

Terminology and Analytical Approaches in Food Flavor Research

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Abstract: The eating quality of a food product, which includes aroma (odor), taste, and texture along with nutritional value, price, and ethnic habits of consumers, is an essential factor in determining the product acceptance and preference in the marketplace. One of the first responses of consumers to a specific food is whether its flavor is liked or disliked. Obviously, if a consumer does not like the flavor of the product, he or she probably will not purchase the product regardless of its price and nutritional value. In other words, an appetizing food is frequently distinguished by its flavor characteristics. Although the term flavor has been given different meanings throughout the literature, it can be defined as the interaction of taste, odor, and textural feelings that provides an overall sensation when a particular food material is consumed. The noses and tongues were the only tools to analyze flavors until recently (sensory analysis). Today, although these human instruments continue to provide the last words on flavor compounds and their impact, the routine availability of purge and trap devices, gas chromatography, mass spectrometry, infrared and nuclear magnetic resonance spectroscopies has enabled flavor researchers to achieve isolation, concentration, separation, and identification at unprecedented resolutions and sensitivities. The developments have been mainly observed in the fields of sampling, including volatile isolation and concentration, and separation and characterization of volatiles in foods.

Keywords: food flavor, taste, odor, flavor research and analysis

Gıda Lezzet Araştırmalarında Terminoloji ve Analitik Yaklaşımlar

Özet: Bir gıda maddesinin tüketim kalitesini aroma (koku), tad, tekstür (yapı), besleyici değer, fiyat ve bölgesel tüketici tercihi belirlediği için, bir gıdanın piyasada kabul görmesinde onun tüketim kalitesi temel faktördür. Tüketicilerin herhangi bir gıdaya karşı ilk tepkilerinden biri gıdanın lezzetinin beğenilip-beğenilmediği şeklindedir. Eğer tüketici gıdanın lezzetini beğenmemişse, gıdanın fiyatı ve besleyici değerine bakmaksızın almaktan vazgeçecektir. Diğer bir ifadeyle, iştah açıcı bir gıdayı o gıdanın lezzeti belirler. Literatürde lezzet terimi değişik anlamlarda kullanılmasına rağmen; gıdanın tüketimi sırasında tat, koku ve tekstür özelliklerinin etkileşimi sonucu bıraktığı his şeklinde tanımlanabilir. Yakın zamana kadar burun ve dil yardımıyla gıdalarda lezzet analizi (duyusal analiz) tek yaklaşımdı. Günümüzde, insan organları gıdalarda lezzet maddelerinin tanımlanması ve etkilerinin değerlendirilmesinde hala son sözü söylemesine rağmen, purge-trap cihazı, gaz kromatografisi, kütle spektrometresi ve infrared ve NMR spektroskopilerinin rutin olarak kullanımı, lezzet maddelerinin iyi bir duyarlık ve çözünürlükte izolasyonu, konsantre edilmesi, ayrılması ve tanımlanmasını sağlamıştır. Bu alandaki gelişmeler özellikle gıdalardan lezzet maddelerinin örneklenmesi, ayrılması ve tanımlanmasında görülmektedir.

Anahtar Kelimeler: gıda lezzeti, tad, koku, lezzet araştırmaları ve analizi

Terminology Associated with Flavor

The term flavor has been given different meanings throughout the literature. According to Belitz and Grosch (1987), flavor is the interaction of taste, odor, and textural feelings that provides an overall sensation when a particular food material is consumed. Hall (1968) defined the term flavor as the sensations produced by a material taken into the mouth, perceived by the senses of taste and smell, and also by the general pain, tactile, and temperature receptors in the mouth. Based on this definition, flavor is a property of both the food material and the receptor mechanisms of the person consuming the food.

The term flavor sometimes refers only to the olfactory (smell or odor) and aromatic

aspect of foods, in which case the term taste means gustation. Sometimes it refers to both gustatory and olfactory sensations together. However, it is proper to include the tactual or feel sensations as a valid and unavoidable component of flavor because tactual sensations have a significant influence on the rate of the release of gustatory and olfactory stimuli, and further have an equal role with gustation and olfaction in the comprehensive consideration of the overall flavor sensation (McGrory and Dirks, 1966). Since tastes and aromas are embedded in such textural matrices as liquid, solid, or semisolid (Christensen, 1977), textural feelings are often incorporated into the definition of the term flavor. In addition, there are other qualities contributing to the overall

flavor sensation, such as smoothness, roughness, granularity, and viscosity as well as hotness, coolness, fullness, astringency, and pungency (DeMon, 1980). In a broad sense, flavor consists of a collection of responses to the stimuli that include gustatory, olfactory, and tactual sensations.

The term taste refers to the sensations perceived by the stimulation of the gustatory receptor cells of taste buds that are located mainly on the tongue (Caul, 1966). Tastes are traditionally classified into four distinct groups: sweet, sour, bitter, and salty (Govindarajan, 1979). Taste is perceived through the receptor cells of tongue upon the interaction of taste compounds with those cells because taste compounds are generally non-volatile at body temperature (Boudreau et al., 1979). It is, therefore, required of a taste substance to be soluble in water or aqueous solutions to some extent in order to provide gustatory sensation (Ramirez, 1991). Taste perception of foods is substantially affected by particle size, texture, consistency, and temperature (Harrigan, 1997).

The term aroma or odor means the sensations perceived by the stimulation of olfactory receptor cells and free nerve endings in the nasal cavity. The olfactory system serves important functions, including perception of smell and flavor. Olfactory perception results from a cascade of events that begin with the arrival of airborne odorant molecules at the periphery of the olfactory system and end in physiological impacts, which in turn leads to a response to the stimuli as a result of the interaction of odorant molecules with the receptors. After the initial binding of volatile molecules to the receptor surface, a chain of second messenger events involving G-proteins takes place resulting in depolarization of the olfactory receptor cells. This electric signal is then transmitted through neurons to the olfactory bulbs and to the receptor cortex of the brain. The olfactory cortex, in turn, transmits the information to the hippocampus which is a primitive brain structure involved in memory storage and retrieval (Cardello, 1996).

Unlike the tastes, odors are not so simply classified, nor are the classifications widely accepted because of the existence of thousands of different odor qualities. Due to such a large number of distinct odor qualities, a variety of odor classification schemes have been

developed (Cardello, 1996). Most aroma materials, in contrast to taste substances, are volatile organic compounds at ambient temperature.

The properties of molecules play a significant role in the arrival of the molecules in the vicinity of receptors and receptor-molecule interactions. Based on this fact, a couple of structure-odor relationships have been proposed. Amoore (1964) proposed the olfactory functioning theory (key and lock relation) which argues the existence of a specific stereo chemical arrangement of stimulant molecules and receptor sites. According to the theory, receptors are sensitive to the shape, size, and electronic status of odorous molecules. Based on the number of specific anosmias reported, primary odor qualities were classified as sweaty, spermous, fishy, camphoraceous, minty, malty, and uranous. Beets (1964) proposed that the shape of volatile compounds and the nature and disposition of their functional groups play a significant role in determining the direction of the molecular dipole vector and the orientation of the molecules. Functional groups may be either polar, such as carbonyl and hydroxyl groups, or non-polar, such as methylene. Another structure-odor relationship proposed by Chastrette and Zakarya (1991) is based on hydrogen bonding and dispersion interactions. In addition to molecular shape and functional groups of volatile molecules, the interactions through hydrogen bonding rather than the orientation of dipoles were emphasized. This structure-odor relationship model correctly predicted the musk odor of various compounds, including benzene and coumarin derivatives. The theory also proved that the odor was closely related to molecular shape, volatility, and electronic properties.

Flavor impressions are conveyed by the senses of smell and taste. According to Moskowitz (1977) and Amato (1988), smell is the most important characteristic of flavor that allows consumers to form an overall impression, but the sense of taste also has a significant contribution to flavor perception.

There is an unavoidable connection between the flavor and texture of a food. Vickers (1977) stated that the texture-flavor interaction can be observed in two different ways. One is the interaction between the

sensory systems perceiving flavor and those perceiving mechanical properties. For instance, stimulating one sense may enhance or depress to some degree the sensitivity of other senses. The other relation is that the changes in the structural and mechanical properties of a food affect the rate and extent of flavor formation and release. In many cases, viscosity modifying agents, such as starches and gums, may directly or indirectly alter the rate and release of taste and odor compounds, thus affecting flavor intensities of solutions to which they are added. This influence is not a direct result of viscosity, but originates from an interaction between taste or aroma compounds and the hydrocolloids. Consequently, flavor molecules may be physically entrapped within the food matrix, or may be bound to certain ingredients specifically or non-specifically (Carr et al., 1996). The structural and mechanical properties of foods are mainly provided by large polymeric molecules, namely starch, proteins, and non-starch polysaccharides. Morris (1987) found that thickened, structured, or viscous systems require a higher concentration of flavorings and sweeteners to produce the desired sensory stimulation than do aqueous or fluid systems. This phenomenon was explained by Godshall and Solms (1992) as the following: either the diffusion of flavor molecules to the taste buds was hindered or taste buds were physically coated and flavor molecules became unavailable for perception as a result of complexing with starch or other polysaccharides. They also acknowledged that starch serves as a carrier and protector of flavor in foods and interacts with sweeteners in baking affecting baking quality and flavor perception.

The science of food flavor has more intensely investigated the volatile compounds in foods and their contribution to the overall flavor profile rather than the contribution of taste and texture to the flavor. The first reason is that volatiles are primarily responsible for the aroma component of significant flavors and the number of possible compounds and their permutations are almost limitless. The second reason for more emphasis on volatiles than taste substances is the ease of their separation and identification. Volatile compounds can be much more easily separated from complex natural products than nonvolatile taste substances (Risch and Ma, 1989). Due to such a trend in

flavor research, the majority of publications cover volatile components of flavors.

Methodology in Flavor Research

Volatile flavor chemicals are one of the major areas of interest among food chemists. The complexity of volatile concentrates of flavors, composed of hundreds of compounds in concentrations ranging from several percent to ppb, has forced and encouraged flavor chemists to develop new techniques. According to Amato (1988), noses and tongues were the only tools to analyze flavors until recently. Today, although these human instruments continue to provide the last words on flavor compounds and their impact, the routine availability of purge and trap devices, gas chromatography (GC), mass spectrometry (MS), infrared spectroscopy (IR), and nuclear magnetic resonance spectroscopy (NMR) has enabled flavor researchers to achieve isolation, concentration, separation, and identification at unprecedented resolutions and sensitivities. The developments have been mainly observed in the fields of sampling, including volatile isolation and concentration, and separation and characterization of volatiles in foods.

Sample preparation for GC, which is an unavoidable tool in the hands of flavor chemists, is one of the critical areas in instrumental flavor analyses. Since it is generally impossible to directly inject a food material into GC, certain sample preparation, component isolation, and concentration are required prior to GC analysis. Trace amounts of compounds in foods are often critical in imparting the proper taste and aroma or causing foods to have an off-taste and odor. Therefore selection of an appropriate sampling technique is essential to obtain reliable and accurate results (Penton, 1996).

The isolation of volatiles can be carried out by a number of procedures. These techniques include headspace analysis (equilibrium or dynamic), vacuum or steam distillation, solvent extraction, adsorption, solid phase microextraction, supercritical fluid extraction, and combination or modification of those methods depending on the nature of the products to be analyzed.

Of those extraction techniques, dynamic headspace sampling through a purge and trap instrument has found wide use in recent years in

flavor analysis. The method involves simply purging the sample with an inert gas and sweeping the headspace vapors onto a porous polymer adsorbent trap, followed by purging of water, then thermal desorption or solvent elution prior to cryofocusing of volatiles at the top of the GC column. Dynamic headspace sampling technique is a simple and useful isolation method and the volatiles collected through a purge and trap concentrator closely resemble those in the original sample (Chen et al., 1982). Chang et al. (1995) cited the use of dynamic headspace method in the analysis of volatile components of peanuts, passion fruit juices, corn roots, sweet potatoes, crayfish tail meat, and crab meat by a number of researchers. This volatile isolation and concentration method has also been used for the analysis of grain volatiles by Seitz (1995) and for bread volatiles by Chang et al. (1995). The properties of headspace sampling and its applications in food flavor analysis have been reviewed in detail by Charalambous (1978).

Much progress has been made in the area of flavor research with the advent of GC, particularly with the availability of various types of capillary columns for gas chromatographic separation. Gas chromatography has become the most widely used analytical separation technique in the analysis of food volatiles since its first appearance in 1952 (Lamkin et al., 1986). Introduction of capillary columns has further expanded its use in food flavor analysis. Gas chromatograph provides rapid qualitative and quantitative analysis of complex mixtures with precision and sensitivity (Dickes and Nicholas, 1976).

Gas chromatographic separation of volatiles is the best available technique for the separation of organic compounds. Volatile organic compounds are separated from one another on the basis of their relative volatility by a special column through which an inert carrier gas is passed. Each compound gives a specific peak on the recording chart. Although GC is highly sensitive, it is not as sensitive as human nose for many flavor and aroma compounds. Furthermore, the instrumental approach does not provide any clue as to whether the flavor is liked or disliked. In other words, qualitative and quantitative analysis of volatile components of food flavors can be

achieved by GC; however, no information on the type and flavor characteristics of volatiles and their contribution to the overall flavor note is provided. For this reason, sensory analysis, such as flavor profile analysis and descriptive sensory analysis, carried out by trained panel people is required. GC effluent sniffing (GC-Olfactometry) is another method widely used to provide odor information about the compounds separated by GC (Fischer and Eijk, 1996).

In addition to GC effluent sniffing and sensory flavor profile analysis, a number of methods that combine the results of the sensory evaluation and GC analysis of food volatiles have been developed in order to figure out the character, quality, importance, and contribution of a specific compound to the overall flavor note. Traditionally, contribution of a GC-separated volatile compound to the flavor is predicted through calculating the ratio of the concentration of the volatile to its odor threshold value. Odor threshold value is defined as the lowest concentration of a compound that can still be directly recognized by its odor (Belitz and Grosch, 1987). The result is then expressed as odor activity value (OAV). Based on the OAV of the volatiles, the character impact compounds are distinguished from those with low or no odor activity. In recent years, a new instrumental and sensory analysis that allows the identification of the volatiles which contribute to the flavor of a food has been developed. The methods, called Charm Analysis (Acree et al., 1984) and Aroma Extract Dilution Analysis (AEDA) developed by Ullrich and Grosch (1987), are based on the evaluation of an extract obtained from a food by GC-olfactometry (GCO). The extract is then diluted in series and each dilution is analyzed by GCO technique. In the AEDA, a flavor dilution (FD) factor, which is the ratio of the concentration of the odorant in the initial extract to its concentration in the most diluted extract in which an odor was detected by GCO, is calculated. As a result, FD factor indicates the relative measure of odor potency of a compound that is proportional to the OAV of the compound in the air (Grosch and Schieberle, 1997).

Qualitative assessment of GC data depends on some measures of the position of peaks, namely retention time and volume or variations of these parameters. Quantitative analysis is, on

the other hand, based on the size, shape, peak areas, and the direct comparison between sample compound and standard material (Reineccius, 1994).

Identification of volatiles upon separation by GC is mostly performed by mass spectrometry (MS). In most cases, particularly in flavor analysis, GC is coupled with MS. Mass spectrometry has long been useful in the structural elucidation of volatile compounds, particularly organic compounds. Mass spectral data, together with GC retention indices, are sufficient for the elucidation of the structure of compounds having only one functional group or low molecular weight. The results are usually confirmed by infrared spectroscopy (IR) and, if necessary, by nuclear magnetic resonance spectroscopy ($^1\text{H-NMR}$) and derivatization analysis (Belitz and Grosch, 1987).

The basic working principle of MS is to produce ions by bombarding organic molecules with high energy electrons, then accelerating those ions in a definite direction, so that they can be separated according to their mass or velocity. The separated ions are then detected and their intensities are measured (Pomeranz and Meloan, 1994). An MS functions as a GC detector when coupled with GC. Since the fragmentation pattern for a given compound is unique under a given set of conditions, mass spectral data can be interpreted by trained people or compared with large mass spectral databases and libraries visually or by computer matching to determine the identity and structure of an unknown compound.

Infrared spectroscopy (IR) is another identification technique for GC-separated organic compounds and to confirm the results obtained through MS. IR radiation is an electromagnetic energy with wavelengths longer than visible light but shorter than microwaves and ranges from 680 to 100,000 nm in wavelength. A molecule can absorb IR radiation if it vibrates in such a way that its charge distribution, therefore its electric dipole moment, changes during the vibration. A molecule can only absorb those frequencies of IR radiation that exactly matches its own vibrational frequencies. Infrared spectroscopy, therefore, deals with the interaction between

molecules and infrared radiation. The atoms in a molecule vibrate in complicated patterns and frequencies depending on the masses of the atoms and the strength of the bonds connecting them to each other. Most important vibrations that produce a change in dipole moment are stretching and bending (scissoring) motions. Because many functional groups have unique patterns of infrared absorption bands, infrared spectra are useful for qualitative analysis of volatiles. Based on this fact, IR can be used as a kind of molecular fingerprint (Wehling, 1994, Pomeranz and Meloan, 1994). Since absorption in the IR region arises mainly from C-H, N-H, O-H, and C-O bonds, organic compounds give strong absorption (Hirschfeld and Stark, 1984).

For qualitative analysis using IR, two approaches are commonly employed: library search and spectrum interpretation. If the IR spectrum of an unknown sample exactly matches that of a known compound, the unknown is then probably that compound, provided that the unknown sample is pure. This is one of the reasons why GC is coupled with IR. Spectrum interpretation can also be employed to identify an unknown sample by its IR spectrum. This approach is based on the vibration and absorption of certain functional groups at certain frequencies. Presence or absence of absorption at these certain frequencies provides clues about the molecular structure of an unknown sample. Spectrum interpretation definitely requires a certain level of expertise (Anonymous, 1993). Infrared spectroscopy is therefore a powerful technique, particularly when combined with MS, for the structural characterization of organic volatile components of flavors.

Of the IR techniques, Fourier transform infrared spectroscopy (FTIR) has gained wide use in the analysis of complex flavors that could match the important contributions of GC/MS (Sanders, 1984). In an FTIR, in contrast to a dispersive IR, the IR radiation is not dispersed, but rather all wavelengths arrive at the detector simultaneously and a mathematical treatment, called Fourier transform, is used to convert the results into a typical IR spectrum. Today, FTIR offers the highest resolution and speed (Pomeranz and Meloan, 1994).

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