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RESEARCH REVIEW

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Flavor Release from Ice Cream during Eating

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Abstract The main purpose of flavor research using conventional extraction methods, such as solvent extraction, distillation, and dynamic headspace, is to effectively extract, identify, and quantify flavor volatiles present in food matrices. In recent flavor research, the importance of understanding flavor release during mastication is increasing, because only volatiles available in the headspace contribute to the perception of food 'flavors'. Odor potency differs among flavor volatiles, and the physicochemical characteristics of flavor volatiles affect their release behavior and interaction with various food matrices. In this review, a general overview of flavor release and flavor-food interactions within frozen dessert systems is given with emphasis on chemical, physiological, and perceptual aspects. Chemical and sensory analysis methods competent for investigating such flavor-food interactions are illustrated. Statistical analysis techniques recommended for data acquired from such experiments are also discussed.

Keywords: flavor release, frozen dessert, flavor-food interaction, chemical analysis, sensory analysis

Introduction

Eating is a complex sensory experience that includes seeing, smelling, first bite sensation, mastication, and swallowing. Food undergoes drastic physical and chemical changes during this process. A series of sensory experiences during eating leads to the creation of textural and flavor perceptions that determine the consumer acceptability of food. Flavor, which is one of the most important factors influencing the acceptability of a food product, is the integrated perception of taste, smell, and tactile sensations during eating. Aroma compounds must initially be released from the food matrix to the buccal headspace and transferred up to the nasal cavity in order for the flavor to be perceived (1). Therefore, the initial headspace concentration of the flavor compounds of food and the interaction of flavor with non-flavor food ingredients are critical in determining the amount and the rate of flavor compounds to be released.

The profile of volatile compounds extracted by conventional methods such as static headspace, solvent extraction, and vacuum distillation, will differ from the profile of volatiles that are released during eating because the releasing conditions differ (2, 3). For instance, the introduction of airflow, physical breakdown, hydration, enzymatic hydrolysis, and mechanical mixing occurs during the mastication of solid food. These series of events during mastication will affect the amount of volatiles released from the food matrix, and the temporal change of volatile release behavior during eating also becomes a critical factor that contributes to the overall flavor balance. For example, the hydrolysis of starch by amylase can occur within less than 10 sec during eating (4), thus in the case of products consisting of carbohydrate based ingredients, enzymatic hydrolysis will significantly influence not only the oral texture of the food but also the release behavior of flavor compounds (5).

Analytical tools that incorporate in-mouth conditions and are, therefore, capable of representing the flavor profile that humans perceive have to be developed. The instrumental approaches currently taken to understand flavor release during eating are direct breath analysis during eating (6-16) and flavor analysis using an in-mouth simulating system (2, 9, 17-22).

Prior to the development of these instrumental tools, sensory analysis was the only effective method to understand flavor-food interactions. It may also be considered as the ultimate method, because humans are the ones who will eventually evaluate the food product. Humans are very sensitive at detecting and differentiating flavors caused by small differences in individual flavor compounds, some of which instruments cannot detect. While sensory analysis alone cannot be used to determine chemical changes, but rather only changes in perception, instrumental analysis is incapable of discerning the effect of a change in chemicals on the change in perceived flavor. The combination of instrumental and sensory analyses forms a powerful tool to understand flavor binding and release in the mouth because the two methods complement each other.

The main purpose of this review is to give a general overview of flavor release and flavor-food interactions in frozen dessert systems with emphasis on the chemical, physiological, and perceptual aspects. The latest developments in analytical technologies, specifically chemical and sensory analysis methods, competent for investigating such flavor-food interactions will be introduced. Finally, statistical analysis techniques recommended for the data acquired from these experiments will be discussed.

Physico-chemical aspects of flavor-food interactions

Flavor is perceived when odor-active volatiles reach the olfactory epithelium after being released from the food matrix in the buccal headspace during eating. The interac-

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tions between the volatiles and the non-volatile food matrix are important in understanding the release of volatiles. McGorrin (23) classified the forms of interaction that occur into three types; binding, partitioning, and release. The binding of a volatile to the food matrix reduces the amount of free volatile that can be released to the gas phase for the subject to perceive (24, 25). Partitioning, which is defined as "the distribution of flavor substances between the oil, water, and gas phases" (23), plays a key role in determining the concentration of the flavor compound in the headspace, water or lipid portion of the food after equilibration (26). The hydrophobicity of the compound and the composition of the food matrix (i.e., water-in-oil or oil-in-water system) will determine the partitioning of the compound in the different phases (27). Partitioning is especially important for the initial flavor impact of products that have had a chance to equilibrate (i.e., packaged product that is stored) (28).

Equilibration between the different phases is hardly ever achieved in a real food system during consumption, and partitioning is not sufficient for delineating the temporal release of flavor. The food matrix is mechanically disrupted by mastication. The solid food is diluted with saliva and air during eating. Studies show that the rate-limiting step of flavor release from the matrix is the resistance to mass transfer in the different interfaces (solid vs. liquid vs. gas) (29-31). The volatiles need to be transferred from the solid food to saliva (liquid) and then to the gas phase to be perceived. The resistance to mass transfer will vary depending on the characteristic of the interface; whether the volatile is transferred from lipid to water, water to lipid, or water to gas, etc. This parameter needs to be well understood to predict the release behavior of a volatile compound.

The role of fat in food systems

Fat serves various functions in food that affect the appearance, mouthfeel, and flavor of the product. Current fat replacer technology has given reduced fat foods a texture very close to that of full-fat foods. However, mimicking other functionalities of fat, especially in regard to flavor, is still a challenge because fat is a suitable solvent for hydrophobic aroma-active compounds. By dissolving flavor compounds, fat controls the flavor intensity and the release rate from food matrices, consequently giving a balanced and rounded flavor characteristic. The affinity of flavor compounds for fat or water is another important factor affecting the flavor-fat interaction. Guyot *et al.* (27) investigated the effect of fat content on the headspace concentration of three aroma compounds varying in the degree of fat affinity in a model emulsion system. An emulsion system containing diacetyl or butyric acid, both of which are hydrophilic odorants, produced a stronger odor when the system had a higher fat content. The opposite observation was made for hydrophobic compounds (i.e., *d*-decalactone). Chain length and the degree of unsaturation of a flavor volatile also affect its interaction with the model food system under investigation (32).

Fat can also mask undesirable flavors (33). It acts as a precursor of many flavor compounds (34) and protects flavor compounds from degradation (35). Therefore, a noticeable change in the total aroma profile occurs when fat is removed or reduced in the original product (36). For example, intense and unbalanced flavor profiles in low-fat frankfurters and low-fat cheddar cheese are some of the flavor defects due to reduction of fat in the system (33, 37). Additionally, fat is involved in controlling the temporal release of flavors during eating (38, 39). Flavors are slowly released as the fat melts during eating, due to the viscosity of fat and the affinity of aroma compounds for fat. Therefore, fat contributes to a balanced overall flavor and desirable aftertaste. When the fat is reduced, the time to reach the maximum intensity is reduced and the maximum intensity is increased, causing an imbalance of flavor in the product (15).

Flavor-matrix interactions in frozen desserts

Ice cream, which is one of the most widely consumed products in the frozen dessert category, is a mixture of milk, cream, sweetener, stabilizer, and air cells frozen to form a semi-solid matrix. The milk fat, milk protein, and carbohydrates from these ingredients construct the matrix of ice cream and interact differently with the flavor compounds in ice cream.

Many studies have investigated fat-flavor interactions in frozen dessert systems (40-43). In frozen desserts, the functionality of fat plays an important role in determining the texture and flavor characteristics of the product. Due to the complexity of the frozen dessert system, sensory analysis is frequently the method of choice to delineate the fat-flavor interactions. Sensory analysis is advantageous in that sufficient integration and differentiation of the attributes occurs in product evaluation by human perception, whereas instrumental analysis can only measure one parameter at a time.

Vanillin, a hydrophilic odor active compound and antioxidant (44) which constitutes the predominant flavor in vanilla and vanilla extract, has been the most frequently studied flavorant regarding flavor–fat interactions in frozen desserts. These studies show varying results for the effect of fat on vanilla flavor depending on the fat level investigated. Perceived vanilla flavor is not affected by fat levels if the milk fat content of the ice cream is higher than 10% (42, 45). However, if the fat is reduced to a great extent (i.e., 2% fat ice cream), vanilla intensity is decreased (46) and the time to reach maximum flavor intensity is decreased (41) compared to full-fat ice cream. Ohmes *et al.* (43) compared the sensory attributes of ice cream containing 5.8% fat versus fat replacers. The perceived intensity of vanillin did not statistically differ with fat or fat replacers, but ice cream containing 5.8% fat received the highest rating. Whey, syrup, and cooked milk flavor were rated higher in ice creams formulated with fat replacers.

Milk proteins are known to interact with flavor compounds and thus affect their release behavior. The most frequently investigated milk protein is β-lactoglobulin. In a study conducted by Guichard and Langourieux (47), the affinity constants of various aroma active compounds with β-lactoglobulin were calculated in model solutions. The study suggested the presence of hydrophobic interactions

between the flavor compounds and β-lactoglobulin since the affinity constant increased as the chain length of the flavor compound increased in the class of 2-alkanones and ethyl esters. Although hydrophobic binding explains a significant portion of the flavor-β-lactoglobulin interaction, it should not be generalized to all aromatic flavor compounds. Limonene showed a salting out effect in the same model solution, and partial covalent bonding was reported for benzaldehyde in a different study (48). Studies have also showed the interaction of α-lactalbumin with flavor compounds such as ketones and aldehydes, however the binding strengths were weaker than with β-lactoglobulin (47, 49). Flavor-protein interactions will be discussed in more detail in the following section on fat replacers.

Carbohydrate based ingredients, often used as stabilizers in ice cream, affect the texture of ice cream significantly (50). Although studies of the effects of carbohydrates on flavor release in frozen desserts have not been conducted comprehensively, several studies have been done in dairy systems involving yogurt or dairy custards (51-53).

Among the various carbohydrate substances, pectin shows the strongest capacity to suppress the release of flavor compounds from food matrices (51, 52). Two hypotheses are proposed to explain this phenomenon: 1) hydrophobic interactions exist between the flavor compounds and pectin; 2) the change of physical properties (i.e., increased viscosity or rigidity of the food matrix due to pectin) may reduce the transfer of flavor compounds from the matrix to the headspace (54). The first hypothesis is more relevant in the case of pectin. For other carbohydrates such as guar gum, locust bean gum, κ-carrageenan, and starch, despite their capacities to increase the thickness of matrices, they either show an increase in the release of flavor compounds or show no interaction effects at all (51-53).

Fat replacers

Hatchwell (34) defined a fat mimetic as "a carbohydrate or protein that replaces one or more of the functions of fat". Fat replacers that are based on carbohydrates which usually function as a bulking agent or water-holding matrix that gives moistness to the food. Since most of the carbohydrate-based fat replacers are polar, hydrogen bonds and/or dipole-dipole interactions are responsible for binding flavor compounds. Therefore, carbohydrate-based fat replacers can interact with and hold water-soluble compounds. However, because most flavor compounds are more lipid- than water-soluble, their application as flavor carriers is limited (55). There are exceptions, such as cyclodextrins, which have been shown to complex with lipophilic compounds and thus have been used to encapsulate flavors.

Protein-based fat replacers have the advantage of forming a matrix that holds water as well as providing hydrophobic-binding sites for flavors (56), and thus is favorably used. Two types of interaction can occur between protein and flavor compounds. One is the reversible adsorption of flavors by van der Waals interaction and the other is irreversible binding by covalent or electrostatic linkage (57). Reversibility of binding between flavor-protein interactions is important (58), because flavor compounds have to be reversibly bound to the protein matrix in order for the flavor to be released and perceived. Therefore, in studying flavor-protein interactions, the reversibility of binding has to be considered due to its critical role in flavor perception.

Among the various types of protein-based fat replacers, microparticulated protein is perceived similarly to fat globules by the tongue (56). Microparticulated protein contains milk, egg or whey protein shaped into small round particles and gives a creamy texture (59). Such protein also has roles that include ice crystal control and foam stabilization which leads to improving the quality of reduced-fat ice cream (34). The structure of the protein is a critical factor in determining the interaction between the protein and flavor. It has to be taken into account that the structure of proteins is highly dependent on the environmental conditions. Consequently, the binding behavior of flavor to protein depends significantly on pH, temperature, and salt concentration (55), causing large variations in flavor-protein interaction. Therefore, the experimental conditions have to be well defined to understand the flavor-protein interactions.

Sensory dimensions of frozen desserts: physiological aspects

As soon as a spoonful of ice cream is put into the mouth, ice cream starts to melt instantaneously. Taste and odor-active compounds, which elicit flavor sensations, are released freely on the tongue surface and up to the buccal headspace to stimulate taste and olfactory receptor cells, respectively (28, 60). Concurrently, the physical deformation during this temporal event stimulates mechano-receptors located on the tongue, resulting in tactile sensations such as smoothness and thickness. The 'cold' thermal sensation of ice cream also plays a critical role in the overall sensory characteristics by interacting with other modalities (61). The important flavor characteristics of frozen desserts will be elaborated separately in the following sections.

Flavor volatiles of ice cream base

Knowing the composition of flavor volatiles that constitute the flavor of the ice cream base is critical, because the overall flavor profile of ice cream is constructed when the flavor volatiles of the ice cream base are combined with the flavoring. Key flavor volatiles characterizing various dairy products have been identified and quantified (62).

To have a general idea of the volatile profile of ice cream, it is necessary to briefly review the important flavor compounds of the dairy ingredients constituting the base of ice cream, namely, milk, heavy cream, and non-fat dry milk. Milk fat is the major source of flavors in milk products. The most abundant flavor compounds found in pasteurized milk and cream are fatty acids, methyl ketones, lactones, dimethyl sulfide, aldehydes, ketones from milk-fat oxidation, and diacetyl (63). Methyl-ketones, although individually present in sub-threshold levels, together give milk a cream like flavor (64). Aldehydes produced by lipid oxidation are responsible for the 'cardboardy' flavor of oxidized milk, while Z-4 heptenal contributes to the fullness of flavor in cream (65).

Lactones and acids are the main flavor contributors of non-fat dry milk (66).

Key attributes of full fat ice cream

In this section, the descriptor creaminess will be reviewed in detail among the various ice cream attributes, because creaminess is a complicated attribute encompassing both texture and flavor characteristics (67, 68), and is the most desirable characteristic of ice cream contributed by fat.

Creaminess: A textural perspective A classic model proposed by Kokini (69) explains perceived creaminess as a combined sensation of smoothness and thickness of a product. In this model, creaminess was strictly defined as a textural attribute. In fluid and semi-fluid dairy products, fat is the main component that contributes to the sensation of creaminess (70). In many studies, researchers attempted to understand the relationship between fat, fatty sensation, creaminess, and components that constitute creaminess. Richardson *et al.* (71, 72) further investigated the factors affecting smoothness, which is one of the sensations that constitute creaminess in fluid dairy products. They hypothesized that the textural attributes of fluid dairy products were generated by mechanoreceptor stimulation due to the physical properties (i.e., viscosity and smoothness) of the fluid and the characteristics of fat globules. The effect of the viscosity, fat content, and globule size of cream were evaluated on the perceived creaminess of dairy creams. These studies showed a positive correlation between viscous force and creaminess (73-76). The size of fat globules also affects the perception of creaminess (75). A microparticulated protein, frequently applied as a fat substitute in dairy products, is perceived as smooth and creamy when the particle size is smaller than 3 μm, but perceived as gritty if the particle size becomes larger than 8 μm (77).

All of these studies hypothesize that fat is directly related to the perception of creamy texture. However, the boundaries of creaminess were still limited to textural attributes, and researchers in general attempt to understand creaminess in relation to the physical properties of fat. The flavor properties of fat and flavor aspects of creaminess were simply ignored in most studies and were considered as confounding variables.

Folkenberg *et al.* (78) reevaluated the assumption that physical properties are the only stimulus affecting the textural perception of food. An experiment was conducted to understand the factors involved in generating the descriptive attribute 'mouthfeel' when evaluating instant cocoa drinks. Mouthfeel correlated positively with viscosity and cocoa flavor but negatively with milk flavor. Unlike the definition of mouthfeel stated in International Organization for Standardization (ISO), which constrains the term only to the tactile sensation, the result showed that the evaluation of mouthfeel is affected by not only the rheological properties of the cocoa drink, but also its flavor characteristics. This study provides evidence of an association between rheological and chemical properties in evaluating the textural characteristics of food.

Creaminess: A flavor perspective The addition of aroma can change the perception of fattiness of food. Visual and olfactory cues alter fat perception. 'Fat' aroma contributes to the overall fatty perception of milk (79). The perceptions of thickness and creaminess of 1% milk increase when a minute amount of vanilla flavor is added to the milk (80).

Tepper and Kuang (70) incorporated flavor components for understanding the creaminess and fattiness of fluid dairy product. In this study, the effects of fat content and odor active volatiles on the perception of milk fattiness was investigated. Similarities between samples were measured based on perceived fat content, mouth coating, and thickness. In this study, added flavors increased the fatty sensation in milk, suggesting that aroma is one of the elements constituting fatty perception in milk.

Frøst *et al.* (81) conducted an experiment investigating the total fattiness perception of milk. The experiment involved varying the levels of thickener (tactile sensation), whitener (visual), cream aroma (flavor), and fat content to understand the factors involved in the fattiness perception of milk. A combination of thickener, whitener, and cream aroma in 0.1% fat milk mimicked 1.3% fat milk, again suggesting multidimensional characteristics for the perception of fattiness in milk. Sweet taste correlated highly with creamy descriptors. Creaminess and residual mouth coating fully reflected the perception of fattiness in milk. Flavor sensation provides another dimension for understanding fat perception (79). The impact of aroma on fattiness is specific to the type of food and quality of aroma used (82). A mixture of methyl ketones at subthreshold levels produces a cream-like flavor, and Z-4 heptenal enhances the sensation of 'fullness' in cream.

Perceptual interactions between different modalities (taste-flavor, flavor-flavor, flavor-texture)

Flavor is generally defined as the combined sensation of taste and aroma when consuming a food or beverage. However, flavor perception is not necessarily confined to the area of aroma and taste, but can also be influenced by pungency, chemical heat, and tactile sensation (83). Interactions between different modalities play an important role in flavor perception during eating. Taste and aroma perceptions are almost inseparable during eating (42, 83, 84). Taste influences the perception of trigeminal stimuli and flavor. The bitterness of caffeine has been shown to increase the overall intensity of menthol (85). Visual (86) and tactile stimuli (81) also influence the perception of flavor. Strong multimodality interaction is observed when the qualities of stimuli are congruent. For example, sweetness is enhanced with fruity aroma or vanilla flavor but not with peanut butter flavor (83).

Multi-modality interactions are also observed in dairy products (81). Creaminess is enhanced by sweetness (76). A large part of the sensory stimulus is perceived as missing when the aroma portion is removed using a nose-clip during sensory evaluation (82). Saltiness and sweetness decreased with the use of a nose clip, showing the impact of aroma on taste perception. High levels of certain flavors may also trigger the false perception of higher fat levels via learned association (82).

The interactions of different modalities can be due to

physico-chemical (salting out effect), physiological (diffusion of compounds to taste and olfactory receptors), and psychological causes (83). Most sensory scientists agree that a large component of flavor interaction occurs at the cognitive and psychological levels (83, 87, 88). Lawless (87) suggests that these observed interactions are more likely due to a 'dumping effect'. For example, panelists show tendencies to rate sweetness intensity high when a fruity flavor is present, but the ballot lacks a category for fruity intensity, i.e., panelists 'dump' fruity intensity into the sweetness category. However, this effect does not completely explain the interactions that are observed in many studies. Unfortunately, with the currently available methodologies, it is impossible to separate the dumping effect from any true flavor interaction.

Instrumental analyses

Analyzing the flavors of food ***Extraction***: Various extraction methods exist for extracting volatile compounds from food products such as the solvent extraction method, headspace analysis, solvent phase microextraction (SPME), etc. Solvent extraction of aqueous samples can be used based on the different partitioning coefficients of volatile compounds relative to non-volatile compounds (89, 90). Headspace analysis is a relatively non-destructive extraction method which can be divided into static (91, 92) and dynamic headspace analysis (93). These solventless methods have the advantage of protecting volatiles from degradation during the extraction procedure. SPME is a rapid solventless extraction method that can concentrate volatiles or semi-volatile organic compounds (94). SPME principally uses the partitioning of organic components between bulk aqueous or vapor phases and thin polymeric films coated onto fused silica fibers. Unfortunately, there isn't a single extraction method that can completely represent the flavor profile of a food sample. Using a combination of different methods will effectively extract volatiles and is recommended (95). When selecting the most appropriate extraction method, the following factors should be considered: the purpose of extraction, the type of food matrix and the target compound to be analyzed. The method also needs to be straightforward in procedure, reproducible, accurate, and robust. It has to be kept in mind that the main purpose of the extraction methods is to completely identify and quantify the flavor compounds present in a food product rather than profiling the flavors that humans perceive.

Separation and Identification: Gas chromatography (GC) is the most frequently used separation method for analyzing volatiles extracted from the food matrix (96). High performance liquid chromatography (HPLC) can also be used for flavor compounds that are relatively hydrophilic (i.e., vanillin analysis) (41). For the identification of isolated compounds, use of a flame ionization detector (FID) and mass spectrometry (MS) are the most favorably used methods in flavor analysis. In flavor research, the fact that volatiles have variable odor potencies should be taken into account. Gas chromatography-olfactometry (GC-O) allows the identification of important odor active compounds and will eliminate the odorless compounds since the GC-O technique uses the human olfactory system as a detector (89, 97).

In-mouth simulating system As mentioned earlier, solid food undergoes a dramatic change during eating. Physical breakdown, hydration by saliva, enzymatic hydrolysis, and warming or cooling to body temperature occur in addition to the release of volatiles. Roberts and Acree (2) investigated the effect of saliva, temperature, and shearing on flavor release from water and oil systems. Shearing and increasing the temperature from 23 to 37°C increased the volatility of compounds. In addition, the addition of synthetic saliva increased the pH and volatility of basic compounds.

Two methodological approaches can be taken in order to understand flavor release from a food matrix during eating: an extraction method that mimics the mouth environment, and a method that directly collects the breath volatiles during eating. Conventional extraction methods, such as the solvent extraction, vacuum distillation, and static headspace methods, do not sufficiently represent the mouth environment. Therefore, the volatiles extracted by conventional methods will have a different profile than those released during eating. Thus, constructing an instrumental tool that can extract volatiles in a manner similar to their release in the mouth is necessary to understand flavor release during eating.

An in-mouth simulating system was initially developed by Lee (17). The system incorporated body temperature, saliva, gas flow, and work-input, and used a headspace extraction method to investigate the effect of different types of fats on the volatility of diacetyl. Although the response time of the instrument was longer than that of time-intensity (T-I) measurements, the pattern of flavor release from oil using this model system was similar to the temporal changes in the T-I curve. More sophisticated in-mouth simulating systems have been developed and frequently employed to understand the binding and release of flavors in both model and actual food systems. Some of the studies use an in-mouth simulator as an extraction method to investigate the total volatile profile of the target system (9, 19, 98). Other studies connected this extractor to mass spectrometry to monitor the volatiles released in real time (18, 20-22). Studies that conducted T-I measurements, including those of Lee (17), Elmore and Langley (18), and Springett *et al.* (21), showed similarities in the release pattern, but the response time was longer for the model system. It was concluded that, once the correlation between the sensory response and the instrumental measurement is established, the in-mouth simulating system can be used to understand and predict flavor release during eating. The advantages of using an in-mouth simulating system are the reproducibility of the system and fewer uncontrollable variables compared to direct in-nose breath analysis. However, this method still suffers from insufficient sensitivity for measuring low detection threshold flavor compounds (18).

Direct in nose/mouth breath analysis Analyzing the volatile compounds in the exhaled breath during eating may be the most idealistic instrumental approach to investigate flavor-food interactions during eating. Breath analysis has been practiced frequently in the medical field

(99), and found its initial application in flavor research by Soeting and Heidema (6), who monitored the continuous release of volatiles from the nostril while consuming a simple model solution using multiple-ion monitoring of a mass spectrometer equipped with a membrane separator. The study was successful in observing the temporal changes in volatile profiles. However, large variation in the release pattern among subjects was a problem in this research. Many studies have adapted the concept of direct in-nose breath analysis to understand flavor-food interactions.

The simplest method is to trap the volatiles coming out of the nose during food consumption in a Tenax trap and analyze the trapped volatile samples. The volatiles can be sampled either in one Tenax trap during the whole consumption period (e.g., sampling for 1 min), which yields an overall volatile profile (12), or sampled for particular time intervals (e.g., 0-10, 10-20, 20-30 sec, and so on), which yields a semi-continuous temporal profile during the time course of mastication (10, 11). The former approach measures the total volatiles released during eating, whereas the latter approach can determine temporal changes in the release behavior of volatiles.

The most advanced techniques for analyzing in-nose breath analysis were introduced by Brauss *et al.* (13, 15) utilizing an atmospheric pressure chemical ionization mass spectrometry (APCI-MS) method (100, 101), and by Buhr *et al.* (102) utilizing proton transfer reaction-mass spectrometry (PTR-MS) (103). Both methods utilize proton transfer from a positively ionized source, preferably water, to the target aroma analyte.

In the APCI-MS method, the analytical conditions are set to optimize the formation of protonated analyte $[M+H^+]$ and to minimize fragmentation (101). Brauss *et al.* (13) employed the APCI-MS to analyze breath samples that came directly from the nose using a "custom-built air-sampling interface" (14). The method was efficient and sensitive enough to monitor flavor release in both fruits and model systems. The studies involving both T-I evaluation and real time breath analysis showed a high correlation between the two measurements in simple food systems (14). However, this approach also showed a wide variation in release profiles among panelists. Another potential problem with this method is that APCI-MS is a soft ionization method, thus the system can only differentiate between compounds of different molecular weights, and the identification of an unknown compound is difficult since APCI does not give a mass spectrum with fragmentation. Despite these weaknesses, APCI-MS techniques have advanced significantly over the past few years. These studies show that the analysis of non-volatile flavors in liquid solution (104) as well as the analysis of flavor compounds in ethanolic systems (101) has been successful.

PTR-MS developed by Lindinger *et al.* (105) is a chemical ionization system based on a proton-transfer reaction using H_3O^+ as the primary reactant. H_3O^+ ions undergo non-dissociative proton transfer to volatile organic compounds but do not react with the natural components of clean air (106). The distinguishing character of PTR-MS is the separate process of generating H_3O^+ ions and ionizing the target analyte, hence it is possible to calculate the absolute concentration of the analyte without any calibration (103). PTR-MS is a fast and sensitive method adequate for real-time chemical analysis and has applications not only in environmental and medical science (106), but also in the flavor research area for in-nose breath analysis and model mouth systems (16, 107).

Both in-mouth simulating systems and direct in-nose breath analysis techniques have become more elaborate allowing their use with simple model solutions as well as real food systems (38, 108).

Comparison of the two methodologies Several studies have compared the flavor profiles of various foods obtained by breath analysis and in-mouth simulating systems (109, 110). Deibler *et al.* (109) verified that the flavor profiles of various foods acquired using a Retronasal Aroma Simulator (RAS) had a high correlation with those acquired using breath-by-breath analysis. Despite the high overall correlation between the two methods, the degree of correlation varied significantly (0-30%) depending on the flavor compounds.

Cheddar cheese volatiles extracted by breath analysis and vacuum distillation methods were shown to be different (110). Aroma compounds having high volatility were better detected through breath analysis than the distillation method. The distillation method was most effective at extracting low-volatility aroma compounds from cheese. The authors explained that vacuum distillation gives a more complete extraction, yet artifacts may form during the extraction. In this study, blank breath volatiles were also analyzed by GC-O. Interestingly, the results showed that the blank breath also contained flavor compounds that are present in cheddar cheese. This observation suggests a potential problem of adapting breath analysis as an instrumental tool for investigating flavor release from the food matrix.

Unfortunately, sensory analysis was not conducted in either of these studies. Thus, both studies were inadequate for validating whether the method extracts the flavor volatiles in a manner similar to their sensory perception during eating. Theoretically, it would be expected that the volatile profile obtained by breath analysis would be a true representation of what we perceive. However, person-to-person and within-person variations lower the sensitivity of breath analysis (38), and interference with background breath may seriously hinder the interpretation of the results.

Sensory analyses

Descriptive analysis (DA) DA is a sensory method that analyzes a food product by describing its sensory attributes and rating the intensity of the food product using trained panelists. It is a powerful and sophisticated method to understand overall sensory characteristics of a product. The panelists are initially trained to communicate with each other to understand the product attributes, and to use the rating scale in the correct manner. The panelists determine and define the attributes to be used. Attributes that can discriminate between products, are not redundant, and relate to consumer acceptance and instrumental measurement should be preferably selected (111). Reference

standards should be developed for each attribute to help tune the panelists. DA is effectively used in identifying the significant attributes that drive consumer acceptance. The method can also facilitate the understanding of the important chemical or physical variables of the product that relate to the sensorial aspects.

Time-intensity (TI) sensory evaluation Time-Intensity sensory evaluation measures the sensory perception of a specific attribute over a course of time. Unlike unipoint sensory evaluation (time average), where the intensity of an attribute is integrated, TI enables the monitoring of changes in perceptual intensity during product evaluation (112, 113). Therefore, it is a useful tool for evaluating products that undergo drastic changes (texture, phase, flavor intensity, etc.) during consumption (114, 115). Parameters frequently used in this method are perceived maximum intensity, time to maximum intensity, rate of increase to maximum intensity, the extinction point, and the total duration of sensation. Traditionally, TI measurement has been favorably used in evaluating the bitterness of beer or sweetness of artificial sweetener (116), in which the attributes tend to linger and persist during the time course.

Because food undergoes oral breakdown and hydration due to saliva during mastication, the TI method is also well suited for understanding the binding and release of flavors from the food matrix during eating. The effects of fats, carbohydrates, and proteins on the binding and release of flavors have been successfully studied by many researchers (19, 27, 34). Valuable information has been obtained by TI measurement regarding the behavior of flavor release in food matrices that may not be revealed with unipoint evaluation (41). Although TI can be a powerful tool to understand food flavor interactions, the method shows large variation between panelists depending on their eating behavior, which can be a potential problem. Consequently, data handling would be another issue to overcome in order to obtain accurate results. Ovejero-Lopez *et al.* (117) have comprehensively reviewed and compared a range of data handling methods for TI data sets covering univariate as well as multivariate approaches.

Statistical analysis

Multivariate analyses are used on data which contain multiple responses for each individual or unit studied. Multivariate analyses have become very useful in consumer research and product development in the food industry due to their effectiveness in various situations. Multivariate techniques are mainly used in data reduction (principal component analysis, PCA; factor analysis, FA; correspondence analysis, CA; etc.), classification (FA, hierarchial cluster analysis, discriminant analysis, etc.), and assessing data relationships – multiple regression, principal component regression, partial least square regression (PLSR) analysis, etc. (118).

The objective of multivariate reduction techniques is to create a few new variables that contain most of the information present in the original, multi-dependent variables. PCA and FA are performed for numerical or interval data, and CA is used when the data are nominal or categorical (119). PCA is performed to simplify the description of a set of interrelated variables (120-122). It can be summarized as a method that transforms the original variables into new uncorrelated variables called principal components (PC). PCA will find linear combinations that will maximize the total variance. PCs are a combination of linear regressions of the original variables. Regression coefficients are estimated such that, within a PC, total variation is maximized and each is independent (123).

Canonical variate analysis (CVA), also known as DA, is frequently used to evaluate the differences between objects or to classify objects into groups. Unlike PCA, CVA finds linear combinations that maximize the F-ratio rather than the total variance (124). CVA compared to other multivariate techniques has the advantage of accounting for the variations among panelists within the data set. Moreover, the confidence interval of a product can be calculated from CVA similar to Fisher's protected least significant difference (LSD) in univariate analysis.

PLSR analysis is frequently used to understand the relationships between data sets. PLSR can be applied in drawing the relationship between sensory (y) and instrumental (x) analysis data. MacFie and Hedderley (125) consider this method as "a hybrid" between multiple regression and PCA, because PLSR provides a compromise between giving weight to the analytical variables and maximizing the variance explained.

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