



**SLOVENSKI STANDARD**  
**SIST ISO 7302:1997**

**01-maj-1997**

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**Žito in žitni proizvodi - Določanje celotnih maščob**

Cereals and cereal products -- Determination of total fat content

Céréales et produits céréaliers -- Détermination de la teneur en matières grasses totales

**Ta slovenski standard je istoveten z: ISO 7302:1982**

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**ICS:**

67.060	Žita, stročnice in proizvodi iz njih	Cereals, pulses and derived products
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International Standard



7302

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INTERNATIONAL ORGANIZATION FOR STANDARDIZATION • МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ • ORGANISATION INTERNATIONALE DE NORMALISATION

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## Cereals and cereal products — Determination of total fat content

*Céréales et produits céréaliers — Détermination de la teneur en matières grasses totales*

First edition — 1982-11-15

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UDC 633.1 : 543.851.2

Ref. No. ISO 7302-1982 (E)

Descriptors : agricultural products, cereal products, tests, determination of content, fats.

## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards institutes (ISO member bodies). The work of developing International Standards is carried out through ISO technical committees. Every member body interested in a subject for which a technical committee has been set up 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.

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 7302 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in June 1981.

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

Australia	India	Poland
Austria	Iran	Portugal
Brazil	Iraq	Romania
Bulgaria	Ireland	South Africa, Rep. of
Canada	Israel	Spain
Chile	Italy	Sri Lanka
Czechoslovakia	Kenya	Tanzania
Egypt, Arab Rep. of	Korea, Rep. of	Thailand
Ethiopia	Malaysia	Turkey
France	Netherlands	United Kingdom
Germany, F.R.	New Zealand	Yugoslavia
Hungary	Philippines	

The member body of the following country expressed disapproval of the document on technical grounds :

USA

This International Standard has also been approved by the International Union of Pure and Applied Chemistry (IUPAC).

# Cereals and cereal products — Determination of total fat content

## 1 Scope and field of application

This International Standard specifies a method for the determination of the total fat content of cereals and cereal products intended for human consumption, including baked products and pasta.

## 2 References

ISO 712, *Cereals and cereal products — Determination of moisture content (Routine reference method)*.

ISO 950, *Cereals — Sampling (as grain)*.

ISO 2170, *Cereals and pulses — Sampling of milled products*.

ISO 6540, *Maize — Determination of moisture content (on milled grains and on whole grains)*.

## 3 Definition

**total fat content** : The whole of the substances extracted by hexane under the operating conditions specified in this International Standard, and expressed as a percentage by mass of the product as received.

## 4 Principle

After any grinding required, hydrolysis of a test portion by hydrochloric acid in the presence of ethanol and formic acid, thus releasing lipids bound to proteins and sugars and producing *in situ* ethyl formate which is a lipid solvent. Extraction of the fat by hexane in a special flask, removal of the solvent and weighing the residue thus obtained.

## 5 Reagents

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

**5.1 *n*-Hexane**, boiling between 68 and 70 °C and having a residue on evaporation of less than 0,001 g per 100 ml, or, failing this, *n*-hexane boiling between 67 and 70 °C and having a residue on evaporation of less than 0,002 g per 100 ml (if necessary, the residue on evaporation should be taken into account in the expression of results).

**5.2 Ethanol**, 95 % (V/V).

**5.3 Formic acid**, 99 % (V/V).

**5.4 Hydrochloric acid solution**.

Dilute 7 volumes of concentrated hydrochloric acid,  $\rho_{20} = 1,19$  g/ml, with 3 volumes of water.

**5.5 Nitrogen**.

## 6 Apparatus

Usual laboratory apparatus and in particular

**6.1 Mechanical grinder**.

**6.2 Sieve**, of aperture size 500  $\mu\text{m}$ .

**6.3 Water bath**, capable of being thermostatically controlled at  $75 \pm 1$  °C.

**6.4 Analytical balance**.

**6.5 Distillation apparatus**, preferably a reduced pressure rotary evaporator.

**6.6 Magnetic stirrer**, with a PTFE coated bar magnet approximately 0,9 cm in diameter and 4,5 cm long.

**6.7 Flask for hydrolysis and extraction**, with a ground neck and a side tube of sufficient capacity to retain the aqueous phase and a small portion of the hexane phase, and of dimensions and shape similar to that shown in the figure. The flask shown in the figure is suitable and has a capacity of 317 ml, the capacity of the side tube being 51 ml.

**6.8 Reflux condenser**, to fit the flask (6.7) (ground joint 29/32).

**6.9 Round-bottomed flask**, of capacity 250 ml, to fit the distillation apparatus (6.5).

## 7 Sampling

See ISO 950 or ISO 2170 as appropriate.

## ISO 7302-1982 (E)

## 8 Procedure

## 8.1 Preparation of the test sample

If necessary, grind the laboratory sample in the previously well cleaned mechanical grinder (6.1) to produce particles such that 95 % will pass through the sieve (6.2). Mix well before taking the test portion.

## 8.2 Preparation of the flask

Dry the flask (6.9) in an oven and allow it to cool to room temperature in the laboratory atmosphere. Then weigh it to the nearest 0,1 mg.

## 8.3 Test portion

Weigh, to the nearest 0,01 g, about 8 g of the test sample (8.1) and transfer it to the flask for hydrolysis and extraction (6.7) containing the bar magnet of the magnetic stirrer (6.6).

## 8.4 Hydrolysis

Spread the test portion in the bottom of the flask. Add 10 ml of the ethanol (5.2), place the flask on the magnetic stirrer (6.6) and operate the stirrer until as homogeneous a paste as possible is obtained.

Add 8 ml of the formic acid (5.3) and 12 ml of the hydrochloric acid solution (5.4) and continue stirring to homogenize (see clause 10).

Fit the reflux condenser (6.8) and place the flask in the water bath (6.3), controlled at  $75 \pm 1$  °C, for 20 min. Remove the reflux condenser, cool the flask and replace it on the magnetic stirrer.

## 8.5 Extraction

Place 18 ml of the ethanol (5.2) and 50 ml of the hexane (5.1) in the flask, and stir the mixture for 5 min at the maximum frequency of rotation of the bar magnet that can be used without risk of ejection of material from the flask. Allow the mixture to stand until the phases are completely separated.

If necessary, accelerate the separation by heating the flask for 20 s on the water bath (6.3), controlled at  $75 \pm 1$  °C, then allow it to cool (see clause 10). Transfer the hexane phase into the prepared flask (8.2), retaining the aqueous phase in the lateral tube of the flask. Rinse the neck of the flask with several drops of hexane.

Place 30 ml of hexane in the flask, stir the mixture for 5 min as above, allow the phases to separate, then transfer the hexane phase into the flask containing the first extract. Repeat the extraction twice more, using 30 ml of hexane each time.

## 8.6 Removal of solvent and weighing the residue

Evaporate the solvent contained in the flask, preferably under reduced pressure, by means of the distillation apparatus (6.5).

Immediately after evaporation, pass a stream of nitrogen (5.5) through the flask for 10 min. Carefully wipe the outside of the flask and allow it to cool to room temperature in the laboratory atmosphere. Then weigh it to the nearest 0,1 mg.

## 9 Expression of results

## 9.1 Method of calculation and formula

The total fat content, expressed as a percentage by mass of the product as received, is equal to

$$\frac{m_2 - m_1}{m_0} \times 100$$

where

$m_0$  is the mass, in grams, of the test portion (8.3);

$m_1$  is the mass, in grams, of the flask (8.2);

$m_2$  is the mass, in grams, of the flask and the residue (8.6).

Express the result to the nearest 0,01 %.

NOTE — The total fat content, expressed relative to the dry matter content of the product, may be calculated from the formula

$$S \times \frac{100}{100 - H}$$

where

$S$  is the total fat content, expressed as a percentage by mass of the product as received (calculated according to the above formula);

$H$  is the moisture content of the product, expressed as a percentage by mass, determined by the method specified in ISO 712 or ISO 6540.

## 9.2 Precision

An inter-laboratory trial, carried out at the international level under the authority of the International Association for Cereal Chemistry (ICC), with the participation of 16 laboratories, each performing five replications, gave the statistical results (determined in accordance with ISO 5725<sup>1)</sup>) given in the table.

NOTE — Results are expressed as percentages by mass of the product as received.

1) ISO 5725, *Precision of test methods — Determination of repeatability and reproducibility by inter-laboratory tests.*

Table

Sample	Wheat flour	Durum wheat semolina	Rye flour	Whole wheat millings	Wheat flour bread	Lipid-enriched wheat flour bread
Number of laboratories after eliminating outliers	12	14	14	14	14	13
Mean	1,65	1,74	1,81	2,61	1,74	4,76
Standard deviation of repeatability ( $s_r$ )	0,02	0,03	0,05	0,04	0,03	0,04
Coefficient of variation of repeatability	1,4 %	1,7 %	2,5 %	1,6 %	1,9 %	0,8 %
Repeatability ( $2,83 s_r$ )	0,06	0,08	0,13	0,12	0,09	0,11
Standard deviation of reproducibility ( $s_R$ )	0,11	0,10	0,12	0,13	0,12	0,21
Coefficient of variation of reproducibility	6,4 %	5,8 %	6,6 %	5,1 %	6,6 %	4,4 %
Reproducibility ( $2,83 s_R$ )	0,30	0,28	0,34	0,38	0,33	0,59

## 10 Notes on procedure

If dispersion of the product in the reagents before hydrolysis (8.4) is not possible, or if the decantation of the upper phase after extraction by hexane (8.5) is difficult, it is recommended to carry out the determination on a test portion of mass less than 8 g, while maintaining the same quantity of reagents.

## 11 Test report

The test report shall show the method used and the results obtained. It shall also mention all operating details not specified in this International Standard or regarded as optional, as well as any circumstance that may have influenced the results.

The test report shall include all the information necessary for the complete identification of the sample.

Dimensions in millimetres

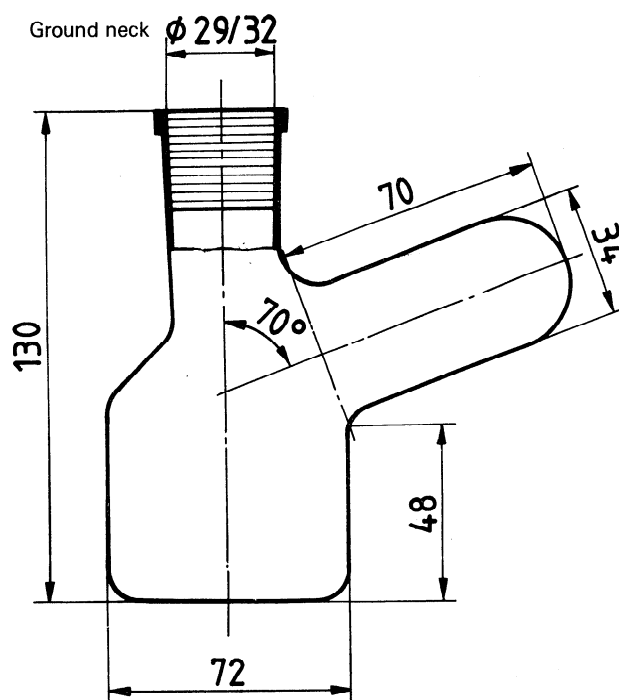


Figure — Flask for hydrolysis and extraction (for information only)