Reference Standards for Beer Flavor Terminology System[1]

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The following is a report of studies carried out by the ASBC and EBC Subcommittees on Sensory Analysis.

ABSTRACT

Twenty-seven reference standards are described for use with the International Beer Flavor Terminology. For each standard, sources of supply and methods of purification were selected that could be shown to lead to acceptable sensory purity. Flavor type and threshold for addition to beer were determined in two or more laboratories, and the distribution of thresholds among the tasters in at least one. A list is given of fifteen additional standards that received insufficient study.

Key words: *ASBC, EBC, Flavor, Reference standard, Taste tests, Terminology*

In 1979 (11), the Subcommittees on Sensory Analysis of the European Brewery Convention and the American Society of Brewing Chemists published the International Flavor Terminology System. As illustrated in Fig. 1, this has 14 classes, 44 first-tier terms and (not shown) 78 second-tier terms. Three basic principles of the system are relevant to the present discussion: each separately identifiable flavor characteristic has its own name; no terms are duplicated[2] for the same flavor characteristic; and the meaning of each term is illustrated with readily available flavor standards.

Harper (7) was the first to introduce a "flavor library" of chemical substances that could be used to illustrate various flavor terms. Clapperton's flavor library (5) consisted of solids, neat liquids, or substances dissolved in odorless nonyl phthalate and was presented in sniff bottles. Williams (15) proposed a set of 50 standards for the evaluation of cider and perry; the standards were made up in 20 g of molten paraffin wax allowed to solidify in a bottle. None of the three researchers attempted to purify or standardize the preparations.

The principal use of the present standards is in the training and testing of members of sensory panels. Work on the selection and testing of standards commenced in 1975, and progress reports appeared in 1980 and 1981 (1,2). It soon became clear that sniff bottles, despite their convenience and reuseability, gave poorer recognition, more errors, and less precise flavor descriptions than did a system of standards dissolved in beer. A system of purification became necessary, as several compounds were found to be misflavored on receipt and to vary among lots.

COMMUNICATION OF SENSORY INFORMATION

The difficulty in effectively communicating to another person the flavor notes present in a food or beverage is perhaps not appreciated. Whereas the basic standards of sight and hearing are taught in elementary school, those of smell and taste are acquired by chance. Semantic misconceptions abound: O'Mahony et al (12) found that one out of seven university students typically will describe citric acid as "bitter," whereas one out of 12 students will find quinine sulfate "sour." Once instructed, however, nearly all learn to use the generally accepted terms.

Another obstacle is the need to find and use a narrow range of concentrations in any demonstration. For comparison, impressions of sight and hearing are instantaneous; the receptors can handle variations of six or seven orders of magnitude, and in testing situations any accidental overdose is easily turned down. The flavor impression, however, must reach and remain for some seconds at the gustatory or olfactory epithelium, which can handle at most two or three orders of magnitude, and sensory fatigue sets in rapidly; any overdose blinds the sensors for minutes or hours.

Visual or auditory aspects of an object can usually be separated in space or time, whereas a flavor note must generally be judged in a mixture with several other notes. As a result, instances have been recorded (1) in which panelists failed to detect a standard added at a concentration 10–100 times above their personal threshold because of unfamiliarity with its flavor. To be successful, therefore, a flavor demonstration requires a large amount of preparation, care, and subsequent control of what the subject perceived.

Any system of standards must account for the variations between individuals. Studies by the ASBC subcommittee (4) have shown that if panels are sufficiently large (eg, 16–25 or more), the most sensitive 10% of panelists will have thresholds approximately four times below those of the group mean, and the least sensitive 10% will have thresholds approximately five times above it. With the exception of anosmics, most healthy persons appear to show normal sensitivity for most substances, but each person tends to show high sensitivity for certain substances and low sensitivity for a few other substances (10). No "super-taster" who had high sensitivity to all substances was found by any of the participating laboratories, nor was any pair of tasters discovered who showed exactly the same pattern of sensitivity.

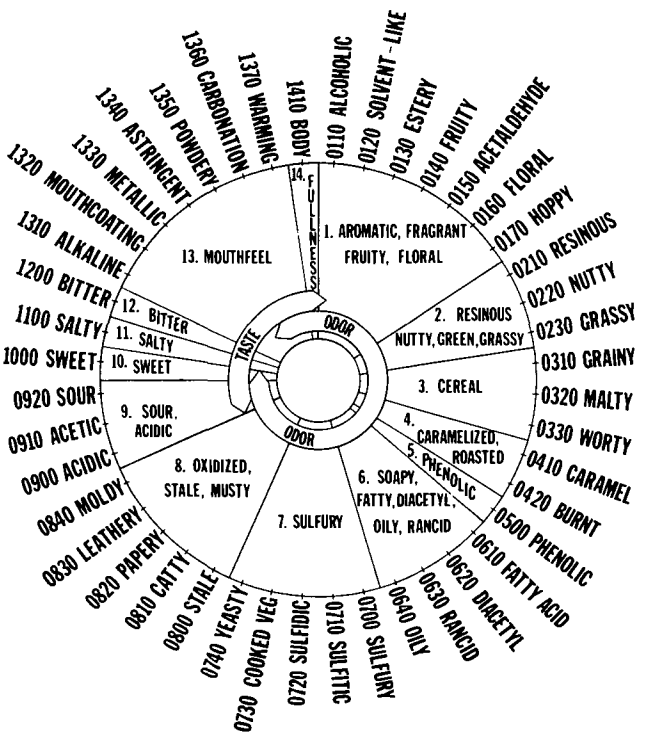

Fig. 1. The Flavor Wheel, showing class terms and first-tier terms.

[1]Presented at the 47th Annual Meeting, Miami, FL, May 1981.

[2]In five cases, we used overlapping pairs of chemical name terms and generally descriptive terms. The five pairs are: 0131 ISOAMYL ACETATE and 0143 BANANA; 0132 ETHYL HEXANOATE and 0142 APPLE; 0133 ETHYL ACETATE and 0120 SOLVENTLIKE; 0613 ISOVALERIC and 0612 CHEESY; and 0732 DMS and 0734 COOKED SWEET CORN.

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TESTING PROTOCOL: THE IDEAL REFERENCE COMPOUND

An ideal reference compound is sodium chloride. It represents the term 1100 SALTY perfectly. It is easily purified but can be used without purification. It is stable and nonpoisonous and does not react with beer. It does not affect foam, color, or clarity, and it is soluble at 9× and even 81× threshold concentration. Its flavor threshold is well defined, and it does not show a highly skewed or bimodal distribution of individual thresholds.

A second ideal reference compound is sucrose, for the term 1000 SWEET. Testing in these two cases could be limited to determinations of the threshold by the A.S.T.M. Ascending Method of Limits (1). An indication of the distribution of thresholds in the population is obtained with this method.

All other substances tested caused one or several problems that required separate investigation. With all little-known flavors, thresholds changed markedly with training so that three or more sittings of the threshold panel were required before useful results could be obtained. Wide variations between tasters were encountered, which necessitated repeated study of the compound by more than one panel, with each panel using the extended form of the A.S.T.M. method to detect tasters showing very high or very low thresholds and to confirm or disprove any abnormal pattern of distribution of thresholds in the population, which would make the compound in question a dubious choice as a reference standard. Some complications were so severe that months or years were required for a solution, or the investigators gave up altogether. Variations between laboratories caused by the use of different panels and different beers are of course real and will be discussed below. Their accurate assessment proved labor-intensive; however, the most difficult problems encountered were the removal of persistent impurities and the associated difficulty of determining when a substance is sensorially pure.

Criteria for Sensory Purity

Chemical purity is of little or no consequence in this connection, as impurities exist (8) that have flavor thresholds in the ng/L or ppt range (carbonyls) or even at the pg/L level (mercaptans). The only viable alternative was "purification to constant flavor and threshold" in analogy with purification to a constant melting point. Compounds were purchased in the highest available purity, examined for flavor and threshold as received, purified by one method, examined for threshold and flavor, purified by a second method, examined for threshold, purified again, and so on, until no change was seen with subsequent purification steps, and until the flavor, examined by perfumers and other experts, was true to type and did not change with purification.

An unexpected problem was that of the variable state of solution (14) of certain surface-active substances (eg, long-chain aliphatic compounds). When first added, they appeared to migrate to the surface of beer, causing a drop in flavor threshold, a lower foam stability, and loss of the compound by adhesion to glass. The problem was largely but perhaps not completely overcome by stipulating: 1) that enough stock solution in ethanol or water be prepared so that all additions to beer could be made directly into the bottles to be used for pouring taste tests, and 2) that bottles be stored between addition and tasting for at least 12 hr to allow time for complete solution of the added substance.

Reaction between beer and an added substance was seen in a few cases. Thresholds were found too high as a consequence, as for H_2S and DMS in beer high in copper or iron and for DMS in a beer accidentally prepared with water that had not been fully dechlorinated.

EXPERIMENTAL

Methods of Purification

Adsorption-1. For each 5 ml to be purified, the following materials are weighed into a 15-ml centrifuge tube: 0.92 g of silica gel (Sigma grade SIL-R, 100–300 mesh); 1.84 g of aluminum oxide (E. Merck Labs., grade 90, neutral, activity grade 1); and 0.65 g of activated carbon (Darco grade S-51 EE, 80–230 mesh). The adsorbent mixture is dried 4 hr at 105°C, cooled in a desiccator, and mixed with the liquid to be purified to form a thick slurry. The slurry is agitated for 1 hr at 50°C and then separated by centrifugation.

Adsorption-2. For each 5 ml to be purified, the following materials are weighed into a 15-ml centrifuge tube: 0.46 g of silica gel (Sigma grade SIL-R, 100–200 mesh); 0.92 g of aluminum oxide (E. Merck Labs., grade 90, neutral, Activity grade 1); 0.33 g of activated carbon (Darco grade S-51 EE, 80–230 mesh); and 2.33 g of silver powder (Alpha Ventron 00678, −1 μ grade). The adsorbent mixture is dried 4 hr at 105°C, cooled in a desiccator, and mixed with the liquid to be purified to form a thick slurry. The slurry is agitated for 1 hr at 50°C and then separated by centrifugation. (NOTE: The specified grades of the adsorbents must be used, as coarser grades show insufficient adsorption, and finer grades do not separate well by centrifugation.)

Solvent Wash. Fifty grams of the substance to be purified (Eugenol) is mixed with 50 ml of 6*N* NaOH, 50 ml of distilled water, and 200 ml of chloroform (Burdick & Jackson, distilled-in-glass). The mixture is shaken vigorously several times, then separated; the organic layer is discarded, and the aqueous layer is freed of chloroform by bubbling with a stream of N_2 for 3 min. Slow addition of 13 g of concentrated HCl to the aqueous layer separates the Eugenol, which is washed three times with water.

Fractional Distillation. Unless otherwise specified, the compound to be purified is distilled at its atmospheric boiling point through a Vigreux column 50–100 cm long. A center cut of 10% or less is collected.

Vacuum Distillation. Unless otherwise specified, the compound to be purified is distilled at 50°C or lower through a Vigreux column 50–100 cm long. A center cut of 10% or less is collected.

Gas Chromatography. Unless otherwise specified, the compound to be purified is chromatographed on a 6-m glass column, 4 mm i.d., containing Chromosorb P 60–80 mesh coated with 20% Carbowax 20M. Temperature conditions are chosen so that the compound will elute at 18–25 min, of which the last 10 min or more shall have been at a constant temperature. A center cut of not more than 50% of the principal peak is collected in a glass U-tube. The temperatures of injector and detector are set at the lowest levels possible with the compound in question so as not to create impurities by thermal decomposition. The progress of the purification is controlled by analytical GC on a 50-m capillary column.

Recrystallization. The substance to be purified is dissolved in hot or boiling solvent. The solution is filtered hot and, if necessary, decolorized with activated carbon. A second, weaker solvent may be added after which the solution is cooled to crystallize. Crystals are filtered off, quickly washed with a small amount of solvent, and dried.

Recrystallization of Calcium Salt. The substance (a fatty acid, 10 ml) is dissolved in 30–35 ml of ammonia solution (specific gravity 0.880, with the bottle opened carefully after cooling to 0°C). Saturated $CaCl_2$ solution (approx. 50 ml) is added until no more precipitate is formed, and the precipitate is filtered on a Buchner funnel where it is washed with 50:50 water-ethanol until no more ammonia is evolved. The calcium salt is recrystallized twice from 50:50 water-ethanol and may be used without regeneration as a flavor standard. For addition to beer, it is dissolved in 50:50 water-ethanol and buffered at pH 4.

Demonstration to Tasters

1. A stock solution of the standard should be prepared in water, ethanol, or aqueous ethanol of a concentration such that no less than 5 μl (the minimum for accuracy using a microsyringe) nor more than 500 μl of ethanol (the maximum before ethanol flavor

becomes apparent) needs to be added per 355-ml bottle of null beer. A relatively bland beer that is well known to the panelist should be used as null beer.

2. Bottles should be prepared for tasting by injecting the required concentration of stock solution below the surface of the beer, which should have been cooled to 4–10° C before uncrowning. The bottle should be tapped with a metal object until a head of foam expels the air in the neck (but not the added substance). The bottle should be recrowned and stored at 4° C overnight before tasting.

3. The panelist should be given a glass of beer to which is added the demonstration standard in a concentration nine times the threshold (Table I). The glass (or the bottle from which it is poured) should be clearly marked with the name of the standard followed by "9× threshold." The panelist is to state the strength and type of added flavor perceived and to discuss the flavor. The panelist should be asked questions such as "Is the flavor more xxx-like or more yyy-like?" and should be guided by the replies to ask further questions. The panelist should be encouraged to sip or sniff the sample, and perhaps also a control, until the flavor and its relationship to other, similar flavors is understood. In rare cases, the panelist may not perceive the added flavor because of low sensitivity, in which case a concentration of "81× threshold" may be tried.

4. The panelist should be given a triangular test comparing the demonstration standard blindly in a concentration of 3× threshold against the control. The panelist should know that these two samples are being served, but the combination used should not be divulged until a written reply has been submitted. The panelist should retaste the samples, then be served a second triangle using a different combination, and then a third.

5. The panelist should be regarded as having understood the flavor if the scoring is correct on both the second and third triangles. A miss on one of those should cause either a return to step 3 or, if the panelist shows lack of interest, disqualification with regard to this particular flavor.

6. Panelists of low sensitivity may be qualified using sets of triangles containing the standard at nine times the threshold.

Purification of Individual Standards

For reasons of clarity, experimental details such as the origin and method of purification of individual standards will be found under the corresponding flavor term below.

RESULTS AND DISCUSSION

A summary of the Subcommittee's recommendations with respect to the selection of standards and methods of purification is given in Table I, which also contains the threshold found for each standard, expressed as a range in beers containing various levels of the compound or flavor in question. For example, the group

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	Non 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
0614	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.

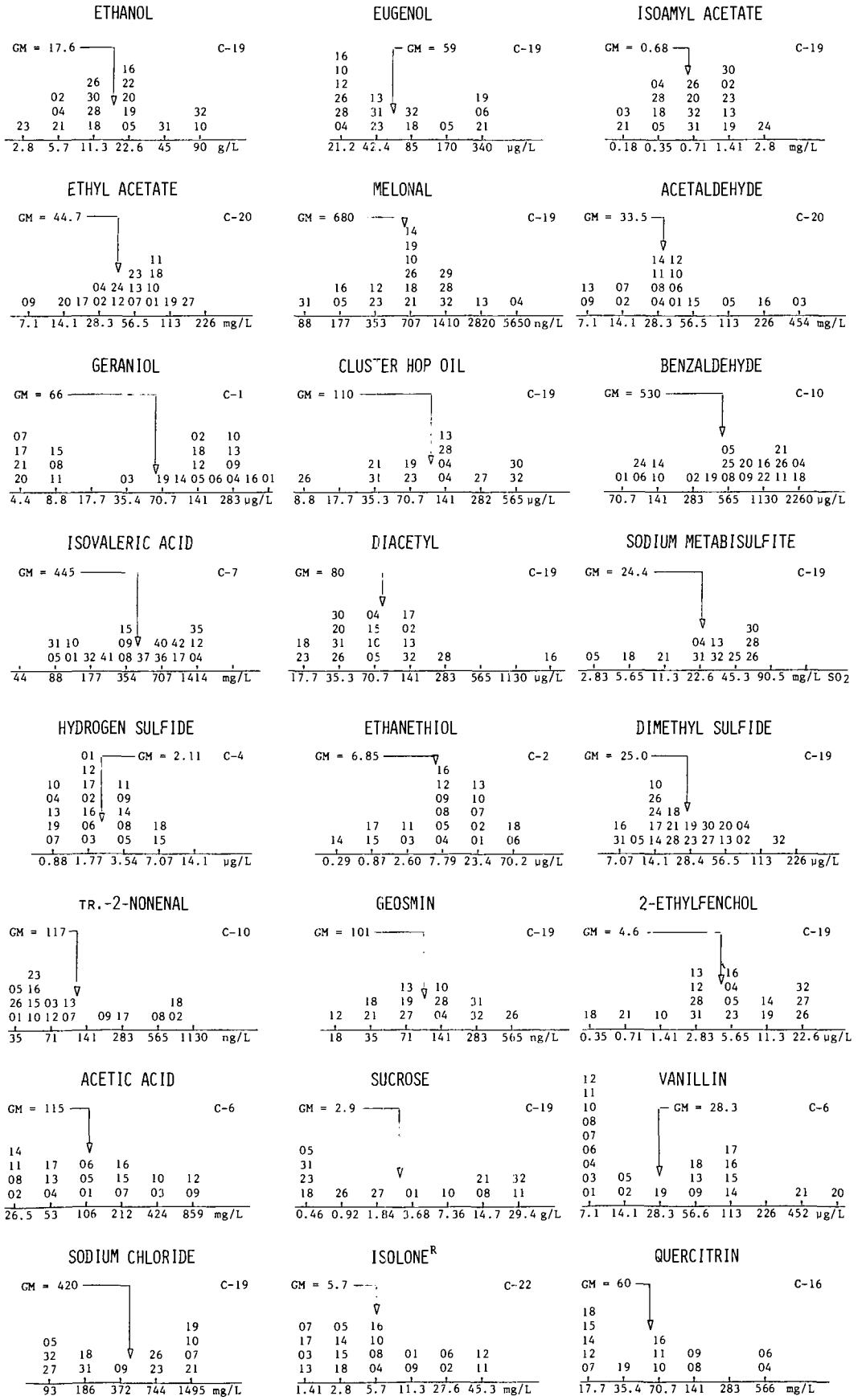

Fig. 2. Examples of the distribution of thresholds among tasters for various reference substances by the A.S.T.M. Ascending Method of Limits (*1*). The panel supplying each set of data is identified by a code (eg, C-5) in the upper right-hand corner of each histogram, which, itself is composed of the identifying numbers of the tasters on that panel.

threshold for dimethyl sulfide (DMS) is given as "25–50 μg/L in beer containing 10–100 μg/L." By interpolation, if a given beer contains 50 μg/L, then the group threshold can be expected to fall near 35 μg/L, and the recommended addition for demonstration purposes is then $3 \times 35 = 105$ μg/L for the average panelist and three times as much for those who show low sensitivity to DMS.

Examples of the distribution of thresholds among members of taste panels (and, by inference, among the population) are shown in Fig. 2. Typical, approximately bell-shaped histograms are seen for sodium sulfide, ethyl mercaptan, and ethanol, among others, indicating the expected log-normal distribution of sensitivities to the compound in question. Histograms that deviate from this pattern indicate an abnormal distribution, often bimodal or one-sided, which would make the compound less suited as a reference standard.

Unless specifically mentioned, the flavor type of standards examined was found to agree with the expected. Examples of compounds whose flavor type proved unsatisfactory for the intended purpose are 5-methylfurfural and *trans*-2-nonenal.

Individual Standards

0110 ALCOHOLIC. Although *n*-propanol or iso-butanol might have been chosen, as they are closer to a compromise between the two main alcoholic-flavored constituents (ethanol and isoamyl alcohol), ethanol was chosen because it represents more than 70% of total alcoholic flavor in the average beer (9). Absolute ethanol, which is available in 99.99% purity, proved too difficult to purify by repeated distillation and adsorption. Perhaps this is not surprising; of the more than 250 compounds examined for their threshold in beer, ethanol gave the highest value and is consequently the compound most easily misflavored. However, the removal of such off-flavors is a well-investigated problem in the manufacture of vodka. Results obtained with three brands are listed in Table II.

Sensory purity may vary between brands, and probably between lots. Purification of vodka by adsorption was attempted but was deemed too costly, as it would have raised the requirements for a test by several gallons; instead, the ASBC Subcommittee decided to recommend the use of vodka as received, with the proviso that a preliminary comparison be made (at 20 g/L in water) of the brands available locally. Strengths vary with locality and must be determined by analysis, after which the calculated weight of vodka should be diluted to the desired volume with beer.

0111 SPICY. Several problems common to many standards were encountered with this term. First, although Subcommittee members agreed that a "spicy" off-flavor occurs sporadically in beers from Europe, Oceania, and the Americas, the origins of this are not known, and several types may exist. After much debate a majority agreed that, for the purpose of training tasters, the clovelike flavor of eugenol would be suitable. Second, commercial samples of eugenol had different flavors, and the collaborators decided to use pure-flavored clove oil as the starting point. This meant that attainment of chemical purity, while preserving the flavor, required a laborious three-step purification process as detailed in Table II. Third, eugenol, in common with other spicelike substances such as geraniol and vanillin (Fig. 2), does not show a normal distribution of thresholds in the population; the purified preparation was tested by three panels, and a proportion of each panel could detect it at concentrations 10–100 times lower than the rest. After repeated testing of two highly purified preparations in the three laboratories, the Subcommittee decided to publish a threshold of 40 μg/L, and consequently a recommended test concentration of 120 μg/L, with a note that each panel is likely to contain 10–40% of tasters who find this a very strong clovelike flavor.

0131 ISOAMYL ACETATE. Although isoamyl acetate is an important contributor to the estery flavor spectrum of all beers, a question about the choice of reference compound arose because the natural ester is a mixture of 2-methylbutyl and 3-methylbutyl acetates. Attempts to determine the relative proportions of the two constituents failed at the time because no collaborator could separate them on available gas chromatography (GC) columns. Instead, one collaborator synthesized and purified 2-methylbutyl acetate, and it was found to have the same flavor and threshold as purified commercial isoamyl acetate. Two preparations of the latter were tested; both, according to the manufacturers, were prepared from isoamyl alcohol produced by fermentation, ie, they are likely to contain 70–80% of the 3-methylbutyl isomer.

Figure 2 shows that thresholds for isoamyl acetate are normally distributed in the population. Fairly rigorous purification is necessary to remove "medicinal" and "fusel-like" off flavors. Thresholds were found to vary considerably with the isoamyl acetate content of the null beer used (Table II), and the range listed in Table I, 0.5–1.7 mg/L for beers containing 1–3 mg/L, is therefore wider than those of the remaining standards. The argument used in establishing these figures is as follows: the range of isoamyl acetate contents in "normal beers" (ie, 80–90% of published analyses) is 1–3 mg/L; the corresponding thresholds for two hypothetical beers of 1 mg/L and 3 mg/L are then estimated by extrapolation from the results in Table II.

0132 ETHYL HEXANOATE. An applelike flavor note is common to the ethyl esters of butyric, hexanoic, octanoic, and decanoic acid. The three preparations of ethyl hexanoate tested (Table II) each showed an additional aniseedlike note which is not found in the other ethyl esters mentioned. The aniseed note was thought to be an impurity, but it could not be removed or even reduced in intensity by any method of purification tested; hence, it appears to be a natural part of the flavor of ethyl hexanoate. This was confirmed by collaborator 11, who reported that an aniseedlike off-flavor had been encountered in commercial beers, and that these, upon analysis, were found to contain 0.5–0.6 mg/L of ethyl hexanoate. Purification by Adsorption-1 plus GC is recommended, but either method alone may suffice. The distribution of thresholds among panel members was not determined; however, no tasters appeared to show either very high or very low sensitivity, and the other short-chain esters examined, isoamyl acetate and ethyl acetate, both showed normal distributions.

0133 ETHYL ACETATE. Although ethyl acetate preparations in the market were found to be quite pure of flavor, small amounts of solventlike off-flavor were detectable. These should be removed using Adsorption-1 once or, if sulfury flavor notes are present, Adsorption-2. Repeated adsorption treatment is not required (Table II).

0145 MELONY. A melony off-flavor is quite rare, and the proposed standard, 2,6-dimethyl-5-hepten-1-al (Melonal), was examined in only one laboratory. The compound, as received, did have a melonlike flavor, but its gas chromatogram showed two peaks close together. Collection of the larger peak produced a pure melonlike flavor and a normal distribution of thresholds among panel members.

0150 ACETALDEHYDE. Acetaldehyde is a difficult standard for several reasons. Its flavor is noncharacteristic and mild, and the threshold is relatively high, which means that misflavoring occurs easily. Common acetaldehyde is never pure since it readily forms dimers, polymers (eg, hexamers), and aldol condensation products. Most fractions obtained during purification of a compound can be considered more nearly pure the higher the threshold because one begins with 95%+ chemical purity, so that removal of any impurity with a weaker flavor than the parent compound can cause, at most, a 5% change in threshold; therefore, any change on purification larger than 5% must be towards higher threshold and must be caused by removal of impurities that are more strongly flavored than the parent compound. This simple rule does not hold for acetaldehyde, as it may contain up to 50% condensation products, some of which are more strongly flavored and others more weakly flavored than the aldehyde itself. Fortunately, the small amount (15 ml) required for a set of taste tests, may be easily distilled using the warmth of a hand as the heating source, and most of the

TABLE II
Group Threshold Values for Proposed Reference Substances, Purified in Various Ways as Indicated, and Added to Pale Lager Beer

Flavor Term	Sample and Method of Purification	Threshold mg/L	Collaborator	In Beer Containing, mg/L	No. of Tasters	Notes
0110	Food grade alcohol, as received	10 g/L[a]	C-8	34 g/L	12	Sl. medicinal
Alcoholic	Same, purified 3× by Adsorption-1	14 g/L[a]	C-8	34 g/L	12	Very sl. medicinal
	Food grade alcohol, p. 3× by Adsorption-1	13 g/L[a]	C-3	0 g/L	14	In water
	Vodka, Tanqueray Gordon	5 g/L[a]	C-3	0 g/L	14	In water; "hospital"
	Same, purified 2× by Adsorption-1	9 g/L[b]	C-3	0 g/L	14	In water
	200 Proof Anhydrous Ethanol, Nat'l Distill.	5 g/L[b]	C-19	36 g/L	16	Phenolic, solvent
	Same, purified 3× by Adsorption-2	10 g/L[b]	C-19	36 g/L	16	Phenolic, solvent
	Vodka, Hiram Walker, Peoria, IL	6 g/L[d]	C-19	36 g/L	16	Sweet, hospital
	Vodka, Hiram Walker, Walkerville, Ontario	10 g/L[d]	C-19	36 g/L	16	Sweet, spicy
	Vodka, Smirnoff, U.S.A.	17 g/L[d]	C-19	36 g/L	16	Av. of three tests
0111	Eugenol (cloves) purified by Solvent Wash	0.043[c]	C-8	<0.02	25	Clovelike
Spicy	+ Vacuum Distillation + Adsorption-1	0.003[d]	C-5	<0.02	14	Mouthfeel effects
	Same	0.059[d]	C-19	<0.02	15	Clove, spicy
	Eugenol (Aldrich) purified as above	0.040[b]	C-8	<0.02	10	Clovelike
0131	Isoamyl acetate (Aldrich) p. by Adsorption-1	2.5[a]	C-8	2.1	12	Untrained panel
Isoamyl	Same, additionally pur. by Gas Chromatogr.	1.6[a]	C-8	2.1	12	Trained panel
Acetate	Isoamyl acetate (Fisher) p. Adsorption-1	1.0[a]	C-19	1.8	12	Sl. medicinal
	Same, additionally pur. by Gas Chromatogr.	1.2[a]	C-19	1.8	12	Free of off-flavor
	As above	0.7[d]	C-19	1.4	16	Free of off-flavor
0132	Ethyl hexanoate (Applied Science), as received	0.12[a]	C-8	0.15	12	Caprylic off-note
Ethyl	Same, purified 4× by Adsorption-1	0.20[a]	C-8	0.15	12	Free of off-flavor
Hexanoate	Same, additionally purif. by Gas Chromatogr.	0.23[a]	C-8	0.15	12	As above
	Ethyl hexanoate, K & K Laboratories, as received	0.10[a]	C-19	0.20	12	Caprylic off-note
	Same, purified by Adsorption-2	0.21[a]	C-19	0.20	12	Free of off-flavor
	Same, additionally purified by Gas Chromatogr.	0.21[a]	C-19	0.20	12	Free of off-flavor
	Ethyl hexanoate (Fluka), purified by GC	<0.3[a]	C-11	0.19	12	
0133	Ethyl acetate (Fluka) p. by Adsorption-1	40[d]	C-20	20	16	Best of three tests
Ethyl	Ethyl acetate (Merck) p. by Gas Chromatogr.	66[c]	C-20	20	25	Half of panel untrained
Acetate	Ethyl acetate (Eastman)	19[b]	C-1	9	20	
	Ethyl acetate (Matheson) p. Adsorption-1	33[a]	C-8	14	12	
	Same, purified by Gas Chromatogr.	33[a]	C-8	14	12	
	Ethyl acetate (J. T. Baker) p. Adsorption-1	30[a]	C-19	17	11	
	Same, purified 3× by Adsorption-1	30[a]	C-19	17	12	
0145	Melonal® (Givaudan), as received	0.00008[d]	C-19	···	6	Few tasters
Melony	Same, purified by Gas Chromatogr.	0.0007[d]	C-19	···	16	
0150	Acetaldehyde (Aldrich) as received	25[a]	C-8	5	5	Few tasters
Acetaldehyde	Acetaldehyde (Merck) p. by Distillation	16[c]	C-20	5	25	
	Same	33[d]	C-20	6	12	Av. of two tests
	Acetaldehyde (Aldrich) p. by Distillation	10[a]	C-19	3	12	
	Same, older preparation	6[d]	C-19	3	16	Pumpkin off-flavor
	Same, p. Adsorp-1 + Dist. + Adsorp-2 + Dist.	14[d]	C-19	3	16	Free of off-flavor
0162	Geraniol (Aldrich), freshly received	0.30[d]	C-14	<0.06	16	
Geraniol	Same	0.26[d]	C-21	<0.06	16	
	Same	0.23[d]	C-18	<0.06	16	
	Same	0.18[d]	C-9	<0.06	16	
	Same	0.28[d]	C-20	<0.06	16	Av. of two tests
	Same	0.11[d]	C-16	<0.06	16	Av. of two tests
	Same	0.07[d]	C-19	<0.06	16	
	Same	0.07[d]	C-1	<0.06	22	Av. of two tests
	Same	0.18[d]	C-15	<0.06	19	
	Same	0.13[d]	C-18	<0.06	18	Av. of three tests
0173	1978 Cluster Hop Oil (S. S. Steiner)	0.007[d]	C-15	<0.05	27	Very low value
Hop Oil	Same	0.11[d]	C-19	<0.05	12	
0224	Benzaldehyde (Aldrich), as received	1.1[d]	C-14	<0.1	16	
Almond	Same	2.4[d]	C-21	<0.1	17	
	Same	1.9[d]	C-18	<0.1	16	
	Same	1.2[d]	C-20	<0.1	16	Av. of four tests
	Same	0.7[d]	C-9	<0.1	16	
	Same	1.4[d]	C-16	<0.1	16	Av. of two tests
	Same	0.7[d]	C-19	<0.1	16	
	Same	0.6[d]	C-10	<0.1	19	Av. of two tests

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(*continued*)

TABLE II

Flavor Term	Sample and Method of Purification	Threshold mg/L	Collaborator	In Beer Containing, mg/L	No. of Tasters	Notes
0611 Caprylic	Octanoic acid (Applied Science) p. Adsorption-1	10[a]	C-8	7	12	
	Same, purified by Gas Chromatogr.	13[a]	C-8	7	12	
	Oct. acid (BDH) p. by crystall. of Ca-salt	4.5[b]	C-5	3	16	
	Oct. acid (Sigma) p. by Gas Chromatogr.	15[a]	C-19	7	12	
0613 Isovaleric	Isovaleric acid (Analabs), as received	1.5[a]	C-19	0.5	12	
	Same, purified by Gas Chromatogr.	1.5[a]	C-19	0.5	12	
	Isov. acid (Sigma) p. by Gas Chromatogr.	1.0[a]	C-19	0.5	12	
	Same, purified 3× by Adsorption-1	1.2[a]	C-19	0.5	12	
	Isov. acid (Fluka Isomers-free), as received	1.5[a]	C-19	0.5	11	
	Same	0.61[b]	C-7	0.4	18	Av. of two tests
	Isov. acid (BDH), as received	0.69[b]	C-7	0.4	18	Av. of four tests
	Isov. acid (Sigma), as received	0.50[b,d]	C-7	0.4	18	Av. of seven tests
	Same, purified by Gas Chromatogr.	0.45[d]	C-7	0.4	18	Av. of three tests
0614 Butyric	Butyric acid (Eastman) p. by Adsorption-1	3[a]	C-19	0.8	12	
	Butyric acid (Sigma) p. by Gas Chromatogr.	2.2[a]	C-19	0.8	12	
	Butyric acid (Merck), as received	3.3[b]	C-10	1.0	20	Av. of two tests
	Same, purified 1× by Fractional Distillation	3.0[b]	C-10	1.0	20	Av. of two tests
	Same, purified 2× by Fractional Distillation	3.3[b]	C-10	1.0	20	Av. of four tests
	Same	2.8[c]	C-10	1.0	25	
0620 Diacetyl	Diacetyl (Quim. Monterrey), p. by Fract. Dist.	0.15[a]	C-8	0.10	12	
	Diacetyl (Aldrich) purif. by Adsorption-1	0.10[b]	C-19	0.04	16	
	Diacetyl (Aldrich) p. by Fract. Dist. + Ads.-1	0.08[d]	C-19	0.03	16	Freshly distilled
0710 Sulfitic	Sodium metabisulfite (Fisher), as SO_2, mg/L	27[d]	C-19	4	12	1 hr after addition
	Same	27[d]	C-19	4	12	4 hr after addition
	Same	20[d]	C-19	4	12	24 hr after addition
	Same	18[d]	C-14	3	16	
	Same	266[d]	C-21	4	17	Naive tasters
	Same	67[d]	C-18	3	16	
	Same	25[d]	C-20	5	16	Av. of two tests
	Same	98[d]	C-9	5	15	Naive tasters
	Same	27[d]	C-16	3	16	Av. of two tests
	Same	16[d]	C-10	2	17	Av. of two tests
0721 H_2S	$Na_2S{\cdot}9H_2O$ (Fisher), as H_2S, mg/L	0.0032[d]	C-4	<0.002	16	Sl. harsh, sl. sour
	$Na_2S{\cdot}9H_2O$ (Mallinckrodt)	0.0022[d]	C-4	<0.002	16	
	Same, selected crystals	0.0023[d]	C-4	<0.002	19	
	Same, recrystallized 2× from water	0.0021[d]	C-4	<0.002	19	
	Hydrogen sulfide gas (Matheson)	0.0047[d]	C-4	<0.002	16	Sl. harsh bitter
0722 Mercaptan	Ethanethiol (Aldrich, 97%)	0.0015[b]	C-14	<0.0005	16	
	Same, purified by Adsorption-1	0.0015[b]	C-14	<0.0005	16	
	Same	0.0009[c]	C-14	<0.0005	25	
	Ethanethiol (Aldrich, 97%)	0.0002[d]	C-15	<0.0005	16	
	Same	0.0017[a]	C-8	<0.0005	12	
	Same	0.0004[d]	C-19	<0.0005	16	
	Ethanethiol (Pierce)	0.0004[d]	C-19	<0.0005	16	
	Same	0.0069[d]	C-2	···	18	
0732 DMS	Dimethyl sulfide (Aldrich)	0.029[b]	C-19	0.040	12	Chemical
	Same, purified by Adsorption-1	0.049[a]	C-19	0.040	12	
	Dimethyl sulfide (Sigma)	0.023[a]	C-19	0.040	12	Oil refinery
	Same, purified by Adsorption-1	0.028[d]	C-19	0.040	12	Oil refinery
	Same, purified by Adsorption-2	0.027[d]	C-19	0.040	12	Oil refinery
	Dimethyl sulfide (Matheson, Coleman, Bell)	0.023[b]	C-19	0.040	12	Sl. Chem. av. of
	Same, purified by Adsorption-1	0.050[a]	C-8	0.030	12	two tests
	Same	0.037[b]	C-19	0.040	12	Av. of three tests
	Same, purified by Adsorption-2	0.030[b]	C-19	0.040	15	Best of six tests
	Same	0.033[c]	C-19	0.040	25	
	Same	0.023[b]	C-5	0.025	16	Best of six tests
	Same	0.040[b]	C-20	0.050	16	Best of three tests
	Same, using BG grade carbon	0.008[d]	C-15	0.030	16	
0820 Papery	5-Methylfurfural (K & K Laboratories)	5[b]	C-16	<0.01	19	Papery; best of three
	Same, purified by Fractional Distillation	9[c]	C-16	<0.01	25	Sl. papery
	Same	6[d]	C-16	<0.01	18	
	Same, purified by Gas Chromatogr.	14[d]	C-19	<0.01	16	Almonds, vanilla
	5-Methylfurfural (Aldrich) p. by Adsorption-1	20[a]	C-8	<0.01	12	Almonds, burnt

(*continued on next page*)

(*continued*) **TABLE II**

Flavor Term	Sample and Method of Purification	Threshold mg/L	Collaborator	In Beer Containing, mg/L	No. of Tasters	Notes
	2-*trans*-Nonenal (Parento), p. by Gas Chromatogr.	0.00011[a]	C-19	0.00003	12	Papery, stale
	2-*trans*-Nonenal (Oril)	0.00010[d]	C-10	0.00004	16	Oily, papery
	Same, purified by Gas Chromatogr.	0.00012[d]	C-10	0.00004	15	Oily
0841	Geosmin (Nat'l. Envir'l. Research Center)	0.00003[a]	C-19		11	In water
Earthy	Same	0.00010[d]	C-19	<0.00003	12	Earthy, musty
	2-Ethylfenchol (PFW, Inc.)	0.005[d]	C-19	<0.0001	16	Earthy, mushroom
0842	2,3,6-Trichloroanisole (Aldrich), recryst. 1×	0.0003[a]	C-19	···	12	Musty
Musty	Same	0.0001[a]	C-19	···	12	Musty (in water)
0910	Acetic acid (Merck GR)	133[b]	C-6	40	17	Av. of three tests
Acetic	Same, purified by Adsorption-1 (MgO omitted)	79[b]	C-6	40	17	
	Same, purified by crystallization @ 16°C	134[b]	C-6	40	17	Av. of two tests
	Acetic acid (BDH Aristar)	186[b]	C-6	40	17	Av. of two tests
	Same, purified by Adsorption-1 (MgO omitted)	150[b]	C-6	40	17	Av. of two tests
	Same, purified by crystallization @ 16°C	199[b]	C-6	40	17	
	Same	166[c]	C-6	40	25	
	Acetic acid (Baker Ultrex)	115[d]	C-6	40	17	
	Same	175[a]	C-19	50	12	
	Same	46[b]	C-17	30	10	
	Acetic acid (DuPont), p. Adsorption-1 (MgO om.)	125[a]	C-19	50	12	
1000	Sucrose (Grocery store)	2600[b]	C-12	100	16	Av. of six tests
Sweet	Same	2900[d]	C-19	80	12	
1003	Vanillin (Fluka)	0.032[d]	C-6	<0.010	21	
Vanilla	Same, recrystallized 1× from water	0.028[d]	C-6	<0.010	21	
	Vanillin (Fluka)	0.052[d]	C-19	<0.010	16	
1100	Sodium chloride (Grocery store)	560[b]	C-12	200	16	Av. of six tests
Salty	Same	420[d]	C-19	250	12	
1200	Isohumulone (Kalsec Isolone)	7.3[b]	C-22	<1	16	Three tests,
Bitter	Same	5.7[d]	C-22	<1	18	unhopped beer
1330	$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (Baker), as Fe^{++}, mg/L	3.0[b]	C-13	<0.1	15	In water, av. of six tests
Metallic	Same	3.3[c]	C-13	<0.1	15	In water
	Same, recrystallized from warm water	3.9[b]	C-13	<0.1	15	In water, av. of five test
	$FeSO_4 \cdot 7H_2O$ (Baker), as Fe^{++}, mg/L	~1[d]	C-13	<0.1	21	See text
1340	Quercitrin (K & K Laboratories)	55[b]	C-16	<2	16	Best of six tests
Astringent	Same, recrystallized from 50% ethanol	80[b-d]	C-16	<2	16	Best of three tests
	Quercitrin (K & K Laboratories)	81[c]	C-20	<2	25	

[a] Series of triangular tests with identification.
[b] Difference Rating Test.
[c] Guadagni Multiple Pairs Test.
[d] A.S.T.M. Ascending Method of Limits Test.

condensation products depolymerize on distillation. Adsorption treatment is also simple, as it must be done at room temperature, and best results were found when two adsorption steps were combined with two distillation steps (Table II). The purification process may be monitored by analytical GC using a capillary column at room temperature. Acetaldehyde showed a normal distribution of thresholds among panel members.

0162 GERANIOL. This flowery, roselike flavor is a part of hop aroma in beer (13). Freshly purchased geraniol was found to be sensorially pure, but the compound turns rancid when stored on the shelf after two to three months. A fresh sample was examined by 10 collaborators as part of the 1979-1980 program of the Subcommittee (1). The results, as illustrated in Fig. 2, showed a bimodal distribution; apparently tasters consist of two populations with thresholds at 18 μg/L (35% of persons studied) and 350 μg/L (65%). Geraniol probably satisfies Amoore's criteria (3) for a primary odorant, but this interesting property makes it less suitable as a flavor reference standard. The Subcommittee decided that the corresponding flavor does occur in beer and that geraniol should be accepted with a recommended test concentration of 1 mg/L.

0173 HOP OIL. In the system of terminology, this term is reserved for the characteristic flavor obtained with commercial hop oil isolated by steam distillation, as distinct from 0172 DRY HOP obtained by adding dry hops to the beer during storage. The Cluster Hop Oil preparation examined produced a high threshold in one laboratory and a low threshold in another. Only the higher concentration appeared to correspond to a true "hop oil" flavor. It was decided to accept the preparation listed (Table II) for demonstration purposes, but not as a reference standard.

0224 ALMOND. The product (Aldrich) was examined as received by eight of the collaborators mentioned under GERANIOL above. The compound has a characteristic almond odor without off-flavor, and the distribution of thresholds both among tasters and among laboratories was normal.

0611 CAPRYLIC. Octanoic acid of commerce carries a small amount of contamination with strongly-flavored substances such as octenone and octenal and must be carefully purified. Crystallization of the calcium salt was selected as the method of purification, but gas chromatography is also effective, and a combination of the two methods probably should be used in some

cases. The distribution of thresholds was not examined but is expected to be similar to those of isovaleric and acetic acids.

Roberts et al (14) found that freshly made solutions of octanoic acid in beer show lower thresholds. When a beer is poured during the first hours after addition, the foam can be observed to collapse more quickly and to leave behind on the surface of the beer or the walls of the glass a thin film of the added substance. Roberts et al also reported that whereas with most compounds two or three presentations were sufficient for training purposes, with octanoic acid tasters slowly learned a technique whereby lower and lower concentrations could be detected at the back of the mouth, after swallowing.

0613 ISOVALERIC. Isovaleric acid preparations available commercially were found relatively free of sensory impurities, and the purest (Sigma) was accepted as the standard. Thresholds were normally distributed among tasters (Fig. 2).

0614 BUTYRIC. Again, preparations on the market are relatively pure, but purification did remove oily and carbonyl-like off-flavors and it was decided to recommend that the product be distilled twice before use. The distribution of thresholds was not determined.

0620 DIACETYL. Diacetyl, like acetaldehyde, forms dimers and possibly ring polymers in solution. Solutions in beer rapidly lost flavor strength, and a standard solution was observed to increase its threshold approximately twofold after two weeks in the refrigerator. Even pure diacetyl lost strength on standing in a bottle on the shelf for some months, according to one Subcommittee member. Another member reported that a stock solution of 2,000 mg/L lost 10% of its strength in four weeks in a refrigerator in the dark, and 70% during the same period if exposed to light. Distillation of diacetyl appeared to regenerate the monomeric substance but also to produce some off-flavor; this could be removed by Adsorption-1. The distribution of thresholds showed that some tasters, such as no. 16 at laboratory C-19 (Fig. 2), are quite insensitive to diacetyl even though they may be among the most sensitive tasters with other standards.

0710 SULFITIC. A pure SO_2 flavor was obtained with commercial sulfites such as sodium or potassium hydrosulfites or metabisulfites. Sodium metabisulfite was the first substance to be used for collaborative testing of the A.S.T.M. Ascending Method of Limits and the results, published by the Subcommittee (1) and summarized in Table II, demonstrate one of the pitfalls of the method: a naive taster who is quite unfamiliar with the substance may easily fail to detect it at concentrations tens of times above his or her threshold. For a flavor to be detected at or near the threshold, it must be known and retained in memory. The present system of standards serves just this purpose.

0721 H_2S. The flavor of H_2S may be produced in beer by adding a sulfide salt or gaseous H_2S. The former, available as $Na_2S{\cdot}9H_2O$, is highly hygroscopic, and commercial samples contain a slurry of crystals in a decomposing mother liquor. H_2S gas is quite pure but is difficult to add to beer with precision. Advantage was taken of the fact that a saturated aqueous solution of H_2S at 0°C contains 7.07 g/L, however, both over- and undersaturation was observed. Most reproducible values were obtained with selected, clear crystals of the sodium salt. These gave a pure H_2S flavor showing normal distribution of thresholds among tasters. Two recrystallizations did not materially alter the threshold.

0722 MERCAPTAN. The thresholds obtained with ethanethiol (Table II) varied widely among laboratories, although in each laboratory they were normally distributed among the tasters. For example, collaborator 2 found a group threshold of 0.0069 mg/L with individual tasters varying from 0.001 to 0.07 mg/L, whereas collaborator 15 found a threshold of 0.0002 mg/L with individual tasters varying from 0.00004 to 0.001 mg/L, ie, virtually no common ground existed between these two panels. Such a situation was not encountered with any other substance. Except for this problem, ethanethiol appeared to be an acceptable standard: preparations were deemed to have the correct flavor type, were chemically pure, and were unchanged after purification. The assumption that the difference between laboratories was caused by the beers used should be tested by an exchange of beers among the panels.

0722 DMS. All commercial preparations examined were misflavored by substances causing "rubbery" or "oil refinery-like" taints that could not be removed by distillation. Various adsorbents were tested as described in detail (10). The importance of using the correct grade of adsorbent was mentioned under EXPERIMENTAL and is illustrated by the low threshold reported by collaborator 2 who had used Darco BG, a coarser grade of carbon. DMS thresholds were normally distributed among the tasters of three laboratories (4), and the group thresholds were similar when allowance was made for differences in preexisting concentrations of DMS in the four beers used.

0820 PAPERY. 5-Methylfurfural (K & K Laboratories) as received had a papery flavor which, however, appeared to diminish in intensity as the compound was purified. A highly purified sample was prepared and tested by three collaborators (Table II) who found that little, if any, papery flavor was present. Attempts at isolating the papery impurity failed. *Trans*-2-Nonenal (Compagnie Parento), received as a 10% solution in ethanol, did have a papery flavor, but showed less than 60% purity by chemical tests. Purification by gas chromatography appeared promising, but the preparation was discontinued by the manufacturer. *Trans*-2-Nonenal (Oril) was 95% pure and could be highly purified by gas chromatography; however, slightly more than half of the tasters found the pure product "oily" rather than "papery." Accordingly, no reference standard could be recommended.

0841 EARTHY. Geosmin is available for scientific purposes from the National Environment Research Center, Cincinnati, OH, but the supply is very limited. This compound has a pure, earthy flavor, and thresholds were normally distributed. 2-Ethylfenchol, available by arrangement with PFW, Inc., Middletown, NY 10940, also has a strong, earthy flavor, perhaps with a slightly mushroomlike secondary flavor note. It was decided to accept both compounds without purification as alternative standards for 0841 EARTHY.

0842 MUSTY. The compound 2,3,6-trichloroanisole, known as the cause of a musty taint in chicken (6), did have a typically musty (or moldlike, enclosed) flavor as received. However, tests were discontinued because of doubts about the toxicological status of the compound.

0910 ACETIC. Acetic acid, like ethanol, has a mild, uncharacteristic flavor and is therefore easily misflavored. The purest preparations on the market (several manufacturers offer ultra-high purity grades) appeared to be free of off-flavors (Table II), but thresholds were somewhat variable. The Subcommittee decided that a certain degree of variation is normal because of the uncharacteristic flavor, and that the best of the ultra-high purity grades is acceptable as a reference standard without purification. Thresholds were normally distributed among tasters.

1000 SWEET. Tests to confirm sucrose as a standard were uneventful. The threshold of sucrose in a given beer depends on several factors in addition to its sucrose content, eg, the levels of bitterness and astringency as well as that of sweetness caused by other sugars, notably maltose, isomaltose, glucose, and fructose.

1003 VANILLA. Vanillin (Fluka) as received was found to have a pure vanilla flavor; neither flavor nor threshold changed after purification. The distribution of thresholds among tasters was uneven (Fig. 2) and may actually be trimodal; if it is, approximately 40% of tasters appear to have thresholds around 100 μg/L, a group of vanilla-insensitive tasters show thresholds above 500 μg/L, and approximately 50% are very sensitive with thresholds below 7 μg/L. These variations must be accepted, as no better standard exists for vanilla flavor than vanillin.

1100 SALTY. As for sucrose, the tests to confirm sodium chloride as a standard were uneventful.

1200 BITTER. The preparation Isolone® (Kalsec Inc.,

Kalamazoo, MI 49005) gave a typical bitter flavor with a minimum of other flavors. Thresholds were normally distributed.

1330 METALLIC. In addition to ferrous salts, ferric and cupric salts were briefly considered but were found to produce untypical metallic flavors. Comparison between ferrous ammonium sulfate and ferrous sulfate gave equivocal results; both compounds gave thresholds varying from 0.4 to 3.5 mg/L (as Fe^{++}), which is also the range of published thresholds in water. The Fe^{++} ion has a weak initial taste and a long-lasting aftertaste, making a 30-min interval necessary between sips. Accurate thresholds could not be obtained, nor was it possible to ascertain whether a sensory difference exists between the two compounds. In the end the simplest compound, ferrous sulfate, was selected and assigned a compromise threshold of mg/L Fe^{++}. The distribution of thresholds in the population could not be determined.

1340 ASTRINGENT. Although quercitrin is both astringent and bitter, it was selected because other astringent compounds showed the same defect and because quercitrin is available in good purity and can be recrystallized from 50% ethanol. A tendency towards bimodal distribution (Fig. 2) was evident in the test shown here but was less in other tests performed with this compound.

STANDARDS UNDER INVESTIGATION

At the time (May 1981) when the Subcommittee voted to terminate its work on flavor reference standards, the compounds listed below were under investigation. For some standards, a compound had been chosen, and only details such as the choice of manufacturer or method of purification, or the determination of thresholds remained. Fifteen standards were investigated: a white wine, Chablis Blanc, Gallo Vineyards, Modesto, CA (for 0112 VINOUS); acetone, Matheson, approx. 200 mg/L (for 0123 ACETONE); 2-phenyl-ethanol, Aldrich, purified by Adsorption-1 plus GC, 100 mg/L (for 0161 2-PHENYLETHANOL); Exaltolide Musk, Firmenich (for 0163 PERFUMY); myrcene + humulene + geraniol + linalool (for 0172 DRY-HOP); *cis*-3-hexenol, Aldrich, 13 mg/L (for 0231 FRESH-CUT GRASS); maltol (for 0410 CARAMEL); guaiacol (for 0423 SMOKY); phenol (for 0503 CARBOLIC); 2,6-dichlorophenol (for 0504 CHLOROPHENOL); iodoform (for 0505 IODOFORM); 3-methyl-2-butene-1-thiol, or thioglycolic acid (for 0724 LIGHTSTRUCK); *p*-methane-8-thiol-3-one (from pulegol), or 4-mercapto-4-methylpentan-2-one (by passing H_2S through mesityl oxide) (for 0810 CATTY); 6-isobutylquinoline (for 0830 LEATHERY); and sodium bicarbonate (for 1310 ALKALINE).

Concentrations listed above are the approximate thresholds; additions for demonstration purposes should be three times higher. The senior author will maintain an active interest in the development of these and other reference standards and will welcome requests for collaboration.

CONCLUSION

After five years of collaborative studies, the Subcommittee has selected 27 flavor reference standards, as compared with a need for 122. Some of the 27 standards, such as DMS, were selected after thorough investigation, whereas others such as geosmin were accepted on less thorough grounds. Three substances, 5-methylfurfural, *trans*-2-nonenal, and 2,3,6-trichloroanisole, were rejected after full examination while 15 substances received insufficient study. Many important flavor terms still need a standard; these terms will continue to have different meanings to different people, a situation which makes any serious study of their causes impossible.

On the positive side, reference standards are now available for one out of every five terms; the Subcommittee has learned what to look for and what to guard against. A pool of international expertise has been assembled. Readers are urged to use the standards now in force and to communicate any suggestions for improvement or proposals for new standards to the ASBC Subcommittee on sensory analysis, M. C. Meilgaard, chairman.

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