

Flavor Quality: Objective Measurement

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FOREWORD

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PREFACE

Over the past 15 years we have witnessed remarkable advancements in areas relating to objective measurement of flavor quality. A look at several of the analytical techniques used today in flavor research provides examples of this. The common use of glass capillary gas chromatographic columns and high pressure liquid chromatographic systems allows separation of labile compounds which were virtually impossible to separate several years ago. Improvements in vacuum systems and in carrier gas separators has made gas chromatographic-mass spectrometric analysis much more efficient. Use of computers with mass spectrometers and other instruments has increased our ability to make correct structural assignments.

These advances have been extremely helpful, and undoubtedly we could also point to similar advances in sensory evaluation, in statistical applications, and in other areas relating to measurement of flavor quality. Perhaps at this point we should remind ourselves that flavor is the sensation perceived when one takes food or beverage into the mouth. Ultimately it is this sensation which we attempt to define and measure, and we usually try to do so by measuring those things in the food which effect, or are responsible for, the flavor sensation. How successful are we? What type of problems do we encounter, and what type of research will be necessary to solve these problems? The papers in this symposium on objective measurement of flavor quality provide information helpful to the solution of these questions.

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March 25, 1977

Objective Measurements of Flavor Quality: General Approaches, Problems, Pitfalls, and Accomplishments

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After I had accepted the invitation to prepare an opening paper for this series, I was filled with a sense of foreboding, and for very good reasons. First, I can think of a number of individuals who beyond any doubt are better qualified to discuss this multi-disciplinary area than I am, and second, the title itself is enough to flash a warning light. While the complexity of this attribute we call "flavor" has been stressed by several workers (e.g. 1), Moncrief (2) argued that taste and odor are the major components. Certainly it is generally agreed that flavor requires the participation of sensory receptors, which makes it an individual, and at least within certain limits, a variable characteristic. Because of this, flavor is necessarily a highly subjective trait. Rereading my title, I find that I'm committed to discussing objective measurements of a subjective characteristic and my first thought is, "I'm in trouble." But as long as we recognize this contradiction and are willing to accept a degree of compromise, hopefully we can make some progress.

An early quest for an objective measurement of quality has been cited from the 13th and 14th centuries, when "ale conners" or "ale tasters" in England were assigned the task of setting the price on batches of brew based on their individual flavor judgments. In an effort to achieve a greater degree of objectivity, this was combined with a test of the ale strength: some of the ale was poured on a bench, and the ale conner sat in it. After a predetermined interval, "he made to stand up"; if his leather breeches stuck to the bench, the ale was of the right quality (3).

To most of us, an "objective measurement of flavor quality" means establishing a chemical or physical method for measuring the amount of a substance responsible for a particular flavor attribute. This requires first establishing which compound or compounds are responsible for a particular attribute, which has been done in relatively few instances, at least in complex mixtures. The problem is further complicated by the fact that synergism and antagonism can exist between compounds that elicit flavor responses. In many cases, flavor is due to an integrated

response to at least several compounds, and as we change the relative ratios and/or absolute amounts of these compounds, the flavor responses change in a manner that is usually unpredictable. Moskowitz (4) argued that the activities of the flavor chemist, who is usually concerned with establishing the contribution of individual components to flavor, diverge from but are complementary to psychometric efforts concerned with the quality of flavor mixtures.

Two general approaches have been utilized in attempts to obtain objective measurements of flavor quality. One has been concerned with the measurement or recording of the minute electrical response of olfactory or taste cells elicited by odor or taste stimuli (e.g. 5, 6), but a great deal of work remains to be done in this area. Another widely used approach has involved sniffing the outlet of a gas chromatograph (e.g. 7). Certain problems are immediately obvious: flavor is an integrated response to a mixture of compounds, and the gas chromatograph provides a differential rather than an integrated response to those compounds. Secondly, it can be most disturbing when the panel reports that interesting odors exist between the peaks (e.g. 8, 9) which emphasizes the fact that for some substances, the gas chromatograph has not yet matched the sensitivity of the human nose.

Even so, useful results can be obtained by this route. Barylko-Pikielna et al. (10) split the effluent from a gas chromatographic column to assign qualitative odor assessments to the individual peaks of a sugar-amino acid reaction mixture. McLeod and Coppock (11) used a similar technique to assign odor evaluations to fractions from boiling beef. A number of workers interested in correlating their analytical results with sensory response have also used the odors of individual peaks, sometimes to good advantage (vide infra).

Tucknott and Williams (12) pointed out that sniffing the effluent from a gas chromatograph suffers from a number of other shortcomings, and suggested that the effluent gas containing peaks of interest be trapped in individual disposable syringes for subsequent odor assessment. Clark and Cronin (13) utilized a novel method; peaks were trapped in short sections of support coated open tubular (SCOT) glass columns, which were then crushed under water to produce a solution for sensory analysis. Parliment (14) bubbled the gas chromatographic effluent into water to prepare solutions for taste-testing, which he reported was in some cases more satisfactory than sniff-testing.

When we resort to gas chromatography, we have of course restricted our objective measurements to volatile compounds; it is all too easy to forget that taste is also a major contributor to flavor (1). Weiss and Schaller (15, 16) studied the influence of several variables--e.g. titratable acidity, pH, dissolved gases--on the sensory properties of apple juice; a number of such studies

on other products have been published. Recently, Noble (17) used gel chromatography to fractionate flavor components of tomato.

Another major problem is obtaining gas chromatographic results that reflect with high qualitative and quantitative fidelity the volatile composition of the material in question. Compositional changes are frequently caused in the preparation of a sample suitable for gas chromatography, and additional errors may be introduced by the gas chromatographic analysis itself.

Many materials require some type of treatment to free the compounds of interest from materials that would otherwise interfere with the analysis (e.g. water and non-volatile components) before the sample can be injected into the gas chromatograph. Also, some type of concentration is frequently required so that the limited amount of sample that can be injected contains detectable quantities of the compounds to be studied. Most of the procedures that are used to achieve these ends involve distillation, extraction and/or evaporation, or adsorption, all of which cause quantitative changes and some of which may engender qualitative changes in the concentrated sample, so that it no longer reflects the composition of the starting material. In efforts to surmount these difficulties, many investigators prefer to use direct injections of headspace gas for analysis, but the chromatogram is then restricted to those components whose partial pressures are relatively high. A great deal of attention has been given to the use of porous polymers in concentrating headspace volatiles by less strenuous means (e.g. 18, 19, 20). Sample preparation remains a critically important area, and forms the subject of another symposium at this meeting.

Another source of problems lies in the gas chromatographic analysis itself; not all compounds are stable to the conditions of the analysis, and the chromatogram may not accurately reflect the composition of the material injected. Additionally, minor components that are not well resolved from larger constituents may be of critical importance to a given flavor attribute, but unless these are unambiguously separated from the other components, we are frequently not even aware of their existence in the sample. Recent developments in wall-coated open tubular glass capillary columns (WCOT: 21, 22, 23) make it possible to resolve many of these previously poorly separated components. Additionally, the more inert character of the glass columns has permitted analysis of some constituents (notably sulfur-containing compounds) that are almost surely of great importance to some flavors and which have resisted packed-column or metal-capillary analysis (e.g. 24, 25, 26). Glass inlet splitters of much higher linearity (27, 28), when combined with the open tubular glass capillary column, are capable of producing chromatograms that reflect much more accurately the composition of the injected sample.

Unfortunately, column efficiency--i.e. the inherent power to separate the components of a mixture--is inversely proportional to column capacity. The maximum theoretical efficiency of WCOT columns, expressed in theoretical plates per meter, is approximately $1000/r$, where r is the inner column radius in mm (29). A compromise is also usually necessary in the thickness of the film of liquid phase, as columns with thinner films have higher efficiencies but impose severe limitations on sample capacity, and columns with thicker films possess higher capacities at the expense of column efficiency (e.g. 29, 30). While the lower capacities of small diameter (e.g. 0.25 mm) WCOT columns are still sufficient for applications such as gas chromatography-mass spectrometry, their use for the sensory analysis of individual fractions poses very difficult problems. Some investigators have compromised on larger diameter WCOT columns (e.g. 31, 32), or columns whose roughened or extended inner surfaces (PLOT or SCOT columns) increase the surface area of the liquid phase (e.g. 33, 34). Because of this capacity requirement, there remains a use and a need for packed columns. It is, however, critically important to duplicate the analysis on a high resolution system, so the investigator is not misled by peaks that may play a crucial role in the sensory qualities and that are not well resolved on the lower resolution high capacity system. The investigator must also frequently compromise in selecting gas chromatographic parameters, balancing the degree of separation desired against the length of time required for the analysis, and, for thermally labile materials, the amount of heat to which the samples are exposed (35). Attention must also be given to the suitability of the system for the separation of the components of a given mixture.

Once the problems of sample preparation and sample analysis are overcome, one has at best a well-resolved chromatogram, without overlapping or co-chromatographing components, which may qualitatively and quantitatively reflect the composition of the original material. It cannot be overemphasized that even this is rarely achieved. Still to be reckoned with are the myriad problems and sources of error inherent in the sensory testing procedures. Most of us would now agree that it is not sufficient for the chemist, untrained in sensory analysis, to casually sniff the outlet of his gas chromatograph and record his impressions. Sensory analysis, too, has come a long way, and a meaningful study should use selected panels of trained personnel utilizing quantitative procedures amenable to statistical evaluation. Martin (36) described procedures used in the selection and training of panelists for various types of sensory evaluations. Larmond (37) emphasized the importance of controlling physical stimuli to which the panelists are subjected. Best (38) described the analysis of taste-test data, and Harries (39) reviewed the complexities of sensory assessment. Stone et al.

(40) described a sensory evaluation technique termed "quantitative descriptive analysis" which uses an interval scale and a panel of at least six trained panelists, and Moskowitz (41) has argued for a method he terms "magnitude estimation", in which numbers are assigned to stimuli so that the ratios of judgments reflect sensory ratios.

Taking all of these factors into account--changes engendered by sample preparation; poor separation, artifacts and errors in the gas chromatographic analysis; sins of omission and sins of commission in the sensory analysis--the investigator may finally be in a position to try and relate variations in the sensory properties to variations in the chromatographic pattern. Here again, however, results can be over-interpreted. Szczesniak (42) and Persson et al. (43, 44) both emphasize that even a significant correlation between a physical measurement and a sensory rating establishes only a predictive relationship, not a causal one.

Two general approaches, which are not necessarily mutually exclusive, have been used in attempts to correlate the analytical data with sensory attributes. One has involved attempts to establish the qualitative and/or quantitative flavor properties of individual compounds, sometimes with attention to the synergism or antagonism that they exhibit in mixtures with other compounds, or in one solvent as opposed to another. Meilgaard (45, 46) and Clapperton et al. (47) have accumulated a large amount of information on the flavor characteristics and threshold values of aroma volatiles in beer. As emphasized by these workers, the odor purity of the compounds tested is critically important to this type of study. They point out, as one example, that Murahashi (48) reported 1-octen-3-ol as possessing a mushroom aroma, while Hoffmann (49) ascribed the odor to 1,3-dioxalans formed on decomposition of the alcohol. Later Meilgaard (46) found that the odor in question was due to contamination by 1-octen-3-one, which had a metallic aroma in fat solutions, and a mushroom aroma in aqueous medium; the purified alcohol had an odor he reported as "spicy, perfumed and grass-like." Similarly, Boelens et al. (50), who identified a large number of compounds in onion oil, suggested that 2,4-dimethylthiophene and 3,4-dimethylthiophene possessed an odor of fried onions. Recently, Galetto and Hoffman (51) synthesized these compounds, and reported that alone or in combination with other compounds they did not make a significant contribution to the fried onion aroma.

The other major approach has involved attempts to correlate flavor with gas chromatographic patterns. Guadagni et al. worked on such correlations for apple (7); these and other efforts have been reviewed by Powers (52), ASTM (53, 54) and von Sydow (55); additional efforts include those of Salo et al. (56) and Salo (57).

Bednarczyk and Kramer (58) reported that four gas chromatographic peaks accounted for 85% of a sensory panel's flavor response to ginger essential oil. Cited as prime contributors to the characteristic ginger attribute were β -sesquiphellandrene and α -curcumene; α terpineol and citrals contributed a lemony attribute, and an undesirable woody or soapy note was caused by nerolidol.

Using linear regression analysis, Fore et al. (59) found a high correlation between the flavor score of stored peanut butter and the ratio between 2-methyl propanal and hexanal as determined gas chromatographically. Dravnieks et al. (60) used stepwise discriminate analysis to classify, by gas chromatographic techniques, the odor of corn. Galetto and Bednarczyk (61) used multiple regression techniques in establishing that the amount of methyl propyl disulfide, methyl propyl trisulfide and dipropyl trisulfide as determined gas chromatographically showed a high degree of correlation with overall onion flavor. Tassan and Russell (62) used a micro olfactometer to evaluate the odors of individual cumin constituents trapped from a gas chromatograph, and reported that variations in four aldehydes influenced the main odor character; 3-p-menthen-7-al was shown to be necessary for the characteristic odor of heated cumin.

Karlsson-Ekström and von Sydow (63) using a psychophysical-statistical approach, found that the sensory changes that occur when black currants are heated could be well correlated with a decrease in monoterpene hydrocarbons and an increase in dimethyl sulfide and aliphatic aldehydes.

Persson and von Sydow (64) examined the sum, differences, ratios, geometric means and vectorial sums of peaks from gas chromatographic analyses for their correlation with flavor scores of frozen and refrigerated cooked sliced beef. A number of different models involving both sensory response and gas chromatographic data exhibited high coefficients of correlation. Studies on canned beef (43) established that a high degree of correlation existed between 15 odor properties and four gas chromatographic peak combinations. In later work the methods were extended to a variety of meat products and found to hold true (44).

Åkesson et al. (65) used headspace sampling techniques and open tubular gas chromatography combined with a flavor profile technique to see if sensory properties and preference values for a variety of food materials could be predicted by gas chromatographic data. Testing a large number of models that used gas chromatographic peak areas in various combinations and in several types of functions, they found that the assessment of sensory qualities was related monotonically to gas chromatographic data, while estimated preference values were in most cases related in a more complex non-monotonic way. Using the proper models, very accurate predictions could be made. Von Sydow (66)

generalized that all compounds present above threshold levels contribute to the aroma of foods, and that the number of important aroma contributors is variable, but is usually high in thermally processed foods or foods of animal origin. He emphasized that the instrumental technique must be gentle, so that the sample investigated represents the true situation in the food, and that the sensory technique must be detailed and sensitive. The development of numerical and psychophysical models capable of relating the physicochemical data with the sensory data is crucial.

It would appear that we may finally have arrived at a point where we have the opportunity to combine improved methods of sample preparation, gas chromatographic separation, sensory analysis and computerized data analysis to achieve some realistic results in the search for objective measurements of flavor quality in at least some products. Success in these efforts will depend to a large degree on how well we combine advances in sample preparation with high resolution gas chromatography, valid sensory procedures and advanced methods of data analysis. This requires judgments and evaluations on the part of the investigator. No single sample preparation procedure can be accepted as uniformly satisfactory; one or another may be superior depending on the sample composition and the compounds of interest. Gas chromatographic parameters must be selected carefully, with attention to the sample composition, its stability under the conditions of analysis and whether one's immediate goal is resolution or capacity. Neither are all procedures for sensory or data analysis--including those cited in this paper--equally valid under all circumstances, nor are all of them acceptable to experts in this field (e.g. 40, 41, 58). And no degree of sophisticated computer analysis can compensate for careless sensory procedures, or for an incompletely resolved chromatogram in which peaks of sensory importance have failed to separate. But we do now have increased capability in all of these areas, and the next few years should see some exciting results.

I would like to conclude by thanking several close friends, especially Erik von Sydow, Morten Meilgaard, Rose Marie Pangborn and Gerry Russell for their help in preparing this information. There are of course many other investigations which could and perhaps should have been included in this presentation. I can only apologize for such omissions, but in a very short time the data I have presented will be largely obsolete, as the papers that follow this brief introduction extend our efforts and knowledge in this very important area.

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Correlation of Odor Intensities and Vapor Pressures with Structural Properties of Odorants

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The relationship of perceived odor intensity to the concentration of odorants in headspace samples of various materials tends to follow a similar mathematical function but with different coefficients for different odorants. The task of obtaining experimental data for all compounds of interest would be overwhelming. It is desirable, therefore, to evolve procedures which would enable generalization and estimation of these coefficients from some molecular properties. (1,2) The most readily accessible properties are those derivable from the structural chemical formulas of the compounds. Attempts in this direction achieved some success (3) but were obtained for a relatively narrow selection of compounds.

The present study expands odor data to include a more diversified selection of odorants. Furthermore, the properties derivable from the molecular structures were selected in such a way that they could be inventoried directly from inspection of the one-line Wiswesser notation formulas of compounds. Such formulas utilize specific symbols for certain structural characteristics of molecules (e.g., U for double bond, R for benzene ring, Q for hydroxyl group), and in principle permit their enumeration by computerized methods.

Odor Intensity Measurements

Concentration of odorants in headspace vapors of a flavor-possessing sample can be measured by appropriate analytical means. The intensity of the odor sensation experienced when smelling the sample can be derived from such data if the dose-response relationship is known; dose is described by the types and concentrations of the odorants in the headspace, and response by the intensity of the resulting odor sensation.

Three principal methods exist to describe how strong an odor is:

- (1) Magnitude estimation: "Smell the odor and give a number or some other type of response (finger span, length of a line) in proportion to the experienced odor intensity". The choice of the numbers is either left to the best judgement of the subject or may be "pegged", prescribing that the intensity of another reference stimulus is given some definite value, frequently 10 (ten). The resulting numbers are proportional to the perceived odor intensities. Figure 1 illustrates results of the magnitude estimation of two odorants at several air dilution levels.
- (2) Category scaling: "Judging the intensity on a scale of zero to five (or some other fixed numerical scale)". The resulting numbers are not proportional to the perceived odor intensity. Typically, an odor one unit higher than another on 0-5 scale is actually perceived to be stronger by a factor of 3 or 4.
- (3) Reference scale: "Compare the odor intensity of a sample to a reference scale consisting of a series of known concentrations of a selected odorant and indicate the best intensity match".

The magnitude estimate method gives the truest representation of the perceived intensity. The reference scale method permits the easiest documentation and transfer of information on the odor intensity.

The following functions approximately relate the magnitude of the perceived odor intensity S , category scale number N and the odorant concentration C

$$N = m \log S \quad 1.$$

$$S = kC^n \quad 2.$$

The coefficients m and k depend on the choice of units for S ; m , also on the choice of units for the category scale. In addition, k and n depend on the type of odorants. Equation 2, known as the psychophysical power function (sometimes referred to as Stevens law) better describes the relation between odor intensity and odorant concentration for the great majority of odorants over a broad range of concentrations than the traditional Weber-Fechners law. For a few odorants, Equation 2 seems to hold true only over a certain range or ranges of concentrations.

Recently, a 1-butanol (n-butanol) reference scale (4) has been established as a recommended practice, ASTM E544, for referencing odor intensity ("sample smells as strong as X ppm vol/vol of 1-butanol in air"). Figure 2 illustrates one physical form of this scale. For n-butanol,

$$S = kC^{0.66} \quad 3.$$

$$\log S = \log k + 0.66 \log C$$

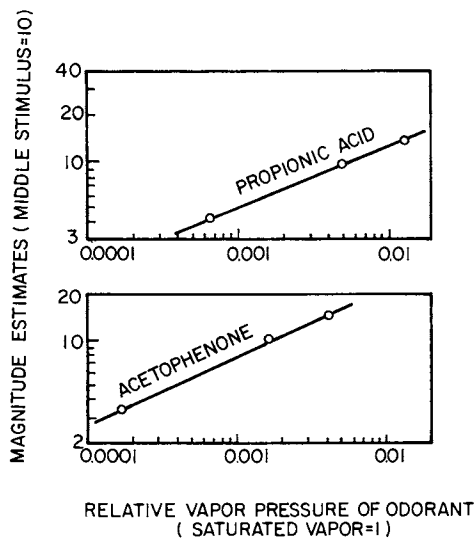


Figure 1. Effect of dilution on odor intensity; obtained by magnitude estimation method

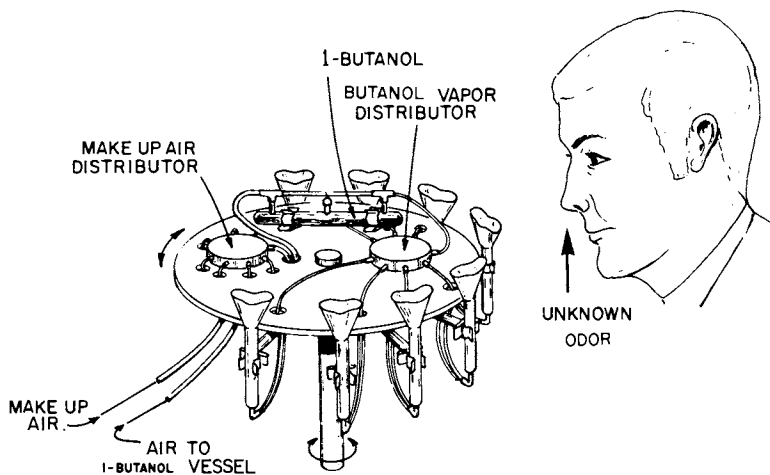


Figure 2. A form of vapor dilution olfactometer to serve as an ASTM 544 odor suprathreshold. Intensity referencing scale based on 1-butanol.

Moskowitz, et al (5) have proposed to define the odor intensity of 250 ppm vol/vol of n-butanol in air as 10. With C taken in ppm (vol/vol) units, Equation 3 becomes:

$$S = 0.261 C^{0.66} \quad 4.$$

It permits translation of 1-butanol referenced values of intensity to an open-ended numerical scale (both ends open) in which the numbers are approximately proportional to the perceived odor intensities. In such a scale, odor intensities <1 approach perception thresholds and occur at odor threshold concentration ranges of the respective odorants.

Relations between the butanol, category, and S-value scales are illustrated in Figure 3.

Measuring several concentrations of a sample odorant vs. n-butanol scale, converting n-butanol reference values to S values by Equation 4, and plotting log S vs. log (odorant concentration) one can obtain values of k and n (position)(slope) for the sample odorant. For odorants, n is usually in the range of 0.2-0.8.

When an odorant stimulus is delivered for smelling as a vapor diluted with air, the flow rate of this mixture of constant odorant concentration becomes an additional variable. The ASTM E544 1-butanol scale is standardized to 160 ml/min air from glass nozzles, cf. Figure 2. Figure 4 illustrates how the odor intensity of the same 1-butanol vapor concentration in air increases as the delivery flow rate from a sniffing port is changed. It is evident that for an equitable comparison of the abilities of different odorants to generate odor of certain intensities, the comparison should be at identical flow rates.

Dose-Response Parameters

From Equation 2, the dose-response relationship for odors can be characterized by a plot of log (response) vs. log (concentration), Figure 5.

$$\log S = \log k + n \log (C) \quad 5.$$

The slope is represented by n, and the position is related to k. The odor threshold is a subsidiary parameter, signifying the concentration at which the response S becomes indistinct at some selected statistical significance level. Because of the difference in the slopes, the odor intensity at the same multiple of odor threshold concentration can be quite different for different odorants. Thus, expressing the odor intensities in terms of multiples of the odor threshold concentrations is a procedure which ignores differences in the slopes of dose/response functions.

For example, an easily noticeable odor intensity for thiophenol is at 100 to 1000 multiples of its odor threshold

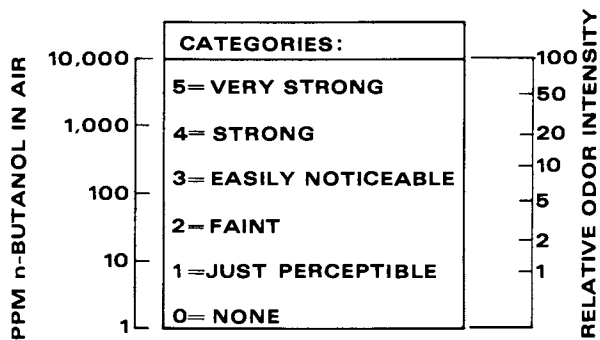


Figure 3. Relations between various odor intensity scales

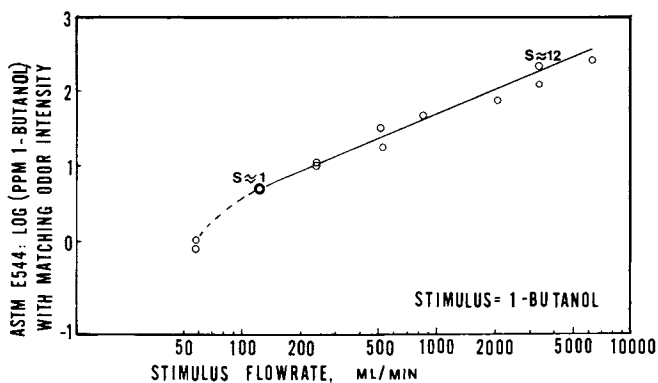


Figure 4. Change of odor intensity with stimulus flowrate from a sniffing port

concentration, while for 2,4-hexadienal it is at 20 to 50 multiples of its own threshold.

Patte, Etcheto, and Laffort (6) have published a list of slopes n for 110 compounds. A calculation from their tables indicated that the mean value of n is 0.41, and the dispersion of the values for different odorants is characterized by a standard deviation of 0.14. Thus, the estimated 95 percent of odorants have n values in the range between 0.15 and 0.70, and most are between 0.25 and 0.55.

Since the flavor industry deals mostly with odors well above their thresholds, parameters k and n may be more suitable for characterizing the dose/response function of an odorant that a single value of threshold value which says nothing about the change in the response with concentration.

Characterization of Molecular Properties of Odorants

A number of molecular or physico-chemically measurable properties of odorants have been considered and sometimes successfully correlated to some characteristics of their odors:*

- a. Molecular volumes (Laffort)
- b. Gas-chromatographic retention volumes in several types of stationary phases (Laffort & Dravnieks)
- c. Hydrogen-bonding, electron acceptor/donor, etc. properties
- d. Adsorptivities at water/oil interfaces (Davies)
- e. Molecular shapes and sizes (Amoore)
- f. Relative arrangement of functional groups (Beets)
- g. Spectral, e.g., far infrared and Raman characteristics (Wright)
- h. Building blocks of molecules (Dravnieks).

Each of these selections is correlated to others in some way. Thus, spectral characteristics reflect types and arrangements of functional groups, GC properties are the result of similar factors and molecular sizes, etc. In some flavor work, the identity of a compound may not be known, while its GC characteristics can be measured. Here, the group under (b) is useful. In many cases, the identity of the compound is known from mass-spectrometric or infrared data.

The most universally available properties are those that can be directly derived from structural formulas. Recently, Wiswesser one-line chemical notation formulas (7) are coming more in use for computerized storage of chemical information, such as on infrared, mass-spectrometric, and odor threshold data. (8) These formulas reflect the structures by utilizing a series of symbols and formal logical rules, so that each compound has one unique sequence of symbols that can be carried by a punched card and used to search

* Names in parentheses indicate researchers that emphasized some specific effects and reflect their work; Ann. N.Y. Acad. Sci., Vol 116 (1964) and Vol 237 (1974).

for various types of data on the same compound in different data collections. As an example, the formula for skatole is:

T56 BMJ D

where T56 represents a heterocyclic ring with 5 or 6 atoms respectively, BM indicates the presence of NH (=M group) in one of the rings in a certain position represented by B, and D indicates a methyl group attached to the ring structure in a position represented by D.

Wiswesser formulas provide an excellent base for scanning for the presence of certain molecular structural characteristics. Thus, if correlations can be found between such characteristics and odors, it should become possible to

- (1) first inspect the data collections to find if experimental odor data already exist for the compound of interest and
- (2) if not, to estimate the most probable odor values for the compound from the correlations based on experimental data.

Selection of Data

Katz and Talbert (9) have published data on odor intensities of warning agents, using category scaling on a 0 to 5 scale. Concentrations to obtain an intensity score of 3 (= easily noticeable) were selected for correlations for 52 compounds from their publication. In our own work, odors of 59 compounds listed in Table I at different concentrations were rated vs. 1-butanol scale, and the concentrations producing an intensity $S = 5$ (Equation 4) obtained by interpolation.

By a comparison of our and Katz-Talbert's data for some compounds, it was estimated that their score of 3 was approximately equivalent to the odor intensity of ppm vol/vol 1-butanol in air. Thus, concentration values to obtain approximately identical odor intensities for 107 (111 data points since 4 substances were evaluated in both studies) were available for correlation.

A supplementary effort was a search for correlations between vapor pressures and Wiswesser notation characteristics. Very frequently, data on vapor pressures of odorants are not available, so that it is impossible to estimate to what extent a saturated vapor of an odorant need to be diluted to obtain concentrations that would yield selected odor intensity level. In these correlations, vapor pressure data on 326 compounds were used and assembled from several sources (11,12). Halogen-containing organic compounds were not included in this data base, since their flavor significance is low.

Method of Correlation

Functional groups may exhibit a different influence on the

Table I. Concentrations of Vapors in Air, PPM Vol/Vol, to Produce Odor Intensity Equivalent to 87 PPM (Vol/Vol) of 1-Butanol (S=5)*

<u>Compound</u>	<u>This work</u>	$\frac{\text{Log}_{10}(\text{PPM})}{\text{Katz-Talbert}}$
Acetone	3.55	
Acetonitrile	3.73	
Acetophenone	-1.19	
Allyl alcohol	1.46 (=29 PPM)	1.56 (=36 PPM)
Benzaldehyde	0.49 (=3 PPM)	1.08 (=12 PPM)
Benzene	2.97	
Butanoic acid	-1.25	
2-Butanone	2.62	
Butylbutanoate	1.01	
Butylether	1.03	
Carbon tetrachloride	3.84	
Chlorobenzene	0.98	
Chloroform	3.49	
Cineole	-1.06	
Citral	-1.42	
Cyclohexane	3.67	
1,2-Dichloroethane	2.84	
Dimethylbenzylcarbinol	0.38	
2,4-Dimethylpentane	3.97	
1,4-Dioxane	2.48	
Ethanol	4.14	
Ethylbutanoate	0.17	
Eugenol	-1.07	
Guaiacol	-1.20	
Hexanoic acid	-0.98	
1-Hexanal	0.43	
1-Hexanol	1.08	
3-Hexanol	0.83	
2-Hexanone	0.67	
Indole	-0.85	
1-Iodobutane	1.90	
d-Limonene	1.27	
Linalool	-1.88	

Table I. (Continued)

<u>Compound</u>	<u>Log₁₀ (PPM)</u>	
	<u>This work</u>	<u>Katz-Talbert</u>
Menthol	0.50	
Mesitylene	1.49	
Methyl isopentanoate	0.82	
Methyl pentanoate	1.12	
2-Methyl-2-propanol	3.79	
Methyl salicylate	0.54	
Nitrobenzene	0.50 (=3 PPM)	1.26 (=18 PPM)
1-Nitropropane	2.67	
1-Octene	1.48	
2-Octene	1.36	
2-Octyne	0.00	
3-Pentanone	1.03	
Phenylethanol	0.14	
Alpha-Pinene	1.34	
Propanoic acid	0.72	
1-Propanol	3.07	
2-Propanol	3.41	
Propylbutanoate	0.97	
iso-Propylpropionate	0.80	
Pyridine	0.84 (=7 PPM)	1.26 (=18 PPM)
Styrene	0.98	
1,1,2,2-Tetrachloroethane	1.62	
Thiophene	0.68	
Toluene	2.37	
Vanillin	-2.30	
m-Xylene	1.37	

* Note: for four of the odorants, Katz-Talbert values were available indicating concentrations needed to produce odor intensity of category 3 on their scale. Values of ppm for these substances are shown in parentheses.

molecular properties depending on (1) their position in the molecule, (2) molecular size, and (3) the presence of other functional groups. Therefore, in characterizing the structure of the molecule it is not sufficient to e.g., classify these by the presence of -OH groups. The size of the molecule needs to be considered as well as the presence of groups that may enhance the exposure of the -OH group, such as being on a ring structure, or in a terminal position, or elsewhere in the vicinity of bulky groups that may obscure the -OH, e.g., next to a branched -CH₃ group.

Molecular weight was used as one of the molecular descriptors, followed by 38 attributes derived from inspection of Wiswesser notations. These reflected:

- presence, number, and positional types of hydroxyl group
- presence of carboxylic acid groups
- presence and positional types of thiol groups
- number and positional types of double bonds
- number and positional types of carboxyl, aldehyde, ester, ether groups
- number of benzene rings
- number of amino, imino, and -N= groups
- number of -S- groups
- number of cyano, nitrile, thiocyanate, and isothiocyanate groups
- number of nitro groups
- number of ternary and quaternary atoms
- number of halogen atoms (Cl, Br, I)
- number of heterocyclic rings
- number of substituents on rings
- longest hydrocarbon chain in the molecule (one of the attributes related to the solubility in oils and insolubility in water)

Odor properties in a homologous series vary with the molecular size and frequently reach a maximum at a certain specific size. Also, they are influenced by the types of the other functional groups present. To permit consideration of these factors, combinations of the enumerated properties were also introduced as potential candidates for correlations. One set of principal combinations consisted of products of molecular weight and the numbers indicating presence of the most of the quoted functional groups. Such terms reflect interaction effects: e.g., significance of hydroxyl group may decrease as the molecular weight increases. The other set included products of the square of molecular weight and functional group indicators. The square-containing term provides for a maximum effect at a certain molecular weight, but a lesser (or in reverse) effect at lower and higher molecular weight. Additional factors were introduced to reflect the possibility of interactions between the presence of functional groups and the presence of ternary and quaternary atoms and double bonds. In total, 118 indices characterizing molecular

characteristics and some of their interactions, most likely to have an effect on odor, resulted.

An updated form of Biomed O2R Stepwise Regression Analysis program and a Univac 1108 were used to search for the correlations.

Results and Discussion

Iso-intensity Concentrations. These are concentrations that produce approximately equal suprathreshold odor intensities. In this case, an odor intensity, $S = 5$, equivalent to that of 87 ppm of 1-butanol for our data set; or an intensity score of 3 for the Katz-Talbert set. A variable to account for a possible systematic difference between these two sets was introduced as a candidate term in the regression equation.

Figure 6 illustrates the correlation found. Logarithm of ppm concentration to exhibit above intensity was best reflected by Equation 6. Coefficient of determination (squared correlation coefficient) between the calculated and the actual value was 0.74. Thus, 74 percent of the variance in the concentrations needed to obtain the defined odor intensity was accommodated by the equation. The value of F-ratio was 18.2: the probability of such correlation by chance is $p \ll 0.001$. Standard deviation for the calculated vs. actual value was 0.78.

There was a systematic difference between our and Katz-Talbert data sets, which is understandable because of different sample presentation and odor intensity rating methods. In their work, the log (ppm) needed to obtain the intensity score of 3 was by 0.6 log units lower than that to obtain $S = 5$ in our work.

Figure 7 illustrates the obtained result. The upper part is a histogram indicating the experimental distribution of concentrations, by ranges, found to exhibit the target intensity $S = 5$ (or score 3 for Katz-Talbert data): e.g., 21 substances exhibited this odor level at concentrations between 0.1 and 1 ppm in air.

The lower part of the figure is a histogram of deviations from the use of Equation 6. The set of numbers above this histogram estimates the resulting odor intensities S for cases when an odorant's experimental iso-intensity concentration value is different from the calculated value. Thus, if the substance disobeyed the calculated value to the extent of 0.5 log units to the left (actual needed concentration to obtain $S = 5$ was 3 (= $10^{-0.5}$) times lower than the calculated value), a stimulus prepared to contain the calculated concentration would exhibit an intensity of 8 instead of 5 (assuming that the odor intensity varies proportionally to 0.41 power of the odorant concentration, see above).

There may be several reasons for the residual data scatter:

- (1) Match vs. 1-butanol scale in repeated measurements of the same odor stimulus by different panels has a standard

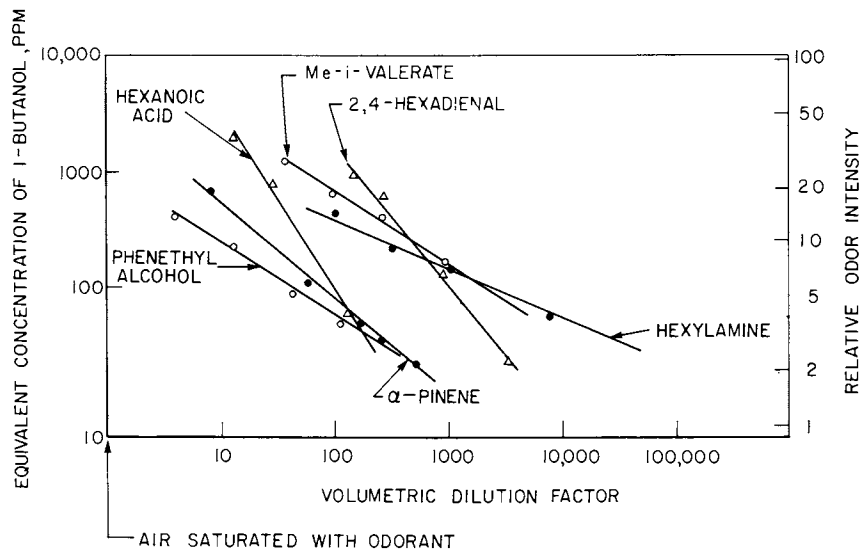


Figure 5. Dose-response plots for several odorants

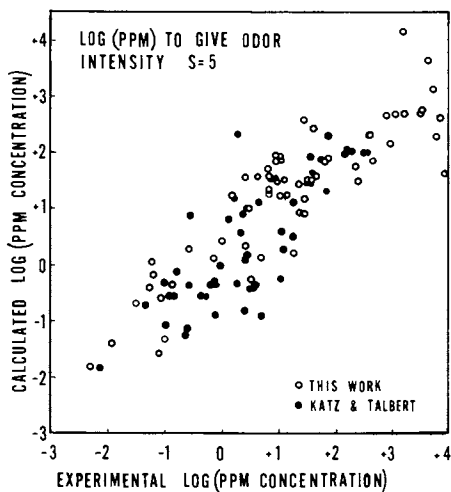


Figure 6. Results of isointensity concentration correlations

Regression Equation for Isointensity Concentrations

(S = 5 for our data;

intensity score 3 for Katz & Talbert Data)

Equation 6

	F-Ratios for Separate Terms
Log (PPM v/v) = 6.903	
-1.3296 (Log MW) ²	115
-0.596 (Log MW) ² (VQ)	24
-1.030 (SH)	21
+0.517 (G)	19
-0.004 (MW) (U)	16
+0.216 (L + T) (max. No.)	15
-1.276 (R) (Q)	13
-0.396 (L + T) (X + Y + L + T + R)	12
-1.116 (R) (V)	9
-0.0002 (MW) ² (CN + NC)	8
+1526 (R) (∅V)	5
+1.816 (I)	5
+0.742 (L + T) (V)	4
-0.599 {Katz-Talbert = 1 Our Data = 0	13

cf. next page for explanation of symbols.

Regression Equation for Vapor Pressure

Equation 7

	F-Ratios for Separate Terms
log (mm vapor pressure at 25°C) =	
4.152	
-0.0354 (MW)	1085
-0.746 (Q)	74
+0.131 (max. No.)	62
+0.719 (X + Y)	61
-0.887 (V in L & T)	30
-0.586 (terminal Q)	21
-0.338 (VH)	15
-0.712 (CN or NC)	10.5
+0.206 (∅)	8.7
+0.237 (∅V)	8.5
-0.298 (X + Y + L + T + R)	8.2
-0.680 (Z in LT)	7.9
+0.082 (No. of Subst.)	5.1
-0.280 (U in L & T)	4.1
+0.174 (L + T)	3.2

See notes to Equation 6 for the meanings of the term symbols.

Symbols in Regression Equations
(for all except molecular weight
the number of the corresponding symbols
in Wiswesser notation formula is used as the variable)

MW = molecular weight
VQ = Carboxylic acid group
SH = mercaptan
G = chlorine
U = double bonds
L = carbocyclic ring (but not benzene)
T = heterocyclic ring
max. No. = largest Arabic numeral in the formula,
denoting the longest methyl group segment
in the molecule
R = benzene ring
Q = hydroxyl group
X = C atom with four bonds extending to separate
atoms which are not hydrogen
Y = same as X but with three bonds extending as above
V = carbonyl group
CN, NC = cyanide and isocyanide groups
ØV = ester linkage (oxygen bridge Ø and carbonyl V)
I = iodine (there is only one iodine compound in the
series - 1-iodobutane)

Other symbols appearing in vapor pressure regression equation,
see below:

VH = aldehyde group
Z = NH₂ group
No. of Subst. = number of substituted position on rings

deviation of the order of 0.2 log (ppm 1-butanol) units.

Expressed in odor intensity units, values of S between 4 and 7 may occur for a stimulus with $S = 5$. This would account in part for the scatter in the lower part of Figure 7.

- (2) The expression of structures from Wiswesser formulas is not yet sufficiently refined to reflect some odor-significant property, or the experimental substance's odor is contaminated by components not represented by the formula.

The largest differences between the calculated and experimental values occurred for the following compounds: benzaldehyde, skatole, propylmercaptan, dithioethylglycol, methylsulfide, alpha-chloroethylsulfide (all these from Katz-Talbert data published in 1930); and allyl alcohol, dioxane, dimethylpentane, 1-hexanal, ethylbutyrate, acetophenone, guaiacol, d-limonene, and carbon tetrachloride in our data. There is a good reason to suspect odor-important impurities in many of these. Also, some substances are the first members (smallest molecules) of several homologous series, usually difficult to fit into physico-chemical correlations developed for the higher members of the same series.

Iso-intensity Concentrations and Vapor Pressures. When saturated vapors of substances are diluted by the same factor, they are at the same thermodynamic state with respect to the corresponding condensed phases. Substances compared for the same property at the same vapor dilution tend to act more similarly than when compared at the same molar concentration (as in the case of concentrations expressed in ppm v/v). It was of interest to compare vapor dilution factors needed to obtain odor iso-intensity concentrations. This was possible only for those compounds for which saturation vapor pressures at 25°C were available from various handbooks.

The upper part of Figure 8 repeats the upper part of Figure 7. The lower part is a histogram by ranges of dilutions from saturated vapor. In transforming from ppm concentrations to dilutions, the range has contracted from 7 to 5 log units. Substances with similar functional group occupy even a lesser range.

It is possible that correlations vs. Wiswesser formula characteristics would considerably improve if dilution to obtain odor intensity $S = 5$ would be introduced as one of the starting independent variables. This, however, would require knowledge of vapor pressures, cf. next subsection.

Figure 8 has some practical applications in olfactometry where odor stimuli frequently are prepared by saturating air with the vapor of the odorant and followed by subsequent dilution with additional air. Diluting the vapor by a factor of 100 would bring a considerable fraction of odorants into the weak to moderate odor intensity range.

Vapor Pressures. Lack of knowledge of vapor pressures for

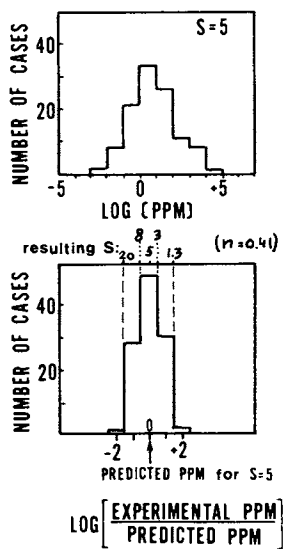


Figure 7. Limitations of obtained isointensity vs. concentrations correlation

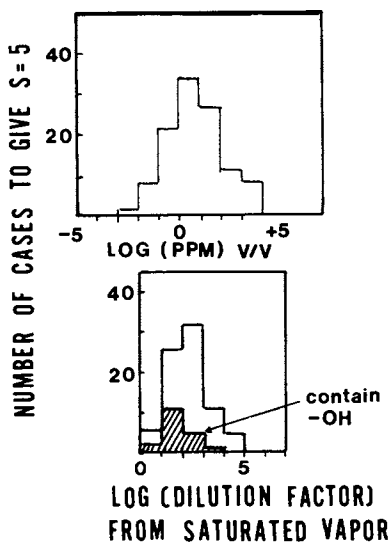


Figure 8. Isointensity concentrations in terms of dilution ratio for saturation vapor pressure

many odorants makes it difficult to estimate their concentrations in olfactometric work. Since Wiswesser notation formulas contain well-organized structural information, a test was conducted to determine if vapor pressures can be correlated to such properties.

Two statistical experiments were conducted. In one, use of interaction terms was minimized -- log (molecular weight), square terms, etc. were not introduced. This procedure was considered proper since log (vapor pressure) for a homologous series follows a negatively sloped linear trend with respect to the molecular weight, and the corresponding members of different homologous series tend to be separated by a constant logarithmic increment.

In the other statistical experiment, all interaction terms used in the odor intensity correlations were introduced as additional candidate terms.

Vapor pressures were used in terms of logarithm of pressures in mm Hg.

The results were as follows:

	Experiment 1 Simpler Properties	Experiment 2 Same Plus Interaction Terms
F-Ratio	200	190
Degrees of Freedom	(15,310)	(26,299)
Coefficient of Determination	0.91	0.94
Residual Standard Deviation	0.52	0.41
Number of Terms in Regression Equation	17	26

Apparently, the addition of interaction terms improved the correlation only somewhat.

It is apparent that the vapor pressure data, not subject to sensory variability effects, correlated to the formula properties considerably better (91 percent of variance accommodated) than the iso-intensity data. However, the relatively large standard deviation (0.52 log units, or by a factor of 3.3) indicates that the properties and their combinations used should be further refined.

Halogen compounds were not represented in the vapor pressure data. Also, for some substances the vapor pressure extrapolations impermissibly extended downward through the fusion point, without considering the effect of the heat of fusion on the vapor pressure. Larger deviations occurred with salicylic structures, musks, vanillin, lactones, skatol, indole, and compounds with several ester groups. For some, such as musks, the literature data may also be inexact, since their vapor pressures are very low and difficult to measure. On the whole, vapor pressures are predicable usually within a factor of 3 (one standard deviation), and most usually better than 9 (two standard deviation).

Slopes. Correlation of slopes n to the Wiswesser formula properties are yet to be developed.

Summary

The values of concentrations of odorants in air that would exhibit odors in a certain odor intensity ("easily noticeable") range can be approximated from data extracted from one-line Wiswesser notation formulas of odorants. Vapor pressures can be approximated considerably better. Thus, the potential exists for using Wiswesser notation formulas not only for a computerized information search, but also for calculations of approximate odor iso-intensity concentrations and of vapor pressures. Improvements in the approximations should be possible by reviewing cases where significant deviations of the experimental calculated values occurred.

Acknowledgement. The assistance of Dr. F. C. Bock in treatment of the computerized data analysis is gratefully acknowledged.

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Flavor Chemical Mixtures—A Psychophysical Analysis

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Gas chromatographic analyses can reveal the components of many mixtures, and are used as a standard tool in flavor analysis by research and development chemists. Yet, despite the impressive evidence which has accumulated concerning the composition of aromas, little information is available on how odors are perceived when presented in concert with each other. This paper concerns the psychophysical evaluation of odors evaluated in pairs.

Odors can be assessed on at least three criteria: intensity, hedonics and quality.

Intensity. Most studies using the method of magnitude estimation (1,2) suggest that odor intensity conforms to a decelerating function of concentration. The function can be approximated by a power equation of the form $S = kC^n$ (n between 0.4 and 0.7 for air dilutions of an odorant, and much lower for liquid dilutions of the odorant; (3, 4, 5, 6)). Magnitude estimation yields numbers which possess ratio properties. A 20 on the magnitude estimation scale indicates an odorant which is twice as strong (sensorically) as an odor rated 10. There are no highest nor lowest numbers on the scale, and only ratios convey information.

Hedonics. Most pure chemicals are unpleasant (5, 6, 7). Even odorants which are reminiscent of actual food aromas are considered unpleasant, and less pleasing than are their natural counterparts, which comprise many components. Complexity of an odor tends to improve its acceptability in those cases. Hedonics is often assessed by means of a scale which recognizes a fixed point of neutrality (0 point), and allows for ratings of liking and disliking on separate parts of the scale (5, 8). Sometimes experimenters force the panelist to use a single continuum of positive numbers, and thus, never know when an odorant becomes unpleasant (9, 10).

Quality. The quality of an odor can best be captured by means of an adjective descriptor list. Numerous lists have been proposed. Recent lists include the Crocker-Henderson system (11) Harper *et al*'s list of 44 descriptor terms for flavor of foods (12), and Dravnieks' list of 136 descriptor terms. The descriptor list is meant as a partial, and not a complete list of nuances which can be perceived, and for specific foods, the list must be augmented (13). Recent developments in multidimensional scaling have added yet another method for evaluating quality. Multidimensional scaling is a method which locates stimuli in a geometrical space of low dimensionality, with the property that stimuli qualitatively similar to each other are located close to each other. Numerous methods exist for converting evaluations of subjective similarity (obtained from panelists) to locations of points in space, with the points corresponding to the stimuli which the panelists had evaluated for similarity (14, 15, 16, 17).

Odor Mixtures. Odor mixtures have been evaluated in terms of intensity, hedonics and quality.

Intensity. Intensity of mixtures is usually lower than the intensity sum of their components. The exact amount depends upon the two components (or more in the mixture). Berglund *et al* (18) have suggested that two component mixtures can be approximated by a vector model of additivity.

$$(\text{Mixture Intensity})^2 = (A)^2 + (B)^2 + 2AB (\cos)\alpha$$

$$(105 \leq \alpha \leq 130)$$

Where: A = odor intensity of odorant 1, B = odor intensity of odorant 2, mixture = odorant 1 and 2 mixed in air, all scaled by magnitude estimation.

They evaluated mixtures of noxious odors, such as dimethyl sulfide and dimethyl disulfide. Berglund (19) suggested a method whereby mixtures of 3, 4 and 5 components could be evaluated for intensity and predicted via the vector model. Cain (20) and Cain and Drexler (21) discussed the nature of odor mixtures, and suggested that for odor masking of a pleasant odor by an unpleasant odor (amyl butyrate masking propanol, linalool and linalyl acetate and lavandin, respectively, masking pyridine), the vector model represents a good approximation of the sensory effects for intensity.

Hedonics. The hedonics of mixtures has not been as thoroughly studied. An early study by Spence and Guilford (22) suggested intermediacy, so that the mixture was neither as pleasant as the more preferred component, nor as unpleasant as the less preferred one. However, these results are at best preliminary, and do not illustrate the large scope of hedonic modifications in mixtures which underlie odor masking and

modification.

Quality. Odor quality of mixtures is the most difficult aspect to study, primarily because there are no adequate models of odor quality with which to investigate, formalize and evaluate quality shifts. Moskowitz (23) has suggested that the method of multidimensional scaling be used to assess the relative positions of odors and their mixtures in space, which would pictorially display the shifts in quality, but not lend verbal descriptions to those qualities. Moskowitz (24) discussed the difficulties which could be encountered when evaluating the aroma of a flavor material by sensory evaluation as well as when correlating the flavor with the array of chemicals which the aroma comprises.

The Scope Of This Paper

The present study concerns the psychophysical evaluation of two component mixtures, evaluated at different concentrations, for odor intensity, odor hedonics and odor quality. The aim is to develop a series of mathematical (or at least quantitative) models which describe the perceptions of odor when unmixed, and relate those perceptions to behavior of the odorants in binary mixtures of known composition. The assessment method of magnitude estimation (2, 25) provides the optimum procedure for determining the relative odor intensities, hedonic values, and quality ratings as well as for evaluating the percentage change in sensory intensity, etc., in mixtures. Magnitude estimation was developed at Harvard University to assess brightness of lights and loudness of tones, with the aim of relating these, through meaningful equations, to measured physical quantities. The method has found wide use in the evaluation of tastes, smells, appearance and color, etc.

Experimental Section

Stimuli. The stimuli were two reagent grade chemicals, with the appropriate and distinct odor characters. The stimuli were heptyl acetate (pear, rose), and ethyl salicylate (wintergreen). (See Table 1)

The Apparatus. An air dilution olfactometer was developed by A. Dravnieks of the Illinois Institute of Technology Research Institute (Chicago). The olfactometer presented panelists with two odorants at four concentrations (saturated odorant diluted to four final levels). It also presented the 16 mixtures, by continuous air flow, so that the panelist had at her disposal 24 different air channels, with two sets of four channels presenting two unmixed stimuli at four concentrations (relative saturations = 1/5, 1/20, 1/80, 1/320). The flow system of the olfactometer was set up to deliver 320 cc/minute of odorous air. In both the

unmixed flows and in the mixtures, the two components were presented at specific concentrations (10%, 2.5%, 0.625%, and 0.153% saturation), and makeup air was added to produce the following:

- a) The components embedded in a total air flow of 320 cc/min.
- b) With respect to the entire flow, the concentration of each component at its proper level.

Moskowitz, Dravnieks and Gerbers (10) discussed the design of a similar olfactometer, which presented panelists with one odorant at 8 concentrations. That design was modified in the present apparatus to accommodate two odorants, and their mixtures, but the basic design of the system was maintained.

Air dilution olfactometry has a distinct advantage over standard sniff bottle techniques, since

- a) The odorants are continuously regenerated;
- b) The mixtures are never in liquid, only in air;
- c) The concentration can be adjusted by adjusting flow rates.

The Experimental Design. Panelists were women residents of the Framingham-Natick (Massachusetts) area, who were trained in the method of magnitude estimation. They were instructed to assign numbers to odors, so that the ratios of the numbers reflected as closely as possible the ratios of odor intensity, odor hedonics, and odor qualities, respectively. For example, an estimate of 30 was to imply an odor twice as strong as an odorant assigned a magnitude estimate of 15. The following provisions were made in the assignments:

- a) For intensity judgements, a 0 was to reflect no odor, and increasing positive numbers were to reflect increasing degrees of odor intensity.
- b) For hedonic judgements, a 0 was to reflect neutrality (neither liking nor disliking), positive numbers were to reflect increasing degrees of liking, and negative numbers were to reflect increasing degrees of disliking.
- c) Quality judgements were treated like intensity judgements. Eight individuals participated in the study. They evaluated every one of the 24 odors in random order, assigning magnitude estimates to odor intensity, odor pleasantness, and odor qualities. Table 1 shows the odor qualities evaluated in each experiment. When possible, a description of the odor quality was provided in depth to assure the panelists of the meaning of that quality.

The panelists replicated the experiment eight times, to produce a total of 64 ratings per sample (8 panelists x 8 replicates = 64 panelist/replicate ratings).

Results

Table 1 contains the average ratings on attributes for different concentrations of the two stimuli.

Odor Intensity. Figures 1a and 1b show how the average perceived sensory intensity varies with concentration, for each odorant, evaluated both alone, (Figure 1a) and in the presence of the other odorant at varying concentration of the second odorant (Figure 1b).

Both coordinates are logarithmically spaced in both figures.

The major finding regarding subjective estimates of odor intensity can be summarized as follows:

- a) Odor intensity grows approximately as a power function of concentration, supporting previous results. However, the range of concentrations was not large enough to exclude other candidate equations (e.g., linear, logarithmic, exponential).
- b) The exponents for the odorants are all lower than 1.0, so that odor intensity grows as a decelerating function of concentration.
- c) Representative power functions are:

Ethyl Salicylate: $S = 8.26 (\%C)^{0.65}$

Heptyl Acetate: $S = 16.9 (\%C)^{0.53}$

Where: $S =$ magnitude estimate of odor intensity

The principal finding for odor intensity of mixtures (Figure 1b) is that pairs of odors suppress each other. The mixture is weaker than would be expected on the basis of additivity of component odor intensity. For some cases, the intensity of the mixture is weaker than the weaker component itself, whereas for the majority of cases, the odor intensity lies between the stronger and the weaker component.

A mathematical model of odor mixtures can be developed by appeal to the rules governing vector summation. Berglund, Berglund, Lindvall and Svensson (18) suggested that mixtures of odorants add together in intensity as if they were vectors, with an estimated angle of $105^\circ - 130^\circ$ separating the vectors. The model of vector additivity was used here, to predict the angular separation between the pairs of odorants. The mean ratings for the components and for the mixtures were used in the following formula, and an estimate of cosine φ was obtained:

$$(MIX)^2 = A^2 + B^2 + 2AB \cos (\varphi)$$

Figure 2 shows how the vector model behaves as a function of the odor intensity of the components.

Table I. Odor Concentrations and Average Ratings on Attributes

<u>LEVEL</u> <u>ETHYL</u> <u>SALI-</u> <u>CYLATE</u>	<u>LEVEL</u> <u>HEPTYL</u> <u>ACE-</u> <u>TATE</u>	<u>INTEN-</u> <u>SITY</u>	<u>PLEAS-</u> <u>ANTNESS</u>	<u>COM-</u> <u>PLEXITY</u>	<u>FRUITY</u>	<u>FRA-</u> <u>GRANT</u>	<u>MINTY</u>
1	0	5.6	3.7	18.1	0.6	0.5	0.2
2	0	14.7	8.0	26.6	1.6	5.8	7.4
3	0	31.6	18.3	36.4	4.4	12.9	22.4
4	0	49.8	26.9	34.1	2.5	23.4	38.6
0	1	2.3	7.1	19.3	2.5	2.6	0.1
0	2	5.6	9.6	36.5	16.3	6.8	1.1
0	3	17.9	3.6	40.5	22.6	12.6	1.6
0	4	32.1	-2.5	45.9	29.7	15.8	4.4
1	1	3.3	3.2	16.5	3.8	3.0	0.0
2	1	6.2	8.1	26.3	1.9	2.2	1.8
3	1	20.9	17.2	27.2	1.7	15.3	26.7
4	1	41.6	26.6	33.5	3.1	26.4	44.0
1	2	11.3	6.6	32.9	7.7	1.8	2.6
2	2	13.7	8.3	38.0	13.1	7.2	2.3
3	2	19.2	10.0	40.7	16.7	7.9	7.1
4	2	30.0	29.6	35.8	3.9	22.5	38.4
1	3	32.0	4.8	37.3	25.0	10.9	3.8
2	3	24.1	6.6	29.6	19.1	8.2	2.3
3	3	25.5	11.1	32.8	16.4	13.1	8.6
4	3	34.2	25.6	34.6	14.4	22.6	30.6
1	4	43.6	0.2	40.3	28.2	12.1	5.6
2	4	43.8	-4.3	39.3	26.8	14.5	4.3
3	4	36.7	-0.5	36.9	23.6	10.9	3.2
4	4	49.9	2.4	42.3	27.0	17.7	13.9

Table I (cont.)

<u>BANANA</u>	<u>SWEET</u>	<u>ARO- MATIC</u>	<u>PEAR</u>	<u>FLOW- ERY</u>	<u>HEAVY</u>	<u>SPEAR- MINT</u>	<u>WINEY</u>	<u>HERBAL</u>
0.0	2.7	1.3	0.5	0.3	2.6	0.3	0.0	0.3
1.0	4.0	8.8	4.7	0.8	2.4	5.2	1.3	1.0
1.6	10.6	19.9	2.2	1.8	6.7	16.1	3.9	5.2
1.7	14.5	27.0	3.3	2.7	7.9	20.5	2.5	6.9
0.8	3.8	6.4	4.9	0.8	2.5	0.3	0.3	0.3
3.6	9.1	13.0	20.8	4.8	10.1	1.6	4.1	4.4
6.6	11.4	16.1	21.6	4.1	19.7	4.1	5.4	7.2
10.5	10.6	20.3	24.5	8.2	28.8	6.6	7.2	9.4
1.9	4.5	4.2	4.3	1.8	2.0	0.6	0.6	0.0
0.7	2.7	9.9	7.6	0.8	3.3	2.4	0.9	1.3
1.1	9.7	19.2	2.7	2.0	8.8	14.4	2.0	5.8
2.7	18.7	27.9	3.7	3.2	11.0	26.5	3.9	6.0
5.9	7.3	12.1	12.1	2.1	6.5	2.7	2.3	3.8
5.3	10.9	15.1	16.6	5.5	8.8	3.7	5.8	3.1
4.0	11.8	11.8	12.0	6.3	12.2	4.1	4.8	4.9
2.2	14.2	27.8	5.3	2.5	7.8	23.1	3.4	6.4
8.0	15.2	12.0	20.2	5.0	17.5	5.6	4.0	6.4
5.5	11.4	11.3	16.9	4.1	19.4	4.5	4.2	8.3
6.1	14.1	13.5	17.3	5.5	14.6	6.8	8.0	7.5
5.2	16.1	25.3	8.1	5.8	13.6	21.0	5.5	7.8
8.7	17.8	15.5	25.3	5.7	29.5	6.7	6.3	11.0
10.0	21.1	17.0	23.9	5.7	23.3	7.5	7.7	11.7
7.0	15.6	11.4	21.1	7.2	27.2	5.4	7.8	8.9
8.3	22.9	20.2	20.3	6.4	31.8	10.0	7.7	13.5

KEY

LEVEL 1 = 0.153% SATURATION

LEVEL 3 = 2.5% SATURATION

LEVEL 2 = 0.625% SATURATION

LEVEL 4 = 10% SATURATION

Figure 1a. Dose-response (psychophysical) function relating percentage saturation of single odorants to perceived odor intensity assessed by magnitude estimation. The coordinates are log-log, in which a straight line implies that the function is a power function.

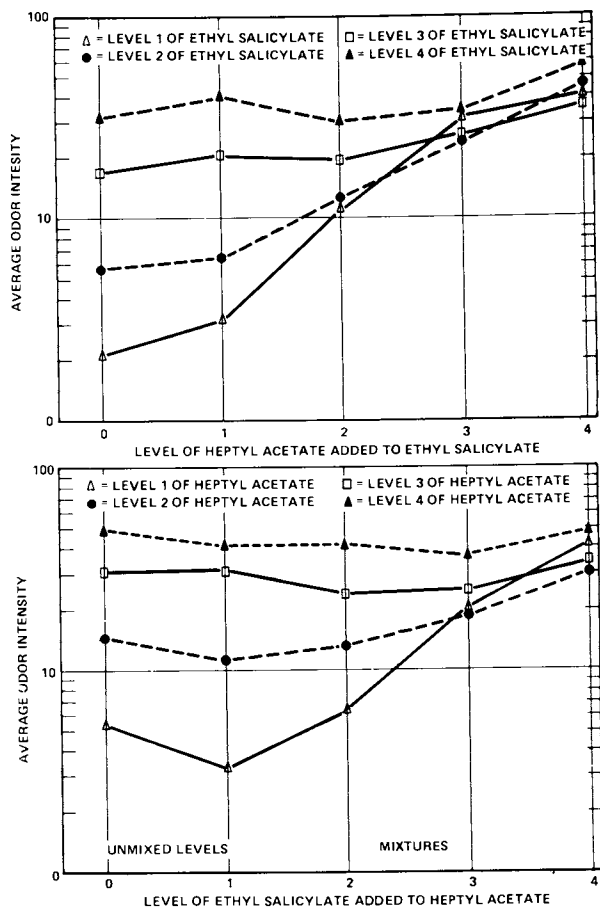
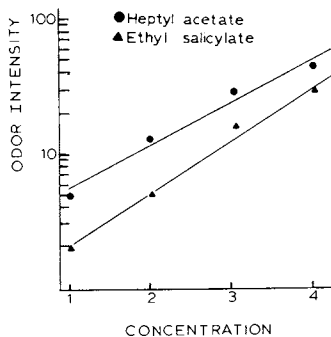


Figure 1b. Dose-response (psychophysical) functions relating percentage saturation of mixtures of odorants to perceived odor intensity. The coordinates are log-log, in which straight lines imply that the function is a power function.

In Figure 2, a 'v' represents the fact that the mixture intensity can be fit by the vector model. A limit on $\cos \alpha$ was set so that $105^\circ < \alpha < 140^\circ$ (or $-0.76 < \cos \alpha < -0.26$). A 'v+' means that the mixture was stronger than would be predicted by the vector model (i.e., $\alpha \leq 105^\circ$ or $\cos \alpha \geq -0.76$). A 'v-' would mean that the mixture was weaker than would be predicted by the vector model (i.e., $180^\circ \geq \alpha \geq 140^\circ$). An 's' reflects the fact that the mixture was weaker than would be predicted, even if $\alpha = 180^\circ$ (or $\cos \alpha = -1$). Synergy refers to the fact that the arithmetic sum of the mixture odor intensities was greater than the sum of the mixture intensities.

Figure 2 suggests that there are three regions of mixture:

- a) An intermediate region of intensities, where the vector model holds. This region is that where the two odorants are similar (although not necessarily equal) in intensity.
- b) A region of enhancement above the level which the vector model predicts. This region encompasses a high level of heptyl acetate and a very low level of ethyl salicylate.
- c) A region of suppression (or diminished additivity). This region comprises low levels of heptyl acetate plus varying levels of ethyl salicylate.

Hedonics. Figure 3 shows the hedonic ratings of the mixture and the components (vs. concentration). The layout of Figure 3 is similar to that of Figure 1b. An attempt was made to relate the hedonic ratings of mixtures (M_H) to the hedonic ratings of the components (A_H & B_H). The equation best fitting the results is:

For mixtures of ethyl salicylate (E) and heptyl acetate (H)

$$M_H = -0.08(E_H) - 1.01(H_H) + 14.24 \quad R = 0.48$$

$$F(2,21) = 3.21$$

Where: R = correlation coefficient

F = F ratio, analysis of variance

The important thing about the hedonic tone of binary mixtures is that they cannot be easily predicted from the hedonic tones of the components. Rules of intermediacy do not necessarily hold for hedonics, possibly because:

- a) The mixture has changed in character;
- b) The hedonic tones of mixtures do not obey algebraic combination rules;
- c) The vector model is inappropriate for positive vs. negative ratings.

Quality Shifts. Quality shifts in mixtures can be assessed by contrasting the profile of the components and of their binary

Figure 2. The nature of additivity of odor intensities in mixtures vs. the component odor intensities. The axes indicate the component, unmixed odor intensities. (v) the mixture can be adequately modeled by the vector model; (v+) the mixture intensity can also be modeled by the vector model, but the angular separation is less than 105° . A 'v-' would mean that the intensity would also be modeled by the vector model but that the angular separation is greater than 140° . (Sup) suppression—the mixture odor intensity is less than the difference obtained by subtracting the weaker odor intensity from the stronger odor intensity. (Synergism)—the mixture odor intensity exceeds the arithmetic sum of the component odor intensities.

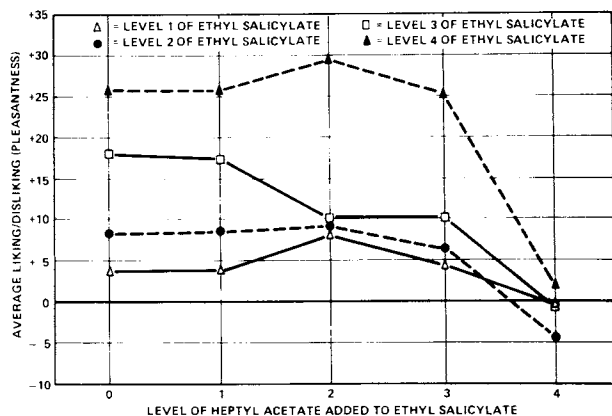
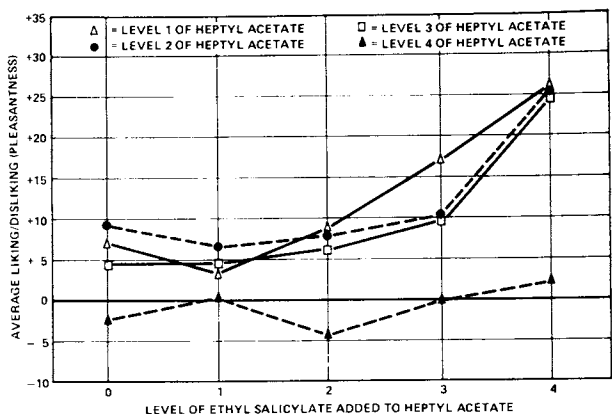
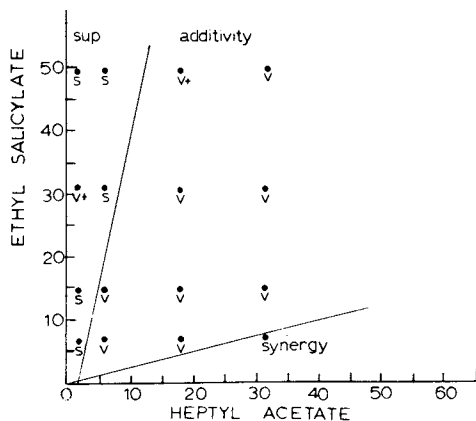


Figure 3. Dose-response functions relating hedonic tone to odor concentration for mixtures. The abscissa reflects the relative level of one odorant added to a constant amount of a background odorant. The ordinate reflects the average liking (+) or disliking (-) magnitude estimate assigned to the mixture.

mixtures. Figures 4 and 5 show some of these mixtures, and their relations to their components. A general rule which emerges from Figures 4 and 5 is that there is no systematic rule that predicts the quality notes of a mixture from the quality notes of its components. Other things which emerge are:

- a) The presence of a quality note in a pure odorant can be completely masked by the addition of a second odorant.
- b) Rarely does an entirely new quality note emerge that was not present in the components evaluated alone. This conclusion must be tempered with the realization that in the experiment an exhaustive list of descriptors was not used. Some descriptors may have applied to the mixture, but not to the components.

Multidimensional Analysis. Graphical 'bar' or similar representations of odor quality in mixtures make an understanding of potential sensory processes difficult to obtain. However, pictorial representations of odors and their mixtures in a 'perceptual space' may illustrate hitherto undiscovered relations between odors and mixtures in a 'perceptual space.' Such an approach was reported by Moskowitz (23) for five component mixtures. Here, a similar approach was taken, in order to visualize the relations between components and mixtures.

The method of factor analysis (26) is particularly well suited to the analysis of mixtures. Factor analysis attempts to determine the number of independent factors (or primaries) which, in concert, reproduce a set of stimuli. The input for factor analysis is usually a series of descriptor terms that pertain to a set of stimuli, and ratings of a number of stimuli on these descriptors. The output of the factor analysis is a set of correlations between pairs of different descriptors (computed across different stimuli which were evaluated on the descriptors), as well as a set of axes. These axes or coordinates are perpendicular to each other, and represent the fundamental dimensions or primaries. Every descriptor comprises some percentage of each primary. Hence, by obtaining a factor analysis solution, the experimenter can see the overlap, communalities, etc., among different descriptor terms, as well as see how many underlying basic terms are really needed.

In this experiment, the analysis was turned around. The different odorants (all 24) were treated as odorants. Ratings for odor intensity were not included in this analysis. The input to the factor analysis was the set of odorants, and the outputs of the analysis were:

- a) The number of primary factors (or underlying odors) that in concert would reproduce the set of 24 odorants.
- b) The contribution of each primary to each of the 24 odorants.

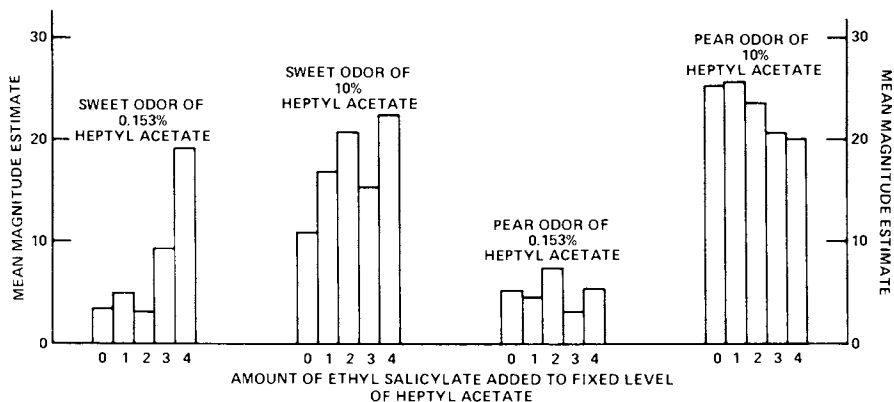


Figure 4. Mixture quality profiles for ethyl salicylate added to fixed levels of heptyl acetate. (Abcissa) the amount of ethyl salicylate added to fixed levels of heptyl acetate. (Ordinate) the mean magnitude estimates for sweetness and pear odor. There are two levels of heptyl acetate (0.153% and 10%) which serve as the base to which ethyl salicylate is added.

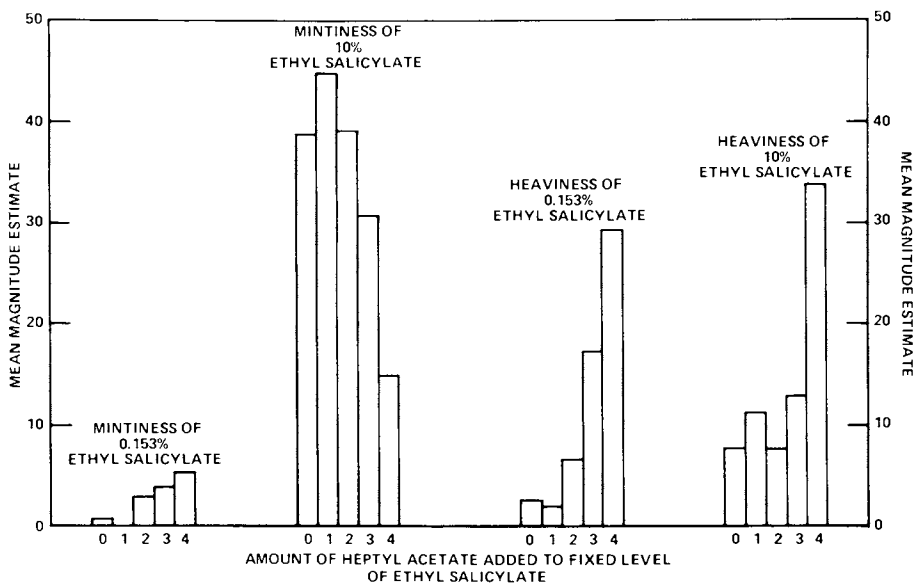


Figure 5. Mixture quality profiles for heptyl acetate added to fixed levels of ethyl salicylate. The attributes of ethyl salicylate are mintiness and heaviness. Those qualities change as different amounts of heptyl acetate are added to fixed levels of ethyl salicylate.

Figure 6 shows a two dimensional solution to the factor analysis. The analysis indicated that there were two basic dimensions (corresponding to the two odors). The pure odorants (unmixed) tend to lie on or near axes, whereas the mixtures lie in between, showing that they comprise some parts of each primary. Note that the units in Figure 6 are relative units only.

Figure 6 can be used to determine the nature of emergent qualities not present in the mixture components evaluated alone. If a line is drawn connecting the two components of a mixture, then ideally the mixtures should lie on that line. The mixture may lie closer to one end or to another (indicating that it is more highly correlated with, or sensorically related to one component, or to another). As the points depart from the line connecting the components it becomes clear that the mixture correlates less well with the components, or even with a linear combination of the components.

Discussion and Conclusions

The present set of studies shows that new techniques must be developed to assess the sensory characteristics of mixtures. Thus far it appears that for evaluating mixture intensity, the vector model provides an initially useful approach with which to evaluate and compare different mixtures. Fortunately, the use of the vector model (or, in fact, any combination rule for intensities) allows the experimenter to formulate questions about potential mechanisms underlying odor mixtures.

With regard to hedonic tone of mixtures, the use of such simple mixture rules as the vector model is not effective, especially when two odorants are mixed over large ranges of concentration, and a general rule for hedonics mixtures is desired. A similar failure to achieve a general rule of hedonic tone of mixtures occurs when tastes are evaluated as well(27). They evaluated the pleasantness/unpleasantness of mixtures of glucose and artificial sweeteners (cyclamate, saccharin). They were unable to develop a model which would adequately predict the overall mixture hedonics from knowledge of the mixture concentrations alone.

It may well turn out that for a large range of mixtures of two components, of varying chemical structure and smell quality, there exists a simple averaging rule for hedonics, so that, in general, the hedonic tone of an odor mixture is in between the hedonic tones of the components. Spence and Guilford (22) found this intermediacy for the evaluation of thirteen odorants. In the micro-analysis of two odorants that intermediacy rule probably no longer holds, because of quality shifts in the mixtures, the fine attention paid to nuances when only two components are mixed in various concentrations, and the overall set or perceptual strategy adopted by the respondent.

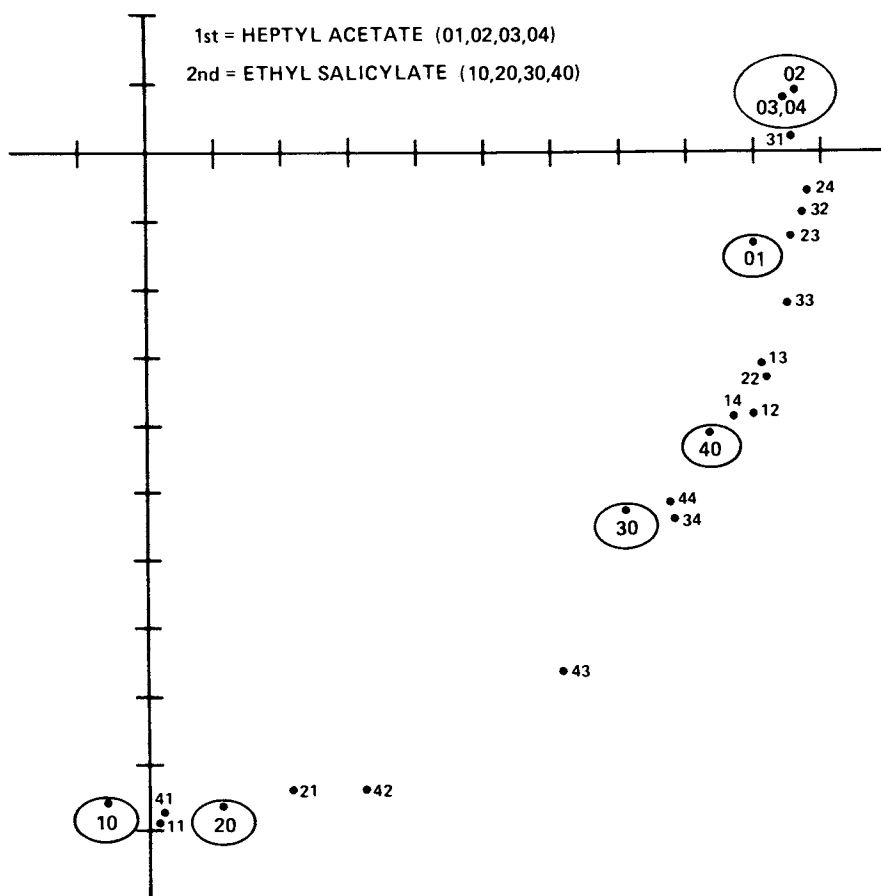


Figure 6. Two-dimensional factor space, obtained from factor analysis of the ratings. The odorants were factor-analyzed. The two-digit number reflects the concentrations of heptyl acetate and ethyl salicylate. The coordinate system is relative, so that the distances between the odorants are meaningful. The results suggest two primaries with projection of each odorant onto each primary shown in Figure 6. The primaries are orthogonal to each other.

Perhaps the most important thing about these studies is the types of quality shifts which are encountered in mixtures, and the difficulty of easily capturing the quality of a mixture, and relating that back to the qualities of the components. The present state-of-the-art requires appeal to one of two different approaches to evaluate quality:

- a) Descriptor checklists (12, 28).
- b) Multidimensional scaling, in which mixtures are embedded in a geometrical space, along with their components (23, 29).

These two approaches only portray the quality shifts for the experimenter, and do not predict them. The picture which is developed may either be easy to understand, or so punctuated by minutiae of detail that the major quality shifts are hidden.

It is possible that today we can only portray quality shifts by these scaling and analytic procedures, but that we have no control over producing a mixture with a specific quality. That is, given the data pertaining to two-component mixtures, we may not yet be able to produce a specific mixture (or determine that it cannot be produced). In contrast, psychophysical analyses of odor intensity and odor hedonics produce descriptive functions, with the property that a curve can be fit to the data, and an intermediate level of intensity or hedonic tone can be obtained by appeal to the curve (or to the descriptive equation).

Abstract

A study with chemicals mixed together pairwise in vapor phase and evaluated by panelists for odor intensity, odor hedonics and odor quality (character) reveals the following general rules:

1. Odor intensity is a power function of odorant concentration for unmixed odorants, with an exponent less than 1.0.
2. Odor hedonics is often a monotonic function of concentration, but cannot be modeled by a power function.
3. Odor quality can be captured by means of a profiling system, using magnitude estimation as the measuring system.
4. In binary mixtures, odor intensity is usually suppressed for more intense component, so that the final mixture intensity is somewhere in between the intensities of the components.
5. In binary mixtures, hedonics are often changed, so that the addition of a pleasing component to a displeasing one makes the mixture more pleasing.
6. Mathematical equations can be developed to model some of the mixture effects.

7. The change in odor quality in mixture is a function of the type of odorants, their quality and their starting odor intensity.

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Structural and Mechanical Indicators of Flavor Quality

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Is the flavor of a food in any way related to its structural and mechanical properties? Most food scientists would say yes. There is some literature on the subject but little, if any, attempts to explain how and why such relationships exist. Many of the newer foods on the market have their texture and flavor constructed or developed separately, making any general relationships between the two difficult to determine. At times it seems possible to combine almost any type and intensity of flavor with just about any texture.

Assuming though, that there is some connection between flavor and texture, how does it occur or why does it exist? Is it due to an interaction between the sensory-systems perceiving flavor and those perceiving mechanical properties? In other words, does stimulating one set of sense organs affect the sensitivity of others? Or do changes in the structural and mechanical properties of a food affect the rate and extent of flavor formation and release? Both suggestions are valid. The first explanation is a phenomenon of an individual's sensory system. The latter is a result of changes occurring in the food.

The sense organs involved in perceiving flavor are the taste buds, olfactory epithelium, and nerve endings responding to chemicals. Those involved in perceiving the structural and mechanical properties of foods are those responding to touch, pressure, position and sound. Peripherally these senses are quite distinct. However, all sensory systems can interact together at higher levels in the brain. If one sense is stimulated it will affect to some degree the sensitivity of other senses. Therefore, if all or part of the sensory system perceiving mechanical properties was stimulated, one would expect some alteration in the perception of flavor.

Stimulating one sense may enhance or depress sensations in another sense modality. Generally, a low level of an accessory stimulus will enhance perception, whereas higher levels of an accessory stimulus probably depress sensations. Little information is available on the enhancement or depression of odors or

tastes by textures or sounds (1), but expecting such effects is reasonable. A crunch reminiscent of the Titanic hitting an iceberg may leave the cruncher with little idea of whether the product was cheese or onion flavored.

The structural and mechanical properties of foods may affect flavor by controlling both the rate of flavor release and the total amount of flavor released. This is fairly easy to understand. A product that breaks down or melts quickly in the mouth will release its flavor relatively rapidly. A product that disintegrates slowly gives up its flavor less rapidly. If two such products contained equal amounts of flavor compounds, the one undergoing rapid breakdown would be perceived as being more intensely flavored.

In many cases viscosity modifying agents such as starches and gums appear to change the taste and odor intensities of solutions to which they are added. This effect does not appear to be due to the viscosity. An interaction between the taste or odor compound and the hydrocolloid is probably responsible (2).

For example, the flavor compound acetaldehyde tastes more intense in a solution of sodium alginate than in plain water. This intensity difference appears to be due to the interaction of acetaldehyde with the alginate. When acetaldehyde was mixed with other gums such as carboxymethyl cellulose or hydroxypropyl cellulose over equivalent ranges of viscosity, there was no significant change in flavor intensity (2).

The same authors found similar results with basic taste compounds and hydrocolloids. Enhancement or depression of compounds, e.g., saccharin and caffeine, also appeared to result from interactions between the taste compound and the viscosity modifying agent. Some gums produced changes in taste intensity whereas others at equal viscosities did not (3).

The results of these studies provide evidence against any interaction between the peripheral sense organs for flavor and those for viscosity.

From the consumer's point of view, the most important way the structural and mechanical properties of a food are related to flavor is through association. We associate certain textures and sounds with certain flavors because through years of eating experience we have learned they always occur together. Given two equally red tomatoes, the softer one will likely be richer in flavor. We expect a softer loaf of bread to be more flavorful than a firmer loaf because the staling process which makes it firmer also makes it less flavorful. A crisp, juicy apple will likely have more flavor than a soft, mealy one. A curdy, rubbery Cheddar cheese has a milder, greener flavor compared to the sharp nutty taste of a more waxy aged cheese.

Products where such associations can be made are generally natural or traditionally processed meats, cheese, fruits, etc., as opposed to fabricated foods. Natural or traditional foods are dynamic systems. Many reactions are occurring simultaneously and

are producing changes in both the structural and mechanical properties and the flavor. These reactions are commonly caused by spontaneous chemical changes, enzyme systems, and heating.

Now, I would like to look at some representative examples of such texture-flavor associations in a few different commodities.

Relationships between the structural and mechanical properties of meat and the flavor of meat are commonly found. Probably the best known are those occurring during aging. Aging meat produces a more tender, flavorful product, whereas unaged meat is relatively tough with a bland, metallic and astringent taste. Why is this increase in tenderness accompanied by an increase in flavor?

If we look at one of the changes occurring in a cut of meat as it ages, we find the cathepsins or proteolytic enzymes beginning to break up the myofibrils. This degradation increases the fragility or tenderness of the muscle because the aged fiber bundles break more easily when subjected to tensile or shear stress. If the myofibrils are not enzymatically degraded, they stretch more when stressed, producing a tougher muscle (4) (5).

The proteolytic breakdown also produces free amino acids. When meat is heated or cooked, these free amino acids may participate in the non-enzymatic browning reactions which produce the lean meat flavor (6). This is an example of an enzymatic process that produces both flavor and texture changes. The proteolytic breakdown of myofibrils contributes to both meat tenderness and flavor.

Browning reactions are also responsible for the formation of volatiles that give freshly baked bread much of its flavor. During staling, this flavor progressively disappears. But the most pronounced change that takes place during staling is an increase in firmness or hardness of the crumb. The extent of firming can be used as an approximate index of flavor loss or deterioration during staling. This is why people shopping for bread judge its freshness and flavor by squeezing the loaf.

Both firmness and flavor loss are time and temperature dependent processes. Furthermore, the basic molecular changes producing an increase in firmness are likely the same ones producing the change or apparent loss of flavor. Most of the increase in firmness during staling is attributed to changes in the starch fraction of the product. The starch in bread increases in crystallinity during aging. The exact nature of this crystallinity is not clear (7).

The loss of flavor during staling does not appear to be through volatilization or through chemical reactions (8). On reheating, when the process of starch retrogradation or crystallization is temporarily reversed, the flavor compounds are released. This suggests the flavor compounds are probably trapped within the crystalline regions of the starch molecules. When trapped, they are prevented from volatilizing or solubilizing and, therefore, can make no contribution to the taste or aroma of the

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product. The molecular process responsible for the increase in firmness during staling is also the mechanism for trapping and releasing flavor compounds.

Experienced cheese graders can usually guess the flavor of a cheese by observing the texture. The structure and mechanical properties of a cheese depend partly on the bacteriological and/or enzymatic treatment of the milk, the processing techniques, any added proteolytic cultures, and the aging process. These factors are also largely responsible for the flavor.

For example, a fresh, unripe Cheddar cheese has a rubbery, curdy texture and a bland flavor. This texture is due to the microstructure of casein micelle aggregates. As the cheese ages, the proteins are broken down and the rubberiness changes into a smooth, plastic texture. To a cheese grader this smooth, silky character indicates favorable flavor development. A dry texture in Cheddar cheese would be associated with a less desirable high acid flavor, and a pasty, sticky texture would indicate a fruity, fermented flavor (9). Calves rennet is currently the most widely used proteolytic enzyme in cheese making. The cheese industry has been engaged in finding a clotting agent to substitute for rennet. Problems arise when other proteolytic enzymes are used because the proteins break down in a different manner. The way proteolysis occurs appears to affect how the casein micelles attach to each other to form a gel or curd. The microstructure of cheese made with proteases other than rennet tends to be more open (10). This alteration in basic microstructure produces cheese with a less plastic structure. The proteolysis also determines the peptides and amino acids available. These not only contribute to the taste of the cheese but may undergo further enzymatic breakdown into other flavor compounds.

In fruits and vegetables, very important changes in both texture and flavor occur during ripening. The texture of most fruits becomes softer and less crisp as it matures. The flavor becomes sweeter and more intense. The biochemical reactions that produce these changes occur independently. Changes in flavor are due to an increase in the synthesis of sugars and an increase in the rate at which volatiles responsible for the aroma are synthesized. Changes in the texture are largely due to reactions taking place in the pectic substances of the middle lamella.

In green fruit, the pectic materials have a high molecular weight and are insoluble. They serve to cement the walls of adjacent cells together, thereby imparting considerable strength to the tissue. During ripening and senescence, enzymes in the plant hydrolyze and otherwise alter these pectic substances, making them more soluble and less effective as cement. As a result, the fruit becomes softer and eventually mushy. Heat also promotes the hydrolysis of pectic materials. This softening is readily seen when fruits or vegetables are cooked.

The strength or cementing power of the middle lamella has important implications for both the flavor and the texture of a

product. If the middle lamella is stronger than the cell wall, which is generally the case with green and slightly underripe fruit, the tissue will tend to fracture or break across the cell walls. If the cementing power of the pectic substances is weakened, whether through enzymatic degradation or heating, the tissue will tend to fracture between the cell walls (11).

From a sensory perspective, this change influences both texture and flavor. If the cells break across the cell walls the cell contents will run out, creating the sensation of juiciness, and also releasing the flavor compounds inside the cell.

The cells of most fresh fruits are turgid, meaning there is an intracellular pressure directed outward against the cell wall. If the product fractures across the cell wall, this turgor pressure is released resulting in the rapid expansion of the cell's contents. This sudden expansion produces the sounds that tell us the product is crisp.

If the product fractures between the cells, they are not broken open. The product appears less juicy, less flavorful, has a mushy or mealy texture, and little, if any, crispness. An example of such a system would be apples. A fresh, slightly underripe or just ripe, crisp apple breaks across the cell walls producing crisp, juicy, and flavorful sensations. During prolonged storage or senescence, the middle lamellar pectins lose their cementing power. The same apple, if stored for several months, would tend to lose its crispness and become mushy or mealy.

The preceding examples illustrate that the processes generating changes in flavor are not necessarily independent of those causing changes in texture. The structural and mechanical properties of foods are mainly due to large polymeric molecules. These molecules, e.g., starch, proteins, cellulose and pectins link together or interact with each other to form the basic structure of the food. Such molecules themselves do not have any inherent flavor properties. Molecules producing flavor sensations are much smaller and generally make no contribution to texture. When the structural molecules degrade or are broken down to smaller flavor-producing compounds, e.g., meat and cheese, or when changes in the molecular structure responsible for texture entrap or release flavor compounds, e.g., starch systems and fruits, the texture and flavor changes will take place concurrently.

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Relations between Sensory and Objective Measurements for Quality Evaluation of Green Beans

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For decades, substantial effort has been expended to develop sound objective methods for quality evaluation of foods. Considerable success has been achieved for color measurement; and for texture, moderate success. As was pointed out by Powers and Quinlan (1), part of this success has come about because some of the same forces or properties that cause us humans to respond to the food could be utilized in developing objective tests. Before the origin of gas-liquid chromatography (GLC) two decades ago, objective measurements of the numerous compounds that make up flavor was nigh impossible. Actually, not until a decade ago when Powers and Keith (2) and Dravnieks et al. (3) described practical means of analyzing GLC patterns could GLC measurements be efficiently correlated with flavor (4, 5). Quinlan et al. (6) and Powers (7) have reviewed most of the literature through early 1974. Recent papers are those of Galleto and Bednarczyk (8), Dravnieks et al. (9), Dravnieks (10), Gianturco et al. (11), Jobbágy and Holló (12), Severnants (13), and Powers (14). In spite of much progress, there are still major problems to be solved. Unlike color and texture, the relation between GLC peaks and flavor sensations is peripheral indeed. As Powers (7) pointed out, the properties that enable us to measure a substance chemically may often not be at all related to the properties that cause us to respond sensorially to that compound. In fact, in most cases, we don't know exactly the properties that do make us respond to a compound.

Approximately two years ago, we turned to the sensory side to learn if better correlations could be obtained if one dealt with specific flavor or taste descriptions rather than the composite term, flavor. In the intervening years since GLC analysis became practical as a tool for flavor evaluation, others (9, 10, 12, 15, 16, 17, 18, 19, 20) have also endeavored to relate specific odor responses to GLC patterns.

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The problems in trying to develop better objective methods for flavor are: (A) We need to learn the properties which make us respond sensorially to taste or odor substances; (B) We probably could use objective/sensory means more efficiently, even though we are a long way from attaining the first objective, if we could relate specific terms on both sides of the objective-sensory equation rather than doing as we generally do now, relate very specific entities (GLC peaks, for example) to a very broad term, flavor; and (C) No one of the sense modalities operates in a vacuum. Each one is affected by the others. We have to learn more about interrelations among the senses, especially as to sensations which encompass more than one sense modality. We have thus turned to the more general field of trying to relate nuances of sensory response to specific objective measurements. Bargmann et al. (21) applied component analysis to descriptors for blueberry attributes and quality, and Wu et al. (22) have recently carried on a factor analysis of wine descriptors to investigate an objective means of developing a suitable terminology for taste and odor sensations. In our laboratory, Hightower (23) has applied a minimax approach (24) to component analysis of potato chip flavors.

This study is a part of a continuing effort to broaden our base of knowledge and methodology so that objective/sensory measurements can be put upon an even firmer foundation.

Experimental

A preliminary trial was carried on in mid-1975 for the purpose of setting up a vocabulary of descriptor terms for appearance, color, mouthfeel, and flavor of canned and frozen beans. Frozen or canned beans were heated for serving and then sampled by approximately 40 individuals who were asked to write down every sensory response they thought pertinent. This list of 53 descriptors was later edited to 27 terms thought to be pertinent and not redundant. Sensory evaluation trials were then made using three commercial brands of canned beans and three of frozen beans, selected from a much larger group of brands to be sure that the brands actually used differed at least moderately in quality. The purpose of this phase was to determine whether the terms which the large group of panelists said were pertinent were in fact actually used and of aid in discriminating among the different lots of beans.

A common problem in acceptability trials is to be able to explain the results. A panelist may rate two products as being equally acceptable; yet, say the products are different, simply because one attribute makes one product desirable whereas a different attribute makes the other product equally acceptable. This, of course, is the reason for trying to go behind the general terms, flavor or mouthfeel, for example, to seek out the specific sensory qualities which make the food desirable.

First sensory trial. The three frozen and canned products were evaluated similarly to the procedure described by Vuataz et al. (25) and Wu et al. (22). The panelists evaluated the products hedonically for acceptability, appearance, color, mouthfeel, and flavor. Hereafter, these five factors will be referred to as "general descriptors" to save having to list them each time. The hedonic terms for the general descriptors were later transposed to a 9-point scale. The panelists were also asked to rate the degree to which each product was stronger or weaker as compared with a reference sample in a particular attribute, using the 27 descriptors chosen from the original list of 53 specific descriptors. One of the brands of canned beans was the reference sample. The same product was also included among the test products as a coded sample to provide a check upon the panelists. The panelists were thus asked to (A) evaluate the quality of the beans, using hedonic terms for the five general descriptors, (B) compare each test product against the reference product for each of the 27 specific descriptors, and then (C) re-evaluate the products, using the five general descriptors. There were 21 panelists and each product was evaluated four times. Before the trials started, a table of random numbers had been used to assign each product to a session so that eventually each product was examined four times. At any one session, the panelists evaluated four samples plus the reference. Sampling was always between 10:30 to 11:30 AM.

Statistical analysis. The sensory data were subjected to analysis of variance to eliminate ineffectual judges or descriptors. By univariate analysis (MUDAID program) (26), the treatment/error F values were computed. Judges or descriptors, not statistically significant, were dropped from further consideration. This procedure is routine in our laboratory (1, 6, 7, 14, 21, 22, 23). The edited results were then analyzed by factor analysis to detect descriptors of major importance and to effect further editing of the descriptor list. The minimax program of Bargmann and Baker (24) was used for this purpose, coupled with the factor analysis by stepwise maximum likelihood solution and rotated (oblique rotation) by Thurstone's Analytical Method of Rotation (Harman) (27).

Analysis of variance and Duncan's multiple range tests were used to test for significant differences between the products, both for the general and 27 specific descriptors.

Main trials. The main experiment consisted of evaluating eight lots of beans, much as above. The trials were carried on in early summer 1976. There were 27 panelists, very few of whom were on the 1975 panel; there were 20 specific descriptors (7 having been dropped as a result of the 1975 trial), and again the trials were replicated four times. The beans consisted of four brands of canned beans, three of frozen beans, and fresh beans.

The fresh beans were used for the reference sample; as before, they were also included as a coded sample. The panelists were asked to evaluate five or six products at each session. The products had previously been assigned to a particular session, using, as before, a table of random numbers. The judges evaluated the beans hedonically for the five general descriptors, rated them against the reference sample for the 20 specific descriptors, then re-evaluated the beans again for general attributes. The fresh beans were cooked by simmering them for 25 minutes, an equal weight of beans with an equal weight of 1% saline solution. The frozen product was prepared by cooking 510 g of beans in 700 ml of 1% saline solution for 15 minutes. The canned beans were heated for 15 minutes in their own packing liquor.

For each product, all samples for sensory and objective tests were withdrawn from the same cooking vessel at the same time. In other words, the beans withdrawn for objective testing were cooked exactly the same as the beans used for the sensory trial. The organoleptic tests were thus strictly comparable to the objective tests in terms of prior treatment.

The panelists were given 15-20 g of sample in a translucent plastic souffle cup. The cups were color coded, a given color was not assigned to the same position, but randomly changed from session to session. Sensory evaluation was done either at 9:30 to 10:30 AM or 3:30 to 4:30 PM.

At the end of the taste-testing sessions, univariate analysis was used, as described above, to eliminate panelists or descriptors not statistically significant as judged by the treatment/error F value.

GLC procedure. The GLC procedure was essentially the same as described in earlier publications (28, 29). Fifty grams of beans were placed in a 2 liter flask with 1 liter of water for the combined steam-distillation-solvent-extraction procedure of Likens and Nickerson (30). Diethyl ether was the solvent. After extraction, dissolved and emulsified water was frozen out at -28°C . The extract was decanted from the ice crystals into a Kuderna-Danish assembly and the volume reduced to 0.5 ml.

For GLC analysis, a single column chromatograph with a 3.66 m, 6.4mm stainless steel column packed with 5% SP-1000 on Chromosorb W-HP, AW, DMCS, 60/80 mesh, was used. Programming was at $6.4^{\circ}\text{C}/\text{min}$ from 25 to 200°C . A 5 μl sample was injected.

Inspection of the chromatograms showed 45 distinct peaks. Several other peaks were also discernible, but only those which were well resolved were used. The 45 peaks were converted to percent area (4).

Liquid-solid chromatography. Non-volatile and pigment components were analyzed for by a LC method. The products were prepared for analysis by extracting 50 g of beans with 100 ml of an extraction solvent composed of acetone, chloroform, and

hexane (1/1/1). The beans and the extracting solvent were placed in a high speed blender for 5 min and then separated by two filtration steps through No. 4 and No. 42 filter paper, respectively. The extract was evaporated at reduced pressure to a dry residue in a rotary evaporator partially submerged in a water bath at 50-60°C. The residue was then re-extracted with 25 ml of hexane. Drying had a two-fold objective; first, to remove volatile compounds which were already being analyzed for by GLC analysis and, secondly, to remove the polar solvents, especially water. The polar solvents and the highly-polar water had to be removed prior to the LC analysis. The components are carried by the injected sample initially, but when separation occurs along the column, partitioning between the stationary phase and the carrier produces such a high affinity for the stationary phase that polar components become non-mobile bands on the column.

For LC analysis, a Waters Associates LSC system composed of a Model UGK, Universal Liquid Chromatography Injector and a Model 6000A solvent delivery system with a model 440 absorbance detector was used. The flow rate was 1.0 ml/min with detection at 365 nm. The sample size was 10 µl. The solvent, an adapted version of Pons' (31) solvent, was 750 ml chloroform, 225 ml cyclohexane, 3 ml acetonitrile. and 2 ml 2-propanol.

Inspection of the chromatograms yielded 11 peaks. These peaks were compared as absolute absorbance values.

Color. The products were analyzed spectrophotometrically over the visible region of the spectrum. The samples used for this analysis were aliquots taken from the hexane-extraction phase of the LC procedure described above. This procedure was quite efficient for the extraction of plant pigments.

A Shimadzu Multipurpose Recording Spectrophotometer, Model MPS-50L, equipped with 1 cm cells was used. All measurements were made in the 0-1 absorbance range with necessary dilutions to provide on-scale readings. (Just before the trials started, the instrument went slightly out of adjustment so that switching from the 0-1 to the 1-2 range did not result in absolute coincidence; rather than delay the trials, the dilution method was used with backward calculation of the values to provide a continuous spectrum.) After analysis, absorbance at 16 different wavelength was selected for comparisons.

Mechanical measurements. Factor analysis of the preliminary trial showed that there was a strong factor consisting of "coarse, fibrous, crisp, juicy, slimy, soggy, and tender." Thought was turned to utilizing some mechanical test which might correspond with this sensory factor. It was therefore decided to measure the coefficient of friction between a moveable plate (32) (sled) and the outer surface of the beans. The beans were oriented in two positions: one was with the beans paralalled to the force

and the second was with the beans perpendicular to the direction of pull. The beans were tightly fitted into an area on the stationary plate of an Instron, Model 1130, apparatus equipped with a 1 lb. cell. The static and dynamic surfaces were covered with aluminum foil. The sled was then pulled on top of the beans until the force became constant. The plate had a surface area of 39.69 cm². Its dimensions were 6.3 x 6.3 x 1.2 cm and it weighed 169.5 g. The force was calculated as Newtons/cm².

Tensile strength. The tensile force required to pull beans apart longitudinally was also measured. The beans were clamped at each end between the jaws of two fixtures. The ends of the beans were wrapped once with cheesecloth so that the clamps could "bite" into the beans slightly; otherwise, the beans tended to slip from between the jaws or else the clamping force caused them to break first where they were clamped instead of intermediate between the two sets of jaws. The crosshead speed was 2 in/min. The tensile strength was taken as the maximum breaking-point force divided by the cross-sectional area of the bean. As before, the tensile strength was ultimately expressed as Newtons/cm².

Shear force. The force required to cut the beans crosswise while in a Warner-Bratzler type knife assembly was also measured. The crosshead speed was 2 in/min. The shear force was calculated from the force resulting in failure of the bean divided by the cross-sectional area of the bean. The values were recorded as Newtons/cm².

Cluster analysis. The procedure of Trivedi (33) was used to carry on "virtual" cluster analysis. Bargmann and Grailey (34) and Trivedi (33) have defined "virtual." Basically, the relation may be likened to the cluster of stars one sees when one gazes at the Pleiades against the vault of the heavens and the actual positions of the stars. If one were in the midst of the Pleiades, the stars would not appear to be clustered at all. The cluster is illusory. We see their positions in three dimensions projected against the surface of the celestial sphere in two dimensions. Similarly, it is well known that random variables can be regarded as spectors embedded in the Euclidian space of infinite dimensions. If the spherical cap which results from the projection of the cluster is compared with the entire surface area, one then has a measure of the density of the cluster. The computer program forms a cluster when the surface area of a spherical cap in k dimension extending to the farthest point inside the cluster is compared with the surface area of the k dimension when one additional point or attribute is added to it. When this ratio suffers a severe drop, a cluster core is considered terminated. Those attributes that have once been included in a cluster core are not used again to form another cluster core. New clusters are formed

from the residual attributes of prior-formed clusters.

In effect, the vectors are projected onto a unisphere where the points on the unisphere are connected by great circles. The cosine of each of the great circles represents the correlation between each pair of random variables. The representation of correlations as cosines of angles between vectors was first introduced by Karl Pearson (35) in 1901.

To organize the data for cluster analysis, the 27 sensory values that existed for each product and replication had to be reduced to one experimental unit to correspond with the one unit which existed for each of the objective measurements. The general descriptors were weighted by the following formula

$$w = \frac{1}{1 + |x_k - x_u|}$$

where x_k was the score the judge gave knowingly to the reference sample and x_u was the score he gave when he did not know he was judging the reference product. If x_k equals x_u , the weight attached to this judgment on this particular attribute will be one. If he makes a rather extreme misjudgment, the difference x_k minus x_u could be, say, 4 points. In this case, his weight would be only 1/5 of that attached to a judge whose rating of the reference product was consistent regardless of whether he evaluated it knowingly or unknowingly.

For the 20 specific descriptors, the weighting attributed to each judge was somewhat different.

$$w = \frac{1}{1 + |x_u - 5|}$$

In this formula, x_u is the score the judge gave to the coded reference sample. Since he was comparing it against the known reference sample and the "no difference" score was 5, he should have assigned a 5. If he assigned any other score, he was being inconsistent and the weight of his score was decreased accordingly. By weighting the general and specific descriptor scores in the fashion above, we then had 32 experimental units (8 products x 4 replications) on which we had all the objective determinations and the composite judgment on each of the 30 descriptors. A correlation matrix was then formed between the 58 variables on the basis of the 32 experimental units. The 28 objective attributes included 17 GLC peaks, 3 spectral ratios, 4 LC peaks, and 4 mechanical measurements.

Results

Factor analysis. Editing of the 27 descriptors used in the 1975 preliminary trials resulted in seven being dropped because

they either were not used or they did not discriminate among the products. Factor analysis yielded the eight factors listed below:

<u>Factor 1</u>		<u>Factor 4</u>		<u>Factor 7</u>	
Off-flavor	0.52	Coarse	0.57	Soggy	0.50
Persistent	0.42	Fibrous	0.57	Slimy	0.47
aftertaste		Crisp	0.53	Juicy	0.43
Bland flavor	0.31	Slimy	-0.53	Buttery	0.41
Pleasant	-0.44	Juicy	-0.55	Tender	0.34
aftertaste		Soggy	-0.63		
		Tender	-0.66	<u>Factor 8</u>	
<u>Factor 2</u>		<u>Factor 5</u>		Color-1	0.55
Color-1	0.69	Off-flavor	0.65	Appearance-1	0.51
Color-2	0.64	Persistent	0.55	Appearance-2	0.49
Appearance-1	0.61	aftertaste		Color-2	0.49
Appearance-2	0.58	Hay-like	0.42	Coarseness	0.36
Bright color	0.33	taste		Fibrous	0.36
Pale color	-0.38	Coarse	0.41	Crisp	0.33
Color off-	-0.43	Fibrous	0.38	Bright color	0.31
shade		Bland	0.36	Juicy	-0.32
		Juicy	0.29	Slimy	-0.34
<u>Factor 3</u>				Pale color	-0.39
Fibrous	0.47	<u>Factor 6</u>		Color off-	-0.46
Coarse	0.46	Coarse	0.50	shade	
Buttery	0.34	Fibrous	0.49	Tender	-0.50
		Off-flavor	0.44		
		Persistent	0.43		
		aftertaste			
		Hay-like	0.33		
		taste			
		Slimy	0.31		

Comment will be reserved until we come to the cluster analysis of the main experiment because the factor analysis and the cluster analysis corroborated each other, except to point out some of the major observations. Factor 7, for example, relates essentially to mouthfeel except the flavor note of "buttery" was a part of that factor. The same thing may be seen for factor 3; buttery again accompanies the mouthfeel sensations. Factor 4 shows the two sets of terms combined except buttery is not sufficiently well correlated to come within the factor.

Sensory contributions to acceptability. One of the problems in devising objective tests to substitute for or to complement sensory evaluations is to know how to weight the objective test so that it will contribute in the same manner to acceptability as does each sense modality. Listed below are the simple and

multiple correlation coefficients for the 1975 and 1976 experiments.

<u>Multiple correlations</u>	<u>1975 Trials</u>	<u>1976 Trials</u>
Acceptability vs. flavor, mouthfeel, appearance & color	0.917	0.917
Acceptability vs. flavor, mouthfeel & appearance	0.915	0.916
Acceptability vs. flavor and mouthfeel	0.906	0.912
<u>Simple correlations</u>		
Acceptability vs. flavor	0.884	0.891
Acceptability vs. mouthfeel	0.801	0.794
Acceptability vs. appearance	0.626	0.607
Acceptability vs. color	0.597	0.555

It is quite obvious that flavor and mouthfeel essentially determine acceptability and that appearance and color are of lesser importance. This does not mean that appearance and color are unimportant. Rather, within the commercial range, remaining variations in color or appearance are apparently of lesser importance in determining acceptability than the variations that occur in textural and flavor qualities.

Emotional vs. analytical judgments. One of the aspects we have been interested in is the effect of analytical thought such as has to go into the rating of each descriptor relative to the reference sample versus the rather low-key thought or emotion involved in rating foods hedonically. Should the samples be judged for general characteristics prior to or after the analytical phase? They cannot be judged apart without a great deal of extra replication to overcome the variation resulting from using different lots and cooking batches. Immediately below are the correlation coefficients between corresponding general descriptors evaluated before and after the analytical comparison phase.

<u>Factor</u>		<u>1975 Experiment</u>	<u>1976 Experiment</u>
Acceptability	1 vs. 2	0.841	0.863
Appearance	1 vs. 2	0.833	0.837
Color	1 vs. 2	0.849	0.850
Mouthfeel	1 vs. 2	0.798	0.847
Flavor	1 vs. 2	0.859	0.834

There was some evidence that after analytical thought the panelists rated the general qualities of the beans somewhat differently than they had when they were merely expressing liking-disliking without a lot of thought as to why.

Cluster analysis. The three strongest clusters included

factors for texture, flavor, and appearance. They are listed below:

<u>Cluster 1--Texture</u>	<u>Cluster 2--Flavor</u>	<u>Cluster 3--Appearance</u>
Coarse	Flavor-1	Color-1
Fibrous	Flavor-2	Color-2
Tender	Acceptability-1	Appearance-1
Juicy	Acceptability-2	Appearance-2
Crisp	Mouthfeel-2	
Buttery	Mouthfeel-1	
Hay-like flavor	Pleasant aftertaste	
Bright color	Off-flavor	
Slimy	Sweet	
Soggy		

The program was set to place terms in the core (form a cluster) if the correlation coefficient was above 0.70. Terms not used then went into a residual group of factors, from which a secondary core set could be extracted and in turn a third core and subsequent cores, denominated "virtual clusters." Actually, 17 different clusters were generated. The first three clusters included only sensory factors; the later clusters consisted predominantly of objective measurements. The 58 x 58 correlation matrix is too massive to reproduce. From Tables I, II, and III, one can observe some of the sensory-objective correlations. As was true for the factor analysis, attributes which we tend to think of as being texture, flavor, or appearance factors are so well correlated with each other that they sometimes appear in a different cluster than one would expect. Note that "buttery," "hay-like flavor," and "bright color" show up in a cluster otherwise relating to texture. To illustrate the numerous sensory-objective correlations that did appear in the 58 x 58 matrix, three lists are given as follows:

Correlation of absorbance ratio 525/610 nm with

Color-1	-0.67	Green veg. taste	-0.87	Crisp	-0.81
Color-2	-0.64	Buttery flavor	0.76	Coarse	-0.75
Color, off-shade	0.69	Hay-like taste	-0.65	Juicy	0.70
Bright color	-0.77	Process flavor	0.71	Slimy	0.64
GC 24	0.64			Soggy	0.71
GC 32	0.67			Fibrous	-0.75
Ratio 467/525	-0.74			Tender	0.78
Ratio 525/665	0.66				

Table I
Cluster No. 1

	Fibrous	Tender	Juicy	Crisp	Buttery	Hay-like taste	Green Veg. taste	Bright Color	Slimy	Soggy
Coarse	.97	-.96	-.95	.94	-.90	.91	.90	.82	-.87	-.87
Fibrous		-.97	-.92	.92	-.94	.93	.91	.87	-.86	-.84
Tender			.95	-.93	.94	-.94	-.90	-.87	.84	.82
Juicy				-.91	.89	-.87	-.83	-.74	.88	.88
Crisp					-.88	.89	.92	.83	-.81	-.88
Buttery						-.92	-.86	-.82	.77	.72
Hay-like taste							.84	.82	-.76	-.71
Green veg. taste								.91	-.78	-.82
Bright color									-.65	-.67
Slimy										.91
Soggy										
Process flavor										
Color, off shade										
525/610										
Shear force										
Sweet										
Flavor										
Mouthfeel										
Acceptability										
Pleasant aftertaste										
Tensile strength										
LC09										
Off-flavor										
GC-24										
GC-32										
Color										
Pale color										
525/665										

Table I (continued) Cluster No. 1

	Process flavor	Color, off-shade	525/610	Shear force	Sweet	Flavor	Mouth- feel	Accept- ability	Pleasant aftertaste
Coarse	-.77	-.74	-.75	.77	-.74	-.70	-.64	-.64	-.60
Fibrous	-.79	-.78	-.75	.79	-.79	-.74	-.65	-.65	-.64
Tender	.80	.81	.78	-.76	.76	.76	.68	.65	.65
Juicy	.75	.77	.70	-.71	.67	.67	.62	.59	.56
Crisp	-.79	-.73	-.81	.68	-.61	-.61	-.55	-.51	-.56
Buttery	.67	.71	.76	-.76	.84	.84	.73	.74	.78
Hay-like taste	-.67	-.71	-.65	.78	-.82	-.85	-.73	-.77	-.74
Green Veg. taste	-.83	-.75	-.87	.68	-.60	-.67	-.60	-.59	-.57
Bright color	-.77	-.76	-.77	.75	-.61	-.71	-.60	-.59	-.60
Slimy	.71	.75	.64	-.58	.58	.50	.39	.42	.39
Soggy	.75	.70	.71	-.53	.49	.43	.37	.36	.35
Process flavor		.87	.71	-.58	.44	.40	.38	.31	.24
Color, off shade			.69	-.62	.46	.48	.36	.31	.30
525/610				-.57	.43	.50	.51	.42	.46
Shear force					-.73	-.62	-.55	-.57	-.49
Sweet						.76	.65	.69	.74
Flavor							.88	.95	.84
Mouthfeel								.90	.74
Acceptability									.82
Pleasant aftertaste									
Tensile strength									
LC09									
Off-flavor									
GC-24									
GC-32									
Color									
Pale color									
525/664									

Table I (continued) Cluster No. 1

	Tensile strength	LC09	Off-flavor	GC-24	GC-32	Color	Pale color	525/665	GC-20
Coarse	.68	.61	.54	-.55	-.58	.57	-.54	-.52	.37
Fibrous	.70	.63	.55	-.54	-.54	.60	-.57	-.53	.45
Tender	-.66	-.72	-.57	.62	.62	-.65	.53	.51	-.43
Juicy	-.71	-.61	-.49	.60	.61	-.56	.47	.49	-.26
Crisp	.58	.57	.50	-.54	-.54	.60	-.50	-.51	.29
Buttery	-.64	-.67	-.67	.57	.52	-.50	.44	.55	-.50
Hay-like taste	.66	.64	.67	-.44	-.48	.55	-.52	-.39	.54
Green Veg. taste	.52	.63	.50	-.55	-.60	.63	-.57	-.56	.48
Bright color	.51	.69	.48	-.57	-.48	.67	-.60	-.43	.64
Slimy	-.58	-.56	-.33	.53	.61	-.55	.59	.50	-.26
Soggy	-.54	-.45	-.31	.47	.59	-.53	.55	.50	-.16
Process flavor	-.47	-.55	-.10	.52	.55	-.74	.56	.48	-.19
Color, off shade	-.45	-.67	-.17	.60	.58	-.82	.53	.45	-.29
525/607	-.37	-.63	-.41	.64	.67	-.67	.40	.66	-.35
Shear force	.71	.47	.35	-.44	-.33	.53	-.55	-.44	.53
Sweet	-.67	-.46	-.63	.37	.32	-.28	.37	.33	-.46
Flavor	-.51	-.56	-.78	.38	.34	-.24	.31	.34	-.60
Mouthfeel	-.56	-.39	-.65	.41	.35	-.17	.08	.29	-.37
Acceptability	-.47	-.43	-.76	.28	.32	-.06	.27	.30	-.54
Pleasant aftertaste	-.31	-.48	-.90	.33	.32	-.06	.19	.24	-.62
Tensile strength		.33	.26	-.34	-.25	.40	-.33	-.43	.22
LC09			.48	-.55	-.57	.65	-.42	-.40	.56
Off-flavor				-.23	-.30	.01	-.17	-.25	.60
GC-24					.74	-.51	.25	.27	-.24
GC-32						-.49	.49	.42	-.14
Color						-.50		-.31	-.22
Pale color								.32	-.44
525/665									-.24

Table III
Cluster No. 3

	Appearance	Color, off shade	Process Flavor	525/610	LC 09	GC 24	GC 32	Shear force	Pale color	GC 37	GC 47
Color	.83	-.82	-.74	-.67	.65	-.51	-.49	.53	-.50	.38	.52
Appearance		-.52	-.51	-.48	.43	-.39	-.31	.18	-.21	.27	.42
Color, off shade			.87	.69	-.67	.60	.58	-.62	.53	-.50	-.42
Process flavor				.71	-.55	.52	.55	-.58	.56	-.58	-.48
525/610					-.63	.64	.67	-.57	.40	-.40	-.41
LC 09						-.55	-.57	.47	-.42	.49	.32
GC-24							.74	-.44	.25	-.38	-.30
GC-32								-.33	.49	-.37	-.07
Shear force									-.55	.34	.47
Pale color										-.33	-.27
GC-37											.34

Table II
Cluster No. 2.

	Ac- cept- abil- ity	Mouth- feel	Pleas- ant after- taste	Off fla- vor	Sweet	Shear force	GC-20	Bland	Tensile strength	GC-33	Uni- form	LC09	LC02	525/ 610
Flavor	.95	.88	.84	-.78	.76	-.62	-.60	-.61	-.51	-.60	.50	-.56	-.50	.50
Acceptability		.90	.82	-.76	.69	-.57	-.54	-.47	-.47	-.59	.43	-.43	.44	.42
Mouthfeel			.74	-.65	.65	-.54	-.37	-.57	-.56	-.46	.36	-.39	.55	.51
Pleasant aftertaste				-.90	.74	-.49	-.62	-.46	-.31	-.51	.33	-.48	.42	.46
Off-flavor					-.63	.35	.60	.43	.26	.54	-.30	.48	-.38	-.41
Sweet						-.73	-.46	-.69	-.67	-.32	.64	-.46	.45	.43
Shear force							.53	.42	.71	.22	-.44	.47	-.18	-.57
GC-20								.25	.22	.63	-.32	.56	-.20	-.35
Bland									.54	.39	-.72	.30	-.58	-.22
Tensile strength										.25	-.42	.33	-.42	-.37
GC-33											-.30	.27	-.41	-.12
Uniform												-.25	.24	.04
LC09													-.41	-.63
LC02														.26

Correlations of shear force with

Soggy	0.79	Hay-like flavor	0.78	Bright color	0.75
Coarse	0.77	Green veg. taste	0.68	Pale color	-0.55
Crisp	0.68	Acceptability-1	-0.57	Ratio 525/610	-0.57
Mouthfeel-1	-0.55	Process taste	-0.58	Color, off-shade	-0.62
Slimy	-0.58	Flavor-1	-0.62		
Juicy	-0.71	Sweet	-0.73		
Tender	-0.76	Buttery flavor	-0.76		

Correlations with LC Peak No. 9

Color-1	0.65	Buttery	-0.67	Coarse	0.61
Color, off-shade	-0.67	Green veg. taste	0.63	Juicy	-0.61
Bright color	0.69	Hay-like taste	0.64	Fibrous	0.63
525/610 nm ratio	-0.63			Tender	-0.72

Discussion

Some of the observations and relations have been commented on above. One of the striking things with regard to the GLC measurements was that most of the GLC peaks were negatively related to flavor indicating that the compounds being measured were detrimental to flavor. Some of the relations with regard to a bright color were also noteworthy. The correlations are listed below:

Correlations of bright color with

Color-1	0.67	Acceptability-1	-0.59	Slimy	-0.65
Green veg. taste	0.91	Flavor-1	-0.71	Soggy	-0.67
		Sweet	-0.61	Tender	-0.87
Hay-like flavor	0.82	Buttery	-0.82	Pale color	-0.60
		Process flavor	-0.77	Color, off-shade	-0.76
Crisp	0.83	Pleasant after-	-0.60		
Coarse	0.82	taste			
Fibrous	0.87	Juicy	-0.74		
GC 20	0.64				

The correlations indicate the risks involved if one confines oneself to one sense modality (as many of us have done in the past). The interrelations between the sense modalities is so strong that preferably all should be evaluated at the same time, especially if one hopes to make informed judgments as to the objective tests which will nearly reflect overall acceptability.

Within the past few years, increased emphasis has been placed on relating descriptor terms to quality or specific chemicals (9-13, 15-23, 36-48). The procedures depend on articulating a list of descriptors based upon "sniffing" or sampling the food with considerable deep and mature thought as to the sensations being perceived. Objective means of evaluating the

descriptors arrived at should be used to seek out main discriminators (49, 50, 51) because our subjective impressions do not always coincide with the way we actually use words (45), as demonstrated by Lehrer (45), Wu et al. (22) and in this study.

The ultimate goal, of course, is to be able to develop a general equation suitable for predicting the quality of the food from objective measurements or a mixture of sensory-objective terms. There are several studies where covariance or multi-regression procedures have been used (13, 52, 53, 54) to establish relations between response to one or a few sense modalities and objective measurements. This study shows that all of the senses should be taken into account because one component often influences one's response to the food in ways not suspected (or at least fully understood) unless the data are evaluated objectively.

The road to a general predicting equation for quality for any given commodity will be long and tortuous. Cluster analysis is but one of the methods applicable in moving along that road. It permits relations among components to be established. Component analysis should have value, too, in demonstrating the strength of the association between particular objective measurements and sensory components. From these analyses, one should be able to reduce the number of variables which have to be tested in the future. Sometimes a test can be eliminated because another test measures the same thing or else the test is of so little value it should be eliminated.

Once one has settled upon appropriate and precise tests, then decisions will have to be made as to how the objective tests are to be weighed so as to make the prediction match that which would have been said about the acceptability of the food if it had been evaluated sensorially for all of the major sense factors. Multiple and simple correlations calculated between the different sense modalities and acceptability, as we did in this study and in prior studies (1, 6, 7, 14), provides information helpful for that phase of the task. To calculate these correlations is quite simple.

As was pointed out in the beginning, we will be handicapped as long as we have to use words to describe sensations, because descriptors are so subjective, rather than being able to measure the particular property that generates the sensation in the first place. The day when we will be able to measure properties instead of having to use words for most sense responses is a long way off. In the meantime, we can be objective in determining the relations among descriptors for sensations by such methods as factor analysis, which procedure should permit us to pick those descriptors most appropriate and pertinent. In turn cluster analysis, component and multi-regression techniques should enable us to quantify the relations between sensory and objective measurements.

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Measurement of Flavor Quality in Apples, Apple Juices, and Fermented Ciders

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Quality may be understood as merely a measure of desirability in a product and, as such, must be closely related to consumer acceptance, something which is important to any producer, whether of fruit products or foods in general, because without it he will soon be out of business. The consumer, whether he buys directly or has his opinion reflected in the eyes of a buyer for a processing industry, is therefore the final key to what constitutes quality. One function of the scientist interested in flavour is to translate this consumer opinion into tangible information, such as the chemical and physical composition of the product, so that eventually he has a better understanding of what the consumer considers as constituting quality and can then devise logical scientific methods for its improvement.

When considering the produce of the apple industry, although it is the sensory responses evoked in the consumer and the pleasure and satisfaction they give, that have the greatest influence on quality, other factors are also important. The keeping quality of the fruit or beverage, its nutritional value, the variety of uses to which it can be put in the home, the ease and convenience of eating and, of course, the price, are some of the more tangible attributes which are also important to the consumer. The fact that man made fertilisers and insecticides may have been used during the growing of the fruit, the image created by the varietal name, advertising and preconceived ideas of the product also have subtle effects on the acceptability of a fruit. Such phrases as 'an apple a day keeps the doctor away' have little scientific backing but may well subconsciously influence the housewife when she shops for apples and apple products.

Other factors besides the sensory quality of the fruit are also important to the apple producer and processor. Crop yields, resistance of the tree to disease, length and uniformity of harvest, ability to transport and storage potential play a major role in the growers' choice of orchard practices and selection of varieties. For apples destined for juice and cider production,

ability to readily produce a high yield of juice is another factor in defining quality. During the last twenty years it is such considerations as these which have almost entirely governed the type of fruit produced. This has been particularly so in the case of eating apples, where the only tangible criteria being imposed by the retailer appear to be those of colour, size, shape and lack of disorders. When traditional methods were being used to grow and market fruit such criteria possibly proved quite adequate. However, as a result of modern technology, greater yields of apples and a longer storage life are now possible and criteria, such as flavour, which are not directly assessed, are beginning to suffer.

In the apple juice and cider industries increased cleanliness, the use of ascorbic acid, sulphur dioxide and pure yeast cultures have given the manufacturers more control over their products. This has meant that the occurrence of completely unacceptable beverages is becoming less frequent and, here again, the manufacturer is becoming more concerned with maintaining those flavour aspects of his product which the customer considers to be desirable.

Measurement of Flavour Quality

Several attempts have been made to formulate sensory, physical and analytical criteria by which quality of both apples and their products can be assessed and compared. Sensory assessments are mainly of the type in which general attributes such as clarity, aroma and flavour are scored and combined, with either the results of simple chemical analyses, or estimates of commercial value to give an indication of quality (1,2). Over recent years, however, a number of more objective approaches have been developed in connection with specific research programmes (3,4,5,6), but, apart from the vocabulary used by Van Sydow *et al.* (5) and Moskowitz and Van Sydow (6) to describe apple juices, these are still of a rather general nature.

From the point of view of physical measurements relationships are claimed to exist between both the colour (7,8,9) and light transmittance (10,11) of apples and their eating quality and use has been made of these in developing instruments for grading purposes (11,12,13,14). Analytically, measurements of the sugar and acid contents have been used for estimating quality of apples (4,15,16) and juices (2,17), although in the case of the latter it is claimed that the polyphenol content should also fall within specific limits (17). In England it has long been the custom to classify ciders, cider apples and their juices on their acid and polyphenol content as a means of indicating their flavour properties and suitability for cider making.

Whether it is an apple, juice or cider, simple, analytical and physical measurements of this nature are only very crude indicators of quality, giving very little indication of the true

flavour effects of individual compounds. For example, scientists are unable to tell from the results of permanganate titrations whether the polyphenols are contributing to bitterness or astringency or whether they are 'soapy' or 'hard'. Also, except by association, such measurements do not take into account the more subtle effects of the volatile components on aroma and flavour, the interaction of both volatile and non-volatile flavour components and the effect of fruit structure on the release of the flavour imparting compounds.

Modern analytical techniques have, of course, been applied to most aspects of apple products. For example, gas chromatography has enabled over 250 volatile components (18,19,20) to be identified and other chromatographic techniques have given information on the non-volatiles. Some of these obviously contribute more to the flavour than others (21,22), but until one can state which are the important compounds and characteristics, such information cannot be used as a guide to quality.

As stated earlier, the customer is the final key to understanding quality. However, although the average consumer, when presented with a number of products, can readily tell you which he prefers, he is often much less precise in saying why he likes the one he has chosen. In evaluating flavour quality it was therefore considered unwise to try, either to get too much information from the consumer or to interpret his preference information directly, particularly in terms of analytical data. The approach adopted therefore was to use a trained panel and well defined terminology to assess objectively the characteristics present in the product, thus obtaining a detailed measure both qualitatively and quantitatively, of the various sensory characteristics contributing to the overall appreciation of the fruit, juice or cider. Correlating this information on the one hand with the consumer's assessment or preference ratings would give an indication of the relative importance of the characteristics for acceptance and quality, whilst comparing it with the chemical and physical data would enable the analytical parameters which give rise to these various attributes to be determined.

This three directional approach involving preference ratings, objective sensory assessment and analytical data has formed the basis of our thinking with regard to quality and flavour evaluation over the past two years and two examples illustrating aspects of this approach, one involving the aroma components of apples and another the phenolics of fermented cider will now be discussed.

Apples

From the point of view of eating quality, the apple cultivar Cox's Orange Pippin, is considered by the majority of

English growers, retailers and consumers to be amongst the most desirable of apples, particularly when considering cultivars which can be kept for any length of time after harvesting. Preference ratings, comparing it with three other varieties, Red Delicious, Spartan and Idared (Table I), even though taken in April when the Cox was nearing the end of its storage life, serve to illustrate the overwhelming superiority of this cultivar. For economical reasons it is becoming desirable to

TABLE I
Mean ranking of four varieties of apples

Cultivar	External aroma* (total score)	Overall* (total score)
Cox's Orange Pippin	2	1.25
Spartan	2.6	3
Idared	2.3	2.5
Red Delicious	2.6	2.9

* 1 = most preferred 4 = least preferred

store Cox for long periods before marketing. Although these late stored Cox apples are free of disorders and have excellent texture and appearance, their flavour is not attractive and the consumer is beginning to complain. The industry is naturally worried because it believes that much of the sales of this cultivar depends on flavour.

The three directional approach described is being adopted to try and discover what is so desirable in the Cox apple and how the important attributes from the sensory and analytical point of view change both during Controlled Atmosphere storage and its shelf life afterwards. The development of an objective language for describing the various sensory attributes of the fruit was the first step in the programme. To accomplish this, terms and ideas were collected from a panel of 21 tasters who assessed apples during the 1973-74 season both as they came from the tree and after removal from storage. Approximately 200 adjectives and phrases were suggested for describing the various aspects of Cox flavour. The most frequently used, together with a number of the other terms which the panel considered significant, were discussed and, where possible, standards in the form of essences, chemicals and natural materials, produced.

The terms derived were grouped into various classes which ultimately formed the basis of an assessment sheet for scoring

variations in the flavour characters from one batch of apples to another. The sheet consisted of eight sections dealing with the external and internal appearance of the apple, its feel to the hand, its external and internal aroma, taste and texture and finally its aftertaste (Table II). Each section contained the

TABLE II

Segregation of flavour attributes for sensory evaluation

1. Appearance	(a) external (b) internal
2. Feel	
3. Aroma	(a) external (b) internal
4. Taste	
5. Texture	
6. Aftertaste	

appropriate derived adjectives and phrases and was scored on the amount of a particular attribute present in the apple using a 0-5 scale. In scoring these attributes panelists were instructed to be objective and not to be influenced by personal preferences, these being indicated separately. In order to reduce errors apples were assessed in a pre-set manner.

Examination of correlation co-efficients between overall ratings and the various individual section ratings (Table III) based on twelve months' tastings indicated that the taste and texture sensations reflected the overall ratings best, internal aroma and external aroma as such only giving relatively low correlation co-efficients. The results of multiple regression

TABLE III

Correlation of individual section rating with overall rating

	Correlation co-efficients with overall rating
External appearance	0.80
External colour	0.43
Internal appearance	0.74
Internal colour	0.77
External feel	0.75
External aroma	0.56
Internal aroma	0.69
Taste	0.87
Texture	0.84

analysis, however, indicated that the rating for taste could not be interpreted entirely in terms of sweetness, sourness and bitterness, but, as expected, was affected by other factors such as the minor character imparting, volatile flavour components not directly defined in this section.

Analytical methods were developed at the same time to give information on the chemical components in the apple. In connection with this it is worth noting that differing qualitative and quantitative pictures were obtained from the volatiles depending on the collection method used and the degree of enzyme action allowed to take place during preparation. As the aim of the work was to interpret results in terms of a sensory effect the problem exists as to which method gave results closest to the composition of the aroma when the fruit was being chewed when, after all, some enzyme action and oxidation must occur. After a number of investigations (23) it was finally decided to adopt two procedures for collecting volatiles. Headspace collection from intact fruit using a simple syringe technique, supplemented by collection on Porapak Q, was used to obtain information for comparison with the external aroma comments. To correlate with the altogether different internal aroma and taste scores information was obtained by an extraction procedure, based on chipping the fruit followed by methanol inhibition of enzymes after 10-15 sec.

Examination of the aroma profiles of the Cox apples (Figure 1) showed that the panelists had selected and were scoring characters which were not the normal fruity, estery ones usually associated with apples. Two of these were the dried leaf and spicy aromas, the former of which often dominated the external aroma pattern. As yet the cause of the dried leaf character has not been determined, but examination of other apple cultivars showed that the spice-like character was present in a number of these (Figure 1), in particular one known as Ellison's Orange, where it was being described not just as spice-like but by some tasters as specifically aniseed-like.

Collection of the volatiles from this variety using the Porapak technique (22) followed by gas chromatographic examination naturally gave a very complex pattern (Figure 2). Odour evaluation of the separated components indicated most of them to have fruity and estery odours. One high boiling peak however had associated with it the spicy aniseed aroma. Gas chromatography-mass spectrometry, preparative gas liquid chromatography and infra red spectroscopy gave information which enabled this compound to be identified as 4-methoxyallylbenzene, a compound with a distinct aniseed smell.

Examination of volatile components from other cultivars by this and other methods showed 4-methoxyallylbenzene to be present in all cultivars examined, the amounts detected, however, corresponding approximately to the level of scoring of the spicy character by the panel (Figure 3).

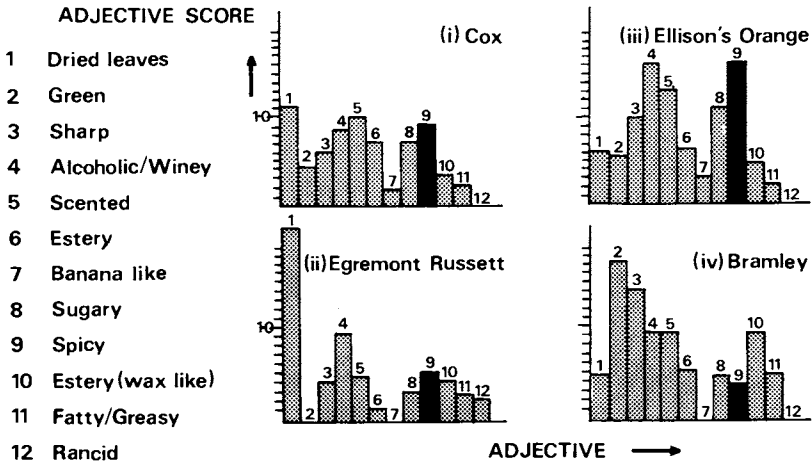
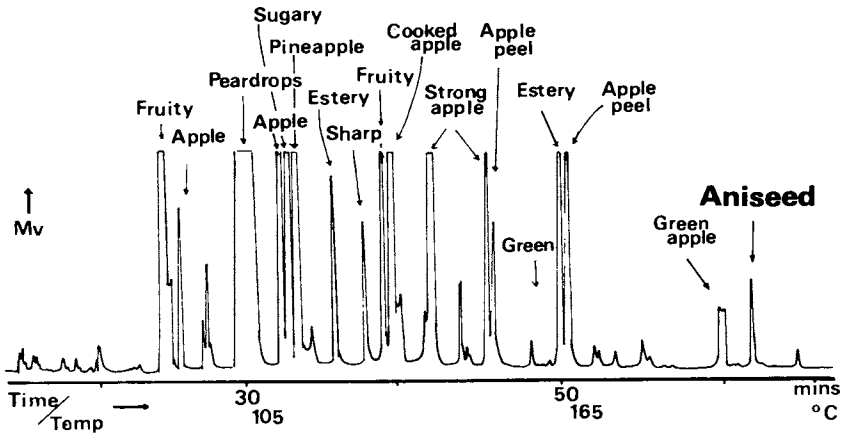


Figure 1. External aroma profiles of apples



Column: 150m x 0.76mm Carbowax 20M; programmed from 65°-210°C at 2-4°C/min

Figure 2. Chromatogram of volatiles from apples (Cultivar Ellison's Orange) Aroma comments.

Threshold measurements in aqueous solution based on triangular assessments in wine glasses indicated 4-methoxyallylbenzene to have a threshold of 0.035 ppm (significant $P=0.01$) which puts it on a par with compounds like hexyl acetate and hexyl 2-methylbutyrate (24,25), two compounds of importance in apples (Table IV). It is however a factor of 100 higher than ethyl 2-methylbutyrate, the significant compound in American Delicious apples (24).

TABLE IV
Threshold values (ppm) of significant aroma compounds in apples

	Our figures	Flath et al. (24)	Jakob et al. (25)
Hexanal		0.005	
2-Hexenal		0.017	
Ethyl 2-methyl butyrate		0.0001	0.00008
Hexyl 2-methyl butyrate			0.06
Hexyl acetate	0.08	0.002	0.085
4-methoxyallylbenzene	0.035		

Distribution measurements between an aqueous solution of 4-methoxyallylbenzene and its vapour indicate that an aqueous concentration of 0.035 ppm gives rise to a vapour concentration of 2.1×10^{-5} ppm. Quantitative figures from the collection of apple volatiles on Porapak Q indicated that the gas stream from Ellison's Orange contained in the region of 1×10^{-4} ppm of 4-methoxyallylbenzene, approximately five times greater than this threshold concentration.

Fermented Cider

Odour evaluation of the gas chromatographic effluent (26), the use of threshold values and odour units (20) and correlation of gas chromatographic data with sensory description of the aroma (27,28) has yielded much useful information on the contribution individual volatile components make to the aroma of ciders. Although important to the overall flavour of the beverage these compounds will not be discussed further in this paper.

In the English cider industry the practice of fermenting completely and adjusting the sugar and acid content before bottling has meant that the ability of the original juice to impart sweetness and acidity is becoming less and less important. This is not so with the compounds responsible for bitterness and astringency, two essential flavour characteristics of English ciders which can only be derived from the fruit. Unfortunately, there is a continuing shortage of apples which can impart these

characters, particularly in relation to demands to increase cider production. The industry can make up some of its fruit deficiencies by using surplus dessert apples, but this can only go so far, as cider made entirely from these varieties lacks the true cider flavour. Before sale such ciders must be blended with a certain amount of material made from bittersweet or bittersharp cider fruit. From the point of view of the industry and consumers it is therefore important that we obtain more knowledge on the compounds responsible for this desirable bitterness and astringency and discover means of increasing them both in the cider apples and the ciders made from them.

The involvement of phenolic material in the bitterness and astringency of English ciders and perries has been recognised since 1801 (29), but not until comparatively recently has it been possible to separate and examine the compounds of the general 'tannin' mixture, and to discover which of them contribute to the characters of bitterness and astringency so desirable in true English cider. In the mid 1950's paper chromatography revealed that the phenolics of cider could be broken down into a number of groups (Figure 4).

Circumstantial evidence derived from tasting pure commercial compounds indicated that it was only the procyanidins which made any direct contribution to bitterness and astringency.

Confirmation of this was obtained when new preparative scale separation techniques, using Sephadex gel as an adsorption medium, allowed large enough quantities of the phenolic fractions to be isolated from cider for tasting trials to be carried out.

Although a total procyanidin fraction, free from other phenolics, could easily be isolated on Sephadex, the problem of separating individual procyanidin species from one another still remained. The readiness of these compounds to both hydrogen bond with or to 'tan' almost any surface or chromatographic support, and to be converted to amorphous brown polymers under the influence of heat, light and oxygen made this very difficult.

The technique which proved more successful than any other in separating bulk quantities of procyanidins was that of counter-current distribution between ethyl acetate and water. By using a modern automated machine which moved both top and bottom phases in opposite directions, it was possible to separate the procyanidins of cider into six fractions (Table V), gram quantities sufficient for both sensory and structural studies thereby easily being obtained.

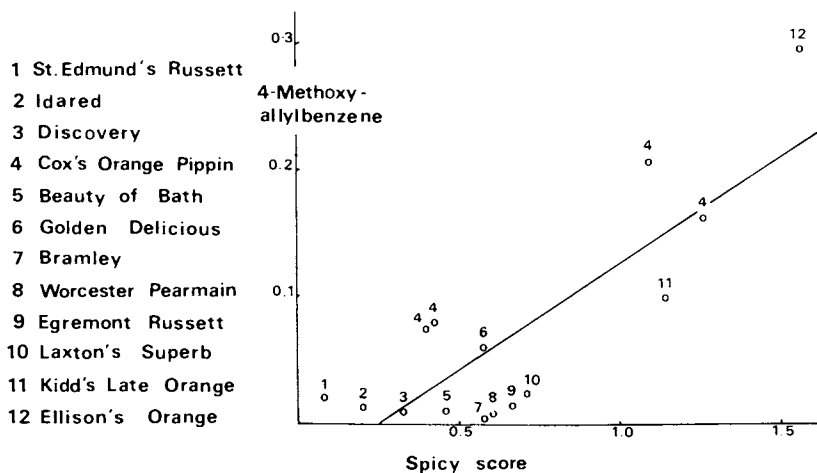


Figure 3. Relationship of spicy scores to amounts of 4-methoxyallylbenzene

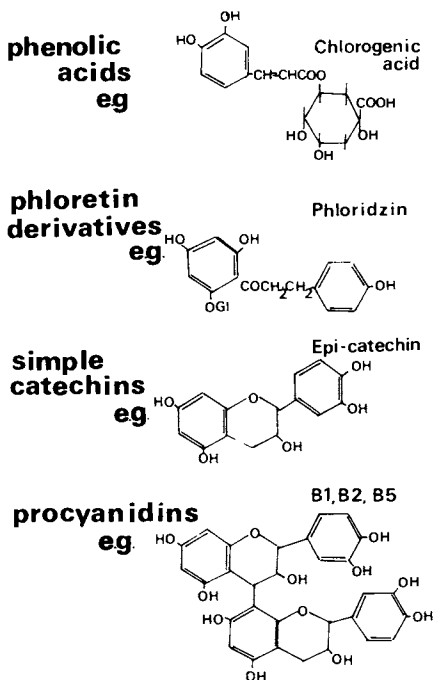


Figure 4. Paper chromatographic separation of cider phenolics

TABLE V
Counter current separation of cider phenolics

Component	Partition co-efficient (Ethyl acetate/ water)	Taste
Polymeric and oxidised procyanidins	0	Most astringent
Pentameric procyanidins	0.05 - 0.10	
Tetrameric procyanidins	0.18	Most bitter
Trimeric procyanidins	0.37	
Dimeric procyanidins	0.67	
Epi-catechin	4.0	

Structural studies, based on techniques developed by Weinges *et al.* in Heidelberg (30) and Haslam *et al.* in Sheffield (31), involved purification of the procyanidins on Sephadex gels, mass spectrometry of the methyl ethers to obtain molecular weights and nuclear magnetic resonance spectroscopy of both the native and acetylated compounds to give stereochemical information. Cleavage of the procyanidins with acid toluenethiol followed by paper chromatography and nuclear magnetic resonance spectroscopy of the fragments also gave valuable information on the constitution of units making up the procyanidins.

By presenting the isolated fractions in aqueous solution to a tasting panel, it was possible to demonstrate that only the materials of partition co-efficient ≤ 0.2 were effectively bitter or astringent at naturally occurring strengths and that, of these, the tetrameric procyanidin was decidedly the most bitter and the more polymeric materials the most astringent. However, all the procyanidins contribute to the total bitterness and astringency, and it is impossible to identify any one component as being solely responsible for these characteristics. It was also apparent that all procyanidins possessed both the characteristics of bitterness and astringency simultaneously rather than there being two classes of compounds each responsible for one or other sensation. It is possible that this dual effect arises from the different action of the procyanidin molecule on different areas of the taste papillae membrane. Current theories imply that astringency is caused by hydrogen-bonding of phenolic groups to protein in the tongue, whereas bitterness results from the interaction of polar molecules with a lipid portion of papillae membranes (32). A large procyanidin molecule (as long as it remains water soluble) would therefore be more astringent than

a small one, whereas for bitterness, increasing the molecular size could well reduce lipid solubility and hence its ability to react with the appropriate receptor.

Unlike the profile procedures described in connection with apples the sensory assessment of bitterness and astringency was relatively simple. As only two attributes were being assessed, and as reasonably appropriate standard samples were available, little training was necessary. However, a number of special problems did exist. Unlike other organoleptic sensations which are generally subject to fatigue effects, the intense physical tanning effect of these compounds causes repeated samples to have a cumulative effect on the palate. When two identical samples are presented in sequence it will thus always appear that the second is the most astringent. For this reason, only two samples could be handled at one session and these had to be presented in random order. In the Long Ashton procedure two ciders were merely compared for bitterness and astringency using a five point centre zero scale (Figure 5) along similar lines to the method originally devised by Scheffé (33).

Although counter-current distribution allowed one to obtain qualitative information on the various procyanidin fractions it was not really a method which lent itself to continuous routine analysis which was necessary if the effect of manufacturing variables and cultural conditions on bitterness and astringency were to be understood. The 'total tannin' methods which survive from the pre-1914 literature, such as permanganate titration, Folin-Denis and Vanillin method, were also not selective enough for the purpose. A modified analytical version of the Sephadex column chromatographic technique, using a methanol-water gradient, has so far given the best information and this has been used routinely over the past three years. Typical of results obtained are illustrated in Figure 6.

With this procedure a cider sample can be applied directly to the column, fractions collected on the basis of continuous UV monitoring, and the concentration of phenolics in each fraction calculated from the UV absorbance. Despite the disadvantage of being time consuming and not really giving adequate resolution in the region of the organoleptically significant compounds (H to J), this method gives more insight than do the traditional analyses. Attempts to use high pressure liquid chromatography to solve these problems have so far been largely unsuccessful due to lack of suitable packings, though in time it is believed that such development will be possible. In the meanwhile, the Sephadex column technique has been used in conjunction with the sensory procedure described, to enable a number of horticultural and manufacturing parameters affecting bitterness and astringency to be investigated.

NAME

DATE

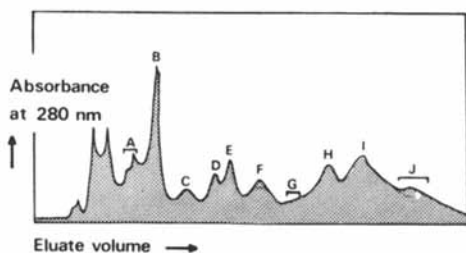
SESSION NO.

GROUP

Please taste Sample ... first

	Bitterness		Astringency	
	<input type="checkbox"/>	Much more	<input type="checkbox"/>	
	<input type="checkbox"/>	Slightly more	<input type="checkbox"/>	
Sample ... has	<input type="checkbox"/>	The same	<input type="checkbox"/>	than sample ...
	<input type="checkbox"/>	Slightly less	<input type="checkbox"/>	
	<input type="checkbox"/>	Much less	<input type="checkbox"/>	

Please state if you find any other major taste difference:

Figure 5. Assessment sheet for evaluating bitterness and astringency

- A p-Coumaroylquinic acid
- B Chlorogenic acid
- C Phloretin xyloglucoside and caffeic acid
- D (-)-Epicatechin
- E (+)-Catechin and phloridzin
- F Procyanidin dimers B2 and B1
- G Minor procyanidins (including B5)
- H Procyanidin trimer
- I Procyanidin tetramer
- J Complex polymeric procyanidins

Figure 6. Analysis of phenolics from Dabinett cider on Sephadex LH20.

The effect of gelatin fining on the bitterness, astringency and polyphenol content of pure variety ciders is typical of the information which has been obtained. This procedure is used to bring down hazes caused by non-flocculating yeasts, but unfortunately it also reduces bitterness and astringency, as our tasting tests showed. Analytical results confirmed the earlier results on the importance of some of the polymeric procyanidins to the taste because it was only those which were effectively removed by the fining, the lower molecular weight compounds being less affected (Figure 7). On reflection this is hardly surprising since if procyanidins are able to hydrogen bond to the tongue protein they will certainly be able to do the same to the gelatin, and conversely if they cannot tan the tongue then they will not react with the gelatin. In practical terms we have shown that gelatin fining may remove up to 25% of the organoleptically important phenolics which the cider industry is desperately trying to retain.

It is interesting to note that traditional methods of estimating polyphenols, because they estimate 'total' polyphenols, much of which is low molecular weight material, showed no significant difference due to the treatment and hence appeared to be at variance with the sensory data.

Similar experiments, some involving 1,000 trees in field trials, have shown that high levels of nitrogen fertilisers in general depress total phenolics in cider apples and that this change is detectable in the finished product (Table VI).

TABLE VI

Effect of nitrogen levels on phenolics, astringency and bitterness in pot-grown cider apples (cultivar Dabinett)

	Fed	Starved	Difference
Leaf N ₂	2.34%	2.00%	-17%
Organoleptically significant procyanidins in cider (mg/100 ml)	133	155	+17%
Total phenolics in cider (mg/100 ml)	350	410	+17%
Bitterness/astringency of cider	Least	Most	

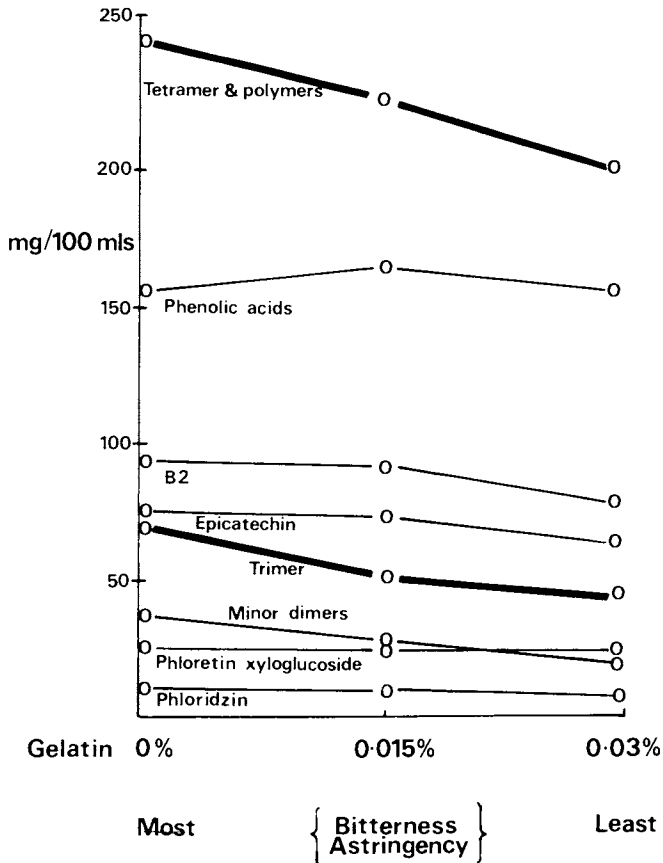


Figure 7. Effect of gelatin fining on phenolics of cider (*Apple cultivar Vilberie*)

It is also clear that different cultivars respond in different ways to the fertiliser treatments, and that differences in amounts of phenolics, whether registered by chromatographic or organoleptic means, is far greater between cultivars than within any given cultivar subjected to various nitrogen levels. Total fruit yields also appear to be depressed by low nitrogen levels, so it is unlikely that nitrogen-deficient orchards will be seriously considered as a means of increasing bitterness, although in traditional grazed cider orchards this is exactly what used to happen.

The amount of organoleptically significant polyphenols in the cider is also affected by the degree of oxidation at the juice pressing stage. It has been shown that use of sulphur dioxide at this stage virtually doubles the quantity of such compounds, similar improvements being noted in juices obtained by hot water diffusion techniques as opposed to the older 'mill and press' procedure.

Experiments involving bitterness and astringency are still, however, in their infancy and a great deal more needs to be done before we can specify to the cider maker how to obtain the taste sensation he desires, but with analytical and tasting tools improving all the time it can only be a matter of time before this objective is accomplished.

Conclusions

The combined use of sensory and analytical procedures has enabled information to be obtained on three important flavour characteristics, the spicy note in apples and bitterness and astringency in cider. It must be stressed, however, that these are only three, albeit important, flavour characters and that the full flavour quality of either of these products is the result of a large number of sensations all acting simultaneously on the brain. To interpret the true significance of characters such as these and their inter-relationship with other aspects of quality requires the integration of such information with consumer opinion. This may be obtained directly as in the case of fresh apples or reflected in the eyes of a manufacturing industry as in the case of cider. Only a minimum use has been made of the consumer in the investigations reported in the present paper and although a great deal of work, both sensory and analytical, is still required before quality of either apples, juices or ciders can be defined, the consumer is inevitably bound to play a bigger and more important role in any future investigations.

Abstract

In order that quality can be defined more precisely use is being made of a three-directional approach in which sensory assessment by panelists is integrated with preference information on the one hand and analytical data on the other. The paper discusses the application of this approach to aspects of the flavour quality of apples and ciders. Illustrations are given in which sensory procedures combined with gas liquid chromatography, gas liquid chromatography-mass spectrometry have enabled the cause of an important spicy character in certain types of apples to be determined, and counter-current and liquid chromatographic techniques again coupled with sensory appraisal methods have been used to give information on the contribution of the procyanidins to the bitterness and astringency of cider.

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Objective Measurements of the Flavor Quality of Beer

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Beer is consumed principally for its pleasant and satisfying sensory effects. The types of beer available around the world vary quite dramatically in sensory characteristics, but within populations consumers select their products on the basis of a general acceptance of beer flavor, subtle distinctive flavor notes, and an absence of unusual or off-flavors. The key role that flavor plays in product advertising and the fact that beer is a reasonably homogeneous liquid system have made the nature of beer flavor the subject of numerous investigations.

Flavor Chemistry of Beer

Most of the flavor chemistry literature describing beer flavor per se and the brewing parameters affecting flavors of finished products resides in the journals and publications that directly serve the brewing industry. Literature surveys confined to the usual sources of flavor chemistry articles will indicate a deceptive lack of information on the subject. While an extensive review of the flavor chemistry of beer is beyond the scope of this paper, excellent summaries have been published by Palamand and Hardwick (1) and Meilgaard (2,3).

Collective considerations of the beer flavor literature indicate that well over 250 compounds have been characterized and reported for beer. These volatile compounds are derived from ingredients (grains, hops), brewing practices (wort boiling), fermentations (yeast, occasionally bacterial infections), and equilibrium reactions (esterifications, staling processes). As with other food flavors, some difficulties and disagreements have occurred in the assignment of roles for individual compounds in the flavor of beer. Undoubtedly many compounds of significance in beer flavor, particularly flavor defects, remain unrecognized at this time. Still, much progress has been made, and attempts are being made to standardize beer flavor terminology (4).

Based on extensive evaluations of quantitative data for

flavor compounds in beer and flavor thresholds of individual and mixtures of compounds, Meilgaard (2) has proposed a tentative scheme for the role of various beer constituents in determining the perceived flavor of beer (Table I). This classification considers those compounds which generally contribute to aroma, taste, and tactual sensations, and it can be concluded that as further information accumulates modifications will be necessary.

The basis for classification in the scheme shown in Table I is that of Flavor Units (F.U. = Constant X Concentration/Threshold) or as it is sometimes referred to, Odor Units (5). Compounds placed in the category of primary flavor constituents are present in concentrations exceeding 2 F.U., and the removal of any one would cause a decisive change in the character of the product. It can be noted that only specialty beers in relation to the usual domestic products and defective beers contain adequate minor volatile flavor components to allow classification in the primary category. Secondary flavor constituents are those that are present between 0.5 and 2.0 F.U., and collectively these contribute much of the characteristic flavor to beer. However, removal of any one would result in only a small change in flavor. Tertiary flavor constituents are present in levels equivalent to between 0.1 and 0.5 F.U., and add subsidiary notes to the flavor of beer. For these compounds, the removal of any one would not produce a perceptible flavor change. The remaining flavor compounds would fall into the category of background flavor constituents (below 0.1 F.U.), and even though a great number of compounds fall into this category, Meilgaard (2) estimates that the group accounts for less than 30 percent of the overall flavor of beer.

Analysis of Beer Volatiles

Most of the classical procedures for the analysis of volatiles in foods and beverages have been employed with varying degrees of success for the determination of beer flavor compounds (6). More complete recoveries of the entire spectrum of compounds are achieved by distillations (7,8), freeze-drying followed by ether extraction (9), and continuous liquid-liquid solvent extractions (10), but these methods are complex and can easily lead to the formation of artifacts.

More recently porous polymer entrainment procedures have been adapted for use in the analysis of beer volatiles (11,12,13,14,15), but as yet these techniques have not been generally applied for routine monitoring of beer flavors. Wohleb (14) used Poropak Q for the entrainment of volatiles from beer samples held under several storage conditions, and subsequently analyzed the isolates with glass capillary column gas chromatography. Computer analysis of quantitative data for 54 beer flavor compounds indicated that 11 peaks (mainly esters and alcohols)

Table I. Tentative Scheme for Role of Constituents in Determining the Flavor of Beer.

-
1. PRIMARY FLAVOR CONSTITUENTS (Above 2 F.U.^a)
 - Ethanol
 - Hop Bitter Compounds (e.g., Isohumulone)
 - Carbon Dioxide

 - Specialty Beers
 - Hop Aroma Compounds (e.g., Humuladienone)
 - Caramel Flavored Compounds
 - Several Esters & Alcohols (High-Gravity Beers)
 - Short-Chain Acids

 - Defective Beers
 - 2-trans-Nonenal (Oxidized, Stale)
 - Diacetyl & 2,3-Pentanedione (Fermentation)
 - Hydrogen Sulfide, Dimethyl Sulfide
and Other Sulfur Compounds (Fermentation)
 - Acetic Acid (Fermentation)
 - 3-Methylbut-2-enylthiol (Light-Struck-Hops)
 - Others (Microbial Infection, etc.)

 2. SECONDARY FLAVOR CONSTITUENTS (Between 0.5 - 2.0 F.U.)
 - Volatiles
 - Banana Esters (e.g., Isoamyl Acetate)
 - Apple Esters (e.g., Ethyl Hexanoate)
 - Fusel Alcohols (e.g., Isoamyl Alcohol)
 - C₆, C₈, C₁₀ Aliphatic Acids
 - Ethyl Acetate
 - Butyric and Isovaleric Acids
 - Phenylacetic Acid
 - Non-Volatiles
 - Polyphenols
 - Various Acids, Sugars, Hop Compounds

 3. TERTIARY FLAVOR CONSTITUENTS (Between 0.1 - 0.5 F.U.)
 - 2-Phenethyl Acetate, *o*-Aminoacetophenone
 - Isovaleraldehyde, Methional, Acetoin
 - 4-Ethylguaiacol, gamma-Valerolactone

 4. BACKGROUND FLAVOR CONSTITUENTS (Below 0.1 F.U.)
 - Remaining Flavor Compounds
-

^a Flavor Units (F.U.) = Constant X Concentration/Threshold.
From Meilgaard (3).

varied significantly with the temperature of storage. Dravnieks (15) utilized Apiezon L on Chromosorb T for the quantitative entrainment of beer aroma constituents and utilized these data for demonstrating the utility of methods for correlating subjective and objective flavor data. However, information on identified samples in actual experimental designs was not reported. To date limitations, including polymer stability, analytical reproducibility, and analysis time, have contributed to the lack of acceptance of porous polymer entrainment procedures for the routine analysis of beer headspace volatiles.

Most of the routine monitoring of beer aroma constituents is accomplished through the use of either static headspace sampling procedures (16,17) or direct carbon disulfide extraction (18), although direct beer injections have been used to some extent (19). These methods employ packed-column gas chromatography, and the number of compounds (peaks) observed is quite limited (Table II). It is possible to observe larger numbers

Table II. Volatile Compounds in Beer Determined by Quantitative Static Headspace Sampling and Carbon Disulfide Extraction Procedures.

Compound	Determined By Method	
	Static Headspace	Carbon Disulfide
Acetaldehyde	+	-
Ethyl Acetate	+	+
Ethanol	+	+
n-Propanol	+	+
Isobutanol	+	+
2-Methylbutene-2	+	-
Isopropyl Acetate	+	-
Ethyl Propanoate	+	-
2-Methyl and 3-Methylbutanol	+	+
m-Xylene (Internal Standard)	+	-
Isoamyl Acetate	+	+
Ethyl Hexanoate	-	+
Ethyl Octanoate	-	+
1-Octanol (Internal Standard)	-	+
Hexanoic Acid	-	+
2-Phenethanol	-	+
Octanoic Acid	-	+
Decanoic Acid	-	+

of peaks during analytical runs by simply operating the gas chromatograph at higher sensitivities, but this is usually not done because of greater analytical variabilities observed under these conditions. Under usual conditions from 10 to 18 peaks are observed in a given run for the static headspace or the carbon disulfide extraction procedures (17,18), and it is accepted that some peaks are not identified and that others may contain more than one compound. Still the data are useful, and many breweries are equipped for automated gas chromatographic analysis of production samples of beer. It can be noted that most volatiles detected by the carbon disulfide extraction and headspace sampling techniques are derived either directly or indirectly from the fermentation (and aging) process (Table II). Therefore, many flavor contributions arising from processing steps, ingredients, and constituent interactions (i.e., staling) go undetected by these methods of analysis.

Sensory Analysis of Beer Flavors

Traditionally, the brewmaster has been delegated nearly absolute authority for the determination and maintenance of flavor quality of beer issuing from the brewery. However, as breweries grew in size and distribution areas increased, shifts in responsibilities have occurred to the point that the brewmaster usually receives sensory data from expert corporate panels (20,21) and trained or selected panels composed of brewery workers (17,21). Expert panels often perform some type of descriptive test function, but the principal concern is quality control for products released from the brewery. As a result, difference testing is useful for monitoring uniformity, and the triangle test apparently is the most widely used type of taste test used by expert and trained groups in the brewing industry (17).

Some breweries utilize quantitative descriptive analysis procedures for characterizing beer flavor properties, and this approach has been reported to assist in determining the degree to which consumers can recognize variations in beer flavors (21). Since it has been reported that under laboratory conditions beer consumption rates increase with hedonic ratings (22), there is an increased interest in relating beer flavor attributes to consumer preferences for products (23).

Objective Analysis of Beer Quality

In addition to the compounds which comprise the volatile aroma fraction of beer, there are a great number of physical and chemical parameters which can be measured and ultimately utilized in describing or evaluating beer quality (15,24,25,26,27). Included are clarity, foam head retention, carbon dioxide, and air which relate directly to quality appearance factors or which

can influence appearance in time. Percent alcohol, real extract, pH, titratable acids, free amino acids, headspace volatiles, formol nitrogen, and trace metals are measured and reflect on the status or success of the fermentation. Since the bitterness contribution to the flavor of beer is principally due to the hop resin components, a special attention is paid to the measurement and control of these compounds, particularly humulone and iso-humulone (α -acids) which are responsible for the major portion of the bitter flavor in beer (28). The list of other analyses is rather extensive, and depending upon the requirements of breweries various tests are used to provide information for production and quality control.

Objective Measurements of Beer Flavor Quality

Objective assessments of food quality are essential for establishing a basis for economic value of foods, but frequently standards defined by measurements are only roughly related to quality (29). Since aroma (i.e., flavor) constitutes a major portion of the overall apparent quality of foods, considerable effort has been expended for developing means for meaningfully using gas chromatographic data in predicting sensory quality (30,31,32). Although classification of foods based on objective flavor data appears promising for a wide range of commodities (33,34,35,36,37), few actual instances of application of this approach are apparent.

Several alternatives for the objective measurement of beer flavors and classification of beers have emerged from research in the brewing industry.

The PTPA Method. Hoff and Herwig (17) have reported a method for the correlation of statistical differences or lack of statistical differences between headspace volatile profiles of beer samples and the results of the widely-used triangle taste tests for the same beers. A static headspace analysis technique employing a Poropak Q column for separation was used for collecting quantitative data for 12 peaks in the volatile fraction (exclusive of the peaks for ethanol and the internal standard). Data from replicate GC analyses were processed by dividing each individual raw peak area by the sum of all raw peak areas, and then statistically analyzing these data between samples. Thus, the procedure was named the "Percent of the Total Peak Area" (PTPA) method.

In the PTPA method, a chronologically updated database was used to calculate pooled standard deviations for peak area values to overcome problems associated with aberrant values sometimes encountered for unexplained reasons or for those instances where the GC column characteristics change with usage. The database consisted of standard deviations for each peak of 24 previous replicate determinations plus the replicate deter-

mination under investigation. The standard deviations of the oldest of the 25 former replicates were eliminated from the database each time a new entry was made.

When two beers were compared, the procedure was repeated for each. Then a two-tailed t-test was performed using the most recent of the pooled standard deviations obtained from the database and the mean peak area values obtained from the replicate headspace determinations of the two beers. If none of the peaks in the volatile profiles of the two beers were significantly different at the 0.005 level, the results of a triangle taste panel of the beers were predicted to be insignificant at the 0.05 level. On the other hand, if one or more of the peaks in the volatile profiles were found significantly different between samples at 0.001 level, the triangle panel were predicted to be significant at the 0.05 level. When one or more peaks were significantly different between samples at the 0.005 level, but were insignificant at the 0.001 level, no prediction was made and more analyses were required to resolve the situation.

In a test of the correlation of the PTPA method with triangle taste panel results (Table III), it was found that the beers in 32 of 69 comparisons of fresh American lager beers were predicted by the PTPA method to give significantly different triangle taste panel results. In actual triangle taste panel testing, beer samples in 27 of these comparisons were found to be significantly different while the beers in 5 of the comparisons were found not significantly different. Conversely, the PTPA method predicted 37 of the 69 comparisons to be not significantly different, and actual taste panels showed that 35 of these predictions were correct.

Table III. Evaluation of Correlation of PTPA Predictions with Triangle Taste Panel Results for Lager Beers.

PTPA Predicted Triangle Panel Results		Actual Triangle Panel Results (0.05)	
Trials Sig. Different	Trials Not Sig. Different	Trials Sig. Different	Trials Not Sig. Different
32	0	27	5
0	37	2	35

From Hoff and Herwig (17).

Hoff and Herwig (17) suggested that routine triangle taste panel testing of normal, fresh American lager beers could be reduced by eliminating those samples which were found to be not different by the headspace analysis procedure. However, it was noted that the PTPA method would not recognize samples of beers containing organoleptically detectable levels of sulfur, staling, or certain hop compounds. Therefore, it appears that some type of small descriptive panel should be employed to detect beers exhibiting these significant, but unusual lager beer flavor characteristics.

Discriminant Analysis of GC Volatile Profile Data. The computer identification and classification of some selected beer samples with the aid of stepwise discriminant analysis (38) of volatile profiles from either carbon disulfide extractables or headspace vapors has been reported by Hoff, Helbert, and Chicoye (39,40). In this work the sensory characteristics of individual beer samples were not determined, but rather it was assumed that competitive American lager beers, beers brewed with different adjuncts (carbohydrate sources), and beers from branch plants within a company would exhibit flavor differences if their volatile profiles differed. To demonstrate the utility of the technique, individual lots of beer from a given source were divided and subsequently analyzed by either headspace or carbon disulfide extraction procedures to provide both a "known" database and "unknown" beer sample data.

In one instance, four competitive American lager beers were classified on the basis of the quantitative amounts of four peaks (isoamyl alcohols, isobutanol, ethyl acetate, and isoamyl acetate) from carbon disulfide extractables which were selected by the stepwise discriminant analysis of the 12 available variables. A canonical plot of the beers showed tight groupings of both known and unknown samples within sample lots.

The technique was shown (39) to be useful for classifying all selected samples in the study on the basis of headspace or carbon disulfide extractable volatiles, including beers brewed with different types and amounts of adjuncts. An interesting aspect which was also demonstrated was that of shifting an original classification of a beer from a branch plant to that of another plant's classification after a brewing modification was made. Utilization of such techniques could be very useful in assessing the uniformity of flavor quality, particularly when flavor compounds reflecting fermentation parameters are measured.

Discriminant Analysis of Physicochemical Variables Including GC Volatile Profile Data. Reiner and Piendl (41) have demonstrated how discriminant analysis of 49 physicochemical variables, including carbon disulfide extractables, could be used to differentiate types of beer, e.g., lager, pilsener,

export, etc. A modified University of California at Los Angeles BMDO7M stepwise discriminant analysis program (38) was used to select variables from the total physicochemical data available which included quantitative information on 14 general beer or brewing parameters, 20 α -amino acids, and 15 metabolic by-products from the fermentation process. Selected variables were then used to demonstrate groupings of beer types in canonical plots, and attempts were made to determine which properties allowed clear differentiation of beer samples evaluated.

Although varying degrees of success in differentiation of beer types on the basis of selected physicochemical variables was achieved by Reiner and Piendl (41), correct classification of all beers within a type was realized only for alt and pilsener diet beers when all of the initial variables were utilized (Table IV). With this approach difficulties were still encountered in the differentiation of the more similar full, light

Table IV. Classification of Beers on the Basis of 14 General Parameters, 20 α -Amino Acids, and 15 Metabolic By-Products of Fermentation.

Actual Beer Type	Classification As				
	Light Lager	Light Export	Pilsener Lager	Alt Beer	Pilsener Diet
Light Lager	30	1	4	0	0
Light Export	0	24	4	0	0
Pilsener Lager	3	4	14	0	0
Alt Beer	0	0	0	17	0
Pilsener Diet	0	0	0	0	12

From Reiner and Piendl (41).

type beers (light lager, light export, and pilsener lager). While the alt beer and pilsener diet beer samples were characterized by distinct physical properties, the full, light beer samples exhibited similar physical characteristics.

In order to differentiate the full, light beer types, it was necessary to expand the available data, and this was done by also utilizing the sums of aliphatic esters, aliphatic alcohols, lactates, and the vicinal diketones which were calculated from the original physicochemical data. The success

of the final differentiation is shown in Table V. All 35 light lager beers were correctly classified, and only one beer in each of the light export and pilsener lager categories was misclassified. However, it was claimed that these samples were actually correctly classified, and that errors in initial labeling or classification led to the apparent false classifications.

Table V. Classification of Three Types of Light, Full Beers with Individual Physicochemical Parameters Plus the Sums of Aliphatic Alcohols, Esters, Lactate, and Vicinal Diketones.

Actual Beer Type	Classification As		
	Light Lager	Light Export	Pilsener Lager
Light Lager	35	0	0
Light Export	0	27	1
Pilsener Lager	1	0	20

From Reiner and Piendl (41).

Further analysis of the data showed that light lager beers were differentiated from light export beers by higher original gravities, soluble nitrogen, anthocyanogens, malate, lactate, proline, and α -amino-nitrogen contents. Pilsener lager beers contained more bitter substances and anthocyanogens, but less pyruvate and higher aliphatic alcohols than pale or light lager beers. Alt beers which are heavier and darker beers were shown to have deeper colors, more anthocyanogens, glycerol, malate, citrate, esters and higher aliphatic alcohols than light lager beers. Pilsener diet beers were characterized by their very high levels of attenuation, high ethanol contents, low pyruvate and citrate contents, and very low viscosities. It was suggested by these workers that inclusion of quantitative data for higher aromatic alcohols and sulfur-containing compounds would greatly facilitate differentiation of beer types. However, methods for routine analysis of these components have not been available.

Discriminant Analysis of Flavor Profile Descriptor Data.

Some workers have taken the position that methods for developing reliable quantitative sensory data must be available before the potential usefulness of chemical and instrumental flavor analysis data will be realized. Along this line, Brown, Clapperton,

and Dalgliesh (42) have shown that beers from different geographical locations (Europe, Britain, and America) can be successfully classified by discriminant (cluster) analysis of quantitative flavor profile characteristics detected by a qualified panel. In this work approximately 40 descriptors were believed to adequately define the flavor of all types of beer, but of these only 27 gave significant scores for typical, light lager beers.

Average profile panel scores for the 27 significant sensory characteristics were obtained for 9 brands of English lager beer, 11 brands of Continental European lager beer, and 13 brands of North American beer. Discriminant analysis of these data gave cluster plots showing very close groupings of the beers within each geographical sampling, and the groups were well-separated in space. Discriminant analysis was used to reduce the variables to 12 (i.e., burnt, bitter, body, drying, viscous, smooth, dimethyl sulfide, cabbagy, high-gravity fullness, warming, toffee-like, and liveliness), and using these data much more diffuse cluster plots were obtained. However, a reasonable degree of classification was still achieved.

Brown, Clapperton and Dalgliesh (42) also discussed the practice of producing useful practical correlations between sensory and instrumental analyses in the brewery laboratory without a computer. Examples were discussed where levels of measured dimethyl sulfide correlated with sensory scores for this compound, and that the type of malt employed directly influenced the levels of dimethyl sulfide encountered. Similar simple correlations have been utilized for other compounds causing off-flavors in beer, and included are diacetyl (butter-milk-like), α -2-nonenal (cardboardy, oxidized), and certain short-chain fatty acids (soapy).

Discriminant Analysis of Analytical (Including GC) and Sensory Data. Research in this category involves the demonstration of differentiation of beers on the basis of either analytical measurements or sensory data, and then subsequent classification of the beers in the other set of data whichever the case may be. Discriminant analysis can then be used to reduce the number of variables to those essential to effect correct classifications.

Moll *et al.* (43) have used this approach to study the feasibility of classifying beers into categories of good, average, or poor as determined by expert tasters. The expert panel first scored 10 flavor and aroma characteristics and the overall flavor impression for 34 beers produced under differing brewing conditions in a French brewery. The same experimental beers were then analyzed for 12 individual as well as total carbon disulfide-extractable volatiles, and four metal ions (K, Mg, Na, Ca). Principal component analysis of each of the data sets (sensory and analytical) yielded classifications of the beers

in each instance which were separated into three distinct groupings (assigned as good, average, or poor). While the groupings obtained for the sensory data evaluation were used as the basis for quality assignments, only two samples were misclassified (changing from average to poor) when the analytical data alone were used. Stepwise discriminant analysis was then employed

Table VI. Classification of Beers into Quality Groupings (Good, Average, Poor) with Stepwise Discriminant Analysis of Physicochemical Data.

Total Number of Variables	Variables Included	% of Beers Within Classes
13	11 Volatiles 2 Mineral Salts	94.2 (32/34)
11	9 Volatiles 2 Mineral Salts	91.2 (31/34)
7	5 Volatiles 2 Mineral Salts	82.4 (28/34)

From Moll *et al.* (43).

to reduce the number of variables required to effect classification into the quality groupings (Table VI), and even when the data from only 5 volatiles and 2 minerals were used, 82.4 percent of the beers were correctly classified into the groups determined by sensory characteristics. This degree of success for these samples is similar to that achieved by the same workers for 58 French and foreign beers using 8 physicochemical variables selected from 38 initial variables, and classification into good or passable categories. In this study 80.5% of the samples were correctly classified by using data for 3 amino acids, 3 volatiles, 1 mineral, and the concentration of bitter substances in beer.

An extension of this approach has been discussed by Dravnieks (15) where odor characteristics of individual GC peaks from beers analyzed by an entrainment technique were quantified and correlated with some GC peak areas for certain aroma types. While the report was limited to descriptions of a few correlations, the approach should prove useful in the future.

In summary, evidence has been accumulated to show that beers can be differentiated and classified with the aid of appropriate statistical analysis of both physicochemical and sensory data.

Initiation of routine analytical monitoring of flavor quality appears feasible with current gas chromatography and computer methodology, but with experience and refinements of procedures greater applications should follow soon.

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8

Use of Regression Models in Objective Flavor Evaluation of Processed Orange Juice during Four Seasons

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Multivariate analysis first found application in food processing in quality areas other than flavor. Mittler in 1962 (1), selected prime size and texture processing tomatoes, while Rolle and Vandercook (2) used three objective analyses to predict natural citric acid content of lemon juices, a value useful in detecting citric acid adulterations.

In 1966, Powers and Keith (3), and Miller (4) working with potato chips, first described application of stepwise discriminant analysis to correlate flavor with chemical analyses. This tool has been refined and applied to a broad range of processed foods (5).

In 1971, Fellers and Buslig (6) and Attaway and Carter (7) applied multiple regression analysis to processed orange juice using a season's data from the Juice Definition Program (JDP), developing models for objective flavor prediction. Models for a second season's JDP data were developed in 1972 (8) and models for a third and fourth JDP season combined were found in 1975 (9).

In 1974, Persson and von Sydow concluded a five-paper study of the aroma of canned beef by relating sensory and chemical data by application of regression models (10).

A general model predicting flavor of processed orange juice produced from any variety during any season would find good application in rapid objective flavor determination during processing, by means of automatic on-stream analysis for a few constituents (variables). Such flavor prediction could indicate necessary processing technique changes instantaneously. The degree of these changes necessary when coupled with the capability of current techniques for adjustment, would optimize quality of the product continuously at the time of juice extraction. The rapid response and increased sampling frequency made possible by this tool would be a valuable supplement to the normal intermittent subjective organoleptic evaluations now a normal function of quality control in the industry.

The purpose of this paper is to report on objective prediction of flavor scores of orange juice by models found by a "forward

selection procedure" of multiple linear stepwise regression analysis of JDP data for 4 annual seasons (1970-71, 1971-72, 1972-73, and 1973-74) using various data sets.

Experimental Procedure

Harvesting, sample preparation, extraction treatments, processing, and sample analysis for each of the 4 seasons' data used here have been described (7, 8, and 9). 'Hamlin,' 'Pineapple,' and 'Valencia' varieties were used each season with several harvests of each variety throughout its maturity season¹. A total of 84 harvests were made. Each harvest was treated with both a soft and a hard extraction (squeeze) method, within the range used commercially. This produced a total of 168 samples, 50 'Hamlin,' 54 'Pineapple,' and 64 'Valencia,' for the 4 seasons studied.

Samples were analyzed for 24 chemical or physical characteristics, including organoleptic flavor score evaluation by a 12-member experienced panel using a 9-point hedonic scale. The data were analyzed by stepwise linear multiple regression, always letting flavor score (F) be a dependent variable and using the "forward selection procedure" judged superior to the "backwards elimination method of a stepwise regression analysis" (11) used in studies of the 1970-71 JDP data (7). Models were limited to 5 variables, according to Dravnieks (12). Ten models and respective coefficients of determination (R^2) were found. Three of the models predict flavor scores specific for each of the 3 varieties. Two models predict flavor scores of juices prepared by each of the 2 treatments, soft and hard extraction. One model appears for each of the 4 seasons studied, and a model found by combining data of all seasons is included.

Results and Discussion

Varietal models. Fourteen variables appear in the 3 varietal models listed below:

$$F = 3.249 + 0.381 \text{ CR} - 0.183 \text{ CY} + 1.433 \text{ Sucrose} + 0.240 \text{ Total Sugar} - 0.148 \text{ Protein} \quad \begin{array}{l} \text{'Hamlin'} \\ n = 50 \\ R^2 = 0.835 \end{array}$$

$$F = 2.949 - 0.052 \text{ Sinking Pulp} + 0.072 \text{ Brix/Acid} - 0.025 \text{ Glycosides} - 0.026 \text{ Limonin} + 1.01 \text{ Ash} \quad \begin{array}{l} \text{'Pineapple'} \\ n = 54 \\ R^2 = 0.870 \end{array}$$

$$F = -13.873 - 0.603 \text{ Acid} - 0.270 \text{ Serum Viscosity} - 16.022 \text{ Oil} - 0.0077 \text{ Glycosides} + 0.575 \text{ Color No.} \quad \begin{array}{l} \text{'Valencia'} \\ n = 64 \\ R^2 = 0.842 \end{array}$$

¹Only one 'Hamlin' harvest 1970-71.

Only one variable, glycosides, appears more than once and appears in both the 'Pineapple' and 'Valencia' models. Six of these variables: protein, sinking pulp, glycosides, limonin, oil, and serum viscosity were previously shown (8) to be highly positively correlated with extraction pressures and highly negatively correlated with flavor. The remaining 8 variables: CR, CY, color no. (derived color functions) (13), sucrose, total sugar, Brix to acid ratio, ash, and acid, were shown to be well correlated with maturity of citrus (9), and 6 of these exhibit positive coefficients. A negative coefficient appears for acid as this variable decreases with maturity and thus is associated with a flavor improvement. CY appears with a negative coefficient which cannot be readily explained in view of this variable's usual positive correlation with flavor (7). After studying the appearance of both the maturity-related and extraction-related variables in the models, it is apparent that 'Hamlin' flavor is influenced most by maturity-related variables, while 'Pineapple' and 'Valencia' flavor are affected most by extraction-related variables. This indicates that soft extraction pressures are more necessary for good flavor scores in 'Pineapple' and 'Valencia' than 'Hamlin.' The complete dissimilarity of the 3 models illustrates a unique influence of variables on flavor for each variety. The R^2 values of the 3 models are strikingly close.

Extraction treatment models. A model found using all seasons data for soft squeeze extraction treatment only, and also a model found using all seasons data for the hard treatment only, are listed below:

$$\begin{array}{rcl}
 & \text{Soft squeeze} & n = 84 \\
 F = 4.409 + 0.130 \text{ Brix/Acid} - 28.784 \text{ Oil} & & \\
 \quad - 0.070 \text{ Limonin} - 0.021 \text{ Cloud} & & R^2 = 0.593 \\
 \quad + 5.104 \text{ Protein} & &
 \end{array}$$

$$\begin{array}{rcl}
 & \text{Hard squeeze} & n = 84 \\
 F = 0.972 + 3.113 \text{ Acid} + 0.326 \text{ Brix/Acid} & & \\
 \quad - 32.222 \text{ Oil} - 0.020 \text{ Glycosides} & & R^2 = 0.725 \\
 \quad - 0.022 \text{ Limonin} & &
 \end{array}$$

Limonin, oil, and Brix to acid ratio variables appear in both these models. The soft squeeze model has the lowest R^2 value. The positive coefficient for acid appearing in the hard squeeze model requires explanation as acid appeared in the 'Valencia' model above with a negative coefficient, and acid has always had a negative simple correlation coefficient with flavor in other regression analyses (9) where data sets are composed of samples treated with both hard and soft squeeze. Hard squeeze orange juice samples always had lower flavor scores and lower acid values than soft squeeze samples (7, 8, and 9). Lower acid may result from dilution of acid in hard squeeze by more lower or non-acid

components originating in the cytoplasm of the cells in the juice vesicle (14). Some of these components may contribute off-flavors accounting for the lower flavor scores of hard squeeze juices. Therefore, the higher acid juices in the hard squeeze set probably indicate less severe treatment and thus higher flavor scores than the lower acid juices in the set.

Season models. Five models were found, one for each season 1970-71 through 1973-74 and one for all 4 seasons combined as follows:

$$F = 2.749 - 0.045 \text{ Sinking Pulp} - 0.098 \text{ Serum Viscosity} - 0.039 \text{ Limonin} + 0.172 \text{ Cloud} - 0.099 \text{ Sucrose} \quad n = 24$$

$$R^2 = 0.956$$

$$F = 1.603 + 0.414 \text{ Brix} + 0.112 \text{ Brix/Acid} - 38.032 \text{ Oil} - 0.926 \text{ Serum Viscosity} - 0.187 \text{ Limonin} \quad n = 36$$

$$R^2 = 0.859$$

$$F = 4.183 + 0.113 \text{ Brix/Acid} - 50.315 \text{ Oil} - 0.230 \text{ Limonin} + 0.059 \text{ CR} - 0.850 \text{ Sodium} \quad n = 36$$

$$R^2 = 0.887$$

$$F = 2.256 + 0.098 \text{ Brix/Acid} - 42.487 \text{ Oil} - 0.042 \text{ Limonin} + 0.051 \text{ CY} - 0.953 \text{ Sodium} \quad n = 72$$

$$R^2 = 0.711$$

$$F = 5.482 + 0.125 \text{ Brix/Acid} - 45.623 \text{ Oil} + 0.047 \text{ Aldehydes} - 0.018 \text{ Glycosides} - 0.025 \text{ Limonin} \quad n = 168$$

$$R^2 = 0.724$$

The latter 3 seasons models and the all seasons model each have 3 variables in common: limonin, oil, and Brix to acid ratio, and these same 3 variables also appeared in both the treatment models discussed earlier. Two of these, limonin and oil, appeared in the 3-variable linear model ($R^2 = 0.958$) reported by Attaway and Carter (7). This model was found by the "backwards elimination method of a stepwise regression analysis" and contained only 3 variables, which may account for its difference from the 1970-71 season model listed above utilizing the same data. The 9-variable model (equation no. 1 where $R^2 = 0.901$) reported by Attaway et al (8) was found by a "forward selection procedure" with data used to find the 1971-72 model listed above. These 2 models developed by the same techniques from the same 1971-72 data have 4 variables in common: limonin, oil, Brix, and serum viscosity. In all models containing limonin and oil, these extraction-related variables appeared with negative coefficients indicating their adverse effects on flavor. Conversely, Brix to acid ratio, a maturity-related variable, always appeared with a positive coefficient.

The appearance of 3 common variables in 3 of the season models is an indication that flavor of orange juice for 3 seasons

can be estimated using the same 3 variables. This indicates a consistency among seasons which is encouraging in looking for a model applicable to orange juice produced in any season.

The R^2 value of the all seasons model times 100% indicates that 72.4% of the variation in flavor in all samples can be explained by the model. The authors felt this model might be useful in multi-season flavor estimation and sought to test the provinciality (12) of the model. This was done by taking 30 random samples throughout the 4 seasons which were not a part of the data set of any of the season models. These samples were prepared in a commercial manner similar to, but not necessarily the same as, treatments used for the samples included in the models above. Each of the 30 samples' values for the 5 variables of the all seasons model were substituted in the all seasons model and a calculated flavor score was then obtained for each of the samples. The range, mean value, and standard deviation of the observed scores and calculated scores are compared below:

	<u>Observed</u>	<u>Calculated</u>
Range	4.0 - 6.5	4.2 - 6.4
Mean Value	5.4	5.5
Standard Deviation	0.74	0.61

The correlation coefficient (r) of the scores was 0.741. Residuals of the observed scores less the calculated scores were determined, and ranged from - 1.2 to + 0.9 flavor score points. The above information indicates that the all season model can be a useful tool in objective flavor evaluation of orange juice in the processing plant, although a model producing more precision between observed and calculated flavor scores is desirable.

The scope of this study precluded the search for curvilinear models and the use of ratios of variable values. Work in these areas is in progress.

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Abstract

Ten models are discussed which were found by multiple linear regression of data for 24 chemical or physical characteristics of 168 samples of processed orange juice produced by both soft and hard commercial juice extraction methods during 4 annual seasons (1970-71, 1971-72, 1972-73, and 1973-74) from 'Hamlin,' 'Pineapple,' and 'Valencia' oranges. Flavor was always the dependent variable.

Models were found using the following data sets: Each season's data, all seasons data, all data from each orange variety, and data from each juice extraction treatment. Coefficients of determination (R^2) for all models varied from 0.593 to 0.956. Limonin, oil, and Brix to acid ratio all appeared in 3 season models and the all season model.

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