Chapter 25

Sampling and sample preparation for food analysis

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25.1 FOOD SAMPLING

25.1.1 Considerations

The term "food" refers to the broad range of edible materials that comprise the essential body nutrients required for life and growth, such as proteins, carbohydrates, fats, vitamins, or minerals. Foodstuffs are described variously as "liquid" or "solid", and "wet" or "dry", depending on the amounts of water and fat they contain. Samples of plant origin are classified for analytical purposes as having a high or medium water content and a lower content of saccharides (from 5% to 15%), very low water content (dry), or a high content of oils [1]. Similarly, food samples can be divided into four main groups based on water and fat content [2]. Food samples of biological origin (liquid or solid) have been divided generally into the five categories described in Table 25.1. This coarse division is important when considering the choice of isolation technique, extraction solvent, and sample clean-up method during an analytical procedure [3].

Moisture content is an important consideration during sampling procedures, in part because it affects the extent of sample heterogeneity. Virtually all foods are heterogeneous, and the analyst should be familiar with their variability in composition and structure. In general, fresh foods of plant origin are more variable in composition than fresh foods of animal origin. The analyst should be also aware of the postmortem or postharvest physiological changes that can occur after a fresh food is sampled and which can affect sample heterogeneity. A combination of cold storage and chemical preservation may be required to maintain sample integrity in the event of prolonged storage.

Although the chemical and physical properties of foods are inherently variable, even between samples that originate from the same breed or strain, the variability in composition of a single food sample can be minimized with proper sampling and sample pretreatment techniques. Two approaches can be used for sampling a food mass that is larger than the amount required for analysis in the

Comprehensive Analytical Chemistry XXXVII
J. Pawliszyn (Ed.)

TABLE 25.1

General classification of food samples according to their content (with permission from Ref. [3])

Sample	Character	Typical analytes
Milk	Aqueous, proteins, lipids	Veterinary drugs, toxic elements, pesticides, industrial contaminants
Eggs	High lipids and albumin content	Veterinary drugs, industrial contaminants, pesticides
Other samples of animal origin (e.g. muscle, liver, fat)	Various fat, proteins, or water	Drugs, industrial contaminants, pesticides
Plant material (e.g. fruits, vegetables, seeds)	Various water, plant pigments, lipids, proteins, essential oils or waxes	Pesticides, toxic elements, industrial contaminants
Food (e.g. meat, fish, milk, cereals, wine, juices, plant oils, sugar)	Various fat, oils, lipids, proteins, sugar, starch, water, or pigments	Pesticides, industrial contaminants, synthetic colorants, additives, synthetic sweeteners, antioxidants

laboratory. Many minute increments of a solid material can be collected and blended to represent the entire foodstuff, or a quantity of material that is large enough to be compositionally representative of the whole can be collected and then reduced to a fine mixture before being subsampled [4]. The first approach is usually avoided, since it is difficult to obtain a statistically representative sample and the sampling time can also be very long. The latter approach is more practical, accurate, and reproducible.

Since virtually no food material can be analyzed in its entirety, careful sampling techniques are required to obtain representative, laboratory-sized primary samples, in addition to subsequent subsamples, or secondary samples [5]. The amount of subsample required for an analytical procedure usually varies from a fraction of a gram to several grams. The sampling techniques discussed in the sections that follow are used to produce small, discrete primary and secondary samples that are representative of the entire food material, with minimal error.

The required sample size is defined in part by the nature of the target compound, that is, to what extent the analyte is retained in the matrix. Xenobiotics are generally present at trace levels, i.e., in $\mu g \cdot g^{-1}$ or $ng \cdot g^{-1}$ concentrations, or even lower. A sufficiently large amount of sample must be collected and analyzed in order to be able to measure minute quantities of the compound of interest and to satisfy the method's limit of detection. Conversely, relatively small samples may be collected for the macro analysis of gross food components, i.e., to measure crude fat, crude protein, crude fiber, or ash. Although proximate analysis of these food components is sometimes sufficient, more exact analyses are usually required.

The sample size is also dependent on the relationship that exists between the mass required to adequately represent a sample and the characteristics of that sample [6]. If a foodstuff consists of some mixture of different-sized particles, enough sample mass needs to be collected in order to adequately represent all of the particles. Because large particles are more difficult to represent than smaller ones, a mass that is large enough to represent the larger particles will also be representative of the smaller ones. The segregation of finer, denser particles to the bottom of the sample container must be recognized during the sampling process to ensure that all particles are represented and to avoid large sampling errors. The theory of sampling along with solutions for correct sampling are well-described in other texts [7–9].

25.1.2 Techniques

Food lots are sampled in either a manual or continuous manner in order to obtain a representative specimen. Containers holding loose foodstuffs can be sampled manually with devices that trap the material in a compartment such as a probe or tube. Slots or openings placed at intervals in the tube allow for simultaneous sampling at different depths of the product. When employing this technique, however, the analyst must consider the segregation effect and ensure that all particle sizes are accessible. The foodstuff may ultimately need to be removed from the sample container and poured onto a flat surface. The amount of material may then be reduced with a coning-and-quartering method [10], and a subsample collected in multiple random increments. No particle size should be excluded during the sampling process since food components or contaminants that collect in certain-sized particles might be omitted from the final analysis, thereby resulting in an increase in sampling error.

Large mixtures may also be reduced with a riffle cutter, which is a box-like device that has equally spaced dividers to divide the sample stream. The sample may be further cut or quartered by passing it through successive riffles. Other proportional dividers are available for reducing a sample, such as the straight-line sampler and the spinning riffle sample divider [10].

Uniformly solid or liquid products are perhaps the most straightforward to sample. Drill-type devices are used to obtain a core from solid products such as cheese or frozen foods. Liquid samples are thoroughly mixed before a subsample is removed with a syringe-type sampler or by submerging a container under the liquid's surface (a so-called "grab" sample) [11]. For obvious reasons, many complex foods such as vegetables, fruit, or animal tissues may require blending prior to being sampled. These blending methods are discussed in the section that follows.

Throughout the sample preparation procedure, it is essential for the analyst to recognize the necessity of utilizing methods that satisfy statistical sampling and analysis requirements. The inherent variability in the composition of raw materials, basic ingredients, and processed foods requires the use of statistical

methods for obtaining representative and replicate samples, and for estimating the error involved in sampling. Measures of the precision of the mean results are also required, in addition to statistical analysis and interpretation of the data obtained [12]. The reader is referred to standard text and reference sources in the field for these purposes [7–9,12,13].

25.2 FOOD PRETREATMENT

25.2.1 Removal of extraneous matter

Before sample blending is done, it is often necessary to wash, remove, or drain irrelevant extraneous matter. Soil or sand that adheres to fresh fruit or vegetables can be removed by washing or wiping the surface of the produce; however, excessive washing should be avoided to prevent the leaching of soluble solids. Depending on the objective of the analysis, fresh produce may be separated into the core and the outer and inner tissues. Shells are usually separated from nut kernels and pits from stone fruits. Large fish are cleaned, scaled, and eviscerated, while small fish can be blended whole. Shellfish are shucked, eggs are broken to isolate the liquid interior, and meat is removed as completely as possible from bone. Suspended matter or sediment present in liquids such as beer, wine, juice, or cooking oil is removed by filtration or separated by centrifugation. Canned fruit and vegetable products may be drained through screens if it is not necessary to analyze the composite sample [14].

25.2.2 Sample reduction

Once a food sample has been collected using the sampling techniques discussed in Section 25.1.2, a suitable method is required to make the material less heterogeneous. Various approaches may be utilized for reducing the particle weight and size in a primary sample, so that smaller subsamples can be taken for a representative analysis of the whole [4]. Finely divided materials also dissolve faster and are easier to extract because of their greater surface area.

Methods for reducing solid or semi-solid foods include mechanical grinding, mixing, rolling, agitating, stirring, chopping, crushing, macerating, mincing, pressing, pulverizing, or any other reasonable means of comminuting the sample. Sample reduction can also be achieved with a Wiley or ball mill, mortar and pestle, mechanical high-speed beaters or blenders (for soft or wet foods), and meat grinders. Liquid samples can be mixed using magnetic stirrers or sonic oscillators. Figure 25.1 demonstrates the importance of selecting the appropriate hardware for sample mixing, and of blending the sample for a sufficient period of time [15].

There are several other factors to consider when reducing a food sample. Food choppers, blenders, and mixers should be constructed of metal alloys that resist corrosion or erosion, and that are inert enough to prevent contamination

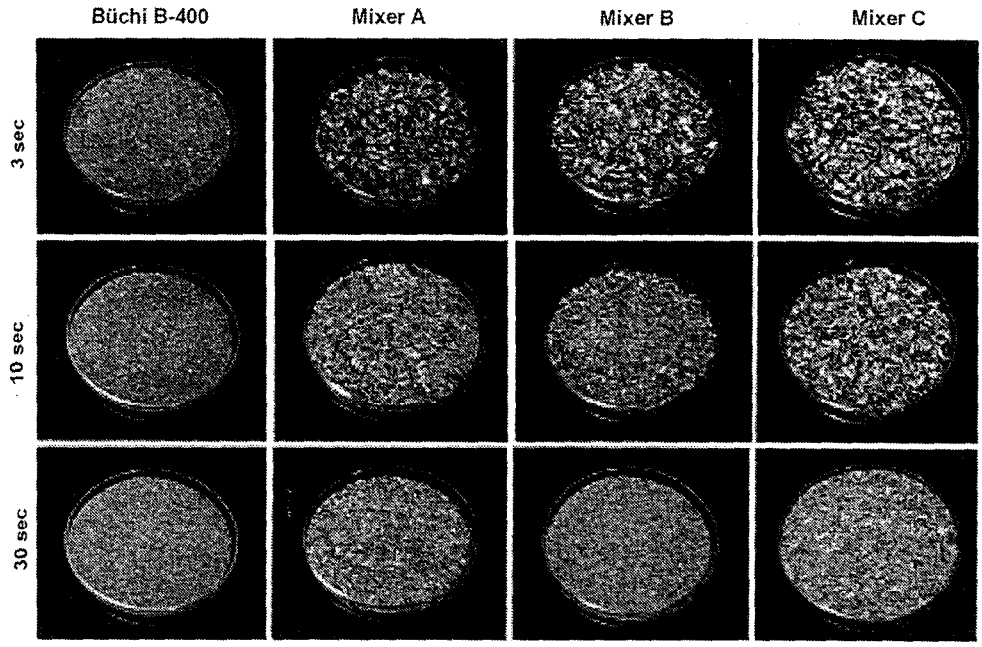


Fig. 25.1. Effect of choice of mixing equipment and blending time on sample heterogeneity for sunflower seeds. Reprinted with permission from Ref. [15].

of the product. Aeration of the product during the blending process should be avoided since this can result in appreciable changes in oxidizable components. It is also important to avoid heating the material during the grinding step since this can accelerate chemical changes in the foodstuff. The surfaces of all mixing equipment should be clean and dry, since changes in sample moisture content can change the chemical and physical nature of the foodstuff. Care should also be taken to prevent the release of volatile constituents during grinding, if this is of concern [14].

The analyst should be aware of the enzymatic changes that can rapidly occur in crushed plant and animal tissues. In animal tissue, rapid enzymatic changes may result in appreciable changes of certain food components, particularly in the case of carbohydrate and nitrogenous compounds [14]. It may also be necessary to inactivate food enzymes, for example by denaturation in boiling methanol–water or ethanol–water mixtures [16].

In conclusion, it is imperative for the analyst to be familiar with the food matrix that is being analyzed. Since it is not feasible to discuss all possible cases here, it is important that the analyst to consult the appropriate sources for information before beginning a new sampling procedure.

25.2.3 Moisture

Recognition of the level of moisture in food samples is important for several reasons. As previously discussed, moisture can contribute to the extent of sample heterogeneity. A sample may also need to be dried prior to being blended

or stored, since the material may lose moisture during blending, or deteriorate during storage. Determining the moisture content through sample drying may be necessary in order to calculate the nutritive value of a food product or to express analytical results on a uniform scale, for example in the determination of the dry matter in flour [17]. Moisture is also important in terms of food quality, since it affects food freshness, preservation, and resistance to deterioration.

Water is present in food samples in three forms [11]: as a solvent or dispersing media; adsorbed on the internal or external surfaces, or as fine capillaries by capillary condensation; and as water of hydration.

Solvent or free water is most easily removed. The rate at which moisture is removed from foods is affected by drying temperature, particle size, vacuum, crust formations on the surface, and surface area of the sample [11]. Bound water is quite difficult to remove and normally requires a vacuum process. Vacuum drying is preferred nonetheless, since this technique significantly reduces the deterioration of samples during heating. For example, plant tissues, which are often dried prior to the analysis step, might undergo extensive enzymatic changes during the drying process, especially when they are exposed to air. Utilizing vacuum drying also accelerates the drying time, which can take up to 16 h under ideal conditions.

Other precautions need to be considered when drying foods at elevated temperatures, since chemical reactions such as hydrolysis can occur and chemical reactions can be accelerated. Moisture determinations can be erroneous if hydrolysis has occurred, since the water of hydrolysis has not been released from the sample. On the other hand, very dry samples may absorb water from the air before the moisture determination has been completed.

A general rule of thumb for sample drying is that it should be as rapid and at as low a temperature as possible. Vacuum methods that can used to dry a sample include vacuum ovens and lyophilization, or freeze-drying. Other methods that can be employed are distillation, microwave drying, and the Fischer titration method. The titration method is particularly applicable to low-moisture foods that give erratic results when heated or under vacuum [11].

Finally, when drying a sample, the analyst should be aware that a certain level of moisture might be required for prolonged food storage, since chemical reactions such as oxidative deterioration can occur when moisture levels are too low, for example in vegetables such as carrots and potatoes, which will develop oxidized flavours or become rancid in two to three weeks at a 2 or 3% moisture content. Oxidative deterioration of these foods is inhibited for several months when they have a 8–10% moisture content [14].

25.2.4 Removal of co-extractives

An inherent difficulty in the extraction of food samples is the co-extraction of matrix components that are also soluble in the extraction solvent. A common example of this is the co-extraction of lipids during supercritical carbon dioxide

extraction (or any other type of extraction) of non-polar compounds from animal and vegetable matrices [18–22]. The presence of matrix interferences in sample extracts can result in a multitude of problems, including the generation of emulsions, sample turbidity, contamination or plugging of equipment, and, perhaps most importantly, the masking of the analytical signal for the target analyte and the consequent increase in the method limit of detection.

Co-extractives are frequently removed during a post-extraction clean-up step that requires passing the liquid extract through a clean-up column for sorption or filtration of the interferences. Commonly used clean-up materials include Florisil, alumina, silica gel, in addition to gel permeation chromatography, solid-phase extraction materials, etc. Solid-phase materials can also be used to exclude co-extractives from the analyte concentration step, that is, the material may only retain the target analyte and not the interferences. This step is also referred to as analyte enrichment, since the analyte concentration is increased over that of the matrix background signal, if indeed any occurs at all. The factors that affect the choice of clean-up material are similar to those considered when choosing a solid-phase for the extraction of liquid food samples. Overviews of both types of applications will therefore be presented together in Section 25.3.

Of particular interest are analytical methods that incorporate an *in situ* or on-line clean-up technique. Sample clean-up in this case can be achieved *in situ*, for example, with a simple and elegant extraction technique called matrix solid-phase dispersion (MSPD) [23,24]. The advantage to MSPD is that it combines sample blending, clean-up, and extraction into one technique. During an MSPD procedure, the sample matrix is mixed with an appropriate polymer resin, such as the reverse-phase chromatographic sorbent, C_{18}. The solid or semi-solid sample is prepared for extraction by grinding it in the presence of the sorbent using a mortar and pestle, which facilitates disruption of the sample matrix. Total disruption is achieved once the cell components are disrupted and the sample is evenly dispersed over the polymer material [23]. The end result is that the entire dispersed sample becomes a unique chromatographic phase from which either the analyte or matrix components can be selectively eluted using an organic solvent or solvent mixture with the appropriate eluent strength. The solvent mixture is usually water-immiscible.

The MSPD technique was originally applied to the isolation of drug residues from animal tissues [25]. It has since been successfully applied to the wide variety of food matrices shown in Table 25.2, including dairy or medical products, animal tissues, vegetables, fruits, and aquatic species. It should be noted that adsorbent consumption can be high for samples with high lipid content, and that an additional clean-up step may be required for an extract obtained from a complex biological matrix.

A particularly interesting application uses a miniaturized and automated MSPD extraction method for the isolation of pesticides from fruit samples [40]. This method was optimized for a variety of organophosphorous pesticides and a pyrethroid from oranges, but satisfactory recoveries were also obtained from

TABLE 25.2

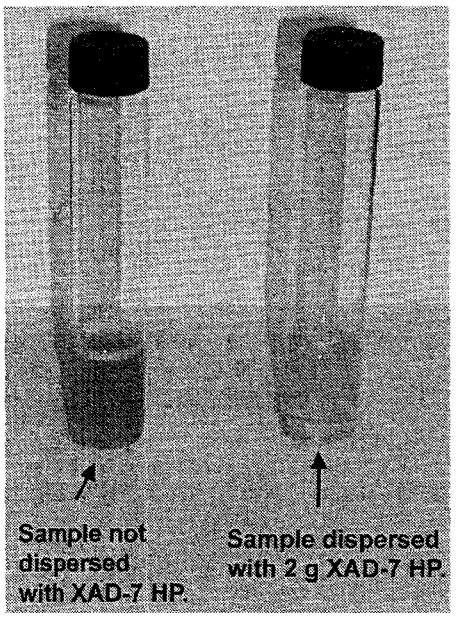
MSPD clean-up and extraction of food matrices

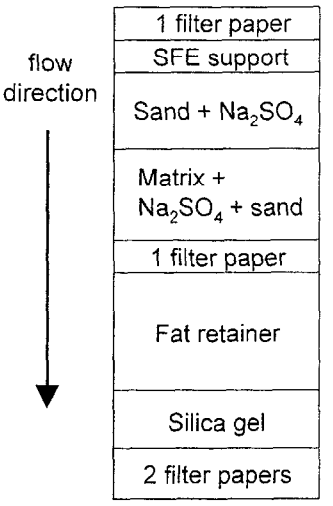
Sample	Analyte	MSPD material	Ref.
Citrus fruit	Various pesticides	C_8	26
Oranges, grape, onions, tomatoes	Carbamate pesticides	C_{18}, C_8, cyano, amine and phenyl solid phases	27
Milk	Organochlorine and organophosphorus pesticides	C_{18}	28
Milk	Veterinary drugs	C_{18}	29
Medical foods	Vitamin K_1	C_{18}	30
Meat, milk, cheese	Tetracyclines	C_{18}	31
Fish	Surfactants	C_{18}	32–34
Fish	Triazine pesticides	C_{18}	35
Beef fat	Chlorinated pesticides	C_{18}	36
Liver	β-agonists	C_8, C_{18}	37
Liver	Clenbuterol	C_{18}	38
Chicken muscle	Sulfonamides	C_{18}	39

pears and grapes. The method requires only 25 mg of sample and 100 μl of organic solvent. Solid-phase C_8 was determined to be the optimum dispersion material for this application.

The technique of matrix–solid phase dispersion can be adjusted to retain particular compounds by choosing an appropriate dispersion material in addition to using a specific eluent. Most applications have utilized the reverse-phase material C_{18}, in part because the solid silica support facilitates sample disruption while silanol groups on the silica surface may associate with polar components in the sample matrix [23]. However, a recent application has demonstrated that clean-up from kidney tissue can be achieved with a cross-linked acrylic polymer [41]. In this case, the acrylic polymer XAD-7 HP was able to retain lipid components, such as fatty acids, sterols, and triglycerides, in addition to protein matter in the presence of an ethanol-modified water eluent at 100°C. Figure 25.2 demonstrates how the kidney sample clean-up was achieved with the XAD-7 HP resin.

A slightly different *in situ* sample clean-up technique has been employed for the selective extraction of polychlorinated biphenyls (PCBs) from lard, fish, fish meal, and cod-liver oil [42,43]. In these cases, a fat retainer was placed in sequence inside an extraction thimble, rather than being dispersed through the sample. The packing of the extraction thimble shown in Fig. 25.3 demonstrates how matrix interferences are initially co-extracted from the sample, but are then trapped by the fat-retainer inside the thimble. Several fat retainers have been investigated, including sulfuric acid, Florisil, and basic, neutral, and acidic alumina, for static extractions performed at 100°C, followed by elution with *n*-hexane. Figure 25.4 demonstrates that the magnitude of fat retention is similar for





Left: Fig. 25.2. Extract from kidney samples pretreated with or without the acrylic polymer XAD-7 HP prior to pressurized liquid extraction with 30% ethanol in water at 100°C and 50 atm. Samples: 0.5 g beef kidney + 2 g diatomaceous earth.

Right: Fig. 25.3. Packing of an extraction thimble with fat retainer. Reproduced with permission from Ref. [42].

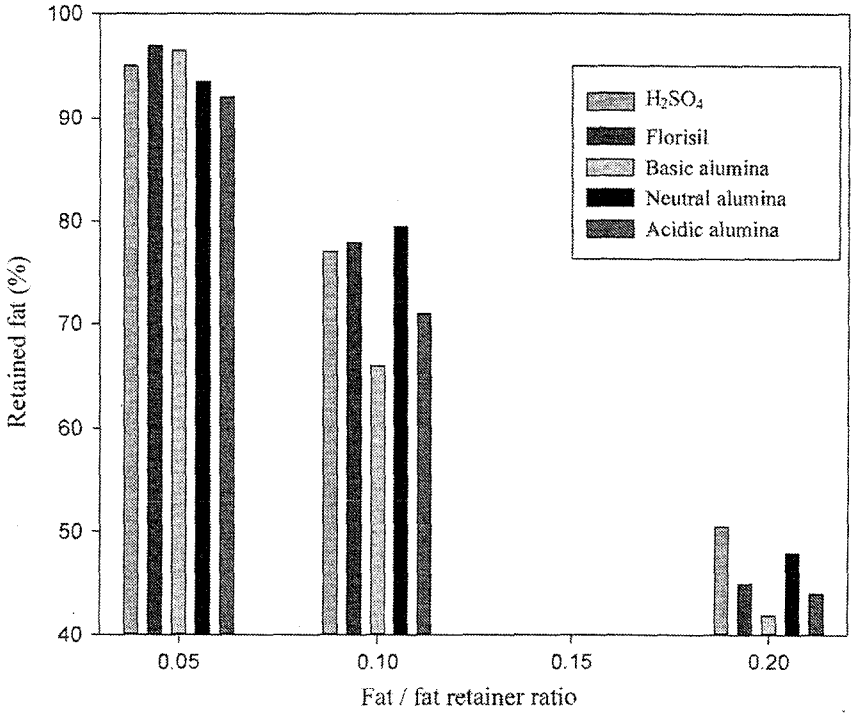


Fig. 25.4. Amount of fat retained for five fat retainers using different fat/fat retainer ratios. Reproduced with permission from Ref. [42].

each retainer, but is dependent on the fat/fat retainer ratio. Fat retainers have been utilized in a similar mode when conducting supercritical fluid extraction (SFE) with CO_2 [44–48].

25.3 LIQUID FOOD SAMPLES AND EXTRACTS

25.3.1 Choice of extraction methods

The analyses of liquid food samples have an advantage over those associated with solid samples in that they usually require one less pretreatment step, due to their liquid form. In some cases, very little sample preparation may be required if the liquid is sufficiently free of matrix interferences. Straightforward techniques that may used to prepare "clean" liquid samples prior to the analysis step include sample dilution, evaporation, distillation, microdialysis, lyophilization, or liquid–liquid extraction (LLE) [49]. Sample drying by lyophilization was discussed in the previous section, and is particularly useful for the analysis of nonvolatile organics. The technique of microdialysis is further discussed in Section 25.3.4.

The technique of LLE is included in this list of "conventional" or straightforward methods since it is well-described in standard texts and references, and has also been described in some recent reviews [50,51]. Further information on LLE methods is also found in Chapter 11. The LLE technique is frequently utilized in the analysis of toxicants, but can also be applied to food components, for example in the extraction of low relative molecular mass compounds from food samples, such as milk, soft drinks, wine, or beer. The extraction procedure generally results in the separation of hydrophilic and lipophilic compounds, such as fat and proteins, following a protein denaturation step with an acid or organic solvent, or following solvent extraction under gentler conditions [16].

Other major techniques for the isolation or purification of liquid food samples are solid-phase extraction (SPE), including immunoaffinity extraction (IAE) and molecularly-imprinted polymers (MIPs); microextraction techniques, including solid-phase microextraction (SPME) and spin bar sorptive extraction (SBSE); and membrane extraction techniques, including dialysis. These methods are characterized by a reduced use of organic solvents, and the associated toxic effects to the laboratory worker and the environment.

25.3.2 Solid-phase extraction

The clean-up and concentration of target analytes in liquid samples or solvent extracts is frequently achieved through sorption onto a solid-phase extraction material that is loaded in a separate cartridge or disk, or placed in-line downstream from the extraction vessel. Table 25.3 presents an overview of select SPE applications for liquid food samples, in addition to examples of the SPE clean-up of solvent extracts from solid foods. Most of the examples cited refer to the

TABLE 25.3

Solid-phase extraction and clean-up of liquid foods and solvent extracts

Sample	Analyte	Solid-phase	Ref.
SPE clean-up of solvent extracts			
Fruits and vegetables	Pesticides	C_{18}, CN	54
Apples, pears	Benzoylurea insecticides	Silica	55
Meat extract	Heterocyclic amines	C_{18}	56
Liver, kidney, muscle	Penicillin antibiotics	Ion-exchange	57
SPE of liquid food			
Red wine	Pigments	Silica, C_{18}, CN, alumina, NH_2, Florisil, carbon black	58
Wine	Various pesticides	C_{18}, PS-DVB, Oasis cartridge	59
Alcoholic beverages	Synthetic colours	NH_2	60
Orange juice	Carotenoids	C_{18}	61
Soft drinks	Caffeine	C_{18}	62
Fruit juices	Phenolic acids	C_{18}	63
Butter (liquid)	Flavour compounds	C_{18}, C_8, NH_2, CN, PS-DVB	64
Immunoaffinity SPE and clean-up			
Orange juice	*s*-triazine pesticides	Immunoaffinity	65
Fruit and vegetables	Phenylurea herbidices	Immunoaffinity	66
Fruit and vegetables	Triazine herbicides	Immunoaffinity	67
Fish	Microcystins	Immunoaffinity	68
Grains	Mycotoxin	Immunoaffinity	69
Herbs	Nitrated polycyclic aromatic hydrocarbon	Immunoaffinity	70
Peanut butter, paprika, pistachios	Aflatoxins	Immunoaffinity	71
MIP SPE and clean-up			
Liver	Clenbuterol	MIP	72
Chewing Gum	Nicotine and analogs	MIP	73
Liver	Triazine pesticides	MIP	74

PS-DVB = Polystyrenedivinyl-benzene.

analysis of xenobiotics or trace components. However, SPE is also amenable to the analysis of lipid classes and related compounds, as described in a recent review [52]. Automated SPE is easily achieved with a dedicated SPE workstation, for example in the determination of resveratrol derivatives in wine [53].

Normal- and reversed-phase chromatographic materials continue to find widespread use in the food industry. However, the use of analyte-specific materials, such immunoaffinity-based solid-phase extraction and molecularly imprinted polymers, is becoming increasingly advantageous. In the case of IAE, the appropriate antibodies are developed against the compound of interest. This technique may also be utilized on-line, for example in the determination of

s-triazines in orange juice, where the cartridge containing the immobilized antibodies is coupled on-line to a gas chromatograph via a reversed-phase cartridge [65].

Table 25.3 also cites examples in which molecularly imprinted polymers were used as solid-phase sorbents for the enrichment of analytes from liquid foods and solvent extracts. MIPs are highly stable polymers that possess recognition sites within the polymer matrix that are specific for the three-dimensional shape and functionalities of the analyte of interest [75]. For example, an MIP material was utilized as part of a two-tier, on-line sample clean-up method performed concurrently with sample extraction for the determination of clenbuterol in liver. The liver samples were first blended with C_{18} in a MSPD clean-up procedure, then a molecularly imprinted solid-phase extraction cartridge was placed in-line after the MSPD cartridge to selectively trap the analyte during elution with acetonitrile [72].

SPE methods may also use ion-exchange materials. For example, anion exchange membranes have been utilized for the determination of glucosinolates in canola and mustard seeds. The analytes in this case were isolated by immersing the membranes in an aqueous suspension of the ground seeds. The membrane was then removed from the suspension, washed, and submerged in an appropriate solvent for elution inside of a shaken vial [76,77].

25.3.3 Microextraction techniques

Two equilibrium-based microextraction techniques serve as alternatives to classical solid-phase extraction: solid-phase microextraction (SPME) and stir-bar sorptive extraction (SBSE). The advantages of utilizing SPME have been well-discussed in previous sections. Table 25.4 lists a few of the many liquid food applications that have been developed utilizing SPME fibers, in addition to the SPME sampling of solvent extracts from solid foods (headspace sampling of solid foods will be discussed in Section 25.4). Each of the examples cited in Table 25.4 utilize a "classical" sampling method consistent with SPME, that is, either by immersing the fiber directly in the sample, by sampling the headspace, or by sampling the effluent from a gas stream (the latter two are classified together as "headspace" in Table 25.4). Alternative SPME sampling methods have been investigated, for example in the determination of catechins and caffeine in tea by utilizing automated in-tube solid-phase microextraction [78].

Stir bar sorptive extraction is a similar equilibrium technique that requires submersion of a stir bar (that is encapsulated in a glass jacket and coated with a solid-phase) into the liquid sample. In this case, the solid-phase is usually a relatively high amount (25–125 μl) of polydimethylsiloxane (PDMS) polymer. The stir bar is then thermally desorbed on-line in the heated injector of a gas chromatograph. The advantage to utilizing SBSE for sampling liquid samples or extracts that are amenable to the PDMS solid-phase technique is that a 500-fold increase in enrichment, and therefore sensitivity, can be achieved compared

TABLE 25.4

SPME sampling of liquid foods and solvent extracts

Sample	Analyte	Fiber	Ref.
Liquid food, headspace			
Beer	Alcohols and esters	PA	79
Wine	Flavours	PDMS	80
Milk	Fatty acids	PA	81
Alcoholic beverages	Flavours	PA	82
Vanilla extracts	Volatiles	PA	83
Orange juice	Flavours	PDMS	84
Vegetable oils	Volatiles	DVB/Carboxene/PDMS	85
Wine	Sulphides	Carboxen/PDMS	86
Wine	Diacetyl	PDMS, CW-DVB	87
Liquid food and extracts, immersion			
Fruit juices	Organophosphates	PDMS, CW-DVB, PDMS-DVB, PA	88
Honey	Pesticides	PA, PDMS	89
Beverages	Caffeine	Silica	90
Strawberries	Pesticides	PDMS-DVB	91
Cheese	Mycotoxin	CW-DVB	92
Kidney	Triazine pesticide	CW-DVB	41

PA = polyacrylate; PDMS = polydimethylsiloxane; CW-DVB = Carbowax divinylbenzene.

with a 100 μm PDMS SPME fiber [93]. However, such a SBSE technique does not have the same selectivity as SPME.

Although the SBSE technique has only been recently developed, it has already seen modest use in the food industry. Stir bar sorptive extraction has been applied to the determination of dicarboximide fungicides in wine [94], organochlorine pesticides and chlorobenzenes in fruit and vegetables [95,96], benzoic acid in lemon-flavoured beverages [97], and flavour compounds in strawberries [98].

25.3.4 Membrane techniques

Membrane extraction methodologies encompass both the non-porous techniques of supported liquid membrane extraction (SLM), microporous membrane liquid–liquid extraction (MMLLE), polymeric membrane extraction (PME), and membrane-extraction with a sorbent interface (MESI), in addition to the porous membrane technique of dialysis [99,100]. Variations of the latter are microdialysis and electrodialysis. Unlike the non-porous membrane methodologies, the porosity-based techniques are not characterized by analyte enrichment. There is no discrimination between small-sized molecules that are similar in size

to the analyte, and only partial sample clean-up is achieved by membrane separation of lower molecular weight species from higher molecular weight matrix components. A dialysis clean-up step is therefore often combined with a subsequent enrichment technique, for example on an automated trace enrichment of dialysates system, also known as ASTED.

Dialysis techniques are strictly not extraction techniques, unlike the nonporous membrane extraction methods. However, they will be discussed here nonetheless, since they are highly effective for the clean-up of liquid foods and solvent extracts. Several on-line microdialysis methodologies have been developed for this purpose. For example, on-line microdialysis clean-up has been coupled with liquid chromatography and programmable fluorescence detection for the analysis of chicken liver fortified with fluoroquinolone antibacterials [101]. Fluoroquinolones in eggs [102] and chicken liver and muscle [103], in addition to sarafloxacin residues in fortified and incurred eggs [104], can be determined in a similar manner.

On-line microdialysis has also been utilized to improve the sensitivity of a disposable lactate biosensor used in the flow-injection mode for the analysis of L-lactate in milk and yoghurt [105]. The measurement of lactulose in milk did not require pre-treatment when a microdialysis probe was used as the sampling system [106]. Glucose determination in milk and juice samples can be achieved with little or no sample pretreatment using a microsystem that integrates a microdialysis probe with a glucose oxidase bioreactor [107].

During a nonporous membrane extraction technique, a liquid or solid (e.g. polymeric) phase is placed between two other phases, which are usually liquid but sometimes gaseous [100]. The sample to be processed may be viewed as part of the donor phase, while an acceptor phase on the other side of the membrane collects the analyte for transfer to the analytical instrument. In this fashion, unparalleled sample clean-up and analyte enrichment can be achieved when compared with classical liquid-liquid extractions.

Nonporous membrane techniques have tremendous potential for the food industry, although they as yet have seen limited use in this field. For example, Vitamin E has been determined in butter samples after dissolution of the butter in a micellar medium. Following on-line saponification, the nutrient was enriched across a silicone membrane and taken up in acetonitrile prior to liquid chromatographic analysis using an electrochemical detector [108]. Continuous extraction of Vitamin E isomers from vegetable oils has been achieved in a similar manner [109]. Another membrane separation device has been coupled to a liquid chromatograph for the enrichment of pesticide multiresidues from egg extracts generated via Soxhlet extraction [110].

The supported liquid membrane (SLM) extraction principle can also be extended to solid or semi-solid samples by incorporating a donor channel unit that permits close contact between the sample and the membrane. Using such a configuration, it has been possible to extract and quantify vanillin in food samples (e.g. chocolate) [111] and caffeine in coffee and tea [112].

25.4 EXTRACTION OF SOLID SAMPLES

25.4.1 Headspace solid-phase microextraction

The application of solid-phase microextraction to the analysis of solvent extracts from solid food samples has already been discussed in Section 25.3.3. Several examples of the direct immersion of a SPME fiber into food extracts were provided in Table 25.4. This section provides a short review of a second approach that may be utilized to sample volatile species from solid food samples, that is, by sampling the headspace above the food with a SPME fiber. Sampling solid foods in such a manner allows the solvent extraction step to be omitted from the analytical procedure. Table 25.5 lists several examples of this approach for a variety of foodstuffs.

25.4.2 Microwave-assisted extraction

The traditional method for the determination of compounds in many foodstuffs is Soxhlet extraction, whereby the solid sample is placed in a porous thimble and is continuously extracted in a glass apparatus with a sub-boiling solvent. The thimble in this case also serves as a filtration medium. Soxhlet methods are fairly simple, standard, and continue to have widespread use in the food industry, for example in the determination of pesticide residues in eggs [110]. However, these methods can also be inefficient and slow, and they can consume large quantities of organic solvents.

Microwave-assisted extraction (MAE) is one of several techniques that have been developed in response to the increased demand for techniques that have a shortened extraction time and reduced solvent consumption, as discussed in a recent review [123]. One of the primary benefits of MAE is the ability to directly

TABLE 25.5

Headspace SPME sampling of solid and semi-solid foodstuffs

Sample	Analyte	Fiber	Ref.
Mustard paste	Flavour compounds	PDMS-DVB	113
Cucumber	Flavour compounds	PDMS	114
Butter	Reduced sulphur compounds	PA	115
Apple	Flavour compounds	PDMS	116
Onion	Volatiles	PDMS	117
Cheese	Volatiles	PDMS, PA	118
Smoked ham	Nitrosoamines	PA	119
Catfish	Off-flavours	PDMS	120
Tomato and strawberry	Flavour compounds	PDMS, PDMS-DVB, CW-DVB	121
Processed poultry	Volatiles produced by bacteria	PDMS	122

heat the sample with the application of microwaves. This type of heating is fast and temperature gradients are kept to a minimum. A drawback to the technique is the requirement for an extraction solvent that is able to absorb microwaves. In addition, a subsequent clean-up step is usually required once the microwave vessel has cooled sufficiently for handling.

Microwave techniques have been applied to biological and food samples quite extensively. The first use of the microwave domestic oven in the laboratory was for the determination of trace metals in biological samples [124]. This was followed by the extraction of crude fat and nutrients from food [125], and such solutes as pyrimidine-glucoside from seeds and fava beans [126]. A patented variation of MAE is the microwave-assisted process [127], or MAP, which was first applied to the extraction of essential oils from plant products [128]. MAP methods mainly concern biological applications ranging from analytical to processing scale.

All the applications cited thus far have utilized closed-vessel systems. Recently, closed-vessel MAE has been used for a number of marine tissue applications. For example, organic contaminants such as polychlorinated biphenyl (PCB) congeners, chlorinated pesticides, and polycyclic aromatic hydrocarbons (PAHs) have been extracted from standard reference materials [129]. In this case, it was determined that the moisture content in the samples greatly influenced analyte recovery, and it was necessary to standardize the moisture content in all samples in a batch prior to extraction. Closed vessel MAE has also been utilized for the determination of PCBs in freeze-dried mussels [130], organochlorine compounds in cod liver and fish fillets [131], xenoestrogens in liver and muscle tissue from rainbow trout [132], and ionic arsenic species in oyster tissue [133], as well as sulphamethazine in swine tissue [134] and fat in chocolate [135].

Several authors have investigated novel microwave extraction applications. For example, a simultaneous extraction-derivatization procedure was developed for the analysis of methylmercury in biological samples [136]. In addition, the determination of the release of dimethyl sulfide from cereals and canola was facilitate by MAE, producing a gaseous sample in the headspace above the microwave-extracted food sample which could be injected to a gas chromatograph [137].

A microwave extraction procedure may also make use of an open vessel, in what is called focused open-vessel microwave-assisted extraction (FOV-MAE). In a closed vessel procedure, an additional sample treatment step may be required to remove co-extracted water from a wet sample. However, in an open vessel procedure, the water may be removed from the sample via azeotropic distillation. This was shown to be the case during the determination of organochlorine compounds in cod liver and fish fillets [131]. Open vessel procedures have also been applied to the determination of polychlorinated biphenyls and chlorinated pesticides in standard reference materials of cod liver oil and freeze-dried mussel tissue [138], and mercury in fish [139].

Most of the MAE applications have concerned the determination of exogenous species. Microwave extraction procedures are also useful for the characterization of food components. The MAE technique has been applied to the determination of the fatty acid profile of mackerel and cod [140], trace element analysis in plant materials [141], and the extraction of free amino acids from foods [142]. During the extraction of lipids from milk samples, it was determined that triglycerides are more stable using an MAE procedure compared with the conventional Weibull–Berntrop extraction procedure, since there is less chemical transformation of the triglycerides via hydrolysis [143].

25.4.3 Pressurized liquid extraction

A second technique that has been employed for the rapid extraction of food samples at elevated temperatures is pressurized liquid extraction (PLE). PLE methods frequently utilize the Accelerated Solvent Extraction (ASE) system developed by Dionex, or any other system that performs static or dynamic solvent extractions at elevated temperatures and pressures. The advantage to performing extractions under pressurized conditions is that the upper extraction temperature is not limited by the boiling point of the solvent, as is the case with the traditional Soxhlet system. A flow-through system such as the ASE is also particularly beneficial in food analysis. Static extractions are performed inside steel extraction vessels that have ample capacity for food samples, from 11–100 ml. The static extraction period is followed by elution of the extraction solvent into a collection vial. PLE extracts are usually cleaner than microwave extracts, since matrix components that do not dissolve in the extraction solvent may be retained inside of the vessel. In addition, *in situ* clean-up methods can be employed during PLE methods. While most food samples are blended with only diatomaceous earth or sand prior to the PLE extraction step, further *in situ* clean-up can be achieved by utilizing the matrix solid-phase dispersion technique (MSPD) [24–41] or by placing fat retainers in series inside of the vessel [42,43]. Further details on these applications have been discussed in Section 25.2.4.

PLE methods have been utilized for both the extraction of food components and the isolation of food contaminants. With regards to food composition, PLE has been particularly useful in the determination of fats and lipids in food samples such as meats [144] and egg-containing food [145]. The PLE technique has also been applied to the determination of the fatty acid composition in cereal, egg yolk, and chicken breast muscle [146]. PLE techniques have been employed for the extraction and speciation of arsenic in freeze-dried carrots [147] and to assess the levels of vitamin K-1 in medical foods [30].

Pressurized liquid extraction has also seen widespread use in the isolation of contaminants from foodstuffs. There has been particular interest in applying the technology to the analysis of lipid-containing foods. For example, organochlorine compounds have been isolated from cod liver and fish fillets [131], and

various pesticides have been removed from baby foods [148]. PLE has also been utilized for the determination of polycyclic aromatic hydrocarbons in smoked fish and pork [149], and polychlorinated biphenyls in cod-liver oil and milk powder [150], as well as fish tissue [151]. Corticosteroid residues in bovine liver have been quantified using a two-step extraction method. Fat components were first removed from the sample with hexane in a defatting step. This was followed by the actual extraction of the analytes with a 1:1 hexane-ethyl acetate mixture [152].

Pressurized liquid extraction has also been useful for the rapid analysis of toxicants in fruits and vegetables. The technique has been applied to the determination of fungicides in oranges and bananas [153], organophosphorous pesticides in apples and carrots [154], and various pesticide residues in fresh pear, cantaloupe, white potato, and cabbage [155].

A discussion on the utilization of hot, aqueous extraction solvents during the analysis of food samples is included in this section. Hot, pressurized water, also called subcritical water, is a novel extraction solvent that has been discussed at length in an earlier chapter. The benefit of utilizing subcritical water for analytical extractions is that the solvent strength can be tuned by varying the extraction temperature and/or through the addition of a cosolvent. Water as a solvent is easily obtained and disposed of, being benign to the laboratory worker and the environment. Aqueous extractions of food samples are also convenient, since the sample matrix does not need to be dried prior to the extraction step.

The application of this relatively new technology to the analysis of foods has been limited thus far. The technique has been largely restricted to the isolation of food components or contaminants from foods of plant origin. This is likely due to the fact that hot water extracts from animal tissues can be quite turbid and highly coloured [41]. Applications to foods of plant origin have included the selective extraction of oxygenates from savory and peppermint [156], essential oils from oregano [157], fennel [158], and marjoram [159], fungicides from various vegetables [160], and organochlorine compounds from strawberries [95,96], kohlrabi, lettuce, and tomatoes [96].

In our laboratory, the problem of coextractives from foods of animal origin was overcome by incorporating an *in situ* MSPD clean-up technique into the extraction procedure. In this case, the pesticide atrazine was isolated from beef kidney that was dispersed with an acrylic polymer [41]. It was necessary to modify the water with another benign solvent (ethanol at 30% v/v) in order to attain the solvent strength necessary to achieve complete recovery of the target analyte from the dispersed matrix.

25.4.4 Supercritical fluid extraction

The use of supercritical fluids in food analysis has grown tremendously in the past decade. Two applications that have had significant development in recent years have been the determination of fat and associated nutrients, as well as the

isolation of pesticides from food matrices. Supercritical fluid extraction (SFE) technology has also been applied to the determination of PAHs and PCBs, drugs, and other food toxicants. Many of these applications are summarized in Table 25.6, all of which utilize supercritical carbon dioxide (SC CO_2). SC CO_2 continues to be the fluid of choice, since its critical parameters (31.1°C, 72.8 bar) are easily achieved with high pressure instrumentation. Further, it is non-toxic and easy to obtain. Some of the SF-based methodologies utilize suitable modifiers to enhance analyte recovery. Protocols have been developed that encompass sample clean-up, derivatization, and automation in the methodology. The clean-up of lipids has been of critical importance during the extraction of non-polar compounds such as pesticides, PAHs and PCBs, due to the propensity of these toxicants to accumulate in the lipid phase.

It is probably fair to say that analytical SFE will be a method of choice for the fat determination of foods in the future. Toward that end, several collaborative and peer-verified methods have been published utilizing SFE for the determination of lipid levels in oilseeds, meats, and food products.

There has been limited use of alternative fluids for the analysis of food samples. For example, three fluids were examined for the removal of ethoxyquin from lean beef and beef fat: carbon dioxide, trifluoromethane, and 1,1,1,2-tetrafluoroethane. While CO_2 appeared to react with the analyte during the extraction, methanol-modified hydrofluorocarbons provided more complete extractions than the pure fluids. Quantitative extraction was achieved at the 0.5 ppm level [197]. Binary mixtures of CO_2 and nitrogen have also been utilized for the selective extraction of organochlorine and organophosphate pesticides from poultry. The binary mixtures provided quantitative recoveries of the analytes while significantly reducing lipid solubility and coextraction [198].

25.5 FINAL REMARKS

In this concise chapter, we have attempted to provide an overview of modern methods that can be used in preparing samples for food analysis. Food analysis is a complex area that has led to a plethora of approaches due to the complexity of the sample matrices. An emphasis has been placed here on recently developed techniques and methods that are rapid and minimize the generation of chemical waste (e.g. organic solvents). The authors hope that the near 200 references that have been cited may aid the analyst in further selecting the sample preparation method that is most appropriate for solving the problem being investigated.

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TABLE 25.6
Supercritical fluid extraction of food samples

Sample	Analyte	Ref.
Fat and Nutrients		
Ground beef	Total fat	161–163
Ground cumin	Volatile oils	164
Egg-containing foods	Cholesterol	165
Chocolate	Triacylglycerols	135
Infant formula	Fat	166
Milk products	Total fat	167
Milk products	Total fat	168
Deep-fried chicken and potato	Lipids	169
Oilseeds	Total fat	162,163
Bakery samples	Total fat	163
Milk powder, infant formula	Fat-soluble vitamins	170,171
Tomato skin	Lycopene	172
Raisins	5-Hydroxymethyl-2-furaldehyde	173
Pesticides		
Butter fat, corn oil	Organochlorine and organophosphate	174
Wheat, maize	Organophosphate	175
Eggs	Triazine	176
Eggs	Organochlorine	177
Sugar cane, oranges	Diuron	178
Garlic	Organochlorine	179
Cereals	Various	180
Beef and chicken tissue	Carbamate	181
Apples	Fenpyroximate	182
Apples	Various	183
Chicken fat, ground beef, lard	Organochlorine and organophosphate	184
Grains	Organochlorine and organophosphate	185
Poultry tissue	Organophosphate	186
Drugs		
Bovine liver	β-agonists	187
Poultry eggs and muscle	Nicarbazin	188
Animal tissues	Steroids	189
Eggs	Sulfamethazine	190
Pig fat	Androstenone	191
PAHs and PCBs		
Smoked meat	PAHs	18
Toasted bread	PAHs	19
Liver	PAHs	20
Fish	PCBs	21
Fat	PCBs	22
Miscellaneous		
Irradiated foods	Hydrocarbons and 2-alkylcyclobutanones	192,193
Cured meats	Nitrosamines	194
Beef Liver	Aflatoxin M_1	195
Corn	Aftatoxin B_1	196

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