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**Meat and meat products — Determination  
of starch and glucose contents —  
Enzymatic method**

*Viande et produits à base de viande — Détermination des teneurs en  
amidon et en glucose — Méthode enzymatique*



## Foreword

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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 13965 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 6, *Meat and meat products*.

Annexes A and B of this International Standard are for information only.

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# Meat and meat products — Determination of starch and glucose contents — Enzymatic method

## 1 Scope

This International Standard specifies an enzymatic method for the determination of water-free starch content and glucose content of all kinds of meat and meat products, including poultry.

The method is suitable for the quantitative determination of starch and glucose contents down to levels of 0,30 % (*m/m*).

The method is not applicable for chemically modified starches or their derivatives.

## 2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the edition indicated was valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*.

## 3 Definitions

For the purposes of this International Standard, the following definitions apply.

### 3.1

#### **starch content of meat and meat products**

starch content determined in accordance with the procedure described in this International Standard, and expressed as a percentage by mass

### 3.2

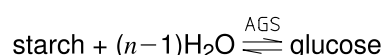
#### **glucose content of meat and meat products**

glucose content determined in accordance with the procedure described in this International Standard, and expressed as a percentage by mass

## 4 Principle

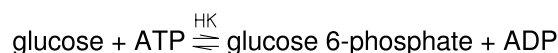
**4.1** Hydrolysis of the starch present in a test portion with the enzyme  $\alpha$ -amylase at pH = 5,0 for 15 min. Determination of the starch content using the following enzymatic reactions.

#### 4.2 Hydrolysis of the solubilized starch to yield glucose using amyloglucosidase (AGS):

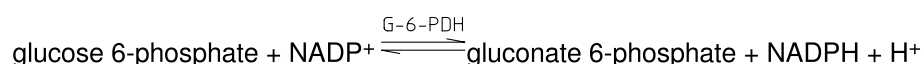


For the determination of the glucose content, this step is omitted.

#### 4.3 Phosphorylation of the glucose generated by means of adenosine 5'-triphosphate (ATP) to yield glucose 6-phosphate (G-6-P) using hexokinase (HK):



#### 4.4 Oxidation of glucose 6-phosphate (G-6-P) by means of nicotinamide adenine dinucleotide phosphate (NADP) to gluconate 6-phosphate using glucose 6-phosphate dehydrogenase (G-6-PDH):



#### 4.5 Spectrometric measurement of the amount of reduced nicotinamide dinucleotide phosphate (NADPH) at a wavelength of 340 nm.

## 5 Reagents

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

#### 5.1 **Water**, complying with at least grade 3 in accordance with ISO 3696.

#### 5.2 **$\alpha$ -Amylase (EC 3.2.1.1)**, enzyme suspension.

A liquid enzyme preparation of a heat-stable  $\alpha$ -amylase produced from *Bacillus licheniformis* (Termamyl® 120L)<sup>1)</sup>.

#### 5.3 **Sodium hydroxide solution**, $c(\text{NaOH}) = 5 \text{ mol/l}$ .

Dissolve 200 g of sodium hydroxide in water. Cool to room temperature, dilute to 1000 ml and mix.

#### 5.4 **Sodium hydroxide solution**, $c(\text{NaOH}) = 0,5 \text{ mol/l}$ .

Dissolve 20 g of sodium hydroxide in water. Cool to room temperature, dilute to 1000 ml and mix.

#### 5.5 **Ammonium sulfate solution**, $c[(\text{NH}_4)_2\text{SO}_4] = 3,2 \text{ mol/l}$ .

Dissolve 422 g of ammonium sulfate in water. Dilute to 1000 ml and mix.

#### 5.6 **Acetate buffer**, $c(\text{CH}_3\text{CO}_2\text{Na}) = 0,1 \text{ mol/l}$ , pH = 5,0.

Dissolve 6,80 g of sodium acetate trihydrate ( $\text{CH}_3\text{CO}_2\text{Na} \cdot 3\text{H}_2\text{O}$ ) in 400 ml of water. Adjust the pH to 5,0 with hydrochloric acid or sodium hydroxide solution with a pH-meter (6.2). Dilute with water to 500 ml and mix.

The solution is stable for at least 3 months at +4 °C in the dark.

#### 5.7 **Citrate buffer**, $c(\text{citrate}) = 0,05 \text{ mol/l}$ , pH = 4,6.

Dissolve 440 mg of citric acid monohydrate ( $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$ ) and 850 mg of trisodium citrate dihydrate ( $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$ ) in water. Dilute with water to 100 ml and mix. Check the pH with a pH-meter (6.2) and adjust if necessary with hydrochloric acid or sodium hydroxide solution.

The solution is stable for at least 3 months at +4 °C in the dark.

1) Termamyl® 120 L is an example of a suitable product available commercially from Novo, Denmark, and Tecator, Sweden. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

**5.8 Triethanolamine buffer**,  $c(\text{triethanolamine}) = 0,75 \text{ mol/l}$ ,  $\text{pH} = 7,6$ .

Dissolve 14,0 g of triethanolamine hydrochloride ( $\text{C}_6\text{H}_{15}\text{NO}_3\cdot\text{HCl}$ ) and 0,25 g of magnesium sulfate heptahydrate ( $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ ) in 80 ml of water. Set the pH at 7,6 with a pH-meter, using the sodium hydroxide solutions (5.3 and 5.4). Dilute with water to 100 ml and mix.

The solution is stable for 4 weeks at  $+4^\circ\text{C}$  in the dark.

**5.9 Nicotinamide adenine dinucleotide phosphate solution**,  $c(\beta\text{-NADP-Na}_2) = 12,7 \times 10^{-3} \text{ mol/l}$ .

Dissolve 100 mg of NADP disodium salt in 10,0 ml of water and mix.

The solution is stable for 4 weeks at  $+4^\circ\text{C}$  in the dark.

**5.10 Adenosine-5'-triphosphate solution**,  $c(5'\text{-ATP-Na}_2\text{H}_2\cdot 3\text{H}_2\text{O}) \approx 81 \times 10^{-3} \text{ mol/l}$ .

Dissolve 500 mg of 5'-ATP- $\text{Na}_2\text{H}_2\cdot 3\text{H}_2\text{O}$  and 500 mg of anhydrous monosodium hydrogen carbonate ( $\text{NaHCO}_3$ ) in 10,0 ml of water and mix.

The solution is stable for 4 weeks at  $+4^\circ\text{C}$  in the dark.

**5.11 Amyloglucosidase (AGS; EC 3.2.1.3)**, enzyme suspension in ammonium sulfate solution (5.5),  $\rho(\text{AGS}) = 10 \text{ mg/ml}$ .

The specific activity of the enzyme shall be 14 units per milligram.

The suspension is stable for 1 year at  $+4^\circ\text{C}$  in the dark.

**5.12 Hexokinase (HK; EC 2.7.1.1)/glucose 6-phosphate dehydrogenase (G-6-PDH; EC 1.1.1.49)**, enzyme suspension in ammonium sulfate solution (5.5),  $\rho(\text{HK}) = 2 \text{ mg/ml}$  and  $\rho(\text{G-6-PDH}) = 1 \text{ mg/ml}$ .

The specific activity of both enzymes shall be 140 units per milligram.

The suspension is stable for 1 year at  $+4^\circ\text{C}$  in the dark.

## 6 Apparatus

Usual laboratory apparatus 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 pH-meter.****6.3 Fluted filter papers**, glucose free, of diameter about 15 cm.**6.4 Pipettes**, calibrated, for enzymatic analysis, or automatic micropipettes of equivalent quality with the following volumes: 20  $\mu\text{l}$ , 50  $\mu\text{l}$ , 100  $\mu\text{l}$  and 200  $\mu\text{l}$ .**6.5 Small plastics spatula**, for mixing the contents of a cuvette (6.9).**6.6 Water bath**, capable of being maintained at  $(60 \pm 2)^\circ\text{C}$ .**6.7 Hot plate.**