AN ABSTRACT OF THE THESIS OF

Bryce K. Lukes for the degree of Master of Science in

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Title: Development of Methods for Analysis and

         Sensory Threshold Determination of Malt

         Derived Flavor Components in Beer

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        Malt, wort and beer samples representing a single
pilot brew were extracted from Celite columns with Freon
114.   Total extracts were analyzed by gas
chromatography/mass spectrometry.   Compounds were
identified that were unique or common to beer, wort or
malt and quantities present were determined for those
common to the three sample types.

        Concentrated extracts were separated by liquid
chromatography on partially deactivated silica gel packed
in Teflon tubes, using Freon 11 as the solvent.
Fractions from liquid chromatography were analyzed by
descriptive sensory evaluation.   Those fractions selected
as bearing cereal-like, grassy, nutty or malty aroma
characteristics were analyzed by gas chromatography.
Freon extracts and fractions were found suitable for
descriptive sensory evaluation and this was used to

advantage in locating fractions with the desired aroma characteristics. Cereal-like, grassy, nutty or malty aroma characters were associated with liquid chromatographic fractions containing primarily alcohols, aldehydes and ketones.

The Ascending Method of Limits for determination of the threshold of added substances in beer was critically reviewed and a new threshold test was developed. The new test design reduces the sample requirement from eighteen to twelve for each session. In contrast to the Ascending Method of Limits, the new test design was amenable to statistical evaluation and statement of a level of confidence for the threshold intervals determined.

Both a nine-point intensity scale of difference from the labeled reference and a category scale were applied to the new test design. Intensity scale data were normalized and subjected to analysis of variance and a series of one-sided t-tests to determine individual and combined panel threshold intervals. Category scale data were analyzed by a rank sums test to determine individual thresholds and the R-index values thus obtained were subjected to analysis of variance and orthogonal contrasts of means to establish the combined panel threshold.

The intensity scale method applied to the new test design provided the lowest and narrowest estimates of the threshold interval in comparative testing with the

category scale method and the Ascending Method of Limits and, in addition, required fewer replications than the category scale method to establish the threshold interval.

Development of Methods for Analysis and Sensory
Threshold Determination of Malt Derived Flavor Components
in Beer

by

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Chapter I.

Introduction

BEER FLAVOR CHEMISTRY. The chemistry of beer flavor has

been the subject of an extensive volume of research over

the years both from the academic standpoint and as a

matter of critical importance at the brewery level in

determination of the quality and consistency of the

product. Beer is an exceedingly complex food

incorporating the natural products malt and hops with

aqueous extraction, heating and fermentation processes.

More than 800 compounds have been identified in beer and

the list continues to grow. Identification of flavor

active compounds responsible for the various aromas and

flavors characteristic of beer has been moderately

successful but there are still large gaps in our

knowledge of compounds responsible for malt and hop

associated characters. Many of the compounds present in

malt and hops contributing to their characteristic

sensory qualities are volatilized and/or transformed

during processing such that these compounds may or may

not be present in sufficient quantity to contribute

similar sensory qualities to beer. Newly formed products

may also have sensory qualities reminiscent of the raw

materials. The number of compounds present in beer makes it difficult to isolate those responsible for a particular aroma or flavor character without some form of initial separation. Compounds present in high concentration may have little or no contribution to beer flavor while some compounds present in only trace quantities can have a major impact on the sensory qualities of beer.

ANALYSIS OF BEER FLAVOR. The analysis of beer flavor has followed and often has provided the impetus for development of new methods of separation and identification of compounds in complex mixtures. Extraction of volatiles from beer has proven difficult due to aqueous dilution and the presence of a large quantity of ethanol and other small chain alcohols. Solvents used with reasonable success have included pentane, carbon disulfide, methylene chloride, liquid carbon dioxide and trichlorofluoromethane with extraction directly from the aqueous phase or column extraction of the aqueous phase dispersed on a relatively inert matrix such as Celite. Dynamic headspace entrainment and reduced temperature vacuum trapping of beer volatiles have also been practiced. Gas chromatography has evolved as a particularly useful method for investigating the

flavor chemistry of natural products and has been used extensively in beer flavor work, particularly when used in combination with mass spectrometry for positive identification of compounds separated. Methods for pre-separation of aroma compounds from beer have included fractionation on cation-exchange resin, silica gel and alumina. Sensory evaluation in beer flavor work has largely been confined to identification of desirable or undesirable characteristics of the finished product or evaluation of individual compounds isolated from beer.

AROMA OR FLAVOR THRESHOLDS. The definition of aroma or flavor threshold has varied with the test methods used and the criteria established by the individual researcher. There are at least four recognized threshold types; the absolute threshold, difference threshold, recognition threshold, and the terminal threshold. The absolute and difference thresholds are often not clearly distinguishable when the media used for testing may include endogenous levels of the particular substance or substances for which the threshold is to be determined.

Difference tests often form the basis for the experimental design used and the probability of detection generally is the criterion that defines the threshold, with various probability levels assigned by individual

researchers. There has been a reluctance to define the threshold as that level of difference that can be detected with statistical significance. This has led to a situation where it is virtually impossible to compare threshold values reported in the literature.

There is no one universally accepted method to determine thresholds for substances added to a particular background medium such as air, water or beer. The Ascending Method of Limits, based on a series of forced-choice triangle tests, has been recognized by the American Society for Testing and Materials and has therefore been widely used although the estimates provided are not verifiable.

Chapter II.

Isolation of Aroma Components from Beer, Wort and Malt

by Combined Sensory and Analytical-

Instrumental Techniques

ABSTRACT

Beer, wort and malt samples were packed in glass columns with Celite and extracted using 1, 2 Dichlorotetrafluoroethane (Freon 114) as the solvent at $1^{O}C$ and atmospheric pressure. Sample extracts were concentrated in a Kuderna - Danish evaporator at reduced temperature and separated on partially deactivated silica gel packed in Teflon tubes with trichlorofluoromethane (Freon 11) as the solvent. Total extracts and isolated fractions were subjected to descriptive sensory evaluation and analysis by Gas Chromatography/Mass Spectrometry. Individual compounds in fractions with cereal-like, grassy, nutty or malty aroma characteristics were identified.

INTRODUCTION

The isolation and characterization of beer flavor constituents has been the topic of a tremendous quantity of research, pursued with increasing sophistication as new analytical methods have become available. Beer, its' primary raw material, malt and processing intermediate, wort, all contain exceedingly complex mixtures of volatile components, many of which are derived from heating and/or fermentation of natural products. Beer is known to contain over 800 compounds (Meilgaard, 1982) at levels from percentage to less than 1 ppb. To further complicate matters, many of the components present at relatively high levels contribute little to the aroma or flavor of beer, whereas minute quantities of certain flavor active compounds can have a major influence.

Gas chromatographic (GC) analysis is the technique of choice in separating volatile flavor compounds for identification or quantification. Beer and brewing materials can be analyzed by direct injection of headspace vapor into the GC (Kepner, et. al, 1963). This technique is not suitable for analysis of minor components since no concentration is effected, which will limit detection and resolution. Some form of preparation

is usually necessary to concentrate the compounds of interest, and to free them from interfering substances that may be present in the original sample. Beer sample preparation has evolved along the path of expediency, as often large numbers of samples are routinely analyzed. Methods that have been used include distillation (Bavisotto, et. al, 1961), solvent extraction (Stenroos, et. al, 1975 and 1985), headspace entrainment (Chen, 1983) and more recently, solvent extraction of samples dispersed on Celite columns (Lam, et. al, 1986, and Irwin and Thompson, 1987). All of these methods can have negative effects on the quality of the aroma concentrate due to thermal decomposition, oxidation, incomplete recovery or contamination (Flath, et. al, 1981, and Sugisawa, 1981). A low- temperature vacuum distillation procedure for non-destructive concentration of beer volatiles was developed by Pickett et. al, 1976, but is very time consuming and requires specially constructed glassware and constant attention. Low temperature extraction with various Freons has been used to concentrate fruit essences (Schultz, et. al, 1967) and various reactive or heat sensitive flavor mixtures (Bullard and Holguin, 1977 and Murray and Stanley, 1968). Freons 11 and 114 are particularly useful in unpressurized extraction systems and offer the advantages

of low toxicity, nearly complete rejection of H_2O and ethanol, and minimal ether-like odor that allows sensory evaluation of extracts. Freon 11 has also found use in liquid chromatographic separations of aroma concentrates (Murray and Stanley, 1968 and Palmer, 1973).

Flavor chemistry research must ultimately relate to the sensory perception of the end user if it is to have any real value. The technological intricacies and impressive capability of new instrumentation has in many cases led to long lists of novel compounds identified in a food or essential oil with little or no sensory evaluation other than perhaps a single person sniffing hot gases at the outlet port of a gas chromatograph. Considerable effort has recently been applied to integrating meaningful sensory evaluation in the analysis of aroma volatiles using techniques such as CHARM (Acree, et. al, 1984).

The brewing industry has been relatively diligent in applying descriptive analysis and threshold testing to compounds after they are identified in beer, but sensory input has generally been restricted to examination of the finished product before analysis or isolated compounds.

Class separation of aroma-bearing constituents in complex mixtures has been used to simplify analysis further, and with the aid of descriptive sensory

evaluation, to isolate volatile constituents that are responsible for a particular aroma (Murray and Stanley, 1968 and Palmer, 1973). A relatively odorless and non-toxic solvent such as Freon is required for descriptive assessment of aroma concentrates or fractions.

In the present study, extraction and separation techniques were developed to minimize degradation and loss of extracted constituents, and at the same time allow sensory input to assist in locating fractions containing aroma components of interest prior to instrumental analysis. Malt, wort and beer samples from a single pilot brew were analyzed using the techniques described to isolate compounds contributing to cereal-like, grassy, nutty or malty aroma.

MATERIALS AND METHODS

SAMPLES. Malt, wort and beer samples were obtained from the Stroh Brewery Company. Portions of the malt sample were pilot brewed with and without addition of hops. Yeast were generated twice in unhopped wort to remove any traces of hop compounds before production of unhopped beer samples. Brewing was carried out under conditions known to approximate commercial processing except that final dilution was not made from 16°Plato original gravity. Hopped and unhopped wort and beer samples were bottled and pasteurized. Samples were stored at $1^{o}C$ from 30 to 90 days before analysis.

EXTRACTION. All extraction operations were carried out at $1^{o}C$. Celite was purified before use by firing at $700^{o}C$ for 16h. Freon 114 (Union Carbide) was redistilled and passed through a column of activity I alumina previously fired for 1h at $600^{o}C$. Beer or wort samples (450g) or 100g of finely ground malt were well mixed with 450g of Celite and tamped into a 55mm x 1m glass column fitted with a Teflon stopcock and coarse porosity sintered glass bottom plate. Freon 114, 1350ml, was applied to the column, and solvent flow to the covered receiving flask was regulated at approx. 7ml/min. The

column was allowed to run dry (5-5.5h). Approx. 900ml of solvent was recovered from the column. Kuderna - Danish concentrators were assembled at room temperature with evaporative columns packed in dry ice. A dry ice - acetone cooled solvent recovery apparatus was attached and concentrator flasks containing the extract were removed from cold storage (-10°C), fitted to the system and immersed in a water bath at 18°C. Samples were concentrated to 10ml and removed from the water bath. The bottom tube containing the concentrated sample extract was packed in ice and fitted with a dry ice packed micro-Snyder column for final concentration under a gentle stream of nitrogen to 0.3ml. A 0.1ml aliquot was taken for liquid chromatography and the remaining sample was brought to 1ml final volume with methylene chloride, (Burdick & Jackson). Samples were sealed and stored at -40°C. Recovery standards 4-Phenyl-3-buten-2-one (benzal acetone) and ethyl heptanoate (1g per 100ml of ethanol) were added (50µl) if required to beer, wort or malt. Napthalene (1g per 100ml methylene chloride) was used as an analysis standard (100µl) in the final concentrate.

LIQUID CHROMATOGRAPHY. Teflon tubing, 5mm i.d. in 230mm lengths was thoroughly washed, rinsed with distilled

water and oven dried. High purity TLC silica gel without
binder (Aldrich) was dried for 1 h at 110°C and partially
deactivated by addition of water (25% w/w). A few
crystals of azobenzene were placed 1cm inside one end of
the Teflon tube, followed by glass wool packing to fill
the 1cm void. The Teflon tube was slid into a glass
tubing support (7mm i.d. x 230mm) and packed with exactly
2g of partially deactivated silica gel with gently
tapping until 3cm of the tube remained unfilled. All
remaining operations were carried out at 1^{O}C. Duplicate
tubes were held vertically and 50µl charges of
concentrated extract from beer, wort or malt were applied
evenly to the surface of the packing, followed by 2cm
additional silica gel packing, and lastly glass wool to
fill the remaining 1cm. Teflon tubes with their glass
tubing supports were inverted and placed in a sealed 2L
erlenmeyer flask with high purity Freon 11 developing
solvent (Aldrich) added to a depth of 2cm. Development
required approximately 6h and was complete when the
orange color of the azobenzene appeared in the upper
glass wool plug. Teflon tubes were removed from the
Freon, chilled to -10°C, extracted from their glass
supports and cut into eight 23mm segments from the point
where the aroma concentrate was introduced. From one set
of duplicate tubes, cut sections were transferred to

vials, sealed and stored at -40°C. Sections from the
second set of tubes were each flooded with 1ml of
methylene chloride and the resulting suspensions filtered
through disposable membrane filter disks, (0.45μ), sealed
in glass vials and stored at -40°C.

GAS CHROMATOGRAPHY/MASS SPECTROMETRY. All compounds were
identified on a Finnigan Model 4023 quadropole mass
spectrometer. Samples were chromatographed on a 0.32mm x
60m Durawax-4 fused silica WCOT column (J & W Scientific)
with helium as the carrier gas at a linear velocity of
20cm/s. The split ratio was 1:100. Oven temperature was
programmed from 60 to 200°C at 5°C/min, with a 5 min hold
at the initial temperature. Samples (0.5μl) were
injected on-column. The mass spectrometer was operated
in electron-impact mode with the electron energy at 50eV.
Mass spectra were taken each second with a scan range of
m/z 40 to 400. Compounds were identified by comparison
of retention time with authentic compounds and also by
comparison of mass spectra with reference spectra from
the National Bureau of Standards, and with a collection
of reference spectra compiled at the Agricultural
Chemistry Department of Oregon State University.

Quantitation of extracted compounds was carried out
on a Hewlett-Packard 5830 gas chromatograph fitted with

an FID detector and a Supelcowax-10 (Supelco, Inc.)
0.25mm x 60m WCOT fused silica column. Helium was the
carrier gas. Column temperature was programmed from 60 -
200°C at 5°C/min, with a 5 min hold at the initial
temperature. A Hewlett-Packard 18850 GC terminal was
used to perform peak area quantitation.

DESCRIPTIVE ANALYSIS. A six member descriptive panel was
assembled from a pool of active beer panel members. A
series of round table sessions was held to evaluate
potential aroma standards characteristic of the
aroma/flavor classes established for beer (Meilgaard,
1982), with particular emphasis on classes 2, 3, and 4,
representing resinous, nutty, green grassy, cereal,
caramelized and roasted characters. Liquid aroma samples
were adsorbed on glass wool in screw-top test tubes
wrapped in aluminum foil. Solid samples were placed
as-is in the foil wrapped tubes. Random 3-digit numbers
were applied to the tubes and assessors were required to
individually apply descriptive terms to each sample, and
then reach a consensus approval of an appropriate
descriptor or descriptors, if possible. Ten aroma
standards were selected that received consensus
characterization by class and common or similar aroma
descriptors (Table II.1). Liquid standards were

dispersed in 0.5ml Freon 11 and presented, as above, to determine any effect Freon might have on the assessors descriptive abilities. Correct class designation and descriptor assignment by assessors was confirmed in blind testing.

Samples from liquid chromatographic fractions were transferred to screw top test tubes containing 0.5ml distilled water at 1^OC. Assessors were instructed to individually apply aroma class designation and descriptors to each coded sample as it was brought to room temperature. Order of presentation was random and tubes were not foil wrapped as the appearance of all samples was identical. Aroma reference standards were available.

RESULTS AND DISCUSSION

CHARACTERISTICS OF FREON EXTRACTS. Aroma concentrates
obtained by Freon extraction were largely characteristic
of the starting material, malt, wort, or of beer, as
verified by the descriptive panel. Extracts did not
require additional treatment to remove water or lower
alcohols as they were not extracted from Celite packed
columns by the solvent. Major objections to the use of
Freon for extraction have been the difficulties
associated with the handling of low boiling solvents and
relatively poor extraction of organics from aqueous
systems (Leahy and Reineccius, 1984). Selection of a
Freon that can be handled in a non-pressurized system at
reduced temperature overcame the first objection and it
was hoped that thin layer dispersion of the aqueous phase
on Celite would improve recovery. Average recovery of
standards was only 63% for ethyl heptanoate and 75% for
4-phenyl-3-buten-2-one (benzal acetone). Recovery might
have been improved with a continuous
extraction-distillation loop. The concentrate obtained
was suitable for instrumental analysis or descriptive
panel evaluation.

ANALYSIS OF FREON EXTRACTS. GC/MS and GC/FID analysis of

aroma concentrates or fractions was relatively straightforward. Attempts were made early in the research to inject concentrates in Freon directly on-column, but were abandoned due to difficulty in handling the solvent. Refinement of sample injection to allow the use of Freon would be useful in analysis of low boiling compounds. Large peaks for amyl alcohols and 2-phenylethanol, that often cause difficulties in analysis of beer volatiles, are also present in Freon extracts. A large number of individual compounds were identified or tentatively identified in Freon extracts of malt, wort, or of beer samples, although no novel compounds were identified with the possible exception of a pyrazine tentatively identified as 2-dimethylamino-3-isobutyl pyrazine. A number of individual compounds isolated by gas chromatography and for which mass spectra were obtained, were not identified. The solvent favored extraction of hydrophobic compounds, including large chain alcohols and aldehydes, organic acids, and cyclic compounds. Compounds identified that were unique to either the malt, wort or beer samples analyzed are listed in Table II.2. The compounds unique to wort and beer are products from the respective heating and fermentation processes involved and are compounds normally found in wort and

beer. Compounds that were common to malt, wort and beer were of particular interest in this study as presumably compounds responsible for cereal-like, grassy, nutty or malty aroma characteristics in beer would have their origin in malt. Table II.3 lists compounds and their concentrations that were identified as common to the malt and pilot wort and beer samples analyzed. Compounds present in the pilot beer analyzed that were above their reported thresholds in beer or water were 2-octanol, 𝜸- -nonalactone and decanoic acid. The aroma descriptions for these compounds are coconut/walnut/oily, coconut, and tallowy/caprylic, respectively (Meilgaard, 1982).

ANALYSIS OF LIQUID CHROMATOGRAPHY FRACTIONS. As shown in Table II.4, fractions from silica gel columns that were assigned to aroma classes 2, 3 or 4 by the descriptive panel were confined to lower segments of columns, where mainly alcohols and aldehydes were retained. Descriptive panel results for fractions from malt, wort and beer are listed in Tables II.5-II.10. Assessor agreement was nearly universal with regard to class assignment for fractions, although descriptors varied somewhat with individuals to express their perception of a particular aroma. The slightly sweet, ether-like aroma of the Freon solvent did not seem to interfere with panelist

assessment of fractions except when the aroma present was very faint. The compounds identified in these fractions are listed in Table II.4. Other compounds of the same class present in lesser quantities may have eluded efforts at identification as a result of dilution in final recovery by methylene chloride extraction. Of the compounds common to malt, wort and beer, it appears that the alcohols and aldehydes contribute to aromas characteristic of classes 2, 3 and 4. The transformation of carbonyl compounds to the associated alcohols and esters was studied by Peppard and Halsey, 1981, following discovery that malt-like aroma in aqueous mashes of malt was nearly completely removed after treatment with 2,4-dinitrophenylhydrazine. The contribution of carbonyl compounds to beer flavor was not ruled out, but it was deemed unlikely that the particular aldehydes and ketones investigated contributed directly to beer aroma or flavor.

A pyrazine tentatively identified as 2-dimethylamino-3-isobutyl pyrazine was also common to the malt, wort and beer samples analyzed. This compound has been synthesized previously and was shown to have a strong cocoa, roasted aroma (Takken, et. al, 1975). It would most likely follow the solvent front in the separation procedures used and could have been missed in sectioning of the Teflon tubes.

CONCLUSIONS

1. The procedures developed in this paper have application in flavor research where aroma volatiles with particular sensory characteristics are targeted in a complex mixture, and may have application for industrial use with certain natural products. Freon extraction of aroma volatiles from apple essence was the subject of a patent by Stanley et. al, 1963. Concentrates in Freon 114 could be added to food without concern over residual solvent.

2. The use of Freon as a solvent allows sensory input in the preparation of samples for instrumental analysis and can simplify separation and identification of compounds responsible for a characteristic aroma.

3. The aroma volatiles characteristic of aroma classes 2, 3 and 4, and representing resinous, nutty, green grassy, cereal, caramelized and roasted characters were found in fractions containing primarily alcohols, aldehydes and ketones from liquid chromatographic separation of Freon extracts of malt, and pilot wort and beer samples.

3. The time consuming nature of the analytical procedures presented in this research would prevent routine use of these techniques for sample screening or quality control purposes.

TABLE II.1

AROMA STANDARDS
DESCRIPTIVE ANALYSIS PANEL
AROMA CLASS ASSIGNMENTS

Sample or Compound	Aroma Class Assigned[a]	Descriptors
1. Iso-amyl acetate	1	Flowery, banana
2. Ethyl hexanoate	1	Green apple, fruity
3. 2-Phenylethanol	1	Roses, floral
4. Butanal	2	Earthy, sour grass
5. Finely ground lager malt	3	Malty, grainy
6. Moistened finely ground lager malt	2	Ground corn, green
7. Moistened whole lager malt	2	Wet straw, hay
8. 1-Hepten-3-ol	5	Plastic
9. ɤ-Nonalactone	9	Sweet
10. Amber malt	4	Burnt, popcorn, toast

[a] Aroma classes (Meilgaard, 1982):

    1 = Fragrant, fruity, floral
    2 = Resinous, nutty, green, grassy
    3 = Cereal
    4 = Caramelized, roasted
    5 = Phenolic
    9 = Sweet

TABLE II.2

COMPOUNDS IDENTIFIED IN FREON 114 EXTRACTS
UNIQUE TO MALT, WORT OR BEER FROM PILOT BREWING

Malt	Wort[a]	Beer[a]
Undecane	Thiazole	Isoamyl acetate
3-Methylbutanol	2-Octanone	Ethyl hexanoate
1-Pentanol	Furfural	n-Heptyl formate
2-Nonenal	5-Methyl furfural	Ethyl octanoate
		Ethyl nonanoate
		Ethyl decanoate
		2-Phenylethyl acetate

[a] Unhopped

TABLE II.3

COMPOUNDS IDENTIFIED IN FREON 114 EXTRACTS
COMMON TO MALT, WORT AND BEER FROM PILOT BREWING

Compound	Malt mg/kg[a]	Wort mg/l[a]	Beer mg/l[a]	Reported Threshold in Beer mg/l[b]
2-Octanol	0.528	0.096	0.081	0.04
Benzaldehyde	---	0.220	0.038	2.0
1-Octanol	0.087	0.031	0.043	0.9
2-Phenylethanol	0.636	0.725	25.38	125.0
2-Acetylpyrrole	---	0.302	0.184	---
४-Nonalactone	---	0.078	0.382	0.001[c]
Octanoic acid	---	0.112	2.67	4.5, 15
Nonanoic acid	---	0.486	0.289	---
Decanoic acid	---	1.21	4.05	1.5, 10
A pyrazine, MW 179	0.863	0.699	0.661	---

[a] Concentration based on average recovery of standards ethyl heptanoate (63%) and benzal acetone (75%).

[b] As reported by Meilgaard, 1982.

[c] In water.

TABLE II.4

LIQUID CHROMATOGRAPHY FRACTIONS
SELECTED BY DESCRIPTIVE ANALYSIS PANEL

Sample Fraction[a]	Aroma Class Assigned	Aroma Descriptors Assigned	Compounds Identified[b]
Malt			
1	2,3	Grainy, green	1-Pentanol 1-Hexanol 1-Octanol 2-Octanol 1-Methyl-1-butanol
2	3	Grainy, malty	1-Octanol 2-Nonenal
3	1	Fruity, green	2-Phenylethanol Phenylacetaldehyde Furfural
Wort			
1	3	Grainy, malty, wet dog	1-Hexanol 1-Octanol 2-Octanol 2-Methyl-1-butanol Carvacrol
2	4	Malty, roasted barley	1-Octanol Furfural ɣ-Nonalactone
3	1	Woody, resinous	Furfural Nonanal Benzaldehyde
Beer			
1	4	Malty, sweet	1-Hexanol 2-Octanol 2-Phenylethanol
4	4	Roasted, grainy	Benzaldehyde ɣ-Nonalactone n-Heptyl formate

[a] Sample fraction numbers correspond to teflon tube segments from the point of sample introduction.

[b] Fractions eluted with methylene chloride and analyzed by gas chromatography.

TABLE II.5

DESCRIPTIVE ANALYSIS
LIQUID CHROMATOGRAPHY FRACTIONS
MALT SAMPLE A

Fraction Number	Panelist	Descriptors	Category Assigned
1	1	straw, hay	2
	2	grain, green	2,3
	3	roasted cereal	---
	4	grain, green	3
2	1	grainy	3
	2	grainy	3
	3	corn, cereal	3
	4	grainy	3
3	1	papery	---
	2	fruity	1
	3	green	2
	4	grainy	3
4	1	solvent	1
	2	fruity	1
	3	fruity	---
	4	fruity	1
5	1	---	---
	2	solvent	1
	3	---	---
	4	solvent	1
6	1	---	---
	2	fruity	1
	3	---	---
	4	slight grainy	---
7	1	apple	1
	2	fruity	1
	3	roses	1
	4	hops	1
8	1	stale	---
	2	solvent	1
	3	stale	8
	4	solvent	1

TABLE II.6

DESCRIPTIVE ANALYSIS
LIQUID CHROMATOGRAPHY FRACTIONS
MALT SAMPLE B

Fraction Number	Panelist	Descriptors	Category Assigned
1	1	strawlike, cereal	2,3
	2	grain, green	2,3
	3	malt	3
	4	grain, green	2,3
2	1	straw, grassy	2,3
	2	grainy	3
	3	malt	3
	4	grainy, malty	3
3	1	grassy	2
	2	grainy, green	2,3
	3	grainy	3
	4	grainy	3
4	1	---	---
	2	floral	1
	3	plastic	1
	4	---	---
5	1	---	---
	2	floral	1
	3	---	---
	4	---	---
6	1	---	---
	2	---	---
	3	---	---
	4	roasted grain	4
7	1	fruity	1
	2	sweet, floral	1
	3	---	---
	4	---	---
8	1	---	---
	2	---	---
	3	---	---
	4	sulfur	---

TABLE II.7

DESCRIPTIVE ANALYSIS
LIQUID CHROMATOGRAPHY FRACTIONS
UNHOPPED WORT SAMPLE A

Fraction Number	Panelist	Descriptors	Category Assigned
1	1	sweet, caramel	4
	2	malty	3
	3	wet dog, malty	3
	4	grainy	3
2	1	caramel, woody	4
	2	roast barley	4
	3	grainy	3
	4	green, grainy	3
3	1	slight earthy	8
	2	woody, roasted	4
	3	grainy	3
	4	solvent	---
4	1	slight sweet	---
	2	---	---
	3	slight sweet	---
	4	solvent	---
5	1	slight sweet	---
	2	---	---
	3	---	---
	4	---	---
6	1	solvent	1
	2	---	---
	3	---	---
	4	---	---
7	1	skunky, sulfur	7
	2	plastic	1
	3	biting	7
	4	sulfur	7
8	1	skunky	7
	2	solvent	1
	3	biting	7
	4	solvent	---

TABLE II.8

DESCRIPTIVE ANALYSIS
LIQUID CHROMATOGRAPHY FRACTIONS
UNHOPPED WORT SAMPLE B

Fraction Number	Panelist	Descriptors	Category Assigned
1	1	caramel	4
	2	grain, molasses	4
	3	wort	3
	4	grainy, caramel	4
2	1	green	3
	2	green	3
	3	grainy	3
	4	green	3
3	1	sweet, malty	4
	2	grainy	3
	3	grainy	3
	4	silage	3
4	1	---	---
	2	---	---
	3	---	---
	4	---	---
5	1	---	---
	2	---	---
	3	---	---
	4	---	---
6	1	---	---
	2	---	---
	3	---	---
	4	---	---
7	1	stale	8
	2	floral	1
	3	sulfur	7
	4	stale	---
8	1	---	---
	2	---	---
	3	green	2
	4	---	---

TABLE II.9

DESCRIPTIVE ANALYSIS
LIQUID CHROMATOGRAPHY FRACTIONS
UNHOPPED BEER

Fraction Number	Panelist	Descriptors	Category Assigned
1	1	banana, apple	1
	2	malty, sweet	10,4
	3	solvent	1
	4	sweet, malty	4
2	1	banana	1
	2	sweet, hop	10,1
	3	stale	8
	4	fruity	1
3	1	---	---
	2	---	---
	3	sulfur	---
	4	solvent	---
4	1	grain	3
	2	roasted	4
	3	---	---
	4	---	---
5	1	---	---
	2	---	---
	3	---	---
	4	---	---
6	1	papery	---
	2	burnt	4
	3	---	---
	4	---	---
7	1	floral, beer	1
	2	sweet, beer	10
	3	beer	1
	4	beer	1
8	1	---	---
	2	---	---
	3	---	---
	4	---	---

TABLE II.10

DESCRIPTIVE ANALYSIS
LIQUID CHROMATOGRAPHY FRACTIONS
HOPPED BEER

Fraction Number	Panelist	Descriptors	Category Assigned
1	1	acetone, solvent	1
	2	malty	3
	3	grainy	3
	4	sweet, floral	1
2	1	fruity	1
	2	---	---
	3	---	---
	4	---	---
3	1	sour, butyric	---
	2	---	---
	3	plastic	---
	4	---	---
4	1	---	---
	2	roasted	4
	3	grainy	3
	4	pungent	---
5	1	---	---
	2	woody	2
	3	plastic	---
	4	solvent	---
6	1	---	---
	2	burnt	4
	3	---	---
	4	---	---
7	1	papery, sour	8
	2	---	---
	3	---	---
	4	alcohol	---
8	1	slightly fruity	---
	2	---	---
	3	---	---
	4	---	---

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Chapter III.

Sensory Thresholds of Added Substances in Beer:

Alternative Test Design and Analyses

ABSTRACT

The Ascending Method of Limits as described by the
American Society for Testing and Materials and adopted by
the brewing industry was critically reviewed. A new test
for absolute or difference threshold determination of
added substances in beer was developed. Samples were
arranged in three groups that each contained a labeled
reference sample and three randomly arranged samples.
Random samples included an unlabeled reference sample and
two samples containing adjacent concentration levels from
a series of six increasing concentrations of added
substance. Groups were presented in ascending order by
concentration. In one version of the test, panelists
rated each sample using an intensity scale of difference
from the labeled reference sample. The resulting data
were normalized and subjected to analysis of variance
followed by a series of paired comparison t-tests to
determine threshold intervals for individual panelists
and the combined panel. A modified signal detection
technique was also applied to the new test design.
Individual panelist thresholds were determined by use of
a rank-sums test. Combined panel thresholds were
obtained by analysis of variance applied to R-Indices
obtained for each panelist followed with a series of one
sided t-tests based on orthogonal contrasts.

INTRODUCTION

Brewing chemists involved in beer flavor work are of
necessity interested in the quantity of a particular beer
constituent or additive that will create a perceptible
change in the sensory characteristics of a base brand or
style of beer. Thresholds for substances added to beer
have not been clearly defined as either difference or
absolute thresholds (Brown, et. al, 1978 and Lundahl, et.
al, 1986) but can be determined for addition of
substances that are normally present in or totally
foreign to the system under study. True difference or
absolute threshold tests, when applied to existing
sensory systems as complex as beer, require inclusion of
a control sample with no added substance against which
all test samples are compared. In this regard, the
principles of Signal Detection Theory are particularly
applicable (Green and Swets, 1966). Beer as a medium for
sensory evaluation elicits a high base level of sensory
response or "noise". The concentration range of an added
substance that is of interest in threshold determination
will provide a sensory signal that is weak or of similar
intensity to other sensory signals characteristic of the
medium. To allow maximum sensitivity in detection of the
added substance at low stimulus levels a known reference

must be supplied to provide a fixed background noise.

There are at least two other recognized sensory thresholds, the recognition and terminal thresholds (ISO Subcommittee, 1978). The actual point estimates or ranges of concentration of a given substance determined for absolute, difference, and recognition thresholds may be similar but are dependent upon the conditions of the test and the criteria used to define the threshold concentration (Brown, et. al, 1978).

Threshold test design and evaluation of data should account for assessor variability to the extent possible. Hidden biases introduced by test design must be avoided and panelist motivational effects accommodated in analysis of the data.

In an effort to standardize the reporting of threshold values in the brewing industry and provide a common basis for comparison of these values, the American Society of Brewing Chemists (ASBC) and European Brewing Congress jointly studied 14 threshold methods and collaboratively examined four of these methods (Brown, et. al, 1978). The ASBC finally accepted the Ascending Method of Limits (AML) as described by the American Society for Testing and Materials (ASTM) as a standard method (ASBC Subcommittee, 1980 and ASTM,1979). The AML consists of series of six triangle tests presented in

total to the assessor in which the different sample within each triangle contains an added substance and the two remaining samples consist of the background medium with no addition. The concentration of added substance increases by a fixed factor for each successive triangle in the presentation. The assessor must correctly identify the sample in each triangle that contains the added substance.

The AML has been criticized for a number of reasons which include the level of difficulty of the test, unavoidable fatigue and sensory adaptation from assessor exposure to six triangle tests in a single session, and inefficiency in terms of glassware, sample requirements, and time required for the assessor to complete the test. No provisions have been made in the recommended form of the AML for separate aroma and taste evaluation of samples which can lead to erratic panelist performance. It has been shown that hidden biases exist that are not accounted for in the test design (Morrison, 1982). An overestimate of the difference threshold will result when errors in panelist judgment occur at concentration levels above the panelists true threshold. The triangle test as presently employed in the AML for evaluation of thresholds of substances added to beer does not include a known control sample for comparison. Recommended

practice for evaluation of data obtained by the AML does not result in a statistically valid estimate of individual or group threshold concentrations. The Best-Estimate Threshold or BET (ASTM, 1979) that is generated is a biased estimator established by convention rather than sound statistical interpretation and uses unproven assumptions regarding assessor performance.

We reasoned that difference threshold determination in beer could be improved by application of standard experimental design techniques (Kempthorne, 1973) to develop a test which would allow examination of major sources of error and at the same time satisfy the requirements we had established for a desirable method. These requirements included a test which was ascending in concentration series, required minimal samples and glassware, allowed maximum sensitivity with minimum assessor effort and would yield statistically valid estimates of individual and group thresholds. It was also desirable to obtain information from the test after a minimum number of replications that would establish the approximate extent of testing required to obtain significant differences in response within the concentrations of added substance tested.

The proposed new difference threshold test relies on an ordered paired presentation scheme with samples

arranged in fixed groups. The test requires 12 samples
and is semi-ascending for a range of six concentrations.
Labeled and unmarked reference samples are included
within each group.

MATERIALS AND METHODS

SAMPLE PREPARATION. Exactly 22.2g of reagent grade
sodium metabisulfite, Mallinckrodt, were dissolved in 4%
ethanol to 100ml final volume. Additions to a commercial
beer, required 7.0μl to 670μl in each 355ml bottle to
obtain the desired range of final concentrations as mg/l
SO_2.

A linalool stock solution was prepared by dissolving
60.7mg of 99% minimum purity linalool, Aldrich, in 95%
ethanol to 100ml final volume. A 1:4 ethanol dilution of
the stock solution was made to accommodate the lowest two
concentrations in the series tested. Addition volumes of
5.0μl to 125μl established the desired range of
concentrations in beer.

A stock solution of 2-hexanol was prepared by
dissolving 3.887g of 99.9% minimum purity 2-hexanol,
Aldrich, in 95% ethanol to 100ml final volume. Volumes
from 2.0μl to 64μl were added to bottled beer as
required.

A complete listing of concentrations in beer for
each of the compounds tested is provided in Table III.1.

All test samples were prepared, stored overnight at
approximately 18°C to equilibrate and served at 18 to
20°C. Control samples were treated the same as test

samples except that no additions were made prior to resealing the bottles.

SENSORY TESTING METHODS. The AML was used as described by the ASBC for determination of thresholds of substances added to beer (ASBC Subcommittee, 1980). Concentration of added substance increased by a factor of 2.5 in successive triangles for SO_2 and linalool and by a factor of 2.0 for 2-hexanol. Results were interpreted for individual and group thresholds by calculation of BET (ASTM, 1979).

The semi-ascending ordered paired difference (SAPD) test under investigation consisted of three groups of four samples as illustrated in the ballot shown in Fig. 1. Three of the samples within each group were randomly presented and comprised of an unknown reference sample with no addition of added substance and two samples containing different levels of an added substance. Panelists were instructed to compare samples only with the labeled reference supplied with each group and in the order given. The lowest two levels of added substance were included in the first group presented, the middle two levels in the next group, and the highest remaining levels in the final group. Concentration of added substance was increased for each successive level by a

constant factor of 2.0 for 2-hexanol and 2.5 for both SO_2 and linalool. The test required initial response based strictly on aroma followed by separate taste evaluation. A minimum of 18 individual sample assessments were required to complete each test for aroma or taste response.

The SAPD presentation scheme was evaluated using two test methods. In one of the methods, a nine-point category scale was used for rating the degree of difference from the labeled reference as shown in Fig. 1. Within each group the response from the unknown reference was subtracted from the response for each sample with the added substance to remove errors due to expectation. The resulting data were analyzed using ANOVA procedures and paired t-tests as described by Lundahl et. al, 1986. Programs were written in SAS and BASIC for an IBM-PC[R] to essentially automate the determination of threshold intervals for individual panelists and the combined panel.

A second test method involved signal detection measures applied to the SAPD presentation scheme. Four response categories were substituted for the nine-point intensity scale of difference. Possible responses for each test sample as compared with the labeled reference were (D) "definitely different", (D?) "probably

different, not sure", (S) "definitely the same", (S?) "probably the same, not sure". A series of response matrices were obtained for each panelist to compare ratings given to the unknown reference sample within each group with those obtained for the test samples containing adjacent levels of added substance. R-indices and Z-values were determined by panelist for each concentration level as described by O'Mahony, 1986 and shown in Fig. 2. Individual panelist thresholds were determined by application of a rank sums test to initially determine the level of added substance where a difference in response could be detected compared to the response to the unlabeled reference and secondly, to establish the threshold interval by sample to sample comparison analogous to the technique described above for intensity scale data. Combined panelist thresholds were located by repeated-measures ANOVA applied to R-index scores from all panelists, followed by successive one-sided t-tests of means based on orthogonal contrasts.

SO_2 and linalool method comparisons were replicated four times for both the AML and the nine-point intensity scale version of the SAPD test. Method comparison tests were replicated ten times with 2-hexanol as the added substance with the AML and both versions of the SAPD test. Order of method was established as a random

drawing with no more than two sessions with a single method allowed in succession. Method comparison series were not concurrent for the three compounds tested.

SENSORY PANEL SELECTION. Panelists were selected by availability to participate from a pool of 15 to 20 active beer panel members. To familiarize panelists with testing procedures and sensory qualities of added substances, reference samples were made available and at least one replication of each test was made prior to data generation for method comparison. All panelists were informed of the design of each test method prior to participation in method evaluation. A nine member panel was used in testing SO_2 and linalool, and a twelve member panel was used for all testing with 2-hexanol.

STATISTICAL ANALYSIS. A detailed example of the analysis for combined panel thresholds is provided in Lundahl et. al, 1986 for the intensity scale version of the new SAPD test.

Threshold intervals for individual panelists were determined by analysis procedures similar to those used for estimating combined panel thresholds. Sample responses were normalized by subtracting the response on the unknown control. As an example, the ANOVA for

panelist 1 data for response to 2-hexanol aroma is listed in Table III.2. The Group Effect is very significant (p-value < 0.01) and the Sample Effect within group 3 was significant (p-value < 0.05) which indicates a difference exists in mean sample responses over the concentrations of added substance tested.

The threshold interval for panelist 1 was then determined from two sets of simultaneous one-sided t-tests, Table III.3. A significant mean deviation from zero was found at concentration level 4 in the first set of t-tests (p-value <0.01). The first significant difference was found in the second set of simultaneous one-sided t-tests between mean sample response at concentration level 4 compared with level 2 (p-value <0.01). By this analysis, the threshold of panelist 1 lies between concentration level 2 (0.438mg/l), and concentration level 4 (1.75mg/l) for the aroma of 2-hexanol added to beer.

In the signal detection version of the SAPD test, R-indices and Z-values were determined for comparison of each sample with the unknown reference within the sample group. Fig. 3 contains an illustration of the analysis for panelist 1, concentration level 1, from the 2-hexanol aroma test series. The analysis is repeated for each sample concentration level. The first significant

Z-value occurred at concentration level 4 which indicates the panelist's responses at this level were significantly different from those obtained for the unknown reference. Level 4 responses were then tested against those for each sample containing a lower concentration of added substance to determine the threshold interval. The first significant difference was found between sample responses at level 4 versus level 2 by rank sums test (p-value < 0.05). The threshold for panelist 1 by this analysis was between concentration level 2 and concentration level 4, which in this instance, is the same interval as determined from the analysis applied to intensity scale data. The test sets required for this determination are shown in Table III.4.

Combined panel thresholds for the signal detection version of the SAPD test were determined by ANOVA applied to panelist R-Index values obtained for each concentration level of added substance as shown for 2-hexanol aroma in Table III.5. This was followed by a series of paired comparisons (Table III.6) based on orthogonal contrasts. The combined panel threshold interval for the aroma of 2-hexanol added to beer was between concentration level 4 (1.75mg/l), and concentration level 5 (3.50mg/l) by this analysis.

RESULTS AND DISCUSSION

Method comparison results are listed in Tables
III.7-III.10 for individual and combined panel
thresholds. Substantial differences are apparent in
thresholds obtained for individual panelists by each
method. The SAPD intensity scale method tends to produce
threshold intervals that are lower in concentration than
the SAPD signal detection method but not necessarily
smaller in concentration span. AML results most closely
approximate those obtained by the SAPD signal detection
method for both individual panelist and combined panel
thresholds. The combined panel threshold for the test
series with 2-hexanol was much lower for the SAPD
intensity scale method as compared to either of the other
methods.

THRESHOLD DEFINITION. The definition for absolute or
difference thresholds reported in the literature has
varied according to convention established by the
experimenter. A common definition is stated as the
concentration of added substance that corresponds to a
50% probability of detection by the individual or panel.
Some experimenters have chosen to use 70% probability as
a criterion or some other arbitrary measure to define the

threshold.

This researcher has assumed the definition of threshold, as the meaning of the word implies, to be the lowest concentration of added substance which can be detected by the individual or panel with statistical significance. The difficulty of applying statistical analysis to sensory testing and in particular threshold testing should not be an excuse to apply some other arbitrary criterion to define the threshold.

The AML uses 50% probability of detection to define the threshold while both the intensity scale and signal detection methods applied to the SAPD test determine the threshold interval containing the lowest concentration of added substance that can be detected with statistical significance.

SAPD INTENSITY SCALE METHOD. The SAPD intensity scale method estimates a threshold interval with the assumptions of normally distributed mean scores and homogeneity of variances in error terms pooled across groups at or below threshold. The degree to which ANOVA and t-tests are robust to violation of these assumptions has not been well established. Sensory data obtained by scaling may not be normally distributed due to end effects, panelists' preferential use of selected segments

of the scale and scale intervals that are not

psychologically equivalent. Subtraction of response to

the unknown control from the responses for samples

containing added substance within each group was seen as

a way to normalize the data by removing variation due to

panelist expectation of an ascending concentration series

of added substance. A density trace (Chambers, et. al,

1983) of these adjusted responses at each concentration

level of added 2-hexanol for the combined panel is given

in Fig. 4. The observed distributions approach the

normal curve in the critical region at or below

threshold, but become skewed at concentrations of added

substance above threshold. Individual panelist threshold

intervals were lowest overall in concentration as

determined by the SAPD intensity scale method.

The combined panel threshold interval covers a lower

range of concentration than would be anticipated from

individual panelist results. The combined panel analysis

includes a much larger number of trials than the analysis

for the individual panelist. As the number of trials

increases due to replication or panel size, the variance

in comparison of sample means should decrease, thereby

providing greater precision in estimating the threshold.

This increase in precision, ie, lowering and narrowing of

the threshold estimate, will become smaller (tending to

zero) as a sample of panelists is attained, with respect to thresholds, that is representative of the true distribution of the population. The ANOVA for the combined panel data matrix is very sensitive to small changes in mean response. An alternative and potentially more useful combined panel analysis may be possible from constructing a model appropriate for the distribution of individual panelist thresholds as a density function over the concentration range tested.

A plot of means for normalized difference response at each concentration level over the threshold range follows the expected logarithmic function for the combined panel as given in Fig. 5 for 2- hexanol aroma by the SAPD intensity scale method and is similar to plots obtained for test series with linalool and SO_2 by the same method.

Threshold estimates by the SAPD intensity scale method are established with minimal replication and are relatively stable with increased replication as shown in Table III.11. Threshold intervals established with 10 replications are generally narrower but within the same range as threshold intervals from separate analysis of the first and last five replications.

SAPD SIGNAL DETECTION METHOD. The SAPD signal detection

method relies on nonparametric tests for determination of individual panelist thresholds and to arrive at R-indices that are amenable to parametric analysis in determination of combined panel thresholds. In general, nonparametric tests are not as powerful as their parametric counterparts but do not require means for sample scores to be normally distributed. Panelist uncertainty is accommodated in the response categories and subsequently taken into account in the analysis.

The analysis to determine individual panelist threshold intervals by the SAPD signal detection method is relatively simple, easily adapted to computer spreadsheet programs and results in a valid confidence statement that the panelist's true threshold is contained within the stated interval.

Combined panel threshold intervals obtain the benefit of parametric analysis applied to R-indices and isolation of panelist effect from the analysis. R-indices should be normally distributed since they are actually probability estimates. T-tests based on orthogonal contrasts provide a series of independent analyses leading to a statistically valid statement concerning the location of the combined panel threshold. Failure to reach significance over the series of comparisons is an indication of insufficient replication

or that the combined panel threshold is above or below
the concentration series tested; the direction indicated
by results of individual panelist analyses. The combined
panel threshold can be determined by the same procedures
used to determine individual panelist thresholds but
error due to panelist effect is not isolated in the
analysis.

The SAPD signal detection method does not account
for day to day differences in panelist sensitivity. Each
response is assumed to be independent when in fact they
may be influenced by the same external effects on any
given day.

Determination of threshold intervals for individual
panelists by the SAPD signal detection method requires a
greater number of replications than the SAPD intensity
scale method as seen in Table III.12. This is a result
of the non-parametric rank-sums test used in which a
significant number of tied scores is possible, requiring
normal approximation of the Wilcoxon Rank-Sum S
distribution.

ASCENDING METHOD OF LIMITS. The AML provides a point
estimate for individual panelist or combined panel
threshold without statistical indication of validity of
the estimate or the associated confidence interval. The

degree of testing required (replication) is determined subjectively by the assessor as is the appropriate concentration range for an individual panelist. Examination of AML results for 2-hexanol testing reveals questionable threshold estimates for panelists 4, 9, 10, and 11. The calculated thresholds with one exception lie within the limits of concentration of added substance tested, although for a number of replications, the highest concentration was not identified correctly or if identified correctly was preceded by an incorrect response at the next lower concentration. The assessor should adjust concentration ranges for these panelists such that incorrect responses occur regularly at concentration level 2 and correct responses occur regularly at concentration level 5. The AML is essentially a test of 4 samples as changes in response from incorrect to correct at the lowest and highest concentration levels will generate a suspect threshold estimate. A plot of % correct response versus concentration level in Fig. 6 for the AML with SO_2 as the added substance shows enhanced ability among panelists to detect the lowest concentration level. Panelists knew that the lowest concentration of added substance was included in the first triangle and may have concentrated their efforts on detecting a difference in that triangle,

possibly resulting in sensory adaptation effects that were carried on to the successive triangles. Similar plots were obtained for AML test series with linalool and 2-hexanol as added substances and may indicate a basic flaw in test design, resulting in threshold estimates higher than the panelists' true thresholds. This would support the lower thresholds obtained by the SAPD intensity scale method where no such shift in apparent panelist sensitivity was evident as illustrated in Fig. 5.

Threshold estimates by the AML are possible with minimal replication as demonstrated in Table III.13, but no level of confidence can be stated concerning the estimate. The number of replications required is left to the discretion of the experimenter.

CONCLUSIONS

1. The SAPD test design we have proposed for determining sensory thresholds of added substances in beer minimizes sample requirements and assessor effort, compares each sample with a known control to maintain independence of sample responses, retains an ascending concentration series to minimize adaptation effects and is amenable to analysis by methods that provide statistically valid threshold estimates.

2. The SAPD intensity scale method appears the most sensitive in detecting individual or combined panel thresholds, but requires the assumptions of normal distribution of mean scores and equal variance in pooled error terms at or below threshold. The ANOVA and t-tests required are complex and require specialized computer programs.

3. The SAPD signal detection method does not require normal distribution of mean scores or homoscedasticity, accommodates panelist uncertainty and eliminates potential errors due to panelist inconsistency in use of a difference scale. The analysis leading to a threshold estimate is relatively simple. The disadvantages of this method are the increased replication required to

establish threshold intervals and inability to isolate
error associated with day to day variation in panelist
sensitivity.

4. The AML does not provide a threshold estimate based
on sound statistical analysis. Selection of testing
ranges for individual panelists is critical to minimize
bias in the threshold estimate. Threshold estimates are
possible with minimal replication but no confidence level
can be attached to the estimate and the number of
replications required is determined subjectively by the
experimenter.

You have received a set of 12 glasses arranged in three rows. Each row contains a labeled reference and three test samples. Test samples may be the same as the reference or may contain various levels of an added substance.

Individually compare the aroma of each test sample with the labeled reference, working from left to right within each row and from front to back by row. Compare test samples only with the labeled reference and not directly with each other.

In the box representing each test sample place the number of the phrase that best describes the size of the difference of the test sample from the labeled reference.

ROW #

3 - [Ref.] [ ] [ ] [ ]

2 - [Ref.] [ ] [ ] [ ]

1 - [Ref.] [ ] [ ] [ ]

0 - No Difference
1 - Just Detectable Difference
2 - Slight Difference
3 - Slight to Moderate Difference
4 - Moderate Difference
5 - Moderate to Large Difference
6 - Large Difference
7 - Large to Extreme Difference
8 - Extreme Difference

Taste the samples and record your results below using the instructions given above for aroma.

ROW #

3 - [Ref.] [ ] [ ] [ ]

2 - [Ref.] [ ] [ ] [ ]

1 - [Ref.] [ ] [ ] [ ]

Figure 1. Semi-ascending paired difference threshold test ballot with nine-point intensity of difference category scale.

Panelist Responses

		D	D?	S?	S
Beer Samples	D	a	b	c	d
	S	e	f	g	h

total = (a+b+c+d) = N_D

total = (e+f+g+h) = N_S

Column Totals: C_1 C_2 C_3 C_k

total responses (N_T) = $N_D + N_S$

response conditions (k) = 4

$$R = \frac{a(f+g+h) + b(g+h) + ch + 1/2(ae+bf+cg+dh)}{N_D N_S}$$

RANK SUMS TEST

$U = a(f+g+h) + b(g+h) + ch$

$U' = be + c(e+f) + d(e+f+g)$

$S = U - U'$

$$K = \frac{2N_T - C_1 - C_k}{2(k-1)}$$

Standard Deviation (SD) = $\sqrt{\dfrac{N_D N_S (N_T^3 - \Sigma C^3)}{3N_T(N_T - 1)}}$

$Z = \dfrac{S - K}{SD}$

Figure 2. Panelist response matrix, calculation of the R-index and a rank sums test for comparison of responses for beer sample D containing an added substance to responses for beer sample S with no added substance.

Panelist - 1
Responses

		D	D?	S?	S	
Beer Samples	D	1	8	1	0	total = (1+8+1) = 10
	S	0	4	4	2	total = (4+4+2) = 10
Column Totals		1	12	5	2	

total responses = 20

R = 0.78

U = 60

S = 56

K = 6.17

SD = 23.20

Z = 2.148

Figure 3. Panelist - 1 response matrix for 2-hexanol aroma, concentration level one versus the unknown reference within group one. R-index value and Z from rank sums test.

SAPD INTENSITY SCALE
2-Hexanol Aroma
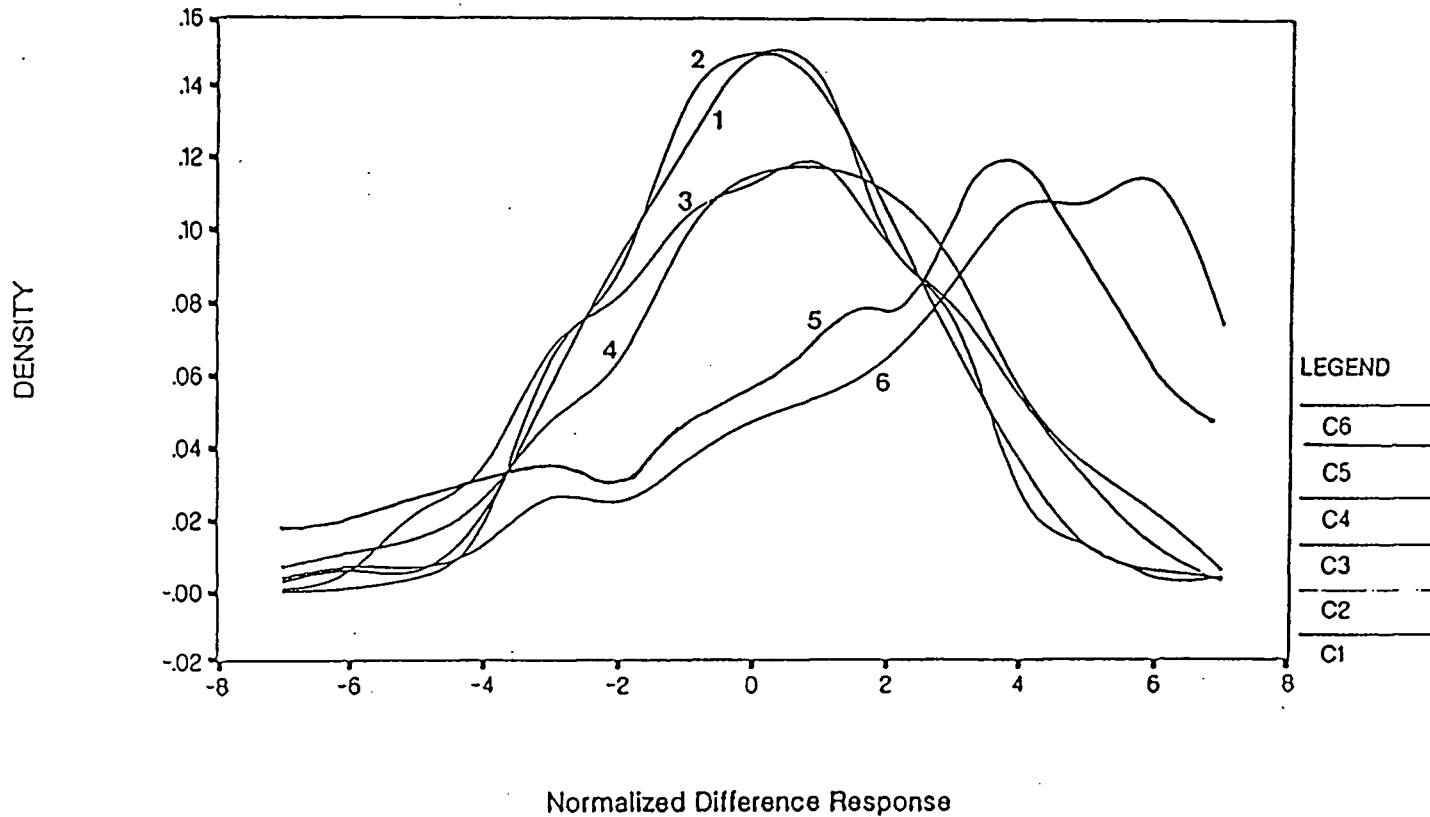
Figure 4. Density trace of normalized difference response from the combined panel to the aroma of 2 - hexanol in beer by the SAPD intensity scale method.
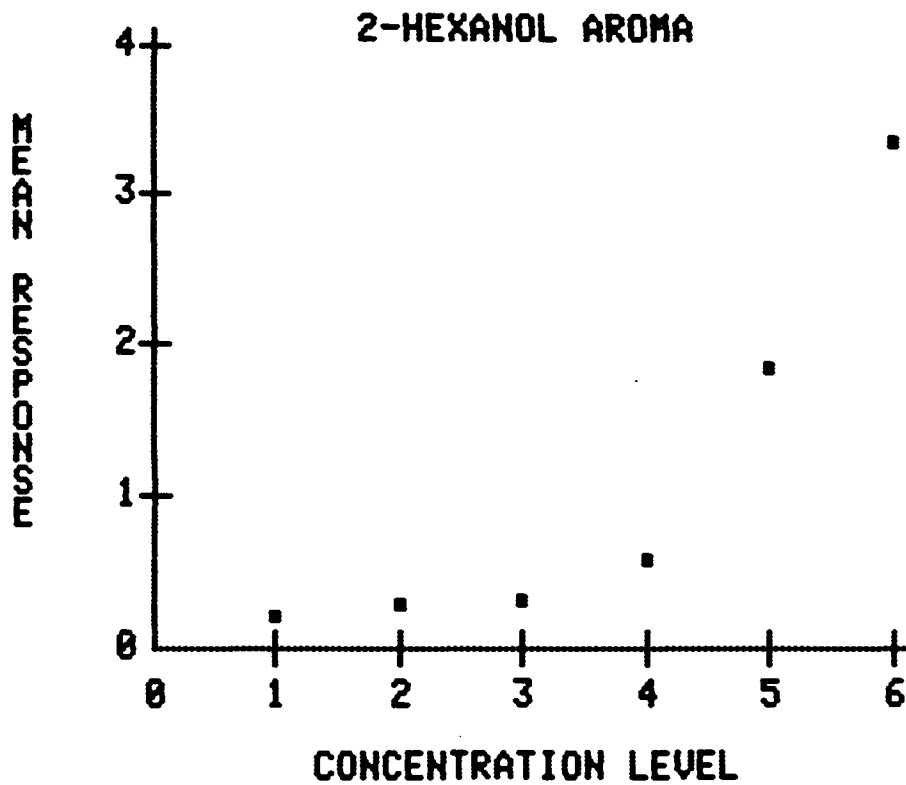
Figure 5. Mean normalized difference response from the combined panel to the aroma of 2 - hexanol in beer at each concentration level by the SAPD intensity scale method.
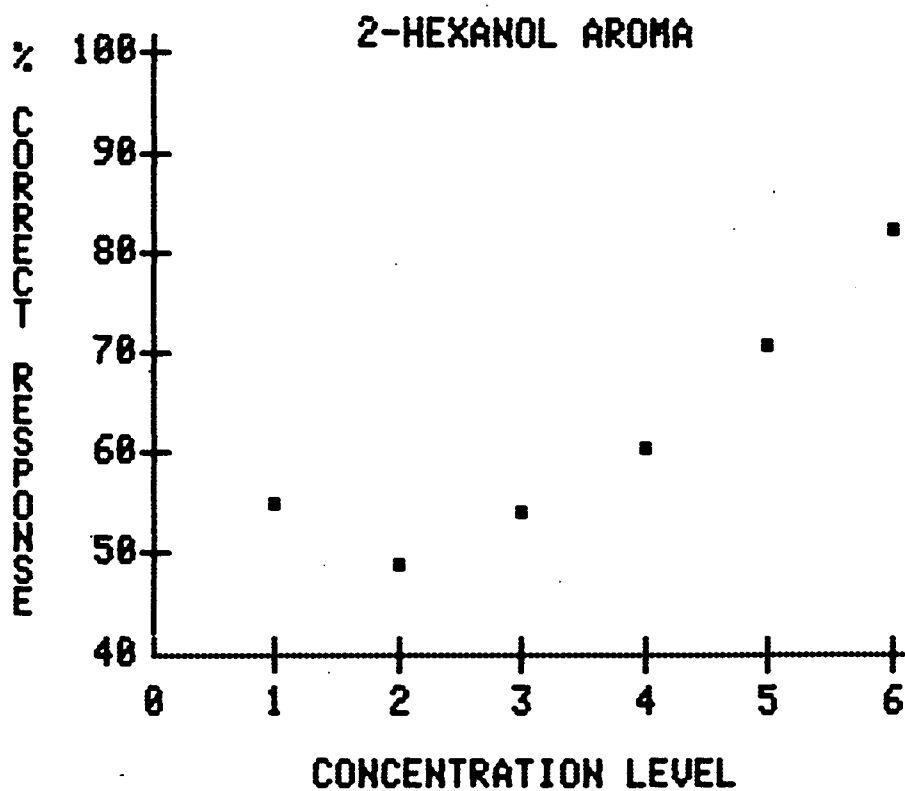
Figure 6. Percentage correct of the total number of responses from the combined panel at each concentration level of 2 - hexanol added to beer, by the Ascending Method of Limits.

TABLE III.1

EXPERIMENTAL PARAMETERS
CONCENTRATIONS OF ADDED SUBSTANCE TESTED

Compound tested	SO_2	Linalool	2-Hexanol
Unit of concentration[a]	mg/l	μg/l	mg/l
Concentrations tested: Factor per step	2.5	2.5	2.0
Level 1	2.83	2.14	0.22
Level 2	7.08	5.56	0.44
Level 3	17.7	13.7	0.88
Level 4	44.2	34.2	1.75
Level 5	111	85.5	3.50
Level 6	276	214	7.00
Panel size, n	9	9	12

[a] Concentration in beer.

TABLE III.2

SAPD INTENSITY SCALE METHOD
ANALYSIS OF VARIANCE FOR 2 - HEXANOL AROMA:

PANELIST - 1

Line	Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F - Value[a]
1	Rep	9	37.083	4.120	2.86 *
2	Group	2	108.033	54.017	37.54 **
3	Error(1)	27	38.850	1.439	
4	Sam/Grp (Samples within Groups)	3			
a	Sam/Grp(1)	1	0.800	0.800	1.71
b	Sam/Grp(2)	1	1.800	1.800	1.45
c	Sam/Grp(3)	1	18.050	18.050	6.93 *
5	Error(2)	27			
a	Error(2)/Grp(1)	9	4.200	0.467	
b	Error(2)/Grp(2)	9	11.200	1.244	
c	Error(2)/Grp(3)	9	23.450	2.606	
6	Total	59			

[a] * = Calculated F significant at the 95% confidence level.
** = Calculated F significant at the 99.9% confidence level.

TABLE III.3

SAPD INTENSITY SCALE METHOD
DETERMINATION OF THE THRESHOLD INTERVAL
SIMULTANEOUS PAIRED COMPARISONS OF SAMPLE DIFFERENCES:

PANELIST - 1

Test Set	Compared Group	Conc. Level	Sample Mean	t - value[a] Mean = 0	Std Error of Mean
	1	1	0.30	1.3887 (NS)	0.2160
		2	-0.10	-0.4629 (NS)	
1	2	3	1.00	2.8347 (NS)	0.3528
		4	1.60	4.5356 **	
	3	5	2.40	4.7018 **	0.5104
		6	4.30	8.4240 **	

Test Set	Comparisons Conc. Levels	Mean Sample Difference	t - value[a] Diff. = 0	Std Error of Diff.
	4 vs 3	0.600	1.2027 (NS)	0.4989
2	4 vs 2	1.700	4.1907 **	0.4137
	4 vs 1	1.300	3.1426 **	0.4137

[a] (NS) = Calculated t not significant at the 95% confidence level.
   ** = Calculated t significant at the 99% confidence level.

TABLE III.4

SAPD SIGNAL DETECTION METHOD
DETERMINATION OF THE THRESHOLD INTERVAL
RANK SUMS TESTS OF SAMPLE RESPONSES:

PANELIST - 1

Test Set	Comparisons Group	Conc. Level	R - Index	Z - Value[a]
	1	1 vs. Control	0.78	2.15
		2 vs. Control	0.67	1.66
1	2	3 vs. Control	0.40	-0.98
		4 vs. Control	0.65	1.03
	3	5 vs. Control	0.90	3.04 **
		6 vs. Control	0.95	3.67 **

Test Set	Comparisons Conc. Levels	R - Index	Z - Value
	5 vs 4	0.54	0.12
	5 vs 3	0.80	2.35 *
2	5 vs 2	0.60	1.10
	5 vs 1	0.73	1.63

[a] ** = Z-value significant at the 99% confidence level.
   * = Z-value significant at the 95% confidence level.

TABLE III.5

ANALYSIS OF VARIANCE FOR R-INDICES
2 - HEXANOL AROMA:

COMBINED PANEL

Line	Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F - value[a]
1	PAN (Panelist)	11	0.250	0.023	1.21 (NS)
2	TRT (Treatment)	5	0.941	0.188	9.89 **
3	ERROR	55	1.045	0.019	

[a] NS = non significant calculated F at the 95% confidence level.
** = Calculated F significant at the 99.9% confidence level.

TABLE III.6

PAIRED COMPARISONS OF R-INDEX MEANS
2 - HEXANOL AROMA:

COMBINED PANEL

	Sample Means						
	1	2	3	4	5	6	
Contrast	0.625	0.600	0.492	0.535	0.672	0.848	t - value[a]
1	-1	1	0	0	0	0	-0.441
2	-1	-1	2	0	0	0	-2.453
3	-1	-1	-1	3	0	0	-0.816
4	-1	-1	-1	-1	4	0	2.428 **
5	-1	-1	-1	-1	-1	5	6.005 **

[a] ** = t-value significant at the 95% confidence level.

TABLE III.7

METHOD COMPARISON
THRESHOLD ESTIMATES FOR 2 - HEXANOL AROMA

| Panelist | SAPD Intensity Scale | | SAPD Signal Detection | | AML |
	Level	Conc.[a]	Level	Conc.	Conc.
1. AM	2 - 4	0.44-1.75	3 - 5	0.88-3.50	0.87
2. AS	4 - 5	1.75-3.50	2 - 6	0.44-7.00	2.15
3. BL	4 - 5	1.75-3.50	2 - 5	0.44-3.50	1.63
4. FH	3 - 6	0.88-7.00	>6	>7.00	3.75
5. LH	4 - 5	1.75-3.50	4 - 6	1.75-7.00	3.04
6. MS	2 - 6	0.44-7.00	4 - 6	1.75-7.00	3.27
7. NY	1 - 4	0.22-1.75	>6	>7.00	2.00
8. TMC	0 - 5	0.00-3.50	5 - 6	3.50-7.00	2.84
9. TMI	0 - 2	0.00-0.44	>6	>7.00	4.02
10. TS	>6	>7.00	3 - 6	0.88-7.00	4.95
11. VC	>6	>7.00	>6	>7.00	5.69
12. VS	2 - 4	0.44-1.75	4 - 5	1.75-3.50	3.05
Combined Panel	0 - 2	0.00-0.44	4 - 5	1.75-3.50	2.79[b]

[a] Concentration of 2-Hexanol in beer, mg/l.
[b] Geometric mean of individual panelist thresholds.

TABLE III.8

METHOD COMPARISON
THRESHOLD ESTIMATES FOR 2 - HEXANOL TASTE

		SAPD Intensity Scale		SAPD Signal Detection		AML
Panelist		Level	Conc.[a]	Level	Conc.	Conc.
1.	AM	0 - 3	0.00-0.88	0 - 5	0.00-3.50	1.00
2.	AS	2 - 5	0.44-3.50	4 - 6	1.75-7.00	1.52
3.	BL	4 - 5	1.75-3.50	4 - 5	1.75-3.50	1.23
4.	FH	4 - 6	1.75-7.00	>6	>7.00	4.62
5.	LH	2 - 3	0.44-0.88	2 - 6	0.44-7.00	3.26
6.	MS	1 - 5	0.22-3.50	5 - 6	3.50-7.00	2.64
7.	NY	0 - 4	0.00-1.75	0 - 4	0.00-1.75	1.41
8.	TMC	2 - 5	0.44-3.50	5 - 6	3.50-7.00	2.47
9.	TMI	1 - 2	0.22-0.44	>6	>7.00	4.95
10.	TS	2 - 4	0.44-1.75	5 - 6	3.50-7.00	2.30
11.	VC	>6	>7.00	>6	>7.00	7.51
12.	VS	1 - 5	0.22-3.50	4 - 5	1.75-3.50	1.87
Combined Panel		0 - 2	0.00-0.44	4 - 5	1.75-3.50	2.42[b]

[a] Concentration in beer, mg/l.
[b] Geometric mean of individual panelist thresholds.

TABLE III.9

METHOD COMPARISON
THRESHOLD ESTIMATES FOR LINALOOL IN BEER

| Panelist | Aroma SAPD Intensity Scale | | Taste SAPD Intensity Scale | | AML |
	Level	Conc.[a]	Level	Conc.	Conc.
1. SH	2 - 4	5.56-34.2	2 - 6	5.56-214	85.5
2. KO	2 - 3	5.56-13.7	1 - 3	2.14-13.7	3.51
3. JW	3 - 4	13.7-34.2	3 - 4	13.7-34.2	21.6
4. UK	2 - 3	5.56-13.7	2 - 5	5.56-85.5	214
5. SK	3 - 6	13.7-214	>6	>214	108
6. LH	1 - 2	2.14-5.56	2 - 3	5.56-13.7	2.73
7. MJ	2 - 4	5.56-34.2	>6	>214	68.1
8. TM	1 - 2	2.14-5.56	0 - 5	0.00-85.5	34.1
9. BL	2 - 6	5.56-214	0 - 4	0.00-34.2	43.1
Combined Panel	2 - 3	5.56-13.7	2 - 3	5.56-13.7	32.5[b]

[a] Concentration in beer, μg/l.
[b] Geometric mean of individual panelist thresholds.

TABLE III.10

METHOD COMPARISON
THRESHOLD ESTIMATES FOR SO_2 IN BEER

Panelist	Aroma SAPD Intensity Scale Level	Conc.[a]	Taste SAPD Intensity Scale Level	Conc.	AML Conc.
1. SH	4 - 6	44.2-276	4 - 6	44.2-276	11.2
2. KO	0 - 5	0.00-111	0 - 5	0.00-111	88.0
3. JW	1 - 5	2.83-111	>6	>276	220
4. UK	1 - 2	2.83-7.08	<1	<2.83	55.7
5. SK	1 - 6	2.83-276	1 - 6	2.83-276	35.2
6. LH	2 - 4	7.08-44.2	3 - 5	17.7-111	17.7
7. MJ	3 - 6	17.7-276	>6	>276	70.0
8. TM	0 - 5	0.00-111	0 - 4	0.00-44.2	55.6
9. BL	4 - 5	44.2-111	2 - 5	7.08-111	17.7
Combined Panel	3 - 4	17.7-44.2	3 - 4	17.7-44.2	43.1[b]

[a] Concentration in beer, mg/l.
[b] Geometric mean of individual panelist thresholds.

TABLE III.11

EFFECT OF REPLICATION
SAPD INTENSITY SCALE METHOD
THRESHOLD ESTIMATES FOR 2 - HEXANOL AROMA

Panelist	Replications 1 - 5		Replications 6 - 10		Replications 1 - 10	
	Level	Conc.[a]	Level	Conc.	Level	Conc.
1. AM	3 - 6	0.88-7.00	2 - 6	0.44-7.00	2 - 4	0.44-1.75
2. AS	3 - 5	0.88-3.50	4 - 6	1.75-7.00	4 - 5	1.75-3.50
3. BL	4 - 6	1.75-7.00	2 - 5	0.44-3.50	4 - 5	1.75-3.50
4. FH	>6	>7.00	0 - 5	0.00-3.50	3 - 6	0.88-7.00
5. LH	4 - 6	1.75-7.00	1 - 5	0.22-3.50	4 - 5	1.75-3.50
6. MS	0 - 2	0.00-0.44	2 - 5	0.44-3.50	2 - 6	0.44-7.00
7. NY	2 - 3	0.44-0.88	0 - 6	0.00-7.00	1 - 4	0.22-1.75
8. TMC	4 - 6	1.75-7.00	5 - 6	3.50-7.00	0 - 5	0.00-3.50
9. TMI	0 - 2	0.00-0.44	>6	>7.00	0 - 2	0.00-0.44
10. TS	>6	>7.00	>6	>7.00	>6	>7.00
11. VC	>6	>7.00	>6	>7.00	>6	>7.00
12. VS	1 - 5	0.22-3.50	5 - 6	3.50-7.00	2 - 4	0.44-1.75
Combined Panel	0 - 2	0.00-0.44	4 - 5	1.75-3.50	0 - 2	0.00-0.44

[a] Concentration in beer, mg/l.

TABLE III.12

EFFECT OF REPLICATION
SAPD SIGNAL DETECTION METHOD
THRESHOLD ESTIMATES FOR 2 - HEXANOL AROMA

Panelist	Replications 1 - 5		Replications 6 - 10		Replications 1 - 10	
	Level	Conc.[a]	Level	Conc.	Level	Conc.
1. AM	>6	>7.00	0 - 3	0.00-0.88	3 - 5	0.88-3.50
2. AS	>6	>7.00	>6	>7.00	2 - 6	0.44-7.00
3. BL	>6	>7.00	2 - 5	0.44-3.50	2 - 5	0.44-3.50
4. FH	>6	>7.00	>6	>7.00	>6	>7.00
5. LH	>6	>7.00	>6	>7.00	4 - 6	1.75-7.00
6. MS	>6	>7.00	3 - 6	0.88-7.00	4 - 6	1.75-7.00
7. NY	0 - 5	0.00-3.50	>6	>7.00	>6	>7.00
8. TMC	4 - 6	1.75-7.00	5 - 6	3.50-7.00	5 - 6	3.50-7.00
9. TMI	>6	>7.00	>6	>7.00	>6	>7.00
10. TS	>6	>7.00	>6	>7.00	3 - 6	0.88-7.00
11. VC	>6	>7.00	>6	>7.00	>6	>7.00
12. VS	1 - 5	0.22-3.50	>6	>7.00	4 - 5	1.75-3.50
Combined Panel	>6	>7.00	5 - 6	3.50-7.00	4 - 5	1.75-3.50

[a] Concentration in beer, mg/l.

TABLE III.13

EFFECT OF REPLICATION
ASCENDING METHOD OF LIMITS
THRESHOLD ESTIMATES FOR 2 - HEXANOL

Panelist	Replications 1 - 5 Conc.[a]	Replications 6 - 10 Conc.	Replications 1 - 10 Conc.
1. AM	1.41	0.54	0.87
2. AS	3.27	1.42	2.15
3. BL	1.41	1.87	1.63
4. FH	4.31	3.27	3.75
5. LH	3.25	2.85	3.04
6. MS	2.84	3.75	3.27
7. NY	2.14	1.86	2.00
8. TMC	2.84	2.83	2.84
9. TMI	4.95	3.27	4.02
10. TS	4.95	4.95	4.95
11. VC	7.51	4.31	5.69
12. VS	5.69	1.63	3.05
Combined Panel[b]	3.30	2.36	2.79

[a] Concentration in beer, mg/l.
[b] Geometric mean of individual panelist thresholds.

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