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Review

# Sample preparation for the analysis of flavors and off-flavors in foods

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**Abstract**

Off-flavors in foods may originate from environmental pollutants, the growth of microorganisms, oxidation of lipids, or endogenous enzymatic decomposition in the foods. The chromatographic analysis of flavors and off-flavors in foods usually requires that the samples first be processed to remove as many interfering compounds as possible. For analysis of foods by gas chromatography (GC), sample preparation may include mincing, homogenation, centrifugation, distillation, simple solvent extraction, supercritical fluid extraction, pressurized-fluid extraction, microwave-assisted extraction, Soxhlet extraction, or methylation. For high-performance liquid chromatography of amines in fish, cheese, sausage and olive oil or aldehydes in fruit juice, sample preparation may include solvent extraction and derivatization. Headspace GC analysis of orange juice, fish, dehydrated potatoes, and milk requires almost no sample preparation. Purge-and-trap GC analysis of dairy products, seafoods, and garlic may require heating, microwave-mediated distillation, purging the sample with inert gases and trapping the analytes with Tenax or $C_{18}$, thermal desorption, cryofocusing, or elution with ethyl acetate. Solid-phase microextraction GC analysis of spices, milk and fish can involve microwave-mediated distillation, and usually requires adsorption on poly(dimethyl)siloxane or electrodeposition on fibers followed by thermal desorption. For short-path thermal desorption GC analysis of spices, herbs, coffee, peanuts, candy, mushrooms, beverages, olive oil, honey, and milk, samples are placed in a glass-lined stainless steel thermal desorption tube, which is purged with helium and then heated gradually to desorb the volatiles for analysis. Few of the methods that are available for analysis of food flavors and off-flavors can be described simultaneously as cheap, easy and good. 

*Keywords:* Sample preparation; Food analysis; Aroma compounds; Off-flavor compounds; Reviews

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## 1. Introduction

### *1.1. General considerations*

To humans, food is more than a biological necessity for survival. Meals are a prime nexus for most social interactions: family, courting, business, grief, etc. Food can serve varied psychological functions as an aphrodisiac, an aesthetic stimulus, an anesthetic or narcotic and a solace [1]. Except during extreme hardship and deprivation, food's ability to serve these subtle human needs is tied inextricably to the sensory values of taste, odor and texture. Moreover, food's failure to meet flavor expectations may often

signal a physical health danger associated with spoilage or contamination [2]. For these as well as commercial reasons, food producers, shippers, buyers, wholesale and retail sellers, and especially consumers need reliable ways to assess food flavor quality.

To the scientist, ''reliable assessment'' of a biochemical mixture – whether at the factory, in a package, or on a dinner plate – suggests the need to make an analytical measurement. But, for several reasons, the measurement required for food samples is often not readily defined or easily accomplished. First, even when a panel of sensory experts can agree, the qualities encompassed in the term ''flavor'' are inherently subjective. A ''reliable assessment'' should be objective: a number, a threshold value, something readily interpretable by non-specialists [3]. Second, a particular food must be defined not only with respect to the combination of biochemical constituents associated with pleasing flavor but also with respect to the possible presence of a variety of chemicals, any of which might contribute to off-flavor. Third, important flavor components may decompose within minutes of homogenization, which is often the first step in sample preparation [4–6]. Fourth, sensory levels of flavor and off-flavor components may be present in much lower concentrations than a multitude of taste- or odor-inactive substances. Foods can be as much as 95% water, a major food component without much contribution to taste or odor [7]. Therefore the required measurement must be able to distinguish significant components (analytical targets) from insignificant components, quantifying all of the former while ignoring, removing, or otherwise distinguishing the latter. The scale of the flavor-analysis task is suggested by the number of already identified flavor-active components, about 6000 as of 1990 [8,9].

The first challenge of flavor analysis is to identify and quantify all of the chemicals associated with the desired flavor. Once the scientist has identified the chemical contributors to good flavor and the range of their relative concentrations in dishes having optimal taste, the remaining challenge is to find a reliable way to quantify those components in a particular sample. Most analytical approaches to flavor analysis require that the flavor components be separated from non-flavor components in the sample prior to their quantitative determination [7,10]. This presumes that there is some way to discard the irrelevant chemicals without risking the loss of significant flavor factors.

Off-flavor analysis typically also requires careful, often laborious sample preparation for the same purpose. The analytical task may be complicated by not knowing in advance the chemicals causing the problem or, for quality assessment of frozen or packaged food products, even whether there is a problem. Off-flavors taint food by several different mechanisms which require different sample-handling strategies.

### *1.2. Origin of off-flavors*

#### *1.2.1. General sources of off-flavors*

Four general sources of off-flavor are discussed below: environmental pollutants, the growth of microorganisms, oxidation of lipids, or endogenous enzymatic decomposition. Several other factors which may contribute in special cases to unpleasant flavor or odor are not discussed here but include species-specific flavor variants, chemicals produced by excess doses of radiation during sterilization, conditions during processing or canning, and the sex condition of the animal or bird [11,12].

#### *1.2.2. Microbial production of off-flavors*

Many off-flavors and off-odors in foods are due to the growth of spoilage microorganisms that produce a variety of biogenic amines and other compounds [2,13]. Other off-flavors, especially in fish, result from the production of odorous microbial metabolites in water that are then assimilated by the fish [14].

Meat and poultry serve as growth media for all sorts of microorganisms [15], many of which will grow and produce off-flavors even at refrigerator temperatures [16]. Several types of volatile compounds, including $NH_3$, amines, indole, skatole and $H_2S$ [15], are produced by microorganisms such as *Pseudomonas* spp. growing in meats. Some spoilage organisms are anaerobic, such as *Clostridium laramie*, which can produce $H_2S$ in beef even at temperatures of 2°C or below [17]. Several other bacteria, including *Brochothrix thermosphacta*, *Carnobacterium* spp., Enterobacteriaceae, *Lactobacillus* spp., *Leuconostoc* spp. and *Shewanella putrefaciens*,

produce off-flavors and off-odors in refrigerated beef and pork [18]. In cured meat products, species of *Brochothrix*, *Carnobacterium*, *Lactobacillus*, *Leuconostoc* and *Weissella* produce sour off-flavors [18]. The amounts of tyramine and putrescine in dry sausages have been shown to increase at the same time that the lactic acid bacteria are developing [19].

Off-flavors in ice-packed fish and seafood are produced during spoilage by a succession of microorganisms, including *Pseudomonas* spp., *Acinetobacter* spp., *Moraxella* spp. and *Shewanella putrefaciens*, evolving several different unpleasant odors [15,20]. A strain of *Lactobacillus sake* being considered for use as an antibacterial agent in cold-smoked salmon was judged unsuitable because it produced sulfurous off-flavors [21]. Even fresh, healthy pond-raised catfish may absorb off-flavors from microbial metabolites in the water and sediment [14]. Geosmin and 2-methylisoborneol (MIB), the principal earthy and musty compounds in water and sediments, are produced by the growth of actinomycetes, cyanobacteria and fungi [22,23]. Different catfish in the same pond may temporarily assimilate different amounts of geosmin and MIB and then release them at different rates [24,25]. Pond-raised marine shrimp also may take up the same kinds of off-flavors from water if the salinity has been reduced [26,27].

Milk and other dairy products are excellent growth media for many types of psychrotrophic off-flavor microbes, such as *Pseudomonas fluorescens*, *P. fragi*, *P. putida* and *P. aeruginosa* [28]. *Pseudomonas fragi* produces ethyl butyrate and ethyl hexanoate in refrigerated milk. Psychrotrophic strains of *Bacillus cereus*, which frequently survive pasteurization and grow in milk at 7°C, produce a sweet curdling and then a bitter off-flavor [29]. Although selected strains of lactic acid bacteria are necessary for producing many cultured dairy products, other strains spoil them by producing off-flavors [30]. For instance, phenolic off-flavors in cheese may be due to subspecies of *Lactobacillus casei* [31]. In the making of Swiss cheese, correlations have been shown between the abundance of *Enterococcus* spp. in milk and tyramine in cheese; between *L. casei* in milk and histamine in cheese, and between coliforms in milk and diamines in cheese [32]. In cream-filled hazelnut cakes, the osmotolerant spoilage yeast *Hansenula anomala* (=*Pichia anomala*) may produce an off-flavor due to ethyl acetate [33].

Vegetables, fruits and juices are frequently spoiled by off-flavors produced by lactic acid bacteria, yeasts and molds [34]. For instance, mung bean sprouts may be spoiled by *Klebsiella pneumoniae* and *Enterobacter cloacae*, which produce cadaverine and putrescine, respectively [35]. *Bacillus stearothermophilus*, *B. coagulans* and *Clostridium thermosaccharolyticum* may produce acids or sometimes $H_2S$ in canned vegetables [34]. Yeasts of the genera *Candida*, *Rhodotorula* and *Trichosporon* may convert ferulic acid, a natural component of orange and apple juices, to 4-vinylguaiacol, an off-flavor compound [36,37]. *Alicyclobacillus acidoterrestris* and *Propionibacterium cyclohexanicum* also grow in fruit juices and may produce off-flavors [38,39]. Fermented vegetable products, including pickles, sauerkraut and olives, may develop off-flavors and odors due to amine formation by *Pediococcus* spp. and other lactic acid bacteria [40]. Microbial butyric and propionic acids are sometimes also troublesome in these fermented products [40]. Fungi in the genera *Aspergillus*, *Fusarium* and *Penicillium* attack stored grains, usually producing volatile metabolites with characteristic off-odors [41].

### *1.2.3. Off-flavors arising from environmental sources*

Hundreds of thousands of chemicals are potential food contaminants through environmental routes. The number of possible off-flavor contaminant targets dwarfs even the number of flavor components. Oil spills have been associated with ''kerosene'' off-flavor in marine fish [42–45]. Pesticides and other agricultural chemicals have been associated with tainted fruits and vegetables [45].

Fat-soluble off-flavor contaminants that end up in lakes or streams bioaccumulate in the fat of freshwater fish and enter the food chain. The bioaccumulation factor for a compound is the equilibrium ratio of concentration in the fat to concentration in the serum (or for seafood, in the water). For environmental toxins, such as polychlorinated biphenyls (PCBs), the bioaccumulation factors range from 10 to 30 thousand [46,47]. Relative oil/water solubility coefficients and membrane permeability determine the partitioning of environmental lipid off-flavor com-

pounds, regardless of toxicity. For fat-soluble pollutants, bioaccumulation can cause flavor defects even in seafood harvested in water that is not obviously polluted.

Under the category of environmental off-flavor sources are chemicals obtained by animals from feeds and forage. The flavor of lamb and mutton varies depending on whether the animals feed on perennial rye-grass or other forage [12]. For sheep grazed on white clover, oats, vetch, rape or alfalfa, the resulting meats are described as intense-flavor, rotten-egg-like, sweetish, nauseating, and (simply) off-flavor, respectively. For veal and beef, the flavors are different depending on whether the animals are foraged or grain-fed; a ''grassy'' flavor may arise from δ-tetradecalactone and δ-hexadecalactone [12]. For farm-raised catfish, flavor is altered by feeding turkey livers rather than the usual cereal diet. ''Petroleum-like'' salmon and ''blackberry off-flavor'' cod are due to sulfur-containing compounds, possibly attributable to environmental contamination [12]. Poultry fed with unsaturated lipids (e.g., from tuna) have a distinct ''fishy'' flavor that can be reduced or eliminated by adding α-tocopherol, an antioxidant, to the feed [12]. This suggests that the odor results not from the original unsaturated oils but from their oxidation products.

Another environmental source of off-flavors is containers or packaging materials. Examples include solvent residues from printing inks, lacquers or glues; monomers and other trace constituents from polyethylene, polystyrene, polyvinyl chloride, polypropylene and other plastics used for packaging; penta- and tetrachlorophenols used as wood preservatives; and other chemicals from pallets, cardboard and jute sacks [11].

### *1.2.4. Oxidative production of off-flavors*

Lipid oxidation is a major cause of off-flavors and loss of nutrients in fat-containing foods; it can occur even during frozen storage [11]. Analytical strategies aimed at measuring the original and subsequent levels of lipids in tissues give the most reliable assessment of lipid oxidation rate. Primary products of the oxidative process include hydroperoxides, whose appearance is concurrent with that of conjugated dienes and trienes. Secondary products, such as propanal, a known off-flavor, can serve as a reliable indicator of flavor deterioration for fish products, while hexanal can serve the same function for meats [48].

Lipid oxidation can occur by either enzymatic or non-enzymatic catalysis [49]. Non-enzymatic reaction mechanisms can involve catalysis by $H_2O_2$, heme iron from myoglobin, non-heme iron, or salts. Anti-oxidants (e.g., nitrite, ascorbic acid, phenols or α-tocopherol) are effective lipid oxidation inhibitors [50].

### *1.2.5. Enzymatic production of off-flavors*

Two types of enzymes responsible for lipid degradation are lipases and lipoxygenases [11]. Direct enzymatic degradation can be difficult to distinguish from microbial degradation. By comparing irradiated raw and cooked meats, significant contributions of enzymes endogenous to the tissue have been distinguished from bacterial ones, because the bacteria have been killed in both cases [51]. For cooked beef, pork and veal, the endogenous enzymes were also denatured, so that these products suffered much less lipid oxidation during storage than the corresponding raw meats. In this case the resulting oxidative off-flavor compounds would be similar to those produced by microbial spoilage.

When squid were stored at 2.5°C for 10 days, the total volatile bases, ammonia and trimethylamine levels consistently increased over time and were consistently correlated with organoleptically assessed quality [52]. The microbial cell counts for tissue samples remained constant, suggesting that spoilage was attributable to autolytic enzymes rather than microbial degradation [3]. Similarly, the same rates of production and breakdown of inosine monophosphate, leading to inosine, were found in both sterile and non-sterile samples of Atlantic cod [53].

As pointed out above, many volatile compounds, including volatile bases, are produced by microbial decomposition of fish. However, indole, a relatively non-volatile amine, along with some $NH_3$ is produced by decomposition of shrimp at room temperature, whereas only $NH_3$ is produced at refrigerator temperatures [54]. Here, production of $NH_3$ seems to be associated with an autolytic process and that of indole possibly with microbial proliferation. In any event, the most prominent decomposition product,

the one causing the off-flavor, differs according to the storage temperature.

Analytical targets such as $NH_3$ and indole require different sample preparation and analysis methods. When the existence of a flavor problem is not known in advance, we propose this strategic approach: the use of a rapid, cheap, non-chromatographic screening method, followed by confirmatory analyses of the suspect samples to identify the chemicals causing the off-flavor.

### *1.3. Analytical requirements of flavor analysis*

The difficulty of the flavor/off-flavor analytical challenge was well illustrated recently by Fay and Staempfli [10], who displayed two high resolution GC–MS total ion chromatograms of contaminated and reference chocolate sweets (Fig. 1) They commented, ''Chemicals responsible for off-flavor in the contaminated sample were almost impossible to detect by visual means in such complex traces.''

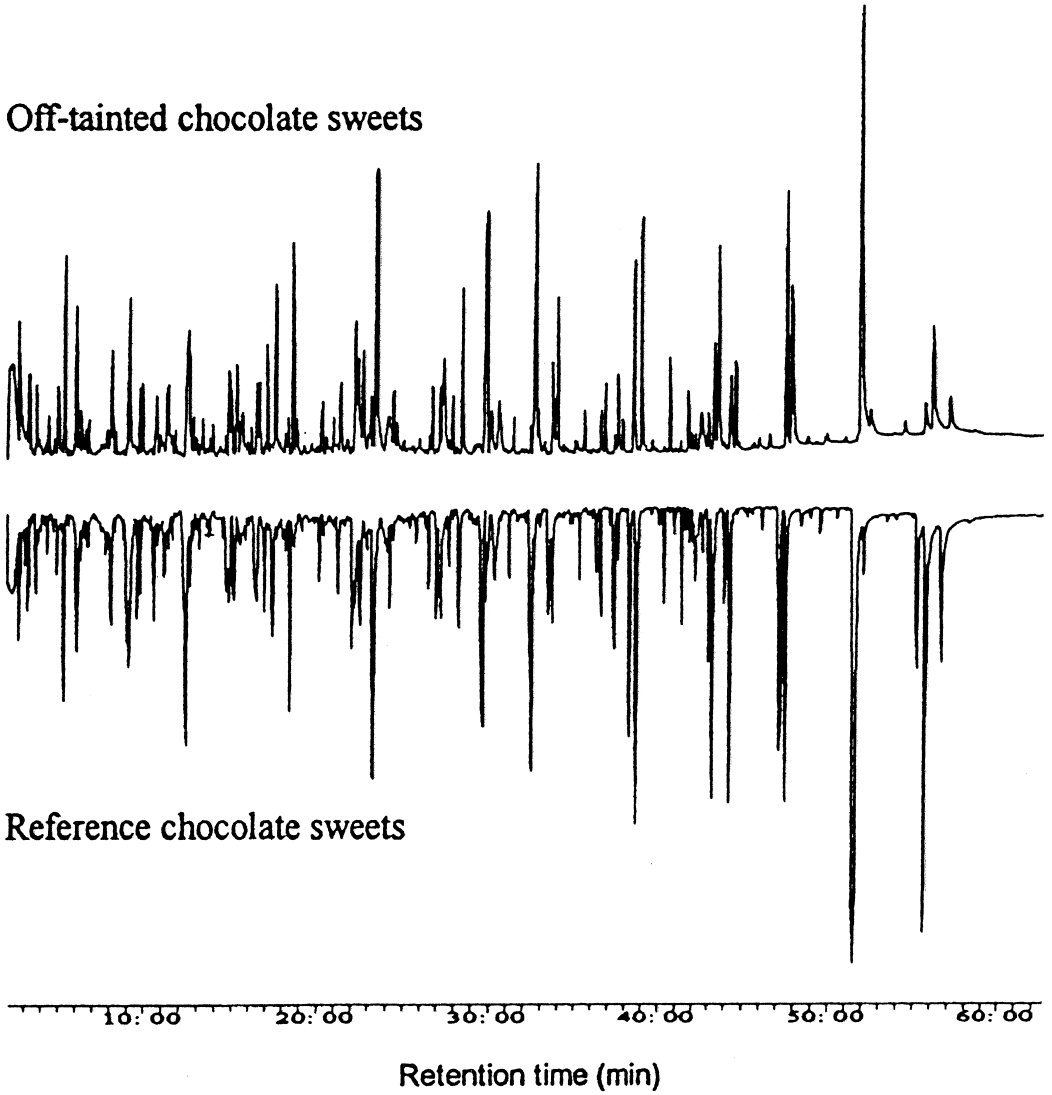


Fig. 1. Total ion chromatograms of contaminated and reference chocolate sweets. Chemicals responsible for off-flavor in the contaminated sample are almost impossible to detect by visual means in such complex samples. (Reprinted by permission from Ref. [10]).

They developed an algorithm for plotting multiple ion traces that could more easily distinguish at a glance ion signals associated with off-flavor components. This system appeared to work well for the specific contaminants in a specific food matrix, chocolate. Their detection system, full scan MS, gave an almost universal response for organic substances. However, they still had to know in advance, or to assume: (1) that the unknown off-flavor components were efficiently recovered by the sample isolation and concentration technique and (2) that these off-flavors would pass through a capillary GC column without thermal degradation or excessive tailing so that the mass spectrometer could detect and identify them. This illustrates how the requirements and constraints of trace analysis in a complex matrix can affect choices, particularly in sample handling, to assess flavor and odor characteristics.

## 2. Sample preparation for non-chromatographic (usually screening) methods

### *2.1. For methods using selective detection*

If the analytical target is known and has distinctive chemical characteristics, it may be possible to determine it directly, without chromatographic separation. Even then, there may be need for sample manipulation to obtain adequate limits of detection and other quantitative figures of merit.

#### *2.1.1. In-laboratory analyses*

While developing a titrimetric method for free fatty acids (FFAs) in homogenized milk, Nath et al. pointed out that off-flavors in milk parallel the increase in FFA concentrations [55]. Since colorimetric assays were not usable for homogenized milk, they developed a titrimetric assay. They extracted the fat from the milk using an acid detergent solution and titrated with ethanolic KOH solution to a thymol blue indicator endpoint, a variation of an official International Dairy Federation (IDF) procedure for extraction of FFAs from milk. The titrimetry required three standard solutions, which had limited shelf life, and an $N_2$ atmosphere. The method was exhaustively optimized to maximize recovery of the larger carbon chain number FFAs associated with the strongest off-flavors. C:4 and C:6 could not be recovered; recoveries for the longer-chain FFAs increased consistently from just over 50% for C:8 to about 85% for C:18.

When the method was judged adequate, Nath et al. tested samples from bulk milk-vending booths in Bangalore, India [55]. Milk from these booths had perceptible flavor defects, but these samples did not have excess FFAs and the off-flavor apparently had another cause. This example, which illustrates the possibility of developing non-chromatographic assays based on selective detection, also illustrates the need to identify the nature of the off-flavor problem before spending time perfecting a method for measuring the wrong analyte.

A microbial sensor specific for short-chain fatty acids has been used by Ukeda et al. to determine FFAs in raw milk [56]. They cultivated the bacterium *Arthrobacter nicotianae* in a butyric acid medium, centrifuged the cells, and re-suspended them in polyvinyl alcohol buffered to pH 7.0. The cells were dried and fixed to the PTFE membrane of an oxygen electrode which was then covered with an $M_r$ 12 000 cut-off dialysis membrane. This ''microbial'' electrode was then set into the flow-through cell of a flow injection analysis (FIA) system. Raw milk could be injected without dilution directly into the FIA system. The resulting sensor response correlated to GC results for short-chain FFAs with $r=0.916$ but for total FFAs with only $r=0.559$. Correlation with a titrimetric assay of total FFAs gave $r=0.78$. These results indicated that the microbial sensor was selective for short-chain FFAs. The system could handle 20 samples/h and was more sensitive than the titrimetric system for the short-chain FFAs. However, if Nath et al. [55] are correct, the short-chain FFAs typically account for only 10% of the total FFAs in milk and also contribute less off-flavor per mole than the medium and longer chain FFAs. Although Ukeda et al.'s method seemed reliable for screening some off-flavor components, it was less valid for the ones that gave the greatest problem.

Optical sensors have been used for detection of volatile compounds, particularly sulfides, from spoiled hams [57]. The optically responsive reagent was a fluorescein mercuric acetate prepared in a membrane and dried onto a glass slide to form an

optrode. As with the previously described microbial sensor for FFAs, use of this sulfide-sensitive optrode involved minimal sample preparation. Several practical issues limit its usefulness for food-screening applications: a 100+ min response time to equilibrium, the necessity for a spectrophotometer to quantify the result, and the potential hazard of placing a toxic mercuric compound in proximity to food.

Fernández et al. have reviewed several methods based on a thiobarbituric acid (TBA) colorimetric test for malondialdehyde (MDA) and other TBA-reactive substances (TBARSs) associated with lipid oxidation causing rancidity in food [58]. Each version of the test has significant limitations: one involves intensive sample preparation by multiple extractions; one requires steam distillation; a solvent extraction method gives incorrect, consistently low values; and a lipid extraction method involves heating that exaggerates the degree of oxidation, leading to consistently high values. All methods are subject to significant colorimetric interferences in real samples. Finally, MDA is not stable, so that advanced lipid oxidation may yield no higher concentrations of MDA than early stages. All versions of these TBARS tests involve wet chemical and other laboratory operations. While their results appear to correlate fairly well with sensory analysis, TBARS tests are not optimal solutions to the problem of rapid screening for lipid-oxidation-induced rancidity.

For several years, the development of rapid, inexpensive, objective techniques for seafood quality evaluation has been a priority [3]. Among the methods is an enzyme-based diagnostic test kit for assaying trimethylamine (TMA) in fish [59]. The kit, which was particularly useful for freshness evaluation of certain species of non-frozen fish, had the enzyme immobilized on a test strip for semi-quantitative determination of TMA in fish press juice [60]. The assay was carried out by color comparison after 5 min. One major disadvantage is the absence of a commercial source for trimethylamine dehydrogenase, the active enzyme. Another disadvantage for field assays and consumer packaging applications is the well-known thermal instability of enzymes. Since the assay for TMA is handled in the liquid phase, it is not suited for use with frozen products unless they are first thawed.

#### *2.1.2. Field assays or intelligent labels for consumer packages*

After a fishy odor was observed in milk when cows grazed on wheat pasture, Kim et al. developed a field assay for TMA in raw milk [61]. Formaldehyde and NaOH were added to a test tube containing the raw milk, the former to complex and sequester $NH_3$ and primary and secondary amines in the milk, and the latter to expel TMA from the milk into the gas phase. An aquarium air pump was used to force the headspace contents (TMA, water vapor and air) out of the test tube into and through a small glass detector tube. The detector tube contained a piece of white acrylic fiber on which a saturated solution of bromocresol green (in its orange form) had been dried. The test tube was submerged in a 28°C water bath for 6 min. The length of green along the fiber was a reliable indicator of the amount of TMA in the milk sample. This simple device could have been used on the farm by minimally trained personnel to determine whether each batch suffered the fishy off-flavor problem, but it was not developed as a commercial product.

Miller et al. have applied for US and international patents on a simple device for determining total volatile bases (TVBs) in seafood and other food applications [62]. Two versions are being marketed, the first for field screening and the second for retail food packages. A color change develops along a dye-coated string and the length of the color change quantifies TVBs in the sample. The first version takes 3 min per sample and is appropriate for screening fish, shrimp and other seafood as well as most red meats, poultry and pork. This version has excellent reproducibility: standard deviations of ±2% for five replicates. The second version can be placed inside an individual retail package. TVB vapors, even at −60°C, diffuse along the string to change its color and indicate decomposition in as little as 1 h. This version provides a continuous assessment of freshness from the factory throughout the transportation, storage, display and purchase of the product. No sample preparation is required, and interpretation of the color is straightforward for anyone, including the consumer. With automated mass production, this version can be fabricated and built into a food container for negligible added cost.

It meets all criteria for food-quality screening by selective detection of an anticipated class of off-flavor agents.

### 2.2. For methods using pattern recognition

#### 2.2.1. Strategies for pattern recognition

An alternative strategy for rapid, objective detection and/or identification of off-flavors is the use of a battery of specific tests, a collection of discrete sensors such as an electronic nose, or a non-specific scanning instrumental detector (such as a mass spectrometer) with or without chromatographic separation. Patterns in the analytical data can be correlated to human sensory analysis [63]. Without chromatography, computerized pattern recognition is necessary to distinguish good from tainted samples. Statistical factor analysis is used to display the significant analytical and sensory features [64].

#### 2.2.2. Sensory analysis

This type of analysis has been used by Porretta in a scheme to distinguish conventional ''commercial'' from ''organic'' tomato products [65]. For 10 representative samples of strained tomato, 26 different chemical and physical properties were measured. Each analytical technique required its own sample preparation. Examples of the qualities measured were total acidity, total solids, color, volatile acidity, mold content, and the levels of NaCl, organic acids, sugars and several pesticides. Added to these chemical and physical factors were scores for seven hedonic attributes obtained by sensory (organoleptic) analysis using a trained seven-member panel. The quantitative values were scaled, relative to each other, by expressing each as the number of standard deviations positive or negative from the average for that variable. These data were subjected to analysis of variance and other statistical interpretation. The most interesting treatments were, (a) principal component analysis, (b) factor analysis based on the first two principal components (PCs) comprising 83% of the total variance, and (c) cluster analysis. The results bunched into two large, distinct clusters that were able to distinguish conventional from organic strained tomatoes, regardless of the brand. Those factors based on analytical measurements that contributing most strongly to the first two PCs were sugar content, total acidity, citric acid, and (negatively) NaCl content. That is, it was possible to distinguish organic tomatoes by objective criteria. Correlation analysis also associated particular sensory panel scores with specific chemical and physical factors, again suggesting the possibility of objective definitions for subjective characteristics. Porretta performed a similar battery of objective and subjective analyses with pattern recognition for tomato purée [66].

In these examples, the use of pattern recognition techniques did not reduce the amount of sample handling but did identify the few measurements most strongly distinguishing organic from conventional tomato products. Future sample handling for evaluation of similar tomato products would be drastically reduced by making only the tests useful in classifying the samples.

#### 2.2.3. Electronic noses

Electronic noses are gas-phase sensor arrays combined with pattern recognition into an instrumental package designed to give an objective basis for odor and flavor identification that correlates with human sensory experience. Two types of chemical sensors have been commonly used in electronic noses: metal oxide sensors and polymer sensors. The instrumental sensor responses are correlated to sensory panel data by using artificial neural network software.

Some practical problems with electronic nose technology appear not yet to have been solved [67]. Just as with the human olfactory sense, the instrumental sensors can become saturated and fail to respond. Particularly for polymer sensors operating at low temperatures, odor causing agents may adsorb irreversibly to a sensor's active surface, causing signal drift, which disturbs the pattern recognition. Irreversible adsorption also eventually requires replacement of the sensor element and re-calibration of the entire system.

A sensor array consisting of eight different amperometric, three-electrode gas sensors, designed to respond to CO, $H_2S$, $SO_2$ and NO, has been used by Schweizer-Berberich et al. to evaluate fish freshness [68]. A temperature-adjustable, catalytic decomposition chamber was inserted between the sample

container and the sensors. Odorous gases passed through the chamber would decompose if the temperature were high enough and give a signal in one or more of the four sensors. The sensors were operated at room temperature. For standard gases (such as TMA, associated with seafood decomposition) each sensor's response was characterized after the gases had undergone catalytic decomposition. The pattern recognition system took into account catalyst temperature as well as individual sensor response and a profile was generated for each standard gas. To test the system, a trout was placed in a polyethylene pouch and stored in a refrigerator for 26 days. The evolving gases were measured each day by pumping out the gas phase over the fish and replacing it with humidity-controlled ''synthetic'' air. No further sample handling was required; most of the experimental effort went into calibrating the equipment.

Results of the experiment of Schweizer-Berberich et al. were encouraging. A principal components (PC1 vs. PC2) score plot for samples taken over 26 days showed a pattern that appeared to be semi-quantitative along a boomerang-shaped curve. The presumption was that if someone, at a later date, put another fish sample of the same species into a similar pouch, the off-gases could be analyzed to determine how many days the sample had been there. These results demonstrate a quantitative use of multivariate statistical pattern recognition [69]. However, because of sensor drift-effects and calibration requirements, we question whether this approach is technically feasible or economically justifiable for quality evaluation of perishable products.

#### *2.2.4. Pyrolysis and other mass spectrometric analyses*

MIB, a cause of muddy off-flavors in catfish, has been detected by thermal desorption in a highly sensitive ion trap mass spectrometer [70]. Collisional dissociation of the protonated molecule ($m/z$ 168) of MIB produces, after a water loss, two product ions ($m/z$ 95 and $m/z$ 109) specific for MIB. The ion trap reduced the 5 h of sample preparation that would be required for a purge-and-trap GC–MS method to 10 min per sample. Neither pattern recognition nor chromatography was required for the analysis.

Goodacre et al. have demonstrated the use of pyrolysis–mass spectrometry (Py–MS) with artificial neural network (ANN) pattern recognition to distinguish extra-virgin olive oil from adulterated oils [71]. Olive oil is typically adulterated using cheaper seed oils that differ from olive oil in the relative amounts of FFAs. The sample handling technique used is not described. In our laboratories, when we use Py–MS for oils, we usually make a 0.1% solution of the oil in methanol, then air-dry 1 μl of the solution onto a wire or foil that can be heated reproducibly and then can be inserted into the mass spectrometer ion source. Nothing more sophisticated than this would be required for olive oil. Goodacre et al. used a small foil strip made of an alloy that loses its magnetic properties at 530°C, the Curie point. Such a foil, placed into a rapidly oscillating electromagnetic field, heats ballistically to 530°C and then stays at that temperature. The sample is thus thermally desorbed from the foil and its vapors are carried through a heated transfer line into the mass spectrometer ion source.

Sample handling for Py–MS is extraordinarily simple: dissolve the oil, dry a drop of solution onto the foil, place the foil into the pyrolyzer chamber, remove the air, start the mass spectrometer acquiring a full-scan spectrum, and initiate the pyrolysis. The instrumental acquisition takes 2 min per sample, even counting sample evacuation before pyrolysis and a waiting period for the clearance of residual pyrolysates from the previous run. The Py–MS system of Goodacre et al. was capable of unattended operation and could handle 300 samples a day; the capacity of the multi-sample carousel limited the productivity more than the analysis time per sample. Samples containing as little as 5% adulteration could be detected and the adulterant could be identified. This result was particularly impressive because a mass spectrometrist, visually comparing pyrolysis–mass spectra for different samples of olive oil, would have had difficulty distinguishing them.

Miller et al. have used Py–MS with statistical pattern recognition to compare extracts from shrimp [72]. Some of the shrimp samples had a corn-like off-odor, but GC–MS total ion chromatograms of the extracts made using an ultra-sensitive ion trap mass spectrometer did not show obvious differences. Although all of the samples had many peaks, visual examination of the complicated chromatographic

traces failed to reveal consistent differences. They did not know whether they had failed to trap the odor components, or whether the odors were in such small concentrations that they could not be seen in a total ion chromatogram. To identify mass spectral features associated with the contaminated shrimp samples, they analyzed replicate samples of the extracts, using a direct exposure probe for sample introduction and Py–MS with statistical pattern recognition. The pattern-recognition with factor analysis identified electron impact (EI) ions (factors) associated with the difference between clear and off-odor samples. They then returned to the original GC–MS ion trap data, plotted single-ion chromatograms of these ions, and found trace odor-associated peaks.

## 3. Sample preparation for direct injection GC analysis

### *3.1. Advanced off-line extraction techniques for natural products*

Three of the isolation procedures for extracting essential oils from spices (distillation, combined distillation–extraction and solvent extraction) require multiple steps, including at least one step that takes 6 to 8 h [73]. Analytical chemists and instrument manufacturers have made great efforts to develop efficient, reliable ways to extract the analytes of interest out of complex matrices such as foods. Their major priorities have been to reduce dramatically the time required by the isolation step, to automate the process for unattended operation, and to reduce consumption of organic solvents. Often, expensive sample preparation equipment has been used to increase productivity and reduce labor costs. *LC·GC* has recently published an issue on current trends and developments in sample preparation, emphasizing the problems of preparing solid samples for analysis [74]. The issue includes an excellent overview by Majors and four articles on supercritical fluid extraction (SFE), pressurized-fluid extraction, microwave-assisted extraction and modern Soxhlet extraction. Features of these techniques pertinent to food off-flavor and flavor analysis are discussed below.

Majors listed the typical sample size reduction techniques for foods as mincing, homogenization, macerating, crushing and blending [75]. Traditional and modern extraction methods were described, with a critical evaluation of the modern ones. Table 1, a simplified version of one from Majors' article, compares characteristics of seven extraction methods.

SFE uses a variety of fluids (typically, $CO_2$ possibly modified with organic solvents), high pressures (2000–4000 p.s.i.≈14 000–28 000 kPa), and elevated temperatures (50–150°C). Since methods using SFE are heavily matrix-dependent, sepa-

Table 1
Comparison of selected extraction methods for solid sample preparation (reprinted in part with permission from Ref. [75])

| Extraction method | Sample size (g) | Solvent volume (ml) | Time[a] (h) | Degree of automation | No. of samples[b] | Cost[c] |
|---|---|---|---|---|---|---|
| Sonication | 20–50 | 100–300 | 0.5–1.0 | None | 1 (serial)<br>Many (batch) | Low |
| Traditional Soxhlet | 10–20 | 200–500 | 12–24 | None | 1 (serial) | Very low |
| Modern Soxhlet | 10–20 | 50–100 | 1–4 | Mostly | 6 (batch) | Moderate |
| SFE | 5–10 | 10–20[d] | 0.5–1.0 | Fully | 44 (serial) | High |
| Pressurized-fluid | 1–30 | 10–45 | 0.2–0.3 | Fully | 24 (serial)<br>6 (batch) | High |
| Closed-vessel, microwave-assisted | 2–5 | 30 | 0.1–0.2 | Mostly | 12 (batch) | Moderate |
| Open-vessel, microwave-assisted | 2–10 | 20–30 | 0.1–0.2 | Mostly | 6 (batch) | Moderate |

[a] Total processing time per sample from weighing to collection.
[b] Maximum number of samples that commercial instruments can handle; serial means one sample processed at a time, and batch means multiple samples at a time.
[c] Very low=less than US$1000; low=less than US$10 000; moderate=US$10 000–20 000; high=more than US$20 000.
[d] Solvent volume when organic modifier is used to affect polarity.

rate method development is required, which requires experienced operators. Nevertheless, SFE is useful for sample preparation for food analyses, including fats [76,77], pesticide residues and toxins [78–80]. SFE generally produces clean extracts with so little residual organic solvent modifier that additional concentration may not be necessary before GC analysis.

Microwave-assisted extraction offers a particularly intriguing feature for analysis of labile food flavor compounds: the extraction of plant or animal tissue in a microwave-transparent solvent [81]. The cells are broken by internal heating at the microscopic scale but the contents immediately spill into the cool, surrounding solvent so that thermal degradation of labile components is minimal. Microwave-assisted extracts may require concentration before analysis.

Pressurized fluid extraction (also referred to as pressurized solvent extraction, accelerated solvent extraction, pressurized accelerated solvent extraction and enhanced solvent extraction) is performed at near-SFE pressures (1500–2000 p.s.i.≈10 000–14 000 kPa) and elevated temperatures (50–200°C). It can be done using modified SFE instrumentation and method development is easy. Solvents appropriate for Soxhlet extraction are substituted for the supercritical fluid, and the extracts may require concentration before analysis.

Results for pressurized fluid extraction are comparable to Soxhlet extraction, but take only 5% of the time and consume only 10% of the organic solvent. Matrix effects are much less prominent than for SFE. Food matrix applications of this technique include pesticides from fruits and vegetables [82,83]; fats from a large variety of foods [84–87]; and fatty acids from egg yolk, chicken and cereal [88]. In some of these applications, direct chromatographic analysis was conducted on the resulting extract. In other cases, fats were analyzed gravimetrically or by generating fatty acid methyl esters followed by GC separation. Clearly, pressurized fluid extraction is applicable for chromatographic analysis of flavors and off-flavors in food. The only issue is whether labile flavor components would be degraded by the extraction's high temperatures and pressures.

### *3.2. Sample preparation for olfactory detection*

Olfactory detection, a variant of sensory analysis, places the nose of a trained expert at the outlet of a GC column. The expert operates a slide rheostat with settings ranging from no odor to moderate odor to extreme odor, and the attached time vs. intensity recorder generates a trace looking like a chromatogram [89]. Odors can be characterized as a function of retention time and the results correlated to responses of an instrumental sensor, such as a flame ionization detection (FID) system or mass spectrometer into which some of the effluent is split. Grosch has reviewed methods for quantifying the strength of odors sensed during olfactory detection [90]. According to him, only a small percentage of compounds in food contribute significantly to odor or flavor, so the ability to assess separated components by their odor strength is a critical first step in odor/flavor analysis. The same would be true for off-odors/off-flavors. ''Charm'' analysis determines a flavor dilution factor (a relative measure of flavor or odor intensity), and aroma extract dilution analysis (AEDA) yields an absolute odor activity value proportional to the component's contribution to flavor. Grosch mentioned the disquieting fact that Charm and AEDA are not corrected for losses of odorants during the isolation procedure. Clearly, the ability to make the desired assessment depends on retaining volatile components chemically unchanged throughout the sample handling and separation. This requirement is as essential for separations using instrumental sensors as for those with olfactory detection.

### *3.3. Sample preparation for GC analysis*

#### *3.3.1. Packed-column GC*

After introducing dimethyl sulfide and 2-pentanone into a tank of cultured catfish, Maligalig et al. compared a packed column GC–FID analysis of cooked and uncooked fish tissue to results of sensory panel taste tests for assessing the effects of concentration and exposure time [91]. Sample preparation was fairly simple. An aqueous fish tissue extract was prepared by homogenizing 1.5 parts of water with 1 part fish tissue, segregating the extract from solids by refrigerated centrifugation, and storing it in a refrigerated flask. A 2-ml volume of extract was combined with 1.2 g of anhydrous sodium sulfate and shaken in a vial. After the vial was equilibrated at 60°C, 1 ml of its gas headspace was sampled with

a syringe and injected onto the packed GC column. The simplicity of these procedures was possible only because the physical and chemical properties of the analytes of interest were known. The two compounds had been selected for their likelihood to occur in real fish samples from contaminated environments, for distinctive odor characteristics that the panel could distinguish from ''fishiness'', and for characteristics amenable to GC separation and analysis.

Lovell and Broce used a packed-column GC method to discover that geosmin was the cause of an earthy-musty flavor in pond-cultured penaeid shrimp [26]. Sample preparation involved mincing 12 g of shrimp tissue, distilling under high vacuum and low temperature, and extracting the distillate with hexane. Because the human sensory threshold for MIB is so low (0.55 μg/kg), they speculated that their method probably would not be able to detect MIB in shrimp even when it made a significant contribution to off-flavor.

A method that achieved 90–96% recovery of lower-molecular-mass FFAs from milk and butter by bromo-phenacyl derivatization was developed in 1975 [92]. However, the IDF FFA extraction procedure followed by methyl esterification, was used by Nath et al. in 1994 to evaluate performance of ''vacreation'', a machine for removal of volatile aldehydes and FFAs from lipid-oxidized butter [55]. As discussed above, the IDF procedure gave poor recoveries for short-chain fatty acids.

An analytical method, using packed column GC, was developed in 1972 for determination of styrene, ethylbenzene, cumene and *o*-xylene at 50 ppb in milk products [93]. Since these polystyrene monomers are fat-soluble, the fat emulsion was destroyed by heating 60 ml of milk with 15 ml of a de-emulsifying reagent in a boiling water bath for 15 min. The fat layer was removed after centrifugation, saponified by adding 15 ml of 0.8 *M* alcoholic KOH, and boiled another 10 min. After cooling, the fat layer was extracted with $CCl_4$, 5 μl of which was injected on-column. Like other analytical methods of this era, there was a fair amount of wet, test-tube chemistry involved in the sample preparation. Unsophisticated instrumentation was used, much time was expended, and dangerous solvents were used, but excellent results often were obtained. In the hands of competent analysts, technology now regarded as obsolete was able to deliver results.

### *3.3.2. Capillary GC, capillary GC–MS, and derivatization capillary GC*

Off-line sample preparation of foods for capillary GC analyses seems to require either laborious multiple liquid–liquid extractions or, for volatile analytes, less labor but more complicated glassware. A sample preparation method of the latter sort was developed for quantitative analysis of orange-juice flavor volatiles [94]. After pulp and seeds had been removed, the filtered juice was vacuum distilled at 150 mmHg (=200 mBar=20 kPa) and 60°C. Condensate was collected for 25 min in a chilled water trap. After this, the vacuum was relaxed and a downstream liquid $N_2$ cold trap was warmed to allow the most volatile components to thaw and drain into the chilled water trap. This produced an aqueous extract that could be injected (10 μl) directly onto a capillary GC–FID system for analysis. Of 24 volatile components detected, 16 were identified. Most of the components detected were more volatile than limonene; less volatile compounds were generally not detected.

An even more complex glassware system, for micro-steam distillation/solvent extraction (μ-SD/SE), has been used to isolate semi-volatile flavor compounds from cinnamon, because the volatility range of the essential oils was too wide for more traditional approaches [95]. Ground cinnamon was placed in a boiling flask with 40 ml of water and heated to boiling with continuous stirring. Distillation vapors were condensed by a chilled-water cold finger so that the condensate, containing water and semi-volatile compounds, dripped into a reservoir holding two liquid phases: pentane and water. The semi-volatile compounds were extracted from the water into the pentane upper layer and evaporated again back up into the cold-finger condenser region. Higher up the cold finger, a vapor-transfer arm led to a small vessel containing only pentane. After the combined steam distillation/solvent extraction process had been continued for 1.5 h, the heat was removed so that refluxing processes in all three vessels stopped. The pentane from the two-phase reservoir was combined with that in the small pentane-only vessel and injected directly for GC–FID and GC–MS analysis. Recoveries ranged from 96% for eugenol to only 4% for coumarin. However, even with poorer recoveries, relative standard deviations (RSDs) were good and quantitation was pos-

sible. For 22 compounds extracted, RSDs varied from 1.4 to 17.6%.

μ-SD/SE has been combined with capillary GC and GC–MS to examine γ-irradiated grapefruit juice for damage [96]. It also has been used effectively with capillary GC–MS to quantify alkylphenols and aromatic thiols in tainted fish from rivers below pulp and paper mills [97].

The preparation of foods for capillary GC analysis begins with mincing, dicing, homogenizing, etc., followed by steps involving multiple liquid–liquid extractions, pH adjustments, precipitation–centrifugation, drying out, or evaporation–concentration. Although the apparatus may be simple, the multiple steps take a long time. For example, when pyrazines, suspected sources of ''earthy'' or ''potato-like'' off-flavors in Canadian maple syrup, were analyzed by capillary GC and GC–MS [98], the preparation involved successive extractions with diethyl ether (3 h, followed by standing overnight), an acidic solution (five times), a basic solution, and finally $CH_2Cl_2$ (five times, then dried over $MgSO_4$, then concentrated almost to dryness under $N_2$). Seven pyrazines were detected and identified, but quantitative characteristics of the method were not reported.

Many of the most common off-flavor compounds, particularly biogenic amines and oxidation-produced aldehydes, show peak tailing in gas chromatograms. The tailing can be caused by thermal decomposition in the injector or by on-column interaction with active sites. Sample preparation can alleviate these problems by adding a derivatization step to give chromatographically stable products. However, it also introduces another layer of complexity into the procedures and another layer of uncertainty into quantitative results. Baker et al. [99] developed a GC method for biogenic amines in cheese and chocolate that involved homogenization in a cold acid suspension, addition of an internal standard, refrigerated centrifugation (15 min), basification of an aliquot from the supernatant, another centrifugation, collection of supernatant, liquid–liquid extraction (10 min), another centrifugation (5 min), discarding the aqueous fraction, back-extraction with strong acid (5 min), another centrifugation (5 min), and basification of the retained acid (aqueous) layer. At this point, a mixture (including benzene) was added that not only extracted the amines from the water but also formed the pentafluorobenzoyl derivatives. The mixture was shaken (15 min), then centrifuged again (5 min). The organic phase was separated, taken to dryness under $N_2$, and reconstituted with toluene. The toluene was washed by shaking with $NH_4OH$ (15 s). After one final centrifugation with separation, the toluene solution was ready for injection.

If done on a routine basis, this analysis would be extremely labor-intensive and environmentally unfriendly due to the benzene. Baker et al. claimed on-column detection limits in the range of 5 to 20 pg [99]. They realized high recoveries for most amines, but histamine recovery was only 66% and 6-hydroxytryptamine was only 42%. Calibration curves were linear over two orders of magnitude. RSDs were not reported, but at levels for 12 amines found in cheeses, standard deviations ranged from 10 to about 90% of the amount detected. For the same 12 amines in chocolates, standard deviations were much smaller relative to the levels measured, many around 5%. Although they summarize their procedure as ''rapid, sensitive'', we can agree only with ''sensitive''.

Chromatographic quality for amines and aldehydes may be greatly improved by pentafluorobenzoyl and 2,4-dinitrophenylhydrazine (DNPH) derivatization, respectively, during sample preparation [100].

#### *3.3.3. High-temperature capillary GC*

Because most flavor and all odor components in food are volatile or semi-volatile and many are also thermally labile, high temperature GC is seldom required or even desirable. However, Lanças and Galhiane used that technique with no evidence of decomposition for determination of limonene, a bitter off-flavor sometimes found in citrus juice [101].

## 4. Sample preparation for HPLC analysis

### *4.1. Problems with HPLC analysis*

Although HPLC has been used as part of the clean-up procedure for food analysis by GC [102], like high-temperature GC, HPLC has not often been used directly for flavor/off-flavor analysis because most flavors and odors are amenable to high-res-

olution GC analysis. On the other hand, problems with on-column thermal instability of labile compounds with GC are eliminated for ambient-temperature HPLC separations. For amines and aldehydes, the problems with GC analysis have caused some investigators to examine HPLC alternatives for the determinative separation. Many biogenic amines have no UV chromophores, and consequently no fluorescence, so considerable effort has been expended to develop pre- or post-column derivatization techniques for attaching chromophores or fluorophores [103–105].

### *4.2. Amines indicative of putrefaction in fish, cheese, sausage or olive oils*

A HPLC method for determining five biogenic amines, regarded as objective quality indicators, has been developed for canned tuna [106]. The method, like the GC methods discussed above, specified multiple extraction steps and a dansyl (5-dimethylaminonaphthalene-1-sulfonyl) derivatization that required several hours to achieve complete reaction. When evaluated recently, the method consumed more than 6 h, too long for same-day extraction and analysis [107]. An alternative method for amines shortened the dansylation step from overnight at ambient temperature to 1 h at 37°C and shortened the HPLC separation from over 30 min to less than 17 min [108]. In a ''simple and rapid'' method for amines, also based on dansyl derivatives, homogenized fish tissue was extracted with 5% trichloroacetic acid for only 2 min [109]. After filtering, the extract was diluted 10-fold with more 5% trichloroacetic acid and then basified with a drop of 4 *M* NaOH. A drop of pH 9 buffer was added, and then the dansylation was conducted at 55°C for 1 h. The time required for sufficient extraction efficiency was estimated, by measuring the recovery of amines spiked into the fish tissue and extracted for different lengths of time, as 2 min. The optimum time for an incurred residue was assumed to correspond to the earliest point on the response plateau for this spiking experiment. Recovery of incurred residues in real samples, however, is often much more difficult and time-consuming than recovery of samples spiked into the tissue [110]. Optimizing efficiency using incurred residues is a more valid way to fine-tune an extraction procedure.

Yet another HPLC method for biogenic amines in canned fish, this time for simultaneous analysis of nine amines, used benzoyl rather than dansyl derivatives, because of the former's chemical simplicity, stability and economy [111]. The reaction time was 20 min, and all sample handling procedures were complete in less than 2 h. HPLC separations of the benzoyl derivatives were complete in less than 10 min each.

A dansyl derivatization method for amines in dry sausage included a step in which excess dansyl chloride was effectively removed after the derivatization was complete [112]. Because unreacted dansyl reagent had been interfering with the analysis of amines by co-eluting with dansyl-cadaverine, excess $NH_3$ was used to react with the remaining reagent, yielding a product that did not interfere with any of the peaks of interest. Unreacted $NH_3$ was not detectable and the HPLC separation was complete in 16 min.

Sample preparation and dansyl derivatization for the simultaneous determination of nine biogenic amines in fish tissue by HPLC yielded a 22-min separation with injections possible every 30 min after equilibration [107,113,114]. Proline was added rather than $NH_3$ to neutralize excess dansyl chloride. Sample handling time was reduced to 40 min. All quantitative figures of merit for the optimized sampling protocol were excellent, and the method was pronounced ready for automated, routine analysis.

Advocates of benzoyl derivatization for HPLC of amines have argued that fluorogenic reaction products are unstable, tosylation reaction procedures are too lengthy, and elution times for dansyl derivatives of some biogenic amines are too great [115]. Comparing results for a $3\times3$ matrix of reaction temperatures and times, optimal benzoylation conditions were determined as 40 min at 30°C. The only other step that took a few minutes was the evaporation to dryness under $N_2$ of 3 ml diethyl ether. Recoveries for four of nine amines approximated 100%, four others fell between 50 and 70%, but agmatine was recovered with less than 5% efficiency. RSDs for the method were not reported, but analysis of a fried marlin fillet implicated in a food poisoning incident showed standard deviations of 3 to 8% for three

amines above the 1 mg/100 g detection limit. Two peaks were associated with unreacted benzoyl chloride. One of these interfered with the tryptamine derivative, but the effect was minimized by the optimal reaction conditions chosen. Linear dynamic range for the method was only slightly greater than one order of magnitude. It appears that this alternative approach offers substantial savings in sample preparation and separation time, but with compromises to the quantitative figures of merit.

Extraction with dansylation for HPLC of eight biogenic amines in fermented table olives showed that the amines were associated with spoilage [116]. Nine combinations of temperature and time for the dansylation reaction were examined and 35°C for 2 h was the best. 5% Trichloroacetic acid (TCA) was slightly less efficient than methanol for initial extraction of amines from olive paste. Moreover, because methanol also extracted oils, TCA was recommended for oily foods. Linear dynamic range for the method approximated three orders of magnitude.

### *4.3. Aldehydes indicative of temperature abuse in fruit juice*

A HPLC method for 2-furaldehyde and 5-hydroxymethyl-2-furaldehyde in fruit juice required the derivatization of carbonyls with DNPH, thus improving analytical sensitivity and selectivity relative to HPLC methods that did not use derivatization [117]. The DNPH was dissolved in a perchloric acid–acetonitrile solution, rather than in the usual aqueous HCl, so that the resulting derivatives could be injected directly into the HPLC. This saved a number of sample handling steps. Derivatization was complete within 25 min and recoveries from spiked juice ranged from 93 to 96%. The linear dynamic range of this method was four orders of magnitude, encompassing all concentration values typically found in juice samples.

## 5. Sample preparation for headspace GC analysis

### *5.1. Food applications*

Compared to solvent extraction, purge-and-trap, and direct-injection GC methods, equilibrium headspace GC dramatically reduces sample preparation time for analysis of volatile flavor and odor components [118]. Commercial instrumentation with autosampler capability exists [119]. However, the method suffers volatility-based discrimination, which necessitates experimentally-developed or theoretically-calculated calibration curves for each component [120]. The required data manipulations can consume all the time saved by easier sample preparation. Also, without trapping and concentration steps, static headspace analysis often has insufficient sensitivity for trace components. With all these limitations, the method has been used for certain food applications, as described below.

### *5.2. Off-flavors in orange juice*

Equilibrium (static) headspace GC has been used to analyze packed orange juice for the volatile flavor components α-pinene, octanal and *d*-limonene, which are absorbed into polymeric packing materials during storage [118]. A 25-ml sample of orange juice was injected into a 38-ml, nitrogen-filled glass vial. The vial was heated for 15 min to 50°C in a water bath to establish thermodynamic equilibrium. After this, 0.5 ml was withdrawn from the headspace and injected into the GC system. In other words, sample preparation was almost nil. Fig. 2 shows a typical headspace GC–FID chromatogram. α-Pinene, octanal and *d*-limonene appear at 11.2, 12.8 and 14.2 min, respectively. From the chromatogram, it is clear that the orange juice headspace also contains a large number of other volatile components. If quantitative determination of other components is necessary, each would require its own calibration curve to be established by injections of an authentic standard.

Shaw et al. used headspace GC with pattern recognition to classify 60 samples of commercial orange juice into four categories: (A) fresh-squeezed, not pasteurized; (B) pasteurized, not from concentrate; (C) reconstituted, from concentrate; and (D) single-strength, aseptically packaged from concentrate [121]. Fig. 3 shows a two-dimensional eigenvector score plot for the first and second principal components of variation. The pattern recognition was based on quantitative values for 19 separate compounds, and each value was determined from a regression for that compound. Each regression was

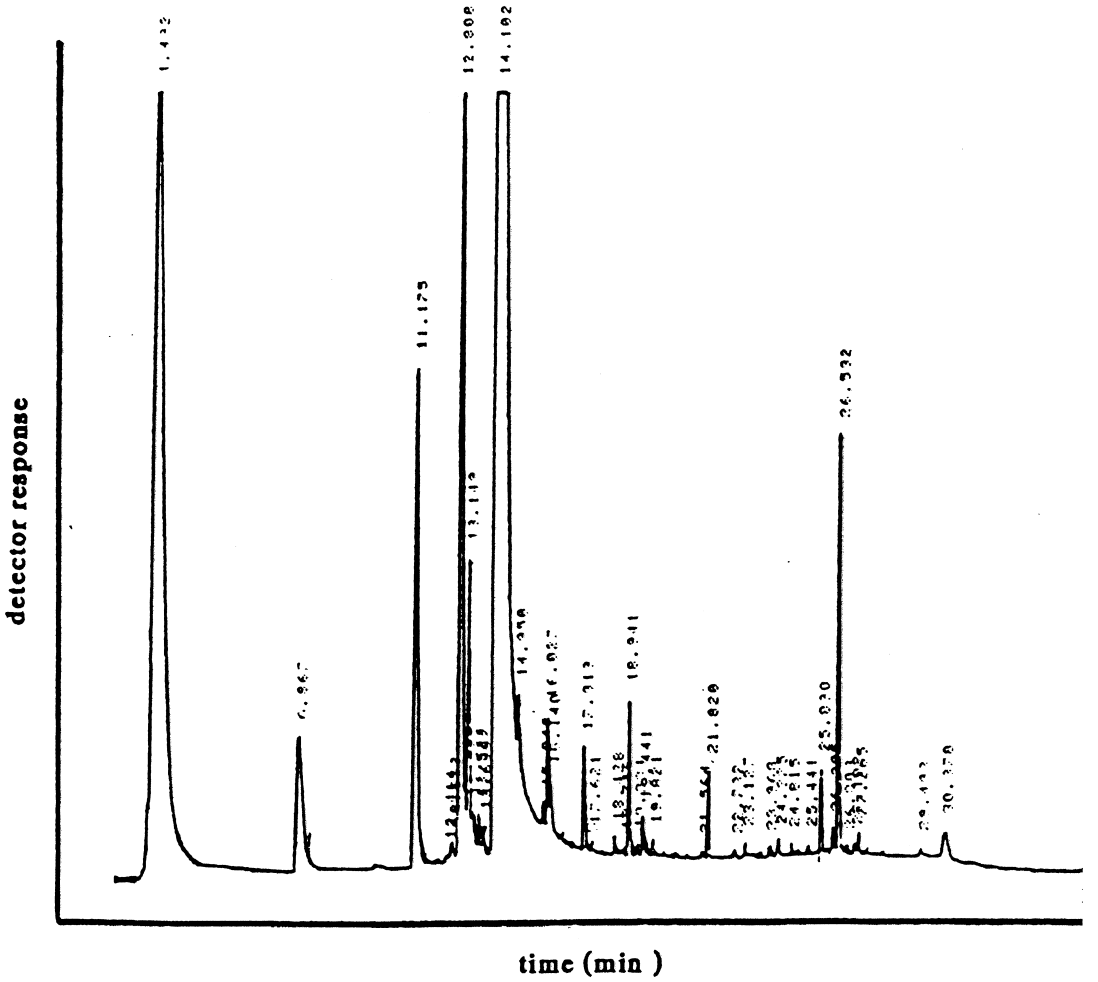


Fig. 2. Typical equilibrium headspace gas chromatogram of orange juice at 50°C. (Reprinted by permission from Ref. [118]).

based on headspace sampling of a reconstituted juice base that had been vacuum-cleaned of all flavor volatiles and to which one of five different concentrations of each standard had been added.

### *5.3. Off-odors or flavors in boiled fish, dehydrated potato or pasteurized milk*

Sampling of decreasing volumes from the headspace over boiled fish samples has been used with olfactory detection to establish AEDA values for individual components contributing to sample odor [122]. To standardize the injection conditions, the sampled volatiles were first trapped downstream and then thermally desorbed at the beginning of each analysis. The AEDA analysis assigns relative strength to odor components by comparing the ability to detect them as samples are serially diluted. It compares the minimum sample concentration for which all analysts agree that they can smell every component to the diluted concentration at which each component can no longer be detected. The strongest odor is the one detectable at the greatest dilution. This kind of measurement yields a significant, but relative and subjective, indicator of sample concentration. It does not require that calibration curves based on multiple data point regressions or liquid phase–gas phase partition coefficients be calculated

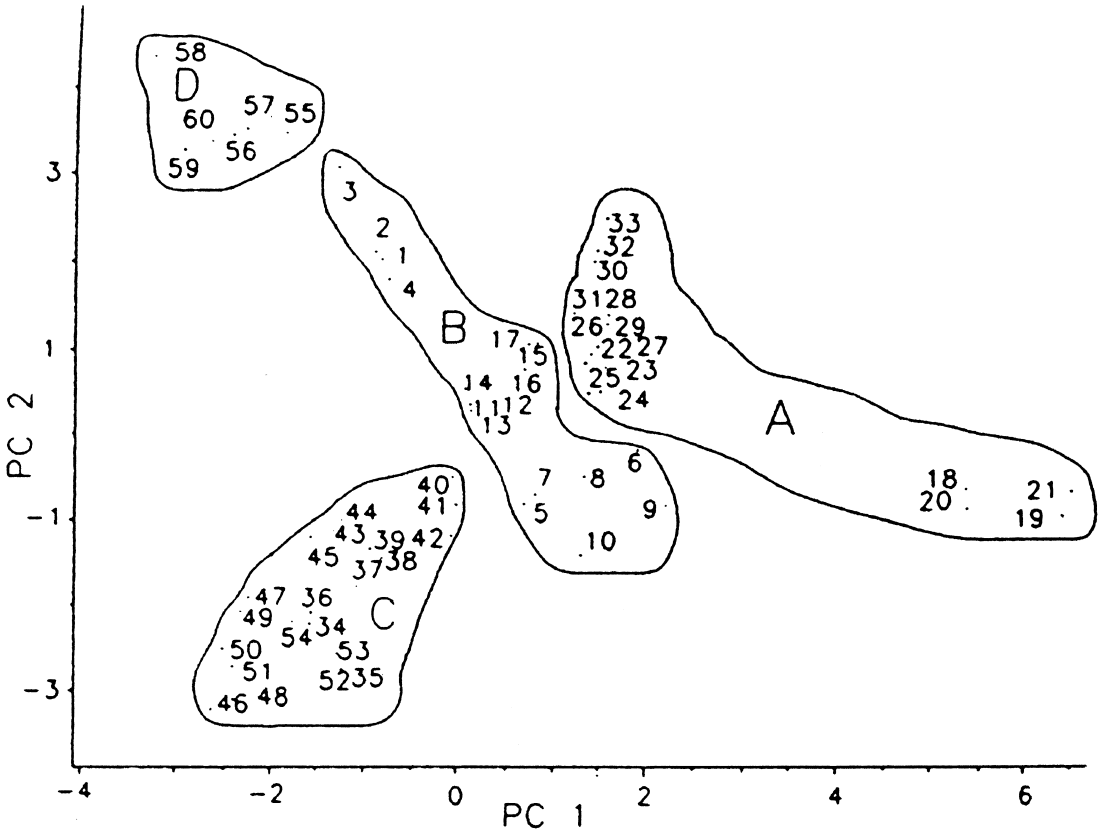


Fig. 3. Two-dimensional eigenvector score plot for the first and second principal components (PCs) of variation, pattern recognition based on headspace GC of orange juice volatiles. Sixty samples plotted into four categories: (A) fresh-squeezed, not pasteurized; (B) pasteurized, not from concentrate; (C) reconstituted from concentrate; and (D) single-strength aseptically packaged from concentrate. (Reprinted by permission from Ref. [121]).

for each component. This experimental design takes full advantage of headspace GC's easy sample preparation and avoids its greatest limitation.

Manual sampling of headspace vapors above dehydrated, ground potatoes was combined with manual injection into a GC system to identify odor components associated with poorly-controlled processing [123]. The procedure worked well, but in the absence of an automated system, the technique required absolute control of experimental variables, an internal standard, and individual calibration curves for all compounds contributing significantly to off-flavor.

Christensen and Reineccius published a method for static headspace GC analysis of volatile sulfur compounds in heated milk [124]. With minimal sample preparation (putting the milk into the vial and capping it), they detected $H_2S$ and dimethyl sulfide, which showed thermal decomposition. Their system included an automatic headspace sampling apparatus, a 3-ml (large volume) sampling loop, head-of-the-column cryo-focusing capability, and a flame photometric detection (FPD) system capable of sulfur-mode operation. They also bypassed the split in the injection port. Although the results were not encouraging, the authors had the honesty to describe them without flinching and did not try to oversell the method. Sensitivity was not good for either $H_2S$ or dimethyl sulfide and other compounds were not detected. Problems included breakthrough of $H_2S$ through the cryo-trap, necessitating a blank run between each pair of data-generating runs and reduc-

ing sample throughput to 1–1.5 runs per hour. Also, the non-linear response of FPD in sulfur mode necessitated a daily multilevel calibration. The authors found that the same amount of dimethyl sulfide spiked into whole and skim milk gave different responses because the fat in whole milk caused a significant matrix effect. Therefore, the simplified sample preparation could only be used for skim milk. The honest description of these problems in this paper is particularly useful in illuminating issues of sample preparation for foods analysis.

## 6. Sample preparation for purge-and-trap or distillation GC analysis

### *6.1. Off-line techniques*

Grob and Habich stated that ''Off-line techniques involving sampling and concentration steps clearly separate from the subsequent GC analysis are the most reliable, but not necessarily the most convenient'' [125].

### *6.2. Dairy products*

#### *6.2.1. Volatiles emitted during ripening of cheese*

Yang and Min used dynamic headspace analysis to determine volatile compounds produced during the ripening of cheddar and Swiss cheese [126]. Their procedure included heating a sample bottle containing cheese cubes to 40°C, purging with $N_2$, trapping the evolved gases onto Tenax, thermally desorbing them at 160°C and cryo-focusing them onto a cryo-trap at the head of the capillary GC column. Because the concentration step is temporally and spatially removed from the injection and can be controlled independently, this technique is off-line. At least 30 compounds were detected, including volatile alcohols, ketones, chlorinated hydrocarbons, aromatics, sulfides and esters. Yang and Min described their method as ''simple, rapid ($<$3 h/sample compared to from 5 h to 4 days for other published techniques), sensitive and reproducible (RSD 3.15%).''

#### *6.2.2. Milk off-flavors*

Wellnitz-Ruen et al. used headspace concentration capillary GC to examine fruity off-flavor (primarily from ethyl butyrate and ethyl hexanoate) that appeared in milk after extended refrigeration [127]. Helium was bubbled through the milk for 40 min and purged volatiles were trapped on Tenax. Since thermal desorption from the Tenax trap coincided with on-column injection for analysis, this was an on-line process. (There was no cryo-focusing apparatus). Reproducibility for 10 replicate analyses of 2% milk samples was excellent; RSDs were 1.4% and 5.1% for ethyl butyrate and ethyl hexanoate, respectively. However, RSDs were around 30% for analyses of low concentrations in whole milk. The authors attributed the much larger variations to the lower levels, but another explanation might be the sequestering effect of fat in whole milk. 2% Milk showed higher concentrations of the esters than either skim or whole milk. This result might be explained if the biochemical source of the esters is milk-fat decomposition, but they are not easily recovered by purging from whole milk. The purge-and-trap methodology, with dynamic bubbling of inert gas through the sample, ought to be less susceptible to fat solubility matrix effects than the direct headspace method. Significant enhancements in sensitivity and resolution were obtained by inserting a cryo-focusing trap.

Purge-and-trap headspace analysis has been used to study aroma compounds in milk and other dairy products [128]. The apparatus used two cold traps in sequence, the first to condense water and the second to trap the organic volatiles. The milk samples were purged for 20 min and the GC separation was initiated by rapidly warming the second trap to 250°C. Differences in chromatographic resolution between compounds directly injected and those desorbed from the second cold trap were negligible. Calibration curves for test compounds showed responses 29 to 219% higher when they were purged from water than when purged from whole milk. However, using the whole-milk calibration curve, recoveries of dimethyl sulfide and isopentanal added to 3% milk were complete within the measurement variability. That is, the purge-and-trap method, when calibrated to compensate for matrix effect, had quantitative integrity.

Dynamic headspace analysis was coupled with multivariate principal component regression analysis

to predict the shelf life of homogenized and pasteurized whole milk [129]. With 18 h of incubation and 2 h of analytical detection and data processing, milk shelf life at 4°C, as judged by trained experts, could be predicted with a standard error of less than 2 days. Each milk sample was purged for 12.5 min at 44°C. Significantly, initial total bacterial cell counts did not reliably predict the shelf life.

Dynamic-headspace-sampling capillary GC for 20 min, with multivariate data analysis, has been used to predict with up to 93% accuracy the type of abuse responsible for off-flavor in milk [130]. Two thermally desorbed Tenax traps (one off-line and one on-line) were used with a cryo-focusing trap. Accurate predictions were made, demonstrating the adequacy of the analytical data.

### *6.3. Seafood*

#### *6.3.1. Volatiles from oyster decomposition*

Volatile compounds in fresh and decomposed oysters have been examined by purge-and-trap GC–MS [131]. Oyster samples (1 g) were homogenized and 1 ml saturated aqueous KCl solution with 100 μl of an internal standard were added. The mixture was purged with He for 4 min at 40 ml/min onto a Tekmar dynamic headspace concentrator with Tenax glass liners. The concentrator cartridge was dry-purged for 3 more minutes to remove water vapor. The cartridge was desorbed for 4 min at 150°C to initiate the chromatographic separation. No cryo-focusing was used. Total ion chromatograms showed significantly poorer resolution than would be expected for direct-injection capillary GC, but mass spectral detection compensated for the poorer resolution of near-co-eluting compounds by retention time differences observed in single-ion chromatograms. That is, a more sophisticated detection system can compensate for a less capable sample handling and separation method.

#### *6.3.2. Rancidity and other off-flavors in fish and clams*

Purge-and-trap GC has been used to determine MIB in water, mud and cooked channel catfish samples [132]. The bioconcentration factor of MIB (concentration in water/concentration in fish) was 28.1±14.0. Purge-and-trap GC–MS has also been used to identify geosmin as the major muddy off-flavor of Louisiana brackish water clam [133].

Przybylski et al. developed a GC system for trapping and analyzing volatiles from canned tuna, broth and mixed tuna/broth samples [134]. They heated the samples to 80°C and purged them for 2 h with He, trapping volatiles on a liquid $N_2$ cooled pre-column. Because the samples contained large amounts of water, they installed a condenser between the sample container and the pre-column cryo-trap. After purging and trapping, that apparatus was removed from the system before the capillary GC separation was initiated. Solid samples were mixed with a dehydrating agent to bind all free water and set aside for an hour to give enough time for binding to occur before purging. This step, necessary to eliminate ice plugging of the cryo-trap, increased recoveries from the range 50–70% to 80–97% and reduced RSDs from 5.1–11.5% to 2.8–5.2%. Use of the dehydrating agent improved contact and penetration between the purging gas and volatiles in the samples. Among the interesting results was the consistent observation that the off-flavors were found at greater concentrations in the fatty tissue than in the broth, even from the same specimen. The most pronounced differences were evident for components with low water solubility. That is, the differential solubilities that produce the fat matrix effect also express themselves in the distribution of the chemicals within the animal.

A dynamic headspace method to sample and concentrate volatile, rancid off-flavor aldehydes from frozen catfish fillets for quantification by capillary GC has been used for purge-and-trap analysis of catfish fillet disks (3 mm thick×12 mm diameter) [135,136]. Samples were distinguished by their site within the fish: lateral, skin-side, visceral-side and internal. Intact tissue samples were purged for 1 h at 50°C. (Presumably, the longer purge time was required because the sample was a solid plug, not homogenized tissue). Tenax traps were thermally desorbed in an external closed-inlet device at 225°C for 5 min. Significant results included discovery that the lateral line was highly susceptible to lipid oxidation after only 2 months storage at −20°C. The authors recommended evaluation of the lateral line, rather than the whole fillet, to assess rancidity.

Conte et al. have described an off-line argon

purge/microwave distillation/solid-phase adsorbent trapping device for determination of geosmin and MIB in catfish tissue [137,138]. The microwave time required to remove the off-flavors efficiently from a ground 20-g catfish tissue sample was 10 min. During passage through a 5°C water-cooled condenser, the volatiles either condensed and ran down into a $C_{18}$ Sep-Pak cartridge or were trapped from gas phase on the cartridge. After distillation, the condenser and Sep-Pak were rinsed with water to remove any polar residues; this rinse was discarded. The analytes were then eluted by rinsing both the condenser and the Sep-Pak twice with 1 ml of ethyl acetate, and then the collected extract was dried over $Na_2SO_4$. A 1-μl volume was injected onto a GC–ion trap MS system focused selectively on three ions each for the geosmin, MIB, and two internal standards. Recoveries for geosmin were 91.3% at 5 ppb and 78.7% at 500 ppb; for MIB they were 92.8% at 5 ppb and 99.6% at 500 ppb [138]. Detection limits for geosmin and MIB were 0.630 ppb and 0.217 ppb, which were 13-fold and four-fold, respectively, below the rejection levels for the two compounds. The simplicity, performance, and rapidity (microwave distillation/trapping/extraction, 20 min/sample; 35 min total/sample) of this method compare favorably with the previous state-of-the-art. Only 2 ml of organic solvent were consumed per analysis and no concentration step was necessary.

### *6.4. Miscellaneous foods*

Two procedures, steam distillation and solvent extraction (SDE) or steam distillation and adsorption, were used to collect volatile components from garlic [139]. For SDE, 50 g of garlic was blended for 2 min with 200 g water and then extracted for 2 h with a mixture of pentane–diethyl ether (1:1). Volatile extracts were then dried over anhydrous $Na_2SO_4$ and concentrated under $N_2$ to 0.2 ml. For the adsorption method, 20 g of garlic and >2 l of water were homogenized for 2 min in the blender, and then distilled for 1 h. The distillate was passed through a Tenax column and the volatiles were desorbed with 40 ml of ether. Again, the ether was dried over $Na_2SO_4$ and concentrated as before. The first method yielded a 31% greater mass of volatiles than the second on replicate samples, but this may have been caused by the use of a shorter distillation time for the second method.

## 7. Sample preparation for solid-phase microextraction–GC analysis

### *7.1. Food applications*

Solid-phase microextraction (SPME) was originally conceived as a variant of traditional headspace analysis in which a stationary phase, commonly poly(dimethylsiloxane) coated on a fused-silica fiber, is placed in the headspace of a sample vial [140]. After equilibration between volatiles in the headspace and on the solid surface of the coated fiber, the analytes are thermally desorbed by insertion into a GC injection port, cryo-focused on the head of the column, and separated by capillary GC procedures. Page and Lacroix examined the performance characteristics of SPME with a model aqueous system containing 33 common volatile and semi-volatile environmental pollutants [141]. They compared conventional headspace and SPME analysis with respect to sensitivity and selectivity for the 33 compounds. They investigated the effect of adding NaCl to the aqueous phase to increase partitioning of volatiles from the liquid into the gas phase. Envisioning fatty-food applications, they also studied the effect of non-polar, non-volatile materials in the aqueous layer on the partition of volatile analytes. Finally, they investigated the differences in partitioning associated with immersing the fiber in the aqueous layer or the headspace above the liquid.

Many of Page and Lacroix's results are relevant to flavor and off-flavor applications in foods [141]. For example, Fig. 4 shows two chromatograms of the model system, with each analyte at 2 ng/ml. The trace at A shows the response for headspace SPME with 15 ml NaCl–saturated water added to the aqueous phase. B shows the corresponding response for conventional headspace analysis. Since the separation was volatility-based, the traces demonstrate the complementary volatility-based discrimination tendencies for the two techniques. Overall, headspace analysis shows much less response than headspace SPME because the former only uses 1 of the ~28 μl of available gas phase, but the latter injects

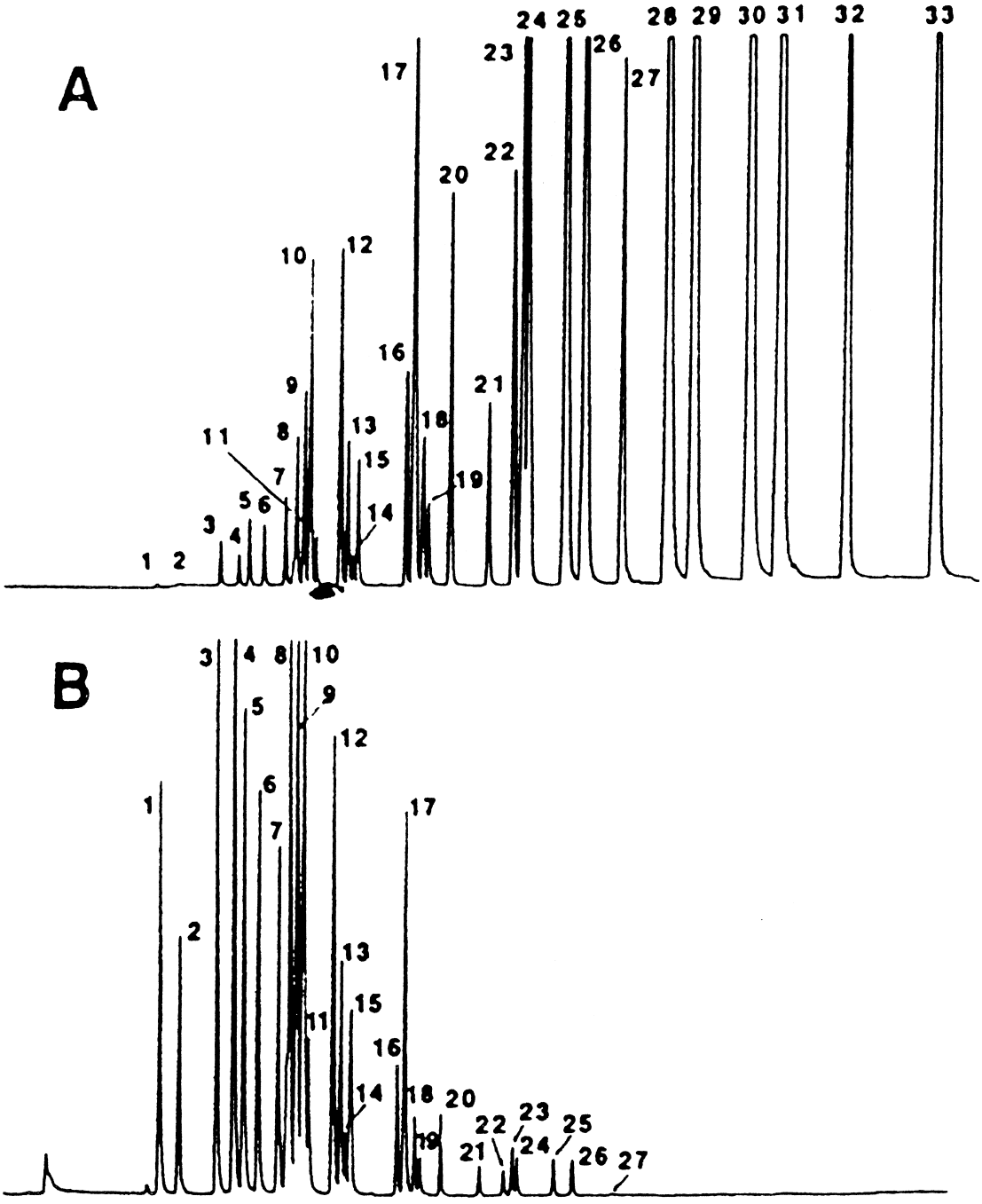


Fig. 4. Chromatograms of a 33 compound model system (A) volatiles (2 ng/ml each) by headspace SPME over 15 ml NaCl–saturated water. (B) as in (A) but with 1 ml headspace injected. (Reprinted by permission from Ref. [141]).

100% of the analytes that are adsorbed on the stationary phase. In water, detection limits for tri- and hexachlorobenzenes (Fig. 4, analytes 28, 29 and 33) by SPME were less than 0.005 μg/kg (=5 parts per trillion). However, lipids in the aqueous phase (such as butterfat in whole milk) markedly reduced the sensitivity, especially for those analytes, like the tri- and hexachlorobenzenes, that have the least volatility. In these cases, partitioning into the gas phase was reduced by a factor of 50–300 by the presence of fats in the food matrices. Note that this effect theoretically could yield, rather than 5 parts per trillion, about a 1 ppb limit of detection. This is still rather good sensitivity if the numbers are reproducible for any particular food matrix. Obviously, individual calibration curves would be required for whole, 3%, 2%, 1%, 0.5% and skim milk.

Other miscellaneous results of interest in this study include the substantially different adsorptive characteristics of two apparently identical SPME fibers, so standards and analytes have to be run using the same fiber. Also, adsorption of analytes by silicone exposed to headspace vapor during septum penetration by the SPME syringe can amount to 5% of the total amount available. A comparison of SPME immersion sampling vs. SPME headspace sampling for a 45-min extraction showed that, for more volatile components, immersion gave >90% of the response of headspace, whereas for less volatile components, it gave responses of only 25–87%. Sampling foods by immersion of the SPME fiber in the liquid could also lead to adsorption of non-volatile food components, which Page and Lacroix regarded as undesirable.

### *7.2. Volatiles in spices and other finely divided solid foods*

Page and Lacroix [141] also described application of the SPME–GC system for analysis of exogenous volatiles (environmental pollutants, but not necessarily off-flavors, that could be found in food) in: fruit juices (pear, orange, apple, grapefruit); soft drinks (orange, cola, ginger ale); fruit drinks (citrus, cranberry/raspberry, lemonade); milk (0.1%, 2%, 3.4% butterfat); spices (paprika, ground pepper, cinnamon, onion flakes, nutmeg); flour (all-purpose and biscuit mix); decaffeinated teas (three brands); and decaffeinated coffees (four types).

The general pattern of partitioning described in the preceding paragraph held true for these examples. All products that had been decaffeinated with dichloromethane showed dichloromethane (analyte 4) residues at concentrations from 13 to over 300 μg/kg.

### *7.3. Off-flavors in milk*

Marsili recently published an application of SPME with mass spectrometric detection followed by multivariate pattern recognition [142]. This type of application is increasingly referred to as an electronic nose, although the term originated to describe pattern recognition based on responses of selective sensors operated at atmospheric pressure rather than on intensities of ions formed inside mass spectrometers under vacuum. Marsili used a novel fiber, coated with porous carbon optimized for SPME analysis, at ultra-trace levels of highly volatile odor components. (As mentioned above, the fat matrix discrimination effect is the lowest for volatile components). He mentioned that SPME, unlike dynamic headspace analysis, does not suffer carryover from previous samples or introduce background (artifact) peaks. Also, he asserted that SPME is more efficient than dynamic headspace for extracting volatile fatty acids associated with milk off-flavors. Finally, SPME has much lower detection limits than several common sample preparation methods, which is important for many flavor and off-flavor analytes. Considering the chemicals expected from various kinds of sample abuse in milk, Marsili selected 30 ions to monitor during desorption of the analytes from the SPME fiber. Intensities for these ions were determined for a large number of milk samples intentionally abused by light, samples exposed to copper, and controls that had not been abused. A principal component score plot of the data showed distinct clusters for the three different types of samples. This use of multivariate statistical pattern recognition works well, in part because it classifies and clusters samples based on differences in ion ratios rather than differences in total signal intensity. That is, if a sample of milk has 0.55% butterfat, rather than 0.45% butterfat, its total ion signal may be more depressed even for volatile

components, but since most ions will be depressed to some extent, the $m/z$ ratios will be less affected by the discrimination. Thus, the ability to distinguish variations in chemical constituents, for qualitative analysis, is not compromised.

### *7.4. Off-flavors in catfish*

SPME has been combined with an electro-deposition device for preparation of shrimp and fish samples for capillary GC analysis of putrescine and cadaverine, semi-volatile amines associated with microbiological decomposition and also occurring in other foods and beverages [143]. The exposed SPME fiber was lowered into the solution to be tested and placed under an electrical potential of −1.70 V vs. a Ag/AgCl reference electrode. The amines were electroosmotically transported to the fiber surface, where they were reacted so that the molecule became neutral in solution. Thus it was adsorbed onto the lipophilic surface. After 1 h, the analyte concentration on the fiber surface reached equilibrium. The voltage was removed and the SPME device was inserted into a GC system for analysis. This system showed a linear dynamic range for both diamines of 3.5 orders of magnitude. Detection limits were about 0.5±0.04 ppb for both.

Microwave-mediated distillation with SPME has been used to analyze MIB and geosmin in shredded catfish tissue by GC–ion trap MS at levels below the human taste threshold [144]. The microwave distillation step was used to concentrate the off-flavors. Then SPME sampling for 15 min at 40°C of the distillate headspace achieved yet more sample concentration, so that a rapid, extremely sensitive technique was available. Detection limits for geosmin and MIB were 0.01 μg/kg, with limits of quantitation of 0.1 μg/kg, which compares well to the limit of olfactory detection of these compounds in catfish, ~0.7 μg/kg [145]. Recovery of spiked samples was only 4.4% for MIB and 5.0% for geosmin.

In another recent method by Zhu et al., the SPME extraction step was performed by immersing the fiber in the distillate rather than sampling the headspace above it [146]. Under these conditions, the required extraction time was 30 min, which for multiple analyses synchronized well with the time used in GC analysis and re-equilibration. Detection limits were 0.043 ppb (=μg/kg) for MIB and 0.008 ppb for geosmin. At 1 ppb each, recoveries of MIB on the SPME fiber were 15.6% and for geosmin were 36.9%. Recoveries from the microwave extraction step for MIB were 81.4% and for geosmin were 30.4%. Thus the overall method recoveries for MIB were 12.7% and for geosmin, 11.2%.

In these examples, a limiting step for either conventional SPME or the electro-deposited version is the length of exposure time needed to achieve equilibrium. This problem could be partially ameliorated by using a large number of fibers and performing the exposure steps simultaneously in a batch mode. However, there is enough variation between individual fibers that batch operation presently seems impractical. This may be the limiting characteristic for a sample handling methodology that otherwise offers significant advantages for some flavor and off-flavor applications.

## 8. Sample preparation for short-path thermal desorption–GC analysis

In a warning about the use of thermal desorption for labile compounds, Grob and Habich [125] wrote, ‘‘The negative role of pyrolysis and other structural alterations owing to thermal desorption can hardly be over-emphasized. . . . The extent of thermal alteration depends on temperature, duration, and surface catalytic effects. It may be less well known how efficiently such effects can be reduced by optimizing the trap geometry with the aim of reducing residence time of sensitive substances in the heated trap.’’

Short-path thermal desorption (SPTD) offers significant advantages in simplicity, speed, economy and sensitivity for analysis of solid and liquid samples [147]. It was developed to provide solventless preparation and facile sample introduction of volatile and semi-volatile components in foods for GC and GC–MS. Flavor-compound lability issues were considered in optimizing the ‘‘short path’’ of the design. Since the apparatus is mounted directly on top of the GC injection port, there is no heated transfer line and therefore no memory effects are associated with a contaminated transfer line or with a reusable, permanent adsorbent bed [148].

For analysis of solids, 1 to 5 mg of sample are

placed into a glass-lined stainless steel thermal desorption tube between two glass wool plugs. This is referred to as direct thermal desorption (DTE). The sample is purged of oxygen with He, after which it is heated to a pre-selected temperature (80 to 400°C) for 5 to 10 min. This thermal desorption step can be understood as a temperature ramp, so that labile compounds can desorb and move on before they are thermally degraded [149]. Evolved volatiles are sparged into the GC injection port, where they are cryo-focused on the head of the GC column. When cryo-trapping solid samples containing a significant percentage of water, modifications can be made to prevent plugging the trap with ice. Examples of solid-food product DTE applications include: (i) spices and herbs (black pepper, oregano, basil, garlic), 1–2 mg, desorbed at 100–250°C [150]; (ii) coffee (regular and decaffeinated), 1.5 mg, desorbed at 250°C for 6 min [150]; (iii) roasted peanuts, 2 mg, 150°C for 6 min [147]; (iv) candy, 2 mg, 150°C for 6 min [147] and (v) mushrooms (six edible species), 1–2 g samples, heated to 90°C, gas extracted onto 100 mg Tenax TA, desorbed at 250°C for 10 min [151]. Some of the peaks are greatly broadened, possibly due to on-column thermal degradation.

To analyze liquid samples of 1 to 25 ml by SPTD, 100 mg of a solid sorbent such as Tenax is placed in the desorption tube. Volatile organics are sparged out of the liquid and collected on the trap. For analysis, the Tenax is dry-purged, concurrent with the sampling purge, to reduce humidity and maintain the sorption effectiveness of the Tenax. After sampling is complete, the Tenax is dry-purged for another 10–15 min to remove any condensed water vapor. For highly aqueous samples, two desorption tubes can be attached in series, the first containing Tenax and the second a more aggressive sorbent, such as Carboxen 569, a hydrophobic carbon molecular sieve with a large surface area. By noting water breakthrough volumes for the two sorbents, it is possible to sample long enough to capture the full volatility range of organic compounds in one or the other of the tubes. The majority of the water is passed on through the second desorption tube and out to waste before the tube is connected to the cryo-trap for desorption and analysis [152].

In the adsorbent-collection mode, the system works similarly to purge-and-trap or dynamic headspace analysis and is referred to as purge-and-trap thermal desorption (P&T TD). The short-path plumbing minimizes thermal decomposition of the trapped analytes, and there are other significant improvements in sensitivity relative to conventional purge-and-trap technology (see Fig. 5).

Table 2 compares some of the performance characteristics of SPTD, P&T TD and DTE modes, to other methods.

Typical food or beverage flavor applications include:

(i) Non-alcoholic fruit drinks (orange, cranberry–apple, raspberry), 25 ml sample, 10 min collection, 10–15 min dry purge, 10 min thermal desorption at 150°C [152]

(ii) Carbonated colas, 25 ml sample, 10 min collection, 10–15 min dry purge, 10 min thermal desorption at 150°C [152]

(iii) Wines and wine coolers, 2.5 ml sample for wines, 25 ml for wine coolers, 10 min collection, 10–15 min dry purge, 20:1 split, 10 min thermal desorption at 150°C [153]

(iv) Olive oil, 5 ml sample, 10 min collection, 10–15 min dry purge, 10 min thermal desorption at 150°C [148]

(v) Honey, 5–6 g sample, heated to 80°C and sparged 45 min, spiked with 100 ng of *d*-14 cymene internal standard, 10 min thermal desorption at 220°C, split 10:1 (Fig. 6) [154]

(vi) Milk, 0.5 ml samples heated at 60°C for 90 min, dry-purged, trapped on 200 mg Tenax, desorption at 250°C, GC–MS [155]

An SPTD system fitted with a microprocessor-controlled auto-sampler (SIS, Ringoes, NJ, USA) has also been used for food analysis [156].

## 9. Perspectives

The relationships among sample preparation methods, separation and detection systems, and the integrity of quantitative results may be summarized in a common modern aphorism: ''Cheap, fast, good . . . choose any two'' [157]. The implication is that it may be possible to have two of the three characteristics, but it is not possible to have all three at once. That is why the instruction reads, ''Choose any two.'' For sample preparation of foods for flavor

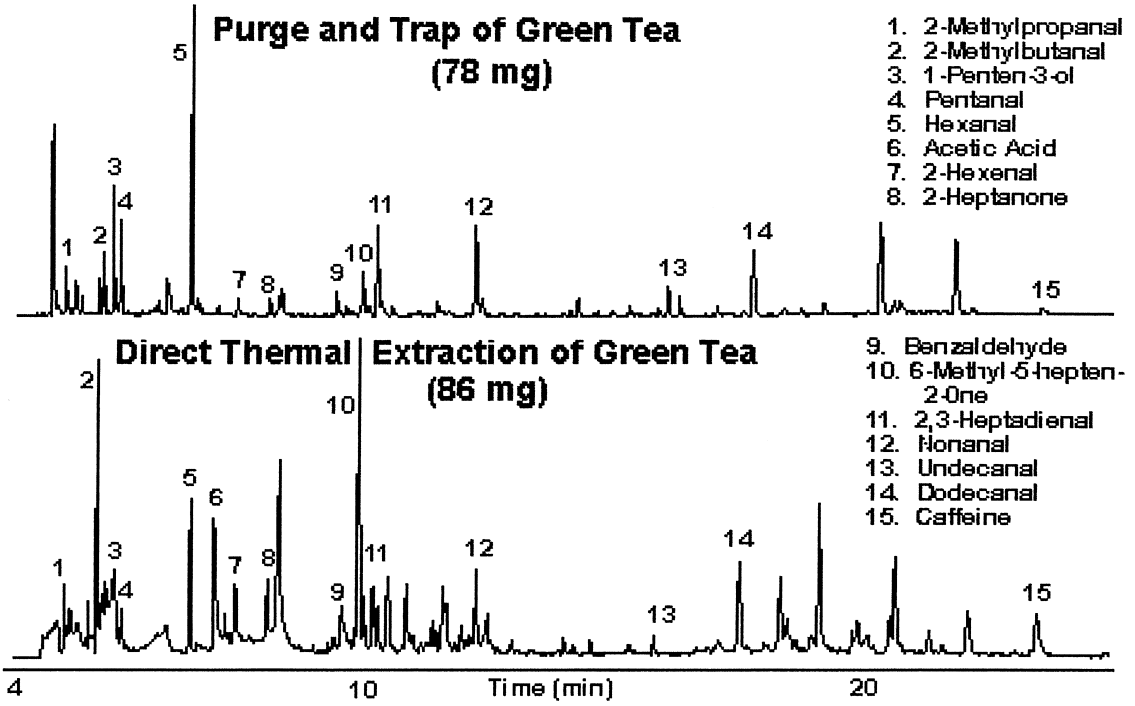


Fig. 5. Chromatograms comparing conventional purge-and-trap to direct thermal extraction (DTE) for green tea leaves. DTE collects a wider variety of components from the same sample, especially higher-boiling compounds appearing later in the chromatogram. (Reprinted with permission from Ref. [152]).

and off-flavor analysis, we might substitute the word ''easy'' for ''fast'' in the aphorism.

The papers reviewed here demonstrate that there are usually several ways to solve a problem in flavor or off-flavor analysis. The analyst can make a successful choice based on the resources available and her/his priorities. This is particularly encouraging when the budget for new equipment is severely limited.

If one of the methods for food flavor/off-flavor

Table 2
Comparison of selected extraction methods for volatile compounds (reprinted with permission from Ref. [152])[a]

| | Sample matrix | Sample size | Sensitivity | Range volatiles analyzed | Sample auto. | Sample prep. |
|---|---|---|---|---|---|---|
| | | | | Gas Volat. Semi-vol. Non-vol. | | |
| Headspace | L/S | 0.1–10 | ppm | | Yes | 5–10 |
| P&T TD | G/L/S | 5–1000 | ppb | | No | 10–30 |
| SPME | G/L | 0.1–10 | ppt | | Yes | 5–15 |
| Sol'v't. Ex. | L/S | 0.1–10 | ppb | | No | 30+ |
| SFE | L/S | 0.1–10 | ppb | | Yes | 10–60 |
| DTE | S | 0.1–10 | ppb | | No | 1–2 |
| | | | | −100 0 100 200 300 400 | | |
| | | (g) | | Boiling point (°C) | | min |

[a] P&T TD is purge-and-trap thermal desorption; DTE, direct thermal extraction. Sensitivity unit ''ppt'' is parts-per-trillion.

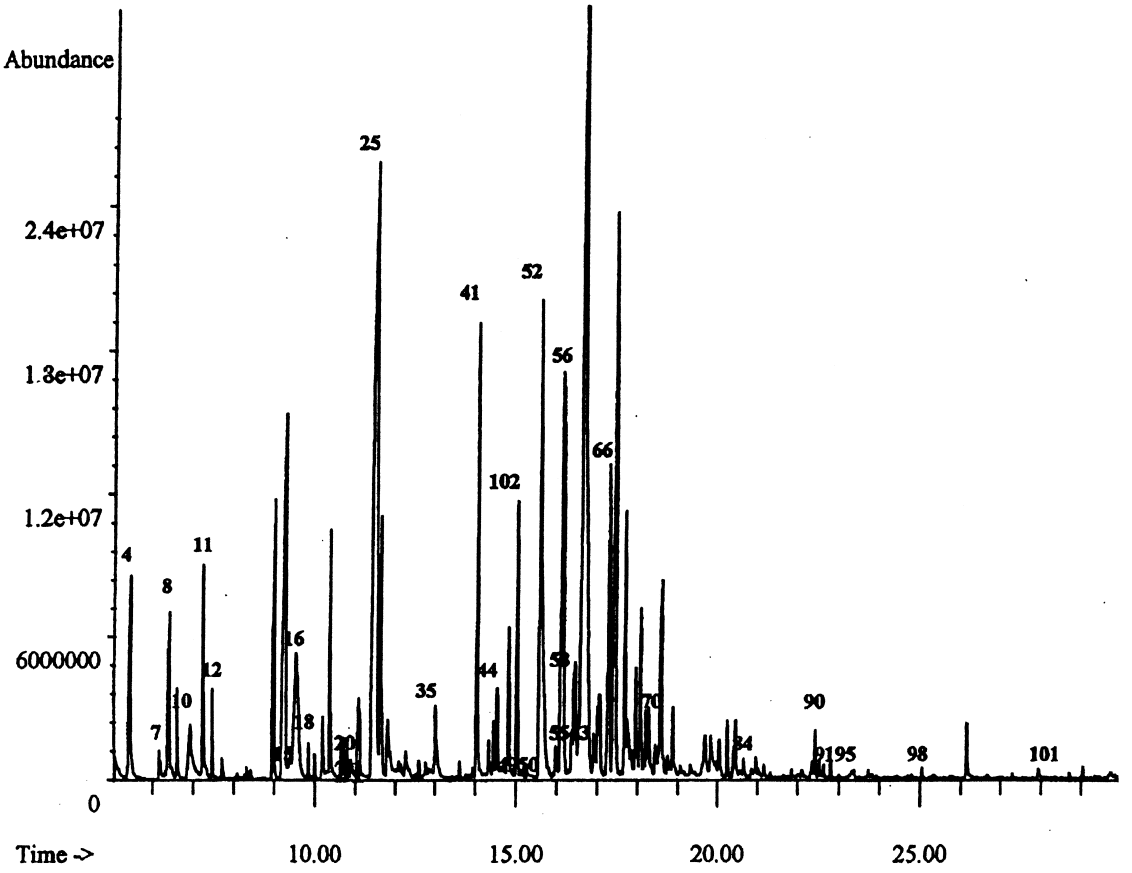


Fig. 6. Chromatogram of wildflower honey (5.14 g). Purged for 45 min at 20 ml/min with 20 ml/min dry purge on Tenax TA adsorbent trap followed by thermal desorption at 220°C for 10 min. (Reprinted by permission from Ref. [154]).

analysis seems to contradict the ''Cheap/fast/good'' aphorism, it may be the DTE mode of SPTD, developed for food and food-packaging analysis. This method is fast, can give excellent quality results with FID, GC or GC–MS detection, and is relatively inexpensive compared to many other modern instruments. Pressurized-fluid extraction and microwave-assisted distillation/trapping/extraction are other particularly promising systems.

## 10. Disclaimer

The opinions expressed in this paper are those of the authors and do not reflect official positions of the United States Food and Drug Administration.

## 11. Nomenclature

| | |
|---|---|
| AEDA | Aroma extract dilution analysis |
| ANN | Artificial neural network |
| dansyl | (5-Dimethylaminonaphthalene-1-sulfonyl) |
| DMA | Dimethylamine |
| DNPH | 2,4-Dinitrophenylhydrazine |
| FIA | Flow injection analysis |
| FID | Flame ionization detection |

| | |
|---|---|
| FPD | Flame photometric detection |
| FFAs | Free fatty acids |
| HPLC | High-performance liquid chromatography |
| IDF | International Dairy Federation |
| MDA | Malondialdehyde |
| MIB | 2-Methylisoborneol |
| μ-SD/SE | Micro steam distillation/solvent extraction |
| PCR | Principal component regression |
| PCs | Principal components of variation |
| PCBs | Polychlorinated biphenyls |
| P&T TD | Purge-and-trap thermal desorption |
| Py–MS | Pyrolysis–mass spectrometry |
| SDE | Steam distillation and solvent extraction |
| SFE | Supercritical fluid extraction |
| SPME | Solid-phase microextraction |
| SPTD | Short path thermal desorption |
| TBA | Thiobarbituric acid |
| TCA | Trichloroacetic acid |
| TBARS | Thiobarbituric acid reactive substance |
| TMA | Trimethylamine |
| TVBs | Total volatile bases |

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