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**Milled cereal products — Determination of  
fat acidity**

*Produits de mouture des céréales — Détermination de l'acidité grasse*

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[ISO 7305:1998](https://standards.iteh.ai/catalog/standards/sist/0dec609f-84c0-40d5-9992-67070c92de63/iso-7305-1998)

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## 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 7305 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 4, *Cereals and pulses*.

This second edition cancels and replaces the first edition (ISO 7305:1986) which has been technically revised.

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

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## Introduction

This International Standard describes a method of estimating the quantity of long-chain, non-esterified fatty acids which are liberated by the action of lipase during the storage of milled cereal products. It therefore provides a sensitive and significant test to characterize the state of conservation and the utilization values of these products.

The solvent used for the extraction, 95 % ethanol, breaks all the low-energy links where fatty acids are involved, and solubilizes the latter rapidly and quantitatively, with the exclusion of the major part of amino acids and mineral salts.

Observation of the colour change at the endpoint of the titration is facilitated by the absence of turbidity in the solution and by the use of a filter that eliminates the yellow coloration of the extract.

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# Milled cereal products — Determination of fat acidity

## 1 Scope

This International Standard specifies a method for the determination of the "fat acidity" of milled cereal products. It is applicable to flours and semolinas obtained from wheat and durum wheat, and also to pasta.

NOTE This method appears to be applicable also to grains, to flours and semolinas obtained from maize, and to rye flour and oat flakes, but a further interlaboratory test is necessary before confirming this extension of the field of application.

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## 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 712:—1), *Cereals and cereal products — Determination of moisture content — Routine reference method*.

## 3 Definition

For the purposes of this International Standard, the following definition applies.

### 3.1

#### fat acidity

conventional term used to express the quantity of acids, essentially non-esterified fatty acids, extracted according to the procedure described in this International Standard

NOTE Fat acidity is expressed in milligrams of potassium hydroxide per 100 g of dry matter. It can also be expressed in milligrams of sodium hydroxide per 100 g of dry matter (see clause 11).

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1) To be published. (Revision of ISO 712:1985)

## 4 Principle

Dissolution of the acids in ethanol at room temperature, followed by centrifuging and titration of an aliquot portion of the supernatant liquid against sodium hydroxide.

Conversion by calculation of the obtained result to express the result with reference to potassium hydroxide.

## 5 Reagents

Use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

### 5.1 Ethanol, 95 % (V/V).

### 5.2 Sodium hydroxide, standard volumetric solution, $c(\text{NaOH}) = 0,05 \text{ mol/l}$ , in 95 % (V/V) ethanol, free of carbonates.

The exact concentration shall be known and checked immediately prior to each series of determinations of fat acidity.

Use a solution prepared at least 5 days in advance and stored in a brown glass bottle, fitted with a rubber stopper. The solution shall be colourless or straw coloured.

If a commercially available solution is not used, it is recommended that the ethanol be purified as follows. Dissolve 5 g to 10 g of sodium hydroxide in 1 l of ethanol and add 0,5 g of aluminium turnings. Boil the mixture under reflux for 1 h, then distil the ethanol. Dissolve the required quantity of sodium hydroxide (i.e. to give a concentration of 2 g/l) in the distillate. Leave to stand for 5 days in order to allow the insoluble sodium carbonate to settle out, then use the supernatant solution.

### 5.3 Phenolphthalein, indicator solution, 1 g per 100 ml of 95 % (V/V) ethanol (5.1).

## 6 Apparatus

Usual laboratory apparatus and, in particular, the following.

### 6.1 Sieves, of wire gauze, of nominal aperture size 1 mm (for flour, if necessary), and 160 $\mu\text{m}$ and 500 $\mu\text{m}$ (for semolina and pasta).

### 6.2 Centrifuge tubes, of borosilicate or neutral glass, of capacity 45 ml, hermetically stoppered.

### 6.3 Centrifuge, capable of a centrifugal acceleration of 2000 $g$ .

### 6.4 Pipettes, of capacities 20 ml and 30 ml.

### 6.5 Conical flask, of capacity 250 ml.

### 6.6 Microburette, graduated in 0,01 ml divisions.

### 6.7 Rotary stirrer, capable of 30 r/min to 60 r/min.

### 6.8 Analytical balance, capable of weighing to an accuracy of $\pm 0,01 \text{ g}$ .

### 6.9 Grinder, capable of grinding without any appreciable heating (for semolina and pasta).

### 6.10 Orange filter, photographic-type cellulose acetate filter, blue absorbing (wavelength 440 nm).

## 7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 13690.

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport and storage.

Acidity increases during storage, therefore the samples shall be stored in sealed bottles at about 4 °C. Allow the sample to return to laboratory temperature in the sealed bottle before taking test portions.

## 8 Preparation of test sample

**8.1** In the case of flour which completely passes a sieve of aperture size 500 µm (6.1) and at least 80 % (*m/m*) passes a sieve of aperture size 160 µm (6.1), take about 50 g of the flour and sift it if necessary, using a sieve of aperture size 1 mm (6.1) so as to break up any lumps present. Mix well before taking the test portion.

**8.2** For other flours and for semolina and pasta, grind about 50 g in the grinder (6.9) until the particle size characteristics specified in 8.1 are achieved. Mix well before taking the test portion.

## 9 Determination of moisture content of the test sample

Determine the moisture content of the test sample in accordance with ISO 712.

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## 10 Procedure

ISO 7305:1998

NOTE If it is required to check whether the repeatability limit (12.2) is met, carry out two single determinations in accordance with 10.1 and 10.2.

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### 10.1 Test portion

Weigh, to the nearest 0,01 g, approximately 5 g of the test sample (clause 8) and place it in a centrifuge tube (6.2).

### 10.2 Determination

**10.2.1** Using a pipette (6.4), transfer 30 ml of the ethanol (5.1) into the centrifuge tube (6.2). Seal the tube hermetically and agitate for 1 h using the rotary stirrer (6.7), working at a temperature of 20 °C ± 5 °C. Then remove the stopper and centrifuge (6.3) for 5 min with an acceleration of 2000 *g*.

**10.2.2** Transfer, by means of a pipette (6.4), 20 ml of the supernatant liquid to a conical flask (6.5). Add 5 drops of phenolphthalein (5.3).

Titrate the solution, using the microburette (6.6), with the sodium hydroxide solution (5.2) until a pale pink colour lasting approximately 3 s appears, using an orange filter (6.10) to eliminate the yellow coloration at the colour change of the indicator. The use of an orange filter, placed to the operator's eye, allows observation of the colour change of the indicator with a greater precision by eliminating the yellow coloration of the ethanolic extract.

### 10.3 Blank test

Carry out a blank test in parallel with the determination, beginning at 10.2.2 and replacing the 20 ml of supernatant liquid by 20 ml of ethanol (5.1).

## 11 Expression of results

### 11.1 Calculation of fat acidity as potassium hydroxide

The fat acidity,  $A_K$ , expressed in milligrams of potassium hydroxide per 100 g of dry matter, is obtained by the following equation:

$$A_K = \frac{8\,415 (V_1 - V_0) c}{m} \times \frac{100}{100 - w}$$

where

- $c$  is the exact concentration, expressed in moles per litre, of the standard volumetric sodium hydroxide solution used;
- $m$  is the mass, in grams, of the test portion (10.1);
- $V_1$  is the volume, in millilitres, of the sodium hydroxide solution used in the determination (10.2)
- $V_0$  is the volume, in millilitres, of the sodium hydroxide solution used in the blank test (10.3)
- $w$  is the moisture content, expressed as a percentage by mass, of the test sample (clause 9);
- 8 415 is a constant to be applied for potassium hydroxide, i.e.  $(56,1 \times 1,5 \times 100)$ .

Express the result to the nearest milligram.

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### 11.2 Calculation of fat acidity as sodium hydroxide

The fat acidity,  $A_{Na}$ , expressed in milligrams of sodium hydroxide per 100 g of dry matter, is obtained by the following equation:

$$A_{Na} = \frac{6\,000 (V_1 - V_0) c}{m} \times \frac{100}{100 - w}$$

where

- $c$  is the exact concentration, expressed in moles per litre, of the standard volumetric sodium hydroxide solution used;
- $m$  is the mass, in grams, of the test portion (10.1);
- $V_1$  is the volume, in millilitres, of the sodium hydroxide solution used in the determination (10.2);
- $V_0$  is the volume, in millilitres, of the sodium hydroxide solution used in the blank test (10.3);
- $w$  is the moisture content, expressed as a percentage by mass, of the test sample (clause 9);
- 6 000 is a constant to be applied for sodium hydroxide, i.e.  $(40 \times 1,5 \times 100)$ .

Express the result to the nearest milligram.

### 11.3 Conversion of the results

**11.3.1** To convert the results expressed as potassium hydroxide to results expressed as sodium hydroxide, multiply the result obtained in 11.1 by 0,7130.



**11.3.2** To convert the result expressed as sodium hydroxide to results expressed as potassium hydroxide, multiply the result obtained in 11.2 by 1,4025.

## 12 Precision

### 12.1 Interlaboratory tests

Details of interlaboratory tests on the precision of the method are summarized in annex A. The values derived from these interlaboratory tests may not be applicable to concentration ranges and matrices other than those given.

#### 12.1 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than 4 mg of potassium hydroxide or 3 mg of sodium hydroxide.

#### 12.2 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than 17 mg of potassium hydroxide or 12 mg of sodium hydroxide.

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## 13 Test report

The test report shall specify <https://standards.iteh.ai/catalog/standards/sist/0dec609f-84c0-40d5-9992-67070c92de63/iso-7305-1998>

- all information necessary for the complete identification of the sample;
- the sampling method used, if known;
- the test method used, with the reference to this International Standard;
- all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);
- the test result(s) obtained;
- if the repeatability has been checked, the final quoted result obtained.