# INTERNATIONAL STANDARD

ISO 5496

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# Sensory analysis — Methodology — Initiation and training of assessors in the detection and recognition of odours

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ISO 5496 was prepared by Technical Committee ISO/TC 34, Food products, Subcommittee SC 12, Sensory analysis.

This second edition cancels and replaces the first edition (ISO 5496:1992), which has been technically revised.

# Introduction

Owing to the complexity of olfaction, assessors who are to make up panels need to undergo a familiarization and training process before undertaking any sensory analysis concerning the detection of odours.

This period of initiation, followed by training, is intended to teach assessors to evaluate and to identify odours, to teach them to use the appropriate vocabulary, and also to allow them to improve their individual aptitude.

This International Standard provides guidance on the existing techniques used for this purpose.

ares or striction in the full policy of the children in t At a later stage, organizers should direct the training according to the procedures or specific areas of use and, where necessary, make a selection of assessors on the basis of certain criteria.

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# Sensory analysis — Methodology — Initiation and training of assessors in the detection and recognition of odours

# 1 Scope

This International Standard describes several types of method for determining the aptitude of assessors and for training assessors to identify and describe odoriferous products.

The methods described in this International Standard are suitable for use by the agri-foodstuffs industries employing olfactory analysis (e.g. perfumery, cosmetics and aromatics).

#### 2 Normative references

The following referenced documents are indispensable for the application 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.

ISO 6658:2005, Sensory analysis — Methodology — General guidance

ISO 8589:— 1), Sensory analysis — General guidance for the design of test rooms

# 3 Principle

Presentation to the assessors of oddriferous substances in various forms and concentrations, in accordance with the procedures specified in this International Standard.

Assessment and identification by the assessors of the odours given off by these substances and recording of the results.

#### 4 Reagents and materials

- 4.1 Water, neutral, tasteless, still and odourless.
- **4.2 Ethanol**, 96,9 % (by volume), free from extraneous odours, even in low concentrations.
- **4.3** Other suitable media, appropriate to the requirements of the industry concerned.
- 4.4 Odoriferous substances, as pure as possible:
- a) substances chosen from those given in Table A.2, and used at the concentrations proposed, and/or
- b) any other substance deemed to be of interest, depending on the aim of the test or the requirements of the industry concerned.

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<sup>1)</sup> To be published. (Revision of ISO 8589:1988)

For the training phase, the collection of odours shall comprise odoriferous substances representative of several groups of odours (e.g. terpinic, floral) and substances which the assessors will examine (to determine that assessors have no anosmia for these substances).

It is also advisable to include odours representative of certain defects (e.g. odours typical of cleaning products, printing inks) which are likely to be encountered by the assessors in the forthcoming evaluations.

Odoriferous substances serving as references shall be chosen from among those having a stable composition and which can be stored for an acceptable length of time without deterioration. These substances shall be stored in a cool place (around +5 °C) and protected from air and light.

NOTE When in aqueous media, the aromatic power of certain substances increases with dilution.

# 5 General test conditions

#### 5.1 Test room

The tests shall be carried out in a room meeting the requirements specified in ISO 8589.

Special precautions shall be taken to remove odours from the test room as much as possible (e.g. by ventilation).

#### 5.2 General test rules

In addition to the general rules which apply to assessors involved in any sensory analysis and given in ISO 6658, the assessors participating in these tests shall not have carried out any other sensory analysis concerned with the detection or assessment of odours or odorferous compounds within the 20 min prior to the test.

To avoid tiring the assessors, it is recommended that no more than 10 odoriferous substances are presented to them per session.

#### 6 Methods

The olfactory assessment can be carried out by direct methods or by retro-nasal methods.

There are currently three direct methods <sup>2)</sup> of smelling, i.e.:

- assessement of odours in flasks (6.1.1);
- assessment of odours on smelling strips (6.1.2);
- assessment of encapsulated odours (6.1.3);

and two retro-nasal (or pharyngo-nasal) methods of smelling, i.e.:

- assessment of odours in the gaseous phase (6.2.1);
- assessment of odours by ingestion of aqueous solutions (6.2.2).

<sup>2)</sup> The assessment of odours using an olfactometer is not considered in this International Standard, as it is not used in initiation and training.

#### 6.1 Direct methods of smelling

#### 6.1.1 Method of assessing odours in flasks

#### 6.1.1.1 Principle

Presentation to the assessors of a series of flasks containing different odoriferous substances at given concentrations.

#### 6.1.1.2 Materials

**6.1.1.2.1** Odoriferous substances, chosen for example from Table A.2, at the specified dilution.

#### 6.1.1.3 Apparatus

**6.1.1.3.1 Individual tinted glass flasks**, of sufficient capacity to hold the products to be tested (generally between 20 ml and 125 ml) and to leave sufficient head space to permit equilibrium of the vapour pressure, equipped with unlubricated ground-glass stoppers.

Alternatively, **beakers**, fitted with a watch-glass, or suitable **disposable containers**, sold commercially. If plastics apparatus is used, it is essential to check that is made of an odour-free material which does not absorb odours and which has no chemical affinity with the substances under test.

#### 6.1.1.4 Preparation of samples

Where necessary, prepare, in accordance with the instructions given in A.2, suitable dilutions of the substances used to obtain the appropriate concentrations given in Table A.1.

Prepare the samples at least 30 min before the test, to allow time for the vapour pressure to reach equilibrium at ambient temperature, as follows.

Code the flasks and stoppers.

Place the appropriate quantities of the substances prepared in the coded flasks, taking care to leave sufficient head in the flasks.

The substances are poured directly into the flasks, placed on a medium (e.g. cotton or absorbent paper) which is already in the flasks, or blended with a medium (e.g. fat).

Close the flasks with the glass stoppers or watch-glasses.

#### 6.1.1.5 Procedure

Present to each assessor, the series of flasks prepared. Instruct the assessor to carry out the evaluation as follows.

The assessor opens the flasks one by one and, with the mouth closed, sniffs the vapour phase in order to identify each odoriferous product. There is no strict methodology, provided that the assessor smells all the flasks at suitable intervals in the same way, e.g. in short sniffs, or deep breaths, etc. Once a decision has been made, the assessor closes the flask and replies to the questions on the answer form (see Clause 7). (See the specimen answer form in Annex B.)

NOTE Depending on whether the assessors are undergoing the initiation phase or the training or selection phase, they may or may not be permitted to smell each product several times, or to return to previously examined flasks.

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#### 6.1.1.6 Interpretation of results

Interpret the results in accordance with Clause 8.

#### 6.1.2 Method of assessing odours on smelling strips

#### 6.1.2.1 **Principle**

Presentation to the assessors of a series of smelling strips impregnated with odoriferous substances.

#### 6.1.2.2 **Materials**

Odoriferous substances, chosen for example from Table A.2, at the specified dilution 6.1.2.2.1

#### 6.1.2.3 **Apparatus**

Smelling strips, i.e. small strips of filter-paper, of variable porosity depending on the 6.1.2.3.1 manufacturer, and of varius shapes (rounded, bevelled, etc.) 3), with a mark at the bottom between 50 mm and 100 mm from the end.

6.1.2.3.2 Strip-holders or tweezers, made of an odourless material.

Tinted glass flasks, of suitable capacity for holding the odoriferous substances (one flask per 6.1.2.3.3 substance).

6.1.2.3.4 **Droppers** (optional).

#### 6.1.2.4 Preparation of samples

Prepare, in accordance with the instructions given in A.2, stock solutions of the substances used.

Prepare one substance at a time and place it in a flask.

Rapidly dip the bottom ends of the strips (61.2.3.1) (for each of the assessors) one by one, in the flask up to the mark or, preferably, using a dropper (6.1.2.3.4), place a drop of the substance on the bottom end of each strip.

The strip shall not be over-impregnated with solution; the migration front of the liquid shall be between 5 mm and 10 mm from the bottom end of the strip.

Place the prepared strip on the strip-holder or pick it up with the tweezers (6.1.2.3.2), taking care that the strips do not come into contact with each other. Leave the strips for a few seconds to allow any evaporation of the solvent to occur

#### 6.1.2.5 **Procedure**

Hand the prepared strips to the assessors and instruct them to proceed as follows.

The assessor performs the assessment of the odour by sniffing the smelling strip, waving it gently a few centimetres from his/her nose. The strip shall under no circumstances touch the nose, a moustache or the skin.

NOTE Because of evaporation, the odour is only fully released for a limited period of time, depending on the odoriferous substance.

<sup>3)</sup> The names of suppliers can be obtained from manufacturers of aromatic products.

Once a decision has been made, the assessor discards the strip and replies to the questions on the answer form (see Clause 7). (See the specimen answer form in Annex B.)

It is essential that the strips are collected and disposed of after use in a sealed container, so as not to saturate the atmosphere of the test room and thus interfere with subsequent assessments.

The assessor then goes on to examine the next substance.

#### 6.1.2.6 Interpretation of results

Interpret the results in accordance with Clause 8.

#### 6.1.3 Method of assessing encapsulated odours

#### 6.1.3.1 Principle

Presentation to the assessors of a series of micro-encapsulated odoriferous substances.

#### 6.1.3.2 Materials

**6.1.3.2.1** Odoriferous substances, chosen for example from Table A.2, at the specified dilution.

#### 6.1.3.3 Apparatus

**6.1.3.3.1 Encapsulated odoriferous substances**, available commercially<sup>4)</sup>, such as encapsulated odours on paper supports which are to be sratched, or on labels which are to be torn, etc.

#### 6.1.3.4 Preparation of samples

No preparation is required, since the samples are ready for use.

#### 6.1.3.5 Procedure

Present the samples, one at a time, to the assessors and instruct them to proceed as follows.

The assessor follows the manufacturer's instructions for releasing the odoriferous substance. The odour is then judged by the assessor using the same procedure as for the smelling strip method (see 6.1.2.5).

NOTE With this presentation, re-assessments of the odours are not possible.

Once a decision has been made, the assessor discards the questions on the answer form (see Clause 7). (See the specimen answer form in Annex B.)

It is esseptial that the microcapsules are collected and disposed of after use in a sealed container, so as not to saturate the atmosphere of the test room and thus interfere with subsequent assessments.

The assessor then goes on to examine the next substance.

#### 6.1.3.6 Interpretation of results

Interpret the results in accordance with Clause 8.

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<sup>4)</sup> Series of ready-to-use micro-encapsulated odours are available commercially, but some manufacturers can provide these microcapsules to order. It should, however, be noted that this type of presentation is at present fairly expensive.

#### Retro-nasal methods of smelling

#### 6.2.1 Method of assessing odours in gaseous phase<sup>5)</sup>

#### 6.2.1.1 **Principle**

Assessment of odoriferous substances in the gaseous phase by inhalation of the gaseous phase into the buccal cavity and assessment of the odour by retro-nasal means.

#### 6.2.1.2 **Materials**

Odoriferous substances, chosen for example from Table A.2, at the specified dilution. 6.2.1.2.1

#### 6.2.1.3 **Apparatus**

The apparatus shall be made of glass (preferably) or plastics material. If plastics apparatus is used, it is withe full PDF of 15 essential to check that it is made of an odour-free material which does not absorb odours and which bears no chemical affinity with the substances under test.

- 6.2.1.3.1 Beakers, of minimum capacity 100 ml.
- 6.2.1.3.2 Plastics films, odourless and non-absorbent.
- 6.2.1.3.3 Straws

#### 6.2.1.4 Preparation of samples

Where necessary, prepare, in accordance with the instructions given in A.2, suitable dilutions of the substances used to obtain the appropriate concentrations recommended in Table A.1.

Prepare one substance at a time.

Place 50 ml of the prepared dilutions in the beakers and cover with plastics film.

#### 6.2.1.5 **Procedure**

Give the beakers, one at a time, to the assessors and instruct them to proceed as follows.

The assessor pierces the plastics film with the straw. The assessor then inhales through the mouth the gaseous atmosphere above the liquid and exhales heavily through the nose. Under no circumstances shall the straw touch the liquid if this happens by accident, give the assessor another beaker.

The assessor identifies the odour and replies to the questions on the answer form (see Clause 7). (See the specimen answer form in Annex B.)

The assessor then goes on to assess the next substance.

#### 6.2.1.6 Interpretation of results

Interpret the results in accordance with Clause 8.

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<sup>5)</sup> There is another method for assessing odoriferous substances in the gaseous phase, which consists of placing smelling strips impregnated with the odoriferous substances inside the buccal cavity. This method is, however, difficult to implement with novice assessors and should be reserved for use with trained panels. It is described in Annex C for information.

#### 6.2.2 Method of assessing odours by ingestion of aqueous solutions

#### 6.2.2.1 Principle

Presentation to the assessors of a series of beakers containing different odoriferous substances.

Assessment of the retro-nasl olfactory sensation produced by the substances following ingestion<sup>6)</sup>.

#### 6.2.2.2 Materials

**6.2.2.2.1 Odoriferous substances**, food grade, chosen for example from Table A.2, at the specified dilution.

#### 6.2.2.3 Apparatus

**6.2.2.3.1 Individual beakers**, fitted if possible with a **lid** and a **straw**.

#### 6.2.2.4 Preparation of samples

Where necessary, prepare, in accordance with the instructions given in A.2, suitable dilutions of the substances used to obtain the appropriate concentrations recommended in Table A.1.

NOTE In this method, the solutions are far less concentrated than in the direct smelling method.

Pour the dilutions into the beakers and fit the lid if used.

#### 6.2.2.5 Procedure

Present, to each assessor, the series of beakers prepared and instruct them to proceed as follows.

If the beakers are not covered, the assessor pinches his/her nose, drinks a mouthful of the solution and then releases the nose when the solution is in the mouth, as soon as the beaker has been removed. The assessor swallows the solution. The odour is assessed during the subsequent expiration. If beakers with lids and straws are used, it is not necessary for the assessor to pinch his/her nose.

The assessor thus makes the assessment of the odour by retro-nasal means.

Once a decision has been made, the assessor replies to the questions on the answer form (see Clause 7). (See the specimen answer form in Annex B.)

#### 6.2.2.6 Interpretation of results

Interpret the results in accordance with Clause 8.

#### 7 Answer form

The following questions appear on the answer form.

Do you perceive an odour?

Do you recognize this odour?

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<sup>6)</sup> Under normal conditions of consumption, a mouthful of product also permits the flavour as a whole to be assessed.

The assessor is also asked to name or describe the odour or to make an association.

In addition, it is useful to leave a space for remarks by the assessors.

NOTE The answer form may be in printed form (as shown in the example in Annex B) or in electronic form.

### 8 Interpretation of results

The results are interpreted in differents ways by the test supervisor, depending on the aim of the test and whether the tests are for the purpose of instruction (or initiation), training or selection of assessors.

The requirements regarding the correct designation of the substances also depend on the purpose of the test.

The correct reply to the instruction to name or describe the odour or to make an association, varies as follows, depending on the case:

- a) at the introductory stage, the reply may be the chemical name (where this is known), the common name, or an association or even an appropriate descriptive expression;
- b) at the training or selection stage, the reply may be the chemical name or the appropriate descriptor.

#### 8.1 Initiation

Once the assessors have recorded their evaluations on the answer form, the test supervisor shall call them together and give the results, designating each substance by its chemical name or descriptor.

The supervisor shall make the substances available to the assessors and reply to any questions raised, in order to help them to memorize the association between the chemical substance and the corresponding odour.

Several sessions are required for teaching the assessors to recognize a large number of odours.

There is no marking of the assessors, but the supervisor may, at this stage, make observations regarding suspected cases of anosmia.

#### 8.2 Training

At the training stage, the test supervisor analyses the answer forms, examining the replies of each assessor.

In this training phase, the assessors shall identify the substance by its chemical name or descriptor.

Following repeated assessments, the progress in the performance of each assessor and the effectiveness of the training shall be judged.

#### 8.3 Selection

The information on performance gained during the training period shall form the basis for the test supervisor to eliminate those assessors who have made repeated errors.

The information can also be used to set up specialized groups adapted to various specific problems.

# Annex A

(normative)

# Preparation of dilutions of odoriferous substances

# A.1 Apparatus

011505496.2006 **A.1.1 Glass pipettes**, preferably disposable, for the preparation of the dilutions.

# A.2 Preparation of dilutions

#### A.2.1 Preparation of the stock solution (SS)

Take 1 g of the substance and make up to 100 g with the ethanol (4.2).

# A.2.2 Preparation of the working solution (WS)

Take 1 g of the stock solution (SS) and make up to 100 g with the ethanol (4.2).

# A.2.3 Preparation of further dilutions

See Table A.1.

# A.3 Examples of odoriferous substances that can be used for training in the detection and recognition of odours

See Table A.2.

Table A.2 also gives the number of dilutions to be used, depending on the method of preparation of the samples.

Table A.1 — Preparation of dilutions

Dilution No.	•	Concentration g/l	
1	0,1 g	of working solution (WS) made up to 1 l of water	10 <sup>-5</sup>
2	0,5 g	of working solution (WS) made up to 1 l of water	5 × 10 <sup>-5</sup>
3	1 g	of working solution (WS) made up to 1 l of water	10 <sup>-4</sup>
4	5 g	of working solution (WS) made up to 1 I of water	5 × 10 <sup>-4</sup>
5	10 g	of working solution (WS) made up to 1 l of water	10 <sup>-3</sup>
6	50 g	of working solution (WS) made up to 1 l of water	$5 \times 10^{-3}$
7 <sup>a</sup>	1 g	of stock solution (SS) made up to 1 I of water	10 <sup>-2</sup>
8 a	5 g	of stock solution (SS) made up to 1 I of water	5 × 10 <sup>-2</sup>

Table A.2 — Examples of odoriferous substances that can be used for training in the detection and recognition of odours

				Dilution	No. from Ta	ible A.1 to k	e used <sup>c</sup>
No.	Chemical name or	Molecular			method	Retro-nasal method	
	abbreviation <sup>a</sup>	formula <sup>b</sup>	association	Flasks	Smelling strips	Gaseous phase	Ingestion
1	D-Limonene	C <sub>10</sub> H <sub>16</sub>	lemon, orange zest	6	SS	7	5
2	Citral (geranial + neral)	C <sub>10</sub> H <sub>16</sub> O	fresh, lemon	5	SS	6	4
3	Geraniol	C <sub>10</sub> H <sub>18</sub> O	rose	5	SS	6	4
4	cis-3-Hexen-1-ol	C <sub>6</sub> H <sub>12</sub> O	crushed grass	6	SS	7	<b>6</b> 5
5	Benzaldehyde	C <sub>7</sub> H <sub>6</sub> O	bitter almond, marzipan	6	SS	7	5
6	Butyric acid	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	rancid butter, cheesy (e.g. over-aged Parmesan), sour milk	5	SS	546	4
7	Ethyl butanoate	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	very ripe banana, strawberry	4	SS	5	3
8	Benzyl acetate	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>	floral, lily of the valley, jasmin, lilac	5	SS	8	6
9	$\gamma$ -Undecalactone	C <sub>11</sub> H <sub>20</sub> O <sub>2</sub>	fruity, peach	6	SS	7	5
10	2-Phenylethanol	C <sub>8</sub> H <sub>10</sub> O	floral, rose	<b>8</b>	SS	8	7
11	Methyl anthranilate	C <sub>8</sub> H <sub>9</sub> O <sub>2</sub>	orange blossom	4	SS	5	3
12	Ethyl phenyl acetate	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	apricot, honey	4	SS	5	3
13	Anethole	C <sub>10</sub> H <sub>12</sub> O	aniseed-flavoured beverages	3	SS	4	2
14	Cinnamaldehyde	C <sub>9</sub> H <sub>8</sub> O	cinnamon	6	SS	7	5
15	Vanillin	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub>	vahilla	5	SS	6	4
16	L-Menthol	C <sub>10</sub> H <sub>20</sub> O	peppermint d	6	SS	8	6
17	Terpinyl acetate	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	spicy, pine	4	SS	5	3
18	Thymol	C <sub>10</sub> H <sub>14</sub> O	spicy, fresh thyme	4	SS	5	3
19	Diacetyl	C <sub>4</sub> H <sub>6</sub> O <sub>2</sub>	butter	4	SS	4	4
20	$\gamma$ -Nonalactone	C <sub>9</sub> H <sub>16</sub> O <sub>2</sub>	coconut	4	SS	4	4
21	Eugenol	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	clove	4	SS	5	3
22	1-Octen-3-0	C <sub>8</sub> H <sub>16</sub> O	mushroom, forest damp soil	4	SS	5	3
23	eta-lonone	C <sub>13</sub> H <sub>22</sub> O	violet	4	SS	4	4
24	Methional	C <sub>4</sub> H <sub>8</sub> OS	mashed potato, grilled onion	5	SS	6	4

a It is necessary to use products which are as pure as possible, since impurities can modify the nature and intensity of the odour.

b See the detailed formulae in Table A.3.

<sup>&</sup>lt;sup>c</sup> The concentrations specified have been chosen after practical tests with all the substances given in the table using panels of inexperienced assessors. The concentrations chosen correspond to the recognition threshold of 70 % of the assessors.

d Also produces a sensation of cold.

Table A.3 — Detailed formulae of odoriferous substances

No.	Chemical substance	Detailed formula
1	D-Limonene	3
2	Citral (geranial + neral)	СНО
3	Geraniol Gick to view to	CH <sub>2</sub> OH
4	cis-3-Hexen-1-ol	CH <sub>2</sub> OH
5	Benzaldehyde	СНО
6	Butyric acid	CH <sub>3</sub> -CH <sub>2</sub> -CH <sub>2</sub> -COOH
7	Ethyl butanoate	CH <sub>3</sub> -CH <sub>2</sub> -CH <sub>2</sub> -COO-CH <sub>2</sub> -CH <sub>3</sub>
8	Benzyl acetate	CH₂COOCH₃
9	γ-Undecalactone	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>5</sub> CH <sub>2</sub> OO
10	2-Phenylethanol	CH <sub>2</sub> CH <sub>2</sub> OH

Table A.3 (continued)

No.	Chemical substance	Detailed formula
11	Methyl anthranilate	$NH_2$ $COOCH_3$
12	Ethyl phenyl acetate	CH2COOCH2CH36
13	Anethole	H <sub>3</sub> CO CH=CHCH <sub>3</sub>
14	Cinnamaldehyde	сн=снсно
15	Vanillin	CHO OCH3
16	STANDARDI-Menthol	ОН
17	Terpinyl acetate	AcO OAc

Table A.3 (continued)

No.	Chemical substance	Detailed formula
18	Thymol	Q E
19	Diacetyl	H <sub>3</sub> CCO-COCH <sub>3</sub> O O II II H <sub>3</sub> C-C-CH <sub>3</sub>
20	$\gamma$ -Nonalactone	CH <sub>3</sub> (CH <sub>2</sub> ) O O
21	Eugenol click to view t	$CH_2CH = CH_2$ $OCH_3$
22	1-Octen-3-ol	OH OH
23	β-lonone	
24	Methional	CH <sub>3</sub> -S-CH <sub>2</sub> CH <sub>2</sub> CHO