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Third edition
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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
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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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

This third edition cancels and replaces the second edition (ISO 4134:1999), which has been technically revised. The main changes compared with the previous edition are as follows:

— a new test method, the light absorption microplate reader method, has been added;

— the order of the clauses has been rearranged;

— the Scope (Clause 1) has been revised to specify free L-(+)-glutamic acid in meat and meat products;

— the Normative references (Clause 2) have been updated;

— the Terms and definitions (Clause 3) have been modified by adding the term "free L-(+)-glutamic acid";

— in Clause 4, 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";

— in 7.1, 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; and the buffer, NAD and enzymes have been labelled with R1, R2, and R3;

— the apparatus list (7.2) has been updated;

— in 7.3, the procedure of the test method of spectrophotometer has been modified by halving the sample mass and solution volume;

— in 7.3.4, the method of judging the absorbance of the reaction end point has been modified and, as a result, the previous Annex B "Example of plotting and extrapolation of absorbance values" has been deleted;

— in 8.4, the formula 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 Bibliography has been updated.

Any feedback or questions on this document should be directed to the user’s national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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

1 Scope

This document specifies the spectrophotometer method and the 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*

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
L-(+)-glutamic acid and glutamate existing in meat and meat products in the form of free state

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, see Formula (1). The resultant reduced nicotinamide adenine dinucleotide (NADH) reacts with iodonitrotetrazolium chloride in the presence of diaphorase, see 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.

$$\text{L-(+)-glutamic acid/glutamate} + \text{NAD}^+ + \text{H}_2\text{O} \xleftarrow{\text{glutamate dehydrogenase}} \alpha\text{-ketoglutamate} + \text{NADH} + \text{NH}_3 + \text{H}^+ \quad (1)$$

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

5 Sampling

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

It is important that the sample received by the laboratory 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 a temperature of 4 °C or frozen at −18 °C if not immediately analysed, so that deterioration and change in composition are prevented.

6 Preparation of test sample

Homogenize the laboratory sample with the appropriate equipment (see 7.2.1). Note that the temperature of the sample should not exceed 25 °C. If a mincer is used, process the sample at least twice with the equipment.

Fill a suitable airtight container with the prepared sample. Seal the container and store it at a temperature of 4 °C or frozen at −18 °C if not immediately analysed, so that deterioration and change in composition are prevented. Analyse the sample as soon as practicable, but always within 24 h after homogenization.

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 and 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 = 1,0 mol/l.

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

Add 8,6 ml of the perchloric acid (70 g/ 100 g, ρ_{20} = 1,67 g/ml) to the bulk of water, diluting to 100 ml.

7.1.2 Potassium hydroxide solution, c = 4 mol/l, 2 mol/l, 0,5 mol/l and 0,02 mol/l.

Dissolve 22,4 g of potassium hydroxide in water. Dilute the solution to 100 ml, c = 4 mol/l, and mix evenly after cooling.

Dissolve 11,2 g of potassium hydroxide in water. Dilute the solution to 100 ml, c = 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 = 0,5 mol/l.

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 = 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), detecting with 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. 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 two months when stored at a temperature of between 0 °C and 6 °C.

7.1.4 Solution R2, the mixed solution of NAD and diaphorase (lipoamide dehydrogenase EC[1)] 1.8.1.4), ρ_{NAD} = 11 mg/ml, diaphorase, approximately 4 IU/ml.

Weigh 110 mg of NAD, and approximately 8 mg (approximately 40 IU) of diaphorase into a stoppered flask. Add 10,0 ml water and mix evenly.

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

7.1.5 Solution R3, iodonitrotetrazolium chloride (INT) solution, 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride, ρ = 0,6 mg/ml.

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

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

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

Pipette 6 ml of solution R1, 2 ml of solution R2 and 2 ml of solution R3 into a stoppered brown glass bottle, and mix evenly before the 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), approximately 900 IU/ml.

Weigh 10 mg (approximately 900 IU) of lyophilized glutamate dehydrogenase (GLDH) into a small stoppered flask. Add 1 ml water and mix.

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

7.1.8 L-(+)-glutamic acid standard stock solution, ρ = 1 000 mg/l.

Weigh, to the nearest 0,000 1 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 a value range from 5 to 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 six months when stored at a temperature 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.

7.1.9 L-(+)-glutamic acid standard solution, ρ = 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 for the current use.

7.1.10 L-(+)-glutamic acid series standard solution, ρ = 5 mg/l, 10 mg/l, 15 mg/l, 20 mg/l, 30 mg/l and 40 mg/l.

Pipette accurately 0,50 ml, 1,00 ml, 1,50 ml, 2,00 ml, 3,00 ml and 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 for the current use.

7.2 Apparatus

The usual laboratory equipment and, in particular, the following shall be used.

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.

7.2.2 Laboratory mixer, a stirrer or an oscillator.

7.2.3 Laboratory centrifuge, with 50 ml or 100 ml centrifuge tubes, operating at a radial acceleration of about 2 000*g* or an equivalent speed (e.g. 3 500 r/min to 4 000 r/min).

7.2.4 Analytical balance, capable of weighing to the nearest 0,001 g, 0,000 1 g.

7.2.5 Constant temperature drying box.

7.2.6 pH-meter.

7.2.7 Filter papers, with a 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, conforming to ISO 1042, class B standard.

7.2.9 Single-volume pipettes, capacities of 50 ml, 25 ml and 1 ml conforming to 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, conforming to ISO 8655-2.

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

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

7.2.13 Cuvettes, of 10 mm optical path length.

7.3 Procedure

7.3.1 General

If it is required to check whether the repeatability requirement is achieved, two separate determinations should be performed.

7.3.2 Test portion

Weigh, to the nearest 0,001 g, approximately 25 g, or other appropriate mass (m_1) of the test sample (see Clause 6).

7.3.3 Preparation of extract

7.3.3.1 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).

7.3.3.2 Transfer the homogenized sample to centrifuge tube (7.2.3). Centrifuge for 10 min at a temperature of 10 °C, at a radial acceleration of about 2 000*g* or an equivalent speed (e.g. 3 500 r/min to 4 000 r/min). Carefully skim off 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.

7.3.3.3 Pipet 25 ml of the solution (which should be only slightly turbid) with pipette (7.2.9) into centrifuge tube (7.2.3). Detecting with the pH-meter (7.2.6), adjust the pH to a value range from 7 to 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 a radial acceleration of about 2 000*g* or an equivalent speed (e.g. 3 500 r/min to 4 000 r/min).

NOTE If the pH is slightly above 10,0, it can be adjusted back to the required pH value (7.1.1) with dilute perchloric acid.

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

7.3.3.5 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.

7.3.3.6 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.

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.

7.3.4 Determination

7.3.4.1 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.

7.3.4.2 Absorbance determination of L-(+)-glutamic acid test solution

7.3.4.2.1 Maintain the temperature of solution R123 (7.1.6), water and the L-(+)-glutamic acid test solution (7.3.3.6) between 20 °C and 25 °C.

Pipette 1,0 ml of solution R123 (7.1.6) into a cuvette (7.2.13) and add 2,0 ml of water. The solution obtained is the blank solution.

Pipette 1,0 ml of solution R123 (7.1.6), 1,8 ml of water and 200 µl of the L-(+)-glutamic acid test solution (7.3.3.6) into another cuvette. The solution obtained is the test solution.

Mix the solutions with the spatula or lid (7.2.11), put it into the spectrophotometer and read the absorbance A_1 of the test solution and A_{b1} of the blank solution at a wavelength of 490 nm against water.

7.3.4.2.2 Pipette 50 µl of the GLDH solution (7.1.7) into each of the cuvettes. Mix the contents of the cuvettes evenly (7.2.11).

Read the absorbance A_1' of the test solution and A_{b1}' of the blank solution at a wavelength of 490 nm against water after keeping still for 10 min to 15 min, and record the absorbance every 2 min until a constant absorbance is obtained. Take the constant absorbance value as the test result.

The exposure of the reaction solution in light should be avoided as much as possible. The temperature of the solution should be maintained between 20 °C and 25 °C.

7.3.4.3 Absorbance determination of L-(+)-glutamic acid standard solution

Repeat the operations described in 7.3.3.3.1, but replace the L-(+)-glutamic acid test solution (7.3.3.6) with the L-(+)-glutamic acid series standard solution (7.1.10). Read the absorbance A_{s1} of the L-(+)-glutamic acid standard solution and A_{b2} of the blank solution. Then, repeat the operations described in 7.3.3.3.2. Read the absorbance A_{s1}' of the L-(+)-glutamic acid standard solution and A_{b2}' of the blank solution.

NOTE If 7.3.3.3 and 7.3.4.3 are detected at the same batch, the blank solution determination of 7.3.4.3 can be omitted. Take the value of A_{b1} for A_{b2} and A_{b1}' for A_{b2}' directly.

7.3.4.4 Moisture content determination of test sample

Determine the moisture content of the test sample in accordance with ISO 1442.

7.4 Calculation and results

7.4.1 Absorbance difference of L-(+)-glutamic acid standard solution

Calculate the absorbance difference for the L-(+)-glutamic acid standard solution by using Formula (3):

$$\Delta A_{s1} = \left(A_{s1}' - A_{s1}\right) - \left(A_{b2}' - A_{b2}\right) \quad (3)$$

where

ΔA_{s1} is the absorbance difference for the L-(+)-glutamic acid standard solution;

A_{s1} is the absorbance of the standard solution, measured in 7.3.4.3;

A_{s1}' is the absorbance of the standard solution, measured in 7.3.4.3;

A_{b2} is the absorbance of the blank solution, measured in 7.3.4.3;

A_{b2}' is the absorbance of the blank solution, measured in 7.3.4.3.

7.4.2 Absorbance difference of L-(+)-glutamic acid test solution

Calculate the absorbance difference for the L-(+)-glutamic acid test solution by using Formula (4):

$$\Delta A_1 = \left(A_1' - A_1\right) - \left(A_{b1}' - A_{b1}\right) \quad (4)$$

where

ΔA_1 is the absorbance difference for L-(+)-glutamic acid test solution;

A_1 is the absorbance of the test solution, measured in 7.3.3.3.1;

A_1' is the absorbance of the test solution, measured in 7.3.3.3.2;

A_{b1} is the absorbance of the blank solution, measured in 7.3.3.3.1;

A_{b1}' is the absorbance of the blank solution, measured in 7.3.3.3.2.

7.4.3 Formula of linear regression of L-(+)-glutamic acid standard curve

Take the concentration of the L-(+)-glutamic acid series standard solution (7.1.10) as the x-coordinate axis, and ΔA_{s1} (7.4.1) as the y-coordinate axis to draw the L-(+)-glutamic acid standard curve. The linear regression formula of the L-(+)-glutamic acid standard curve is shown by Formula (5):

$$y = a \times x + d \quad (5)$$

where

y is the absorbance difference for the L-(+)-glutamic acid series standard solution;

x is the concentration of the L-(+)-glutamic acid series standard solution, in milligram per litre;

a is the coefficient of the equation, round the numerical value to the nearest 0,000 1;

d is the coefficient of the formula.

7.4.4 L-(+)-glutamic acid concentration of the test solution

Calculate the L-(+)-glutamic acid concentration in the test solution (7.3.3.6) by using Formula (6):

$$c_1 = \frac{\Delta A_1 - d}{a} \quad (6)$$

where

c_1 is the numerical value of the concentration of L-(+)-glutamic acid test solution (7.3.3.6), in milligram per litre;

ΔA_1 is the absorbance difference for the L-(+)-glutamic acid test solution in 7.4.2;

A is the coefficient of the equation in 7.4.3;

D is the coefficient of the equation in 7.4.3.

7.4.5 L-(+)-glutamic acid content of test sample

Calculate the L-(+)-glutamic acid content of the test sample by using Formula (7):

$$W_1 = \frac{c_1}{10\,000 \times m_1} \times \frac{50 + \frac{W_m}{100} \times m_1}{\frac{V_1}{50} \times \frac{25}{50}} \tag{7}$$

where

W_1 is the numerical value of the L-(+)-glutamic acid content of the test sample, in grams per 100 g;

c_1 is the numerical value of the concentration of L-(+)-glutamic acid test solution (7.3.3.6), in milligram per litre;

V_1 is the numerical value of the volume, in millilitres, of filtrate taken in 7.3.3.6;

m_1 is the numerical value of the mass, in grams, of the test portion (7.3.2);

W_m is the numerical value of the moisture content (7.3.4.4), in grams per 100 g;

$\frac{V_1}{50}$ is the dilution factor of filtrate taken in 7.3.3.6;

$\frac{25}{50}$ "25" is the numerical value of the volume, in millilitres, of the filtrate transferred in 7.3.3.3 for pH adjustment and "50" is the numerical value of the volume, in millilitres, of the solution in 7.3.3.4.

Round the result to the nearest 0,01 g/100 g.

7.5 Precision

7.5.1 Interlaboratory test

The precision of the method was established by an interlaboratory test carried out in accordance with ISO 5725-1 and ISO 5725-2. The results of these tests have been published (see Reference [4]). The values derived from these tests may not be applicable to concentration ranges and matrices types other than those given.

7.5.2 Repeatability

The value less than or equal to which the absolute difference between two test results obtained under repeatability conditions can be expected to be 0,55 % with a probability of 95 % of cases exceeding 0,159 2 g/100 g up to 0,305 0 g/100 g for L-(+)-glutamic acid contents.

7.5.3 Reproducibility

The value less than or equal to which the absolute difference between two test results obtained under reproducibility conditions can be expected to be 0,82 % with a probability of 95 % of cases exceeding 0,159 2 g/100 g up to 0,305 0 g/100 g for L-(+)-glutamic acid contents.

7.6 Detection limit

When W_m (the moisture content of test sample in 7.3.4.4) is 70 and $V_1/50$ (the dilution factor of filtrate taken in 7.3.3.6) is 1/15, the detection limit is 0,02 g/100 g of L-(+)-glutamic acid contents.

8 Test method of light absorption microplate reader

8.1 Reagents

The reagents are the same as those used in 7.1.

8.2 Apparatus

In addition to the equipment from 7.2.1 to 7.2.9, the following apparatus should be also included.

8.2.1 Single channel or multi-channel transferring pipettes and tips, of capacities 1 000 µl, 200 µl, 100 µl and 20 µl conforming to ISO 8655-2.

8.2.2 Light absorption microplate reader, of wavelength 490 nm.

8.2.3 Microplate, of 96 holes (recommended), flat and transparent.

8.2.4 Brown glass vial, with caps, of volume not less than 1,5 ml.

8.3 Procedure

8.3.1 General

If it is required to check whether the repeatability requirement is achieved, two separate determinations should be carried out.

8.3.2 Extraction of L-(+)-glutamic acid in the test portion

The extraction method is the same as 7.3.2 and 7.3.3.

8.3.3 Determination

8.3.3.1 Preparation of detection instrument

Set up the light absorption microplate reader (8.2.2), preheat the instrument according to the instrument specification until equilibrium conditions achieved. Set the detection parameters: the detection wavelength of 490 nm, the microplate reading mode by row and by hole, and the reading time interval of approximately 0,5 s per hole.

8.3.3.2 Absorbance determination

8.3.3.2.1 Maintain the temperature of solution R123 (7.1.6), water, the L-(+)-glutamic acid series standard solution (7.1.10), the L-(+)-glutamic acid test solution (8.3.2) between 20 °C and 25 °C.

Pipette 300 µl of solution R123 (7.1.6) and 600 µl of water into a brown glass vial with caps (8.2.4). Mix by inverting or shaking the vial slowly. The solution obtained is the blank solution.

Pipette 300 µl of solution R123 (7.1.6), 540 µl of water and 60 µl of the L-(+)-glutamic acid test solution (8.3.2) into a brown glass vial (8.2.4). Mix by inverting or shaking the vial slowly. The solution obtained is the test solution.

Pipette 300 µl of solution R123 (7.1.6), 540 µl of water and 60 µl of the L-(+)-glutamic acid series standard solution (7.1.10) into a brown glass vial (8.2.4). Mix by inverting or shaking the vial slowly. The solution obtained is the standard solution.

8.3.3.2.2 Pipette 200 µl of the blank solution, the standard solution and the test solution (8.3.3.2.1) into different holes of the same microplate (8.2.3).

Put the microplate into the light absorption microplate reader and swing for 5 s to 10 s. Read the absorbance A_{b3} of the blank solution, A_{s2} of the L-(+)-glutamic acid series standard solution and A_2 of the L-(+)-glutamic acid test solution at a wavelength of 490 nm.

8.3.3.2.3 Pipette 10 µl of the GLDH solution (7.1.7) into each of the brown glass vials in 8.3.3.2.1. Mix by inverting or shaking the vial slowly.

Take the microplate out and pipette 200 µl of the solution from each brown glass vial into different holes. Each vial is recommended to be determined three times and the numerical average can be used as the test result.

Put the microplate into the light absorption microplate reader and swing for 5 s to 10 s. Read the absorbance A_{b3}' of the blank solution, A_{s2}' of the L-(+)-glutamic acid series standard solution and A_2' of the L-(+)-glutamic acid test solution at a wavelength of 490 nm after waiting for 10 min to 15 min, and keep reading the absorbance every 2 min thereafter until a constant absorbance is obtained. Take the constant absorbance value as the test result. If three determinations are performed, take the numerical average of the three constant absorbance as the result.

It should be pipetted or mixed slowly to avoid bubbles. The exposure of the reaction solution in light should be strictly avoided as much as possible. The temperature of the solution should be maintained between 20 °C and 25 °C.

8.3.3.3 Moisture content determination of test sample

The method for determining the moisture content is the same as 7.3.4.4.

8.4 Calculation and results

8.4.1 Absorbance difference for L-(+)-glutamic acid standard solution

Calculate the absorbance difference for the L-(+)-glutamic acid standard solution by using Formula (8):

$$\Delta A_{s2} = \left(A_{s2}' - A_{s2}\right) - \left(A_{b3}' - A_{b3}\right) \quad (8)$$

where

ΔA_{s2} is the absorbance difference for the L-(+)-glutamic acid series standard solution;

A_{b3} is the absorbance of the blank solution, measured in 8.3.3.2.2;

A_{b3}' is the absorbance of the blank solution, measured in 8.3.3.2.3;

A_{s2} is the absorbance of the standard solution, measured in 8.3.3.2.2;

A_{s2}' is the absorbance of the standard solution, measured in 8.3.3.2.3.

8.4.2 Absorbance difference for L-(+)-glutamic acid test solution

Calculate the absorbance difference of the L-(+)-glutamic acid test solution by using Formula (9):

$$\Delta A_2 = \left(A_2' - A_2\right) - \left(A_{b3}' - A_{b3}\right) \quad (9)$$

where

ΔA_2 is the absorbance difference for the L-(+)-glutamic acid test solution;

A_{b3} is the absorbance of the blank solution, measured in 8.3.3.2.2;

A_{b3}' is the absorbance of the blank solution, measured in 8.3.3.2.3;

A_2 is the absorbance of the L-(+)-glutamic acid test solution, measured in 8.3.3.2.2;

A_2' is the absorbance of the L-(+)-glutamic acid test solution, measured in 8.3.3.2.3.

8.4.3 Formula of linear regression for L-(+)-glutamic acid standard curve

Take the concentration of the L-(+)-glutamic acid series standard solution (7.1.10) as the x-coordinate axis, and ΔA_{s2} as the y coordinate axis to draw the L-(+)-glutamic acid standard curve. The linear regression equation of the L-(+)-glutamic acid standard curve is shown by Formula (10):

$$Y = A \times X + D \tag{10}$$

where

Y is the absorbance difference for the L-(+)-glutamic acid series standard solution;

X is the concentration of the L-(+)-glutamic acid series standard solution;

A is the coefficient of the equation, round the numerical value to the nearest 0,000 1;

D is the coefficient of the formula.

8.4.4 L-(+)-glutamic acid concentration of the test solution

Calculate the L-(+)-glutamic acid concentration in the test solution (8.3.2) by using Formula (11):

$$c_2 = \frac{\Delta A2 - D}{A} \tag{11}$$

where

c_2 is the numerical value of the concentration of L-(+)-glutamic acid test solution (8.3.2), in milligrams per litre;

ΔA_2 is the absorbance difference for the L-(+)-glutamic acid test solution in 8.4.2;

A is the coefficient of the equation in 8.4.3;

D is the coefficient of the equation in 8.4.3.

8.4.5 L-(+)-glutamic acid content of test sample

Calculate the L-(+)-glutamic acid content of the test sample by using Formula (12):

$$W_2 = \frac{c_2}{10\,000 \times m_1} \times \frac{50 + \frac{W_m}{100} \times m_1}{\frac{V_1}{50} \times \frac{25}{50}} \tag{12}$$

where