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DRAFT EAST AFRICAN STANDARD

Mustard seed — Specification

EAST AFRICAN COMMUNITY

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The Community has established an East African Standards Committee (EASC) mandated to develop and issue East African Standards (EAS). The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the public and private sector organizations in the community.

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East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

The committee responsible for this document is Technical Committee EASC/TC 006, *Spices, condiments and culinary herbs*.

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Mustard seed — Specification

1 Scope

This draft East African Standard specifies requirements, methods of sampling and tests for seeds of white mustard (*Sinapis alba* or *Brassica hirta*), brown and yellow mustard (*Brassica juncea*) or black mustard (*Brassica nigra*)”.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

Codex online guideline for pesticide residues in food

CODEX STAN 193, *Codex general standard for contaminants and toxins in food and feed*

CODEX STAN 228, *General method of analysis for contaminants*

EAS 38, *Labelling of pre-packaged foods — General requirements*

EAS 39, *Hygiene in the food and drink manufacturing industry — Code of practice*

EAS 803, *Nutrition labelling — Requirements*

EAS 804, *Claims — General requirements*

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 4833-1, *Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 °C by the pour plate technique*

ISO 6579 (all parts), *Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of salmonella*

ISO 21527-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds — Part 2: Colony count technique in products with water activity less than or equal to 0.95*

ISO 16649-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta –glucuronidase-positive Escherichia coli — Part 2: Colony count technique at 44°C using 5-bromo-4-chloro -3-indolyl beta –D-glucuronide*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at <http://www.iso.org/obp>

3.1

immature or shriveled seed

mustard seed that has indications of lack of maturity or full development

3.2

damaged seeds

seeds that are damaged mechanically, or by mould or weevils, or those showing internal discoloration of seeds materially affecting the quality

4 Requirements

4.1 Description

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

- a) *Sinapis alba* or *Brassica hirta* — White mustard;
- b) *Brassica nigra* — Black mustard;
- c) *Brassica juncea* — Brown and yellow mustard

4.2 General requirements

4.2.1 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.

4.2.2 Freedom from moulds, insects, etc.

The seeds shall be free from living insects, mites and moulds, dead insects, insect fragments and rodent contamination visible to the naked eye.

4.3 Specific requirements

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

Table — Requirements for mustard seed

S/No.	Characteristics	Requirement	Test method
i)	Loss in mass at 103 °C, % (m/m) max.	10	Annex A

ii)	Total ash, % (m/m) on dry basis, max.	6.5	ISO 928
iii)	Acid-insoluble ash, % (m/m) on dry basis, min.	1.0	ISO 930
iv)	Non-volatile ether extract, % (m/m) on dry basis, min.	28	ISO 1108
v)	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	
vi)	p-hydroxybenzyl isothiocyanate, % (m/m) on dry basis in <i>Sinapis alba</i> , min.	2.3	Annex C or Annex D
vii)	Extraneous matter, m/m	0.7 %	ISO 927
viii)	Damaged or shrivelled seeds (m/m).	2 %	

5. Contaminants

5.1 Pesticide residues

Pesticide residues in mustard seed shall not exceed maximum residue limits as established in the Codex online guideline for pesticide residues in food.

5.2 Heavy metals

Heavy metals in mustard seed shall not exceed maximum heavy metal limits as stipulated in CODEX STAN 193.

6 Hygiene

Mustard seed shall be manufactured and handled in a hygienic manner in accordance with EAS 39 and shall comply with the microbiological limits stipulated in Table 2 when tested in accordance with the methods specified therein.

Table 2 — Microbiological requirements for mustard seed

S/No	Characteristic	Requirement	Test method
i.	Total plate count, cfu/g, max.	10 ⁵	ISO 4833-1
ii.	Yeast and moulds, cfu/g, max.	10 ⁴	ISO 21527-2
iii.	<i>Salmonella spp.</i> , per 25 g	Absent	ISO 6579
iv.	<i>E coli</i> , MPN/g, max.	Absent	ISO 16649 -2

7 Packaging

The mustard seed shall be packaged in food grade packaging material that secures the integrity and the safety of the product.

8. Labelling

In addition to the requirements of EAS 38, EAS 803 and EAS 804, each container shall be legibly and indelibly labelled with the following information:

- a) name of the product as “mustard seed”;
- b) trade name or brand name if any;
- c) name, physical and postal address of manufacturer and / or packer;
- d) batch or code number;
- e) net weight in metric units;
- f) storage conditions;
- g) manufacturing date;
- h) expiry date; and
- i) instructions for use.

8 Sampling and test

8.1 Sampling

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

8.2 Methods of test

8.2.1 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.

8.2.2 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, ash less filter paper and wash thoroughly. Transfer the filter paper and contents to the dish (6.11, 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 weighing is less than 0.002 g. Record the lower mass.

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

Annex A (normative)

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 of results

A.3.1 Method of calculation and formula

The loss in mass at 103 °C, *l*, 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 (normative)

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 thiocyanate, 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

$$4.95 \frac{(10 - V) \times 100 \times 100}{10^3 m (100 - H)}$$

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.

Annex C (normative)

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 [(CH₃COO)₂Zn] in water, add 3 ml of glacial acetic acid (CH₃COOH) 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, 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 ± 2 °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 °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 hexa-cyanoferrate(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).

C.4.6 Calibration curve

Transfer, by means of a pipette (C.3.3), 5 ml of the standard volumetric potassium, or ammonium, thiocyanate solution (C.2.8) to a 1 000 ml volumetric flask and dilute to the mark with water.

Into a series of five 50 ml volumetric flasks, transfer the volumes of this diluted potassium, or ammonium, thiocyanate solution indicated in the following table:

Volume of diluted potassium, or ammonium, thiocyanate solution	Corresponding mass of thiocyanate ion
ml	µg
5	145.2
10	290.4
15	435.6
20	580.8
25	726

Add to each flask, 5 ml of the ammonium iron (III) sulphate solution (C.2.7), dilute to the mark with water, shake, and measure the absorbance as indicated in C.4.5.

Plot a calibration curve, giving the absorbance as a function of the number of micrograms of thiocyanate.

C.4.7 Matching test

Carry out a matching test in the same conditions as the actual test, but adding 2 drops of the mercury (III chloride solution (C.2.2) to correct for errors due to the reaction of phenols with the iron(III) salts.

Note on the calibration curve the difference in absorbance between the test solution, containing the thiocyanate and phenols, and the matching test solution.

C.4.8 Number of determinations

Carry out two determinations on the same prepared sample.

C.5 Expression of results

C.5.1 Method of calculation and formula

The *p*-hydroxybenzyl isothiocyanate content, expressed as a percentage by mass on the dry basis, is equal to

$$\frac{2.84 m_1}{10^6} \times \frac{250}{5} \times \frac{100}{m_o} \times \frac{100}{100 - H}$$

where

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

m_1 is the mass, in micrograms, of thiocyanate read from the calibration curve;

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

2.84 is the conversion factor from thiocyanate ion (CNS-) to *p*-hydroxybenzyl isothiocyanate.

Take as the result the arithmetic mean of the two determinations (C.4.8), provided that the requirement for repeatability (see C.5.2) is satisfied.

C.5.2 Repeatability

The difference between the results of the two determinations (C.4.8), carried out simultaneously or in rapid succession by the same analyst, shall not exceed 2 % of the mean value.

C.6 Note on procedure

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.

Annex D (normative)

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

D.0 Introduction

Laboratories not in possession of a colorimeter may determine thiocyanate by argentimetry. In this case it is necessary either

- a) to carry out a preliminary check for the absence of Cl⁻ ions in the seed (no reaction with silver nitrate on the ash of the mustard seed); or
- b) to provide for correction by carrying out the determination of Cl⁻ ions on an aliquot portion of the filtrate.

This method may be substituted for the colorimetric method by agreement between the parties concerned.

D.1 Principle

Decomposition, by enzymic hydrolysis, of sinalbin (glucoside of *Sinapis albas* into glucose, the hydrogen sulphate of sinapin and *p*-hydroxybenzyl isothiocyanate, the last-mentioned giving *p*-hydroxybenzyl alcohol and thiocyanate. Determination of the thiocyanate thus formed, by argentimetry in nitric acid medium; back titration of the excess of silver nitrate using standard volumetric potassium thiocyanate solution in the presence of ammonium iron(III) sulphate.

D.2 Reagents

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

The reagents necessary for hydrolysis and clarification (see annex C), together with

D.2.1 Nitric acid, $e_{20} = 1.40$ g/ ml.

D.2.2 Silver nitrate, standard volumetric solution, $c(\text{AgNO}_3) = 0.1$ mol/l.

D.2.3 Potassium thiocyanate, standard volumetric solution, $c(\text{KCNS}) = 0.1$ mol/l.

D.2.4 Ammonium iron (III) sulphate solution, saturated when cold.

D.3 Apparatus

The apparatus necessary for the preparation of the sample hydrolysis and clarification (see annex C), together with

D.3.1 One-mark pipettes, of capacities 5 and 100 ml, complying with the requirements of ISO 648, class A.

D.3.2 Burette, graduated at every 0.05 ml, complying with the requirements of ISO/ R 385, class A.

D.4 Procedure

D.4.1 Preparation of test sample, test portion, hydrolysis and clarification

Proceed as specified in C.4.1 to C.4.4 of annex C.

D.4.2 Titration

Add to the beaker, shaking after each addition:

- 100 ml, by means of a pipette (D.3.1), of the filtrate (F) (see C.4.4), and approximately equivalent to 2 g of mustard seed;
- 1 ml of the nitric acid (D.2.1);
- 5 ml, by means of a pipette (D.3.1), of the standard volumetric silver nitrate solution (D.2.2);
- 2 ml of the ammonium iron(III) sulphate solution (D.2.4).

Shake the flask to coagulate the precipitate, and titrate with the potassium thiocyanate solution (D.2.3) until a persistent faint red colour is obtained.

D.4.3 Number of determinations

Carry out two determinations on the same prepared sample.

D.5 Expression of results

D.5.1 Method of calculation and formula

The *p*-hydroxybenzyl isothiocyanate content, expressed as a percentage by mass on the dry basis, is equal to

$$0.0165 (5 - V) \times \frac{250}{100} \times \frac{100}{m} \times \frac{100}{100 - H}$$

Where

m is the mass, in grams, of the test portion (see annex C, C.4.2);

V is the volume, in millilitres, of the standard volumetric potassium thiocyanate solution (D.2.3) 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 specified in clause D.2, a suitable correction factor should be used in calculating the result.

Take as the result the arithmetic mean of the two determinations (D.4.3), provided that the requirement for repeatability (see D.5.2) is satisfied.

D.5.2 Repeatability

The difference between the results of the two determinations (D.4.3), carried out simultaneously or in rapid succession by the same analyst, shall not exceed 0.1 g of *p*-hydroxybenzyl isothiocyanate per 100 g of dry matter in the sample.

Annex E (informative)

Recommendations relating to storage and transport

E.1 The packs of mustard seed should be stored in covered premises well protected from the sun, rain and excessive heat.

E.2 The store room should be dry, free from objectionable odours and proofed against entry of insects and vermin. The ventilation should be controlled so as to give good ventilation under dry conditions and no ventilation under damp conditions. In a storage warehouse, suitable facilities should be available for fumigation.

E.3 The packs should be so handled and transported that they are protected from the rain, from the sun or other sources of excessive heat, from objectionable odours and from cross-infestation, especially in the holds of ships.

Bibliography

[1] ISO 1237: *Mustard seed*

