INTERNATIONAL STANDARD

ISO 4134

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

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

Reference number
ISO 4134:1999(E)

Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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 4134 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 4134:1978), which has been technically revised.

Annex A is a normative part of this International Standard. Annexes B and C are for information only.

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

1 Scope

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

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 648, *Laboratory glassware — One-mark pipettes.*

ISO 835-2, *Laboratory glassware — Graduated pipettes — Part 2: Pipettes for which no waiting time is specified.*

ISO 1042, *Laboratory glassware — One-mark volumetric flasks.*

ISO 1442, *Meat and meat products — Determination of moisture content (Reference method).*

3 Terms and definitions

For the purposes of this International Standard, the following term and definition apply.

3.1
L-(+)-glutamic acid content of meat and meat products
mass fraction of L-(+)-glutamic acid determined according to the procedure described in this International Standard

NOTE The L-(+)-glutamic acid content is expressed in percent.

4 Principle

The L-(+)-glutamic acid present in a test portion is extracted with perchloric acid solution at a temperature of 0 °C. The extract is centrifuged, decanted and filtered and the pH is adjusted to 10,0. Nicotinamide adenine dinucleotide (NAD) is reduced by the L-(+)-glutamic acid in the presence of glutamate dehydrogenase [equation (1)]. The resultant reduced nicotinamide adenine dinucleotide (NADH) is reacted with iodonitrotetrazolium chloride in the presence of diaphorase [equation (2)]. The resulting formazane is measured at a wavelength of 492 nm and the L-(+)-glutamic acid content is calculated.

$$\text{L-(+)-glutamate} + NAD^+ + H_2O \xrightleftharpoons{\text{glutamate dehydrogenase}} \alpha\text{-ketoglutamate} + NADH + NH_3 + H^+ \quad (1)$$

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

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

Except for the solutions of inorganic compounds (5.2 and 5.3), store all solutions in stoppered brown glass bottles which have been scrupulously cleaned and steamed or sterilized.

5.1 Water, 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 microorganisms. Metal ions may decrease the activity of enzymes, while microorganisms may give rise to an aspecific enzymatic background activity that might adversely affect the results of analysis.

5.2 Dilute perchloric acid, $c(HClO_4)$ = 1,0 mol/l.

WARNING — Contact with oxidizable or combustible materials or with dehydrating or reducing agents may result in fire or explosion. Persons using this acid should be thoroughly familiar with its hazards. See safety practices listed in annex A.

Dilute 8,6 ml of perchloric acid, 70 % (by mass), ρ_{20} = 1,67 g/ml, to 100 ml with water (5.1). Add the perchloric acid to the bulk of the water before the final dilution to volume and mix.

5.3 Potassium hydroxide solution, $c(KOH)$ = 2 mol/l.

Dissolve 56,1 g of potassium hydroxide in water (5.1). Dilute when cooled to 500 ml and mix.

5.4 Triethanolamine phosphate buffer solution, pH = 8,6.

Dissolve 1,86 g of triethanolamine hydrochloride in water (5.1) and adjust the pH to 8,6 with the potassium hydroxide solution (5.3) using a pH-meter. Add 0,68 g of octylphenol decaethyleneglycol ether (e.g. Triton X-100). Dilute to 100 ml with water and mix (solution A).

Dissolve 0,86 g of dipotassium hydrogen phosphate (K_2HPO_4) and 7 mg of potassium dihydrogen phosphate (KH_2PO_4) in water (5.1). Dilute to 100 ml with water and mix (solution B).

Mix 20 ml of solution A with 5 ml of solution B.

The solution is stable for 2 months when stored at a temperature of between 0 °C and 6 °C.

5.5 Nicotinamide adenine dinucleotide (NAD) solution.

Weigh 25 mg of NAD in a small, stoppered flask. Add 5,0 ml of water (5.1) and mix.

The solution is stable for 2 months when stored in the dark at a temperature of between 0 °C and 6 °C.

5.6 Iodonitrotetrazolium chloride (INT) solution, 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride.

Weigh 30 mg of INT in a small, stoppered brown flask. Add 50 ml of water (5.1) and mix.

The solution is stable for 4 weeks when stored in the dark at a temperature of between 0 °C and 6 °C.

5.7 Diaphorase solution (lipoamide dehydrogenase, EC[1)] 1.8.1.4).

Dissolve 3 mg of lyophilized diaphorase in 1 ml of water (5.1) and mix.

The solution is stable for 4 weeks when stored at a temperature of between 0 °C and 6 °C.

5.8 Glutamate dehydrogenase (GLDH) solution (EC[1)] 1.4.1.3), 10 mg/ml, free from ammonium sulfate, ethylene-dinitrilotetraacetic acid (EDTA) and glutaminase.

This solution is supplied as such (for example in quantities of 1,0 ml) and is stable for 12 months when stored at a temperature of between 0 °C and 6 °C.

5.9 L-(+)-glutamic acid, **standard solution.**

Dissolve 50,0 mg of L-(+)-glutamic acid ($C_5H_9O_4N$) in 25 ml of water (5.1). Adjust the pH to 7,0 with potassium hydroxide solution (5.3). Dilute to 50 ml and mix.

Keep this solution at a temperature of between 0 °C and 6 °C and dilute 1 + 49 shortly before use.

6 Apparatus

Usual laboratory equipment and, in particular, the following.

6.1 Mechanical or electrical equipment, capable of homogenizing the laboratory sample.

This includes a high-speed rotational cutter, or a mincer fitted with a plate with apertures not exceeding 4,0 mm in diameter.

6.2 Laboratory mixer.

6.3 Laboratory centrifuge, with 50 ml or 100 ml centrifuge tubes, operating at a radial acceleration of about 2 000 g_n.

6.4 pH-meter.

6.5 Fluted filter papers, of diameter about 15 cm, high or moderate speed.

6.6 One-mark volumetric flasks, of capacities 100 ml and 250 ml, complying with ISO 1042, class B.

6.7 One-mark pipettes, of capacities 100 ml, 50 ml and 25 ml, complying with ISO 648, class B.

6.8 Graduated (automatic) pipettes, for delivering 2,50 ml, 0,50 ml, 0,20 ml and 0,05 ml, complying with ISO 835-2, class A.

6.9 Small plastics spatula, bent at 90°, for mixing the contents of the cuvettes.

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

6.11 Cuvettes, of 10 mm optical path length.

6.12 Analytical balance, capable of weighing to the nearest 0,1 mg.

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1) The EC number refers to the Enzyme Classification number as given in: The International Union of Biochemistry, *Enzyme nomenclature*, Elsevier, Amsterdam, 1965.

7 Sampling

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

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

Start 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 Preparation of test sample

Homogenize the laboratory sample with the appropriate equipment (6.1). Take care that the temperature of the sample material does not rise above 25 °C. If a mincer is used, pass the sample at least twice through the equipment.

Fill a suitable airtight container with the prepared sample. Close the container and store in such a way that deterioration and change in composition are prevented. Analyse the sample as soon as practicable, but always within 24 h after homogenization.

9 Procedure

NOTE If it is required to check whether the repeatability requirement is met, carry out two single determinations in accordance with 9.1 to 9.3.

9.1 Test portion

Weigh, to the nearest 0,01 g, approximately 50 g of the test sample (see clause 8) and transfer this test portion to the jar of the laboratory mixer (6.2).

9.2 Preparation of extract

9.2.1 Add 100 ml of dilute perchloric acid (5.2) at a temperature of 0 °C to the test portion and homogenize the mixture.

9.2.2 Transfer a portion of the homogenate to a centrifuge tube (6.3). Centrifuge for 10 min at a radial acceleration of about 2 000 g_n. Carefully move aside the fat layer and decant the supernatant liquid through a fluted filter paper (6.5) into a 200 ml conical flask. Discard the first 10 ml of the filtrate.

9.2.3 Transfer by pipette (6.7) 50 ml of the solution (which should be only slightly turbid) into a 100 ml beaker and adjust the pH to 10,0 with the potassium hydroxide solution (5.3) using the pH-meter (6.4).

9.2.4 Transfer the contents of the beaker quantitatively into a 100 ml volumetric flask (6.6). Dilute to the mark with water (5.1) and mix.

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

9.2.6 Pipette 25 ml, or some other appropriate volume (V), of the filtrate into a 250 ml volumetric flask (6.6) and dilute to the mark with water.

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

9.3 Determination

9.3.1 Bring the buffer solution (5.4) and the filtrate (9.2.6) to a temperature of 20 °C to 25 °C.

Pipette into each of two cuvettes (6.11) 2,50 ml of the buffer solution (5.4), 0,20 ml of the NAD solution (5.5), 0,20 ml of the INT solution (5.6) and 0,05 ml of the diaphorase solution (5.7).

After the addition of INT, restrict exposure of the reaction mixture to light to an unavoidable minimum.

Into one of the cuvettes, pipette 0,50 ml of the filtrate (9.2.6); the solution obtained is the test solution.

Into the other cuvette, pipette 0,50 ml of water (5.1); the solution obtained is the blank solution.

Mix the solutions with the spatula (6.9) and read the absorbance A_1 of each cuvette at a wavelength of 492 nm against water. The temperature of the solution shall be 20 °C to 25 °C.

9.3.2 Pipette 0,05 ml of the GLDH solution (5.8) into each of the cuvettes. Mix the contents of the cuvettes by swirling or by moving the spatula up and down.

Read the absorbance A_2 of each cuvette at a wavelength of 492 nm against water after 10 min 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 B).

9.3.3 Repeat the operations described in 9.3.1 and 9.3.2, but replace the 0,50 ml of filtrate (9.2.6) in the first cuvette by 0,50 ml of the L-(+)-glutamic acid standard solution (5.9).

9.3.4 Determine the moisture content of the test sample according to ISO 1442.

10 Calculation and expression of results

10.1 Absorbance difference for standard solution

Calculate the absorbance difference for the standard solution using the following equation:

$$\Delta A_S = (A_{2s} - A_{1s}) - (A_{2b} - A_{1b})$$

where

ΔA_S is the absorbance difference for the standard solution;

A_{1b} is the absorbance of the blank solution, measured in 9.3.3 in accordance with 9.3.1;

A_{2b} is the absorbance of the blank solution, measured in 9.3.3 in accordance with 9.3.2;

A_{1s} is the absorbance of the standard solution, measured in 9.3.3 in accordance with 9.3.1;

A_{2s} is the absorbance of the standard solution, measured in 9.3.3 in accordance with 9.3.2.

10.2 Absorbance difference for test solution

Calculate the absorbance difference for the test solution using the following equation:

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

where

ΔA is the absorbance difference for the test solution;

A_1 is the absorbance of the test solution, measured in 9.3.1;

A_2 is the absorbance of the test solution, measured in 9.3.2;

A_{1b} is the absorbance of the blank solution, measured in 9.3.1;

A_{2b} is the absorbance of the blank solution, measured in 9.3.2.

10.3 L-(+)-glutamic acid content

Calculate the L-(+)-glutamic acid content using the following equation:

$$w_g = \frac{\Delta A}{\Delta A_s \times V}\left(\frac{100}{m} + \frac{w_m}{100}\right)$$

where

w_g is the numerical value of the L-(+)-glutamic acid content, as a percentage (by mass) of the dry test sample;

ΔA is the absorbance difference for the test solution (see 10.2);

ΔA_s is the absorbance difference for the standard solution (see 10.1);

V is the numerical value of the volume, in millilitres, of filtrate taken in 9.2.6;

w_m is the numerical value of the moisture content (9.3.4), as a percentage (by mass) of the sample;

m is the numerical value of the mass, in grams, of the test portion (9.1).

NOTE A full explanation of the derivation of this equation is given in annex C.

Round the result to the nearest 0,01 %.

11 Precision

11.1 Interlaboratory test

The precision of the method was established by interlaboratory tests carried out in accordance with ISO 5725 [2)].

The results of these tests have been published (see references [5] and [6]). The values derived from these tests may not be applicable to concentration ranges and matrices other than those given.

11.2 Repeatability

The absolute difference between two independent single 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, will in not more than 5 % of cases exceed 0,02 % (by mass) for L-(+)-glutamic acid contents up to 0,14 %.

11.3 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, will in not more than 5 % of cases exceed 0,04 % (by mass) for L-(+)-glutamic acid contents up to 0,14 %.

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2) ISO 5725:1986 (now withdrawn) was used to obtain the precision data.

12 Test report

The test report shall specify:

— all information necessary for the complete identification of the sample;

— the sampling method used, if known;

— the test method used, with reference to this International Standard;

— all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

— the test result obtained, or the two test results obtained if the repeatability has been checked.

Annex A
(normative)

Safety practices

Safety practices in handling perchloric acid ($HClO_4$) shall include following.

a) Remove spilled $HClO_4$ by immediate and thorough washing with large amounts of water.

b) Hoods, ducts and other devices for removing $HClO_4$ vapour shall be made of chemically inert materials and so designed that they can be thoroughly washed with water. Exhaust systems should discharge to a safe location and fans shall be accessible for cleaning.

c) Avoid the use of organic chemicals in hoods or other fume-removal devices used for $HClO_4$ digestions.

d) Use goggles, barrier shields and other devices as necessary for personal protection; use polyvinyl chloride, not rubber, gloves.

e) In wet combustions with $HClO_4$, treat the sample first with nitric acid to destroy easily oxidizable organic matter unless otherwise specified. Do not evaporate to dryness.

f) Contact of $HClO_4$ with strong dehydrating agents such as phosphorus pentoxide or concentrated sulfuric acid may result in formation of anhydrous $HClO_4$ which reacts explosively with organic matter and with reducing agents. Exercise special care in performing analyses requiring use of $HClO_4$ with such agents. It is extremely sensitive to shock and heat when the concentration is 72 % (by mass).

NOTE Taken from AOAC Official Methods of Analysis (1995), Laboratory Safety, Appendix B, p. 3

Annex B
(informative)

Example of plotting and extrapolation of absorbance values

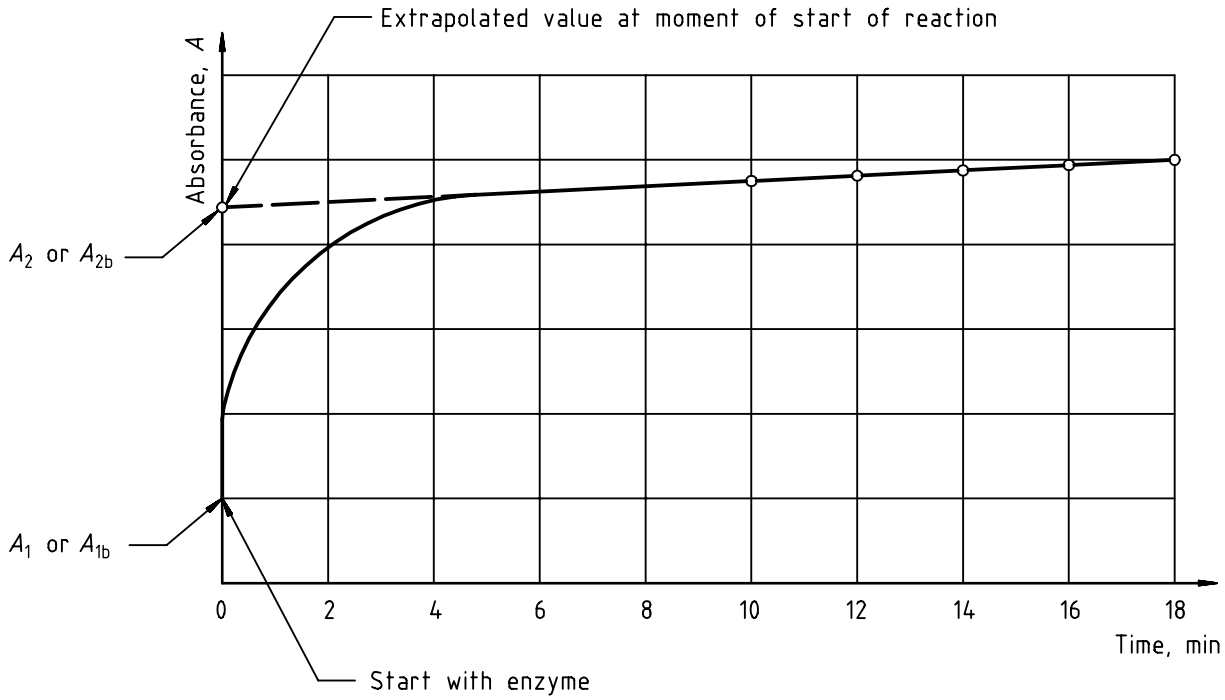

Figure B.1

Annex C
(informative)

Derivation of equation for calculation of L-(+)-glutamic acid content

C.1 Molar absorption coefficient of formazane

Calculate the numerical value of the molar absorption coefficient of the formazane using the following equation:

$$\kappa = \Delta A_S \times \frac{3,5}{0,5} \times \frac{50}{1000} \times 147,1 = 51,485 \times \Delta A_S$$

where

κ is the numerical value of the molar absorption coefficient, in litres per millimole centimetres, of formazane at a wavelength of 492 nm;

ΔA_S is the absorbance difference for the standard solution.

C.2 L-(+)-glutamic acid content

Calculate the numerical value of the L-(+)-glutamic acid content of the dry test sample using the following equation:

$$w_g = \Delta A \times \frac{3,5 \times 147,1}{\kappa \times 0,5 \times 1000} \times \frac{250}{1000} \times \frac{100}{V} \times \frac{\left(100 + \frac{w_m \times m}{100}\right)}{50} \times \frac{100}{m} = 51,485 \times \frac{\Delta A}{\kappa \times V \times m}\left(100 + \frac{w_m \times m}{100}\right)$$

where

w_g is the numerical value of the L-(+)-glutamic acid content, as a percentage (by mass) of the dry test sample;

ΔA is the absorbance difference for the test solution;

κ is the numerical value of the molar absorption coefficient, in litres per millimole centimetres, of formazane at a wavelength of 492 nm;

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

V is the numerical value of the volume, in millilitres, of filtrate taken in 9.2.6;

w_m is the numerical value of the moisture content (9.3.4), as a percentage (by mass) of the sample;

m is the numerical value of the mass, in grams, of the test portion (9.1).

Bibliography

[1] ISO 3100-1, *Meat and meat products — Sampling and preparation of test samples — Part 1: Sampling*.

[2] ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests*.

[3] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*.

[4] ISO 5725-2:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*.

[5] Amtliche Sammlung von Untersuchungsverfahren nach Par. 35 LMBG. *Bestimmung von L-Glutaminsäure (L-Glutamat) in Fleischerzeugnissen.* L07.00-17, November 1981.

[6] HATTULA, M.T. and WALLIN, H.C. *J. AOAC International*, **74**, 1991, pp 921-925.

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