Prediction of Flavor Differences between Beers from Their Chemical Composition

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Approximately 250 compounds were purified and added to beer singly and in groups for determination of flavor thresholds. Results are used to propose a set of hypotheses and formulas for determining which compounds are flavor active and how they interact to produce the totality of beer flavor and how differences between beers can be predicted. Primary flavor constituents of typical U.S. lagers are ethanol, carbon dioxide, and hop bitter substances. Characteristic secondary flavor constituents are oxygenated humulenes, banana esters, fusel alcohols, dialkyl sulfides, and fatty acids. Flavor interaction tests with 26 groups showed that compounds of similar flavor tend to be additive while compounds of different flavor acted independently. Formulas were derived for predicting differences in intensity of 16 flavor notes, e.g. "alcoholic", "banana", "caprylic", "bitter", and "papery". Variations in flavor perception among 180 individuals studied were surprisingly large; often the least sensitive 10% of a group shows thresholds 20 times higher than the most sensitive 10% thereof. A total of 850 compounds found in beer are referenced and cataloged.

The purpose of this paper will be apparent to anyone who has attempted to brew beer at home. The resulting flavor often falls much short of expectation. In a brewery, flavor variations may be less extreme, but their consequences are greater. When profile panels are called upon to define the deviation, verdicts are often ambiguous or contradictory, and the panels are unable to cope with a flood of repeat samples. A double necessity is born, one of sharpening the panel's verdicts and another of detecting and defining flavor effects by chemical analysis. This paper is a summary of a 15-year study aimed at those two needs; i.e., it is a study of those factors that determine the flavor of a beer as a function of its chemical composition.

The attempt to improve panel response began with the identification, naming, and cataloging of each separate flavor note in beer. This was accomplished via a 5-year collaborative study under the auspices of the European Brewery Convention and the American Society of Brewing Chemists (Meilgaard et al., 1979). The resulting standard vocabulary required the development of a system of flavor reference substances (American Society of Brewing Chemists, 1981; Meilgaard, 1982). The choice of methods of flavor testing was studied (Meilgaard, 1981a; Meilgaard and Siebert, 1982).

The chemist's approach started with an inventory of the 850-odd constituents reliably identified in beer to date (Meilgaard, 1981b). Purification studies and threshold determinations were carried out for 250 compounds, after which the properties of most of the rest could be estimated from the relationships between structure and activity. Interactions (synergism, additivity, masking) between various constituents were studied by determining the threshold and flavor type of pairs and multiples of the purified compounds. Equations attempting to predict flavor could then be formulated. Such equations hold for panels as a whole; finally, the extent to which they can be applied to individual beer drinkers was investigated by determining the variations among flavor thresholds in the population for seven compounds and 16–172 panelists.

EXPERIMENTAL SECTION

Terminology of Beer Flavor. A master list of 800-odd terms was circulated in 1974 and gave rise to a first report (Clapperton et al., 1975) and an intermediate system (Clapperton et al., 1977). These were discussed at annual meetings of the European and American organizations and

were extensively revised by a process of mail votation involving 110 brewing professionals, resulting in the system now in force (Meilgaard et al., 1979).

Threshold of Added Substances. Subcommittees of the two organizations examined 13 methods and conducted collaborative experiments with 4. A preliminary report (Brown et al., 1978) examined the variation in sensitivity among brewery panel members, the effects of training and of sensory fatigue, the motivation factor, and the effect of preexisting concentrations of the substance under test in the beer under study. A subsequent collaborative study in which three substances were tested by panels in nine locations (American Society of Brewing Chemists, 1980) led to adoption of the Ascending Method of Limits test. The individual threshold was defined as that added concentration an individual can detect 50% of the time and the group threshold as the geometric mean of individual values. Thresholds were determined over a 15-year period (1966–1981). Judges were well-trained members of brewery panels. During the first 8 years a method consisting of a series of three-glass forced-choice tests was used (Meilgaard, 1975b), and the threshold was taken as that concentration producing seven correct identifications with 12 judges. The two methods agree within the accuracy of either, ±20%, for a given panel and substance. Thresholds were determined in a pale lager "null beer" (Table I).

Source of Test Compounds. The chemicals used were the purest on the market, usually 98%+. For important or suspect compounds the products of several suppliers were compared. The compounds were examined by GC or HPLC and then tasted in beer as received and again after each purification step (Meilgaard, 1975b). Testing was continued until no further increase in the threshold or change in flavor was observed after purification. Most compounds were dissolved in 50% aqueous ethanol, a maximum of 0.5 mL being added per 355-mL bottle of beer. The treated bottles were kept at 4 °C for 12–24 h before tasting. The precise threshold was not determined for compounds normally present in beer at levels below 0.1 FU (see below), unless the compound was of special academic interest.

This experimental section is not exhaustive; the original references should be consulted.

MEASUREMENT OF FLAVOR STRENGTH

Flavor Unit, FU. The basis of the classification of flavors and compounds used here is the flavor unit (Meilgaard, 1975a):

$$FU = concentration/threshold$$

The Stroh Brewery Co., Detroit, Michigan 48226.

Table I. Composition of the Null Beers Used in Tables II, VI, VII, and VIII

compound	beer no. 1,[a] mg/L	beer no. 2,[b] mg/L
original gravity, °Plato	11.0	11.3
color, °Lovibond 0.5 in.	3.0	3.1
pH	4.0	4.1
bitterness index, BU	25.5	14.0
ethanol, g/100 g	3.4	3.6
1-propanol[c]	12	7
2-methyl-1-propanol[c]	7	11
2-methyl- plus 3-methyl-1-butanol	51	62
2-phenyl-1-ethanol	34	22
ethyl acetate	14	17
2-methylpropyl acetate	0.4	0.2
2-methylbutyl plus 3-methylbutyl acetates	2.1	1.8
ethyl hexanoate	0.15	0.23
ethyl octanoate	0.26	0.30
ethyl decanoate	n.d.[d]	0.06
ethyl dodecanoate	n.d.	0.03
2-phenylethyl acetate	n.d.	0.07
acetic acid	n.d.	50
propanoic acid	n.d.	1.3
2-methylpropanoic acid	n.d.	0.7
butanoic acid	n.d.	0.8
2-methylbutanoic acid	n.d.	0.5
pentanoic acid	n.d.	0.03
hexanoic acid	3.0	1.3
heptanoic acid	n.d.	0.02
octanoic acid	7	7
nonanoic acid	n.d.	0.05
decanoic acid	5	3
dodecanoic acid	n.d.	0.2
tetradecanoic acid	n.d.	0.13
pentadecanoic acid	n.d.	0.02
hexadecanoic acid	n.d.	0.03
heptadecanoic acid	n.d.	0.06
octadecanoic acid	n.d.	0.06
9-octadecenoic acid	n.d.	0.17
acetaldehyde	3	2
2,3-butanedione plus 2,3-pentanedione	0.1	0.07
dimethyl sulfide	0.03	0.04

[a] Cerveceria Cuauhtemoc, S. A.   [b] The Stroh Brewery Co.   [c] Actual concentrations are lower as the GC peaks in question contain other compounds in addition to those listed.   [d] n.d. = not determined.

For example, 1 FU equals the threshold, 2 FU is twice as much, etc. Use of the FU as a measure of flavor strength is permissible provided the conditions A and B set out below are satisfied.

(A) Condition A is that the standard psychophysical power function

intensity of perception = (constant)(concentration)^n

(Engen, 1971) can be simplified by setting the exponent, n, equal to 1. Evidence was presented (Meilgaard and Reid, 1978, 1979) that the error resulting from doing this would not exceed ±20% if only a limited range, e.g., from 0.2 to 2 FU, was considered and if an exception was made for ethanol, which shows direct solvent effects in the mouth at high concentrations (such as 7.5% = 5 FU).

(B) Condition B is that both thresholds and concentrations are determined in beer rather than in air or water. Thresholds vary from beer to beer depending, e.g., on the preexisting concentration of the compound in question. Table II shows that, as the "endogenous" concentration increases, so does the threshold. The error committed by ignoring the endogenous concentration is tolerable in the region below 1 FU, but above this level the error soon

Table II. Difference Threshold for 2,3-Butanedione Added to a Null Beer Already Containing Various Added Levels of the Compound[a]

sample used for the determination	difference threshold found for added 2,3-butanedione, mg/L
null beer containing 0.03 mg/L naturally present 2,3-butanedione	0.20
null beer plus 0.30 mg/L added 2,3-butanedione	0.30
null beer plus 2.0 mg/L added 2,3-butanedione	1.5

[a] From Brown et al. (1978).

becomes large. Strictly speaking, this means that calculated FUs apply only to the difference between two beers, one of which is the null beer used for the determination. In practice, the FUs listed in the tables below have been found to be applicable with reasonable accuracy to comparisons involving beers that do not differ more than ±50% in composition from the two null beers used (Table I).

It was found (Meilgaard et al., 1971) that the following approximate relation exists between the concentration of a substance and its perceived flavor strength: a difference of 0.5 FU or less is not perceptible unless there is interaction between several components of related flavor; a difference of 0.5–1.0 FU can sometimes be detected by the average taster, but there is difficulty in identifying the precise nature of the flavor change; a difference of 1–2 FU corresponds to a major change of flavor.

It is emphasized that FUs determined as described cannot be used to estimate flavor differences between water and beer nor between beer and other beverages. In actual fact this is not a serious limitation as most brewing research is aimed at improving a given brand or creating new brands that differ in certain specified ways from those in existence.

Panels used should have at least 12 and preferably over 20 members. Comparison between two panels is permitted, but results should be corrected for any large differences in thresholds. Typically such differences do not exceed 2× (Brown et al., 1978; American Society of Brewing Chemists, 1980), and they are smaller the larger the panels.

RESULTS AND DISCUSSION

Nature of Differences between Beer Types. It is generally agreed that the senses of smell and taste are "analytical" like pitch discrimination and not "synthetic" like color vision (Mozell, 1971). A complex flavor can be mentally analyzed (Amerine et al., 1965), and consciously or unconsciously, we perceive it in the manner in which we hear a symphony. Systematic taste testing has shown that the beer types of the world can be described in terms of the nine "groups of instruments" shown in Table III. For example, the Pilsen type is high in bitterness, hop character, and DMS flavor and low in sweetness and fruity/estery flavor. U.S. lagers are high in fruity/estery and alcoholic flavors and low in bitterness and caramel flavor. München beers have medium bitterness and hop character but are high in caramel flavor. Pale ale resembles Pilsner in its hop character, but it has more caramel flavor and less DMS flavor. Clapperton and Piggott (1979) have shown that ales and lagers may be distinguished on the basis of the latter two flavor notes. Stout, of course, is highest in caramel flavor but is also high in hop character, bitterness, and acidity, and Lambic differs by its acidic

Table III. Principal Flavor Elements of Various Beer Types

	intensity of flavor (in FU)					
flavor group	Pilsner	U.S. lager	Münchener	pale ale	Stout	Lambic
bitterness	6-10	2-4	3-6	5-8	6-10	3-6
alcoholic flavor	3-4	3-5	2-4	3-4	3-5	3-6
carbonation	3-4	4	3-4	1-3	3-4	3-5
hop character	6-10	0.5-4	2-6	5-8	6-10	3-6
caramel flavor	0.5-2	0.5-1	4-8	3-5	6-100	1-3
fruity/estery flavor	1-1.5	2-3	1-2	1-2	2-3	3-5
sweetness	1-2	2-3	2-3	1-2	1-2	1-2
acidity	1-2	1-2	1-2	1-2	2-3	3-20
DMS flavor	1-3	1-3	1-2	0.2-0.8	0.2-0.8	1-10

Table IV. Eight Odor Classes and Corresponding Aroma Volatiles

class no.	flavor groups included in class	corresponding aroma volatiles
1	aromatic, fragrant, fruity, floral	aliphatic alcohols and esters; terpenes
2	resinous, nutty, green, grassy	the above, including aldehydes; plus many compounds unknown at present
3	cereal, e.g., grainy, malty, worty	long-chain aldehydes; O-, N-, and S-heterocyclic compounds
4	caramelized, roasted	O-, N-, and S-heterocyclic compounds produced by Maillard reactions
5	phenolic	phenols
6	soapy, fatty, diacetyl, oily, rancid	fatty acids, hydrocarbons, other long-chained aliphatic compounds; vicinal diketones
7	sulfury	volatile sulfur compounds
8	oxidized, stale, musty	a variety of compounds causing flavor defects, often flavor active at low concentrations

flavors caused by lactic acid fermentation. Note that the comparisons in Table III are semiquantitative only as they extend outside the range in which FUs can be used to express flavor strength in a quantitative manner.

Flavor Terminology System. The complete system (122 terms) is available as supplementary material (see paragraph at end of paper regarding supplementary material). Figure 1 shows how the principal flavor notes may be represented as spokes in a "flavor wheel". The taste of beer is described in terms of the four basic tastes, from 0900 acidic to 1200 bitter, plus certain other taste impressions that are grouped under class 13, mouthfeel, and class 14, fullness. The system divides the more complex part of beer flavor, the odor (or aroma), into eight classes in an approximate order from pleasant to unpleasant (Table IV).

Flavor Reference Standards. Twenty-seven standards were chosen (Table V); for each standard, a source and a method of purification are specified. For each purified compound, the threshold for addition to beer was determined by a minimum of two panels with a minimum of 30 tasters in all. Tests showed that a suitable addition for demonstration and training purposes is 3 times the threshold.

The ideal reference compound is sodium chloride. It represents the term 1100 salty very satisfactorily, and it is widely available in high sensory purity. It is stable and nonpoisonous and does not react with beer. It also does not affect the appearance of beer in any way. Most other compounds cause a number of complications such as instability, formation of haze, wide variations of thresholds between laboratories, presence of persistant impurities, or difficulty in determining whether sensory purity has been obtained. For the 27 substances, these difficulties have been solved, but many more reference substances would be desirable to cover the 122 standard terms.

Flavor-Active Constituents in Beer. A table, listing the compounds reliably reported in beer, their concentrations, and their flavor type, is available as supplementary material (850 compounds). Flavor thresholds for 250 compounds are included, permitting their classification as primary, secondary, tertiary, or background flavor con-

Figure 1. Flavor wheel showing class terms and first-tier terms of the internationally agreed upon terminology. Reprinted with permission from Meilgaard et al. (1979). Copyright 1979 American Society of Brewing Chemists.

stituents. Table VI gives the primary constituents and a selection from the other three groups.

Primary flavor constituents are present above 2 FU. Removal of any of these constituents would produce a decisive change in flavor. Hop bitter substances, ethanol, and carbon dioxide are the only constituents of typical, pale lagers that can be classified in this category. In specialty beers, the arbitrary limit of 2 FU may be exceeded by various distinctive flavor elements such as dry hop, kettle hop, caramel, etc.

However, when any (or several) of the remaining flavor constituents in Table VI exceed 2 FU or even 1 FU, we are concerned with a flavor defect. Most flavor defects in brewery practice are caused in this manner. Typical defects are diacetyl flavor, DMS flavor, and papery (trans-2-nonenal) flavor.
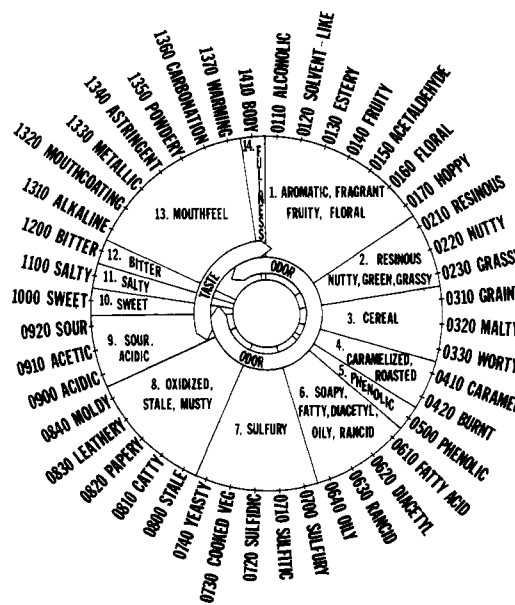
Table V. The 27 Official Flavor Reference Standards of the European Brewery Convention and the American Society of Brewing Chemists [from Meilgaard (1982)]

standard term	compound	supplier	difference threshold[a]	in beer containing
0110 alcoholic	ethanol	high-quality vodka[b]	17 g/L	33-42 g/L
0111 spicy	eugenol	Aldrich[c]	40 μg/L	0-20 μg/L
0131 isoamyl acetate	isoamyl acetate	Aldrich[d]	0.5-1.7 mg/L	1-3 mg/L
0132 ethyl hexanoate	ethyl hexanoate	K & K Laboratories[d]	0.15-0.25 mg/L	0.2-0.4 mg/L
0133 ethyl acetate	ethyl acetate	Fluka[e]	20-40 mg/L	10-30 mg/L
0145 melony	Melonal	Givaudan[f,g]	1 μg/L	
0150 acetaldehyde	acetaldehyde	Merck[h]	10-20 mg/L	2-10 mg/L
0162 geraniol	geraniol	Merck[f,i]	ca. 150 μg/L	0-60 μg/L
0173 hop oil	Cluster hop oil[j]	S. S. Steiner[f]	0.1 mg/L	0-0.5 mg/L
0224 almond	benzaldehyde	Aldrich[f]	1 mg/L	0-0.1 mg/L
0611 caprylic	octanoic acid	Sigma[k]	5-10 mg/L	2-8 mg/L
0613 isovaleric	isovaleric acid	Sigma[f]	0.5-1.5 mg/L	0.5-1.5 mg/L
0614 butyric	butyric acid	Merck[l]	2-3 mg/L	0.5-1.5 mg/L
0620 diacetyl	2,3-butanedione	Aldrich[m]	70-150 μg/L	30-300 μg/L
0710 sulfitic	sodium metabisulfite	Fisher Scientific[f]	20 mg/L SO_2	1-10 mg/L SO_2
0721 H_2S	sodium sulfide	Mallinckrodt[n]	4 μg/L H_2S	0-2 μg/L H_2S
0722 mercaptan	ethanethiol	Aldrich[f]	1 μg/L	0-0.5 μg/L
0732 DMS	dimethyl sulfide	Matheson Coleman and Bell[e]	25-50 μg/L	30-100 μg/L
0841 earthy	geosmin	National Environment Research Center[f]	0.1 μg/L	0-0.03 μg/L
0841 earthy	2-ethyl fenchol	PFW, Inc.[f]	5 μg/L	
0910 acetic	acetic acid	J. T. Baker "Ultrex"[f]	60-120 mg/L	30-200 mg/L
1000 sweet	sucrose	grocery[f]	2.6 g/L	0-1 g/L
1003 vanilla	vanillin	Fluka[f]	40 μg/L	0-10 μg/L
1100 salty	NaCl	grocery[f]	0.6 g/L	0-300 mg/L
1200 bitter	isohumulone	Kalsec Isolone[f,n]	7-15 mg/L	0-30 mg/L
1330 metallic	$FeSO_4 \cdot 7H_2O$	J. T. Baker[f]	1 mg/L Fe	0-0.5 mg/L Fe
1340 astringent	quercitrin[o]	K & K Laboratories[p]	80 mg/L	0-10 mg/L

[a] The standard recommended addition for reference purposes is 3 times the threshold. [b] Smirnoff or equivalent. Strength varies with locality and must be determined by analysis before use. Add to beer by weight, not volumetrically. [c] Purify by fractional distillation plus solvent wash plus adsorption. [d] Mixture of 2-methylbutyl and 3-methylbutyl acetates. Purify by adsorption plus preparative GC. [e] Purify by adsorption. [f] No purification required. [g] Melonal is a trade name for 2,6-dimethyl-5-hepten-1-al. Store refrigerated. [h] Purify by adsorption plus distillation plus adsorption. [i] Geraniol turns rancid: use fresh supply. Thresholds of this compound 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. [j] Not a reference standard; recommended for demonstration purposes. [k] Purify by recrystallization of calcium salt. [l] Purify by 2× fractional distillation. [m] Purify by fractional distillation plus adsorption. [n] Select colorless crystals. [o] Quercitrin is both astringent and bitter. [p] Purify by recrystallization from 50% ethanol.

Secondary flavor constituents are those that are present between 0.5 and 2 FU. Removal of any one constituent from this group would produce a minor change in flavor. Collectively, the secondary constituents contribute the major part of a beer's flavor. Table VI ranks them approximately in order of importance, beginning with hop oil components. Maltol and other caramel-like flavors may be prominent, especially in dark beers. In a very pale beer that is not hoppy, the fermentation esters rank first. These can be divided into "banana esters", which are the acetates of the fusel alcohols, and the "apple esters", which are the ethyl esters of butyric, hexanoic, octanoic, and decanoic acids. Ethyl acetate forms a group of its own with a light, solvent-like flavor.

Surprisingly, the fusel alcohols 2-methyl-1-propanol, isoamyl alcohols, and phenylethanol were rated warm and pleasant flavored once they were fully purified. The unpleasant flavor notes in commercial compounds and in fusel oils appear to be caused by impurities, probably carbonyls that are formed in the still. Dialkyl sulfides are cabbage or cooked sweet corn like and are characteristically present in lager beers at just under 1 FU. Also just below 1 FU are found the caprylic acids (hexanoic, octanoic, and decanoic) and also acetic, isovaleric (old hops), and butyric acids. The simple acidic flavor comes from a group of hydroxy acids (citric, malic, lactic, pyruvic, etc.). 2,3-Butanedione and pentanedione produce a buttery aroma that is necessary for a full, beer-like flavor but should remain well below the threshold. The same is true for the three classes of nonvolatile constituents with which the sec-

ondary group ends: the polyphenols (astringent, tea-like), the sugars (sweet), and the amino acids and peptides (broth-like, bitter, and sweet).

Tertiary flavor constituents occur at levels of 0.1-0.5 FU where they cannot be perceived individually; together they add subsidiary flavor notes. The lactones produce fruity flavors (raspberry, strawberry. Stale-flavor compounds that are present at low levels in fresh beer include long-chain aldehydes (trans-2-nonenal), acetaldehyde, and stale-flavor esters (grape-like). Other tertiary flavor constituents are the volatile phenols, hydrogen sulfide, mercaptans, inorganic salts, metals, C-4 to C-6 aldehydes, furans, heterocyclic N and S compounds, and many more.

Background flavor constituents are present below 0.1 FU and number over 750. Theoretically, approximately 20 similarly flavored compounds, each present at a level near 0.1 FU, may produce a noticeable flavor effect, and the same may be true of 30 or 40 dissimilarly flavored compounds (see below). In practice, background flavor effects must be estimated from the difference between total beer flavor and the sum of known flavor contributions. I estimate that background constituents account for less than 30% of total flavor.

Interaction between Flavor Constituents. There is agreement that regardless of the approach taken in the measurement of flavor strength, interaction should be measured in terms of the observed flavor intensity of the mixture (R_{mix}) divided by the sum of the intensities of the components ($R_1 + R_2 + ... + R_n$) (Guadagni et al., 1966; Guadagni, 1968; Teranishi et al., 1971; Harper, 1972;

Table VI. Examples of Principal and Subsidiary Flavor Constituents of Beer

flavor term	flavor-active compound or group of compounds	typical or distinctive member of group
	(A) Primary Flavor Constituents, Present above 2 FU	
	In Typical, Pale Lager Beers	
1200 bitter	hop bitter substances	*trans*-isohumulone
0110 alcoholic	ethanol	
1360 carbonation	carbon dioxide	
	In Specialty Beers (e.g., Dark, Hoppy, Strong, Naturally Fermented, Primed)	
0171 kettle hop	hop oil transformation products	oxygenated humulenes
0172 dry hop	hop oil constituents	myrcene, humulene
0410 caramel	O-heterocyclic ketones	maltol and isomers
0130 estery	esters	3-methylbutyl acetate
1000 sweet	sugars	sucrose
	(B) Secondary Flavor Constituents, Present between 0.5 and 2 FU	
	Compounds Listed under Specialty Beers Above, Plus the Following	
0131 isoamyl acetate	banana esters	3-methylbutyl acetate, 2-methylpropyl acetate
0132 ethyl hexanoate	apple esters	ethyl hexanoate, ethyl octanoate
0110 alcoholic	fusel alcohols	3-methylbutanol
0732 DMS	dialkyl sulfides	dimethyl sulfide
0611 caprylic	C-6 to C-12 fatty acids	octanoic acid
0910 acetic	acetic and propanoic acids	acetic acid
0133 ethyl acetate	ethyl acetate	
0620 diacetyl	vicinal diketones	2,3-butanedione
0613 isovaleric	3-methylbutanoic and 1-pentanoic acids	3-methylbutanoic acid
0612 butyric	butanoic and 2-methylpropanoic acids	butanoic acid
0920 sour	other organic acids	citric, malic acids
1340 astringent	polyphenols	leucocyanidin
1000 sweet	sugars	maltotriose
1410 body and others	amino acids, small peptides, nucleic acid derivatives	proline
	(C) Tertiary Flavor Constituents, Present between 0.1 and 0.5 FU	
	Numerous Compounds Such as the Following	
0140 fruity	lactones of hydroxy acids	λ-decalactone
0161 2-phenylethanol	2-phenylethanol	
0820 papery	long-chain aldehydes	*trans*-2-nonenal
0500 phenolic	volatile phenols	4-vinylguaiacol
0721 H₂S	hydrogen sulfide	
0724 light struck	3-methyl-2-butene-1-thiol plus methanethiol	3-methyl-2-butene-1-thiol
1100 salty	inorganic salts	sodium chloride
1330 metallic	metals	iron
	(D) Background Flavor Constituents, Present below 0.1 FU	
	All Other Flavor Constituents (Probably Thousands)	

Berglund, 1974). This ratio will be called *the degree of flavor interaction*

$$d_{int} = R_{mix}/(R_1 + R_2 + ... + R_n)$$

It is seen that flavors which are additive will give d_{int} = 1.00. Synergism (or enhancement) will result in d_{int} > 1.00: for example, a d_{int} = 2.00 equals a 2-fold synergism: the flavor of the mixture is twice as strong as the sum of the parts. At d_{int} < 1.00 we have either independence (compensation) or antagonism (suppression, masking). The lowest possible value, d_{int} = 0.00, is obtained when flavored compounds produced a flavorless mixture (cancellation, extinction).

Three studies of flavor interaction have been published in the field of brewing. Palamand and Hardwick (1969) found no significant departure from additivity (d_{int} = 1.00) in eight pairs of flavor components added to beer. Engan (1972) also found perfect additivity between 10 pairs of added alcohols and esters. Meilgaard (1975a) tested 26 groups of two to five components and found that compounds of similar flavor are approximately additive (d_{int} = 0.7–1.3), whereas compounds of different flavor show partial independence (d_{int} = 0.4–0.7; Table VII). The banana esters, 2-methylpropyl acetate and the isoamyl

acetates, which have very similar flavors, show d_{int} = 1.00. The two apple esters, ethyl hexanoate and ethyl octanoate, show a small degree of apparent synergism: 0.54 FU of one plus 0.25 FU of the other produced 1.00 FU of combined flavor. However, the combined errors of three threshold measurements are at least ±20%, and the d_{int} =1.27 found for this mixture is therefore not significantly different from 1.00. In the same tests, two different flavors, octanoic acid and ethyl acetate, were almost independent: more than 0.9 FU of each, or a total of 1.85 FU, was added before the mixture could be tasted (d_{int} = 0.54). Slight evidence of actual suppression by hexanoic acid was shown in the mixture with 3-methylbutanol: the threshold concentration of the mixture included slightly more than 1.0 FU of the latter.

The results in Table VII can be explained if it is assumed that similarity of flavor is a stronger factor than similarity of chemical structure. For example, butanoic acid and 2,3-butanedione, a fatty acid and a carbonyl compound, are additive because they have similar flavors; in contrast, acetic and octanoic acids are independent owing to their different flavors. A general conclusion from the tests is that most beer flavor compounds were partly additive: typically 0.5 FU of one compound plus 0.5 FU

Table VII. Flavor Threshold (Milligrams per Liter) of Sensorially Pure Compounds Added to Beer Individually and in Mixtures to Beers No. 1 and 2 in Table I

(I) Threshold of Compounds Added Individually

ethanol, g/100 g	1.3	decanoic acid	10
1-propanol	800	ethyl acetate	33,[a] 30[b]
2-methyl-1-propanol	200	2-methylpropyl acetate	1.6
2-methyl-1-butanol	65	isoamyl acetates[c]	1.6,[a] 1.2[b]
3-methyl-1-butanol	70	ethyl hexanoate	0.23,[a] 0.21[b]
cis-3-hexenol	13	ethyl octanoate	0.9
2-phenylethanol	125,[a] 100[b]	acetaldehyde	10
acetic acid	175	2-methylbutanal	1.25
butanoic acid	2.2	3-methylbutanal	0.6
3-methylbutanoic acid	1.5	trans-2-nonenal	0.00011
hexanoic acid	8	2,3-butanedione	0.15,[a] 0.10[b]
octanoic acid	15	dimethyl sulfide	0.045,[a] 0.030[b]

(II) Threshold and Degree of Interaction (d_{int}) for Added Mixtures

2-methyl-1-propanol	4.5	2-methyl-1-butanol	14.5
2-methyl-1-butanol	14.1	3-methyl-1-butanol	48.7
3-methyl-1-butanol	53.4	2-phenylethanol	22.6
mixture A-1[d] d_{int} = 1.00	72[b]	mixture A-2 d_{int} = 0.87	85.8[b]
2-methyl-1-propanol	10.1	acetic acid	73.3
2-methyl-1-butanol	11.1	butanoic acid	1.18
3-methyl-1-butanol	40.4	3-methylbutanoic acid	0.73
2-phenylethanol	28.4	octanoic acid	10.2
mixture A-3 d_{int} = 0.92	90[a]	mixture B-1 d_{int} = 0.47	85.5[a]
hexanoic acid	7.17	acetic acid	109
octanoic acid	3.00	butanoic acid	1.37
decanoic acid	0.53	mixture B-3 d_{int} = 0.81	110[b]
mixture B-2 d_{int} = 0.85	10.7[a]	butanoic acid	0.69
		3-methylbutanoic acid	0.47
butanoic acid	0.83	octanoic acid	4.68
3-methylbutanoic acid	0.57	mixture B-5 d_{int} = 1.07	5.84[b]
mixture B-4 d_{int} = 1.32	1.40[b]	ethyl hexanoate	0.12
2-methylpropyl acetate	0.69	ethyl octanoate	0.23
isoamyl acetates[c]	0.92	mixture C-2 d_{int} = 1.27	0.35[a]
mixture C-1 d_{int} = 1.00	1.61[a]	ethyl acetate	6.52
ethyl acetate	7.50	2-methylpropyl acetate	0.49
isoamyl acetates[c]	0.90	isoamyl acetates	0.65
ethyl hexanoate	0.10	ethyl hexanoate	0.09
mixture C-3 d_{int} = 0.68	8.50[b]	ethyl octanoate	0.18
		mixture C-4 d_{int} = 0.67	7.93[a]
acetaldehyde	6.25	2-methylpropanol	19.2
3-methylbutanal	0.15	2-methylbutanol	21.2
trans-2-nonenal	0.000028	3-methylbutanol	77.1
2,3-butanedione	0.038	ethyl acetate	30.9
mixture D-1 d_{int} = 0.66	6.44[b]	mixture I-1 d_{int} = 0.40	148.5[a]
2-methylpropanol	4.37	2-methylpropanol	6.90
2-methylbutanol	4.81	2-methylbutanol	7.58
3-methylbutanol	17.5	3-methylbutanol	27.6
2-methylpropyl acetate	0.53	ethyl hexanoate	0.16
isoamyl acetates[c]	0.70	ethyl octanoate	0.30
mixture I-2 d_{int} = 0.90	27.9[a]	mixture I-3 d_{int} = 0.64	42.5[a]
3-methylbutanol	21.5	3-methylbutanol	32.4
isoamyl acetates[c]	0.88	hexanoic acid	9.73
ethyl hexanoate	0.14	mixture Z-1 d_{int} = 0.60	42.2[a]
mixture I-4 d_{int} = 0.68	22.5[a]	cis-3-hexenol	9.75
isoamyl acetates[c]	1.06	2-methylbutanal	0.65
ethyl hexanoate	0.16	mixture Z-3 d_{int} = 0.79	10.4[b]
hexanoic acid	8.00		
mixture Z-2 d_{int} = 0.42	9.22[a]		

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Table VII (*Continued*)

butanoic acid	1.26	octanoic acid	13.9
2,3-butanedione	0.08	ethyl acetate	27.7
mixture Z-4	0.91[b]	mixture Z-5	41.6[b]
$d_{int} = 0.91$		$d_{int} = 0.54$	
ethyl hexanoate	0.13	octanoic acid	11.4
acetaldehyde	6.23	dimethyl sulfide	0.037
mixture Z-6	6.36[b]	mixture Z-7	11.4[b]
$d_{int} = 0.81$		$d_{int} = 0.63$	
ethyl hexanoate	0.14	acetaldehyde	8.02
dimethyl sulfide	0.032	dimethyl sulfide	0.038
mixture Z-8	0.17[b]	mixture Z-9	8.06[b]
$d_{int} = 0.73$		$d_{int} = 0.61$	

[a] In beer no. 1 (Table I). [b] In beer no. 2 (Table I). [c] Mixture of 25:75 2-methylbutyl acetate and 3-methylbutyl acetate. [d] Example of calculations for mixture A-1: this mixture was found to have a threshold in beer no. 2 of 72 mg/L. That amount (72 mg/L) consisted of 4.5 mg/L 2-methyl-1-propanol, 14.1 mg/L 2-methyl-1-butanol, etc. These amounts correspond to 4.5/200 = 0.023 FU of 2-methyl-1-propanol, 14.1/65 = 0.217 FU of 2-methyl-1-butanol, etc., a total of 1.003 FU. $d_{int} = 1/1.003 = 1.00$.

of another produce 0.8 ± 0.2 FU of combined flavor. No obvious examples of synergism or antagonism were observed in beer and may not exist. Similar results were found in many nonbrewing studies of flavor interaction [see Meilgaard (1975a)].

Flavor Type of Mixtures. In almost all cases the mixture was perceived as a simple combination; i.e., the flavors of the original constituents could still be recognized in the mixture. No unexpected flavors were found to arise from interaction of any set of components. Occasionally the flavor of a minor component was clearly perceived in the mixture; for example, in mixture A-3, the combination of 0.23 FU of phenylethanol with over 1 FU of C-4 and C-5 alcohols showed almost as much rose as alcohol flavor.

Estimation of Flavor Differences from Chemical Composition. Table VIII contains a set of equations by which intensity differences (in FU) can be calculated for 16 flavor notes in beer no. 2 of Table I. These equations, which are meant as nothing more than a rough estimation of differences expected, were derived as follows: for a flavor note caused by a single compound, FU = concentration/threshold; for a flavor note caused by two compounds, $(FU_1 + FU_2)d_{int}$ or, alternatively, $FU_{1+2} = $ (concentration$_1$ + concentration$_2$)/threshold$_{1+2}$, in which threshold$_{1+2}$ is that of a mixture of the two compounds in the proportion in which they usually occur in beer; for a flavor note caused by a major compound and a minor one of related flavor, $FU_1 + Q \cdot FU_2$, where Q is a factor between 0.6 and 1.5, chosen so as to take account of the known interaction between the two compounds (see further below).

The calculations use thresholds determined in a null beer, and the results refer to the likely flavor differences between the sample and the null beer. It might be thought that results of greater general applicability could be obtained by using water (or dilute ethanol) as the null beer, but in practice this gives meaningless results as shown by Meilgaard (1975a). An approximation may be obtained by using a set of progressively weaker null beers.

The results of the calculations may be interpreted by reference to the scale presented above and reprinted in footnote *a* of Table VIII. For example, the papery flavor that develops with heat treatment of packaged beer depends almost fully on the amount of 2-nonenal present (Wang and Siebert, 1974) so that it may be calculated in step 38 without consideration of other compounds present. Diacetyl flavor is obtained in step 32 as the sum of FUs from 2,3-butanedione and 2,3-pentanedione. For caprylic
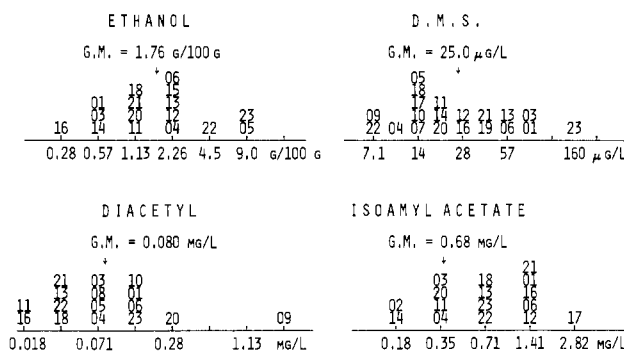
Figure 2. Personal threshold values for 16–20 assessors and for four purified substances (D.M.S. = dimethyl sulfide; Diacetyl = 2,3-butanedione). The A.S.T.M. Ascending Method of Limits was applied 2–4 times for each assessor and sample. Concentrations represent amounts added to beer no. 2 (Table I). G.M. = geometric mean. Redrawn with permission from Meilgaard and Reid (1978). Copyright 1978 Institute of Brewing, Australia and New Zealand section.

flavor (step 27), a formula based on the combined threshold of the four acids appeared to improve prediction. The same approach is used for the C-4 and C-5 alcohols in step 6, whereas 1-propanol is treated as a separate, additive constituent. Examples of flavor interaction are seen, e.g., in steps 9 and 22 in which the FU sums of two or more flavor notes are multiplied by the corresponding d_{int} values, which in turn are estimates; for example, the $d_{int} = 0.66$ in step 22 is a composite of the values obtained with mixtures I-1, I-2, I-3, and I-4. The equations may of course be modified or augmented as more knowledge is gained about flavor-active compounds and their interaction. For example, acetaldehyde (threshold = 10 mg/L), when tested together with ethyl hexanoate, shows $d_{int} = 0.8$; consequently an acetaldehyde content of 5 mg/L would contribute (5/10)·0.8 = 0.4 FU of apple-like flavor in step 18. Other possibilities will be apparent from Table VII.

The column "deg dif." predicts, e.g., in steps 20–21 that the sample beer is perceptibly and identifiably more banana-like and fruity/aromatic than the null beer. Such calculations should not be taken for more than what they are, a "best estimate". They may be a good guide for individual flavor notes, e.g., if a brewer wishes to alter the degree of caprylic flavor, but they should not be used to decide more complex questions such as the effects of a change of yeast strain.

Table VIII. Procedure for Prediction of Noticeable Flavor Differences: Calculated Example

step		instruction	amount present, mg/L null beer	amount present, mg/L sample	FU present null beer	FU present sample	deg dif.[a]	corresponding flavor
1	L[b]	ethanol in % by wt	3.53	3.70				
2	C[c]	[1][d]/1.3			2.72	2.85	0	0110 alcoholic
3	L	1-propanol	11.0	9.0				
4	L	2-methylpropanol	3.0	4.7				
5	L	isoamyl alcohols	60.6	93.8				
6	C	C-3 to C-5 alcohols: ([4] + [5])/72 + [3]/800			0.90	1.38	0	0110 alcoholic
7	L	2-phenylethanol	22.1	46.6				
8	C	[7]/100			0.22	0.47	0	0161 phenylethanol
9	C	fusel alcohols: ([6] + [8])·0.9			1.01	1.67	1	0110 alcoholic, 0160 floral
10	C	all alcohols: [2] + [9]			3.73	4.52	1	0110 alcoholic
11	L	ethyl acetate	15.6	21.2				
12	C	[11]/30			0.52	0.71	0	0137 ethyl acetate
13	L	2-methylpropyl acetate	0.05	0.13				
14	L	isoamyl acetates	1.6	2.4				
15	C	banana esters: ([13] + [14])/1.2			1.38	2.11	1	0143 banana
16	L	ethyl hexanoate	0.30	0.24				
17	L	ethyl octanoate	0.34	0.36				
18	C	apple esters: ([16] + [17])/0.35			1.83	1.71	0	0142 apple
19	C	banana esters + apple esters: ([15] + [18])·0.7			2.25	2.67	0	0140 fruity
20	C	all esters: [19] + [12]·0.6			2.56	3.10	1	0140 fruity, 0130 estery
21	C	fusel alcohols + banana esters: ([9] + [15])·0.9			2.15	3.40	2	0143 banana, aromatic
22	C	all alcohols + all esters: ([10] + [12] + [15] + [18])·0.66			4.92	5.97	2	flavorful, aromatic
23	L	hexanoic acid	1.10	2.51				
24	L	octanoic acid	7.90	7.35				
25	L	decanoic acid	3.34	4.37				
26	L	dodecanoic acid	0.61	0.82				
27	C	caprylic acids: ([23] + [24] + [25] + [26])/10			1.29	1.50	0	0611 caprylic
28	L	3-methylbutanoic acid	0.32	1.12				
29	C	[28]/1.5			0.21	0.72	1	0613 isovaleric
30	L	2,3-butanedione	0.07	0.12				
31	L	2,3-pentanedione	0.05	0.10				
32	C	vicinal diketones: [30]/0.1 + [31]/0.9			0.76	1.31	1	0620 diacetyl
33	L	H_2S in ppb	4.3	3.5				
34	C	[33]/5			0.86	0.70	0	0721 H_2S
35	L	dimethyl sulfide in ppb	22	38				
36	C	[35]/30			0.73	1.27	1	0732 DMS
37	L	2-nonenal in ppb	0.04	0.06				
38	C	[37]/0.11			0.36	0.55	0	0820 papery
39	L	bitterness in BU	14.9	17.8				
40	C	[39]/5			2.98	3.52	1	1200 bitter

[a] Deg dif. = degree of flavor difference as judged on the following scale: 0–0.49 FU = "0" = not perceptible; 0.50–0.99 FU = "1" = perceptible but not identifiable by the average taster; 1.00–1.99 FU = "2" = perceptible and identifiable flavor difference; 2.00 FU and over = "3" = major change of flavor. [b] L = list. [c] C = calculate flavor caused by performing the indicated instruction. [d] [x] = amount listed or result obtained in step x.

Variation among Individuals. Figure 2 shows frequency distributions of the individual thresholds of 16–20 panel members for four compounds. A second test series with 172 panelists and three compounds gave similar results (American Society of Brewing Chemists, 1980) that may be generalized by saying that, for each compound, the most sensitive 10% of the group shows a threshold approximately 20 times lower than the least sensitive 10%. Interestingly, persons showing low thresholds for one compound usually showed high thresholds for one or two other compounds and vice versa. This means that two persons showing equal overall sensitivity may nevertheless receive entirely different flavor impressions from a given group of compounds in beer. It also means that the calculations in Table VIII are valid only for the average observer: the extent to which they are valid for an individual will be governed by the degree to which he or she differs from the average.

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Flavor of Fermented Fish Sauce

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Forty-three previously unidentified compounds have been found in Nam-pla fermented fish sauce. This included 8 acids, 10 alcohols, 6 amines, 7 other nitrogen-containing compounds, 4 lactones, 3 carbonyls, and 5 sulfur-containing compounds. The fish sauce was solvent extracted in order to obtain a flavor isolate. This isolate was fractionated into acidic, neutral, and basic fractions via pH adjustment and liquid–liquid extraction in order to facilitate gas chromatography and mass spectrometry. All gas chromatography was done by using fused silica Carbowax 20M capillary columns.

Fish sauces constitute an important part of the diet of more than 250 million people in Southeast Asia (Van Veen, 1965). Fish sauce provides a substantial part of the protein requirements of these people.

Fermented fish sauces are made by mixing fish with salt and allowing natural fermentation and leaching to occur. For Nam-pla, a Thai sauce, small whole fish (Stolephorus or Sardinella species) are mixed with marine salt in the ratio of 3 parts of fish to 1 or 2 parts of salt. Normally 24–48 h elapse between catching the fish and the salting step during which the fish are not refrigerated. This actually initiates the fermentation process. The salt–fish mixture is transferred to large concrete tanks and left to ferment. The tanks are usually built into the ground, which maintains the temperature in the range of 35–40 °C. After 6–12 months, the brown liquid is decanted and filtered. The filtrate may be sun-ripened in earthenware for up to 3 months or bottled directly for consumption. The residue is covered with fresh brine and held for about 3 months to produce a lower quality sauce that may be blended with the first-run filtrate. Alternatively, the residue from the first fermentation may be ground and sold as paste (Saisithi et al., 1966).

During fermentation, proteolysis of fish proteins results in increasing the soluble protein. For Budu, a sauce from Malaysia, approximately 56% of the total fish protein is converted to soluble protein (Beddows et al., 1976). The yield of liquid is about 75 mL/100 g of fish. Analysis of various sauces finds total organic nitrogen ranging from 1.7% to 2.3% of which 50–60% is in the form of free amino acids (Uyenco et al., 1952; Truong-van-Chom, 1958; Saisithi et al., 1966; Beddows et al., 1979). Volatile nitrogen (mostly ammonia) comprises 7–12% of the total organic nitrogen. The pH of fish sauce is approximately 5.6 and the salt content is about 270 g/L.

Earlier investigators have found the aroma of fish sauce to be composed of a blend of ammonical, meaty, and cheesy notes (Dougan and Howard, 1975; Beddows et al., 1976). The ammonical note has been attributed to ammonia, a variety of amines, and other basic nitrogenous compounds (Saisithi et al., 1966; Dougan and Howard, 1975). Low molecular weight volatile fatty acids (VFA), in particular formic, acetic, propionic, n-butyric, isobutyric, n-valeric, and isovaleric acids, have been identified as contributing to the cheesy notes (Truong-van-Chom, 1958; Saisithi et al., 1966; Dougan and Howard, 1975; Beddows et al., 1976). The compounds responsible for the meaty aroma have not been identified.

A variety of rapid methods have been investigated in an attempt to shorten the time required to produce fish sauce. Processes involving of higher fermentation temperatures (Amano, 1962), addition of proteolytic enzymes, or use of acids (Amano, 1962; Beddows et al., 1976; Beddows and Ardeshir, 1979a), at ambient temperatures (Beddows and

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