INTERNATIONAL STANDARD 4133

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Meat and meat products — Determination of glucono-delta-lactone content (Reference method)

Viandes et produits à base de viande — Détermination de la teneur en glucono-delta-lactone (Méthode de référence)

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FOREWORD

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International Standard ISO 4133 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in June 1977.

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

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No member body expressed disapproval of the document.

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Meat and meat products — Determination of glucono-delta-lactone content (Reference method)

1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a reference method for the determination of the glucono-delta-lactone 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

glucono-delta-lactone content of meat and meat products : The glucono-delta-lactone content determined according to the procedure described in this International Standard and expressed as a percentage by mass.

4 PRINCIPLE

Extraction of the glucono-delta-lactone present in a test portion with ice-cold perchloric acid solution. Centrifuging, decantation and filtration, followed by hydrolysis of the glucono-delta-lactone in a portion of the filtrate with potassium hydroxide, into gluconate.

Transformation of the gluconate in the extract by the following reactions 1), with adenosine-5-triphosphate (ATP), and 2), with concomitant reduction of an equivalent amount of nicotinamide adenine dinucleotide phosphate (NADP) :

1) $\text{gluconate} + \text{ATP} \xrightarrow[\text{kinase}]{\text{gluconate}} \text{6-phosphogluconate} + \text{ADP}$

2) $\text{6-phosphogluconate} + \text{NADP}^+ \xrightarrow[\text{dehydrogenase}]{\text{6-phosphogluconate}}$

$\text{NADPH} + CO_2 + H^+ + \text{ribulose-5-phosphate}$

Photometric measurement of the amount of nicotinamide adenine dinucleotide phosphate (reduced) (NADPH) 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, 0,4 M.

Dilute 17,3 ml of perchloric acid, 70 % (*m/m*), ρ_{20} 1,67 g/ml, to 500 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 Buffer solution

Dissolve 2,64 g of glycylglycine ($C_6H_{11}N_2O_3$) and 0,284 g of magnesium chloride hexahydrate ($MgCl_2.6H_2O$) in 150 ml of water. Adjust the pH to 8,0 with the potassium hydroxide solution (5.2), using a pH meter. Dilute to 200 ml with water.

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

5.4 Nicotinamide adenine dinucleotide phosphate (NADP) solution.

Weigh 50 mg of nicotinamide adenine dinucleotide phosphoric acid disodium salt ($NADP\text{-}Na_2$) in a small, stoppered flask and add 5,0 ml of water.

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

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5.5 Adenosine-5-triphosphate (ATP) solution.

Weigh 250 mg of adenosine-5-triphosphoric acid disodium salt (ATP-Na_2) and 250 mg of sodium hydrogen carbonate ($NaHCO_3$) in a small, stoppered flask and add 5,0 ml of water.

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

5.6 6-Phosphogluconate dehydrogenase (6-PGDH) (EC* 1.1.1.44) suspension, containing 2,0 mg of 6-PGDH from yeast per millilitre and not more than 0,05 % each of gluconate kinase and reduced form of nicotinamide adenine dinucleotide phosphoric acid oxidase.

This suspension is supplied as such and can be kept for at least 6 months at 4 °C.

5.7 Gluconate kinase (GK) (EC* 2.7.1.12) suspension, containing 1,0 mg of GK from *E. Coli* per millilitre, and not more than 0,05 % of reduced form of nicotinamide adenine dinucleotide phosphoric acid oxidase.

This suspension is supplied as such and can be kept for at least 6 months at 4 °C.

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 200 ml, complying with ISO 1042, class A.

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

6.8 Graduated pipettes for delivering 2,5 — 0,2 — 0,1 — 0,05 and 0,01 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 365 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

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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.

8.3.2 Transfer a part of the homogenate to a centrifuge tube. 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).

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

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

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

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 20 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 D-(+)-gluconate is less than 400 mg/l.

8.4 Determination

8.4.1 Pipette into each of two photometric cells (6.11) 2,50 ml of the buffer solution (5.3), 0,10 ml of the NADP solution (5.4), and 0,10 ml of the ATP solution (5.5).

Into one of the cells pipette 0,20 ml of the extract (8.3.6); the solution obtained is the test solution.

Into the other cell pipette 0,20 ml of water; the solution obtained is the blank solution.

Pipette 0,05 ml of the 6-PGDH suspension (5.6) on the plastic spatula (6.9). Mix thoroughly 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 365 nm against air after 5 min.

Note the absorbance as :

A_1 = absorbance of the test solution;

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

8.4.2 Pipette 0,01 ml of the GK suspension (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 365 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.

8.5 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 glucono-delta-lactone content of the sample, expressed as a percentage by mass, using the formula

$$0{,}908\,\Delta A \times \frac{2{,}96 \times 196{,}1}{\kappa \times 0{,}2 \times 1\,000} \times \frac{100}{1\,000} \times \frac{100}{V} \times \frac{\left(100 + \frac{M \times m}{100}\right)}{50} \times \frac{100}{m}$$

$$= 52{,}705 \times \frac{\Delta A}{\kappa \times V \times m} \times \left(100 + \frac{M \times m}{100}\right)$$

where

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

196,1 is the relative molecular mass of D-(+)-gluconic acid;

κ = 3,5 $cm^2/\mu mol$ at 365 nm and 6,23 $cm^2/\mu mol$ at 340 nm;

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 glucono-delta-lactone 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 NOTES ON PROCEDURE

10.1 Measurements may also be performed at 340 nm.

10.2 Inorganic salts (phosphate, sodium, potassium and ammonium ions) can retard the enzymatic reactions. In such cases more enzyme should be added. Normally, however, for meat products with added phosphate, there is no need to increase the amount of enzyme added.

10.3 The stability of the enzymes is mostly guaranteed by the producer until the expiration date mentioned on the label.

Enzyme preparations and buffer solution should be stored in a refrigerator. The enzyme solutions should also be kept

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cool on the laboratory bench. This can be achieved by ice-cooling of a metal block provided with holes, in which the flasks with enzyme solutions can be placed.

11 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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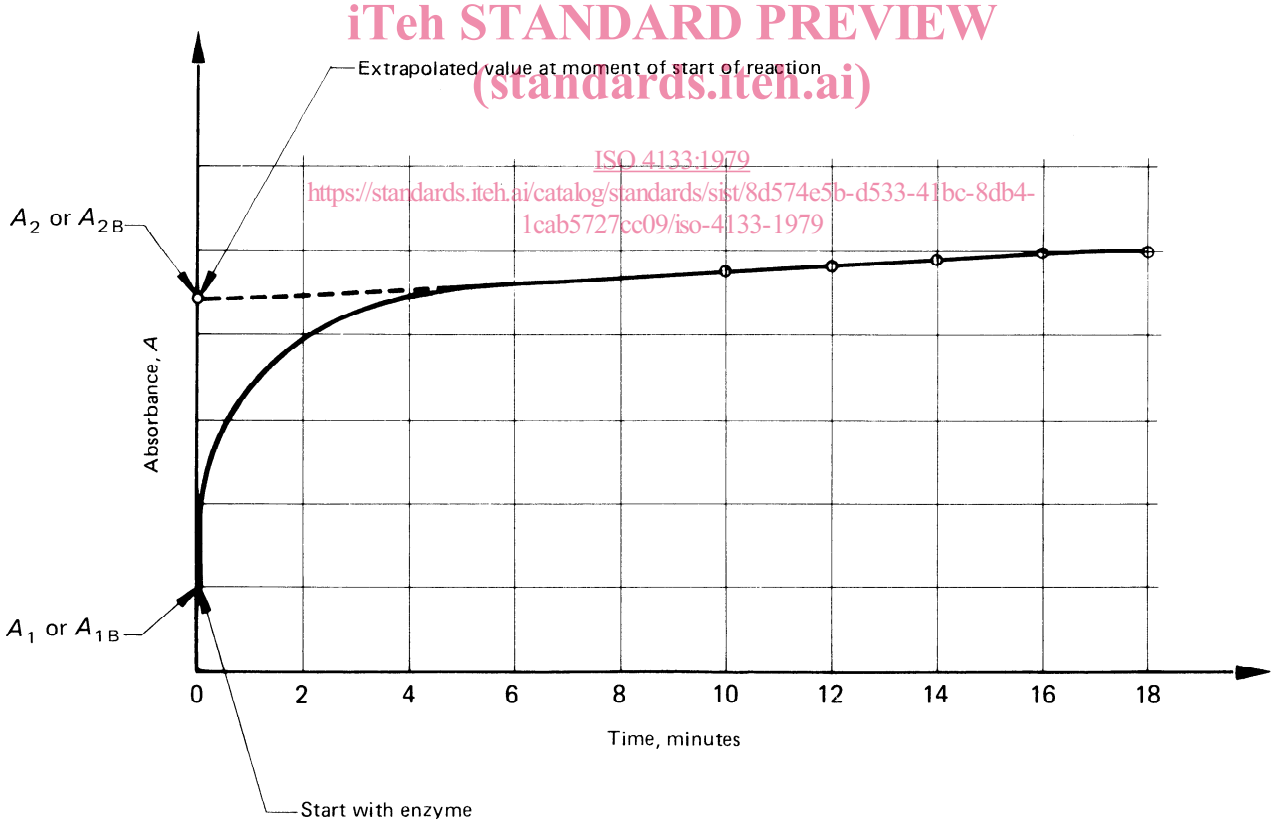

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