
International Standard



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Mustard seed — Specification

Graines de moutarde — Spécifications

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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 1237 was developed by Technical Committee ISO/TC 34, *Agricultural food products*.

This second edition was submitted directly to the ISO Council, in accordance with clause 5.10.1 of part 1 of the Directives for the technical work of ISO. It cancels and replaces the first edition (i.e. ISO 1237:1974), which had been approved by the member bodies of the following countries :

Brazil	Germany, F.R.	Netherlands
Bulgaria	Hungary	Poland
Czechoslovakia	India	Romania
Egypt, Arab Rep. of	Iran	Turkey
France	Israel	United Kingdom

This International Standard had also been approved by the Association of Official Analytical Chemists (AOAC).

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

Austria

Mustard seed — Specification

1 Scope and field of application

This International Standard specifies requirements for mustard seed.

Recommendations relating to storage and transport conditions are given in annex E.

2 References

ISO 927, *Spices and condiments — Determination of extraneous matter content.*

ISO 928, *Spices and condiments — Determination of total ash.*

ISO 930, *Spices and condiments — Determination of acid-insoluble ash.*

ISO 948, *Spices and condiments — Sampling.*

ISO 1108, *Spices and condiments — Determination of non-volatile ether extract.*

ISO 2825, *Spices and condiments — Preparation of a ground sample for analysis.*

3 Requirements

3.1 Description

Mustard seed is the dried clean seed of one or more of the following plants :

- *Sinapis alba* Linnaeus — White mustard, yellow mustard;
- *Brassica nigra* (Linnaeus) W.D.J Koch — Black mustard;
- *Brassica juncea* (Linnaeus) Czernajew and Cosson in Czernajew — Indian mustard.

3.2 Odour and flavour

The odour and flavour of the seeds when ground and moistened shall be fresh and pungent, and free from rancidity and mustiness.

3.3 Freedom from moulds, insects, etc.

The seeds shall be free from living insects, mites and moulds, and shall be practically free from dead insects, insect fragments and rodent contamination visible to the naked eye (corrected, if necessary, for abnormal vision), using such magnification as may be necessary in any particular case. If the magnification exceeds X 10, this fact shall be stated in the test report.

3.4 Extraneous matter, shrivelled and damaged seeds

The seeds shall be whole and mature and shall not contain more than 0,7 % (m/m) of extraneous matter or other vegetable material, determined by the method specified in ISO 927. Extraneous seeds include charlock (*Sinapis arvensis* Linnaeus), rape (*Brassica napus* Linnaeus), and *Mellilotus* species. The proportion of damaged or shrivelled mustard seeds shall not exceed 2 % (m/m).

3.5 Chemical requirements¹⁾

The mustard seed shall comply with the requirements given in the table.

Table — Requirements for mustard seed

Characteristics	Requirement	Method of test
Loss in mass at 103 °C, % (m/m) max.	10	Annex A
Total ash, % (m/m) on dry basis, max.	6,5	ISO 928
Acid-insoluble ash, % (m/m) on dry basis, max.	1,0	ISO 930
Non-volatile ether extract, % (m/m) on dry basis, min.	28	ISO 1108
Allyl isothiocyanate, % (m/m) on dry basis		Annex B
a) in <i>B. nigra</i> , min.	1,0	
b) in <i>B. juncea</i> , min.	0,70	
<i>p</i> -hydroxybenzyl isothiocyanate, % (m/m) on dry basis in <i>Sinapis alba</i> , min.	2,3	Annex C or Annex D

1) Limits for toxic substances will be included later, in accordance with the recommendations of the FAO/WHO Codex Alimentarius Commission.

4 Sampling

Sample the mustard seed by the method specified in ISO 948.

5 Methods of test

5.1 The samples of mustard seed shall be tested for conformity with the requirements of this International Standard by the methods of test referred to in 3.4 and the table.

5.2 For the determination of total ash, use the method specified in ISO 928, with the following modifications to sub-clause 8.3.2 :

Leach the ash with hot water, filter through a medium-fine, ashless filter paper and wash thoroughly. Transfer the filter paper and contents to the dish (6.1), dry and heat again in the muffle furnace (6.2) for 1 h. Cool and add 5 to 10 drops of analytical quality nitric acid, evaporate to dryness on the steam bath (6.4) and heat in the muffle furnace for 30 min. Repeat the addition of 5 to 10 drops of the nitric acid, again evaporate to dryness and heat in the muffle furnace for 1 h. Cool the dish, add the filtrate, and evaporate to dryness on the steam bath. Heat again in the muffle furnace for 1 h, cool and weigh. Repeat these operations until the difference between two successive weighings is less than 0,002 g. Record the lower mass.

The total ash may be retained for the determinations of water-insoluble ash and acid-insoluble ash.

6 Packing and marking

6.1 Packing

The mustard seed shall be packed in clean, hygienic bags of a material which does not affect the seed and prevents it from absorbing moisture.

6.2 Marking

The following particulars shall be marked or labelled on each package :

- a) name of the material (botanical name), and the trade name or brand name, if any;
- b) name and address of the manufacturer or packer;
- c) batch or code number;
- d) net mass;
- e) grade of the material (if graded), according to the national standard of the producing country;
- f) producing country;
- g) year of harvest, if known;
- h) date of packing;
- i) any other marking required by the purchaser.

Annex A

Determination of loss in mass at 103 °C¹⁾

A.1 Apparatus

A.1.1 Dish, of corrosion-resistant metal, with a suitable tight-fitting lid.

A.1.2 Constant temperature oven, controlled at 103 ± 2 °C.

A.1.3 Desiccator, provided with an efficient desiccant.

A.1.4 Analytical balance.

A.2 Procedure

A.2.1 Test portion

Weigh, to the nearest 0,001 g, about 2 g of the sample in the tared dish (A.1.1).

A.2.2 Determination

Heat the dish and contents, with the lid alongside the dish, in the oven (A.1.2) at 103 ± 2 °C for 3 h. Fit the lid, cool in the desiccator (A.1.3) and weigh. Heat in the oven for 1 h, cool in the desiccator and weigh again. Repeat the operations of heating for 1 h in the oven, cooling and weighing, until the difference between two successive weighings does not exceed 0,001 g.

A.2.3 Number of determinations

Carry out two determinations on the same sample.

A.3 Expression or results

A.3.1 Method of calculation and formula

The loss in mass at 103 °C, H , expressed as a percentage by mass of the sample, is equal to :

$$(m_0 - m_1) \times \frac{100}{m_0}$$

where

m_0 is the initial mass, in grams, of the test portion;

m_1 is the mass, in grams, of the test portion after heating in the oven.

Take as the result the arithmetic mean of the two determinations (A.2.3), provided that the requirement for repeatability (see A.3.2) is satisfied.

A.3.2 Repeatability

The difference between the results of the two determinations (A.2.3), carried out simultaneously or in rapid succession by the same analyst, shall not exceed 1 % of the mean value.

1) For the purpose of calculating the results of tests on the dry basis, it is assumed that the loss in mass at 103 °C, as determined by the method described in annex A, is equal to the moisture content.

Annex B

Determination of allyl isothiocyanate

B.1 Principle

After two successive soakings of the sample, the first in water at a temperature of 70 °C and the second in alcoholic medium, distillation of the allyl isothiocyanate liberated into an alcoholic ammonium hydroxide solution, addition to the distillate of a standard volumetric silver nitrate solution, and titration of the excess silver nitrate with standard volumetric potassium, or ammonium, thiocyanate solution in the presence of ammonium iron(III) sulphate.

B.2 Reagents

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

B.2.1 Ethanol, 95 % (V/V).

B.2.2 Ammonium hydroxide solution, $\rho_{20} = 0,925$ g/ml.

B.2.3 Nitric acid, $\rho_{20} = 1,40$ g/ml.

B.2.4 Silver nitrate, standard volumetric solution, $c(\text{AgNO}_3) = 0,1$ mol/l.

B.2.5 Potassium thiocyanate or ammonium thiocyanate, standard volumetric solution, $c(\text{KCNS})$ or $c(\text{NH}_4\text{CNS}) = 0,1$ mol/l.

B.2.6 Ammonium iron(III) sulphate solution, saturated when cold.

B.3 Apparatus

Usual laboratory apparatus, and in particular

B.3.1 Grinding mill.

B.3.2 Entrainment distillation apparatus (see the figure for a suitable example).

B.3.3 Burette, graduated at 0,05 ml intervals, complying with the requirements of ISO/R 385, class A.

B.3.4 Analytical balance.

B.4 Procedure

B.4.1 Preparation of the sample

After very careful mixing of the sample, take 15 to 20 g and grind it (see ISO 2825).

B.4.2 Test portion

Take about 2 g of the ground sample and weigh it to the nearest 0,001 g.

B.4.3 Determination

Transfer the test portion to the pear-shaped flask of the distillation apparatus, add 80 ml of water previously heated to 70 ± 2 °C, close the flask with its ground glass stopper and leave to stand for 15 min. Then add 20 ml of the ethanol (B.2.1) and allow to soak for 45 min.

After the soaking, connect the flask quickly to the distillation apparatus. Distil, and collect the distillate in a conical flask containing a mixture of 5 ml of the ammonium hydroxide solution (B.2.2) and 10 ml of the ethanol (B.2.1). (Entrainment distillation lasts, on average, for 5 min.) The quantity of the distillate should be at least 100 ml.

Add to the distillate 10 ml of the standard volumetric silver nitrate solution (B.2.4) and leave for 12 h at ambient temperature (the operation will be faster if the conical flask is placed for 1 h in a water bath heated to 70 to 80 °C).

Filter through a fine filter paper, rinse the flask and residue several times with hot water (approximately 90 °C).

To the bulked filtrate and washings add 10 ml of the nitric acid (B.2.3) and then titrate with the standard volumetric potassium, or ammonium, thiocyanate solution (B.2.5) using the ammonium iron(III) sulphate solution (B.2.6) as indicator, until a persistent pink colour is obtained.

B.4.4 Number of determinations

Carry out two determinations on the same prepared sample.

B.5 Expression of results

B.5.1 Method of calculation and formula

The allyl isothiocyanate content, expressed as a percentage by mass on the dry basis, is equal to

$$4,95 \frac{(10 - V)}{10^3} \times \frac{100}{m} \times \frac{100}{100 - H}$$

where

m is the mass, in grams, of the test portion;

V is the volume, in millilitres, of the standard volumetric potassium, or ammonium, thiocyanate solution used in the titration;

H is the moisture content of the sample, expressed as a percentage by mass, determined by the method specified in annex A.

NOTE — If the standard volumetric solutions used are not of the exact concentrations indicated in clause B.2, a suitable correction factor should be used in calculating the result.

Take as the result the arithmetic mean of the two determinations (B.4.4), provided that the requirement for repeatability (see B.5.2) is satisfied.

B.5.2 Repeatability

The difference between the results of the two determinations (B.4.4), carried out simultaneously or in rapid succession by the same analyst, shall not exceed 1 % of the mean value.

B.6 Notes on procedure

B.6.1 During the analysis, all contact with copper or rubber shall be avoided, especially in the distillation apparatus. Use cork or, preferably, ground glass stoppers.

B.6.2 The enzymic activity of the mustard seed diminishes with age; thus, it may be necessary to modify the analytical method in the case of old seed.

After a preliminary determination giving particularly low figures for allyl isothiocyanate, add to the distillation residue 5 g of *Sinapis alba* (take care to check that sulphur-containing volatile substances are not present in it) and then proceed to a second determination.

The addition of the two results will give the real figure of allyl isothiocyanate that may be formed in the sample. However, the actual quality of the sample should be considered as very reduced and it is recommended that the two figures, found before and after the addition of *Sinapis alba*, should be given in the test report.

B.6.3 The enzymic activity of the seeds increases during certain periods of the year (particularly in spring); thus, identical results are not always found with a given lot of seed, according to the season in which the analysis is carried out.

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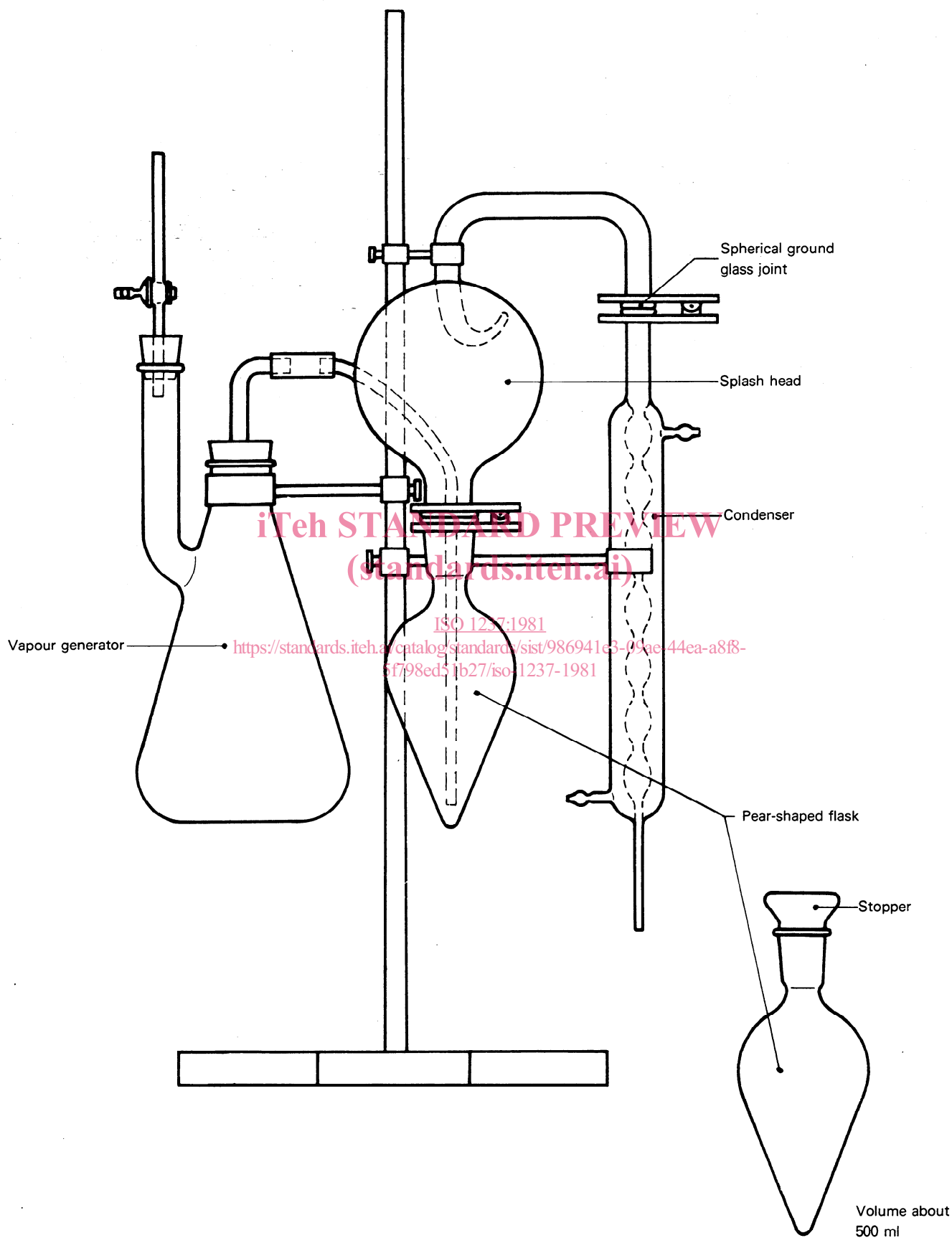


Figure — Entrainment distillation apparatus

Annex C

Determination of *p*-hydroxybenzyl isothiocyanate (Colorimetric method)

C.1 Principle

Decomposition, by enzymatic hydrolysis, of the sinalbin (glucoside of *Sinapis alba*) into glucose, the hydrogen sulphate of sinapin and *p*-hydroxybenzyl isothiocyanate, the last-mentioned giving *p*-hydroxybenzyl and thiocyanate. Colorimetric determination of the thiocyanate so formed.

C.2 Reagents

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

C.2.1 Calcium carbonate, pulverized.

C.2.2 Mercury(II) chloride, 50 g/l solution.

C.2.3 Potassium hexacyanoferrate(II), 106 g/l solution.

C.2.4 Zinc acetate solution.

Dissolve 21,9 g of zinc acetate $[(\text{CH}_3\text{COO})_2\text{Zn}]$ in water, add 3 ml of glacial acetic acid (CH_3COOH) and dilute to 100 ml with water.

C.2.5 Nitric acid, approximately 1 mol/l solution.

C.2.6 Sodium hydroxide, approximately 1 mol/l solution.

C.2.7 Ammonium iron(III) sulphate, 200 g/l solution in approximately 0,5 mol/l sulphuric acid solution.

C.2.8 Potassium thiocyanate or ammonium thiocyanate, standard volumetric solution, $c(\text{KCNS})$ or $c(\text{NH}_4\text{CNS}) = 0,1 \text{ mol/l}$, i.e. containing 5,808 g of CNS^- per litre.

C.3 Apparatus

Usual laboratory apparatus, and in particular

C.3.1 Grinding mill.

C.3.2 One-mark volumetric flasks, of capacities 50, 250 and 1 000 ml, complying with the requirements of ISO 1042, class A.

C.3.3 Pipettes, delivering 2 ml and 5 ml, complying with the requirements of ISO 648, class A, or ISO/R 835.

C.3.4 Colorimeter, suitable for measurements at a wavelength of 450 nm.

C.3.5 Analytical balance.

C.4 Procedure

C.4.1 Preparation of test sample

Carefully render the sample homogeneous, then take a portion of 20 to 25 g of the mustard seed and grind it (see ISO 2825).

C.4.2 Test portion

Take approximately 5 g of the ground sample and weigh it to the nearest 0,001 g.

C.4.3 Hydrolysis

Transfer the test portion to a 250 ml beaker.

Add 100 ml of water at $70 \pm 2 \text{ }^\circ\text{C}$ and at least 100 mg of the calcium carbonate (C.2.1). Cover the beaker with a watch glass. Leave to soak for 15 min at $70 \text{ }^\circ\text{C}$, cool, add 20 ml of the sodium hydroxide solution (C.2.6), and leave in contact for 15 min.

C.4.4 Clarification

Add a sufficient quantity of the nitric acid solution (C.2.5), to bring the contents of the beaker to a pH of about 6,0 to 6,5. Pour the contents of the beaker into a 250 ml volumetric flask and, shaking the flask, add 2 ml of the potassium hexacyanoferrate(II) solution (C.2.3) and then 2 ml of the zinc acetate solution (C.2.4).

Dilute to 250 ml with water and add 2 ml of water by pipette (C.3.3) (to take into account the volume of the precipitate). Shake, and filter through a rapid filter shaded from bright light. The filtrate (F) should be clear and colourless.

C.4.5 Determination

Add to a 50 ml volumetric flask :

- 5 ml of the filtrate (F),
- 5 ml of the ammonium iron(III) sulphate solution (C.2.7).

Dilute to 50 ml with water, shake, and measure the absorbance at a wavelength of 450 nm by means of the colorimeter (C.3.4).