

FLAVOR CHARACTERIZATION

1. Introduction

Flavor is the collection of sensations from the taste, olfactory, and trigeminal sensory systems. Taste perceptions include the currently recognized basic tastes (sweet, sour, salty, butter, and umami). Olfactory perceptions provide nearly limitless specific characterization of objects that smell, and trigeminal sensations provide the qualities such as coolness, pungency, and hot pepper burn. All three perceptual systems contribute to the flavor of a food. Within each perceptual system

individual sensations interact sometimes enhancing each other, sometimes suppressing each other, and sometimes fusing to produce a character that cannot be mentally separated into its more basic components. More detailed descriptions of the mechanisms of taste and odor can be found in Fischetti.

Both sensory and chemical analyses are necessary for thorough flavor characterization. While chemical analyses can tell us the flavor compounds present in a food and their concentrations, the only way we can tell if a collection of chemical components has a strawberry flavor is to have it evaluated by people that can recognize strawberry flavor. Only people can tell you how sweet a food tastes. Analytical chemistry can tell you how much of which sweeteners are present in a product, which is certainly related to sweetness, but accompanying bitter or sour tastes can suppress the perceived sweetness. Adding lemon juice to a sugar solution will make it less sweet; adding strong coffee to a sugar solution will make it taste less sweet even though the concentration of the compounds producing the sweetness remain the same. Methional alone smells like boiled potatoes, but this potato characteristic disappears when methional becomes a component of Cheddar cheese flavor.

The “Holy Grail” in terms of chemically characterizing flavor would be to have characterized the flavor stimuli in a food such that chemical data could predict human judgments. While one can point to a few examples where chemical data can predict a given sensory note (most commonly an off-note resulting from a single aroma component), this goal is far from being attained.

With the above considerations in mind, there is a compelling need to use chemical data to provide an understanding of the stimuli provided to an individual when eating. The end goal may be to provide the chemical basis of an off flavor in a food, understand how desirable flavors are formed biologically or through processing, or understand how flavor changes during storage. While only humans can tell if a food tastes like strawberry or chocolate, or “good” or “bad”, they are quite inept at describing the chemical basis for this judgment. Herein lies the value of chemically characterizing the flavor of a food, and thus explaining the fundamental bases of human flavor perception.

Liking, acceptability, or preferences for a flavor do not characterize the flavor. The identical flavor may be liked by some and disliked by others. Liking of most flavors is a matter of opinion and opinions are heavily influenced by socialization and previous experiences with a specific or similar flavor. Preferences for flavors, like preferences for politicians, are only partly due to the flavor (or politician). Experiences and beliefs of the individuals evaluating the flavors (or politicians) are also extremely important to measures of preference or acceptability.

In this chapter, we will outline how flavor is characterized both by sensory and instrumental means. It is impossible to provide much detail to this task since space is limiting. We have, therefore, included key references for the reader throughout this article.

2. Sensory Methods for Flavor Characterization

Quantitative sensory methodology can be grouped into two categories, difference tests and scaling tests. Difference tests are used when the goal is to determine

whether people can discriminate between two samples. Scaling tests are used when the goal is to measure the intensity of a sensation or an opinion.

2.1. Difference Tests. Some methods, such as the triangle test and the duo–trio test, are designed to determine whether people can detect any difference between two samples. Others, such as the paired comparison test or the three-alternative forced choice test, are designed to determine whether people can detect a specific difference (eg, sweetness, intensity, staleness, or preference) between two samples. And others, such as the R-Index test, can be used to determine whether people categorize the samples differently (ie, Cheddar vs. not Cheddar). All these methods yield proportions of the various responses that serve as a measure of people's abilities to discriminate the samples. Data from all methods can be analyzed statistically given a sufficiently large number of judgments.

The triangle test, a method used to determine whether people can detect *any* difference among samples, consists of giving three samples to a judge. Two of these samples are the same and one is different; the judges' task is to select the different sample. If judges cannot tell a difference they must guess. If none of the judges can discriminate between the samples, the proportion of correct responses will be one-third. As the size of the difference among samples increases the proportion of correct responses will increase.

The paired comparison test, a method for determining whether two products differ in the intensity of a specific attribute, involves giving judges two samples and asking them to select the sample having more of the specific attribute (eg, more sour, more Italian flavor, or more strawberry flavor). If the judges cannot tell a difference in intensity they must guess. If none of the judges can discriminate a difference in intensity the proportion of responses for each product will be one-half. If half the judges think that product A has more of the attribute than product B, and the other half cannot tell a difference in intensity, then 75% of the judges will select product A. The three-alternative forced choice test is similar to the paired comparison test except the judge selects one of the three products as the sample having more of the specific attribute.

The R-Index test, a method that can be used for determining whether people categorize products differently, involves giving judges the samples and asking them to respond if the sample falls in category A, sure; in category A, but they are not sure; in category B, but they are not sure; or in category B, sure. Category B may be simply defined as not being in category A — in which the test gives similar information to the triangle test. A value for the R-Index between any two products is computed (1) and will range from 0.5 (indicating the samples are completely categorized together) to 1.0 (indicating the samples are not categorized together).

2.2. Scaling Tests. The overwhelming majority of sensory tests used to characterize flavor involve scaling the intensity of a flavor or flavor attribute(s). Scaling techniques include ranking, category scaling, magnitude estimation, and the relatively newer labeled magnitude scales (2–4). Further details of their use and statistical analysis of the data produced can be found in these references and the sensory texts listed in Section 1.2.6.

Ranking. Ranking tests order samples according to some attribute or criteria (eg, most-to-least liked, most-to-least spicy, and closest-to-farthest from the

target flavor). Data from ranking have ordinal scale properties and are commonly analyzed by examining frequency distributions or by using nonparametrical statistical tests.

Category Scaling. Category scaling techniques include most types of scales with which people are familiar. The term category scaling also includes scales that appear to have no (or infinite) categories such as line scales that are labeled only at the ends. Category scales vary in the number of categories they have and whether categories other than the end categories have verbal labels. Category scales can be used to rate objective sensory properties such as bitterness intensity and vanilla aroma intensity or subjective attributes such as liking or the extent to which a flavor matches expectations. Some category scales combine both objective and subjective perceptions such as just-about-right scales that have “much too little” of the attribute at one end, “much too much” of the attribute at the other, and “just right” in the middle. Data from category scales are typically ordinal or interval in nature and are most commonly analyzed statistically using parametric statistics after any appropriate transformations.

Magnitude Estimation. Judges are presented with a number of samples and are asked to assign a number to each based on their perception of the intensity of some quality. For example, suppose judges were asked to assign numbers corresponding to the perceived saltiness of several solutions of salt in tomato juice. They could start by assigning an arbitrary number to the first sample, then assign numbers to the remaining samples that reflect the intensity of those samples in proportion to the first sample. Because the judges assign numbers based on ratios or proportions, magnitude estimation data are considered to be on a ratio scale. Magnitude estimation is less used than category scaling because of the need for at least two simultaneously presented samples for a proportionate comparison and because frequent values of zero can be problematic.

Labeled Magnitude Scales. Labeled magnitude scales have been designed to be category scales with interval and perhaps ratio properties (2,3). They are typically drawn as line scales with verbally labeled points. The location of the verbal labels along the lines has been determined experimentally. The general labeled magnitude scales (gLMSs), where the upper end of the scale is labeled “strongest imaginable sensation of any kind”, can be used for comparisons between groups of people in cases where the absolute perceived intensities indicated by the scale labels differ among the groups. [This is the case among 6-*n*-propylthiouracil (PROP) nontaster, taster and supertaster groups (5)].

Scaling Biases. With the exception of ranking, all scaling methods are vulnerable to some well-known psychological scaling biases. People come to sensory tests with frames of reference based on their past experiences and their expectations for the test. The specific samples presented in a sensory test also create their own frame of reference. Two of the most studied psychological biases are the range and frequency effects (6,7). The range effect predicts that subjects will distribute their ratings of the samples over most of the scale. The frequency effect predicts that subjects tend to use each response category with equal frequency. Both effects are reliably present for both trained and untrained judges and for most all types of scales (category, labeled magnitude, magnitude estimation).

Descriptive Analysis. Since its beginning as the A. D. Little Flavor Profile Analysis, published in the 1950s, descriptive analysis has become the most frequent methodology of choice for characterizing the sensory components of a food (8). Descriptive analysis provides a list of all the sensory attributes perceived in a food and a measure of their sensory intensity. The effects on the flavor of a food due to changes in the formulation of a food, changes during storage, comparisons with competitor products, etc, can all be measured using descriptive analysis. For example, Table 1 shows descriptive analysis results of the flavor of two samples of whey stored for 12 months. One was stored at ambient temperatures and one stored at -20°C . The sample stored at ambient temperatures can be characterized as having more astringency, more sourness and more bitter aftertaste than the frozen sample. The remaining 16 sensory attributes did not differ between the samples (9). Civille and Lyon (9) have compiled lists of flavor descriptors, definitions and examples for many food products. In addition, many articles are published each year detailing the descriptive analysis results for specific food products.

The preceding descriptions of sensory testing methodologies are brief. Those wanting additional details about how to conduct these tests and how to analyze the data statistically should consult any of the following books [Meilgaard and co-workers (10), Lawless and Heymann (11), Stone and Sidal(12); O'Mahony (1), Chambers and Wolf (13)].

Table 1. Mean ($n = 10$) Intensity Ratings of 19 Flavor Descriptors for Samples of White Cheddar Whey Stored For 1 Year Under Ambient and Frozen (-20°C) Storage^a

Attribute	Ambient	Frozen
astringency	2.2 ^b	0.9 ^b
bitter	0.9	0.7
bitter aftertaste	1.5 ^b	0.8 ^b
butter	1.1	1.1
caramel	1.7	2.4
cooked	1.5	1.6
diacetyl	1.3	1.1
milky	3.2	2.6
oxidized aftertaste	1.1	1.5
oxidized	1.9	1.5
pungent	1.0	1.2
salty	1.2	1.8
sour	1.6 ^b	0.9 ^b
stale	1.7	1.5
sulfur	0.9	0.8
sweaty	1.5	1.1
sweet	1.4	1.6
umami	1.0	0.9
volatile acid	1.7	1.3

^aIntensity was measured on line scales digitized to 10 units. Lower numbers represent less intensity.

^bSamples within a row having different letter superscripts differ significantly.

2.3. Perceptual Abilities. People make sensory measurements, and people differ markedly in their sensory physiology, their knowledge of sensory attributes and their social and cultural experiences.

Perceptions Differ. People can be divided into three groups: nontasters, tasters, and supertasters, based on their ability to taste the bitter compound PROP (4). These three “taster groups” differ in their perceived intensity of other taste compounds as well. Generally the supertasters experience greater taste intensity when given many other taste compounds (eg, sucrose tastes sweeter and caffeine tastes more bitter). Supertasters also perceive trigeminal sensations, such as the hot-pepper burn, black pepper burn, and alcohol burn, more intensely than do nontasters. More females than males are supertasters (4).

Although relatively few people are anosmic (have no ability to smell) many have specific anosmias and are thus unable to smell one or more specific compound(s), but otherwise have normal smelling abilities. Specific anosmias have been documented for androstenone, cineole, some small branched-chain fatty acids, diacetyl, trimethyl amine, isobutyraldehyde, and carvone (11).

Together these differences in PROP taster status and these specific anosmias suggest that different people will perceive flavors differently. Thus expecting members of a group to agree on the character and intensities of the many attributes present in a flavor is unreasonable. These differences in perceptual abilities also dictate that several people must be involved in the characterization of a flavor to avoid having the results reflect the peculiarities of individual’s perceptual abilities.

Knowledge Differs. Numerous paths can be followed to a food flavor education. They all involve thoughtful engagement with foods and/or their components. Expert tasters have apprenticed themselves to other initially more knowledgeable people for long periods of time during which they have been exposed to many flavor variations, described or labeled those flavor variations, and compared—confirmed their perceptions with other experts. Expert tasters are those that can claim to be able to tell the country of origin and the botanical basis of a wine, a coffee, a tea, etc.

Participants in descriptive analysis are usually trained to identify and rate the intensity of many sensory attributes of many products. During the training process they develop or are introduced to the vocabulary necessary to distinguish among similar products. Vocabulary terms are often illustrated by examples or references chosen because the specific vocabulary term is a major or key component of their flavor. For example, a buttery aroma could be illustrated by diacetyl, by creamy Havarti cheese, by cultured butter, by fresh sweet cream butter, etc (9). In addition to learning which flavor perceptions match which vocabulary terms, descriptive analysis participants also practice rating the intensities of these attributes on scales. Ratings can be calibrated to standards (eg, specific concentrations of chemicals, such as citric acid that illustrate the intensity of specific points on a scale, such as sourness).

Expert tasters and people with descriptive analysis training are assumed to no longer represent consumers. Learning that a specific flavor note is produced by fat oxidation changes the meaning of that sensory attribute from part of a desirable complex flavor associated with, say eating fried food at a fair, to an indicator of deterioration.

Mixtures. All flavors are mixtures of chemicals. Thus, understanding the perceptions of chemicals in mixtures is essential. The vast amount of research on mixtures of tastes and odorants has focused on binary mixtures. However, flavors are almost always much more complex than a mixture of two chemicals. When three or more odorants are mixed together, they often form a new coherent quality, and the individual qualities of the individual odorants may no longer be apparent (14,15). This synthetic aspect of smell is similar to the synthetic nature of color perception. When we mix two or more colors together, we are often unable to perceive the original colors comprising the mixture. This feature of the sense of smell makes the interface between flavor chemistry and sensory evaluation difficult as will be elaborated upon later.

3. Chemical Methods for Flavor Characterization

3.1. Introduction. The task of chemically characterizing the flavor of a food involves developing methods to isolate, identify, and quantify the food components that contribute to food flavor, notably taste, trigeminal, and aroma stimuli. This process includes the analysis of volatile (aroma contributors) and nonvolatile (taste and trigeminal stimuli) food components. It must be recognized that not all volatile and nonvolatile components of a food contribute to flavor and thus, this task must include methodologies to distinguish between those food components that contribute to our definition of flavor and those that do not. This may be illustrated by considering the aroma of coffee. To date, >700 volatile compounds have been identified in brewed coffee. Of these volatiles, it is estimated that a typical coffee aroma can be reproduced by using only 25–30 of these components. Our analytical and data interpretation tasks become much more manageable if we are considering 25–30 chemicals compared to 700, thus determining those compounds that truly contribute to flavor is very important. With this discussion in mind, we will present an overview of hurdles and approaches used in the task of chemically defining the flavor of a food.

3.2. Chemical Characterization of Aroma. Hurdles. Isolating and identifying aroma compounds in a food matrix is one of the most formidable tasks faced by an analytical chemist. A primary obstacle is that laboratory instrumentation is not as sensitive to many odors as is the human olfactory system. Stuiver (16) calculated that as few as eight molecules of a potent odorant can trigger one olfactory neuron and that only 40 molecules may provide an identifiable sensation. By making a few assumptions about air concentration versus absorption on the olfactory membrane, it is postulated that the nose has a theoretical odor detection limit of $\sim 10^{-19}$ mol, which surpasses even the most sensitive analytical instrumentation. The low concentrations at which these analytes may be present in a food and have sensory significance requires that they be isolated from the food system and concentrated to permit instrumental analysis.

The fact that trace quantities of aroma components are distributed throughout a food matrix further complicates the aroma isolation–concentration process. The isolation of exceedingly low concentrations of aroma compounds from food systems containing sugars, complex carbohydrates, lipids, proteins, and water is problematic. Aroma isolation methods based on volatility are complicated by the fact that water is the most abundant volatile in a food. Thus, any procedure

that draws a vacuum or involves distillation will also extract–isolate the water from the sample. Isolation methods based on solubility (most aroma compounds are lipophilic), eg, solvent extraction, will not only extract aroma compounds but lipids. Proteins are great emulsifiers and foam stabilizers, which complicate a simple aroma extraction process using organic solvents. Carbohydrates often add viscosity, foaming or emulsification properties to a product thereby complicating aroma isolation. Food matrices greatly complicate this endeavor.

Aroma isolation and analysis are made difficult also by the fact that aromas comprise a large number of chemical classes. If they were comprised of one or just a few classes of compounds, isolation methods could focus on molecular properties characteristic of a given class of compounds. Rather, the chemist must attempt to effectively extract and concentrate alcohols, aldehydes, acids, ketones, amines, carbonyls, heterocyclics, aromatics, gases, nonvolatiles (or nearly so), etc.

The absolute number of aroma compounds in a food further complicates aroma analysis. It is a rather simple, natural aroma that has <200 identified constituents. In fact, those with <200 identified constituents probably have not been adequately researched. It is not uncommon for the browning aromas (eg, meats) to be comprised of nearly a 1000 volatile constituents. To date, >7000 volatile substances have been found in foods (17).

A final problem complicating the instrumental study of aroma is instability. The food product being examined is a dynamic system, readily undergoing aroma changes while being stored awaiting analysis to begin. The aroma isolation process may initiate chemical reactions (eg, thermally induced degradation or oxidations) that alter the aroma profile and introduce artifacts. Thus, we have to be very cautious that the volatile components we find in a food product are truly native to that product.

Unfortunately, once we have considered each of the points above and obtained some instrumental profile of the aroma compounds in a food, we are left with the huge question of attempting to determine the importance of each volatile to the perceived aroma. This has been the topic of countless research articles over the past 30 years. Unfortunately, analytical instrumentation has no sense of taste or smell. Instrument response for the flame ionization detector (most commonly used detector in gas chromatography) is related to the number of carbon–carbon bonds, whereas the human olfactory system varies greatly in response to different odorants. For example, 2-methoxy-3-hexyl pyrazine has an odor threshold of 1 part in 10^{12} parts water, while pyrazine has an odor threshold of 175,000 parts/ 10^{12} parts water (18). On pyrazines alone, the human threshold varies by nearly 2×10^8 . It could be that the smallest component in an analytical profile may be more important to aroma than the largest component. It must also be recognized that the instrument is providing no appreciation for aroma character of each component. It is not apparent, for example, that component three is buttery while component 48 contributes oxidized aroma notes. There is no question that aroma analysis offers a most challenging analytical problem.

The remainder of this article will discuss the basis of the methods used in the isolation and analysis of food aroma components. It will be pointed out repeatedly that there is no single method of aroma isolation or analysis that provides a complete view of the aroma compounds found in a food. The goal is to find

an analytical method that can measure those components that are of *interest* to the analyst. They may be the compounds that give an off-aroma or those that give a desirable character to a food. Unfortunately, any aroma profile will be a partial view of the overall picture.

Aroma Isolation and Analysis. Most of the techniques used in aroma isolation take advantage of solubility or volatility of the aroma compounds. Inherently, aroma compounds must be volatile to be sensed and thus, it is logical that volatility is a common basis for separation from a food matrix. Likewise, aroma compounds tend to be more soluble in an organic solvent than in an aqueous solution (eg, a food matrix) and thus, aroma isolates may be prepared by solvent extraction processes. There are numerous techniques of applying either isolation principle to the task at hand. The simplest approach is a headspace analysis.

In headspace analysis, a food is placed in a container and time is allowed to permit aroma components in the food to come to equilibrium with the air (headspace) in the container. The headspace gas is sampled either with a gas-tight syringe (<5 mL of gas) or a sorptive fiber (solid-phase microextraction, SPME) and subjected to instrumental analysis for aroma components. While sampling with a syringe is a very simple method, it lacks the sensitivity needed to determine the majority of aroma compounds present in foods and obviously, it selectively isolates the most volatile constituents. The SPME method offers some concentration since the aroma compounds in the sample headspace will be extracted into, and therefore concentrated, in the SPME fiber depending on the affinity of the compounds for the fiber material. Desorption of the loaded fiber into an instrument affords substantial gains in sensitivity over direct headspace sampling and thus, has become popular in the field. However, the aroma isolate reflects the biases of the absorption process, ie, the solubility of each aroma compound in the fiber.

Other headspace concentration methods have been developed. However, when ones uses larger headspace volumes to improve on sensitivity, water vapor in the headspace (from the food) becomes problematic. Water is the most abundant volatile and it complicates any concentration efforts. For example, one can pass a purge gas through a food and collect all of the volatiles stripped from the food by passing the volatile laden gas through a cold trap. Unfortunately, the cold trap collects the aroma components AND a relatively large amount of water. This water precludes the direct analysis of the trap contents and dictates a secondary step to isolate the aroma compounds from the water. This may be done by solvent extraction (assuming the aroma compounds of interest are not polar in nature) or by using a selective trapping material to remove the aroma compounds from the stripping gas, but yet allow the passage of water. Unfortunately, each additional step (selective trapping materials or solvent extraction) changes the aroma isolate composition and thereby weakens the data.

Solvent extraction is often used for aroma isolation when the food does not contain any lipid. When lipid is present, then solvent extraction will extract the lipid as well, providing an aroma isolate in a fat matrix. This extract cannot be concentrated or analyzed without further processing to separate the lipid:aroma fractions. This may be done by a distillation process or chromatographic processes, both of which will alter the composition of the aroma isolate.

Distillations are commonly used for aroma isolation from foods that contain lipid. The combination of a steam distillation of the food and simultaneous solvent extraction of the distillate has been widely used in the field (Likens-Nickerson apparatus). Alternatively, a high vacuum distillation of the food (or a solvent extraction of the food) is used. However, these approaches also provide aroma isolates that reflect method biases. The bottom line is that every method used in aroma isolation–concentration provides a biased view of the aroma composition of that food. It is generally acknowledged that one must use several methods to get a complete view of the aroma compounds found in any food. With this fact in mind, one then moves on to the analysis of these aroma isolates.

The instrumental analysis of aroma isolates always begins with gas chromatography. Gas chromatography (gc) offers the sensitivity and resolution needed for the separation of complex aroma mixtures. Gas chromatography is interfaced with mass spectrometry (ms), which provides identifications, and olfactometry, (sniffing of the column effluent) that provides human judgments of the character and intensity of separated components.

The hardware used to make the human interface varies greatly. In many laboratories the interface involves passing the gc analytical column through a heated exit port in the gas chromatographic oven wall. The analyst stands next to the instrument, sniffs the gc column effluent, and records his–her impressions of the odors eluting from the gc. In other laboratories, more elaborate setups use heated eluant transfer lines, controlled column dilution, comfortable chairs with headphones, and computerized data collection all in environmentally controlled rooms. There is little question of the differences in human comfort between settings but there is no data to suggest one setting affords superior data to the other setting.

While the gc and gc-ms protocols are reasonably consistent across laboratories, the method used in collecting and interpreting olfactory data are laboratory dependent. These data are the first step in the process of determining the aroma compounds needed to chemically define the aroma of a food. The earliest work in this area is now >45 years old (19). Patton and Josephson (19) proposed estimating the importance of an aroma compound to the sensory character of a food by calculating the ratio of the concentration of a compound in a food to its' sensory threshold in that food. This ratio is known as the odor activity value (OAV) (also as: odor value, odor unit, flavor unit, or aroma value). They suggested that compounds present above their sensory threshold concentrations in a food are significant contributors to its aroma, whereas those occurring below their threshold are not. Patton and Josephson (19) proposed this method as a guide "that may not hold in some instances".

Since the introduction of the OAV concept, various approaches have been extensively used to screen for "significant" odorants in food. Two major screening procedures for determining the key odorants in food are based on this concept: the Aroma Extract Dilution Analysis (AEDA) developed by Ullrich and Grosch (20) and a variation, the Aroma Extract Concentration Analysis (AECA) by Kerschler and Grosch (21), and CHARM Analysis developed by Acree and Barnard (22). These two methods evaluate by gc/olfactometry a dilution (or concentration) series of an original aroma extract from a food (Fig. 1). Note is taken of the occurrence of an aroma (its retention time or Kovats index) in each

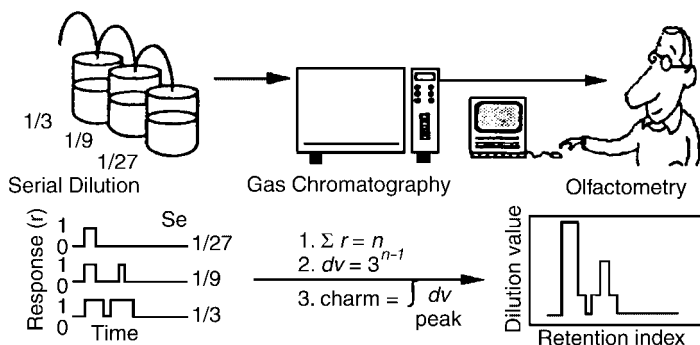


Fig. 1. Schematic of the gc/O system used in obtaining CHARM data (23).

dilution. One then adds the occurrences of an odorant across dilutions. The greater the number of dilutions in which an odorant is sensed, the higher its CHARM or Dilution Value. One generates a plot of Dilution or CHARM values as a function of gc elution time (i.e., retention index, Fig. 1, lower right). Both AEDA and Charm methodologies originally proposed that the larger the dilution value (number of dilutions until an odorant cannot be perceived at the sniffing port), the greater the contribution of that compound to the overall aroma.

Two other gc/O methods have also found application for this purpose. One is called OSME and the other NIF (nasal impact frequency) or SNIF (surface of nasal impact frequency). OSME was developed by McDaniel and co-workers (24) and has been applied to wine aroma studies. In this method, a panelist evaluates the aromas eluting from a gc column and responds by moving a variable resistor as aroma intensity changes (Fig. 2). Thus, one is obtaining intensity and duration measurements of each gc peak. There are no dilutions made of the sample, which facilitates the use of a larger number of judges as opposed to being limited to two or three judges when using dilution methods

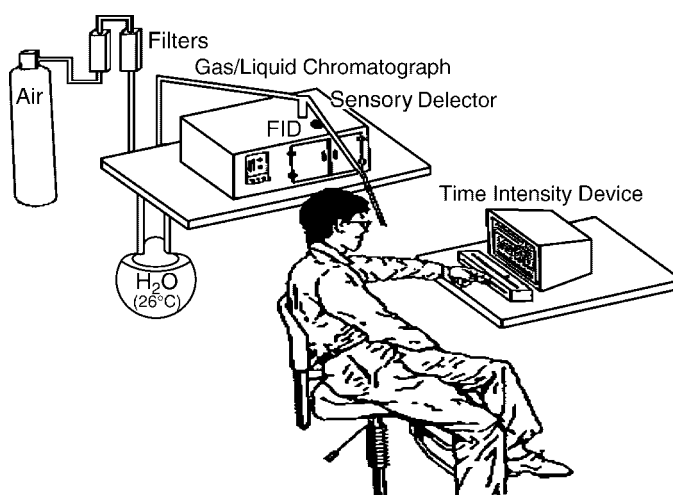


Fig. 2. The gc/O system used by McDaniel for obtaining OSME data (24).

(very tedious). This adds further validity to the method. The importance of an odorant to the overall aroma is judged based on relative sensory intensities during sniffing. This is a fundamental difference between the dilution methods (AEDA, OAV, and CHARM) vs. OSME. The dilution methods are based on the principle that compounds present at the greatest multiple of their threshold are most important to aroma. This violates a basic law of sensory science in that there is a power function relationship between concentration and sensory intensity, and that relationship is different from one aroma compound to another. Thus, ranking compound importance based on OAV, CHARM, or AEDA value has only a weak theoretical basis. This weakness in dilution methods is recognized and these values are now considered as screening as opposed to “hard” numbers, ie, compounds with the highest values are candidates for further study to evaluate their true contribution.

The NIF (or SNIF) method was developed by Pollien and co-workers (25) [see also (26)]. In this method, 8–10 untrained individuals sniff the gc effluent (one at a time). They simply note when they smell an odor. The aroma isolate used is adjusted in strength such that in a single gc run, ~30 odorants are perceivable to the sniffers. This adds an element of selection in that only the more intense aroma compounds will be evaluated. The number of sniffers detecting an odorant is tabulated and plotted. Those odorants (gc peaks) being detected by the greatest number of individuals are considered likely to be the most important odorants. This method also suffers from weaknesses. One problem is that for two compounds in an aroma isolate, one may be barely over the sensory threshold of all sniffers while another may be a great distance above its sensory threshold for all sniffers, and yet both of these compounds would be viewed as being equal by this methodology.

There is no clear choice in methodology to use when determining key aroma components of a food. All methods are complicated by biases in preparing aroma isolates for analysis, by anosmia amongst panelists, human variability and bias, as well as problems interpreting the contribution of an aroma compound singly and out of the food matrix as opposed to being in a food and part of a complex aroma mixture. These weaknesses are acknowledged but there is no alternative, “correct” methodology. Ultimately, one must do sensory studies to determine what aroma compounds are needed to reproduce the aroma of a food. This involves recombination studies involving sensory analysis. Since there must be some preselection of aroma compounds to use in the sensory studies, any of these selection methods may suit the purpose.

Ideally the end result is that one has chemically defined the aroma compounds needed to reproduce the aroma of a food. However, despite >40 years of “modern” aroma research, no food has been chemically characterized in the sense that the key aroma components AND their required concentration limits have been defined. This is primarily due to the complexity of conducting sensory studies involving 20–30 variables (ie, aroma compounds).

3.3. Analysis of Taste Substances. Taste has generally been thought of as a relatively simple sense being composed of salt, sweet, sour, bitter, and umami sensations. This simplification is not justified since it is clear that each basic taste sensation has many nuances. Sour can be used to illustrate such nuances. The sour sensation is different for lactic acid (sour milk), tartaric

acid (grapes), citric acid (citrus), acetic acid (vinegar), or hydrochloric acid. While all of these acidulants are sour, they each differ in sensory character and temporal effects. There is no single sour perception any more than there is a single sweet, bitter or salt sensation. Each taste compound yields a different and unique taste character that complicates this basic sensory component.

Furthermore, it is worthwhile to note that taste influences aroma perception. For example, if one uses citric acid in a food system, the citrus aroma of the flavor will be enhanced. Phosphoric acid is intimately associated with certain cola flavors. Tartaric acid supports grape flavors. Thus, while each acidulant gives a unique sensory character (taste), it also influences our overall flavor perception (interaction with aroma to give an overall flavor perception).

From a chemical analysis perspective, taste can readily be accounted for through well-established analytical techniques. For example, sour can be readily determined through organic (or inorganic) acid analysis via high pressure liquid chromatography (hplc). Sweet or salt can likewise be accounted for through the analysis of known mono and disaccharides or high potency sweeteners (eg, aspartame and acesulfame k), or inorganic salts, respectively. Umami is due primarily to monosodium glutamate or the 5'-nucleotides (some peptides are considered to have a umami character). Bitter is more difficult for there are many diverse compounds known to cause a bitter sensation (27). There is little analytical challenge in analyzing these taste compounds except for bitter. A general overview of methods can be found in basic food analysis texts (28).

Nonvolatile components (as a whole—taste and nontaste) in foods play a greater role in food flavor than just defining the taste sensation. Nonvolatiles in foods are known to interact with some aroma compounds (chemically “bind”) thereby exerting an additional influence on flavor ((29–31). For example, Hoffmann and co-workers (32,33) reported that melanoidins in coffee reduce the intensity of the roasty-sulfury aroma notes in coffee. They found that melanoidins promote the degradation of 2-furfurylthiol (FFT), 3-methyl-2-buten-thiol, 3-mercapto-3-methylbutyl formate, 2-methyl-3-furan-thiol, and methanethiol—all key odorants in coffee. Likewise, Ebeler and co-workers (34–36) have found an interaction between the polyphenolics in wine and certain aroma compounds. Thus, nonvolatile components in foods that might be considered to have no taste per se may still exert an influence on the flavor of a food. (We might also hypothesize that there may be unrecognized cognitive effects between nonvolatiles and overall flavor perception.) Thus, we may be interested in the analysis of nonvolatiles in foods beyond those that contribute to taste perception.

Our interest in the analysis of nonvolatiles, thus, may involve taste substances or substances that indirectly influence taste or aroma. As mentioned earlier, in the first case, we are interested in the analysis of substances that impart sweetness, tartness, bitterness, saltiness, or umami sensations. The analysis of these substances is reasonably well defined. In the latter case, the analyses employed are less well defined and are unique to the components one wishes to analyze. For example, we may wish to measure substances (melanoidins) that interact with sulfur aroma compounds (in coffee). There are no standardized methods for the analysis of melanoidins in foods and thus, the protocols need to be developed. In this article, we will only briefly discuss the established methods for the analysis of taste substances. Due to the specificity of methods for the

analysis of nonvolatiles that may indirectly influence flavor perception, we will only refer the reader to the references cited above.

Sweeteners. Sweeteners used in the food industry typically are limited to the bulk sweeteners, sucrose, fructose, glucose, and corn syrups, or the high potency sweeteners, saccharin, aspartame, sucralose, and acesulfame k. While various enzymatic and colorimetric methods may be used, high-performance liquid chromatography (hplc) is the most commonly used technique in this analysis (37). Hplc offers speed, sensitivity, accuracy and precision to the analyst. Several types of hplc columns (anion and cation exchange, normal phase and reversed phases) may be used in conjunction with a suitable detector. Traditionally, refractive index detectors have been used but they lack sensitivity and cannot be used with gradient elution programs. Thus, electrochemical detectors (eg, a pulsed amperometric detector) have found recent application.

Salt. One may be primarily interested in the determination of table salt (NaCl) or one of the salt substitutes (NH_4Cl or KCl) depending on the product application. While there are numerous methods for the determination of minerals (eg, salt) in foods, table salt is most easily analyzed using a specific ion electrode (38). The simplicity and sensitivity of this method are very attractive with detection limits being <0.1 ppm and response time <30 s.

If one wishes to measure other salts, the choice is generally ion chromatography (39) or atomic absorption–emission spectroscopy (40). The chromatographic approach uses an ion exchange column coupled with a conductivity detector.

Acidulants. Both organic and inorganic acids are broadly used in foods. The organic acids used include citric, malic, tartaric, acetic, and lactic while hydrochloric and phosphoric acids constitute the commonly used inorganic acids. These taste components are commonly measured using ion chromatography (39).

Umami. Monosodium glutamate (MSG) and the 5'-nucleotides are generally recognized as the primary food components that provide the umami sensation (41,42). MSG is readily measured by ion chromatography or reverse phase hplc (39). The 5'-nucleotides are most commonly determined by hplc as well (43,44), but other methods have found use, eg, derivative spectrophotometry (44).

Bitter Substances. As was mentioned earlier, bitter substances are composed of a broad range of chemical structures, thus, there often is little commonality in structure to permit the utilization of a single analytical approach. Methods for this analysis have to be designed for each bitter component (or group of components) to be analyzed. There are numerous methods in the literature most depending on hplc since bitter substances are typically nonvolatile (34,45,46).

Selection of Taste Substances. While the analysis of taste substances be may less problematic than aroma substances, the determination of their contribution to flavor is no less complicated. Presently, methods for this purpose have been developed analogous to those for aroma substances. Taste substances are isolated from foods, they are separated into individual components and then literally tasted to determine if they have a sensory component. If they have a sensory component, they are quantified in the food and their concentration can be compared to their sensory threshold in a similar food matrix. If they are present above their sensory threshold, they are considered likely to make a

sensory contribution to the flavor of that food. If not, perhaps they do not. The task of determining contribution to flavor is extremely complex and again demands very complex sensory studies. To date, this task has not been approached in great detail. Research articles by Engel and co-workers (47,48), Preininger and co-workers (49), Yang (50) and Warmke and co-workers (51) outline current attempts at approaching this problem in cheeses.

3.4. The Chemical Analysis of Trigeminal Stimuli. The chemical characterization of substances in food that give a trigeminal response is less problematic than that of aroma or taste primarily because of the limited number or compounds known to elicit a trigeminal response. Also, these substances in any given food are typically closely related compounds and they have been well researched. For example, the capsaicinoids are common to the capsicum spices (peppers), gingerone and shogaol (ginger), piperine (black pepper), isothiocyanates (mustards), carbon dioxide (natural or added), thiopropanol-S-oxide (onion) and menthol (mint) are the primary members of this group of flavorants. Compounds that elicit a trigeminal response can do so in the mouth or olfactory region. While most of these compounds elicit a pain response, menthol also provides a cooling effect to subsequent air or liquid stimuli.

As noted above, these compound are generally known entities and methods have been developed for their analysis in foods. In the case of the capsaicinoids, instrumental data have been correlated to sensory intensity. For example, the AOAC has published a standard method to determine the “heat” of these compounds in chilis and chili products.

In this method the three major “heat” compounds, nordihydrocapsaicin, capsaicin, and dihydrocapsaicin, are quantified and using a formula, sensory intensity is predicted. This has not been done for the other trigeminal compounds.

The major problem in the chemical characterization of this flavor component is for taste interactions. While we have a good understanding of these compounds in isolation, their effect in complex food systems where numerous interactions may occur becomes problematic. We lack an understanding of these complex interactions both at receptor and cognitive levels.

4. Summary

If we consider our overall capability to chemically characterize the flavor of a food, we are sorely lacking. In the 40+ years that we have had the sophisticated instrumentation needed to even approach this problem, we have progressed in a slow manner. As with all fields of knowledge, the process has been evolutionary: as we have achieved success in one area, it has only moved us up to the next barrier. Forty years ago we thought that we could chemically determine all of the aroma compounds in a food and then have flavor defined. This was not the case. Years of additional effort involved linking this identification work to sensory evaluation to determine the true “key” components of aroma. This got us only a little closer to chemically defining the flavor of a food because recombinations of these “key” aroma compounds did not reproduce the desired sensory

responses. We came to realize that it is not only the chemical compounds in a food, but how they are released from that food during eating (a subject not discussed in this article). Although research has focused on flavor release for >10 years we are only coming to recognize that this is still not the answer. Flavor perception is truly multimodal. We must consider ALL of the stimuli contributing to flavor perception. This involves not only the sensory perceptions of flavor (taste, aroma and trigeminal sensations) but nonflavor sensations such as appearance and texture plus the cognitive input of experience, situation, emotional state, etc.

Chemical characterization of food flavor will continue to be sought as a "Holy Grail" and undoubtedly progress will be made that brings us additional understanding that will be useful to the academic and industrial communities. However, it is highly doubtful that flavor will be chemically characterized within the next several generations.

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