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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

ICS: 67.120.10

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Foreword

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This document was prepared by Technical Committee ISO/TC 34,*Food products*, Subcommittee SC 6, *Meat, poultry, fish, eggs and their products.*

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This third edition cancels and replaces the second edition (ISO 4134:1999), which has been technically revised.

The main changes compared to the previous edition are as follows:

— A new test method - light absorption microplate reader method is added.

— The determine scope of the document has been further clearly defined as free L-(+)-glutamic acid in meat and meat products.

— The clauses order of the document has been rearranged.

— The introductory texts for "Foreword", "Normative references" and "Terms and definitions" have been modified in accordance with ISO/IEC Directives, Part 2.

— The title of the document has been modified.

— The Normative references has been updated.

— The terms and definitions has been modified with adding the terms of "Free L-(+)-glutamic acid".

— The description of "extraction of L-(+)-glutamic acid of test portion" has been modified and the detection wavelength has been changed from "492 nm" to "490 nm".(Clause 4)

— The identification of enzyme activity units for diaphorase and glutamate dehydrogenase has been supplemented; the concentration of KOH, NAD has been modified; the NAD and diaphorase have been mixed into a solution; the buffer, NAD and enzymes have been labelled with R1, R2, and R3.(Clause 7.1)

— The apparatus list has been updated.

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— The "Procedure" of "Test method of spectrophotometer" has been modified with halving the sample weight and solution volume.

— The method of judging the absorbance of the reaction end point has been modified. Accordingly, the previous Annex B "Example of plotting and extrapolation of absorbance values" has been deleted. (Clause 7.3.3)

— In the "Calculation and results", the equation and symbol description of spectrophotometer has been modified.

— The previous Annex C "Derivation of equation for calculation of L-(+)-glutamic acid content" has been deleted.

— The documents list of "Bibliography" has been updated

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Introduction

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

1 Scope

This document specifies spectrophotometer method and light absorption microplate reader method for the determination of the free L-(+)-glutamic acid content of meat and meat products.

This document is applicable to meat and meat products, including livestock and poultry products.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 648, *Laboratory glassware — Single-volume pipettes*

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

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

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 8655-2, *Piston-operated volumetric apparatus — Part 2: piston pipettes*

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3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
Free L-(+)-glutamic acid
The L-(+)-glutamic acid and glutamate exist in meat and meat products in the form of free state

3.2
L-(+)-glutamic acid content of meat and meat products
The mass fraction of the free L-(+)-glutamic acid in meat and meat products determined according to the procedure described in this International Standard

4 Principle

The free L-(+)-glutamic acid present in a test portion is extracted with perchloric acid solution. The extract is centrifuged, decanted and filtered and diluted to appropriate concentration with water, and the pH is adjusted to 10. Nicotinamide adenine dinucleotide (NAD) is reduced by the L-(+)-glutamic acid in the presence of glutamate dehydrogenase [Formula (1)]. The resultant reduced nicotinamide adenine dinucleotide (NADH) reacts with iodonitrotetrazolium chloride in the presence of diaphorase

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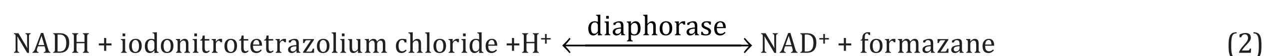
[Formula (2)]. The resulting formazane is measured at a wavelength of 490 nm and the free L-(+)-glutamic acid content of the test sample is calculated.



5 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in CAC/GL 50-2004.

It is important that the laboratory-received sample 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 at such a temperature of 4°C or lower that deterioration and change in composition are prevented.

6 Preparation of test sample

Homogenize the laboratory sample with the appropriate equipment (7.2.1). Take care that the temperature of the sample does not exceed 25 °C. If a mincer is used, pass the sample at least twice through the equipment.

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Fill a suitable airtight container with the prepared sample. Close the container and store at such a temperature of 4°C or lower that deterioration and change in composition are prevented. Analyse the sample as soon as practicable, but always within 24 h after homogenization.

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7 Test method of spectrophotometer

7.1 Reagents

Only reagents of recognized analytical grade and only water of at least grade 2 purity as defined in ISO 3696 shall be used. Except for the solutions of inorganic compounds (7.1.1 to 7.1.2), store all solutions in stoppered brown glass bottles which have been scrupulously cleaned and steamed or sterilized.

7.1.1 Dilute perchloric acid, c(HClO4) = 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.

Add 8.6 mL of the perchloric acid (70 % (by mass), ρ_{20} = 1.67 g/mL) to the bulk of water, diluting to 100 mL.

7.1.2 Potassium hydroxide solution, c(KOH) = 4 mol/L, 2 mol/L, 0.5 mol/L, 0.02 mol/L

Dissolve 22.4 g of potassium hydroxide in water. Dilute the solution to 100 mL, c(KOH) = 4 mol/L, and mix evenly after cooling.

Dissolve 11.2 g of potassium hydroxide in water. Dilute the solution to 100 mL, c(KOH) = 2 mol/L, and mix evenly after cooling.

Transfer 2.5 mL of 2 mol/L potassium hydroxide solution to 10 mL volumetric flask, dilute to the mark with water and mix, c(KOH) = 0.5 mol/L.

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Transfer 0.1 mL of 2 mol/L potassium hydroxide solution to 10 mL volumetric flask, dilute to the mark with water and mix, c(KOH) = 0.02 mol/L.

7.1.3 Solution R1, triethanolamine phosphate buffer solution, pH = 8.6.

Dissolve 1.86 g of triethanolamine hydrochloride in approximately 25 mL of water, adjust the pH to 8.6 with 2 mol/L potassium hydroxide solution (7.1.2), detected by 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. Dilute to 100 mL with water and mix evenly (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.

7.1.4 Solution R2, the mixed solution of nicotinamide adenine dinucleotide (NAD) and diaphorase (lipoamide dehydrogenase EC[1] 1.8.1.4): c(NAD) = 11 mg/mL, c(diaphorase) approximately 4 U/mL.

Weigh 110 mg of NAD, and approximately 8 mg (approximately 40U) of diaphorase in a stoppered flask. Add 10.0 mL water and mix evenly.

The solution is stable for 1 week when stored in dark at a temperature of between 0 °C and 6 °C.

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7.1.5 Solution R3, iodonitrotetrazolium chloride (INT) solution, 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride, c(INT) = 0.6 mg/mL.

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Weigh 6 mg of INT in a small, stoppered brown flask. Add 10 mL of water and mix evenly.

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

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7.1.6 Solution R123, the mixed solution of solution R1, solution R2 and solution R3

Pipette appropriate volume of the solution R1, the solution R2 and the solution R3, and mix evenly at a volume ratio of 3:1:1 before test.

The mixed solution is stable for 1 h in stoppered brown glass bottles at room temperature.

7.1.7 Glutamate dehydrogenase (GLDH) solution (EC[1] 1.4.1.3): c(GLDH) approximately 900 U/mL.

Weigh 10 mg (approximately 900 U) of lyophilized glutamate dehydrogenase (GLDH) in a small stoppered flask. Add 1 mL water and mix.

Free from ammonium sulfate, ethylene-dinitrilotetraacetic acid (EDTA) and glutaminase, this solution is stable for 12 months when stored at a temperature of between 0 °C and 6 °C.

7.1.8 L-(+)-glutamic acid standard stock solution, c($C_5H_9O_4N$) = 1000 mg/L

Weigh, to the nearest 0.0001 g, approximately 50.0 mg of L-(+)-glutamic acid ($C_5H_9O_4N$). Dissolve it in approximately 25 mL of water.

Adjust the pH to 5-6 with a few drops of 2 mol/L potassium hydroxide solution (7.1.2). Then adjust the pH to 7.0 slowly with 0.02 mol/L potassium hydroxide solution (7.1.2). Dilute to 50 mL with water and mix evenly.

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

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

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7.1.9 L-(+)-glutamic acid standard solution, c($C_5H_9O_4N$) = 100 mg/L.

Pipette accurately 5.0 mL of L-(+)-glutamic acid standard stock solution (7.1.8) into a 50 mL volumetric flask (7.2.8), dilute to the mark with water and mix evenly.

The solution is with the current use.

7.1.10 L-(+)-glutamic acid series standard solution, c($C_5H_9O_4N$) = 5 mg/L, 10 mg/L, 15 mg/L,20 mg/L, 30 mg/L, 40 mg/L.

Pipette accurately 0.50 mL, 1.00 mL, 1.50 mL, 2.00mL, 3.00 mL, 4.00 mL of L-(+)-glutamic acid standard solution (7.1.9) into each of six 10 mL volumetric flasks (7.2.8) separately, dilute to the mark with water and mix evenly.

The solution is with the current use.

7.2 Apparatus

The usual laboratory equipment and, in particular, the following.

7.2.1 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.

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7.2.2 Laboratory mixer, stirrer or oscillator

7.2.3 Laboratory centrifuge, with 50 mL or 100 mL centrifuge tubes, operating at a radial acceleration of about 2 000 gn or equivalent speed (e.g. 3 500-4 000 rpm).

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7.2.4 Analytical balance, capable of weighing to the nearest 0.001g.

7.2.5 Constant temperature drying box

7.2.6 pH-meter

7.2.7 Filter papers, diameter of about 15 cm, high or moderate speed.

7.2.8 One-mark volumetric flasks, capacities of 10 mL, 50 mL and 100 mL, complying with ISO 1042, class B standard.

7.2.9 Single-volume pipettes, capacities of 50 mL, 25 mL and 1mL complying with ISO 648, class B standard.

7.2.10 Single channel or multi-channel transferring pipettes and tips, of 5 mL, 1 000 µL, 200 µL and 100 µL complying with ISO 8655-2.

7.2.11 Small plastics spatula or lid, for mixing the content evenly by stirring with spatula in the cuvette or shaking the cuvette covered with lid .

7.2.12 Photoelectric colorimeter, provided with a filter having a transmittance maximum at a wavelength of 490 nm, or spectrometer.

7.2.13 Cuvettes, of 10 mm optical path length.

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7.3 Procedure

NOTE If it is required to check whether the repeatability requirement is met, two individual determination should be performed.

7.3.1 Test portion

Weigh, to the nearest 0.001 g, approximately 25 g, or other appropriate weight (m_1) of the test sample (see Clause 6).

7.3.2 Preparation of extract

7.3.2.1 Add 50 mL of dilute perchloric acid solution (7.1.1) to the test sample, homogenize the mixture with the laboratory mixer (7.2.2).

7.3.2.2 Transfer the homogenized sample to centrifuge tube (7.2.3). Centrifuge for 10 min at 10°C, 2 000 gn or equivalent speed (e.g. 3 500~4 000 rpm). Carefully move aside the fat layer and decant all the supernatant liquid through a filter paper (7.2.7) into a 100 mL conical flask. Discard the first 10 mL of the filtrate.

7.3.2.3 Transfer 25 mL of the solution (which should be only slightly turbid) with pipette (7.2.9) into centrifuge tube (7.2.3). With detected by the pH-meter (7.2.6), adjust the pH to 7-8 with 4 mol/L potassium hydroxide solution (7.1.2), then adjust the pH to 10.0 slowly with 2 mol/L and 0.5 mol/L potassium hydroxide solution (7.1.2). Centrifuge for 3 min at 2 000 gn or equivalent speed (e.g. 3 500~4 000 rpm).

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NOTE If the pH is slightly above 10.0, it could be adjusted with dilute perchloric acid back to the required pH value (7.1.1).

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7.3.2.4 Transfer all the supernatant into a 50 mL volumetric flask (7.2.8). Dilute to the mark with water and mix.

7.3.2.5 Cool the solution in ice for 10 min, and filter through a filter paper (7.2.7). Discard the first 10 mL of the filtrate.

7.3.2.6 Pipet 5 mL, or some other appropriate volume (V_1) of the filtrate into a 50 mL volumetric flask (7.2.8). Dilute to the mark with water and mix. The solution obtained will be used to determine the content of free L-(+)-glutamic acid in the test portion.

NOTE The volume V_1 should be chosen so that the L-(+)-glutamic acid content of the solution is between 8 mg/L and 40 mg/L.

7.3.3 Determination

7.3.3.1 Preparation of detection instrument

Set up the spectrophotometer (7.2.12) and preheat the instrument according to the instrument specification until equilibrium conditions are achieved. Set the detection wavelength to 490 nm. Adjust the baseline of the equipment to zero with pure water.

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