INTERNATIONAL STANDARD ISO 5554

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Meat products — Determination of starch content (Reference method)

Produits à base de viande — Détermination de la teneur en amidon (Méthode de référence)

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FOREWORD

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Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 5554 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in September 1976.

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

Australia	Germany	Portugal
Austria	India	Romania
Bulgaria	Ireland	South Africa, Rep. of
Chile	Korea, Rep. of	Spain
Czechoslovakia	Netherlands	United Kingdom
Egypt, Arab Rep. of	New Zealand	U.S.S.R.
France	Poland	Yugoslavia

No member body expressed disapproval of the document.

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Meat products — Determination of starch content (Reference method)

1 SCOPE

This International Standard specifies a reference method for the determination of the starch content of meat products.

2 FIELD OF APPLICATION

This International Standard applies only to products which do not contain added substances, other than starch, that yield reducing sugars on hydrolysis.

3 REFERENCE

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

4 DEFINITION

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

5 PRINCIPLE

Heating of a test portion with ethanolic potassium hydroxide solution until the meat components are totally dissolved. Decantation, washing of the remaining residue with hot ethanol, filtering, dissolution in hydrochloric acid, and hydrolysis. Titrimetric determination of the glucose formed.

6 REAGENTS

All reagents shall be of recognized analytical quality. The water used shall be distilled water or water of at least equivalent purity.

6.1 Potassium hydroxide, ethanolic solution.

Dissolve 50 g of potassium hydroxide in 800 ml of 95 % (*V/V*) ethanol and dilute to 1 000 ml with the same ethanol.

6.2 Ethanol, 80 % (*V/V*).

6.3 Hydrochloric acid, 1,0 M solution (chlorine-free).

6.4 Bromothymol blue, 10 g/l solution in 95 % (*V/V*) ethanol.

6.5 Sodium hydroxide, 300 g/l solution.

6.6 Solutions for precipitation of proteins.

Solution I

Dissolve 106 g of potassium hexacyanoferrate(II) trihydrate $[K_4Fe(CN)_6.3H_2O]$ in water in a 1 000 ml one-mark volumetric flask and dilute to the mark.

Solution II

Dissolve 220 g of zinc acetate dihydrate $[Zn(CH_3COO)_2.2H_2O]$ in water in a 1 000 ml one-mark volumetric flask. Add 30 ml of glacial acetic acid, and dilute to the mark with water.

6.7 Copper reagent

Prepare the following solutions :

a) 25 g of copper(II) sulphate pentahydrate ($CuSO_4.5H_2O$) in 100 ml of water;

b) 144 g of sodium carbonate (Na_2CO_3) in 300 to 400 ml of water at 50 °C;

c) 50 g of citric acid monohydrate ($C_6H_8O_7.H_2O$) in 50 ml of water.

Add solution c) slowly and carefully, stirring continuously, to solution b). Continue stirring until evolution of carbon dioxide ceases.

Add solution a) to this mixture, stirring continuously.

Allow to cool to room temperature, transfer quantitatively to a 1 000 ml one-mark volumetric flask, dilute to the mark and filter after 24 h.

The pH of the solution, after 1 + 49 dilution with freshly boiled and cooled water, should be 10,0 ± 0,1.

6.8 Starch indicator solution.

Add a mixture of 10 g of soluble starch, 10 mg of mercury(II) iodide (as a preservative) and 30 ml of water to 1 litre of boiling water. Continue boiling for 3 min and cool.

6.9 Sodium thiosulphate, approximately 0,1 N standard volumetric solution.

6.9.1 *Preparation*

Dissolve, in 1 000 ml of freshly boiled and cooled water, 25 g of sodium thiosulphate pentahydrate ($Na_2S_2O_3.5H_2O$) and add 0,2 g of sodium carbonate decahydrate ($Na_2CO_3.10H_2O$). Allow the solution to stand for one day before standardizing.

6.9.2 *Standardization*

Weigh 150,0 mg of dried potassium iodate, dissolve it in 25 ml of water and add 2 g of potassium iodide and 10 ml of the hydrochloric acid solution (6.3).

Titrate with the solution thiosulphate solution while stirring continuously. Add 1 ml of the starch indicator solution (6.8) when the solution has become pale yellow, and continue the titration until the blue colour disappears. The normality T of the sodium thiosulphate solution is then calculated from the formula :

$$T = \frac{6\,m}{214{,}0\,V}$$

where

m is the mass, in milligrams, of the potassium iodate;

V is the volume, in millilitres, of the sodium thiosulphate solution added to the potassium iodate solution;

$\frac{214{,}0}{6}$ is the relative molecular mass of potassium iodate.

6.10 Potassium iodide, 100 g/l solution.

Dissolve 10 g of potassium iodide in water, and dilute to 100 ml. Store the solution in a dark brown bottle.

6.11 Hydrochloric acid, 25 % (*m/m*) solution (chlorine-free).

Dilute 100 ml of concentrated, chlorine-free hydrochloric acid (ρ_{20} 1,19 g/ml) with 60 ml of water.

7 APPARATUS

Usual laboratory apparatus not otherwise specified, and in particular :

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

7.2 Boiling water bath.

7.3 Fluted filter paper, diameter 15 cm, starch-free.

7.4 Asbestos plate with a circular hole, fitting the bottom of the conical flask (7.5).

7.5 Conical flask, capacity 250 to 300 ml, with ground neck, and provided with a glass stopper.

7.6 Condenser, air-cooled, with conical joint fitting the conical flask (7.5).

7.7 Boiling aids (for example pumice stone or glass beads).

7.8 Burette, capacity 50 ml, complying with class A of ISO/R 385.

7.9 pH meter.

8 SAMPLE

8.1 Proceed from a representative sample of at least 200 g. See ISO 3100.

8.2 Store the sample, if necessary, in such a way that deterioration and change in composition are prevented.

9 PROCEDURE

9.1 Preparation of the test sample

Homogenize the sample by passing it at least twice through the meat mincer (7.1) and mixing. Keep it in a completely filled, air-tight, closed container and store it, if necessary, in such a way that deterioration and change in composition are prevented. Analyse the sample as soon as possible after homogenization, but always within 24 h.

9.2 Test portion

Weigh into a 500 or 600 ml beaker, to the nearest 0,1 g, about 25 g of the test sample (9.1). If the mass of starch in this test portion is expected to be more than 1 g, reduce the mass of the test portion accordingly.

9.3 Isolation of starch

Add to the test portion, while stirring with a glass rod, 300 ml of hot ethanolic potassium hydroxide solution (6.1) and cover the beaker with a watch glass. Heat on the boiling water bath (7.2) for 1 h, stirring occasionally. Decant the solution through a filter paper (7.3) and then wash the starch quantitatively on the filter paper using hot ethanol (6.2) and with the aid of a rubber-tipped glass rod. Keep the filter moist.

NOTE — In some cases, centrifuging may be more advantageous than filtration.

9.4 Hydrolysis

Immediately loosen the precipitate from the paper by means of a glass rod. Pierce a hole in the filter paper and wash the starch through it into a 250 ml beaker, using 100 ml of hot hydrochloric acid solution (6.3). Cover the beaker with a watch glass and immerse it in the boiling water bath for 2,5 h, stirring the solution occasionally with a glass rod.

Cool the solution and neutralize by adding the sodium hydroxide solution (6.5) drop by drop, taking care that the pH does not exceed 6,5; check this with the pH meter (7.9).

Transfer the mixture quantitatively into a 200 ml volumetric flask, washing with water, add 3 ml of Solution I (6.6) and, after mixing, 3 ml of Solution II (6.6) and dilute to the mark.

Mix and filter through a fluted filter paper (7.3). Immediately before pipetting an aliquot portion for the next stage, make the filtrate alkaline to bromothymol blue (6.4) by adding 1 or 2 drops of the sodium hydroxide solution (6.5).

9.5 Determination of glucose

If the approximate starch content of the sample is unknown, carry out a preliminary trial analysis to estimate it.

Dilute an aliquot portion (V_2) of the filtrate (9.4) with water to a known volume (V_3) so that 25 ml of the diluted solution contain preferably 40 to 50 mg of glucose and in no circumstances more than 60 mg of glucose.

Mix and pipette 25,0 ml of the diluted solution into the conical flask (7.5). Pipette 25,0 ml of the copper reagent (6.7) into the flask and add some boiling aids (7.7).

NOTE — It is essential that the total volume of liquid at this stage is always 50,0 ml.

Fit the condenser (7.6) to the flask. Place the flask and condenser on a metal wire gauze surmounted by the asbestos plate (7.4).

Bring the liquid to the boil over a gas flame in about 2 min and continue to boil gently for exactly 10 min. Then cool quickly to room temperature. Remove the condenser and add 30 ml of the potassium iodide solution (6.10) and next, carefully but as quickly as possible, 25 ml of the hydrochloric acid solution (6.11). Stopper the flask until titration.

Titrate the liberated iodine with the standard volumetric sodium thiosulphate solution (6.9). When the solution has become pale yellow, add about 1 ml of the starch indicator solution (6.8) and continue the titration until the blue colour disappears.

9.6 Blank determination

Carry out a blank determination, following the same procedure as in 9.5, taking 25,0 ml of water instead of 25,0 ml of the diluted filtrate.

10 EXPRESSION OF RESULTS

10.1 Calculation and formulae

Calculate the difference between the volumes noted in the two titrations, expressed in millilitres of exactly 0,1 N sodium thiosulphate solution, from the formula

$$10\,T \times (V_0 - V_1)$$

where

T is the normality of the standard volumetric sodium thiosulphate solution (see 6.9.2);

V_0 is the volume, in millilitres, of the standard volumetric sodium thiosulphate solution (6.9) needed for the blank determination (9.6);

V_1 is the volume, in millilitres, of the standard volumetric sodium thiosulphate solution (6.9) needed for the diluted filtrate (9.5).

Calculate the starch content, as a percentage by mass, from the formula

$$\frac{m_1}{1\,000} \times 0{,}9 \times \frac{V_3}{25} \times \frac{200}{V_2} \times \frac{100}{m_0} = 0{,}72 \times \frac{V_3}{V_2} \times \frac{m_1}{m_0}$$

where

V_2 is the volume, in millilitres, of the undiluted aliquot portion (see 9.5);

V_3 is the volume, in millilitres, of the diluted aliquot portion (see 9.5);

m_0 is the mass, in grams, of the test portion (9.2);

m_1 is the mass, in milligrams, of glucose as determined from the expression $10\,T \times (V_0 - V_1)$ by reference to the table (page 4) or the graph (page 5);

0,9 is the factor for conversion of the mass of glucose m_1 to the corresponding mass of starch.

Report the result to the nearest 0,1 %.

10.2 Precision

10.2.1 *Repeatability*

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst shall not be greater than 0,2 g of starch per 100 g of sample.

10.2.2 *Reproducibility*

The difference between the results of two determinations carried out in two laboratories on the same sample shall not exceed 0,3 g of starch per 100 g of sample.

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 required for complete identification of the sample.

TABLE – **Conversion of millilitres of 0,1 N sodium thiosulphate solution to milligrams of glucose**

$10\,T \times (V_0 - V_1)$	Corresponding mass of glucose	
ml of 0,1 N sodium thiosulphate solution	m_1	Δm_1
	mg	mg
1	2,4	
		2,4
2	4,8	
		2,4
3	7,2	
		2,5
4	9,7	
		2,5
5	12,2	
		2,5
6	14,7	
		2,5
7	17,2	
		2,6
8	19,8	
		2,6
9	22,4	
		2,6
10	25,0	
		2,6
11	27,6	
		2,7
12	30,3	
		2,7
13	33,0	
		2,7
14	35,7	
		2,8
15	38,5	
		2,8
16	41,3	
		2,9
17	44,2	
		2,9
18	47,1	
		2,9
19	50,0	
		3,0
20	53,0	
		3,0
21	56,0	
		3,1
22	59,1	
		3,1
23	62,2	

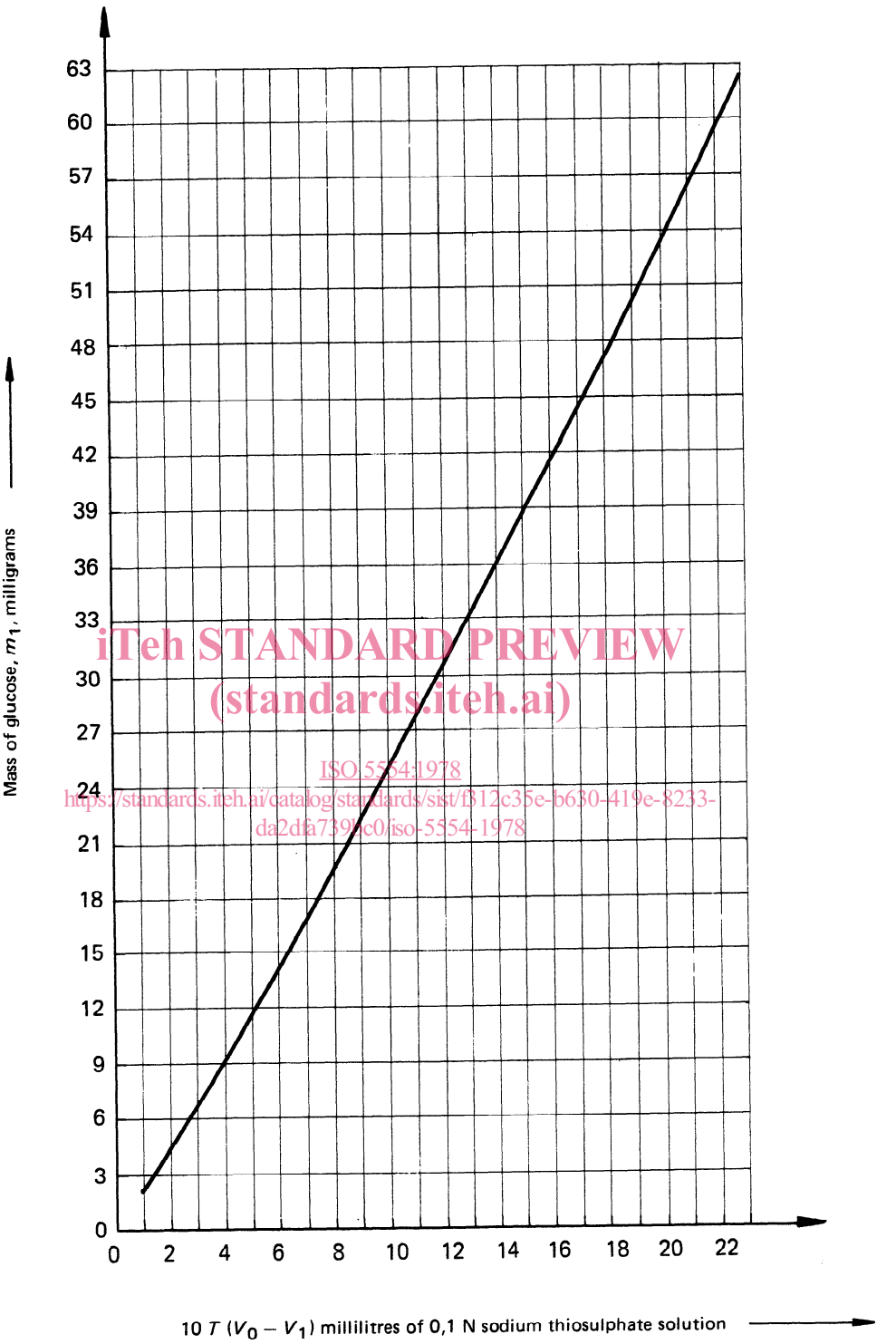

FIGURE – **Graph for conversion of millilitres of 0,1 N sodium thiosulphate solution to milligrams of glucose**

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