INTERNATIONAL STANDARD 4134

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Meat and meat products — Determination of L-(+)-glutamic acid content (Reference method)

Viandes et produits à base de viande — Détermination de la teneur en acide L-(+)-glutamique (Méthode de référence)

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FOREWORD

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International Standard ISO 4134 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in June 1977.

It has been approved by the member bodies of the following countries :

Australia	Hungary	Philippines
Austria	India	Poland
Bulgaria	Iran	South Africa, Rep. of
Chile	Ireland	Spain
Czechoslovakia	Israel	Thailand
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Germany, F. R.	Netherlands	Yugoslavia

No member body expressed disapproval of the document.

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Meat and meat products — Determination of L-(+)-glutamic acid content (Reference method)

1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a reference method for the determination of the L-(+)-glutamic acid content of meat and meat products.

2 REFERENCES

ISO 1442, *Meat and meat products — Determination of moisture content.*

ISO 3100, *Meat and meat products — Sampling.*

3 DEFINITION

L-(+)-glutamic acid content of meat and meat products : The L-(+)-glutamic acid content determined according to the procedure described in this International Standard and expressed as a percentage by mass.

4 PRINCIPLE

Extraction of the L-(+)-glutamic acid present in a test portion with ice-cold perchloric acid solution. Centrifuging, decantation and filtration, followed by adjustment of pH and transformation of the L-(+)-glutamate in a portion of the filtrate by the following reactions 1) with nicotinamide adenine dinucleotide (NAD), and 2), with concomitant oxidation of an equivalent amount of nicotinamide adenine dinucleotide (reduced) (NADH) :

1) $$\text{L-(+)-glutamate} + \text{NAD}^+ + \text{H}_2\text{O} \xrightleftharpoons{\text{glutamate dehydrogenase}} \alpha\text{-cetoglutamate} + \text{NADH} + \text{NH}_3 + \text{H}^+$$

2) $$\text{NADH} + \text{iodonitrotetrazolium chloride} + \text{H}^+ \xrightarrow{\text{diaphorase}} \text{NAD}^+ + \text{formazane}$$

Photometric measurement of the amount of formazane formed.

5 REAGENTS

All reagents shall be of analytical quality. Except for the solutions of inorganic compounds (5.1 and 5.2), all solutions shall be stored in stoppered brown glass bottles which have been scrupulously cleaned and steamed or sterilized.

The water used shall be double-distilled or demineralized and distilled water, obtained by carrying out the final distillation in an all-glass apparatus.

NOTE — Water distilled only once may contain metal ion traces, and demineralized water may contain micro-organisms. Metal ions may decrease the activity of enzymes, while micro-organisms may give rise to an aspecific enzymatic background activity that might adversely affect the results of analysis.

5.1 Perchloric acid solution, 1,0 M.

Dilute 8,6 ml of perchloric acid, 70 % (*m/m*), ρ_{20} 1,67 g/ml, to 100 ml with water.

5.2 Potassium hydroxide solution, 2 M.

Dissolve 56,1 g of potassium hydroxide in water and dilute to 500 ml.

5.3 Triethanolamine-phosphate buffer solution, pH 8,5.

a) Dissolve 1,86 g of triethanolamine hydrochloride in water and adjust the pH to 8,6 with the potassium hydroxide solution (5.2) using a pH meter. Add 0,68 g of octylphenol-decaethyleneglycolether (for example Triton X-100) and dilute to 100 ml with water.

b) Dissolve 0,86 g of dipotassium hydrogen phosphate (K_2HPO_4) and 0,007 g of potassium dihydrogen orthophosphate (KH_2PO_4) in water and dilute to 100 ml.

Mix 20 ml of solution a) with 5 ml of solution b).

Keep the solution at 4 °C.

5.4 Nicotinamide adenine dinucleotide (NAD) solution.

Weigh 0,025 g of NAD in a small, stoppered flask and add 5,0 ml of water.

The solution can be kept for at least 4 weeks at 4 °C.

5.5 Iodonitrotetrazolium (INT) chloride [2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride] solution.

Weigh 0,030 g of INT in a small, stoppered brown flask and add 50 ml of water.

The solution can be kept for at least 2 months at 4 °C in the dark.

5.6 Diaphorase (lipoamine-dehydrogenase, EC* 1.6.4.3) solution.

Dissolve 0,003 g of lyophilized diaphorase in 1 ml of water.

The solution can be kept for at least 3 weeks at 4 °C.

5.7 Glutamate dehydrogenase (GlDH) (EC* 1.4.1.2) solution, 10 mg/ml, free from ammonium sulphate, ethylenedinitrilotetraacetic acid (EDTA) and glutaminase.

This solution is supplied as such (for example in quantities of 1,0 ml) and can be kept for at least 12 months at 4 °C.

5.8 L-(+)-glutamic acid, standard solution.

Dissolve 0,050 0 g of L-(+)-glutamic acid in 25 ml of water. Adjust the pH to 7,0 with the potassium hydroxide solution (5.2) and dilute to 50 ml.

Keep this solution at 4 °C and dilute 1 + 49 shortly before use.

6 APPARATUS

Usual laboratory equipment not otherwise specified, and the following items :

6.1 Mechanical meat mincer, laboratory size, fitted with a perforated plate with holes not exceeding 4 mm in diameter.

6.2 Laboratory mixer.

6.3 Laboratory centrifuge with 50 or 100 ml centrifuge tubes.

6.4 pH meter.

6.5 Fluted filter papers, diameter about 15 cm.

6.6 One-mark volumetric flasks, capacity 100 and 250 ml, complying with ISO 1042, class A.

6.7 One-mark pipettes, capacity 100, 50 and 25 ml, complying with ISO 648, class A.

6.8 Graduated pipettes, for delivering 2,5 – 0,5 – 0,2 and 0,05 ml, complying with ISO/R 835, class A.

6.9 Small plastic spatula, bent at 90°, for mixing the contents of the photometric cell.

6.10 Photoelectric colorimeter, provided with a filter having a transmittance maximum at 492 nm, or **spectrophotometer.**

6.11 Photometric cells of 10 mm optical path length.

7 SAMPLING AND LABORATORY SAMPLE

7.1 Sampling

See ISO 3100.

7.2 Laboratory sample

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.

8 PROCEDURE

8.1 Preparation of test sample

Make the sample homogeneous by passing it at least twice through the meat mincer (6.1) and mixing. Keep it in a completely filled, air-tight, closed container; store it, if necessary, in such a way that deterioration and change in composition are prevented.

Analyse the sample as soon as possible, but always within 24 h.

8.2 Test portion

Weigh, to the nearest 10 mg, approximately 50 g of the test sample (8.1) and transfer this test portion to the jar of the laboratory mixer (6.2).

8.3 Preparation of extract

8.3.1 Add 100 ml of ice-cold perchloric acid solution (5.1) and homogenize.

* The EC number refers to the Enzyme Classification number as given in :

– The International Union of Biochemistry, "Enzyme nomenclature", Elsevier Publ. Co. Amsterdam 1965.

8.3.2 Transfer a part of the homogenate to a centrifuge tube (see 6.3). Centrifuge for 10 min at 3 000 min^{-1}* and, after having carefully moved aside the fat layer, decant the supernatant liquid through a fluted filter paper (6.5) into a 200 ml conical flask, discarding the first 10 ml of the filtrate.

8.3.3 Transfer 50 ml of the solution (which should be only slightly turbid) into a 100 ml beaker and adjust the pH to 10 with the potassium hydroxide solution (5.2).

8.3.4 Transfer the contents of the beaker quantitatively into a 100 ml volumetric flask, dilute to the mark with water and mix.

8.3.5 Cool the solution in ice for 10 min, and filter through a fluted filter paper (6.5), discarding the first 10 ml of the filtrate.

8.3.6 Pipette 25 ml, or some other appropriate volume (V ml), of the filtrate into a 250 ml volumetric flask and dilute to the mark with water.

NOTE — The volume V should be chosen so that the concentration of L-(+)-glutamic acid is less than 30 mg/l.

8.4 Determination

8.4.1 Bring solutions 5.3 and 8.3.6 to a temperature of 20 to 25 °C.

Pipette into each of two photometric cells (6.11) 2,50 ml of the buffer solution (5.3), 0,20 ml of the NAD solution (5.4), 0,20 ml of the INT solution (5.5) and 0,05 ml of the diaphorase solution (5.6).

Into one of the cells pipette 0,50 ml of the extract (8.3.6); the solution obtained is the test solution.

Into the other cell pipette 0,50 ml of water; the solution obtained is the blank solution.

Mix with the plastic spatula (6.9) and read the absorbance of each cell at 492 nm against air. The temperature of the solution should be 20 to 25 °C.

Note the absorbances as :

A_1 = absorbance of the test solution;

A_{1B} = absorbance of the blank solution.

8.4.2 Pipette 0,05 ml of the GIDH solution (5.7) on the plastic spatula (6.9). Mix with the contents of one of the cells by moving the spatula up and down.

Repeat this operation with the second cell.

Read the absorbance of each cell at 492 nm after 10 to 15 min and every 2 min thereafter until a constant increase in absorbance is obtained. Plot the absorbance against time. Extrapolate the absorbance values to the moment of start of the reaction (see annex).

Note these extrapolated absorbance values as :

A_2 = absorbance of the test solution;

A_{2B} = absorbance of the blank solution.

8.4.3 Determine the micromolar absorptivity of the formazane by repeating the operations described in 8.4.1 and 8.4.2, but replacing the 5,0 ml of extract in the first photometric cell by 0,5 ml of the standard L-(+)-glutamic acid solution (5.8).

Note the absorbances corresponding to the operations carried out in accordance with 8.4.1 as :

A'_1 = absorbance of the standard solution;

A'_{1B} = absorbance of the blank solution;

and the extrapolated absorbance values corresponding to the operations carried out in accordance with 8.4.2 as :

A'_2 = absorbance of the standard solution;

A'_{2B} = absorbance of the blank solution.

8.5 Duplicate determination

Carry out two independent determinations starting with different test portions taken from the same test sample (8.1).

9 EXPRESSION OF RESULTS

9.1 Method of calculation and formula

Calculate the L-(+)-glutamic acid content of the sample, expressed as a percentage by mass, using the formula

$$\Delta A \times \frac{3{,}5 \times 147{,}1}{\kappa \times 0{,}5 \times 1\,000} \times \frac{250}{1\,000} \times \frac{100}{V} \times \frac{\left(100 + \frac{M \times m}{100}\right)}{50} \times \frac{100}{m}$$

$$= 51{,}485 \times \frac{\Delta A}{\kappa \times V \times m}\left(100 + \frac{M \times m}{100}\right)$$

where

$$\Delta A = (A_2 - A_1) - (A_{2B} - A_{1B})$$

* A rotational frequency of 3 000 min^{-1} corresponds to 3 000 revolutions per minute.

147,1 is the relative molecular mass of L-(+)-glutamic acid;

κ is the micromolar absorption coefficient of the formazane, in square centimetres per micromole, given by the formula

$$\kappa = \Delta A' \times \frac{3,5}{0,5} \times \frac{50}{1\,000} \times 147,1$$

$$= 51,485\ \Delta A'$$

where $\Delta A' = (A'_2 - A'_1) - (A'_{2B} - A'_{1B})$

V is the volume, in millilitres, of filtrate taken in 8.3.6;

M is the percentage moisture content in the sample, determined according to ISO 1442;

m is the mass, in grams, of the test portion (8.2).

Take as the result the arithmetic mean of the two determinations, provided that the requirement for repeatability (see 9.2) is satisfied. Report the result to the nearest 0,01 g of glutamic acid per 100 g of test sample.

9.2 Repeatability

The difference between the results of two determinations carried out almost simultaneously or in rapid succession by the same analyst shall not exceed 10 % of their arithmetic mean.

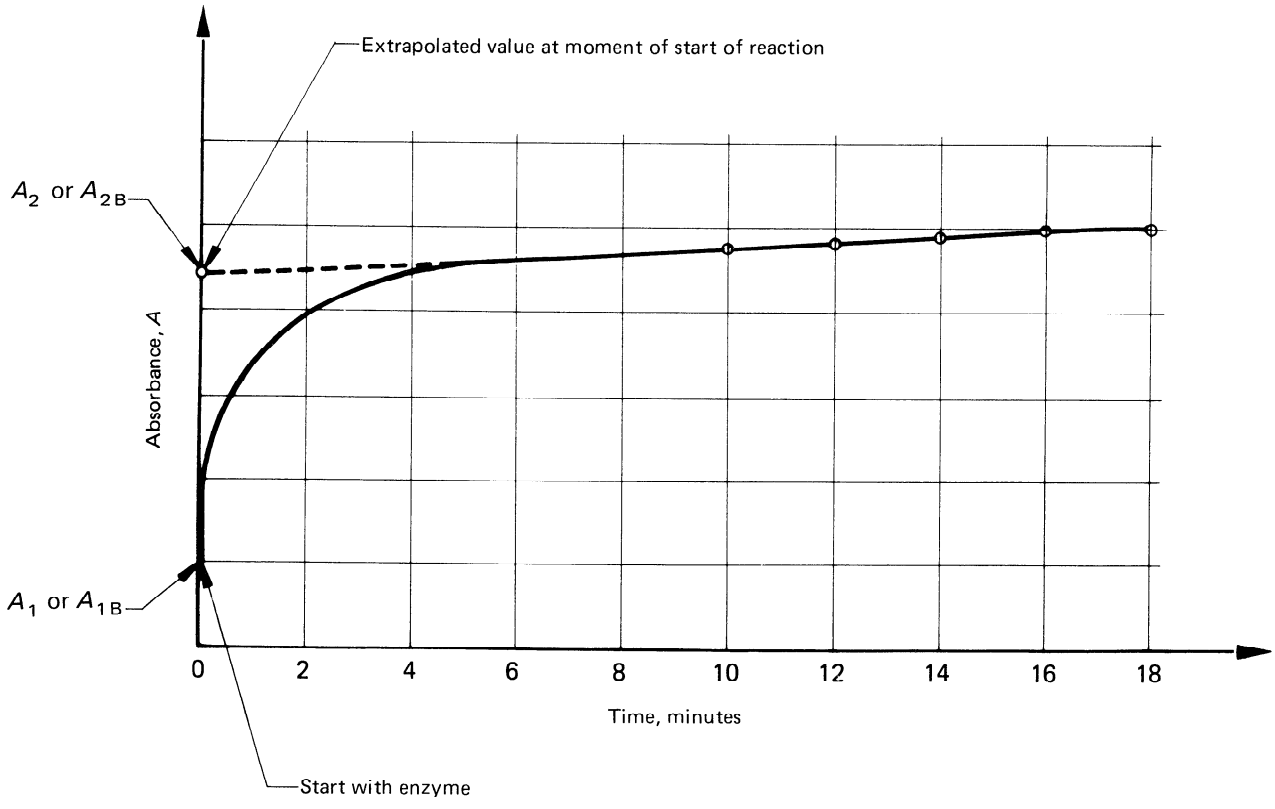
10 TEST REPORT

The test report shall show the method used and the 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 result.

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

ANNEX

EXAMPLE OF PLOTTING AND EXTRAPOLATION OF ABSORBANCE VALUES



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