International Standard ISO 5565

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Vanilla [*Vanilla fragrans* (Salisbury) Ames] — Specification

*Vanille [*Vanilla fragrans *(Salisbury) Ames] — Spécifications*

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Foreword

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No member body expressed disapproval of the document.

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Vanilla [*Vanilla fragrans* (Salisbury) Ames] — Specification

1 Scope

This International Standard specifies requirements for vanilla belonging to the species *Vanilla fragrans* (Salisbury) Ames syn. *Vanilla planifolia* Andrews and for certain forms of vanilla obtained from seeds, which may be hybrids of *Vanilla fragrans* (Salisbury) Ames.

This vanilla is known commercially under various names such as Bourbon vanilla, Mexican vanilla, Indonesian vanilla and Seychelles vanilla.

Methods for the determination of the aromatic constituents of vanilla and for the determination of vanillin are given, for information only, in annexes A and B, respectively. These annexes do not form a mandatory part of this International Standard.

2 Field of application

This International Standard is applicable to vanilla in pods, bulk, cut or powder form. It is not applicable to vanilla extracts.

3 References

ISO 939, *Spices and condiments — Determination of moisture content — Entrainment method.*

ISO 948, *Spices and condiments — Sampling.*

ISO 3493, *Vanilla — Vocabulary.*

4 Definitions

See ISO 3493.

5 Commercial forms

Four commercial forms are established by this International Standard :

a) **vanilla pods,** consisting of whole pods which may be split;

b) **cut vanilla,** consisting of parts of pods, split or not, and deliberately cut or broken;

c) **vanilla in bulk,** consisting of vanilla in pods and cut vanilla.

d) **vanilla powder,** obtained by grinding vanilla pods without additives after drying.

6 General characteristics

6.1 Vanilla pods

Vanilla pods shall :

a) have the characteristics corresponding to their qualitative category (see clause 7);

b) have undergone a suitable treatment with a view to developing their flavour;

c) have a maximum moisture content conforming to that of their qualitative category.

The pods may be rimy, and may have a mark at the bottom one-third of their length.

They shall not :

a) have undergone any treatment which could induce a change in their natural vanillin content or in the content of any other constituent of the flavour;

b) be moth-eaten, mouldy, creosoted, "poiquées" (blistered), oxidized;

c) have an odour which is not typical of vanilla.

6.2 Cut vanilla

Cut vanilla shall :

a) be prepared from vanilla pods meeting the requirements specified in 6.1;

b) be sound and of good specific flavour;

c) have a maximum moisture content of 30 %;

d) be chocolate brown to dark brown in colour.

6.3 Vanilla in bulk

Vanilla in bulk shall :

a) be obtained from vanilla pods meeting the requirements specified in 6.1 or from pieces of pods meeting the requirements specified in 6.2;

b) be sound and of good specific flavour;

c) have a maximum moisture content of 30 %;

d) be chocolate brown to dark brown in colour.

Pods or pieces are generally wooded, and may have several large stains.

6.4 Vanilla powder

Vanilla powder shall :

a) be obtained from vanilla pods meeting the requirements specified in 6.1, from cut vanilla meeting the requirements specified in 6.2 or from vanilla in bulk meeting the requirements specified in 6.3;

b) have a maximum moisture content of 20 %;

c) be sufficiently fine to pass through a sieve of aperture size 1,25 mm;

d) be brown or dark brown in colour;

e) have the natural and very marked flavour of vanilla.

It shall not :

a) have undergone any treatment which could induce a change in its natural vanillin content or in the content of any other constituents of the flavour;

b) contain any extraneous matter;

c) have a musty, creosote or any other odour which is not typical of vanilla.

7 Qualitative classification of vanilla pods

7.1 Category 1

7.1.1 A_1 Non-split

Pods which are whole, sound, supple and full, of typical flavour, of uniform chocolate brown to dark brown colour, and without any other stain than the mark.

Maximum moisture content : 38 %.

7.1.2 B_1 Split

Pods of the same characteristics as those of category A_1, but split.

7.2 Category 2

7.2.1 A_2 Non-split

Pods which are whole, sound, supple and full, of typical flavour, of uniform chocolate brown to dark brown colour, and which may have a few stains, the total length of which does not exceed one-third of the length of the pod.

Maximum moisture content : 38 %

7.2.2 B_2 Split

Pods of the same characteristics as those of category A_2, but split.

7.3 Category 3

7.3.1 A_3 Non-split

Pods which are whole, sound, more or less supple, of typical flavour, chocolate brown to dark brown in colour, and which may have numerous stains the total length of which does not exceed half the length of the pod, as well as a few red filaments which do not exceed one-third of the length of the pod.

Maximum moisture content : 30 %.

7.3.2 B_3 Split

Pods of the same characteristics as those of category A_3, but split.

7.4 Category 4

7.4.1 A_4 Non-split

Pods which are whole, sound, dry or wooded, of typical flavour, reddish in colour and which may have several stains the total length of which does not exceed half the length of the pod.

Maximum moisture content : 25 %.

7.4.2 B_4 Split

Pods of the same characteristics as those of category A_4, but split.

8 Chemical characteristics [1)]

The total ash content and the acid-insoluble ash content will be specified later together with the contents of the main constituents of the flavour.

Methods for the determination of the aromatic constituents of vanilla and for the determination of the vanillin content are given, for information only, in annexes A and B, respectively.

9 Sampling

Proceed as specified in ISO 948.

Each laboratory sample shall have a minimum mass of 100 g.

In the case of vanilla pods, the pods taken as increments shall be representative of the packets contained in the packages chosen for sampling.

The sample shall be stored in an airtight container.

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

10 Methods of test

Carry out the determination of moisture content in accordance with ISO 939 except that

a) in the case of vanilla pods, cut vanilla and vanilla in bulk, prepare the test sample by cutting the vanilla into fragments of maximum size about 5 mm, taking care not to change the moisture content, and use a test portion of about 20 g;

b) in the case of vanilla powder, prepare the test sample by thoroughly mixing the laboratory sample.

11 Packing and marking

11.1 Packing

11.1.1 Vanilla pods

Vanilla pods shall be put in packets of pods of the same length, and shall then be put in clean, sound, watertight containers of material that will have no effect on the product (for example tin-plate boxes).

Each of these elementary containers of packets of pods shall be uniform from the point of view of category (according to clause 7).

A series of these elementary containers, the contents of which are homogeneous, constitutes a lot; a consignment is constituted by either a homogeneous lot or by several lots belonging to different categories.

11.1.2 Cut vanilla

Cut vanilla shall be put in packets of pods of the same length when they are sufficiently long, and in bulk when they cannot be put in bundles.

They shall then be placed in clean, sound and watertight containers of material that will have no effect on the product.

Cut vanilla shall be uniform from the botanical point of view.

11.1.3 Vanilla in bulk

Vanilla in bulk shall be put in clean, sound and watertight containers of material that will have no effect on the product.

11.1.4 Vanilla powder

Vanilla powder shall be put in clean, sound and watertight containers of material which will have no effect on the product.

11.2 Marking

11.2.1 Vanilla pods, cut or in bulk

The following indications shall be inscribed on each container or on a label :

a) name of the product (corresponding to the botanical species);

b) grade;

c) producing country;

d) code, batch or test certificate number, or similar means of identification;

e) any other information required by the purchaser.

11.2.2 Vanilla powder

The indications in 11.2.1 shall be inscribed on every elementary container and on every container to be dispatched.

If glass containers are used, the words "*fragile — glass*" shall be indicated on each container to be dispatched. If possible, the year of harvest shall be indicated.

11.2.3 Vanilla for retail trade

Marking shall be in conformity with the rules and regulations of the country in which the vanilla is to be sold.

Annex A

Qualitative investigation for the main aromatic constituents of vanilla

(This annex does not form part of the standard.)

A.0 Introduction

The method described in this annex is only one of the methods which can be used for determining the aromatic constituents of vanilla. It is simple and, thus, is used particularly for routine laboratory control.

More accurate methods by gas chromatography may be standardized later.

The main aromatic constituents of vanilla are aldehydes, alcohols and phenolic acids. The method also enables detection of the synthetic aroma ethyl vanillin and its derivatives.

This method can be used for vanilla pods and is also applicable to :

a) vanilla flavoured products (after extraction of the constituents of the aroma);

b) vanilla extracts (by carrying out the operations directly on the extract) (clause A.4, from A.4.4 onwards).

A.1 Principle

Extraction of the useful elements and examination for the main aromatic constituents of vanilla by thin-layer chromatography.

Detection of aldehydes by hydrazine sulphate, of alcohols and phenolic acids by diazotized *p*-nitroaniline, and of anisic alcohol by concentrated sulphuric acid.

A.2 Reagents

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

A.2.1 Elution solvents

A.2.1.1

Toluene	90 volumes
Dioxane	25 volumes
Acetic acid	4 volumes

A.2.1.2

Propanol, 1 mol/l solution	3 volumes
Ammonium hydroxide solution, $c(NH_4OH) = 8$ mol/l	1 volume

A.2.1.3

Chloroform	9 volumes
Ethyl acetate	1 volume

A.2.2 Reference solutions

A.2.2.1 Reference solution I

Vanillin	0,1 g
p-Hydroxybenzaldehyde	0,1 g
Anisic aldehyde	0,1 g
Protocatechic aldehyde	0,1 g
Piperonal	0,1 g
Ethyl vanillin	0,1 g
95 % (*V*/*V*) ethanol	To make up the volume to 100 ml

A.2.2.2 Reference solution II

Vanillic acid	0,1 g
p-Hydroxybenzoic acid	0,1 g
Protocatechic acid	0,1 g
Ethyl vanillic acid	0,1 g
95 % (*V*/*V*) ethanol	To make up the volume to 100 ml

A.2.2.3 Reference solution III

Vanillic alcohol	0,1 g
p-Hydroxybenzyl alcohol	0,1 g
95 % (*V*/*V*) ethanol	To make up the volume to 100 ml

A.2.2.4 Reference solution IV

Anisic alcohol	0,1 g
95 % (*V*/*V*) ethanol	To make up the volume to 100 ml

A.2.3 Indicators

A.2.3.1 Dingemans' reagent

Saturated aqueous solution of

Hydrazine sulphate	9 volumes
Hydrochloric acid, $c(HCl) = 4$ mol/l	1 volume

A.2.3.2 Diazotized *p*-nitroaniline

Prepare this reagent at the time of use.

Mix :

— 2 ml of a 0,5 % solution of *p*-nitroaniline in hydrochloric acid, $c(HCl) = 2$ mol/l,

— 3 to 4 drops of 5 % sodium nitrite solution,

— 8 ml of 20 % sodium acetate solution.

A.2.3.3 Potassium hydroxide, approximately 0,5 % ethanolic solution.

A.2.3.4 Sulphuric acid, concentrated.

A.2.4 Diethyl ether.

A.2.5 Ethanol, 95 % (*V*/*V*) solution.

A.2.6 Ethanol, 50 % (*V*/*V*) solution.

A.2.7 Anhydrous sodium sulphate.

A.3 Apparatus

Ordinary laboratory equipment, and in particular

A.3.1 Airtight grinder.

A.3.2 Extraction apparatus.

A.3.3 Separating funnel.

A.3.4 Reduced pressure evaporator.

A.3.5 Micropipettes, graduated at every 1 µl, of capacity 10 µl, or capillary tubes.

A.3.6 Silica plates, for chromatography, without fluorescence indicator.

A.3.7 Cellulose plates, for chromatography, without fluorescence indicator.

A.3.8 Cells, for ascending chromatography.

A.4 Procedure

A.4.1 Global extraction

Extract, in the extraction apparatus (A.3.2), about 5 g of vanilla, which has been cut into small pieces or ground, for 6 h, with 100 ml of the ethanol (A.2.6).

Evaporate the ethanolic solution using the evaporator (A.3.4), at low temperature, so as to expel the ethanol and to obtain about 25 ml of extract.

A.4.2 Elimination of resins

Add, to the vanilla extract obtained, three times its volume of water, and then add hydrochloric acid until the solution has a pH of approximately 2.

After precipitation of the resins, allow them to settle and centrifuge.

A.4.3 Extraction of aromatic constituents

Transfer the aqueous solution into a separating funnel (A.3.3).

Extract this solution with 50 ml of the diethyl ether (A.2.4). Separate the diethyl ether and collect in a second separating funnel.

Repeat this extraction operation twice, using 25 ml of diethyl ether each time.

Combine the diethyl ether solutions in the second separating funnel and wash with a few millilitres of water. Separate and reject the washing solution. Dry the diethyl ether solution over anhydrous sodium sulphate (A.2.7). Filter, evaporate at room temperature, in a glass dish, preferably anti-creeping, and then in air, under a hood.

Dissolve the residue in the ethanol (A.2.5) and make up to 10 ml, or to 50 ml if the product is highly concentrated.

A.4.4 Identification of aldehydes

By means of a micropipette (A.3.5), deposit 1 µl of the solution to be examined (or of commercial vanilla extract) on a silica plate (A.3.6), at a distance 2 cm from the lower edge.

Frame the spots to be examined with 2 µl of reference solution I (A.2.2.1).

Put the plate in the cell in such a way that the base of the plate is immersed in about 1 cm of the solvent (A.2.1.1).

Develop, and when the solvent front is about 12 cm above the starting line, remove the plate and allow it to dry in air.

Spray with the Dingemans' reagent (A.2.3.1).

The aldehydes appear in the form of yellow spots on the white background. However, if the *p*-hydroxybenzaldehyde content is low, the spot will be visible only under ultraviolet light as a green spot.

The results are shown in table 1.

Table 1 — Approximate R_f values and colouration of spots for aldehydes

Compound	R_f approximately	Colour in daylight	Colour in ultraviolet
Protocatechic aldehyde	0,12	Yellow	Yellow
p-Hydroxybenzaldehyde	0,46	Yellow	Green
Vanillin	0,64	Yellow	Yellow
Ethyl vanillin	0,75	Yellow	Yellow
Anisic aldehyde (*Vanilla tahitensis* exclusively)	0,87	Yellow	Light blue
Piperonal, according to species	0,87	Yellow	Yellow

NOTE — Examination for anisic aldehyde is useful for differentiating *Vanilla tahitensis* from *Vanilla fragrans*.

A.4.5 Identification of alcohols and phenolic acids

By means of the micropipette (A.3.5), deposit 5 µl of the solution to be examined on the cellulose plate (A.3.7). Frame the stains with 2 µl of reference solution II (A.2.2.2) and reference solution III (A.2.2.3).

Put the plate in the cell containing the solvent (A.2.1.2).

Develop, and when the solvent front is about 12 cm above the starting line, remove the plate. Allow to dry in air. Wait until the ammonium hydroxide has completely evaporated.

Spray with the diazotized *p*-nitroaniline (A.2.3.2). Allow to dry in air and then spray with the potassium hydroxide solution (A.2.3.3).

The results are shown in table 2.

A.4.6 Identification of anisic alcohol

Deposit 5 µl of the solution to be examined on the silica plate (A.3.6). Frame the stains with 5 µl of reference solution IV (A.2.2.4).

Put the plate in the cell containing the solvent (A.2.1.3).

Develop, and when the solvent front is about 12 cm above the starting line, remove the plate and allow the solvent to evaporate.

Develop with the sulphuric acid (A.2.3.4). Anisic alcohol appears in the form of a red stain on a white background at R_f 0,40.

A.5 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 incidents likely to have affected the results.

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

Table 2 — Approximate R_f values and colouration of spots for alcohols and phenolic acids

Compound	R_f approximately	Colour with diazotized *p*-nitroaniline	Colour with diazotized *p*-nitroaniline + potassium hydroxide
Protocatechic acid	0,07		Grey-blue
Vanillic acid	0,25	Yellow	Violet
p-Hydroxybenzoic acid (more abundant in *Vanilla tahitensis*)	0,31	Yellow	Red
Ethyl vanillic acid	0,39	Yellow	Violet
Vanillic alcohol	0,75	Yellow	Violet
p-Hydroxybenzyl alcohol	0,82	Yellow	Red

Annex B

Determination of vanillin content — Ultraviolet spectrophotometric method

(This annex does not form part of the standard.)

B.0 Introduction

This annex describes a method for the determination of the vanillin content of vanilla.

The method is applicable to vanilla powder obtained from the species of vanilla described in this International Standard.

B.1 Principle

Extraction, with ethanol, of the vanillin contained in a test portion.

Spectrophotometric determination of the vanillin in the ethanolic solution.

B.2 Reagents

The reagents used 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) solution, for UV spectrophotometry.

B.2.2 Sodium hydroxide solution, $c(NaOH) \approx 1$ mol/l.

B.2.3 Vanillin.

B.3 Apparatus

Ordinary laboratory equipment, and in particular

B.3.1 Airtight grinder.

B.3.2 Volumetric flasks, one-mark, of capacities 100 and 250 ml, complying with the requirements of ISO 1042.

B.3.3 Pipettes, to deliver 10, 20, and 25 ml.

B.3.4 Desiccator, containing an efficient desiccant.

B.3.5 Extraction apparatus.

B.3.6 Spectrophotometer, suitable for making measurements in the ultraviolet region of the spectrum.

B.3.7 Silica cells, for spectrophotometry, having optical path lengths of 1 cm.

B.3.8 Weighing bottle, with an airtight stopper, of capacity 25 ml.

B.4 Procedure

B.4.1 Determination of specific absorbance of vanillin

B.4.1.1 Preparation of standard solutions

In the weighing bottle (B.3.8) weigh, to the nearest 0,1 mg, about 30 mg of the vanillin (B.2.3), which has been previously dried in the desiccator (B.3.4). Dissolve in about 20 ml of the ethanol (B.2.1) and transfer quantitatively to a 250 ml volumetric flask (B.3.2). Rinse the weighing bottle several times with the ethanol and pour the washings into the volumetric flask. Make up to the mark with the ethanol and mix well (solution A1).

Pipette 25 ml of solution A1 into a 100 ml volumetric flask (B.3.2). Make up to the mark with the ethanol and mix well (solution B1).

Pipette 10 ml of solution B1 into a 100 ml volumetric flask. Add about 60 ml of the ethanol and 2 ml of the sodium hydroxide solution (B.2.2). Mix well. Make up to the mark with the ethanol and mix well (solution C1).

B.4.1.2 Preparation of reference solution

Prepare a reference solution by pipetting 2 ml of the sodium hydroxide solution (B.2.2) into a 100 ml volumetric flask, and making up to the mark with the ethanol. Mix well.

B.4.1.3 Determination

Record the spectrum of solution C1 relative to that of the reference solution (B.4.1.2) over the wavelength range 250 to 420 nm, using the spectrophotometer (B.3.6) and the cells (B.3.7).

B.4.1.4 Calculation

Maximum absorption occurs at 350 ± 3 nm and its absorbance should be between 0,2 and 0,8.

Draw a baseline from about 270 nm to 380 nm.