Sensory Analysis

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Key words: *Flavor reference standards, Triangular test.*

CONCLUSIONS

1. The subcommittee has added five reference standards, bringing the total to 27. A publication is in preparation.
2. The subcommittee has determined that the Triangular, the Duo-trio, and the Two-out-of-five tests are forced-choice procedures in which "no-difference" verdicts have no place. Panelists should understand that the response being sought is the percentage incorrect replies. This is in contrast to the Paired Comparison method in which panelists who are uncomfortable with a forced-choice procedure should be allowed to submit "no-difference" verdicts.

RECOMMENDATIONS

1. Adopt as international flavor reference standards the compounds listed in Table I, which includes the previously accepted standards.
2. Adopt the Triangular test procedure for inclusion in ASBC "Methods of Analysis."
3. In collaboration with the organizations listed below, continue the study of methods of sensory analysis, notably the Duo-trio and the Two-out-of-five tests, and complete the "Methods of Analysis" section on general methodology and choice of methods.
4. As soon as the Triangular test procedure is issued, discontinue the current BEER-30, Testing for Taste Difference Between Two Beers.

TABLE I
Compounds Recommended for Use as Flavor Reference Standards

Term		Compound	Supplier	Method of Purification	Difference Threshold[a]	In Beer Containing
0110	Alcoholic	Ethanol	High-quality vodka[b]	None required	17 g/L	33–42 g/L
0111	Spicy	Eugenol	Aldrich	Solvent wash + fractional distillation + adsorption	40 μg/L	···
0131	Isoamyl acetate	Isoamyl acetate	Aldrich	Adsorption + GC	0.5–1.7 mg/L	1–3 mg/L
0132	Ethyl hexanoate	Ethyl hexanoate	K & K Laboratories	Adsorption + GC	0.15–0.25 mg/L	0.2–0.4 mg/L
0133	Ethyl acetate	Ethyl acetate	Fluka	Adsorption	20–40 mg/L	10–30 mg/L
0145	Melony	Melonal®[c]	Givaudan	None required	1 μg/L	···
0150	Acetaldehyde	Acetaldehyde	Merck	Adsorption + distillation + adsorption	10–20 mg/L	2–10 mg/L
0162	Geraniol	Geraniol	Merck	Use fresh supply	~150 μg/L[g]	0–60 μg/L
0173	Hop oil	Cluster hop oil[d]	S. S. Steiner	None required	0.1 mg/L	···
0224	Almond	Benzaldehyde	Aldrich	None required	1 mg/L	···
0611	Caprylic	Octanoic acid	Sigma	Recrystallization of calcium salt	5–10 mg/L	2–8 mg/L
0613	Isovaleric	Isovaleric acid	Sigma	None required	0.5–1.5 mg/L	0.5–1.5 mg/L
0.614	Butyric	Butyric acid	Merck	2 × Fractional distillation	2–3 mg/L	0.5–1.5 mg/L
0620	Diacetyl	2,3-Butanedione	Aldrich	Fractional distillation + adsorption	0.07–0.15 mg/L	0.03–0.3 mg/L
0710	Sulfitic	Sodium meta-bisulfite	Fisher Scientific	None required	20 mg/L SO_2	1–10 mg/L SO_2
0721	H_2S	Sodium sulfide	Mallinckrodt	Select colorless crystals	4 μg/L H_2S	0–2 μg/L
0722	Mercaptan	Ethanethiol	Aldrich	None required	1 μg/L	0–0.5 μg/L
0732	DMS	Dimethyl sulfide	Matheson, Coleman and Bell	Adsorption	25–50 μg/L	30–100 μg/L
0841	Earthy	Geosmin	Nat'l Environment Research Center	None required	0.1 μg/L	···
0841	Earthy	2-Ethyl fenchol	PFW, Inc.	None required	5 μg/L	···
0910	Acetic	Acetic acid	J. T. Baker "Ultrex"	None required	60–120 mg/L	30–200 mg/L
1000	Sweet	Sucrose	Grocery	None required	2.6 g/L	···
1003	Vanilla	Vanillin	Fluka	None required	40 μg/L	0–10 μg/L
1100	Salty	NaCl	Grocery	None required	0.6 g/L	···
1200	Bitter	Isohumulone	Kalsec "Isolone"[e]	None required	7–15 mg/L	0–30 mg/L
1330	Metallic	$FeSO_4 \cdot 7H_2O$	J. T. Baker	None required	1 mg/L Fe	0–0.5 mg/L
1340	Astringent	Quercitrin[f]	K & K Laboratories	Recrystallization from 50% ethanol	80 mg/L	···

[a] The standard recommended addition for reference purposes is three times the threshold.
[b] Smirnoff or equivalent. Strength varies with locality and the vodka must be analyzed before use. Addition to beer should be by weight, not volumetrically.
[c] Trade name for 2,6-Dimethyl-5-hepten-1-al. Store under refrigeration.
[d] Not a reference standard; recommended for demonstration purposes.
[e] A solution of varying strength, usually 17%.
[f] Quercitrin is both astringent and bitter.
[g] Thresholds of geraniol added to beer show a distribution with two maxima at 18 μg/L (35% of persons studied) and 350 μg/L (65%). Recommended addition for reference purposes = 1 mg/L.

0361-0470/81/03010804/$03.00/0

As in previous years, this subcommittee was charged with developing a system of flavor reference standards to go with the previously published system (3) of flavor terminology. A second duty was to write a set of standard methods for sensory analysis of beer. In both of these projects, the subcommittee collaborated with the International Organization for Standardization (ISO), the American Society for Testing and Materials (ASTM), the Institute of Food Technologists (IFT), and the European Brewery Convention (EBC).

FLAVOR REFERENCE STANDARDS

The subcommittee encountered increasing lack of interest in the purification and testing of reference standards, and although the terminology (3) has 122 terms and many more standards would be desirable, it was decided to discontinue this project. The study of five standards was completed: geraniol (Aldrich) for 0162 Geraniol; sodium sulfide (9 H_2O) (Mallinckrodt) for 0721 H_2S; ethyl mercaptan (Aldrich, 97%) for 0722 Mercaptan; vanillin (Fluka) for 1003 Vanilla; and ferrous sulfate (7 H_2O) (J. T. Baker) for 1330 Metallic.

Twenty-one compounds, which were under consideration as standards when the project was discontinued, will be reconsidered if interest should reappear; a list is available from the chairman. Table I lists 27 compounds that are now recommended as standards. A report giving methods of purification, distribution of thresholds in the population, and other particulars, will be published in this *Journal.*

TRIANGULAR TEST

Publication of this well-known and much-used method was delayed by international disagreement whether "no-difference" verdicts should be permitted. There is no disagreement that the statistical treatment is simpler if a forced-choice procedure is employed, but many assessors object to having to invent a difference when they find that none exists. At the time of writing, the ISO still permits "no-difference" verdicts in all tests. The IFT, the ASTM, and this subcommittee have agreed, however, that the panelist's objection is only justified in the Paired Comparison test and in preference tests. In contrast, the Triangular, the Duo-trio, and the Two-out-of-five tests were originally conceived as forced-choice test situations and should be kept as such. Members have found that assessors readily accept this when it is explained that a proportion of incorrect responses is normal, and that this proportion is what the test attempts to determine.

Although the Triangular test has been the most used of all sensory tests in the brewery laboratory, subcommittee members agree that the method suffers from two drawbacks. One is simple fatigue which is greater with three samples than with two. The other is often described as "psychological complications." The interval between the first and third sample is, of necessity, so long that a complex flavor impression can no longer be carried in short-term memory. The assessor is forced to memorize and attempt to commit the flavor impressions to longer-term memory. But the assessor does not know which flavor notes may prove to be the important ones. As mentioned above, there may be over 100 separately identifiable flavor notes in a sample of beer, and it is more than likely that the assessor finds the need to retaste, and retaste again, because of a deficiency in memory of those flavor notes that might have shown a difference. Accordingly, in many situations, the Triangular test is less efficient than other methods. For a discussion, see Amerine et al (1).

Currently the subcommittee is attempting to define the optimal area of application for various difference tests. For example, it has been confirmed that the Duo-trio is the more efficient test whenever a product familiar to the assessors (such as the company's standard product) can be used as the reference, provided the panel has more than eight members. If it has eight or less members, the Triangular test is the more efficient, but such small panels should not be used if possible, as the results lack reproducibility. If a difference to be studied is well known to the assessors (eg, sweetness or bitterness), then the Paired Comparison test is most efficient. The Triangular test is especially suited for selection and training of assessors, and is the method of choice when other difference tests do not apply (eg, when both samples are unfamiliar).

A common error in the Triangular test is to assume that if there is no significant difference, then the samples have the same flavor. This is incorrect: if nonrecognition is to be established, a different null hypothesis is required. It becomes necessary to determine arbitrarily what percentage of the panel must be unable to taste a difference (eg, 70, 80, or 90%). A triangular test for nonrecognition was proposed by Ferdinandus et al (2) and formed part of early versions of the present text, but it was found too bulky and complex and is now given as a reference (see Appendix).

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APPENDIX

SENSORY ANALYSIS: TRIANGULAR TEST

Scope

Use this test to determine whether a sensory difference is apparent between two samples (see Note).

The method described is the simple Triangular test which uses a forced-choice, unilateral test situation.

Field of Application

Apply the test to any two samples of beer, wort, water, etc., whether the difference(s) to be expected involves all sensory attributes or one specific attribute (odor, sweetness, etc.).

Although statistically efficient, the method is somewhat limited in application because of psychological complications and sensory fatigue. Apply the method in particular when the number of assessors is limited (eg, 6, 7, or 8)[1]; when there are no problems of sensory memory or sensory fatigue (eg, visual comparison); to select, train, and monitor the performance of assessors; and when other difference tests do not apply.

Principle of the Test

Simultaneous or successive presentation to the assessors of a set of three samples, two of which are identical. After tasting, assessors

[1] A wider selection of methods apply to panels above eight members, see SENSORY ANALYSIS-3: Choice of Method (in preparation).

NAME ..... DATE ..... (year/month/day)
PRODUCT SUBMITTED TO TEST .....
PROBLEM: Three samples are presented to you; circle the number of that which is different from the other two.
SET OF THREE SAMPLES: ... ...
Comments

Fig. A-1. Specimen answer form for the Triangular test.

designate in writing the sample perceived to be different and the results are interpreted.

General Test Requirements

Test Room. Conduct the tests in a room that complies with the requirements in SENSORY ANALYSIS-2; Test Room, Equipment, Conduct of Test.

Assessors. Choose the number of assessors with reference to the degree of difference expected between the samples, the aptitude of the assessors, and the risk of error that can be accepted (eg, 5, 1, or 0.1%).

As a general rule, the minimum required is six assessors, but discrimination is much improved if 20, 30, or a larger number can be employed.

Preliminary Discussion and Test. Ascertain that assessors are fully familiar with any particular characteristic studied and with the mechanics of the test. Arrange, if necessary, a preliminary general discussion concerning the test problem and nature of samples. Direct such discussion in a manner that cannot influence subsequent judgments.

Present and discuss a few samples typical of the series to be analyzed. Limit the number to two or three. If the test concerns the detection of off-flavors, include in the preliminary test a sample free from any off-flavor, or a demonstration of the off-flavor to be detected.

In general, inclusion of controls (reference substances) may be advisable.

Procedure

Preparation of Samples. Take care that assessors cannot draw conclusions about the nature of samples from the way in which they are offered. Use colored glasses. Serve samples at uniform temperature; 12°C is suitable for full perception of flavor. Ascertain that the temperature of each sample in a triangle is identical, also that of samples offered in succession. This applies not only at the time of presentation but also throughout the tests. Code glasses using three-digit random numbers or key the placement of the glasses in advance.

Test Portion. A suitable quantity is 50–100 ml in a 250-ml (8-oz) glass.

Test Technique. Offer the three samples simultaneously for tasting. Prepare equal numbers of the six possible combinations ABB, BAA, AAB, BBA, ABA, and BAB and allocate them at random among the assessors. Instruct assessors to examine sets in a specified order (eg, always from left to right) leaving, however, the opportunity of making repeated tests of any sample while tasting of a set is in progress.

An example of an answer form is shown in Fig. A-1. Space for several triangles may be provided, but do not add supplementary questions (eg, asking the degree or type of difference or the assessor's preference) as these may introduce bias. Choose the appropriate separate test for each separate question.

Expression and Interpretation of Results

Total the number of correct replies and refer to Table A-I. A

TABLE A-I
Minimum Numbers of Correct Replies to Establish Significance at Various Probability Levels for the Triangular Test (Unilateral, $p = 1/3$)[a]

Number of Replies	Minimum Number of Correct Replies for a Significance Level of $\alpha =\leqslant$			Number of Replies	Minimum Number of Correct Replies for a Significance Level of $\alpha =\leqslant$			Number of Replies	Minimum Number of Correct Replies for a Significance Level of $\alpha =\leqslant$		
	0.05	0.01	0.001		0.05	0.01	0.001		0.05	0.01	0.001
5	4	5	···	37	18	20	22	69	31	33	36
6	5	6	···	38	19	21	23	70	31	34	37
7	5	6	7	39	19	21	23	71	31	34	37
8	6	7	8	40	19	21	24	72	32	34	38
9	6	7	8	41	20	22	24	73	32	35	38
10	7	8	9	42	20	22	25	74	32	35	39
11	7	8	10	43	20	23	25	75	33	36	39
12	8	9	10	44	21	23	26	76	33	36	39
13	8	9	11	45	21	24	26	77	34	36	40
14	9	10	11	46	22	24	27	78	34	37	40
15	9	10	12	47	22	24	27	79	34	37	41
16	9	11	12	48	22	25	27	80	35	38	41
17	10	11	13	49	23	25	28	81	35	38	41
18	10	12	13	50	23	26	28	82	35	38	42
19	11	12	14	51	24	26	29	83	36	39	42
20	11	13	14	52	24	26	29	84	36	39	43
21	12	13	15	53	24	27	30	85	37	40	43
22	12	14	15	54	25	27	30	86	37	40	44
23	12	14	16	55	25	28	30	87	37	40	44
24	13	15	16	56	26	28	31	88	38	41	44
25	13	15	17	57	26	28	31	89	38	41	45
26	14	15	17	58	26	29	32	90	38	42	45
27	14	16	18	59	27	29	32	91	39	42	46
28	15	16	18	60	27	30	33	92	39	42	46
29	15	17	19	61	27	30	33	93	40	43	46
30	15	17	19	62	28	30	33	94	40	43	47
31	16	18	20	63	28	31	34	95	40	44	47
32	16	18	20	64	29	31	34	96	41	44	48
33	17	18	21	65	29	32	35	97	41	44	48
34	17	19	21	66	29	32	35	98	41	45	48
35	17	19	22	67	30	33	36	99	42	45	49
36	18	20	22	68	30	33	36	100	42	46	49

[a] The values in this Table were calculated from the exact formula: binomial law for parameter $p = 1/3$ with n repetitions (replies). When the number of replies is larger than 100, numbers of required correct replies may be obtained from the following formula based on the approximation binomial law by the normal law, with a maximum error equal to one unit: $X = 0.4714\, z\sqrt{n} + [(2n+3)/6]$ where $z = 1.64$ for $\alpha \leqslant 0.05$; 2.33 for $\alpha \leqslant 0.01$; and 3.10 for $\alpha \leqslant 0.001$ (4). The minimum number of correct replies is X if X is a whole number, or the next higher integer if X is not a whole number. Tables for significance levels other than those listed here may be found in references 2 and 4.

significant difference is established if the number in question is equal to or larger than that shown in the Table.

Any "no-difference" verdicts should be considered invalid. The Triangular test is a forced-choice technique; assessors should guess if necessary and should be informed that a proportion of incorrect replies is normal, and that this proportion is what the test attempts to determine.

Example

Test beer "B" is brewed using a new lot of malt, and the test supervisor wishes to know if it can be distinguished from control beer "A" taken from current production. A 5% risk of error is accepted and eight trained assessors are available. Twelve glasses of "A" and 12 glasses of "B" are prepared to make eight sets which are distributed at random among the assessors as follows: ABB, BAA, AAB, AAB, BBA, BBA, ABA, and BAB.

Six assessors correctly identify the odd sample. In Table A-I, the conclusion is that the two beers are different at the 5% level of significance.

Note

If the purpose is to prove similarity between two samples, the present test does not apply. A test of nonrecognition is required; see Ferdinandus et al (2).

Expression of Results

Make reference to this method and give the following information:

(a) Test problem
(b) Full identification of the samples
(c) Test parameters adopted and, in particular, the number of presentations of sets of three samples
(d) Whether or not reference substances were used
(e) Any other recommendations given during the test (eg, information relating to the type of beer)
(f) Number of tests and number of trained or untrained assessors
(g) Any test conditions differing from the recommendations given in this method
(h) The results, with their probability levels
(i) Date and time of test
(j) Name of test supervisor

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