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Review

Recent applications of sample preparation techniques in food analysis

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Abstract

Even with the emergence of advanced techniques of separation and identification, it is rarely possible to analyse food without manipulation. The traditional techniques for sample preparation are time consuming and require large amount of reagents, which are expensive, generate considerable waste, contaminate the sample and can enrich it for analytes. The more analytical techniques have become highly developed, the more has sample clean-up become important in order to fully take advantage of them. Due to the multiplicity of food matrices, it is not possible to use one sample preparation technique, so many methods have been proposed for meeting all the requirements. The newest variations of wet digestion, solvent and sorbent extraction and membrane separation are summarised and their most recent applications to food analysis are provided.

Keywords: Reviews; Food analysis; Sample preparation; Solvent extraction; Membrane separation; Sorbent extraction; UV photolysis

Contents

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1. Introduction

The science of food analysis is rapidly developing; the number of related articles and papers is increasing daily. Almost without exception, food is a complex non-homogeneous mixture of a staggering range of chemical substances that makes it hard to isolate and determine analytes of interest. Even with the emergence of advanced techniques of separation and identification, it is rarely possible to analyse food without manipulation. After sampling, it is necessary to prepare the sample for the determination of analytes through its dissolution, trace enrichment and interferents' removal. For the preparation of an appropriate number of samples, the methods have to be selected not only on the basis of the expected concentration of the analytes in the solution that results from the decomposition process, but also according to general requirements such as multi-element analysis and dynamic range of the determination, number and mass of samples, laboratory equipment and the experience of the analytical staff.

These procedures often take up most of the total analysis time, contributing highly to the total cost of analysis, and affect error generation through incomplete dissolution of the matrix or volatilisation of the analytes and contamination from reagents or vessels. The more sensitive and accurate analytical techniques have become, the more attention has been drawn to sample clean-up. The detection limit of advanced analytical techniques is, sometimes, practically established by the attainable blank value which is dependent on the sample preparation stages.

In food analysis, the traditional techniques for sample preparation are time consuming and require large amount of reagents, which are expensive, generate considerable waste and contaminate the sample.

The growing concern over food safety necessitates more rapid and automated procedures to take into account the constant increase in the number of samples to be tested, so interest in procedures that are fast, solventless, inexpensive and amenable to automation for on-line treatment is ongoing [1].

This review describes some of the most interesting sample preparation procedures. Coverage is not intended to be comprehensive but rather to outline those developments which are of particular relevance to food analysis, especially when using chromatographic techniques. Readers seeking more detailed discussion are referred to reference texts [2,3].

2. Sample digestion techniques

Wet digestion (WD) in closed systems, with the addition of reagents to solubilize and/or oxide organic samples, has been known about since 1834 when Henry [4] and Zeise [5] determined sulphur in organic matrices. So far, it is one of the most experienced techniques to disaggregate food and obtain free ionic species [6]. Generally speaking, closed decomposition systems have been found better than open ones, reducing systematic errors and leading to correct analytical results with a higher probability. With the passing of years, the detection limits have been improved by reducing the analytical blank (with the use of pure reagents and advanced vessel materials), by improving the signal-to-noise ratio (with more efficient sample oxidation) and by diluting the decomposition residue as little as possible. This technique is strictly food dependent: carbohydrates are easily mineralised with nitric acid at 180 °C [7], while fats, proteins and amino acids cause incomplete digestion, due to the relatively low oxidation potential of nitric acid at 200 °C, and require the addition of perchloric acid with all the problems related to its use at high temperature and pressure [8].

2.1. Sample contamination problems

A major error source is frequently related to the material used for the vessels involved in the de-

Table 1
Average content (in μg/kg) of current elements in the materials most commonly used for decomposition and storage vessels

Element	PTFE[a]	Ultra-pure quartz	Quartz	Pyrex glass
Al		100	30 000	Main constituent
As		0.1	50	500–20 000
B		10	100	Main constituent
Ca		100	500–3000	1 000 000
Cd		0.1	10	1000
Co	1	1	1	100
Cr	20	2	5	3000
Cu	10	10	50	1000
Fe	10	100	1000	100 000
Hg	10[b]	1	1	10–100
Mg		10	10	500 000
Mn		10	10	5000
Na	25 000	10	1000	Main constituent
Ni				2000
Sb	0.5	1	2	10 000
Si		Main constituent	Main constituent	Main constituent
Ti		100	1000	3000
Zn		50	50	3000

[a] Isostatic moulded polytetrafluoroethylene.
[b] Strongly dependent on cleaning conditions.

composition process and in the solutions' storage, even if the ratio of the sample weight to the vessel surface is as reduced as possible. Table 1 shows the average content of current elements in the materials most commonly used for decomposition and storage vessels.

Isostatic moulded PTFE and ultra-pure quartz appear best suited for trace analysis, but they must be scrupulously cleaned before use by steaming processes normally performed in commercially available equipment [9]. After this procedure the surface is perfectly cleaned and the absorption of species is opposed during the subsequent use.

2.2. Microwave digestion

A true quality jump in sample digestion was the combination of pressurized dissolution with microwave-enhanced chemistry [10]. Many fundamental mechanistic differences separate microwave heating from the other heating methods. Solutions are heated so efficiently that reaction timescales are dramatically reduced and process control offered by microwave heating is better than any other heating method. As a result, microwave oven digestion (MOD) is highly amenable to standardization and automation. In the last 10 years the developments in microwave unit technology and in vessels for closed systems went through several stages of evolution; now materials typically used for microwave vessels are fluoropolymers, combined with advanced composite materials, and quartz, which are chemically inert to a wide range of reagents, so that equipment can handle acid decomposition at temperatures up to 320 °C and pressures of 13–20 MPa [11].

Microwave energy absorption in a solution is caused by molecular dipole rotation and ionic conductance. In the first mechanism, dipole align with the applied electric field that oscillates at high frequency (usually 2.45 GHz), forcing the dipole molecules to move so that the resulting friction heats the solution. In the ionic conduction mechanism, ions migrate as a function of the polarity of the electromagnetic field and the resulting resistance to their flow heats the solution. These two mechanisms heat solutions much faster than conduction and convection, while vessels (made by microwave transparent and chemically resistant materials) remain at lower temperature. Unique temperature and pressure relationships are involved in closed-vessel microwave oven digestion. The gas pressure inside a microwave closed-vessel is not determined by the liquid-phase

temperature, but depends on the gas-phase temperature, the vessel volume and its transparency to microwaves. When heating nitric acid (the most common reagent used for food digestion) it becomes a mixture of nitric acid, nitrogen oxides and water vapour; the gas-phase pressure during heating is lower than expected because of the lower temperature of the liner and of the outer casing of the vessel. The colder vessel walls are, the more efficient they are in removing molecules from the vapour phase. The condensation process results in lower internal pressure at high temperature and in reduced ionic conductance heating (reduced microwave heating efficiency) in the gas phase, so the pressure increase is minimal. The condensation rate varies as a function of several parameters, such as the vessel material and shape, the dissipation of energy in the system, the type and quantity of reagents and sample used, so it follows that pressure control is not suitable for standardizing microwave oven digestion. However, the digestion conditions are accurately controlled by monitoring the liquid-phase temperature ($\pm 2\,^{\circ}C$) whose ramp is settled as needed by food dissolution.

Microwave energy is also useful in post-digestion sample processing when a concentration step is required. Microwave-assisted heating in open vessels allows lower temperatures and the sample is not overheated, because the decrease of dipole molecules via evaporation is associated with the decrease in heating. Complete retention of volatile analytes after tissue digestion has been recently shown [12].

When solubilizing food, a factor worthy of particular care is the mass of sample to be digested. Generally, the sample mass can be calculated from its carbon content. For optimum digestion, the ratio of the sample carbon content to the liner volume should not exceed 1.5 mg C ml^{-1} in order to have a slight excess of nitrogen oxides. Without the slight excess of nitrogen oxides, the extent of the digestion, measured by the conversion of carbon to carbon dioxide, would be adversely affected.

The carbon content of some freeze-dried food is reported in Table 2; when dealing with fresh samples, it is important to take into consideration their proper water content and increase the limit accordingly.

Data reported in Table 2 evidence that if, for instance, the vessel liner volume is 35 ml, 200 mg of meat (40–50% C), 200 mg of vegetables (40–50% C), 300 mg of carbohydrates (35–40% C) or 100 mg only of fats (75–80% C) are allowable for microwave oven digestion. Typical conditions for the microwave oven digestion of fats and meat are shown in Table 3.

Table 4 summarizes some recent application of wet digestion to food analysis. The suitability of this technique is evident when considering the wide range of matrices handled and of advanced analytical techniques used.

When taking into account the whole dissolution procedure, microwave assisted digestion is several times faster than conventional digestion because of the rapid heating process that increases the temperature of the sample/acid solution without interacting with the vessel walls. This effect means the materials involved are subject to less stress than in convective heating, so that porous surfaces are less likely to form resulting in less memory effects. It great care

Table 2
The carbon content of some freeze-dried food

Food	Carbon content (%)	Food	Carbon content (%)
Butter, oils (vegetable), fats (vegetable)	70–80	Oyster (edible part)	46
Milk powder (whole)	52	Wheat	45
Fish (fillet)	52	Milk powder (skimmed)	42
Pig (blood)	52	Sugar (saccharose)	42
Liver (cattle)	51	Lactose	42
Beef (lean)	50	Mussels (tissue)	41
Egg (hen)	50	Peach (fruit flesh)	40
Pig (kidney)	49	Spinach	38
Starches	40–50	Glucose (monohydrate)	37

Table 3
Practical conditions for the microwave oven digestion of fat food and meat

Fat food	
Sample size	100 mg
Reagent addition	2 ml 67–70% superpure HNO_3
Digestion conditions	420 W, 2 min
	560 W, 8 min
Cooling phase	2 min
In case of uncompleted dissolution	
Reagent addition	1 ml 30% m/m H_2O_2
Digestion conditions	560 W, 3 min
Cooling phase	2 min
Meat	
Sample size	200 mg
Reagent addition	2 ml 67–70% superpure HNO_3
Digestion conditions	560 W, 8 min
Cooling phase	2 min

must be taken with the contribution of reagents over blanks (and their variation) and the presence of thermo-resistant food components, that may influence the analytical performance [48]; when available, the permanent use of certified reference materials is highly recommended.

2.3. UV photolysis digestion (UVPD)

When organic compounds interfere in food analysis, oxidative UV photolysis has distinct advantages over traditional dissolution techniques, owing to its very low blank values.

The digestion is not based on direct interaction between the UV radiation and the organic matrix, but rather on a radical mechanism [49]. $HO^{\cdot}$ radicals are photolytically generated and react with organic compounds, degrading them. The greater the number of $HO^{\cdot}$ formed per unit time, the faster UV photolysis progresses. The presence of H_2O is sufficient for producing $HO^{\cdot}$, however in the case of food, where the organic content of the matrix is very high, the introduction of small quantities of H_2O_2 will accelerate radical formation, resulting in less oxidation time.

Usually high-pressure mercury lamps, with high intensity as well as large radiant flux, are used and a broad band spectrum from about 200- to 435-nm wavelength is obtained. This spectrum results from the asymmetric dispersion of the typical mercury lines at 254, 313 and 366 nm in conjunction with powerful radiant energy that is converted to thermal radiation which speeds up the digestion. Digestion is directly proportional to UV intensity, irradiation time and to temperature of the sample, while it is inversely proportional to the organic concentration in the matrix. Heat generation must be controlled because it can lead to loss of the samples through evaporation. UV digesters have cooling systems which permit oxidation to be performed, without any losses, at a sample temperature not above 90–95 °C. Fig. 1 shows a schematic of UV photolysis equipment for sample digestion.

It must be noted that UV radiation is mirrored into the samples, by means of a mirror surface that surrounds the quartz vessels compartment, in order to enhance UV intensity. The lamp-cooling fan and the fluid-cooling coil placed outside the mirroring surface permit temperature control of the sample compartment. Safety locks for avoiding operator interaction with UV radiation are considered.

Oxidative UV photolysis permits the simultaneous digestion of different samples and requires only a small amounts of reagents resulting in very low blank values and no disposal problems. The technique suffers from the disadvantage of relatively long oxidation times. Table 5 shows some recent application to food analysis.

3. Solvent extraction

In 1879 Franz Soxhlet drew the extraction apparatus that was named after him, to separate fats from food. This equipment aroused a great deal of interest because lengthy extractions could be performed unattended. Since then, the extraction of the compounds of interest into a suitable organic solvent is one of the most common methods of separation in food analysis because no filtration is necessary, the extraction temperature is higher than room temperature, the sample is repeatedly brought into contact with fresh solvent, and both polar and non-polar solvents can be used. The disadvantages of this technique are that it requires large amounts of solvent (namely 300–500 ml), the solvent must be evaporated to concentrate analytes before determination and the process is a single sample run that takes several hours or days to complete.

Table 4
Wet digestion in closed systems and microwave assisted dissolution of food

Food	Digestion[a]	Reagents	Analytes	Ref.
Shellfish tissues	WD	HNO_3, HF, $HClO_4$	Al, Ca, Fe, K, Mg, Mn, Na, Zn	[13]
Seafood	MOD	HNO_3	As	[14]
Seafood	MOD	HNO_3, NaF, $Na_2S_2O_8$	As	[15]
Mussels (canned)	MOD	HNO_3	Cu, Fe, Zn	[16]
Liver	MOD	HNO_3	Se	[17]
Mussels	MOD	HNO_3	Cu, Fe, Zn	[18]
Fish	MOD	HNO_3 (V_2O_5 catalized)	As	[19,20]
Mussels	MOD	HNO_3	Fe, Zn	[21]
Vegetables	MOD-WD	HNO_3	Co	[22]
Vegetables	MOD-WD	HNO_3	As, Ba, Be, Cd, Co, Cr, Cu, Ga, Mn, Mo, Ni, Pb, Sb, Se, Sn, V, Zn	[23]
Vegetables, tea	MOD	HNO_3, HF	Ca, Cu, Fe, Mg, Mn, Zn	[24]
Vegetables, corn, sugar	MOD	HNO_3	Hg	[25]
Vegetables	MOD	HNO_3, HF	57 elements	[26]
Vegetables	MOD	H_2SO_4, HNO_3	F	[27]
Vegetables	MOD	HNO_3	Ca, Cu, Fe, K, Mn, Na, Zn	[28]
Vegetables, sugar cane	MOD	HNO_3 (V_2O_5 catalized)	As	[29]
Almonds	MOD	HNO_3, H_2O_2	Ca, Cu, Fe, K, Na, Mg, Mn, P, S, Zn	[30]
Mushrooms	MOD	HNO_3, H_2O_2, $AgNO_3$	Br	[31]
Tomato paste, fruit juice	WD	HNO_3	P	[32]
Fruit juice	MOD	HNO_3	B, Na, Si, Sn	[33]
Vegetables (canned), beverages, milk	WD	H_2SO_4, HNO_3	Cu, Fe, Sn	[34]
Tea	MOD	HNO_3	Ba, Cu, Fe, Pb, Zn	[35]
Wine	MOD	HNO_3	Pb	[36]
Wine	MOD	HNO_3	Pb	[37]
Wine	WD	H_2SO_4, H_2O_2	Ni	[38]
Wine	WD	H_2SO_4, H_2O_2	Cd, Cu, Pb, Zn	[39]
Wine	MOD	HNO_3, H_2O_2	Pt	[40]
Wine, alcoholic beverages	WD	HNO_3, H_2SO_4	Fe	[41]
Dairy products	WD	HNO_3, $HClO_4$, HCl	Cd, Co, Cr, Cu, Fe, Ni, Pb, Zn	[42]
Dairy products	MOD	HNO_3	Cd, Pb	[43]
Flours, bread, canned food	MOD	H_2SO_4, H_2O_2	Al	[44]
Flours, starches, meat, dairy products, eggs	WD	HNO_3	Al, Ca, Cd, Co, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sr, Zn	[45]
Various food matrices	MOD	HNO_3, H_2SO_4, H_2O_2	Ca, Cu, Fe, K, Mg, Mn, Na, Zn	[46]
Vegetable oils, sugar, tea	WD	HNO_3	Cd, Cu, Pb, Zn	[47]

[a] MOD microwave oven digestion, WD wet digestion.

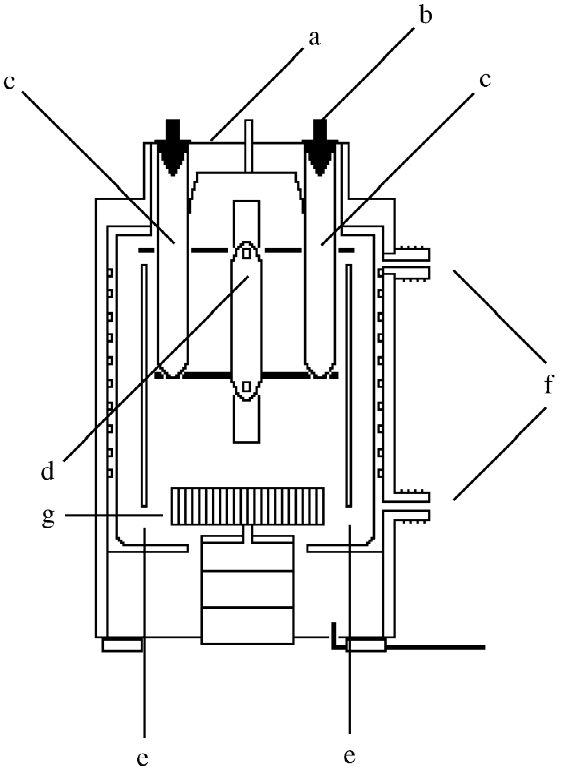

Fig. 1. Schematic of UV photolysis apparatus for sample digestion: (a) UV operator protection, (b) black PTFE stopper, (c) quartz sample vial, (d) high-pressure UV lamp, (e) reflecting shield, (f) coolant circulation and (g) cooling fan.

Recently, pressurised fluid and supercritical fluid extraction, which use extractant at higher temperature (80–200 °C) and pressure (10–20 MPa), have emerged as viable improvements of the traditional soxhlet method.

3.1. Pressurized fluid extraction (PFE)

This technique is like soxhlet extraction, the only difference being the use of solvents that are raised to the near-supercritical region, where they show better extraction properties [59]. At high temperature, the rate of extraction increases because the viscosity and the surface tension of the solvent drop, while its solubility and diffusion rate into the sample increase. Pressure keeps the solvent below its boiling point and forces its penetration into the pores of the sample. The combination of high temperature and pressure results in better extraction efficiency, so minimizing solvent use and expediting the extraction process. The time required for extraction is practically independent of the sample mass and the efficiency of extraction is mainly dependent on temperature. In Fig. 2 a schematic of PFE apparatus is shown.

The sample is loaded in a stainless steel extraction cell into which solvent is pumped and brought to a specified temperature and pressure. The temperature is normally kept between 80 and 200 °C and the pressure ranges between 10 and 20 MPa. These

Table 5
Wet digestion of food by means of UV photolysis

Food	UV (min)	Reagents	Analytes	Ref.
Honey	30–60	H_2O_2, HNO_3	Cd, Co, Cu, Fe, Ni, Pb, Zn	[50]
Olive oil (wastes)	60–120	H_2O_2	Cl^-, NO_2^-, NO_3^-, PO_4^{3-}, SO_4^{2-}	[51]
Wine	30–90	H_2O_2	Cd, Co, Cu, Fe, Ni, Pb, Zn	[52]
Wine	240	H_2O_2, NH_4OH	Cr	[53]
Wine	15	H_2O_2	Ni	[54]
Wine	30	H_2O_2	Pb	[55]
Vegetable oils and fats	30–60	H_2O_2	Cl^-, PO_4^{3-}, SO_4^{2-}	[56]
Vegetables	60–120	H_2O_2	Cd, Co, Cu, Fe, Ni, Pb, Zn, Br^-, Cl^-, PO_4^{3-}	[57]
Vegetables	0.5	$K_2S_2O_8+Na_2B_4O_7$	Dimethylarsinic acid	[58]

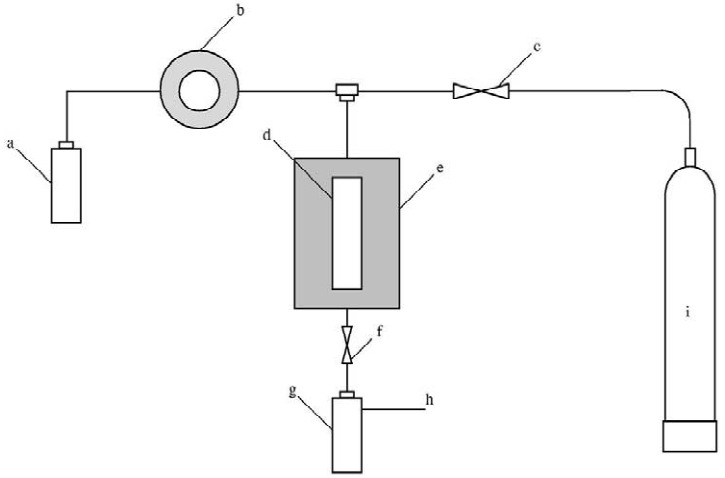

Fig. 2. Schematic of PFE apparatus: (a) solvent supply, (b) pump, (c) purge valve, (d) extraction cell, (e) furnace, (f) static valve, (g) collection vial, (h) vent and (i) inert gas tank.

conditions are kept constant for some minutes in order to permit the static transfer of analytes from the sample into the solvent. The extract is pushed into the collection vial by a second aliquot of solvent inserted into the extraction cell and this second aliquot is then collected into the same vial by pushing it with an inert gas flow. The whole process takes 15–20 min.

When handling moist food, to avoid lump formation, diatomaceous earth (kieselguhr) is used. Calcined diatomite contains ~65–87% SiO_2, 2.3–11.7% Al_2O_3, up to 3% Fe_2O_3 and CaO, MgO, Na_2O, and K_2O traces.

Method set-up is generally straightforward because the conditions are similar to those in soxhlet extraction.

Pressurized fluid extraction, mainly applied to pollution control, has recently been used for food pre-treatment [60–62]; Table 6 shows some applications.

Comparing pressurized fluid to soxhlet extraction, the advantage of reducing solvent consumption and extraction time contrast with the disadvantage of having to use very expensive specialised equipment in order to comply with safety provisions concerning the hazards of superheated solvents and high pressure vessels.

3.2. Supercritical fluid extraction (SFE)

The supercritical fluid extraction of food has occurred for many years on an industrial scale, but it has not been applied to analytical scale sample preparation until recently. Its growing role in separation science is pointed out by up-to-date reviews [79,80].

This technique resembles soxhlet extraction, but the solvent used is a supercritical fluid, namely a substance above its critical temperature and pressure, which provides an unusual combination of properties. Supercritical fluids diffuse through solids like gases, but dissolve analytes like liquids, so that the extraction rate is enhanced and less thermal degradation occurs [81,82]. In addition, many sample pre-treatments can be done with non-polluting, non-toxic supercritical fluids, such as carbon dioxide, which are an excellent alternative to the potentially hazardous and expensive solvents used in soxhlet extraction.

The high rate of penetration of the supercritical fluid in food, even if slightly porous, permits fast back-diffusion of analytes, reducing extraction time. The complete step is performed in less than 20 min instead of several hours as required in traditional liquid-solid extraction. The technique can also be

Table 6
Pressurised fluid extraction of analytes from food matrices

Food	Analytes	Solvent	t (°C)/p (MPa)	Ref.
Vegetables, flour	Pesticides, herbicides	Hexane+10% acetone, acetonitrile	125/10	[63]
Vegetables, fruit juices, flour	Organophosphorus pesticides	Ethyl acetate	100/10.4	[64]
Vegetable, fruit	*n*-Methylcarbamate	Acetonitrile	100/13.8	[65]
Fruits	Polyphenols	Methanol	40/6.9	[66]
Medical food	Vitamin K1			[67]
Baby food	Chlorpyrifos, malathion, 4.4′-DDE, 4,4′-DDT	Acetonitrile	80/13.8	[68]
Smoked meat	Polycyclic aromatic hydrocarbons	Methylene chloride+ 10% acetonitrile	80/10	[69]
Fish	Lipids, polychlorinated biphenyls	Hexane	125/10	[70]
Fish	Arsenobetaine, arsenocholine, dimethylarsenic acid	Methanol+50% H_2O		[71]
Fish	Musk aroma compounds	Hexane-ethyl acetate	80/10	[72]
Meat	Fats	Hexane	125/10	[73]
Cereals, chicken, eggs	Fatty acids	Isopropyl alcohol+ hexane-methanol+ chloroform	120/0.8	[74]
Milk powder	Fats	Hexane+methylene chloride+methanol	80/10.4	[75]
Cod-liver oil, milk powder, feeds	Polychlorinated biphenyls	Hexane	100/10.3	[76]
Dairy products, meat, cereals, vegetable oils and fats	Fats	Petroleum ether	100/13.8	[77]
Malt	Proanthocyanidins	Acetone+20% H_2O	60/100	[78]

applied to thermally unstable analytes when selecting supercritical fluids with low critical temperature.

One of the most interesting properties of these fluids is the direct relationship of solvent strength to density. Since the density of the fluid is a function of its temperature and pressure, precise control of these parameters allows a solvent with a narrow window of solvating strength to be obtained. It is possible, therefore, to substitute a variety of conventional solvents with a single supercritical fluid. For instance, supercritical carbon dioxide at 7.515 MPa and 80 °C (d 0.15 g ml^{-1}) is characterised by a solvating strength similar to gases, such as pentane, while at 38.265 MPa and 40 °C (d 0.95 g ml^{-1}) its solvating strength resembles liquids, such as methylene chloride, carbon tetrachloride, toluene or benzene. When selecting the extraction pressure, it should be kept in mind that as the pressure increases, higher molecular weight compounds become soluble, while as the pressure decreases, the supercritical fluid loses some of its solvent strength. If the pressure is reduced to atmospheric values, the fluid loses practically all of its solvating ability and the extracted compounds fall out of the solution.

Water is a poor choice for this technique because of its high critical temperature and pressure. The most widely used supercritical fluid is carbon dioxide which is characterized by low critical values and low chemical reactivity. Carbon dioxide is easily obtained in extremely pure form at a reasonable cost, it

is environmental friendly and it can be separated from the collected analytes without trouble or disposal problems.

Nitrous oxide would be a good supercritical fluid, but it is very flammable. Ammonia is a polar substance with good solvent strength, but it is chemically reactive and corrosive. The hydrocarbons are usually flammable and are not viable for analytical SFE.

A common practice in supercritical fluid extraction, which must be mentioned in connection with the physicochemical properties of supercritical fluids, is the use of modifiers (co-solvents). These are compounds that are added to the primary fluid to enhance extraction efficiency. For example the addition of some percent (1–10%) of methanol to carbon dioxide expands its extraction range to include more-polar analytes.

Supercritical fluid extraction utilises fairly simple equipment, as outlined in Fig. 3.

The sample is introduced in an inert extraction cell in which fluid is pumped at a pressure above its critical point. The temperature of the cell is increased to overcome the critical value of the fluid. The quantity of food required is ~1–3 g per cell. In the case of solid food it is necessary to homogenise it, while liquid food must be absorbed onto a porous and inert substrate because of the problem of handling two phases under pressure. The water content of the sample must be strictly controlled because water, as a very strong co-solvent, alters the supercritical fluid extraction strength and, in addition, it freezes as the fluid is evaporated, blocking flow restrictors and valves.

Extractions can be performed in static, dynamic or recirculating mode. When performing static extraction, the cell is filled with the supercritical fluid, pressurised and allowed to equilibrate. In the dynamic mode, the supercritical fluid is passed through the extraction cell continuously. In the recirculating mode, the same fluid is repeatedly pumped through the sample and, after the required number of cycles, it is pumped out to the collection vial.

During extraction, the soluble analytes are partitioned from the bulk sample matrix into the supercritical fluid which is decompressed, without losses, through a flow variable restrictor, into the collection vial. The vial can be empty or fitted with a suitable absorbent or the necessary standards for analysis validation, as required by the determination step [83]. In food analysis, fats and oils are usually collected in an empty vial or in a solvent. Similarly pesticides and fat-soluble vitamins are collected in a solvent or can be absorbed on a solid-phase extractor bed, while flavours and fragrances are collected in a cryogenically cooled vial.

The supercritical fluid nature, temperature and pressure, the extraction time, the shape of the

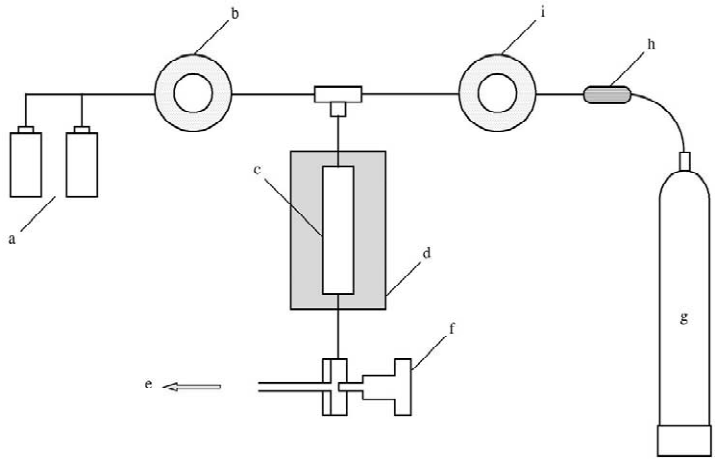

Fig. 3. Schematic of SFE equipment: (a) modifier supply, (b) pump, (c) extraction cell, (d) furnace, (e) to collection, (f) flow restrictor, (g) fluid supply, (h) filter and (i) dual high-pressure piston pump.

extraction cell, the sample particle size, and the type of substrate used for collecting analytes, are all factors that influence the extraction.

From the first, this extraction technique has been extensively used for the separation of organic species in food analysis, particularly for the extraction of fats and oils. From time to time the use of SFE in food analysis is reviewed [84–86] and so, Table 7 summarizes only a few recent separations in food, flavours and spices.

Supercritical fluids offer considerable advantages as extraction solvents in food analysis. The extracts are cleaner than those obtained with organic solvents and can be obtained minimising thermal degradation. No concentration step is needed prior to chemical analysis. In spite of the demonstrated advantages and of its considerable industrial application in specific separations, this technique has failed to become a mainstream separation tool because the selection of supercritical fluids and modifiers is largely empirical, due to the existence of very little analyte solubility data, and, in addition, the interactions between supercritical fluid target analytes and sorptive sites on food are still poorly understood.

Table 7
Supercritical fluid extraction of analytes from food

Food	Analytes	Fluid+modifier	t (°C)/p (MPa)	Ref.
Pork	Fats	CO_2+$(CH_3)_2CHOH$	120/62	[87]
Fried pork	Cholesterol	CO_2	50/34	[88]
Lard fat	Polychlorinated biphenyls			[89]
Lean beef	Warmed-over flavour volatiles	CO_2	40/10.3 and 30	[90]
Meat, eggs	Nicarbazin residues	CO_2+C_2H_5OH	85/27.6	[91]
Eggs	Atrazine and other triazine herbicides	CO_2	50/69	[92]
Eggs	Chloramphenicol	CO_2	80/69	[93]
Eggs	Chlorinated pesticides	CO_2	40/0.72	[94]
Dairy products, meat	Vitamin A	CO_2+5% C_2H_5OH	60/26	[95]
Milk powder	Vitamins A and E	CO_2+5% CH_3OH	80/37	[96]
Corn bran oil	Ferulate-phytosterol esters	CO_2	80/69	[97]
		CO_2+5% CH_3OH	40/34.5	
Seed oils, margarine	Phytosterols	CO_2	80/55.2	[98]
		CO_2+10% $(CH_3)_3COCH_3$	80/13.8, 27.6 and 41.4	
Fats and oils	Organochlorine and organophosphorus pesticides	CO_2+3% CH_3CN	60/27.6	[99]
Apples	Fenpyroximate	CO_2	90/20	[100]
Grapes	Glycosides	CO_2+10% CH_3OH or CO_2	40/69	[101]
Grapes	5-Hydroxymethyl-2-furaldehyde	CO_2+20% CH_3OH	35/38.5	[102]
Paprika	Paprika oil, β-carotene, red carotenoids	CO_2	40/13.8 and 41.4	[103]
Spices	Flavour volatiles	CO_2	40/12	[104]
Rice bran	Lipids and gamma-oryzanol	CO_2	50/69	[105]
Wheat, maize	Organophosphorus pesticides	CO_2	70/24.5	[106]
Malt sprouts	Tocopherols	CO_2	80/25	[107]
Dietary food	Carotenoids, β-carotene, β-cryptoxanthin and zeaxanthin	CO_2+15% C_2H_5OH	75/35	[108]
Baby food	Atrazine, carbofuran, chlorpyrifos, metolachlor	CO_2+10% CH_3CN	70/17.3	[109]
Wheat germ	Vitamin E	CO_2	40–45/27.5–34.5	[110]
Fish	30 VOC	CO_2	45/10	[111]
Tomato	Lycopene, β-carotene, α-carotene, α-tocopherol, γ-tocopherol, δ-tocopherol	CO_2		[112]

3.3. Microwave-assisted extraction (MAE)

Organic extractions using microwave energy incorporate the advantages of the above methods while eliminating most of their disadvantages. The differential temperature between solvent and sample facilitates the transport of the analyte from the sample to the solvent. Closed-vessel microwave heating allows the adoption of well established solvent-analyte-matrix couples instead of the SFE trial-and-error approach, and organic solvents can be heated two to three times hotter than their atmospheric boiling points without the associated high pressure as found in PFE. The technique has proven to be better than soxhlet extraction by cutting solvent consumption and extraction time [113,114]. Usually sample sizes range from 0.5 to 10 g and 10 ml of solvent are sufficient for the extraction that may require from less than 1 to 10 min. The same laboratory microwave unit previously described for digestions is used, so reducing costs; the simultaneous extraction of several samples of different kinds is also possible without any mutual interference.

Analysts have a choice when considering microwave-assisted extraction because a broader spectrum of compounds can be extracted.

It is known that chemical compounds absorb microwaves roughly in proportion to their dielectric constant: the higher the value of dielectric constant, the higher the level of microwave absorption. When using a high dielectric constant solvent, the sample is kept in a closed PTFE vessel. The technique resembles PFE because microwaves heat the solvent far above its atmospheric pressure boiling point and the analytes are rapidly extracted from the sample, which remains at a lower temperature. The use of a high dielectric constant solvent is best for the separation of vitamins, pesticides, or polynuclear aromatic hydrocarbons in food. In the case of low dielectric constant solvent, the sample can be extracted in an open PTFE vessel with microwaves heating the portion of the sample which has higher dielectric components (water, etc.) than the solvent. This localised heating causes analytes to be facilitated in exiting the food and being trapped by the surrounding cold solvent, which is selected according to analyte solubility characteristics. In our experience, this mode of extraction is particularly useful for heat sensitive and thermally labile analytes such as sulphur compounds in garlic, lipids in fishes and chlorinated pesticides in vegetables.

In addition MAE limits contamination or absorption from the vessel, due to direct heating of the sample. The main advantages of microwave pretreatment are the low temperature requirement, high extraction rate, complete automation and the possibility of simultaneously extracting different samples at the same time without interferences. The technique suffers from the disadvantages of using only microwave transparent materials for vessels.

Even if most applications of microwave-assisted extraction are devoted to pollution control [115–117], the technique is widely used in food analysis [118,119] and Table 8 shows some recent applications.

Integrated microwave extractions allow food to be dried, extracted and concentrated with a single piece of equipment and no sample manipulation. Microwave laboratory apparatus is becoming a necessary instrument, joining chromatographs and spectrometers as laboratory-essential analytical instrumentation.

4. Sorbent extraction

The use of a solid adsorbent material to extract analytes from a solution was developed in the 1980s and is now widely applied to many matrices, including food. A sorbent with strong affinity towards some target analytes will retain and concentrate those compounds from the sample solution. Many sorbents are specifically suited for the extraction of different analytes with various degrees of selectivity.

4.1. Solid-phase extraction (SPE)

One widely used sorbent technique is solid-phase extraction (SPE) which involves the use of disposable cartridges to trap analytes and separate them from the bulk of the matrix. As the sample solution passes through the activated sorbent bed, analytes concentrate on its surface, while the other sample components pass through the bed (or vice versa, if necessary to clean-up). The equilibrium between

Table 8
Microwave-assisted solvent extraction of different analytes from food

Food	Solvent	Analytes	Ref.
Beans	Acetone/*n*-hexane (1:1)	Fenitrothion	[114]
Mussels	Acetone/*n*-hexane (1:1) or 1 *M* KOH in methanol	Polychlorinated biphenyls	[120]
Mussels	Polyoxyethylene-10 -luryl ether	Polychlorinated dibenzofurans	[121]
Mussels, oysters	Methanol/water	Arsenobetaine, monomethylarsenic acid, dimethylarsenic acid, arsenite, arsenate	[122]
Fish	Methanol/water	Arsenobetaine, monomethylarsenic acid, dimethylarsenic acid, arsenite, arsenate	[123]
Fish	Methanol/dichloro-methane (1:2)	4-*tert*.-Octylphenol, bisphenol A	[124]
Fish	Tetramethylammonium hydroxide	Dimethyl-, ethylmethyl-, diethylmercury, mercury	[125]
Fish	Ethyl acetate/ cyclohexane (1:1)	Chlorinated hydrocarbons	[126]
Mussels	Methanol/water	Arsenobetaine, monomethylarsenic acid, dimethylarsenic acid, arsenite, arsenate	[127]
Fish	Tetramethylammonium hydroxide	Methylmercury, mercury	[128]
Meat	Methanol	Sulphamethazine	[129]
Meat	Propan-2-ol	4-Hydroxy-3-nitrophenylarsonic acid (roxarsone I)	[130]
Chicken, cooked	Propan-2-ol	Salinomycin	[131]
Oranges	Acetone/*n*-hexane (1:1)	Atrazine, organophosphorus pesticides	[132]
Must	Acetone/dichloro-methane (1:1)	Terpenic compounds	[133]
Must	Dichloromethane	Terpenic compounds	[134]
Crops	Water	Imidazolinone herbicides	[135]
Vegetables, cheese, salami	7% Perchloric acid	Amino acids	[136]
Cheese	Hexane	Fatty acids	[137]
Eggs	Acetonitrile/propan-2-ol	Chloramphenicol	[138]
Beverages, fried chips	Water	Flavours	[139]
Peaches	Isooctane	Thiram, ziram	[140]

analyte and sorbent is rapidly reached because of the large interface.

Many types of sorbent, such as alumina, magnesium silicate and graphitised carbon, are commercially available, but the most common material is silica because it is reactive enough to permit its surface to be modified by chemical reaction and yet stable enough to allow its use with a wide range of solutions. Polymer based sorbent beds are very popular too, and lately their molecular imprinting [141] holds out considerable promise to complement the repertoire of SPE materials for food analysis [142,143]. Molecular imprinting is capable of producing polymeric sorbents that recognize the template molecules used. It starts from the formation of a complex between the template molecules and monomers bearing functional groups. The spatial assembly of the interaction sites is then fixed through polymerisation in the presence of a cross-linking agent. After removal of the template, cavities capable of recognizing and re-binding the template molecules are obtained.

Usually, the size of the sorbent particles ranges from 10 to 60 μm. As the materials are similar to those currently used in liquid chromatography (except for particle size), the whole range of chemistries used in modern packing technology can be applied to prepare solid-phase cartridges. Sorbents fall into three general classes: non-polar, polar and ion-exchange and their activity is dependent on the properties of the bonded phase and of any active site not end capped on the sorbent. The choice of the sorbent is dependent on the food matrix, analytes of interest and their interferents.

The extraction is performed in four steps: conditioning (the functional groups of the sorbent bed are solvated in order to make them to interact with the sample), retention (the analytes are bound to the bed surface), selective washing (undesired species are removed) and elution (the analytes are desorbed and collected for analysis). Great care must be taken regarding fines or small particulates that can be present in extract, when resulting from the sorbent bed, because they cause irreproducible and low recoveries for compounds that are particularly adsorptive to active surfaces.

Method development in SPE is accomplished by investigating pH, ionic strength, polarity and flow-rate of the elution solvent and physico-chemical characteristics of the sorbent bed.

A recent innovation has been the introduction of a disk format which is less subject to channelling problems, in comparison with packed cartridge, by offering larger flow area and lower bed mass. Actually disks are available in a limited number of sorbents, but it is envisaged that the use of disks will become more prevalent in the future [144] not only because of their better performance but also because of their faster extraction speed.

Using SPE, multiple samples can be treated in parallel using relatively small quantities of solvent, while the clean-up procedure can be performed on-line with many analytical techniques, such as ion chromatography. Fig. 4 shows a schematic of the equipment as assembled in the authors' laboratory.

The sample is dissolved and inserted in the autosampler, from which it is pumped to the SPE cartridge, while the chromatographic column is kept in a steady flow of the chromatographic eluent selected for analysis. Interferents are selectively washed off the SPE cartridge by means of a selective solvent, then the cartridge is connected to the chromatographic loop and target analytes are washed out by means of a proper solvent. The loop is then connected to the column and the chromatographic eluent flows. This technique offers many improvements over liquid–liquid extraction and permits simultaneous removal of interfering substances and concentration of analytes.

Many books [145,146] and reviews [147,148] provide in-depth coverage of SPE and readers are referred to these reference texts for detailed information. Many food matrices have been cleaned up by solid-phase extraction for the determination of such a large number of analytes that it is not possible to conduct a comprehensive survey of the literature; usually reviews deal with the separation of specific components from food [149–151]. Table 9 only reports some of the latest applications in food analysis.

4.2. Solid-phase microextraction (SPME)

A direct derivative of SPE is solid-phase microextraction (SPME) [152], in which a fused-silica micro fibre supports a minute quantity of polymeric

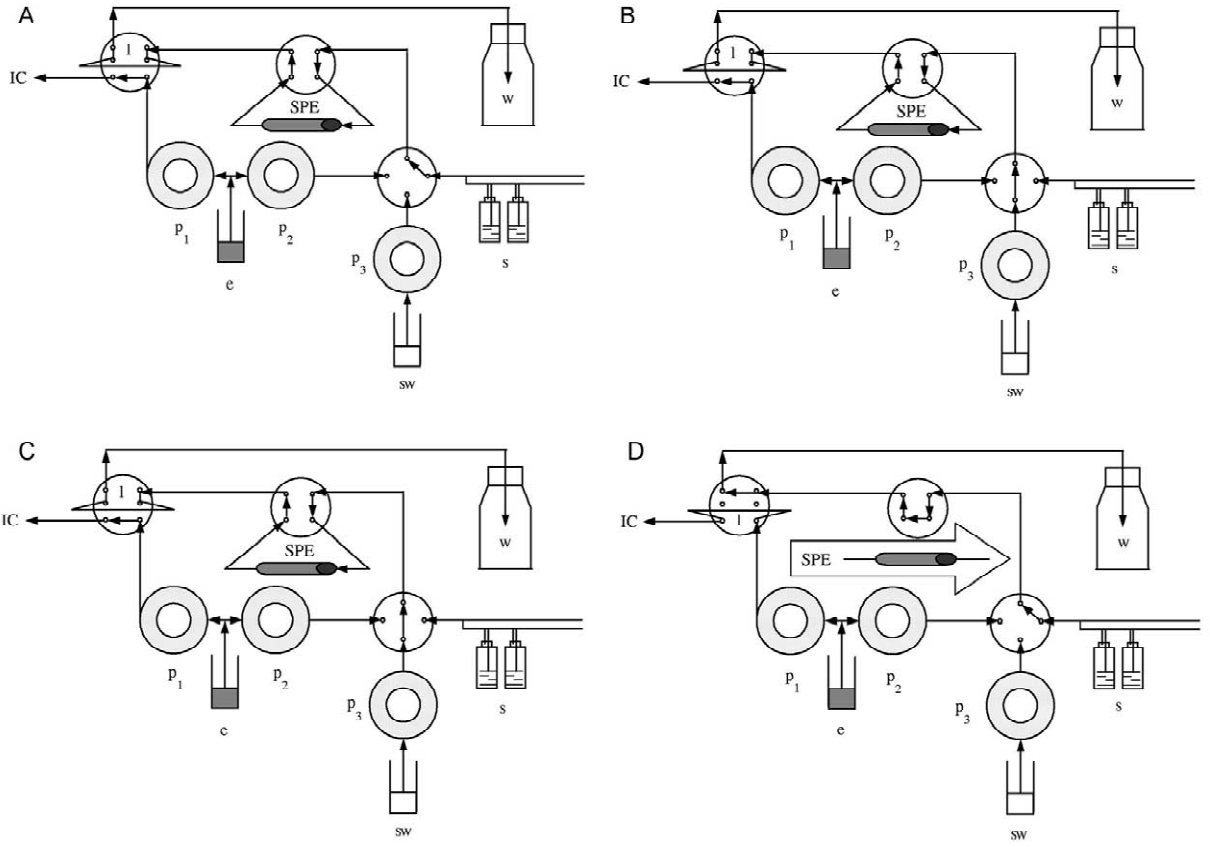

Fig. 4. Operative sequence of SPE unit coupled on-line to an ion chromatograph. (A) Sample is transferred from the autosampler (s) to the SPE cartridge (SPE); the IC column is kept in a steady flow of eluent (e) from pump p_2; (B) interferents are selectively washed off the cartridge (SPE) to the waste reservoir (w), by means of the proper solution (sw) pressurised from pump p_3; the IC column remains in the same conditions as step A; (C) analytes are eluted from cartridge (SPE) by means of the eluent (e) and the loop (l) is filled; the IC column is in the same condition as in steps A and B; (D) loop (l) is connected to the IC column and analytes are eluted by means of the eluent (e); at the same time a new cartridge (SPE) is inserted on-line for the pre-treatment of the sample that follows.

extracting phase, where analytes are adsorbed. SPE and SPME are different sorbent extraction techniques because the former is expected to extract all the target analytes from the sample solution while the latter is a partition process. SPME is used to extract analytes from both liquid and solid food [118,153,154].

A fibre of silica or other appropriate material, coated with a minute quantity of the extracting phase, is exposed to the sample or its headspace; organic compounds present in the sample solution are partitioned into the fibre coating. The process is controlled by the diffusion of analytes through the static layer surrounding the fibre. Diffusion into the polymeric film is aided by sample agitation which helps to establish equilibrium. Equilibration time is dependent on the thickness and type of the coating and the distribution constant of the analytes. The partitioning of analytes into the coating is analogous to that which occurs in liquid–liquid extraction. Similarly, the sensitivity of the extraction depends on the affinity of analytes for the coating and its capacity. To date, the most successful phases have been the relatively non-polar polydimethylsiloxane and the polar polyacrylate moiety. Sometimes fibres also incorporate adsorbents, such a divinylbenzene or carbon molecular sieve, whose adsorbing properties increase analyte retention by reason of the higher

Table 9
Applications of SPE and SPME to food samples clean up

Food	Analytes	Technique	Coating (thickness and functional group) and eluent (or desorption) conditions	Ref.
Malt	Proanthocyanidins	SPE	40–80 μm PA6, eluent $HCON(CH_3)_2/H_2O$ (8.5:1.5)	[78]
Alcoholic beverages	β-Carbolines and tetrahydro-β-carboline-3-carboxylic acids	SPE	40–50 μm PRS or SCX, eluent: CH_3OH+0.2 N K_2HPO_4 (pH 8.8) (1:1); 40–50 μm C_{18}, eluent CH_3OH	[155]
Wine	Anthocyanins	SPE	40–50 μm C_{18}, eluent CH_3CN	[156]
Wine	Pesticide residues	SPE	40–70 μm PS-DVB, eluent $CH_3CO_2C_2H_5$	[157]
Wine	Stilbenes	SPE	40–70 μm PS-DVB, eluent C_4H_8O	[158]
Wine	3,4′,5-Trihydroxystilbene	SPME	100 μm PDMS or 85 μm PA, 7 min at 280 °C	[159]
Wine	Cyprodinil, fludioxonil fungicides	SPME	50/30 μm CAR-DVB-PDMS, 5 min at 240 °C	[160]
Wine	35 VOC	SPME	100 μm PDMS, 3 min at 250 °C	[161]
Wine	16 Sulphur compounds	SPME	50/30 μm CAR-DVB-PDMS, 1 min at 270 °C	[162]
Wine	Pyrazines	SPME	65 μm DVB-PDMS, 1 min at 250 °C	[163]
Alcoholic beverages, fruit juices, sauces, fish, bread	Tetrahydro-β-carboline-3-carboxylic acids	SPE	SCX, eluent CH_3OH+0.4 *M* phosphate buffer (pH 9.1) (1:1)	[164]
Alcoholic beverages, fruit juices, vegetables	Proanthocyanidins	SPE	45 μm C_{18}, eluent $CH_3COCH_3/H_2O/CH_3COOH$ (140:59:1)	[165]
Fruit juices	Folic acid	SPE	45 μm IC-OH, eluent 0.1 *M* CH_3COONa+10% NaCl	[166]
Honey	Acaricide residues	SPE	40–50 μm C_{18}, eluent C_4H_8O	[167]
Strawberries	9 VOC	SPME	100 μm PDMS, 2 min at 240 °C	[168]
Olive oil	Phenolic compounds	SPE	40–50 μm C_{18}, eluent CH_3OH	[169]
Margarine, oils, cheese, sauces	Antioxidants, preservatives	SPE	50–100 μm PS-DVB, eluent $(CH_3)_2CHOH$	[170]
Cheese	70 VOC	SPME	75 μm CAR-PDMS, 5 min at 280 °C	[171]
Milk	Volatile fatty acids	SPME	75 μm CAR-PDMS, 3 min at 275 °C	[172]
Meat, eggs	Spiramycin, tilmicosin, tylosin	SPE	45 μm IC-H, eluent CH_3OH	[173]
Meat	96 VOC	SPME	75 μm CAR-PDMS and/or 50/30 μm DVB-CAR-PDMS, 3 min at 250 °C	[174]
Meat	Heterocyclic amines	SPE	40–50 μm PRS+40–50 μm C_{18}, eluent CH_3OH/NH_4OH (9:1)	[175]
Meat	Aminoazaarenes	SPE	KIE, eluent CH_2Cl_2 and 40–50 μm PRS and 45 μm C_{18}, eluent CH_3OH/NH_4OH (9:1)	[176]

Table 9 (*continued*)

Food	Analytes	Technique	Coating (thickness and functional group) and eluent (or desorption) conditions	Ref.
Meat, fish, honey, milk, eggs	Tetracycline antibiotics	SPE	SCX, eluent CH_3OH	[177]
Bread	Bromate		45 μm C_{18}, eluent CH_3OH/H_2O	[178]
Mustard sauces	11 VOC	SPME	65 μm PDMS-DVB, 5 min at 250 °C	[179]
Wine	3-Alkyl-2-methoxypyrazines	SPME	65 μm PDMS-DVB, 1 min at 250 °C	[180]
Vinegar	29 Aroma compounds	SPME	75 μm CAR-PDMS, 2 min at 280 °C	[181]
Rum	Fatty acid ethyl esters	SPME	100 μm PDMS, 1 min at 250 °C	[182]
Wine	Ochratoxin A	SPE	45 μm C_{18}, eluent CH_3OH	[183]
Wine	4-Ethylphenol, 4-ethylguaiacol	SPME	85 μm PA, 3 min at 250 °C	[184]
Wine	Butyltin compounds	SPME	100 μm PDMS, 1.5 min at 250 °C	[185]
Coffee	16 Aroma compounds	SPME	100 μm PDMS, 10 min at 230 °C	[186]
Banana	7 Flavour compounds	SPME	100 μm PDMS, 15 min at 200 °C	[187]
Honey	35 VOC	SPME	75 μm CAR-PDMS, 30 min at 270 °C	[188]
Fruit juices	Organophosphorus insecticide residues	SPME	100 μm PDMS, 2 min at 240 °C	[189]

C_{18}, octadecyl; CAR-PDMS, carboxen-poly(dimethylsiloxane); CAR-DVB-PDMS, carboxen-poly(divinylbenzene)-poly(dimethylsiloxane); CW-DVB, poly(ethyleneglycol)[Carbowax]-poly(divinylbenzene); IC-H, strongly acid cation exchanger; IC-OH, strongly basic anion exchanger; KIE, kieselguhr; PA, polyacrylate; PDMS, poly(dimethylsiloxane); PDMS-DVB, poly(dimethylsiloxane)-poly(divinylbenzene); PA6, polyamide 6; PRS, propylsulphonic acid; PS-DVB, polystyrene-poly(divinylbenzene); SCX, benzenesulphonic acid; VOC, volatile organic compounds.

surface area. The fibre probes are frequently coupled with gas chromatography or gas chromatography–mass spectrometry techniques; hence the analytes can be thermally desorbed from the fibre coating in the injection port of those equipments, while separation and quantitation take place. The technique suffers from the disadvantage of relatively long equilibration times during the extraction procedure.

Applications of SPME vary in evaluation of food quality or flavour volatiles; Table 9 reports some of the latest applications only.

5. Membrane separation

The selective nature of membranes has made them a unique alternative to solvent extraction for sample clean up, especially if coupled with chromatographic techniques [190,191]. The relative sizes of different molecules largely determine the permeation selectivity of a membrane, in the absence of strong specific interactions. The main advantages over solvent extraction are the use of high ratio between surface area and volume, the lack of emulsions and no phase separation step.

The process is the result of differences in the transport rates of the species through the membrane interface: the separation is achieved when some species are transported to a greater extent than others. The forces able to generate transport through membranes are directly related to differences in pressure, according to Hagen-Poiseuille's law (micro and ultra filtration), in concentration, according to Fick's law (dialysis), or in electrical potential, according to Ohm's law (electro dialysis).

While micro and ultra filtration are based on a size exclusion process only, dialysis and electro dialysis can be based not only on a difference in size, but also on a difference in ionic charge, depending on the type of membrane used.

Membranes are usually made of synthetic polymeric materials, although natural substances, such as

cellulose, or inorganic materials, such as glass fibres or alumina, are also used. First of all, the membrane serves to retain molecules larger than a critical, material-related size. The maximum size of a solute that can pass through the membrane is called the cut-off value and it is normally given as the molecular weight of the smallest compound of which more than 90% is retained (MWCO, molecular weight cut-off in Daltons units). In food analysis, membranes are frequently used to separate salts and low molecular weight analytes from high molecular weight species, such as in desalting protein extracts, so a molecular weight cut-off three to six times different from that of the analyte is required. A correct MWCO permits rapid transport of the analytes through the membrane, with sufficient retention of the interfering compounds. Fig. 5 gives a rough idea of the dimensions of some food constituents.

When selecting the membrane material, its chemical compatibility with the feed (the solution containing the analytes, whose concentration is depleted, also called the donor) and the fluid receiving the analytes (also called the acceptor) is to be considered. The feed pH and its ionic strength must be carefully selected for favouring separation. In addition to chemical compatibility, many physical and chemical characteristics such as pore size and pore size distribution, thickness, extractable material, hydrophobic/hydrophilic character, non-specific binding properties, pyrogenicity, gas- and liquid-flow rate, particulate retention and so on, determine the membrane suitability for a particular application; for instance, proteins are separated better with a hydrophobic PTFE membrane, where they adsorb, than with a hydrophilic membrane.

To obtain compound specificity in sample preparation, functionalised or sorbent impregnated membranes are used. In these membranes, a specific functionality is imposed by chemical bonding reactions; in ion-exchange membranes, for instance, positively or negatively charged groups are covalently attached to the polymeric membrane material.

The extraction efficiency of the process is controlled by the contact time between the membrane and the donor and acceptor fluids and the rate constant of analyte transport between the two solutions, which depends on the properties of the membrane, the fluid channel geometry and the fluid point speed.

Finally, a completely different class of mem-

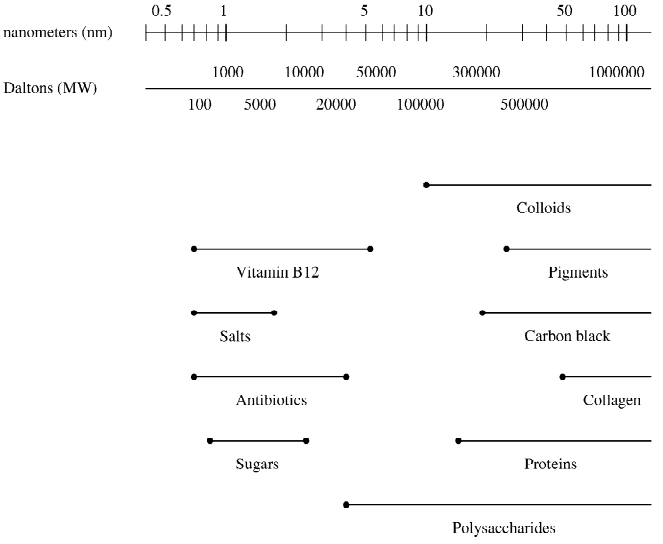

Fig. 5. Molecular size of common compounds in food. Upper scale is logarithmic and it is given in nanometers; below it the corresponding molecular weights (MW) are expressed as Daltons.

branes, so-called non-porous membranes, are used for membrane extraction. They consist of a liquid or polymer film into which the compound must dissolve in order to pass through.

5.1. Dialysis

The use of dialysis for the on-line separation of high molecular mass components from lower molecular size analytes is very interesting in food analysis. Dialysis is a membrane barrier separation process in which differential concentration forces one or more species to transfer from the sample solution to another fluid through a semi-permeable membrane, which allows contact between the fluids without mixing. The critical parameter is the maximum size of the species able to cross the selected membrane. Obviously, the sample must be in a liquid and almost homogeneous form. The separation is usually performed in a cell that consists of two blocks with the membrane in between, to separate the donor (sample) phase from the acceptor (dialyzed) phase. All molecules of appropriate size diffuse through the membrane pores from the donor into the acceptor solution, as the result of the concentration gradient of the compounds. The operative sequence of a dialysis unit on-line coupled to an ion chromatograph is reported in Ref. [51].

Membranes can be used for separation in four different donor–acceptor modes of operation: stagnant–stagnant, stagnant–flowing, pulsed–flowing and flowing–flowing. Because dialysis involves molecular diffusion across the membrane, it is obvious that the stagnant–stagnant mode is time consuming and $>50\%$ recovery cannot be achieved (for equal donor and acceptor volumes). In practice a flowing acceptor stream is almost invariably selected to obtain both a higher speed and a higher recovery. If relatively large sample volumes are available, as in milk analysis for instance, the process is accelerated by inserting sample in pulses, so restoring each time the initial maximum concentration gradient, or by using a continuously flowing sample stream. In these ways the time required for completing sample dialysis is reduced to only a few minutes.

An important parameter to be taken into account for membrane dialysis evaluation, is the analyte enrichment factor, which is the ratio of the maximum attainable concentration in the acceptor to the concentration in the sample. The enrichment factor is particularly relevant when the concentration of the analytes in the sample is undetectable and when only a small portion of the total acceptor phase is used in the final determination.

Apart from the previously mentioned membrane parameters, it must be also considered that analytes can bind to the membrane material. These interactions can be deliberately caused to obtain compound specificity, as in the particular case of ion-exchange membranes, but sometimes unwanted electrostatic or hydrophobic interactions have been observed. Therefore, an incomplete recovery in the acceptor phase may result if no precautions are taken, such as the addition of surfactants or displacers to the sample solution.

In food analysis liquid samples can be directly dialysed because the process does not suffer from the presence of particulate matter or macro compounds such as proteins, lipids, polysaccharides, etc.; solid samples require the addition of water and homogenisation before dialysis. Table 10 shows some applications of dialysis to food sample clean up.

5.2. Membrane extraction

Extraction can be performed with porous impregnated or non-porous membranes that separate two aqueous phases. Sample pH is adjusted in order to have the analytes of interest in uncharged form and therefore easily extracted into the membrane. The acceptor solution must have a pH able to effect ionisation of the analytes as they pass across the membrane, so preventing their back extraction into the donor. This technique has been applied to the determination of triazines in vegetable oils [211], vitamin E in butter [212], vanillin in sugar and chocolate [213], and pesticide residues in eggs [214].

All these membrane-based separation techniques are characterized by advantages in the clean-up of food because they require very small volumes of solvents compared to alternative processes, the laboratory devices are very simple and cheap and their

Table 10
Applications of dialysis to food samples clean up

Food	Analytes	Dialysis membrane material	Ref.
Olive oil wastes	Cl^-, NO_2^-, NO_3^-, PO_4^{3-}, SO_4^{2-}	Cellulose triacetate	[51]
Egg	Tetracyclines	Regenerated cellulose	[192]
Egg	Dexamethasone	Regenerated cellulose	[193]
Egg	Sarafloxacin resides	Regenerated cellulose	[194]
Chicken	Flumequine, oxolinic acid	Regenerated cellulose	[195]
Chicken liver	Fluoroquinolone antibacterials	Regenerated cellulose	[196]
Milk	Lactulose	Polyacrylonitrile metallyl-sulphonate	[197]
Milk	Proteins	Cellulose triacetate	[198]
Milk	Cl^-	Cellulose triacetate	[199]
Milk, fruit juices	Cl^-, HPO_4^{2-}, SO_4^{2-}, Na, K, Ca, Mg	Cellulose triacetate	[200]
Milk, fruit juices	Ascorbic acid	Polyacrylonitrile metallyl-sulphonate	[201]
Milk, fruit juices	Cl^-, HPO_4^{2-}, SO_4^{2-}, HCO_3^-, organic acids	Regenerated cellulose	[202]
Wine, fruit juices	25 Amino acids	Cellulose triacetate	[203]
Fruit juices	Cl^-	Polyetherimide	[204]
Fruit juices	Patulin	Regenerated cellulose	[205]
Wine	Lactic acid, malic acid	Cellulose triacetate	[206]
Wine	Total acidity, tartaric acid	Cellulose triacetate	[207]
Wine	Amino acids, organic acids, sugars	Cellulose triacetate	[208]
Beer	Proteins	No specification	[209]
Tomato	Cd, Cr, Cu, Ni, Pb	Polyether sulphone or polysulphone	[210]

automation and on-line connection to analytical instruments is very easy [215].

6. Conclusions

The rapid development of sample pre-treatment techniques indicates the need for methods which are compatible with modern analytical techniques. The continuously increasing number of samples to be tested, related to the growing concern over food safety, require methods that must be simple, reliable, cheap and, not least, take in account chemical laboratory waste problems. In addition the heavy routine makes absolutely necessary the choice of dissolution and clean up techniques that are similar to the most common analytical techniques, in order to minimize errors and develop expert systems that work without staff supervision. The acceptance by regulatory bodies of up-to-date sample pre-treatment techniques would surely help to give a substantial boost to the their widespread use in food analysis.

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References

[1] M. Peris, Anal. Chim. Acta 454 (2002) 1.
[2] M. Stoeppler (Ed.), Sampling and Sample Preparation, Springer, Berlin, 1997.
[3] I.D. Wilson (Ed.), Encyclopaedia of Separation Science, Academic Press, London, 2000.
[4] E.O. Henry, J. Pharmacie (1834) 29.
[5] W. Zeise, J. Prakt. Chem. 1 (1834) 458.

[6] E. Jackwerth, M. Wuerfels, in: M. Stoeppler (Ed.), Sampling and Sample Preparation, Springer, Berlin, 1997, p. 142, Chapter 12.
[7] M. Wuerfels, E. Jackwerth, Fresenius J. Anal. Chem. 322 (1985) 354.
[8] Analytical Methods Committee, Analyst 84 (1959) 214.
[9] P. Tschoepel, in: M. Stoeppler (Ed.), Hazardous Metals in the Environment, Elsevier Science, Amsterdam, 1992, p. 73.
[10] K. Ganzler, A. Salgo, K. Valko, J. Chromatogr. 371 (1986) 299.
[11] H. Matusiewicz, Anal. Chem. 71 (1999) 3145.
[12] D.D. Link, H.M. Kingston, Anal. Chem. 72 (2000) 2908.
[13] S. Sakao, H. Uchida, Anal. Chim. Acta 382 (1999) 215.
[14] K. Julshamn, A. Maage, E.H. Larsen, Fresenius J. Anal. Chem. 355 (1996) 304.
[15] S. Ringmann, K. Boch, W. Marquardt, M. Schuster, G. Schlemmer, P. Kainrath, Anal. Chim. Acta 452 (2002) 207.
[16] F.J. Copa Rodriguez, M.I. Basadre Pampin, Fresenius J. Anal. Chem. 348 (1994) 390.
[17] L.C. Medeiros, R.P. Belden, E.S. Williams, J. Food Sci. 58 (1993) 731.
[18] R.M. Soto Ferreiro, P. Bermejo Barrera, Analusis 21 (1993) 197.
[19] M. Navarro, H. Lopez, M.C. Lopez, M. Sanchez, Anal. Chim. Acta 257 (1992) 155.
[20] M. Navarro, H. Lopez, M.C. Lopez, M. Sanchez, J. Anal. Toxicol. 16 (1992) 169.
[21] R.M. Soto Ferreiro, C. Casais Laino, P. Bermejo Barrera, Anal. Lett. 24 (1991) 2277.
[22] H. Polkowska-Motrenko, B. Danko, R. Dybczynski, A. Koster-Ammerlaan, P. Bode, Anal. Chim Acta 408 (2000) 89.
[23] H. Lachas, R. Richaud, A.A. Herod, D.R. Dugwell, R. Kandiyoti, Rapid Commun. Mass Spectrom. 14 (2000) 335.
[24] I. Lavilla, A.V. Filgueiras, C. Bendicho, J. Agric. Food Chem. 47 (1999) 5072.
[25] M. Navarro-Alarcon, M.C. Lopez-Martinez, M. Sanchez-Vinas, H. Lopez-Garcia-de-la-Serrana, J. Agric. Food Chem. 39 (1991) 2223.
[26] B. Madeddu, A. Rivoldini, At. Spectrosc. 17 (1996) 148.
[27] D.P. Stevens, M.J. McLaughlin, A.M. Alston, Commun. Soil Sci. Plant Anal. 26 (1995) 1823.
[28] I. Matejovic, A. Durackova, Commun. Soil Sci. Plant Anal. 25 (1994) 1277.
[29] M. Navarro, M.C. Lopez, H. Lopez, J. Assoc. Off. Anal. Chem. 75 (1992) 1029.
[30] S. Prats-Moya, N. Grane-Teruel, V. Berenguer-Navarro, M.L. Martin-Carratala, J. Agric. Food Chem. 45 (1997) 2093.
[31] F. Di Narda, R. Toniolo, G. Bontempelli, Anal. Chim. Acta 436 (2001) 245.
[32] C.F. Oduoza, S.A. Thomas, Fresenius J. Anal. Chem. 342 (1992) 449.
[33] R. Rezaaiyan, S. Nikdel, J. Food Sci. 55 (1990) 1359.
[34] S.W. Chung, W.C. Tsai, At. Spectrosc. 13 (1992) 185.
[35] J. Mierzwa, Y.C. Sun, Y.T. Chung, M.H. Yang, Talanta 47 (1998) 1263.
[36] X.D. Tian, H. Emteborg, M. Barbaste, F.C. Adams, J. Anal. At. Spectrom. 15 (2000) 829.
[37] C.M. Mena, C. Cabrera, M.L. Lorenzo, M.C. Lopez, J. Agric. Food Chem. 45 (1997) 1812.
[38] S. Sanllorente, M. Cruz Ortiz, M.J. Arcos, Analyst 123 (1998) 513.
[39] M.T. Arcos, M.C. Ancin, J.C. Echeverria, A. Gonzalez, J.J. Garrido, J. Agric. Food Chem. 41 (1993) 2333.
[40] C. Cabrera-Vique, P.L. Teissedre, M.T. Cabanis, J.C. Cabanis, J. Assoc. Off. Anal. Chem. 80 (1997) 57.
[41] M. Olalla, M.C. Gonzalez, C. Cabrera, M.C. Lopez, J. Assoc. Off. Anal. Chem. 83 (2000) 189.
[42] L. Vuchkova, L. Margitova, S. Arpadjan, Anal. Lab. 5 (1996) 41.
[43] C. Cabrera, M.L. Lorenzo, M.C. Lopez, J. Agric. Food Chem. 43 (1995) 1605.
[44] Q. Yang, W. Penninckx, J. Smeyers Verbeke, J. Agric. Food Chem. 42 (1994) 1948.
[45] M. Ihnat, R.W. Dabeka, M.S. Wolynetz, Fresenius J. Anal. Chem. 348 (1994) 445.
[46] P.J. Oles, W.M. Graham, J. Assoc. Off. Anal. Chem. 74 (1991) 812.
[47] A. Sattar, N. Ahmad, L.A. Khan, Nahrung 37 (1993) 220.
[48] H.J. Reid, S. Greenfield, T.E. Edmonds, Analyst 120 (1995) 1543.
[49] M. Kolb, P. Rach, J. Schaefer, A. Wild, Fresenius J. Anal. Chem. 342 (1992) 341.
[50] P.L. Buldini, S. Cavalli, A. Mevoli, J.L. Sharma, Food Chem. 73 (2001) 487.
[51] P.L. Buldini, A. Mevoli, A. Quirini, J. Chromatogr. A 882 (2000) 321.
[52] P.L. Buldini, S. Cavalli, J.L. Sharma, J. Agric. Food Chem. 47 (1999) 1993.
[53] O. Dominguez, S. Sanllorente, M.J. Arcos, Electroanalysis 11 (1999) 1273.
[54] S. Sanllorente, M. Cruz-Ortiz, M.J. Arcos, Analyst 123 (1998) 513.
[55] P.L. Buldini, J.L. Sharma, S. Cavalli, Agro-Food Ind. Hi-Tech. 3 (5) (1992) 25.
[56] P.L. Buldini, D. Ferri, J.L. Sharma, J. Chromatogr. A 789 (1997) 549.
[57] P.L. Buldini, S. Cavalli, A. Mevoli, J. Chromatogr. A 739 (1996) 167.
[58] T. Pérez-Ruiz, C. Martinez-Lozano, V. Tomas, J. Martin, Anal. Chim. Acta 447 (2001) 229.
[59] B.E. Richter, B.A. Jones, J.L. Ezzell, N.L. Porter, N. Avdalovic, C. Pohl, Anal. Chem. 68 (1996) 1033.
[60] A.K.D. Liem, Trends Anal. Chem. 18 (1999) 499.
[61] B.E. Richter, LC·GC (Europe) 17 (6) (1999) S22.
[62] F. Schroeter, M. Anastassiades, E. Scherbaum, Chem. Lab. Biotech. 50 (1999) 4.
[63] Application Note No. 332, Dionex, Sunnyvale, CA, USA, 2000, p. 3.
[64] H. Obana, K. Kikuchi, M. Okihashi, S. Hori, Analyst 122 (1997) 217.
[65] M. Okihashi, H. Obana, S. Hori, Analyst 123 (1998) 711.
[66] R.M. Alonso-Salces, E. Korta, A. Barranco, L.A. Berrueta, B. Gallo, F. Vicente, J. Chromatogr. A 933 (2001) 37.
[67] G.W. Chase Jr., B. Thomson, J. Assoc. Off. Anal. Chem. 83 (2000) 407.

[68] J.C. Chuang, K. Hart, J.S. Chang, L.E. Boman, J.M. van Emon, A.W. Reed, Anal. Chim. Acta 444 (2001) 87.
[69] G. Wang, A.S. Lee, M. Lewis, B. Kamath, R.K. Archer, J. Agric. Food Chem. 47 (1999) 1062.
[70] Application Note No. 337, Dionex, Sunnyvale, CA, USA, 2000, p. 3.
[71] J.W. McKiernan, J.T. Creed, C.A. Brockhoff, J.A. Caruso, R.M. Lorenzana, J. Anal. At. Spectrom. 14 (1999) 607.
[72] R. Draisci, C. Marchiafava, E. Ferretti, L. Palleschi, G. Catellani, A. Anastasio, J. Chromatogr. A 814 (1998) 187.
[73] Application Note No. 334, Dionex, Sunnyvale, CA, USA, 2000, p. 5.
[74] K. Schaefer, Anal. Chim. Acta 358 (1998) 69.
[75] Application Note No. 340, Dionex, Sunnyvale, CA, USA, 2000, p. 4.
[76] A. Mueller, E. Björklund, C. von Holst, J. Chromatogr. A 925 (2001) 197.
[77] D. Hauffe, F. Hoeffer, D.E. Knowles, J. Clark, J.L. Ezzell, B.E. Richter, in: G. Sontag, W. Pfannhauser (Eds.), Proc. Euro Food Chem VIII, Vienna. Austrian Chem. Soc., (Austria), Vol. 3, 1995, p. 766.
[78] M. Papagiannopoulos, B. Zimmermann, A. Mellenthin, M. Krappe, G. Maio, R. Galensa, J. Chromatogr. A 958 (2002) 9.
[79] R.M. Smith, J. Chromatogr. A 856 (1999) 83.
[80] J.M. Levy, LC·GC (Europe) 13 (3) (2000) 174.
[81] J.W. King, J. Chromatogr. Sci. 27 (1989) 355.
[82] J.W. King, J. Assoc. Off. Anal. Chem. 81 (1998) 9.
[83] C. Turner, C.S. Eskilsson, E. Bjorklund, J. Chromatogr. A 947 (2002) 1.
[84] M. Valcarcel, M.T. Tena, Fresenius J. Anal. Chem. 358 (1997) 561.
[85] E. Anklam, H. Berg, L. Mathiasson, M. Sharman, F. Ulberth, Food Addit. Contam. 15 (1998) 729.
[86] C. Turner, J.W. King, L. Mathiasson, J. Chromatogr. A 936 (2001) 215.
[87] W. Reichardt, U. Mueller, W. Peschke, P. Oppermann, M. Schuster, S. Mueller, B. Eckert, Fleischwirtschaft 79 (1999) 79.
[88] T.Y. Lin, Y.J. Wang, P.Y. Lai, F.J. Lee, J.T.S. Cheng, Food Chem. 67 (1999) 89.
[89] M. Jaremo, E. Bjorklund, N. Nilsson, L. Karlsson, L. Mathiasson, J. Chromatogr. A 877 (2000) 167.
[90] A. Thongwong, L.M. Fernando, I.U. Grun, A.D. Clarke, J. Food Sci. 64 (1999) 387.
[91] D.K. Matabudul, N.T. Crosby, S. Sumar, Analyst 124 (1999) 499.
[92] J.W. Pensabene, W. Fiddler, D.J. Donoghue, J. Agric. Food Chem. 48 (2000) 1668.
[93] J.W. Pensabene, W. Fiddler, D.J. Donoghue, J. Am. Off. Anal. Chem. 82 (1999) 1334.
[94] W. Fiddler, J.W. Pensabene, R.A. Gates, D.J. Donoghue, J. Agric. Food Chem. 47 (1999) 206.
[95] C. Turner, J.W. King, L. Mathiasson, J. Agric. Food Chem. 49 (2001) 553.
[96] C. Turner, L. Mathiasson, J. Chromatogr. A 874 (2000) 275.
[97] S.L. Taylor, J.W. King, J. Chromatogr. Sci. 38 (2000) 91.
[98] J.M. Snyder, J.W. King, S.L. Taylor, A.L. Neese, J. Am. Oil Chem. Soc. 76 (1999) 717.
[99] M.L. Hopper, J. Chromatogr. A 840 (1999) 93.
[100] B.L. Halvorsen, C. Thomsen, T. Greibrokk, E. Lundanes, J. Chromatogr. A 880 (2000) 121.
[101] M. Palma, L.T. Taylor, B.W. Zoecklein, L.S. Douglas, J. Agric. Food Chem. 48 (2000) 775.
[102] M. Palma, L.T. Taylor, J. Agric. Food Chem. 49 (2001) 628.
[103] M. Jaren Galan, U. Nienaber, S.J. Schwartz, J. Agric. Food Chem. 47 (1999) 3558.
[104] M.C. Diaz-Maroto, M.S. Pérez-Coello, M.D. Cabezudo, J. Chromatogr. A 947 (2002) 23.
[105] Z.M. Xu, J.S. Godber, J. Am. Oil Chem. Soc. 77 (2000) 547.
[106] K.N.T. Norman, S.H.W. Panton, J. Chromatogr. A 907 (2001) 247.
[107] G. Carlucci, P. Mazzeo, S. Del Governatore, G. Di Giacomo, G. Del Re, J. Chromatogr. A 935 (2001) 87.
[108] M. Careri, L. Furlattini, A. Mangia, M. Musci, E. Anklam, A. Theobald, C. von Holst, J. Chromatogr. A 912 (2001) 61.
[109] J.C. Chuang, M.A. Pollard, M. Misita, J.M. van Emon, Anal. Chim. Acta 399 (1999) 135.
[110] Y. Ge, H. Yan, B. Hui, Y. Ni, S. Wang, T. Cai, J. Agric. Food Chem. 50 (2002) 685.
[111] T. Aro, C. Brede, P. Manninen, H. Kallio, J. Agric. Food Chem. 50 (2002) 1970.
[112] N.L. Rozzi, R.K. Singh, R.A. Vierling, B.A. Watkins, J. Agric. Food Chem. 50 (2002) 2638.
[113] V. Lopez Avila, R. Young, N. Teplitsky, J. Am. Off. Anal. Chem. 79 (1996) 142.
[114] R.G. Diagne, G.D. Foster, S.U. Khan, J. Agric. Food Chem. 50 (2002) 3204.
[115] C.S. Eskilsson, E. Bjorklund, J. Chromatogr. A 902 (2000) 227.
[116] V. Camel, Trends Anal. Chem. 19 (2000) 229.
[117] V. Lopez Avila, Crit. Rev. Anal. Chem. 29 (1999) 195.
[118] J.G. Wilkes, E.D. Conte, Y. Kim, M. Holcomb, J.B. Sutherland, D.W. Miller, J. Chromatogr. A 880 (2000) 3.
[119] A.K.D. Liem, Trends Anal. Chem. 18 (1999) 499.
[120] G.H. Xiong, X.Q. He, Z.X. Zhang, Anal. Chim. Acta 413 (2000) 49.
[121] A. Eiguren Fernandez, Z. Sosa Ferrera, J.J. Santana Rodriguez, Luminescence 15 (2000) 94.
[122] J.L. Gomez Ariza, D. Sanchez Rodas, I. Giraldez, E. Morales, Analyst 125 (2000) 401.
[123] K.L. Ackley, C. B'Hymer, K.L. Sutton, J.A. Caruso, J. Anal. At. Spectrom. 14 (1999) 845.
[124] S.N. Pedersen, C. Lindholst, J. Chromatogr. A 864 (1999) 17.
[125] S. Slaets, F. Adams, I. Rodriguez Pereiro, R. Lobinski, J. Anal. At. Spectrom. 14 (1999) 851.
[126] W. Vetter, M. Weichbrodt, K. Hummert, D. Glotz, B. Luckas, Chemosphere 37 (1998) 2439.
[127] T. Dagnac, A. Padro, R. Rubio, G. Rauret, Anal. Chim. Acta 364 (1998) 19.

[128] I.R. Pereiro, A. Wasik, R. Lobinski, J. Anal. At. Spectrom. 13 (1998) 743.
[129] M.H. Akhtar, M. Wong, S.R.H. Crooks, A. Sauve, Food Addit. Contam. 15 (1998) 542.
[130] L.G. Croteau, M.H. Akhtar, J.M.R. Belanger, J.R.P. Pare, J. Liq. Chromatogr. 17 (1994) 2971.
[131] M.H. Akhtar, L.G. Croteau, C. Dani, K.A. ElSooud, Spectroscopy (Amsterdam) 13 (1997) 33.
[132] A. Bouaid, A. Martin Esteban, P. Fernandez, C. Camara, Fresenius J. Anal. Chem. 367 (2000) 291.
[133] N. Carro, C.M. Garcia, R. Cela, Spectroscopy (Amsterdam) 13 (1997) 61.
[134] N. Carro, C.M. Garcia, R. Cela, Analyst 122 (1997) 325.
[135] S.J. Stout, A.R. daCunha, G.L. Picard, M.M. Safarpour, J. Agric. Food Chem. 44 (1996) 3548.
[136] A. Kovacs, K. Ganzler, L. Simon Sarkadi, Z. Lebensm.-Unters.-Forsch. 207 (1998) 26.
[137] L.E. Garcia Ayuso, J. Velasco, M.C. Dobarganes, M.D. Luque de Castro, J. Agric. Food Chem. 47 (1999) 2308.
[138] Y.W. Wang, M. Bonilla, H.M. McNair, M. Khaled, J. High Resolut. Chromatogr. 20 (1997) 213.
[139] M.H. Akhtar, L.G. Croteau, Analyst 121 (1996) 803.
[140] Z. Vryzas, E.N. Papadakis, E. Papadopoulou-Mourkidou, J. Agric. Food Chem. 50 (2002) 2220.
[141] F. Lanza, B. Sellergren, Chromatographia 53 (2001) 599.
[142] J. Jodlbauer, N.M. Maier, W. Lindner, J. Chromatogr. A 945 (2002) 45.
[143] A. Molinelli, R. Weiss, B. Mizaikoff, J. Agric. Food Chem. 50 (2002) 1804.
[144] J.S. Fritz, J.J. Masso, J. Chromatogr. A 909 (2001) 79.
[145] E.M. Thurman, M.S. Mills, in: Solid Phase Extraction: Principles and Practice, Wiley Interscience, New York, 1998, p. 384, ISBN 0-471-61422-X.
[146] J.S. Fritz, in: Analytical Solid Phase Extraction, Wiley–VCH, New York, 1999, p. 224, ISBN 0-471-24667-0.
[147] M.C. Hennion, J. Chromatogr. A 856 (1999) 3.
[148] M.E. Leon-Gonzalez, L.V. Perez-Arribas, J. Chromatogr. A 902 (2000) 3.
[149] F.J. Dos Ramos, J. Chromatogr. A 880 (2000) 69.
[150] V. Ruiz-Gutierrez, M.C. Perez-Camino, J. Chromatogr. A 885 (2000) 321.
[151] A. Sides, K. Robards, S. Helliwell, Trends Anal. Chem. 19 (2000) 322.
[152] J. Pawliszyn, Solid Phase Microextraction, Theory and Practice, Wiley–VCH, New York, 1997.
[153] H. Kataoka, H.L. Lord, J. Pawliszyn, J. Chromatogr. A 880 (2000) 35.
[154] J. Pawliszyn (Ed.), Applications of Solid Phase Microextraction, Royal Society of Chemistry, London, 1999, p. 349, Chapters 26–32.
[155] J. Adachi, M. Asano, Y. Ueno, J. Chromatogr. A 881 (2000) 501.
[156] E. Mataix, M.D. Luque de Castro, J. Chromatogr. A 910 (2001) 255.
[157] J.J. Jimenez, J.L. Bernal, M.J. del Nozal, L. Toribio, E. Arias, J. Chromatogr. A 919 (2001) 147.
[158] C. Dominguez, D.A. Guillen, C.G. Barroso, J. Chromatogr. A 918 (2001) 303.
[159] T. Luan, G. Li, Z. Zhang, Anal. Chim. Acta 424 (2000) 19.
[160] R. Rial Otero, C. Yague Ruiz, B. Cancho Grande, J. Simal Gandara, J. Chromatogr. A 942 (2001) 41.
[161] E. Marengo, M. Aceto, V. Maurino, J. Chromatogr. A 943 (2001) 123.
[162] M. Mestres, O. Busto, J. Guasch, J. Chromatogr. A 945 (2002) 211.
[163] C. Sala, M. Mestres, M.P. Martì, O. Busto, J. Guasch, J. Chromatogr. A 880 (2000) 93.
[164] T. Herraiz, J. Chromatogr. A 871 (2000) 23.
[165] S.A. Lazarus, G.E. Adamson, J.F. Hammerstone, H.H. Schmitz, J. Agric. Food. Chem. 47 (1999) 3693.
[166] D.E. Breithaupt, Food Chem. 74 (2001) 521.
[167] E. Korta, A. Bakkali, L.A. Berrueta, B. Gallo, F. Vicente, J. Chromatogr. A 930 (2001) 21.
[168] R.U. Holt, J. Chromatogr. A 937 (2001) 107.
[169] L. Liberatore, G. Procida, N. d'Alessandro, A. Cichelli, Food Chem. 73 (2001) 119.
[170] M. Gonzales, M. Gallego, M. Valcarcel, J. Chromatogr. A 848 (1999) 529.
[171] C. Peres, C. Viallon, J.L. Berdagué, Anal. Chem. 73 (2001) 1030.
[172] R.T. Marsili, J. Agric. Food Chem. 48 (2000) 3470.
[173] P. Edder, A. Cominoli, C. Corvi, Mitt. Lebensmittelunters. Hyg. 91 (2000) 172.
[174] J.S. Elmore, D.S. Mottram, E. Hierro, J. Chromatogr. A 905 (2000) 233.
[175] F. Toribio, E. Moyano, L. Puignou, M.T. Galceran, J. Chromatogr. A 880 (2000) 101.
[176] J. Janoszka, U. Blaszczyk, L. Warzecha, M. Strozyk, A. Damasiewicz-Bodzek, D. Bodzek, J. Chromatogr. A 938 (2001) 155.
[177] H. Nakazawa, S. Ino, K. Kato, T. Watanabe, Y. Ito, H. Oka, J. Chromatogr. B 732 (1999) 55.
[178] K. Himata, M. Noda, S. Ando, Y. Yamada, J. Assoc. Off. Anal. Chem. 83 (2000) 347.
[179] J. Cai, B. Liu, Q. Su, J. Chromatogr. A 930 (2001) 1.
[180] C. Sala, M. Mestres, M.P. Martì, O. Busto, J. Guasch, J. Chromatogr. A 953 (2002) 1.
[181] R.C. Mejias, R.N. Marin, M. de Valme, G. Moreno, C.G. Barroso, J. Chromatogr. A 953 (2002) 7.
[182] J. Pino, M.P. Martì, M. Mestres, J. Perez, O. Busto, J. Guasch, J. Chromatogr. A 954 (2002) 51.
[183] A. Leitner, P. Zoellner, A. Paolillo, J. Stroka, A. Papadopoulou-Bouraoui, S. Jaborek, E. Anklam, W. Lindner, Anal. Chim. Acta 453 (2002) 33.
[184] M.C. Monje, C. Privat, V. Gastine, F. Nepveu, Anal. Chim. Acta 458 (2002) 111.
[185] M. Azenha, M.T. Vasconcelos, Anal. Chim. Acta 458 (2002) 231.
[186] C. Bicchi, C. Iori, P. Rubiolo, P. Sandra, J. Agric. Food Chem. 50 (2002) 449.
[187] T.T. Liu, T.S. Yang, J. Agric. Food Chem. 50 (2002) 653.
[188] R.A. Perez, C. Sanchez-Brunete, R.M. Calvo, J.L. Tadeo, J. Agric. Food Chem. 50 (2002) 2633.
[189] D.A. Lambropoulou, T.A. Albanis, J. Agric. Food Chem. 50 (2002) 3359.

[190] B. Moreno Cordero, J.L. Perez Pavon, C. Garcia Pinto, M.E. Fernandez Laespada, R. Carabias Martinez, E. Rodriguez Ponzalo, J. Chromatogr. A 902 (2000) 195.
[191] J.A. Jonsson, L. Mathiasson, J. Chromatogr. A 902 (2000) 205.
[192] G. Zurhelle, E. Mueller Seitz, M. Petz, J. Chromatogr. B 739 (2000) 191.
[193] P.E. Walser, Mitt. Geb. Lebensmittelunters. Hyg. 90 (1999) 751.
[194] R.J. Maxwell, E. Cohen, D.J. Donoghue, J. Agric. Food Chem. 47 (1999) 1563.
[195] G.Y. Eng, R.J. Maxwell, E. Cohen, E.G. Piotrowski, W. Fiddler, J. Chromatrogr. A 799 (1998) 349.
[196] E. Cohen, R.J. Maxwell, D.J. Donoghue, J. Chromatogr. B 724 (1999) 137.
[197] D. Moscone, R.A. Bernardo, E. Marconi, A. Amine, G. Palleschi, Analyst 124 (1999) 325.
[198] S. Sabbadin, R. Deraglia, G. Allegri, A. Bertazzo, P. Traldi, Rapid Commun. Mass Spectrom. 13 (1999) 1438.
[199] F.V. Silva, G.B. Souza, L.F.M. Ferraz, A.R.A. Nogueira, Food Chem. 67 (1999) 317.
[200] B.M. De Borda, J.M. Brewer, J. Camarda, J. Chromatogr. A 919 (2001) 59.
[201] S. Mannino, M.S. Cosio, Analyst 122 (1997) 1153.
[202] P. Kuban, B. Karlberg, Anal. Chem. 69 (1997) 1169.
[203] D. Heems, G. Luck, C. Fraudeau, E. Verette, J. Chromatrogr. A 798 (1998) 9.
[204] S. Morais, M.I. Alcaina Miranda, F. Lazaro, M. Plata, A. Maquieira, R. Puchades, Anal. Chim. Acta 353 (1997) 245.
[205] F. Sheu, Y.T. Shyu, J. Agric. Food Chem. 47 (1999) 2711.
[206] J.L.F.C. Lima, T.I.M.S. Lopes, A.O.S.S. Rangel, Anal. Chim. Acta 366 (1998) 187.
[207] A.O.S.S. Rangel, I.V. Toth, Analyst 123 (1998) 661.
[208] C. Linget, C. Netter, D. Heems, E. Verette, Analusis 26 (1998) 35.
[209] S. Gorinstein, M. Zemser, F. Vargas Albores, J.L. Ochoa, O. Paredes Lopez, C. Scheler, J. Salnikow, O. Martin Belloso, S. Trakhtenberg, Food Chem. 67 (1999) 71.
[210] N. Torto, J. Mwatseteza, G. Sawula, Anal. Chim. Acta 456 (2002) 253.
[211] R. Carabias Martinez, E. Rodriguez Gonzalo, E. Hernandez Fernandez, J. Hernandez Mendez, Anal. Chim. Acta 304 (1995) 323.
[212] M.M. Delgado Zamarreno, A. Sanchez Perez, M. Bustamante Rangel, J. Hernandez Mendez, Anal. Chim. Acta 386 (1999) 99.
[213] M. Luque, E. Luque-Perez, A. Rios, M. Valcarcel, Anal. Chim. Acta 410 (2000) 127.
[214] R. Carabias Martinez, E. Rodriguez Gonzalo, P.H. Paniagua-Marcos, J. Hernandez-Mendez, J. Chromatogr. A 869 (2000) 427.
[215] L. Bovanova, E. Brandsteterova, J. Chromatogr. A 880 (2000) 149.