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Solid-Phase Microextraction in the Analysis of Food Taints and Off-Flavors

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Abstract

Selected food taints and off flavors, for which solid phase microextraction (SPME) has been used as a method for volatiles isolation, are the subject of review. Compounds responsible for musty and earthy odor off-flavors and taints in foods are discussed. This group contains haloanisoles, geosmin, and methylisoborneol. Chlorophenols are discussed as precursors of chloroanisoles and compounds impairing the flavor of food. Also described are volatile phenolic compounds responsible for medicinal off flavors, mainly ethyl phenols and vinyl phenols. Sulfur compounds that contribute to off-flavor are also discussed. Finally, a group of volatile compounds being the products of lipid oxidation are summarized. A short review of the formation, occurrence, and information on odor properties of all of these groups of compounds is given. Examples of SPME use for the analysis of compounds belonging to all described groups are shown. Elaboration of method parameters, fiber selection, experimental conditions, and quantitation of compounds are subjects of interest. Also, applications of SPME as a method for introduction of volatiles in mechanical olfaction technologies are shortly outlined.

Introduction

Food taints and off-flavors generate severe problems in the food chain, affecting products from the farm to the table. Economic losses can be associated with loss of production, loss of consumer confidence, and destruction of a brand image. Flavor is one of the most important sensory characteristics of food. Consumers can easily reject products with an inappropriate flavor impression. Food flavor very rarely depends on the presence of a single compound or several compounds. Normally, tens or even hundreds of compounds react with the human olfactory system to form a characteristic for a certain product flavor. In such complicated mixtures, any flavor compound may be considered undesirable when present at concentrations that are too high. Therefore, off-flavors in food are a matter of compounds concentration and odor thresholds (OTs). Apart from many compounds that are associated with such unpleasant odor notes (such as putrid, musty, rotten, skunk, etc.), there are many more flavor compounds in foods that can become off-flavors when their typical concentration is exceeded.

Taints and off-flavors are not synonymous, their definitions reflect the origin of compounds responsible for these impressions. Taints are defined as unpleasant odors or flavors imparted to food through external sources, whereas off-flavors are defined as unpleasant odors or flavors imparted to food through internal deteriorative changes.

Though the compounds that cause off-flavors and taints in food represent various groups and classes, this review describes only the main groups of chemicals, for which solid-phase microextraction (SPME) has been used as a method for their isolation. Use of SPME in the field of food taints and off flavors is most explored for compounds that are crucial for the food industry and occur most frequently, such as those that cause musty, earthy off-flavors [haloanisoles, halophenols, geosmin (GEO), and methylisoborneol (MIB)] and those that cause medicinal off-flavors (mainly phenolic compounds, sulfur-containing compounds, and on carbonyl compounds), which spoil food mainly because of the oxidative changes of lipids. The text will not discuss particular groups of products affected by taints and off-flavors. It will be focused instead on compounds and one technique that is used for their isolation: SPME.

Discussion

SPME as an extraction method in the analysis of flavor compounds

Analysis of food flavor compounds reflects the progress in sample preparation techniques in analytical chemistry. When a survey was done in 1969 (1) on the methods used for isolation of food flavor compounds, 300 papers published between 1960 and 1967 were checked. Distillation methods prevailed in 78% of papers, followed by extraction methods, chemical reactions

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to isolate volatiles, adsorption, and, finally, freeze concentration. Developments in static headspace (HS) analysis and the introduction of dynamic HS (2) offered new perspectives for the analysis of food flavor compounds. HS sampling is now a widely used method for volatile compound isolation. A number of papers related to HS–gas chromatography (GC) exceeds 6000, of which over 1400 are related to dynamic HS (3). In addition to the well-established methods of static (4) and dynamic HS in food flavor analysis, SPME emerged in the beginning of the 1990s as the third technique.

SPME is one of the relatively novel methods of sample preparation in food analysis, along with membrane separation techniques, pressurized fluid extraction, supercritical fluid extraction, or microwave-assisted extraction (5). SPME was developed by Pawliszyn et al. (6,7). It represents a new approach to accumulate analytes by diffusion that combines the advantages of both static and dynamic HS. SPME is the first sampling technique based on analyte diffusion that has successfully been applied to such a number of fields. Sixteen years since its invention, SPME is now one of the most widely used methods for the isolation of volatiles and semivolatiles. Searching available databases for SPME-related application shows that a majority of the results refer to the environmental studies, where analysis of contaminants in water prevails, followed by food applications, forensic and toxicological studies, and analysis of natural products, respectively. Of the 494 works on SPME from the food analysis field searched, 143 works were on soft drinks, alcoholic beverages, and wines; 64 on fruits and vegetables; 48 on milk and dairy products; 33 on meat; and 27 on plant oils—to name only the main group of investigated products. Numerous review papers appeared in the literature in recent years related to various aspects of SPME used in volatiles analysis. They refer to the developments in extraction techniques (8–11), some are also focused on food applications (12,13). Because of its properties, SPME is an efficient tool for monitoring volatiles in biological samples in which measurements can be done in vivo (14). Although there are reviews discussing SPME in food analysis, the analysis of taints and off-flavors has not been explored in a single review paper.

SPME, because of its simplicity, robustness and low cost, is very popular as a fast screening method for qualitative analysis. As shown in many applications, it is also a reliable method in quantitative analyses. It is characterized by high sensitivity and is often compared in this aspect to dynamic HS. In several works, both techniques were used in the analysis of such food products as cola (15), milk, butter, and cheeses (16–19). For flavor compounds isolation, SPME has potent advantages over distillation/extraction (SDE) methods in that isolation is performed at lower temperatures and usually for a shorter period of time, which prevents decomposition of thermally labile compounds. Formation of artefacts in the injection port caused by fiber coating reactions depends on the inertness of the coating material. (20).

Compounds causing taints and off-flavors in foods

Compounds responsible for the occurrence of food taints and off-flavors belong to various chemical classes. A majority represent haloanisoles, chlorophenols, bromophenols, phenolic compounds, sulfur compounds, alcohols, carbonyl compounds, fatty acids, esters, and amines. They are often classified from the point of view of their origin—microbially derived off-flavors, compounds originating from packaging materials and from cleaning agents, compounds resulting from oxidative changes of fats, and Maillard reactions products. Two reference books have been written in which the detailed description of the occurrence and formation of taints and off-flavors in food are provided (21,22).

In the part of the review devoted to the application of SPME, several groups of compounds responsible for main off-flavors will be discussed. Therefore, similar classification will be provided in this chapter.

Chloroanisoles were detected as compounds responsible for the musty taint first in eggs and broilers (23). Products contaminated with chloroanisoles and chlorophenols include poultry, canned vegetables, fruits and juices, beer, peanuts, beans, chocolate, ice cream, packaged flour and rice, dried fruit, gin, meat, and wine. Maarse (24) listed compounds involved in these cases and their origin.

The most frequently detected compound involved in the formation of musty taints was 2,4,6-trichloroanisol (TCA). It was detected also in Brazilian coffee, which had a characteristic Rio off-flavor (25). Off-flavor described as cork taint, or moldy taint, has been a problem for mainly the wine industry. For the first time, Buser et al. (26) correlated the occurrence of this kind of off-flavor with the presence of TCA. It is estimated that cork taint is responsible for total loss in the world wine market of approximately $1 billion per year (24).

It is believed that trichloroanisoles are formed through a process of detoxification (methylation) of corresponding chlorophenols by fungi. The presence of chlorophenolic precursors in corks may come from the use of fungicides, herbicides, wood preservatives, and washing products, which usually contain 2,4,6-trichlorophenols (TCP), 2,3,4,6-tetrachlorophenols (TeCP), and pentachlorophenol (PCP). Chloroanisoles, identified as causative agent of a musty taint in chicken carcasses, were formed from corresponding chlorophenols used for preservation of wood shavings (27). In a group of com-

Table I. Odor Threshold Values for 2,4,6-Trichloroanisol in Various Media*

Medium	Odor threshold (mg/L or mg/kg)
Water	7.6×10^{-8}
Ethanol–water 20%	5.6×10^{-6}
Ethanol–water 40%	3.6×10^{-5}
Beer	7×10^{-6}
Wine	1×10^{-5}
Edible oil	7×10^{-3}
Egg yolk	2.4×10^{-3}
Dried fruit	0.12×10^{-3}
Fruit bun	0.21×10^{-3}
Plain bun	1.4×10^{-3}
Air (mg/m^3)	3.7×10^{-6}

* Based on the literature (24).

pounds that are causative agents for musty taint, di-, tri-, tetra-, and pentachloroanisoles (PCAs) are included together with 2,4,6-tribromoanisole, GEO, 2-MIB, 1-octene-3-ol, octa-1,3-diene, α-terpineol, 4,4,6-trimethyl-1,3-dioxan, 2,6-dimethyl-3-methoxypyrazine, and 2-methoxy-3-isopropylpyrazine (21). Mustiness caused by chloroanisoles can be distinguished from that of GEO and 2-MIB.

MIB and GEO are compounds related to the occurrence of muddy and musty off-flavor in water. According to Zimba and Grimm (28), based on analysis of water samples from 485 channel catfish production ponds, off-flavor related to MIB presence (and, to a lesser extent, GEO) occurred in approximately 20% of samples. These compounds are responsible for delayed harvesting of as much as 30% of harvest-size fish. Mainly *Strepromyces, Nocardia*, and *Oscillatoria* produce both MIB and GEO in aquatic environments. They were also reported as a source of musty and earthy off-odors in stored grain, in which they are produced by storage fungi (29,30). GEO was identified as a metabolite of *Penicillium vulpinum* and *P. aethiopicum*, and MIB was detected in cultures of *P. aurantiogriseum* and *P. expansum* (31,32). GEO is also a compound characteristic for the earthy flavor of red beet (33), whereas MIB contributes to the musty and earthy notes of Brie and Camembert cheeses (34).

Phenolic compounds can be a source of taints in foods, mainly as a result of the activity of microorganisms. It is known that vanillin can be microbiologically degraded to guaiacol (2-methoxyphenol), which is a cause of smoky off-flavor in ice cream or candies. It is known that ethylphenols [4-ethylphenol (EP) and 4-ethylguaiacol (EG)] are responsible for animal and smoky odors, and vinyl phenols (4-vinylphenol and 4-vinylguaiacol) can be responsible for heavy pharmaceutical odors (21). Excessive amounts of EP and EG can cause a wine defect known as "Brettiness" (35), which is described as a barnyard, cider-like, stable, or band-aid odor. These compounds can be produced from hydrocinnamic acid during thermal decarboxylation (36). Many microorganisms, mainly yeasts of the genus *Dekkera/Brettanomyces* (37), can mediate decarboxylation of *trans* ferulic or *trans p*-coumaric acids into vinyl phenols. Guiacol can be produced by *Alicyclobacillus acidoterrestris*, which is a spoilage that occurs worldwide and is a nonpathogenic bacteria of fruit juices. It can also produce 2,6-dibromophenol and other phenol-type substances, resulting in medicinal, phenolic off-flavor, formation of white sediments in the package, and increase of the juice turbidity. Because of its smoky/phenolic odor, guaiacol contributes to many roasted foodstuffs, including Arabica coffee and barley malt. In roasted products, guaiacol is formed by thermal decomposition of phenolic precursors.

Sulfur compounds are an important group of volatiles contributing to characteristic flavor of many vegetables, some fruits, meat, spices, coffee, roasted products, cheeses, wine, and beer (38). There are numerous sulfur-containing volatiles in beer that influence its flavor, and if present in excess of normal concentrations, they can contribute to a formation of off-flavor that is usually described as rotten eggs, putrefaction, rubber, cooked, rotten vegetables, raw potatoes, cabbage, or skunk (39). Dimethyl sulfide (DMS) in beer, when present at high concentrations, gives a distinct cooked vegetable or cabbage-like aroma. DMS comes from the degradation of malt-borne *S*-methylmethionine formed in proteolysis during malting. For red wines of Cabernet Sauvignon, the DMS contribution to flavor is assumed as positive, whereas it is highly negative even at trace levels for red Pinot wines. DMS in wine often exceeds its OT of 27 µg/L for red wine, particularly after aging. DMS has been identified also as the cause of taint in other food products, including chicken and nuts. Highly flavor active 3-methyl-2-butene-thiol (MBT) in beer is derived from bitter iso-α-acids that result from photochemical degradation. It develops rapidly in beer that is exposed to light. Its odor is

Table II. Odor Threshold Values of Different Volatile Compounds*

Compounds	Odor thresholds (ppm)
Hydrocarbons	90–2150
Substituted furans	2–27
Vinyl alcohols	0.5–3
1-Alkenals	0.02–9
2-Alkenals	0.04–2.5
Alkanals	0.04–1.0
trans,trans-2,4-Alkadienals	0.04–0.3
Isolated alkadienals	0.002–0.3
Isolated *cis*-alkenals	0.0003–0.1
trans,cis-2,4-Alkadienals	0.002–0.006
Vinyl ketones	0.00002–0.007

* Based on the literature (24).

Table III. Odor Properties of Selected Aroma Compounds from Lipid Oxidation*

Compound	Odor description	Odor threshold (ppb)		
		Water	Oil (nasal)	Oil (retronasal)
Pentanal	Pungent, bitter almonds	18	240	150
Hexanal	Tallowy, green leafy	12	320	75
Heptanal	Oily, fatty	5	3200	50
E-2-Hexenal	Apple	316	420	250
Z-3-Hexenal	Green leafy	0.03	1.7	1.2
E-2-Nonenal	Tallowy, cucumber	0.25	900	65
2,4-(*E,E*)-Decadienal	Frying odor	0.2	180	40
1-Penten-3-one	Hot, fishy	–	0.73	3
1-Octen-3-one	Mushroom-like, fishy	0.05	10	0.3
(*Z*)-1,5-Octadien-3-one	Geranium, metallic	1.2×10^{-3}	0.45	0.03

* Based on the literature (46).

decribed as skunky or leek-like. Hydrogen sulfide can originate in alcoholic beverages as a result of the reduction of sulfite or sulfate, breakdown of *S*-containing amino acids as a result of yeast autolysis, or microbial infection with *Zymomonas* bacteria. Sulfur compounds in wines are often classified based on the boiling point (BP) of 3-methyltiopropanol (BP, 90°C) as light sulfur compounds (BP < 90°C) and heavy sulfur compounds (BP > 90°C) (40). It is assumed that the light sulfur compounds are responsible for the off-odors in wine resembling rotten eggs, cabbage, garlic, and onions.

Volatile lipid oxidation products contributing to the rancidity of fat-containing foods and a mixture of volatiles, mainly aldehydes, are responsible for this off-flavor. Oxidation of lipids is a process, which can have a purely chemical nature (free radical autooxidation or photosensitized oxidation) or can be mediated by enzymes. In the free radical auto-oxidation process, free radicals react with triplet oxygen-forming peroxy free radicals and hydroperoxides. In photo-oxidation, certain compounds (e.g., riboflavin, phaeophitins, myoglobin, and erytrosine) convert triplet oxygen into singlet oxygen, which can react directly with fatty acid. Hydroperoxides are odorless compounds that can break down in many ways to form odoriferous compounds. The relative amounts of secondary oxidation products (volatiles) produced are highly dependent on the fatty acids profile of oxidized lipid. The formation of different hydroperoxides in photo-oxidation leads to the formation of their decomposition products in different amounts compared with free radical mechanism (41).

The decomposition of these precursors into volatile secondary oxidation products forms a mixture of aldehydes, hydrocarbons, alcohols, and ketones. In food products, the main precursor of volatile compounds is linoleic acid because of its high content in fats and susceptibility to oxidation. The main volatiles formed are hexanal and 2,4-decadienal, in which *trans,trans*-2,4-decadienal is formed from *trans,trans* 9-hydroxyperoxide (9-OOH) or in isomerization of 2-*cis*,4-*trans*-decadienal. 2-heptenal is formed by β-scission of 12-OOH (42). 2-octenal can be formed from 9-OOH or by oxidation of 2,4-decadienal (43). Similarly, 6-*trans*-nonenal can be formed from 10-OOH of linoleic acid. Because of their lability, unsaturated aldehydes can be a source of other volatile compounds. In oxidation of 2,4-heptadienal, C2–C4-alkanals, glyoxal, α-ketoaldehydes (C5–C9), and *cis*-2-buten-1,4-dial are formed (43). At room temperature, 2,4-decadienal can undergo auto-oxidation to a mixture of compounds including butenal; hexanal; 2-heptenal; 2-octenal; benzaldehyde; glyoxal; furan; ethanol; acrolein; pentane; benzene; and acetic, hexanoic, 2-octenoic, and 2,4-decadienoic acids (44). Vinylketones usually have a distinct off-odor: 1-octene-3-one is responsible for the off-flavor of rancid butter, and 1-pentene-3-one, having a characteristic fishy off-flavor, is formed from linoleic or linolenic acid. Important 8-carbon off-odorants (1,5-*cis*-octadiene-3-one and 3,5-octadiene-2-one) are formed from linolenic acid (45,46). Though long-chain aliphatic alcohols play a minor role in the formation of off-flavor, some develop characteristic odor as 2-alken-1-ols (C8–C12) and 1-octene-3-ol, which were identified as oxidation product of methyl linoleate in butter and soy oil (47, 48).

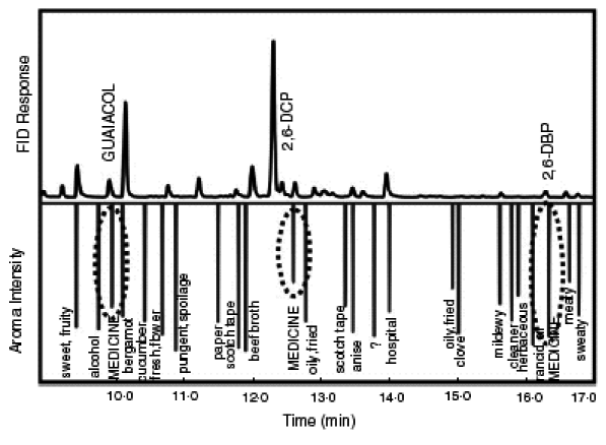

Figure 1. Chromatogram and aromagram of orange juice inoculated with *Alicyclobacillus cycloheptanicus*.

Morales (49) concluded that the marker for a beginning of oxidation of olive oil could be nonanal and also hexanal-to-nonanal ratio, which in the course of oxidation goes down to 1. Also, Jimenez (50) stated that nonanal and 2-decenal can provide discrimination between extra virgin olive oils and defective olive oil.

Off flavors in fat-containing foods are also related to the presence of methylketones, which are responsible for the off-flavor of desiccated coconut (51). This musty, stale off-flavor is known as ketonic rancidity and caused usually by oxidation of medium-chain fatty acids by molds (48). Molds such as *Eurotium amstelodami* can degrade triacylglycerols in the presence of air and water. In subsequent reactions, free fatty acids undergo β-oxidation, which produces methylketones.

Sensory properties of food off-odorants

The term "flavor" involves taste, odor (or aroma), and trigeminal stimuli. Compounds causing off-flavor often impair taste, but the main sense involved in the perception of off-flavor is olfaction. Odorant molecules are sensed by the olfactory epithelium located in the nasal cavity, which can be reached entering a nasal passage via the nose or via the mouth (retronasal path). Introduction of odorant above a certain threshold into the nasal cavity triggers the response of human organism to stimulus (21).

OT is defined as the concentration of a compound in a specified medium that is detectable by 50% of a specified population. In flavor analysis, two thresholds are used: (*i*) detection threshold defined as the lowest physical intensity at which a stimulus is perceptible and (*ii*) the recognition threshold, which is the lowest intensity at which the stimulus could be correctly defined/identified.

Perception of chemicals responsible for taints and off-flavors depends on the chemical structure, concentration of compound in a specific food matrix, and sensitivity of the human subject. Food taints are detectable usually at very low parts-per-million (ppm), parts-per-billion (ppb), and often parts-per-trillion (ppt) levels. Because of the difficulties in the determination of OTs in the literature, different thresholds are often cited for the same compound. For instance, TCA is reported in various

publications to have an OT in air varying between 0.021, 0.001, 0.0016, and 0.00016 mg/m^3. 2,4-Dichloroanisol OTs in water range in various papers from 0.0003 to 0.21 mg/kg, as demonstrated by Maarse (24).

The most crucial factor to be considered is the type of matrix (food) that is affected by the taint. OTs are highly dependent on the matrix that determines distribution constants and release of flavor compounds into HS. For example, OTs for TCA can vary to a high extent, as presented in Table I. Depending on the type of wine used, the OT for TCA ranges from 1.4 to 10.0 ng/L. TCA concentration that causes wine defect is in the range of 10–40 ng/L (52). Apart from TCA, 2,3,4,6-tetrachloroanisole (TeCA) and PCA are also suspected to be causative agents of a musty taint in wine. TCA degradation products have higher OTs: 2,4-dichloroanisole is 0.5 µg/L (53) and 2,6-dichloroanisole is 40 ng/L. Chatonnet (54) detected the presence of bromoanisoles in wines of foul flavor. A potent odorant, 2,4,6 tribromoanisole has an OT of 0.03 ng/L.

OTs for GEO and MIB in water were initially reported at approximately 35 and 40 ng/L, respectively (55), but recent reports tend to estimate them below 10 ng/L (56). Other sources report OTs for GEO at 0.0038 µg/L and for MIB at 0.015 µg/L (53). In the case of fish tissue, GEO and MIB often have detected OTs that are substantially higher: for GEO in trout, an odor threshold of less than 0.9 µg/kg was reported (57), although values even several times higher (6.5 µg/kg) were reported for rainbow trout (58). For MIB in catfish, 0.7 µg/kg is assumed as its OT. The level of MIB or GEO in fish tissue above the limit of 0.7 µg/kg renders fish unfit for retail sale (55). MIB is known to appear in Robusta and Arabica coffees in amounts of 100 ppt and up, is presumed to come from external sources, and causes musty off-flavor (59,60).

Fallico (63) determined OTs for *p*-vinyl phenol and *p*-vinylguaiacol in water and in orange juice. The vinyl phenol OT in water was 0.022 µg/L and in juice was 0.045 µg/L, whereas for vinylguaiacol the values were 0.012 ad 0.033 µg/kg, respectively.

Sulfur compounds exhibit very characteristic odors mainly described as vegetable-like, cabbage, onion, rotten egg, and sulfur. OTs reported for sulfur compounds vary. Lenthionine, present in mushrooms, has an OT of 0.27–0.53 mg/kg. Ethanethiol has a threshold of 4.3 mg/kg (38). However, the OTs are much lower for a majority of sulfur-containing compounds. Methanethiol has an OT in water ranging from 2.0×10^{-3} mg/kg to 2.0×10^{-5} mg/kg, and dimethyl disulfide (DMDS) ranges from 1.2×10^{-2} to 3.0×10^{-3}. Dimethyl trisulfide has a threshold in water of 1.0×10^{-2} mg/kg (38).

DMS, one of the main sulfur compounds present in beer (10–140 µg/L), has an OT estimated at 30–45 µg/L (62). In other references, OT for DMS ranges from 6.0×10^{-2} to 3.0×10^{-4} mg/L (38). When present at high concentrations, DMS gives a distinct cooked vegetable or cabbage-like off flavor. Highly flavor active allylic thiol, MBT has an OT of 10 ng/L.

OTs for compounds that are formed as a result of lipid oxidation vary substantially between classes. Table II shows classes of compounds that can result from lipid oxidation. It can be clearly seen that some of the compounds will have a high impact on the flavor of rancid food even if present at a low concentration. Large differences in air–water and air–oil partition coefficients for listed compounds result in significant differences in their OTs. Because of the different physical properties of oil as a matrix compared with water, the OTs in oil are generally substantially higher than in water. Table III shows selected volatile compounds present in rancid oils and their OTs in water and oil. Noteworthy are the differences in OTs of some compounds measured nasally and retronasally, which influences their perception during food consumption.

Volatile aldehydes formed in small quantities, such as 2-*cis*-nonenal or *trans*-4,5-epoxy-2-*trans*-decenal, have a low OT of 4.5 and 1.3 ppb, respectively (46). Degradation products of linolenic acid, such as 3-*cis*-hexenal, 2-*trans*, 6-*cis*-nonadienal, and 1,5-*cis*-octadien-3-one, have OTs of 14.4 and 0.45 ppb, respectively. Fatty acids, which occur in lipids in low concentrations, such as octadeca-*cis*-11 or *cis*-15-dienoic acid in beef, mutton, or butter can yield 4-*cis*-heptenal (OT = 2 ppb) with an unpleasant putty odor. OTs of unsaturated aldehydes illustrate the dependence of flavor on the structure of the molecule: 3-*cis*-hexenal and 4-*cis*-heptenal have a more intensive flavor (OT = 0.09–0.11 and 0.0005–0.0016 mg/L in paraffin oil) than their corresponding *trans* isomers (0.6–2.5 and 0.1–0.32 mg/L, respectively) (63).

Analytical strategy in identification and quantitation of taints and off-flavors in foods

Identification of compounds responsible for food off-flavors involves several steps, all of them crucial for the success of analysis. They involve: gathering information on sample origin; profile sensory analysis of the sample; isolation and concentration of volatile compounds; GC–olfactometry (O) analysis to select compounds responsible for off-flavor; GC–mass spectrometry (MS) to identify compounds detected by GC–O; quantitation of compounds responsible for off-flavor; spiking reference food product with identified off-odorant to prove its role in the formation of off-odor, analysis by GC–O and sensory profile analysis; and answering the question of the origin of identified compound.

As in the analysis of unknown samples, maximum information has to be gathered on the sample history. This includes technology of the product, its storage history, transportation, and all stages at which taint or off flavor might arise.

To identify compounds of interest, their isolation must be performed. The method selected for isolation of compounds should not produce artefacts and should not cause decomposition of labile compounds. Extraction, distillation, and combined steam distillation and extraction methods are used for this purpose and were discussed in detail elsewhere (8,21). Because of the extremely low concentrations in which compounds of interest are often present, preconcentration of volatiles is required. Solventless methods have become increasingly popular. They involve dynamic HS, closed loop stripping analysis (CLSA) with subsequent thermal desorption (TD), or SPME. Usually more than one method of isolation has to be applied to obtain the full spectrum of volatile constituents. HS–SPME is often used complementary to other techniques such as vacuum distillation (64).

Solventless methods work well for the qualitative and quan-

titative purposes. However, for GC–O, flavor extract dissolved in low-boiling solvent is usually used (HS–GC–O is an exception). GC–O yields an aromagram being the response of human nose to perceived flavors. It is created in addition to a chromatogram obtained from the instrument detector (Figure 1). This method works well in the identification of compounds responsible for the off-odors, which can be quantitated using one of available approaches: aroma extract dilution analysis, Charm, or OSME (65–67). Recent developments in SPME are reflected also in GC–O applications. Deibler and et al. (68) used polydimethylsiloxane (PDMS) fibers of different coating thicknesses and exposed different lengths of the fiber to the HS of volatile compounds, achieving different volumes of PDMS. Linear plots of peak areas versus exposed fiber volume were obtained for all compounds. Ulrich (69) used SPME exposition at different times for this purpose. Brunton (70) used GC–MS for identification and GC–O for determination of compounds crucial to the off-flavor of stored turkey meat. To prepare serial dilutions, meat slurry was diluted with water and sampled using SPME, contrary to approaches described previously. Successive dilution of samples for GC–O experiments was applied also by Pilar Marti (71). Interesting application of nasal impact frequency (NIF) and surface of NIF procedures in GC–O enabled detection of parts-per-trillion odorants as demonstrated for orange juice (72). However, it must be stressed that SPME, which is not an exhaustive extraction method, provides at certain extraction conditions (fiber, time, temperature) a "profile" of volatile compounds that does not reflect the real proportions of volatiles in the matrix.

Quantitation of off-odorants should be carried out using a method that guarantees limits of detection and quantitation lower than an OT of the analyzed compounds. As the matrix in the case of food products is often a heterogenous liquid or solid, appropriate precautions must be taken in the quantitative analysis. Matrix influence is especially important in SPME analysis, and it should be taken into consideration in the process of method development. Internal standard (IS) addition prior to extraction is strongly recommended. Stable isotope dilution analysis (SIDA) (73,74) is a reliable and elegant way to quantitate off-odorants, though in the case of solid, heterogenous matrices, its uniform addition is almost not possible, and the release of an added standard does not reflect the release of analyte from the food product. Stable isotope standards guarantee, in the case of complicated flavor mixtures such as coffee, that they will not be present in the matrix. Segurel (75) utilized SPME for the analysis of DMS in wine using $[^2H_6]$-DMS as IS, which was quantitated in selected ion monitoring (SIM) mode based on *m/z* 62 for DMS and *m/z* 68 for its isotopomer. Evans (76) used deuterated IS ($[^2H_5]$TCA) for quantitation of TCA in wine HS. Pollnitz (77) used deuterated analogues of determined compounds (guaiacol-d_3, ethylphenol-d_4) in SPME analysis of wine and noticed that even when using SIDA, artefacts may occur. In situations in which more compounds responsible for the off-flavor have to be quantitated, as in rancid fats and oils, SIDA procedure becomes more complicated as often commercially unavailable standards of quantitated compounds have to be synthesized (78,79).

SPME in the analysis of compounds causing musty and earthy off-flavors

Haloanisoles and their precursors

The majority of papers describing analysis of haloanisoles are devoted to detection of TCA in wine and, to a lesser extent, in water and cork stoppers. Also, halophenols as haloanisoles precursors are analyzed.

Selectivity for certain analytes and also limits of detections are influenced by the type of SPME fiber coating chosen for analysis. To provide the lowest limits of detection for haloanisoles, different fibers were tested. When polyacrylate (PA) and PDMS fibers were compared by Riu et al. (80), the latter performed better. PDMS was also used by Alzaga (81). Similarly, Evans observed that PDMS adsorbed roughly two times more analyte than PA fiber (76). Lizarraga (84) tested 100 µm PDMS, 7 µm PDMS, PDMS–divinylbenzene (DVB), PA, and carboxene (CAR)–PDMS fibers. The most appropriate fibers were 100 µm PDMS and PA fibers, with the latter providing greater peak areas but requiring longer extraction times. Therefore, PDMS was assumed to be the best choice for determination of TCA. Diaz (83) selected PDMS fiber for the analysis of mixed chloro and bromo anisoles in water after comparison with PA and DVB–CAR–PDMS fibers. Both chloroanisoles and chlorophenols were a subject of investigation by Malleret et al. (84), who evaluated several DVB-based fibers. Better performance was observed for haloanisoles using non-polar DVB–CAR–PDMS fiber and for halophenols using polar carbowax (CW)–DVB fiber. PDMS fiber was selected for the detection of chlorophenols in wine after comparison with PDMS–DVB, CAR–PDMS, and DVB–CAR–PDMS fibers (85). Moreover, PDMS is not associated with displacement effects like PDMS–DVB. Direct extraction of halophenols from water provided higher peak areas than HS extraction (84). On the other hand, in the case of wine, HS–SPME yielded an approximate four-fold increase in peak areas compared with direct extraction (86). Submersion of the fiber in wine also decreases the fiber lifetime. Because of the specificity of the matrix in the analysis of TCA in wines, HS extraction is preferred. At equilibrium, the amount of analyte extracted on the fiber should be the same, whether the fiber is immersed in liquid matrix or extraction is performed from the HS. Therefore, differences in the amounts of extracted analytes in direct extraction and HS extraction can be a result of the various time required to reach equilibrium for both extraction methods. In HS extraction, molecules of analytes have to diffuse from liquid to gaseous phase, then from gaseous phase to the fiber coating. In direct extraction, they have to move from liquid phase into the fiber coating.

Experiment design models to optimize analysis parameters were used in few works on SPME. Bianchi (87) used a three-factor, two-level full factorial design (FFD) for experiments designed to optimize parameters for the analysis of haloanisoles in wine. The Doehlert design was used to evaluate conditions for derivatization SPME of phenols in wine (85), demonstrating that the main variability effect was the choice of the derivatization reagent.

Reports on the influence of temperature on the extraction efficiency vary in their conclusions. A decrease in absorption of TCA from HS on SPME fiber at elevated (40°C and 60°C) tem-

peratures, compared with extraction at ambient temperature of 20°C, was observed (86). To reach equilibrium, a 60-min extraction time was needed. The increase of extraction temperature (40°C) worsened the limit of quantitation (LOQ) of TCA because of a decrease of the *K* value (81). On the contrary, Evans (76) reported an increase in sensitivity at elevated (45°C) temperature. Although extraction yield increases with the increase of temperature because of the enhancement of mass transfer, high temperature decreases the efficiency of extraction because of a decrease of the distribution constant. For direct extraction, 60°C was an optimal temperature providing higher extraction efficiency (84). In addition to this, stirring resulted in a three- to five-fold increase of extraction efficiencies for all examined haloanisoles. Stirring accelerates the transfer from liquid matrix to HS, and the equilibrium in the stirred system is achieved much faster than in nonstirred ones. Moreover, a constant increase in peak area was observed in a time span of up to 240 min. This can be attributed to a slow migration of compounds from the liquid phase into HS and then into a fiber, or it is noticed in fibers where adsorption processes take place and may be related to displacement of compounds on the fiber surface. Diaz (83) observed the highest intensities for more volatile chloroanisoles in water at 50°C and at 65°C for the less volatile PCA. An increase in peak area was observed when extraction time was increased from 20 to 60 min for TCP, TeCP, and PCP (88). For a constant extraction time of 30 min, responses varied with temperature; for TCP, peak areas increased with the increase of temperature from 25°C to 55°C and, at 65°C, decreased below the area noted at 25°C. Similar behavior was observed for TeCP. However, for PCP peak area increased throughout all temperatures tested, which can be related to differences in boiling points of these compounds (150°C for TeCP and 310°C for PCP).

For quantitation of haloanisoles and halophenols, an electron capture detector (ECD) (sensitivity to chlorine) or mass selective detector (MSD) is used. Various ISs were used for quantitation of haloanisoles and halophenols, compounds chemically related or isotopomers. In an interesting work, Alzaga et al. (81) tested four compounds as IS for TCA determination: 2,4,6-tribromoanisole (TBA), 1,3,5-trichlorobenzene (TCB), 2,3,6-trichlorotoluene, and 2,4,6-trichlorophenylethylether (TCPEE). They found that, when using the PDMS fiber, TBA and TCPEE compensate the TCA recovery decrease observed when ethanol concentration in the matrix increased. The authors estimated that 15% of total TCA, TCB, or TCPEE present in the sample was extracted into the fiber. TBA was assumed to work best as an IS for ECD, and the use of IS compensated, to certain extent, for the matrix effect (white, red early, red vintage wines). To minimize the matrix effect, multiple SPME, based on the exhaustive analyte extraction from the sample, was also proposed (89). Diaz (83) used *p*-iodoanisole as an IS in MSD of mixed chloro and bromo anisoles in water using HS–SPME. For 2,4,6- and 2,3,6-trichloroanisol, limits of detection (LODs) were 0.03 and 0.04 ng/L, respectively. For tribromo analogues, the value for both compounds was 0.09 ng/L and for PCA was 0.15 ng/L. The authors found their method competitive with the CLSA–large-volume injection–GC–MS and stir-bar sorptive extraction–TD–GC–MS currently used for the analysis of haloanisoles.

Deuterated IS ($[^2H_5]$TCA) was used for quantitation of TCA in wine HS (76). TCA/IS peak ratios plotted versus the concentration ratios for 5–250 ng/L resulted in a coefficient of determination of 1.000, and quantitation was reliable down to 5 ng/L. The signal-to-noise ratio was influenced by column noise, which was lower for nonpolar columns (CP SIL-5) compared with polar columns (DB or Stabilwax). To detect chloroanisoles in cork stoppers, Bianchi (87) used ion trap MS in electron impact (EI) and chemical ionization (CI) mode. They analyzed TCA and also its degradation products 2,4-dichloroanisole and 2,6-dichloroanisole. Dichloroanisoles showed better sensitivity in CI, whereas TCA was best using EI.

Melleret (84) analyzed a group of tri- and tetrachlorophenols, chloroanisols, bromophenols, and bromoanisols. LOD of < 0.1–0.3 ng/L and LOQ of 0.1–0.8 ng/L were achieved (s/n = 3:1 and 10:1, respectively) depending on a compound type. LOD for TCP, TeCP, and PCP equal to 0.020, 0.016, and 0.003 µg/L, respectively, using ECD were reported (85). In situ acetylation of chlorophenols in cork macerate and wine samples helps to decrease polarity of compounds, avoid peak tailing, and improve detection (88). Limits of detection were 1.5, 0.8, and 1.0 ng/L for TCP, TeCP, and PCP, respectively, in a model solution.

Specificity of wine as a matrix requires special care during calibration. Parameters that are influenced by matrix are analyte recoveries, linearity, and limits of detection and quantitation. Alzaga (81) observed that method sensitivity decreased from ethanol in water (12%) to red vintage wine. White wine was assumed to be a matrix with low TCA interaction, whereas red vintage wines were assumed to exhibit high matrix effect. Linearity in a range of 0.1–150 ng/L with IS was $r > 0.995$ for wine matrices. LOD and LOQ ranged from 0.15 to 5.4 ng/L and 0.5 to 18.0 ng/L, respectively, depending on a matrix type. Riu (80), using SPME–GC with ECD, achieved LOD of 1.0 ng/L for TCA. LOQs were 4.0 and 8.0 ng/L for white and red wine, respectively. TeCA (2,3,4,6-tetrachloroanisole) is also a causative agent of musty taint in wine. Detection and quantitation limits for TCA and TeCA were 0.18 0.4 and 0.06 and 0.3 ng/L, respectively, when red wine was used for spiking. For TCA, relative standard deviations (RSDs) of 2.8% and 3.8% were found for repeatability and reproducibility (three different wine samples and three identical wine samples) for TeCA the respective values were 3.4% and 8.4% (82). Limits of detection achieved by SPME methods are usually in low-ppb range and are similar to those obtained using liquid–liquid extraction (26,90).

Although TCA is mainly associated with taints in wine it can also affect other food products. Microbial *o*-methylation of polychlorophenols into chloroanisoles provides a significant source of taints in fruit. Aung and Jenner (91) analyzed TCA in micro-organism-free irradiated raisins using 2.0-cm DVB–CAR–PDMS fiber for the extraction of TCA at 25°C for more than 1 h. To overcome the matrix effect, quantitation was based on the amount of TCA adsorbed on the fiber coating.

GEO and MIB

Cases of taint occurrence with these compounds are mainly

associated with water quality and the development of taint in fish meat. Several SPME methods for the analysis of GEO and MIB in water were described. Lloyd (92) observed recovery of 7.4% for MIB and 12.2% for GEO after a 20-min extraction using PDMS fiber compared with direct injection. When sample temperature was increased from 22°C to 40°C, no significant change was observed for MIB peak area, and a 15% increase was observed for GEO. At 60°C and 80°C, a decrease in both analytes was observed. Standard addition method was utilized for the quantitation and a high correlation was obtained between results obtained by the SPME and purge-and-trap (P&T) methods. Watson (93) used MSD for detection of MIB and GEO using naphthalene-d_8 (m/z = 136) or biphenyl-d_{10} (m/z = 164) as IS. The highest recoveries for MIB and GEO from HS were noted when using PDMS–DVB fiber. Lower recoveries were observed when PDMS, PA, and even 2-cm PDMS–CAR–DVB fibers were used. Using low concentrations of MIB and GEO (80 and 100 ng/L) they observed reaching equilibrium after 1 h for MIB and 2 h for GEO. Applying the developed method to water samples, GEO was found in a range of less than 1 ng/L up to 894 ng/L and for MIB 120–160 ng/L.

More challenging is the determination of GEO and MIB in the fish tissue. Because of the lipophylic nature of both MIB and GEO, their partition from fish tissue into HS makes SPME for direct analysis ineffective.

Several authors proposed isolation of GEO and MIB from fish tissue using microwave-mediated extraction of volatiles. Generated heat allows analytes to partition rapidly from sample matrix into the extraction solvent, which poses a perspective for shortening analysis time. In a microwave oven, radiation is applied to a sample, and steam formed in the process extracts volatiles, with subsequent condensation of distillate outside the microwave oven. Aroma compounds release during microwave heating at different times that are related to their air–water partition coefficients (94). The disadvantage of this method is the difficulty in controlling the amount of energy received by the sample, which can result in a lack of reproducibility. The other disadvantage is the possibility of the formation of artefacts during microwave heating.

Initially, solid-phase extraction was used to trap MIB and GEO after microwave distillation (MD) (95). SPME was used for this purpose by several authors for the analysis of GEO and MIB in catfish tissue (96–98). After a short microwave extraction was performed (6 min at 120°C), NaCl was added to the distillate, and compounds were extracted for 25 min using PDMS fiber (96). Tissue was spiked with *cis*-decahydro-1-naphtol (DHN) at 1 ppb as IS prior to extraction. Using an ion trap detector (ITD), the limits of detection for MIB and GEO were found to be 0.043 and 0.008 ppb, respectively, and 12.43 ppb of MIB and 0.19 ppb of GEO were detected in channel catfish, which were from a commercial catfish pond with detectable muddy and musty off-flavor. Lloyd and Grimm (97) and Grimm et al. (98) trapped volatiles in a cold trap, and then extracted MIB and GEO using SPME. Using an ITD, they used deuterated MIB (MIB-d_3) and GEO (GEO-d_3) as ISs for spiking samples prior to extraction and estimated quantitation limits for both compounds at 0.1 µg/kg. In catfish fillets, they detected concentrations of MIB ranging from 0.53 to 1.46 µg/kg and for GEO ranging from 0.07 to 0.20 µg/kg. By comparing the MD–SPME method with P&T–solvent extraction (SE) and MD–SE, they observed a 2- to over 10-fold increase of MIB and GEO peak areas in SPME. Comparison of detection limits obtained for MIB and GEO in water with that of fish tissue shows the role of matrix influence on analyte release in SPME. Another advantage of SPME was the shortened analysis time: 30 min (or even 15 min with multiple samples prepared/analyzed) compared with 3.5 h for P&T–SE and 1 h for MD–SE. Grimm et al. (99) observed high correlation (R = 0.9) between the instrumental assessment and the flavor checkers scores for 204 fish with an instrumental cutoff of 0.1 µg/kg for MIB and a cutoff value of 0.25 µg/kg used for GEO.

GEO and MIB were quantitated in wheat grain with musty and earthy off-flavor (30). Of the four fibers tested, a 2-cm DVB–CAR–PDMS fiber yielded peaks of largest areas. GEO was found in malodorous samples in concentrations ranging from 3.19 to 7.57 µg/kg. However, even samples perceived as sound by a sensory panel contained low levels of GEO (0.01–0.06 µg/kg)—obviously less than the OT in grain for this compound. Such parameters as water content can substantially influence recoveries of musty compounds from solid matrices and must be considered in method development (100).

SPME in the analysis of volatile phenolic compounds

Phenolic compounds, with their distinct odor, create problems mainly in fruit juices production, but they also influence other products in which precursors exist. Of volatile phenolic compounds analyzed using SPME, EP, EG, 4-vinylphenol, and 4-vinylguaiacol are most often investigated. Though in some products (such as wine) phenolic compounds contribute to its characteristic flavor, their presence in higher concentrations can impair sensory properties of this product. Papers discussed in this section on the analysis of phenolic compounds in wine can also give hints for their determination in other products where their presence is undesirable.

Different fibers were examined for the determination of volatile phenolic compounds: PA (101), CAR–PDMS (102), CW–DVB, PDMS (77), DVB–PDMS (103), or DVB–CAR–PDMS (104). DVB–CAR–PDMS gave higher peak areas when compared with CAR–PDMS in the extraction of musty compounds and volatile phenols (101). Meijas (106) compared PDMS, CAR–PDMS, CW–DVB, PA, PDMS–DVB, and DVB–CAR–PDMS fibers. The highest responses were generally noted for CW–DVB and PA fibers. A two-level factorial design that was expanded further to central a composite design was used to determine the optimal conditions of extraction using CW–DVB fiber. Four factors were explored: temperature, time of extraction, sampling volume, and ionic strength. Extraction temperature was, in general, the most important parameter with a positive effect for all the phenols studied. Generally, 4-vinylguaiacol and 4-vinylphenol have higher boiling points than corresponding ethyl phenols, so for these compounds extraction efficiency increased with temperature. In kinetically controlled extraction, an increase of temperature can increase recovery. However, a competing process of desorption from the fiber to HS can result in lower recoveries for low-boiling compounds. The next most influential factors were

sampling, time with a positive effect, and sample volume, with a negative effect, on the four volatile phenols (extraction increases as the HS volume decreases). The NaCl addition effect was significant only for EP. For vinylphenols, the NaCl concentration increase resulted in lower peak responses. Na_2SO_4 used for salting out guaiacol can increase its extraction of Na_2SO_4 over three-fold when compared with NaBr and over two-fold when compared with NaCl (105). Sampling temperature and sampling time had a strong positive influence on EG and 4-vinylphenol recovery (106). Detection limits for phenolic compounds are reported usually in a ppb range; they were 0.018, 0.019, 0.015, and 0.005 mg/L for EG, EP, vinylguaiacol, and vinylphenol, respectively (106). Their LOQs were 0.080, 0.081, 0.068, and 0.015 mg/L, respectively. Monje (101) used flame ionization detection (FID) for EP and for EG detection in wine, and the LODs were in low µg/L. Linearity was in the range of 5–5000 µg/L. Similar results were obtained using LLE: 25–10,000 µg/L linearity and 1 µg/L LOD. However, the RSDs were higher for LLE method compared with SPME: 15% and 2% for EP and 12% compared with 5% for EG. For musty and phenolic compounds analyzed in apple juice by Zieler (105), the LOD was the lowest for 2,6-dibromophenol (0.08 µg/L) and highest for 2,3-dimethylpyrazine (7.73 µg/L)—the majority of compounds had an LOD of less than 1 µg/L. Guaiacol was found to be responsible for the smoky/phenolic taint in refrigerated full cream chocolate milk. Detected concentrations were 0.7–0.9 mg/L. The odor threshold of guaiacol in chocolate milk was determined at 43 µg/L, and the detection limit was estimated at 0.05 µg/L in the elaborated SPME method, which was much less than its OT (102).

An elevated temperature and relatively long exposure time are often used for the extraction of phenolic compounds. Orr (107) used a fiber exposure time of 60 min and extraction temperature of 60°C to extract guaiacol produced by *Alicyclobacillus acidoterrestris* in apple juice. The highest extraction efficiency for *Actinomycetes* and *A. acidoterrestris* was obtained at 60°C (30°C, 40°C, and 60°C were tested) (105). A temperature of 40°C and preheating time of 15 min, followed by a 30-min extraction, was used to isolate compounds responsible for medicinal off-flavor in orange juice (104). Apart from guaiacol, major contributors to this off-flavor were 2,6-dichlorophenol for *A. hesperidum* and 2,6-dibromophenol. Two phenolic compounds (EP and EG) were found to be responsible for watercolor paint/phenolic/clove off-odors of strawberries infested with *Phytophthora cactorum* (103). When determination of phenolic compounds in hering extracted by SPME (PA fiber) was compared with results obtained by SDE, a high correlation (R^2 = 0.96) was obtained (108). Compounds with a high concentration in the product can quickly saturate the fiber, preventing sufficient adsorption of compounds with low concentrations. In the case of apple juice, the genuine flavor compounds can negatively influence the detection sensitivity. The highest yield of the off-flavors in apple juice could be achieved when the juice was diluted 10-fold (105).

SPME in the analysis of volatile sulfur compounds

Sulfur compounds in food comprise numerous constituents of different volatility and include H_2S, thiols, thioesters, and sulfides. Though sulfur compounds contribute significantly to the flavor of vegetables, fruit, and meat (38), they can be a source of food off-flavors. The majority of papers in which the use of SPME is described in the analysis of volatile sulfur compounds refers to wine. Reviews concerning the specificity of wine volatiles (109), sample preparation, and analysis of sulfur compounds in wine (40) are available. Apart from low concentrations of sulfur compounds causing off-flavors, the biggest challenge in their analysis is their reactivity, which can take place in the sample preparation step or sample injection. These processes may involve oxidation of DMS to dimethyl sulfoxide or oxidation of MeSH to DMDS, thus the appropriate precausions (deactivation of injection port, flushing samples with inert gas, and use of appropriate columns) are required. It was observed that SPME minimizes thiols oxidation during sampling (110). Low extraction temperatures are advised because water adsorbed by CAR-based SPME fibers can cause shifts in a baseline of a pulsed-flame photometric detector (PFPD). The other problem encountered is the interferences from SO_2 added to wine when using sulfur detectors, which can be eliminated by the addition of acetaldehyde. Alternatively, the binding of free SO_2 pyruvic acid or 2-ketoglutaric acid may be added.

The most frequently occurring compounds in wine and other beverages, which were a subject of investigations using SPME, are listed in Table IV. For a majority, the CAR–PDMS fiber was chosen for isolation and flame photometric detection (PFD or PFPD) were used for detection and quantitation. Fang and Qian (113) observed that a CAR–PDMS fiber gives very high responses for disulfides and trisulfides compared with other sulfur compounds. However, it was also observed that quantiation may be erratic because of displacement of low-molecular-weight compounds by higher-molecular-weight compounds in the competition for active sites on the CAR–PDMS fiber. Moreover, relative proportions of volatile sulfur compounds adsorbed into the fiber depend on their ratio in the mixture (111).

Ethyl methyl sulfide (EMS), isopropyl disulfide (IsoProDS), or 4-(methylthio)butanol were used as ISs. Mestres (112) used ethyl-methyl sulfide and tiophene as ISs for more and less volatile compounds, respectively. Majcenovic (110) used SIDA with the standards $[^2H_5]$ethanethiol and $[^2H_6]$diethyl disulfide for the quantitation of ethanethiol and diethyldisulfide.

Because of the high volatility of many of the analyzed sulfur compounds, relatively low temperatures are used for extraction: CAR–PDMS was used for extraction at room temperature for 30 min (110, 114), and room temperature was used also by Segurel (75). When comparing temperatures of 30°C, 45°C, and 60°C, Mestres (112) observed a decrease in peak areas as temperature increased. Thus, the optimum value was assumed to be 30°C. A relatively long extraction time is sometimes needed for isolation of less volatile compounds; 2 h at 35°C was observed by Mestres (115), and equilibrium was not reached even after 2 h.

In many papers on the quantitation of sulfur compounds, a synthetic wine is used in model experiments apart from white rose or red wines (115). In the work of Fang and Quian (113),

synthetic wine used as the matrix provided recoveries of approximately 100%, whereas for real white or red wines it varied from 80% to 120%. For mimicking real wine for model experiments, tartaric acid is added to water and ethanol, and the pH is adjusted to 3.5 with sodium hydroxide (75,110).

Sulfur compounds are a cause of off-flavors in other products. High levels of DMS in beer are a cause of distinct cooked-vegetable or cabbage-like off-odor. Scarlata and Ebeler (116) used CAR–PDMS fiber to extract DMS from beer at 30°C for 15 min using EMS as an IS. When compared with static HS, differences between results obtained by these two methods were within 3.9% and 7.8%. Quantitation of sulfur compounds by SPME in solid matrices is more difficult. Fan (117) found CAR–PDMS to be the most effective of those tested to extract sulfur compounds from precooked, ready-to-eat turkey breast after irradiation. The calibration curve was prepared in water. Some of sulfur flavor compounds can be bonded to heated proteins, which results in loss of disulfides and formation of free thiols.

SPME in the analysis of volatile lipid oxidation products

Different fibers were examined in methods used for extraction of volatiles from plant oils. One of the most frequently used fibers is DVB–CAR–PDMS. Vicci (118) used it to monitor compounds characteristic of olive oil undergoing a process of oxidation in accelerated storage tests run at 60°C. Vichi et al. (119) tested four fiber coatings: PDMS, CAR–PDMS, PDMS–DVB, and 2-cm DVB–CAR–PDMS. DVB–CAR–PDMS fiber had the highest ability to adsorb 6-carbon alcohols. For 28 compounds tested, 18 showed the highest peak areas when extracted using this fiber. They observed that PDMS fiber and PDMS–DVB fibers reached saturation at 10 and 30 min, respectively, but the remaining fibers did not attain equilibrium within 40 min. This can be attributed to the different mechanisms of extraction for the examined fibers, in which compounds were absorbed into the coating for PDMS, whereas the compounds were adsorbed on fibers surface for CAR-based fibers. The greatest responses were attained for DVB–CAR–PDMS and CAR–PDMS fibers when an extraction time of 30 min was used, and the latter was more selective for some of the most volatile compounds. Four fibers were tested by Cavalli (120): PDMS, CAR–PDMS, CW–PDMS and DVB–CAR–PDMS, the last one being chosen for subsequent analyses. DVB–CAR–PDMS fiber was used also by Mildner-Szkudlarz (121) and and Jeleń (122). Kanavouras (123) used two fibers, PDMS and PDMS–DVB, with the latter extracting more compounds. Keszler and Heberger (124) compared two fiber types covered with the same coating for the analysis of volatiles in sunflower oil (7 and 100 µm PDMS) and noticed that levels of absorbed aldehydes were higher in all cases when the 100-µm fiber was used.

Contini et al. (125) evaluated the effect of matrix composition on the quantitative extraction using PDMS–DVB fiber and 44 standard compounds in the range of 0.012–2.3 ppm for individual compounds (0.5–100 ppm for total volatiles). Only ten analytes were not influenced by the presence of other compounds in the mixture (ethanol, *Z*-3-hexenyl acetate, nonanal, acetic acid, *E*-2-nonenal, and 1-nonanol), whereas the linearity of the remaining compound was lost, which indicates that the capacity of PDMS–DVB fiber may be inadequate for the extraction of all volatiles from virgin olive oil at an overall concentration of approximately 10–50 ppm. They suggest that the loss of linearity for compounds present in high concentrations can be attributed to fiber saturation, and in low concentration can be attributed to compound displacement. To

Table IV. Selected Sulfur Compounds Analyzed by SPME in Various Matrices

Compound	Matrix	Fiber type	Detector	LOD (µg/L)	Reference
Methanethiol	Wine	CAR–PDMS	FPD*	0.50	116
		CAR–PDMS	PFPD	0.50	115
Ethanethiol	Wine	CAR–PDMS	FPD	1.00	116
		CAR–PDMS	PFPD	0.5	115
		CAR–PDMS	MSD	0.3	112
DMS	Wine	CAR–PDMS	FPD	4.00	116
		PDMS	FPD	2.00	114
		PA	FPD	3.00	114
		CAR–PDMS	PFPD	0.5	115
		CAR–PDMS	MSD	2.0	77
	Juice	CAR–PDMS	MSD	0.1	77
	Beer	CAR–PDMS	FPD	1.0	118
Diethyl sulfide	Wine	CAR–PDMS	FPD	0.15	116
		PDMS	FPD	0.25	114
		PA	FPD	0.50	114
		CAR–PDMS	PFPD	0.5	115
DMDS	Wine	CAR–PDMS	FPD	0.07	114
		PDMS	FPD	0.20	114
		PA	FPD	0.20	114
		CAR–PDMS	PFPD	0.01	115
		DVB–CAR–P	FPD	0.25	117
Diethyl disulfide	Wine	CAR–PDMS	FPD	0.05	116
		PDMS	FPD	0.05	114
		PA	FPD	0.10	114
		CAR–PDMS	PFPD	0.01	115
		DVB–CAR–P	FPD	0.04	117
		CAR–PDMS	MSD	0.05	112
Methional	Wine	DVB–CAR–P	FPD	2.5–12.5	117

* Flame photometric detector.

overcome these problems, dilution of analyzed oil with deodorized olive oil, reduction of the exposure time, and reduction of the oil volume were suggested. The 1:7 dilution allowed a stable estimation of target analytes. However, dilution of samples may reduce the quantity of some analytes present in low concentration.

When static HS (SHS), SPME, HS sorptive extraction (HSSE), and direct TD were compared for the analysis of olive oil in a more than 1-h extraction, HSSE showed higher concentration capacity than the SPME fiber, entirely due to the high volume of PDMS coating (55 µL for PDMS stir bar used in HSSE vs. 5 µL for DVB–CAR–PDMS). Similar number of compounds was detected using SPME and HSSE (~ 30 identified), whereas direct TD allowed extraction of more than 60 volatile and semivolatile compounds from the very low quantity of olive oil (5 µL) without any sample preparation. Contrary to HS techniques, the main identified compounds were the semivolatile components (120).

Precautions should be taken when volatile compounds resulting from lipid oxidation are extracted using SPME at elevated temperature. Because of prolonged extraction time and high temperature, decomposition of hydroperoxides can take place. A resulting increase in peak areas of adsorbed compounds can be caused by longer exposure time, as well as the oxidation process. It can be eliminated by either extraction at ambient temperature or addition of antioxidants. Usually, extraction temperatures described in literature were 40°C and lasted for 30 min (118), 60 min (127), or 90 min (125). Extraction at ambient or near ambient temperatures were also used: 25°C (90 min) (126) or 20°C (122). The rate of extraction increased the most until 30 min, and then the increase to 90 min was less pronounced. The temperature of extraction of 50°C provided higher responses in area of peaks compared with sampling at 20°C—more than two-fold, in some cases (122).

Equilibrium for oil flavor compounds is attained at different times. For low-boiling heptanal, 20 min was optimal, but even after 40 min, the equilibrium had not been achieved for 2-undecenal (124).

Often, no quantitation is performed in publications related to the analysis of oils in volatile compounds; relative increases in compound peak areas are measured, which illustrate the process of auto-oxidation. The compounds for which the most rapid increase during oil storage were noted were nonanal, hexanal, heptanal, octanal, octane, 2-pentylfuran, 2-ethylfuran, isomers of 2,4-heptadienal, 1-pentene-3-ol, and 1-octene-3-ol. Vicci (118) and Cavalli (126) monitored volatiles of olive oil stored at ambient temperature in the darkness and observed an increase of (*E*)-hex-2-enol and hexanol, (*Z*)-hex-3-enol, and pentan-2-one and pentan-3-one. In the same time, they observed a decrease of (*E*)-hexen-2-enal during oxidation. Guillen (127) observed changes in the volatile compounds of sunflower oil that was oxidized for 11 days at 70°C with oxygen access and detected mainly aldehydes and ketones, but no quantitative analysis was performed.

Contini (125) carried out quantitative analysis of volatiles resulting from oil oxidation and found calibration curves in a range of 0.1 to 20 ppm to be linear (r^2 = 0.99) for 27 compounds. The upper limit of linearity for the remaining compounds was between 7 and 18 ppm (FID detector). The LOQ (ppb) for the analyzed compounds ranged from 0.4 ppb (hexanal) to 74.9 ppb (ethanol). The CV values in all cases were lower than 9%. In a previous work (122), limits of detection for 11 out of 14 quantitated fatty acids oxidation products in oil were below 1 µg/L. For concentrations at 10 µg/L, RSDs were generally below 7.5%. At 10 mg/L for all analyzed compounds, RSDs ranged between 0.78% and 5.92%. Depending on the analyzed oils, the amount of aldehydes ranged from 76.8 to 814.3 ppb. Keszler and Heberger (124), using SIM for detection of aldehydes, estimated their limits of detection at 50–500 pg/µL in sunflower oil compared with 5–50 pg/µL in water.

Auto-oxidation is a sensory problem that is related not only to edible oils. Other types of food, often containing very low levels of fat, are also affected by these changes. The partition of lipid oxidation products between the oil phase, aqueous phase, and HS can affect the sensory perception of food emulsion. Oil/water emulsions were investigated by Beltran (128) and Pan (129). The latter used SPME to study volatile compounds formed by photosensitized oxidation and auto-oxidation of cod liver oil in emulsion systems without quantitation of detected compounds. A 100-µm PDMS fiber was used for sampling of oil/water emulsion for 15 min at 50°C. Fifty-two compounds were identified: 31 aldehydes, 5 alcohols, 6 ketones, 6 acids, and 4 other compounds. Hydrophobic compounds can be perceived at lower concentrations in water than in oil beecause of the differences in partition coefficients. Many of the lipid oxidation products show higher solubility in the oil phase. Therefore, for a given concentration of volatile compounds, their concentration in the HS of an emulsion decreases as the oil concentration increases. Therefore, low fat emulsions can often be perceived as more oxidized than a high fat emulsion, whereas they can contain the same amount of volatile compounds. Jo and Ahn (130) quantitated the main aldehydes (pentanal, hexanal, *t*-2-hexenal, nonanal, *t*-2-nonenal, *t*-2-decenal, 2,4-decadienal, and *t*-2-undecenal) in oil/water emulsion. Depending on the compound, their concentrations ranged from 0.01 to 3.57 ppm, and the concentration of total aldehydes ranged from 61.3 to 70.0 ppm.

Even more complicated in terms of flavor release and quantitation of volatiles are solid matrices of food containing lipids. Brunton (131) analyzed cooked turkey meat, which has been shown to be more susceptible to lipid oxidation than meat from other species. Moreover, minimally processed turkey products or turkey meat are much more susceptible to lipid oxidation than more heavily processed meats. CAR–PDMS fiber was used for the analysis of volatiles. To facilitate analysis, a homogenate of meat in water (20 g in 100 mL water) was prepared, then sampled for 30 min at 70°C. Sixty-five compounds of 101 detected were identified by GC–MS. Addition of water to meat simplifies the quantitation. However, it influences the release of volatiles from the matrix (water solubility). Therefore, the relative amounts of compounds extracted may not reflect their proportion in a meat sample. GC–O was performed to determine the compounds that were crucial for the formation of off-flavor. The most significant compounds for stored

meat at 4°C for 3 days were 1-octene-3-one (metallic mushroom), (*E*,*Z*)-2,6-nonadienal (oily, cucumber), and (*E*,*E*)-2,4-decadienal (oily, fatty).

Hexanal, a popular indicator of lipid oxidation in foods, is formed during oxidation of linoleic acid. Hexanal was determined in cooked meats (131,132). Fernando (133) used SPME for the analysis of hexanal in the oxidation process in raw pork. Deionized water was added to pork, the samples were heated for 30 min at 75°C, and they were then removed and cooled to room temperature prior to extraction of volatiles using a CAR–PDMS fiber. Using external calibration linearity of 0.999 was observed for a range of 0–0.814 mg/L of hexanal. Precision of the method was 6.7% RSD for hexanal at 0.1 µg/g of muscle sample. Concentration of hexanal in investigated raw meet samples was low, below 1 mg/kg. Andrés (134) used hexanal as an indicator of rancidity in dry-cured hams. Certain levels of compounds with rancid notes is needed in these products to achieve the typical flavor, but an excess of such notes leads to an overall unpleasant flavor. Minced sample was mixed with water and sampled with CAR–PDMS fiber at 40°C for 30 min. For the standard curve, a solution of hexanal was used and the quantity expressed in nanograms of hexanal per gram of muscle. The amount of hexanal varied in hams from 0.07 to 13.0 ng/g. A different approach was presented for the quantitation of hexanal based on the preparation of hexanal vapor standard from hexanal diluted in squalene and allowed to evaporate in flask for the quantitation of hexanal in freeze-dried chicken myofibrils (135). Two methods were compared for the determination of hexanal in potato chips: SPME–GC and high-performance liquid chromatography (HPLC) (136). Samples for SPME–GC were ground and mixed with water, and sampled at 70°C for 20 min using 50:30 µm DVD–CAR–PDMS fiber. HPLC determination of hexanal involved derivatization with 2,4-DNPH prior to analysis and UV detection. The detection limit for SPME–GC–MS was 1 ng/mL compared with 9 ng/mL using HPLC–UV, and the repeatability was, respectively, 7.56% and 2.40% recoveries that were 102% ± 10%.

Various fibers were used for the analysis of lipid oxidation volatiles in products other than oils: PDMS–DVB was used for the analysis of light-induced volatile compounds in goat's milk cheese (137), and identification of volatile compounds in oxidized porcine liver (138). Brunton (131) evaluated three types of SPME fibers using hexanal and pentanal, and CAR–PDMS was the fiber providing the highest peak areas and acceptable reproducibility. It was also selected by Mortensen (139) for the monitoring of light-induced oxidation in semihard cheeses. Sanchez-Silva (140) profiled volatile flavor compounds of potato chips during storage using PDMS–DVB, CAR–PDMS, and DVB–CAR–PDMS. Variations between samples were the smallest for DVB–CAR–PDMS (2%), and this fiber was selected. One of the products, which is highly prone to oxidation because of its high fat content and the presence of large amounts of iron and a low amount of natural antioxidants, is liver pâté. Esteves (141) used DVB–CAR–PDMS fiber to extract volatiles from 1 g of pâté at 60°C for 30 min. Some of the detected compounds were linked to the formation of off-flavors that are characteristic of oxidized liver: *Z*-4-heptenal, which has a fishy off-flavor (OT = 0.04 ppb); (*E*,*E*)-2,4-heptadienal, with a similar note; 2,4-nonadienal, which has a rancid odor; (*Z*)-2-nonenal, with a cardboard-like odor; (*E*,*Z*)-2,4-decadienal, which has a rancid odor; and (*E*,*E*)-2,4-decadienal, with warmed-over flavors. 1-Octene-3-one was the compound primarily responsible for the metallic off-flavor of porcine liver, whereas (*E*,*E*)-2,4-heptadienal, (*E*,*Z*)-2,4-heptadienal, and (*Z*)-4-heptenal contributed to fishy notes (138). Esteves (142) used SPME for the analysis of volatiles in meat from pigs. Samples were homogenized with water and extracted with DVB–CAR–PDMS fiber for 30 min at 37°C. SPME was also used for the determination of oxidative stability of roasted high oleic and normal oleic peanuts (143). The most important compounds produced after the roasting of peanuts are pyrazines (144), whereas off-flavor is formed because of aldehydes generation. A 100-µm PDMS fiber was used to extract volatiles. The aldehydes responsible for the off-flavor were pentanal, hexanal, octanal, and nonanal. Nonanal was pointed as a marker for the oxidation process as a compound originating from the oxidation of oleic acid. DVB–CAR–PDMS fiber was used to sample volatiles from whole sardines put into a specially designed glass vessel, and trimethylamine and 3-methylnonane-2,4-dione were related to overall odor changes (145).

As short-chain aldehydes give poor response on FID, a way to increase sensitivity of analytical methods derivatization of carbonyl compounds is often performed both in HPLC and GC analysis. Derivatization can also be performed when SPME is used for compounds isolation (146). (*E*)-2-nonenal, a potent off-odorant in beers, contributes to a cardboard off-flavor. Because of its low OT, it is difficult to detect in the beer complex matrix. On-fiber derivatization of beer aldehydes with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBOA) was proposed by Vesely (147). For this purpose, a DVB–PDMS fiber and an MS were used as the detector. (*E*)-2-nonenal could be quantitated using this method in concentrations down to 0.01 ppb.

SPME in sampling for artificial olfaction

Rapid progress in electronic noses technology has taken place in recent years. Electronic noses are used for food product differentiation, determination of food origin, detection of adulterants, monitoring freshness of products, and detecting off-flavors (148,149). Instruments can be grouped into two sections. One group of instruments is based on a sensor technology that uses an array of metal oxide sensors (MOS), conducting polymers (CP) as sensors or those based on bulk acoustic wave (BAW), and surface acoustic wave (SAW) sensors (150). The other group contains instruments based on MS. The most popular methods for the introduction of volatiles into the electronic nose have been static HS. Pumps or compressed air is used to sweep volatiles into sensor chamber. However, SPME offers a cheap and simple preconcentration method for sample introduction into electronic noses.

SPME was used as a tool for obtaining a signal, in an FID or nitrogen phosphorus detector, of the whole mixture of volatiles without compounds separation (151). Data collected from such a "sensor" have the a form of single peak with its area proportional to the concentration of HS volatiles.

SPME–MS was used to differentiate infant formulas during storage and monitor oxidative changes. Principal component analysis enabled rapid differentiation of samples. High throughput (100 samples in 24 h) is an additional advantage of this method (152). SPME–MS was used also for the analysis of light-induced lipid oxidation products in milk. Marsili (153) used SPME–MS–multivariate analysis for the study of off flavors in milk using CAR–PDMS fiber. He used this type of device for the shelf-life prediction of pasteurized and homogenized milk stored for over a 7-month period (154). *E*-nose based on MS was used also for the detection of rancidity in potato chips (155). SPME–MS based electronic nose is proposed as an alternative tool for traditional methods such as Rancimat or ADV test to assess lipid stability and the degree of rancidity in chips (156). Sampling based on SPME showed much better performance than sampling based on static HS (157). SPME was also applied to a classical electronic nose with a SAW sensor array and used to distinguish lampante from other virgin olive oils. The model was able to classify 90% of samples correctly (158).

SPME also started to play a role in the sensory assessment of food products. Apart from the elaborated SPME–GC–O technique already described, an interesting approach was proposed by Rega (159) to assess the flavor of orange juice. By replacing the GC analytical column with uncoated fused silica capillary, panelists could sniff the overall orange juice odor obtained using SPME. However, recoveries of compounds at a given time vary from compound to compound because of their partition coefficients in the phases involved in extraction process (sample, HS, and SPME coating). Therefore, the odor of the SPME extracts may be a poor representation of the real product flavor.

Conclusion

The human nose was for a long time an unrivaled detector in the recognition of off-flavors in food. In many instances it still is. Recent applications, which enable detection of flavor, significant compounds in concentrations lower than the OTs make allow for a good perspective in the analysis of taints and off-flavors. Apart from sensitive and selective detectors required for off-odors, extraction and preconcentration play a crucial role in the development of sensitive methods of taints and off-flavors analysis. SPME has substantial advantages, which predispose this technique for off-odors analysis. It is an extraction technique that is oriented towards quantitation of target analytes. Its selectivity can be manipulated by appropriate fiber selection, based on the polarity of the analyte, analyte molecular weight, and character. SPME is very sensitive and is also fiber-dependent. Extraction times are usually much shorter than with competitive techniques because of a small volume of fiber coating. Because of its simplicity, SPME can be used without instrument modification. Its hyphenation with fast chromatography and fast MS (160,161) can also result in a faster tool for analysis of food flavors and off-flavors.

The biggest challenges in SPME method development are related to the specificity of matrix (which can dramatically influence volatiles release), appropriate choice of standards, and quantitation methods. When using fibers in which volatile binding is based on the adsorption on their surface, analyte displacement has to be considered.

SPME already proved to be a reliable method in food volatiles analysis. The increasing number of papers published every year is evidence of this.

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