prepare a saturated solution of *p*-dimethylaminobenzaldehyde in hot 70 % (*V/V*) ethanol. Cool, first at room temperature and finally in a refrigerator. After about 12 h, filter on a Buchner funnel. Wash with a little 70 % (*V/V*) ethanol. Again dissolve in hot

70 % (*V/V*) ethanol. Add cold water and agitate thoroughly. Repeat this procedure until a sufficient quantity of milk-white crystals has been formed. Place in the refrigerator overnight. Filter on the Buchner funnel. Wash with 50 % (*V/V*) ethanol and vacuum dry over phosphorus(V) oxide.

4.5 Hydroxyproline, standard solutions.

Prepare a stock solution by dissolving 50 mg of 4-hydroxypyrrolidine-α-carbonic acid (hydroxyproline) in water in a 100 ml one-mark volumetric flask. Add 1 drop of the sulfuric acid solution (4.1) and make up to the mark with water. This solution is stable for at least 1 month at 4 °C.

On the day of use, transfer 5 ml of the stock solution to a 500 ml one-mark volumetric flask and make up to the mark with water. Then prepare four standard solutions by diluting 10 ml, 20 ml, 30 ml and 40 ml of this solution to 100 ml with water to obtain hydroxyproline concentrations of 0,5 µg/ml, 1 µg/ml, 1,5 µg/ml and 2 µg/ml respectively.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Electric meat mincer, with horizontal blades, capable of rotating at high speed.

5.2 Round- or flat-bottomed hydrolysis flasks, of capacity about 200 ml, wide-necked.

5.3 Drying oven, capable of being operated at 105 °C ± 1 °C

5.4 Filter paper discs, of diameter 12,5 cm.

5.5 pH-meter.

5.6 Aluminium or **opaque plastic foil**.

5.7 Water bath, capable of being maintained at 60 °C ± 0,5 °C.

5.8 Spectrometer, suitable for use at a wavelength of 558 nm ± 2 nm, or a **photoelectric colorimeter** with an interference filter with an absorption maximum at 558 nm ± 2 nm.

5.9 Glass cells, of 10 mm optical path length.

5.10 Analytical balance, capable of weighing to an accuracy of ± 0,001 g.

5.11 Volumetric flasks, of capacity 250 ml.

5.12 Watch glasses, of diameter 5 cm to 6 cm.

6 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 3100-1.

Proceed from a representative sample of at least 200 g.

Store the sample in such a way that deterioration and change in composition are prevented.

7 Preparation of test sample

7.1 Raw meat and meat products

Reduce intact meat into small cubes (approx. 0,5 cm^3, i.e. sides of length approx. 8 mm) by cutting it while it is cold (just below 0 °C), using a sharp knife. Either place the sample in a container and seal the latter hermetically, or vacuum-pack the sample in heat-resistant plastic film. Then heat the container and sample so as to maintain a temperature of at least 70 °C for at least 30 min. Cool and proceed as in 7.2.

During the remaining stages of preparation of the test sample and the weighing out of the test portions, ensure that the sample is kept well mixed and, in particular, that any fat or fluid is kept evenly distributed.

NOTE 1 The heat treatment softens the raw connective tissue and makes it less resistant to homogenization by mincing. However, it may also lead to separation of a fluid containing gelatine. The presence of fat may also demand special attention for the production of a homogeneous test sample.

7.2 Cooked meat and cooked meat products

Homogenize the sample in the meat mincer (5.1). Keep the homogenized sample in a completely filled, air-tight, closed container and store it in such a way that deterioration and change in composition are prevented. Analyse the test sample as soon as possible, but always within 24 h.

8 Procedure

8.1 Test portion

Weigh, to the nearest 0,001 g, about 4 g of the test sample into a hydrolysis flask (5.2). Ensure that no sample material adheres to the side walls of the flask.

8.2 Hydrolysis

8.2.1 Add 30 ml ± 1 ml of sulfuric acid solution (4.1) to the flask. Cover the flask with a watch glass (5.12) and place in the oven (5.3) for 16 h (conveniently, overnight) at 105 °C.

8.2.2 Filter the hot hydrolysate through filter paper (5.4) into a 250 ml one-mark volumetric flask (5.11). Wash the flask and filter paper three times with 10 ml portions of hot sulfuric acid solution (4.1) and add the washings to the hydrolysate. Make up to the mark with water and mix.

8.3 Colour development and measurement of absorbance

8.3.1 Using a pipette, transfer to a one-mark 250 ml volumetric flask (5.11) a volume (V) of the hydrolysate (8.2.2) so that, after dilution to 250 ml, the hydroxyproline concentration will be within the range 0,5 µg/ml to 2 µg/ml. Make up to the mark with water.

NOTE 2 In most cases, V will be in the order of 5 ml to 25 ml depending on the amount of connective tissue present in the sample.

8.3.2 Transfer 4,00 ml of this solution (8.3.1) to a test tube and add 2,00 ml of chloramine-T reagent (4.3). Mix and leave at room temperature for 20 min ± 1 min.

8.3.3 Add 2,00 ml of the colour reagent (4.4), mix thoroughly and cap the tube with aluminium or plastic foil (5.6).

8.3.4 Transfer the tube quickly into the water bath (5.7), set at 60 °C, and heat for exactly 20 min.

8.3.5 Cool the tube under running tap water for at least 3 min and leave at room temperature for 30 min.

8.3.6 Measure the absorbance at 558 nm ± 2 nm in a glass cell (5.9) against water, using the spectrometer or the photoelectric colorimeter equipped with an interference filter (5.8).

8.3.7 Subtract the absorbance measured for the blank solution (8.4) and read the hydroxyproline concentration of the diluted hydrolysate from the calibration graph obtained as described in 8.5.

8.4 Blank test

Carry out in duplicate the procedure described in 8.3.2 to 8.3.7 inclusive, substituting water for the diluted hydrolysate.

NOTE 3 If the absorbance of the blank exceeds 0,040, a fresh colour reagent (4.4) should be prepared and, if necessary, the *p*-dimethylaminobenzaldehyde should be purified (see note 1 in 4.4).

8.5 Calibration graph

8.5.1 Carry out the procedure described in 8.3.2 to 8.3.7 inclusive, substituting in turn 4,00 ml of each of the four diluted standard hydroxyproline solutions (4.5) for the diluted hydrolysate.

8.5.2 Plot the measured absorbance values, corrected for the blank value, against the concentrations of the standard hydroxyproline solutions. Construct the best-fitting straight line through the plotted points and the origin.

Prepare a new calibration graph for each series of analyses.

9 Calculation

For each test portion, calculate the hydroxyproline content, as a percentage by mass, from the formula

$$w_{\mathrm{h}} = \frac{6{,}25c}{m \times V}$$

where

w_{h} is the hydroxyproline content, expressed as a percentage by mass, obtained from the formula;

c is the hydroxyproline concentration, in micrograms per millilitre, of the diluted hydrolysate as read from the calibration graph;

m is the mass, in grams, of the test portion (8.1);

V is the volume, in millilitres, of the aliquot portion of the hydrolysate taken for dilution to 250 ml (see 8.3.1).

Report the result to the nearest 0,01 %.

10 Precision

The precision of this method has been established by an international interlaboratory test carried out in accordance with ISO 5725.

For the values obtained for repeatability and reproducibility, a probability level of 95 % holds.

10.1 Repeatability

The absolute difference between two independent test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, should not be greater than the value of r given by the formula:

$$r = 0{,}013\,1 + 0{,}032\,2\,\overline{w}_h$$

where $\overline{w}_h$ is the mean of the two test results for the hydroxyproline content, expressed as a percentage by mass.

Reject both results if the difference exceeds the above value and carry out two new single determinations.

10.2 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, should not be greater than the value of R given by the formula:

$$R = 0{,}019\,5 + 0{,}052\,9\,\overline{w}_h$$

where $\overline{w}_h$ is the mean of the two test results for the hydroxyproline content, expressed as a percentage by mass.

11 Test report

The test report shall show

— the method in accordance with which sampling was carried out, if known,

— the method used,

— the test result obtained, and

— if the repeatability has been checked, the final quoted result obtained.

It shall also mention any operating conditions not specified in this International Standard, or regarded as optional, as well as any circumstances that may have influenced the test result.

The report shall include all details necessary for the complete identification of the sample.