International Standard 1208

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Spices and condiments — Determination of filth

Épices — Détermination des impuretés

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Foreword

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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.

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

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Ethopia	Malaysia	Sri Lanka
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The member bodies of the following countries expressed disapproval of the document on technical grounds:

Canada
USA

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Spices and condiments — Determination of filth

0 Introduction

International Standards specifying requirements for spices and condiments prescribe, *inter alia,* that spices and condiments shall be practically free from dead insects, insect fragments and rodent contamination. For testing compliance with this requirement, hand lens examination is adequate only in the case of whole spices and condiments. For ground spices and condiments, the method to be used is that specified in this International Standard, especially in cases of dispute.

The method is applicable to most spices and condiments. In view of the number and variety of such products, however, it may be necessary in particular cases to modify the method or even to choose a more suitable method. Such modifications and other methods will be indicated in the International Standards appropriate to the spices and condiments concerned.

1 Scope and field of application

This International Standard specifies a method for the quantitative determination of filth in spices and condiments. As no limits have been prescribed for filth in International Standards on spices and condiments, this method should be used for collecting more data and for settling international disputes.

2 Reference

ISO 948, *Spices and condiments — Sampling.*

3 Definition

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

filth: Mineral matter (sand, soil) and matter of animal origin (insect fragments, rodent hairs and excreta) separated from the product under the conditions specified.

4 Principle

Washing the product with chloroform (after, if necessary, preliminary extraction with light petroleum) and examination of the washings for heavy filth and sand. Washing the product with water, with or without treatment with pancreatin enzyme, and agitation with light petroleum, the light filth collecting at the interface between the liquids after separation. Transference of the light filth to a filter paper and microscopical examination for contaminants such as insect fragments and rodent hairs.

5 Reagents

The water used shall be distilled water or water of at least equivalent purity.

5.1 Chloroform and, if required (see 8.3), **chloroform/carbon tetrachloride mixtures.**

5.2 Pancreatin solution.

Use pancreatin complying with the requirements of the annex, and which has been kept at about 10 °C. Use a solution which has been freshly prepared as follows.

Mix 10 g of pancreatin with 100 ml of warm water (temperature not exceeding 40 °C). Stir mechanically for 10 min or allow to stand for 30 min with intermittent stirring. Pour the solution through a loosely packed pad of cotton wool, 100 mm thick, in a 60° funnel of diameter 100 to 125 mm. Repeat the filtration through the same pad. If filtration is slow in either case, filter with suction through a fast filter paper using a Buchner funnel. If the filtration is still slow, pour the solution through a slightly compressed cotton wool plug in the 60° funnel. Repeat if necessary, until the solution filters rapidly through paper. (Soluble pancreatin may be filtered directly through paper with suction.) Dilute the filtrate to 100 ml for each 10 g portion.

5.3 ***Tri*sodium orthophosphate,** 50 g/l solution.

5.4 Formaldehyde solution.

5.5 Light petroleum, boiling range 40 to 60 °C.

5.6 Light petroleum, boiling range 100 to 120 °C.

6 Apparatus

Usual laboratory apparatus, and

6.1 Trap flask (Wildman), consisting of a 1 000 ml conical flask into which is inserted a close-fitting rubber stopper carried on a rigid metal rod, 5 mm in diameter and approximately 100 mm longer than the height of the flask. (A rod of a greater diameter is not desirable because of its greater displacement of liquid.) The rod is threaded at its lower end and furnished with nuts and washers to hold it in place on the stopper. The lower nut and washer are countersunk in the rubber to prevent them from striking the flask. See the figure.

Alternatively, a separating funnel, of capacity 1 000 ml, may be used.

6.2 Beakers, of capacity 600 ml.

6.3 Buchner funnel, of diameter 15 cm with a filter paper.

6.4 Buchner funnel, of diameter 7 cm with a filter paper ruled with parallel lines 5 mm apart.

6.5 Filter paper, ashless.

6.6 Crucible, tared.

6.7 Oven, capable of being controlled at 80 °C.

6.8 Oven, capable of being controlled at 103 °C.

6.9 Petri-dish, of diameter 80 mm.

6.10 Suitable magnifying device (microscope, binocular magnifier, etc. or preferably a wide-field stereoscopic microscope).

6.11 Balance.

7 Sampling

Sample the material by the method specified in ISO 948.

8 Procedure

NOTE — To achieve a satisfactory separation of the light filth from spice tissue, it may be necessary to remove most of the volatile oil and fat, or to treat the test portion with pancreatin enzyme to digest starch and protein, or both.

If removal of volatile oil or fat is required, proceed as described in 8.2; otherwise, proceed directly as described in 8.3.

8.1 Test portion

Ensure that the test portion is representative of the laboratory sample (final lot sample).

8.1.1 Whole and broken spices

Break the material into small pieces, weigh, to the nearest 0,1 g, 25 g of the sample and transfer it to a breaker (6.2).

8.1.2 Ground spices

Weigh, to the nearest 0,1 g, 25 g of the sample and transfer it to a beaker (6.2).

8.2 Preliminary removal of volatile oil and fat

Add 200 ml of the light petroleum (5.5) to the test portion (8.1) in the beaker. Boil gently on a warm water bath for 15 min. Remove the light petroleum by decanting, taking care not to lose any of the material under test.

8.3 Separation of heavy filth and sand

Add 400 ml of the chloroform (5.1) to the test portion (8.1) or to the residue from the operation described in 8.2. Allow the beaker to stand for at least 1 h with occasional stirring. Transfer the material and the solvent to the Buchner funnel (6.3), leaving the heavy residue of sand and soil in the beaker. Drain. If appreciable spice tissue remains on the bottom of the beaker, add successive portions of chloroform mixed with carbon tetrachloride to give increasingly higher relative densities until practically all spice tissue has been removed by flotation. Transfer the heavy residue from the beaker to the ashless filter paper (6.5) and wash it with water to remove any sodium chloride present in the spice. Examine this residue. If there is an appreciable residue, place the filter paper in the tared crucible (6.6), ignite, and weigh the sand and soil.

8.4 Treatment of residue retained on the Buchner funnel

Dry the material retained on the Buchner funnel (see 8.3) for 1 h in the oven (6.7), controlled at 80 °C.

8.4.1 Procedure without enzyme treatment

Transfer the residue to the trap flask (6.1). Add about 150 ml of water, heat to boiling and simmer for 15 min with stirring. Wash down the inside of the flask with water and cool to below 20 °C, diluting to about 600 ml with water.

8.4.2 Procedure with enzyme treatment

Transfer the dry residue to a beaker (6.2). Add 300 ml of water and stir until homogeneous. Add 50 ml of the pancreatin solution (5.2) and mix. Adjust to pH 8 with the *tri*sodium orthophosphate solution (5.3). Readjust the pH after about 15 min, and again after about 45 min. Add 5 drops of the formaldehyde solution (5.4) and allow to digest overnight at 37 to 40 °C. Cool and transfer to the trap flask (6.1), making up to about 600 ml with water.

8.5 Separation of light filth

8.5.1 Add, down the stirring rod of the flask, 25 ml of the light petroleum (5.6). Tilt the trap flask at about 45° to the ver-

tical and mix for 1 min at the rate of 4 strokes per second with a brisk rotary motion so that the liquid is brought to a roll. Avoid splashing through the surface of the liquid in the trap flask with the rubber stopper.

Allow to stand for 5 min. Then fill the flask with water and allow to stand for 30 min. Stir every 5 min.

8.5.2 Spin the stopper to remove sediment and raise the stopper as far as possible into the neck of the flask, making sure that the light petroleum layer and at least 1 cm of the liquid below the interface are above the stopper. Holding the stopper in place, transfer the trapped liquid to the Buchner funnel (6.4). Filter.

8.5.3 Add 15 ml of the light petroleum (5.6) to the contents of the trap flask and mix thoroughly. After 15 min, repeat the operations described in 8.5.2. If this second extraction yields an appreciable quantity of filth, decant most of the liquid from the flask, add another 15 ml of the light petroleum (5.6) and carry out a third extraction.

8.6 Microscopical examination of light filth

Remove the filter paper from the Buchner funnel and place it on the petri-dish (6.9). Place the petri-dish in the oven (6.8), controlled at 103 °C, for 30 min. The dried filter paper should be tightly stuck to the petri-dish.

Examine the entire area of the filter paper, using the magnifying device (6.10) in reflected light, scraping and probing with a mounted needle.

Carry out the examination from left to right, from top to bottom for the first interval, from bottom to top for the second interval, from top to bottom for the third interval, etc.

9 Expression of results

9.1 Heavy filth (see 8.4)

Note the presence of mineral matter.

If the amount of sand and soil is such as to require calcination and weighing of these impurities, the mineral impurities content of the product, expressed as a percentage by mass, is equal to

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

where

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

m_1 is the mass, in grams, of the residue obtained (8.3).

9.2 Light filth (see 8.6)

Note the presence of matter of animal origin (see 8.6).

If required, record separately the numbers of insect fragments, rodent hairs and other items of animal origin in the test portion, i.e. the numbers per 25 g of sample.

10 Test report

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

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

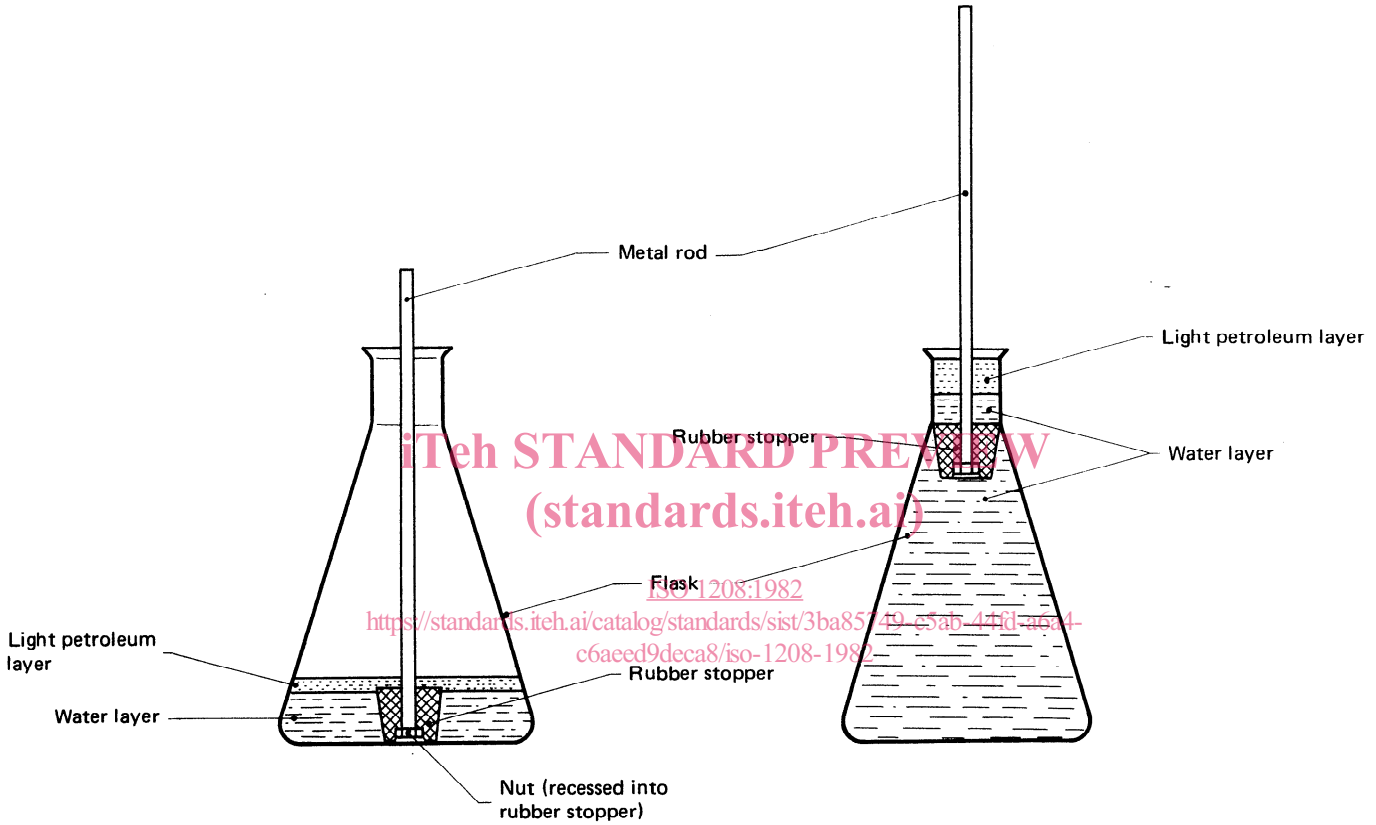

Figure — Wildman trap flask (6.1), showing method of use

NOTE — The upper nut and washer have been omitted.

Annex

Pancreatin — Specification

A.0 General

Pancreatin is a substance containing enzymes, principally pancreatic amylase, trypsin, and pancreatic lipase, obtained from the pancreas of the hog, *Sus scrofa* Linne var. *domesticus* Gray (Fam. Suidae), or of the ox, *Bos taurus* Linne (Fam. Bovidae). Pancreatin converts not less than 25 times its mass of N.F.* Potato Starch Reference Standard into soluble carbohydrates, and not less than 25 times its mass of casein into proteoses. Pancreatin of a higher digestive power may be brought to this standard by admixture with lactose, or with sucrose containing not more than 3,25 % of starch, or with pancreatin of lower digestive power.

A.1 Description

Pancreatin occurs as a cream-coloured, amorphous powder, having a faint, characteristic, but not offensive odour. Pancreatin changes protein into proteoses and derived substances, and converts starch into dextrins and sugars. Its greatest activities are in neutral or faintly alkaline media; more than traces of mineral acids or large amounts of alkali hydroxides render it inert. An excess of alkali carbonate inhibits its action.

A.2 Assay for fat

Introduce 2 g of pancreatin into a flask of capacity about 50 ml, add 20 ml of diethyl ether, stopper, and set it aside for several hours, mixing by rotating at frequent intervals. Decant the supernatant diethyl ether by means of a guiding rod into a plain filter paper of diameter about 7 cm, previously moistened with diethyl ether, and collect the filtrate in a tared beaker. To the residue remaining in the flask, add a further 10 ml portion of diethyl ether, proceeding as before, then add a third 10 ml portion of diethyl ether, and transfer the diethyl ether and the remainder of the pancreatin to the filter. Allow to drain, evaporate the diethyl ether spontaneously, and dry the residue at 105 °C for 2 h: the residue of fat shall weigh not more than 60 mg (3,0 %).

The fat may also be determined by the use of a Soxhlet continuous extraction apparatus.

A.3 Assay for starch digestive power

Determine the percentage of moisture in N.F. Potato Starch Reference Standard by drying about 500 mg of it, accurately weighed, at 120 °C for 4 h. Boil a sufficient quantity of water for 10 min, and cool to room temperature. Use this water for all subsequent dilutions specifying water.

Thoroughly mix a quantity of the N.F. Potato Starch Reference Standard, equivalent to 3,75 g of dry Reference Standard, with 10 ml of water. Add the mixture, with constant stirring, to 75 ml of water, previously heated to about 55 °C, and contained in a tared 250 ml beaker.

Rinse the remaining starch into the beaker with 10 ml of water. Heat the mixture to boiling, and boil it gently, with constant stirring, for 5 min. Add sufficient water to bring the mass of the mixture to 100 g, cool the paste to 40 °C, and place the beaker in a water bath maintained at 40 °C. Suspend 150 mg of the pancreatin to be tested in 5 ml of water in a 250 ml beaker and add the suspension to the starch paste, mixing it well by pouring the mixture from beaker to beaker for 30 s, noting the time when the pancreatin suspension was first added to the paste. Maintain the mixture at a temperature of 40 °C for exactly 5 min. Stir, at once add 0,1 ml of this mixture to a solution previously made by adding 0,2 ml of iodine solution, $c(1/2\ I_2)$ = 0,1 mol/l, to 60 ml of water, at a temperature of 23 to 25 °C, and mix: no blue or violet colour shall be produced.

A.4 Assay for casein digestive power

Place 100 mg of finely powdered casein in a 50 ml volumetric flask, add 30 ml of water, and shake well to bring the casein into suspension. Add 1,0 ml of sodium hydroxide solution, $c(NaOH)$ = 0,1 mol/l, and heat the mixture at 40 °C until the casein is completely dissolved, which should not require more than 30 min. Cool, dilute with water to 50 ml, and mix. Dissolve 100 mg of the pancreatin to be tested in 500 ml of water. Also dissolve 100 mg of N.F. Pancreatin Reference Standard for Casein Digestive Activity in another 500 ml of water. Mix 1 ml of glacial acetic acid with 9 ml of water and 10 ml of ethanol. Place 5 ml of the casein solution in each of two test tubes. To one of the test tubes add 2 ml of well-shaken pancreatin solution and to the other test tube add 2 ml of the well-shaken Reference Standard solution. Add 3 ml of water to each tube, mix by gentle agitation, immediately immerse the test tubes in a water bath at 40 °C and keep at this temperature for 1 h. Then remove the tubes from the bath, and add 3 drops of the acetic acid mixture to each tube.

The amount of turbidity in the test tube containing the solution of pancreatin being tested shall not be greater than that in the test tube containing the Reference Standard solution.

A.5 Packaging and storage

Preserve pancreatin in tight containers, preferably at a temperature of about 10 °C but in no circumstances above 30 °C.

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* National Formulary (of USA).

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