Recent achievements and applications of solid — phase microextraction in pesticide residues analysis in food

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Abstract: The current trend in sample preparation methods is devoted to minimizing or eliminating the volume of extractive solvent. In this review, the focus on solid phase microextraction (SPME) as a solvent free sample preparation method for the isolation of pesticides residues in different food matrices is given. To achieve satisfactory extraction efficiency, selection of the fiber coating is an important step in the method development. Here, recent trends in new supporting materials and new fiber coatings development are discussed. Finally, applicability of SPME for the pesticide residues analysis in various food matrices using mainly chromatographic methods is also reviewed.

Keywords: sample preparation, solid phase microextraction, pesticide residues analysis, food

Introduction

Pesticides are synthetic chemicals widely used to prevent and control pest and disease and their applications leads to higher production yields and quality of agricultural crops. One-third reduction in crop yield would be happened if pesticides are not used against pest. However, this process contaminates food and environment by these toxic chemicals (Bagheri et al., 2012). As a result, people are exposed to pesticide residues at low concentrations through the environment, their diets, etc. The extensive and indiscriminate use of pesticides is a major concern because of the potential harm that these compounds can cause to the environment and because of the known or suspected toxic effects in humans. By transformation through the food chain, pesticide residues bioaccumulate in animal and human body and eventually show their adverse effects, like cancer, hormone disruption, disturbances in the immune system, disturbances in the reproductive and endocrine systems, chronic kidney diseases, birth defect, neurological effects and other diseases (Bagheri et al., 2012; Chowdhury et al., 2013; Masiá et al., 2014). Food analysis is very important for the quality control of products and the determination of additives and toxic contaminants such as pesticides (Viñas et al., 2009). For food samples, pesticide residues have been regulated by several legislative authorities throughout the world, basically concerned with the quality, efficacy and safety in the use of pesticides, however, there is not a global harmonized legislation. Maximum residual limits (MRLs) for pesticides in fruit and vegetables were established by European Union (EU, Regulation (EC) No. 839/2008) (EC Directive 2005). In general, the MRLs are in the range of 0.01—10 mg/kg, depending on the combination of commodity and pesticide. The MRL value of banned compounds is considered as the minimum limit of detection (LODs) achievable (Masiá et al., 2014).

Food is a very complex matrix, and several procedures are typically used in order to prepare a food sample for the final gas chromatography (GC), liquid chromatography (LC) or other techniques (Kudlejova et al., 2012). Sampling and sample preparation prior to chromatographic analysis is one of the most critical steps of the whole analytical processes, depends on the type of matrix, especially for the analysis of trace levels of analytes in complex matrices (Zhao et al., 2011; Kudlejova et al., 2012). Consequently, there is a need to develop, time saving, fast, green and accurate analytical procedures capable of detecting low concentrations of pesticides in a variety of food matrices (Souza-Silva et al., 2013). In order to prepare a representative sample, all kind of matrices need to be homogenized prior to the isolation of the target analytes from the examined matrix (Kudlejova et al., 2012).

Efficient sample preparation requires minimal analyte loss; analyte recovered in a good yield; efficient removing of coexisting components; to avoid problems in chromatography systems; the procedure can be performed conveniently and quickly; and inexpensive. Furthermore, use of harmful chemicals and large amounts of solvent in the sample preparation step cause environmental pollution and health hazards for operators, and extra-operational costs for waste treatment (Kataoka et al., 2009; Martins et al., 2012).
Among conventional methods of sample preparation at the determination of pesticide residues in food, liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are the most conventional and still frequently used sample preparation method (Filho et al., 2008). LLE is time-consuming, tedious, and often requires large quantities of toxic organic solvents. SPE is difficult to be applied widely in complex sample matrix. SPE may be faster than LLE, but in the elution step, SPE column still requires to be washed by organic solvents, and the presence of particulate matter in food samples or biological samples easily results in plugging of the cartridges (Wang et al., 2008). Recent trends in the sample preparation have focused on miniaturization, automation, high throughput performance, on-line coupling with analytical instruments and low-cost operations through extremely low or no solvent consumption in order to minimize the environmental effect (Moliner-Martínez et al., 2009; Andraščíková et al., 2015). A number of sample preparation methods have been developed for the isolation and enrichment of analytes from different samples. In recent years, many applications in the field of pesticide residues analysis have been performed using liquid phase microextraction (LPME) such as single drop microextraction (SDME), dispersive liquid-liquid microextraction (DLLME) and hollow fiber liquid phase microextraction (HF-LPME) (Andraščíková et al., 2015). As an alternative, SPME has important advantages because it is a simple and rapid technique which minimizes the sample handling (Ruiz del Castillo et al., 2012). SPME requires little or no organic solvents, is easily automated, and can also improve the limits of detection. SPME encompasses sampling, extraction, preconcentration and introduction of the sample extract into the system of analyses in a single uninterrupted process, thus avoiding contamination of the sample extract (Filho et al., 2008).

The objective of this work is to present an overview of the recent achievements of SPME for the isolation of pesticides from different food matrices. New supporting materials and techniques for the preparation of SPME fiber coatings are mentioned and discussed together with different extraction and desorption modes of the SPME method. Combination of SPME with analytical instrumentation for pesticide residues analysis of food in order to reach fast and simple solventless “green” method is evaluated.

**Solid phase microextraction principle**

Solid-phase microextraction was introduced and developed by Pawliszyn (Arthur et al., 1990). The technique is based on the partition of the analyte between the sample matrix and a stationary phase that is a fibre coated with an extracting liquid (polymer) or solid (sorbent) phase and housed in the commercial holder (Melo et al., 2012). Equilibrium is reached between the concentration of the analyte in sample and the concentration of analyte sorbed on the fiber, depending on the distribution coefficient. After that, desorption of the analytes can be performed thermally into a heated injector for GC determination or with a solvent, mainly when LC is used (Pereira et al., 2014). Fiber is portable and the whole technique is easily automated. Except for special applications, SPME methods use existing injectors on GC instruments, and analytes are thermally desorbed into the inlet. In LC analysis, the desorption interface typically consists of a standard six-position valve with a special fibre desorption chamber and desorption is performed into the mobile phase (Kudlejova et al., 2012).

The main advantage of the SPME procedure with respect to other techniques is the fact that it uses no or little volume of extraction solvent. The SPME approach is relatively simple (sampling, extraction and sample concentration are integrated in one step), fast and cheap. SPME methods are suitable for liquid, solid and gaseous samples and typically use only small sample volumes (Kudlejova et al., 2012). SPME is an alternative extraction method to traditional techniques, due to solvent consumption, blanks reduction and a decrease in extraction time. This method does not require complete removal of the analyte from the liquid matrix and can be used for a wider range of applications than other techniques such as solid phase extraction (SPE), which requires an exhaustive extraction (Kataoka et al., 2009). Owing to its convenience, almost solvent-free operation and low cost, it has gained wide applicability as an analytical technique (Melo et al., 2012).

**Parameters affecting SPME performance**

Several factors can influence the SPME efficiency. During the extraction step, the nature and the thickness of the fiber, the ionic strength and the pH of the aqueous sample, the stirring conditions and the temperature and time of the extraction must be optimized. During the desorption step, temperature of the GC injector and desorption time must be considered (Martins et al., 2012).

**Fibers coating in SPME**

Selection of a suitable fiber coating is the first step in SPME method development. The SPME fiber comprises a supporting substrate and thin layer of
sorbent material. Fused-silica fiber has been successfully used as supporting substrate since the first introduction of SPME technology (Feng et al., 2013). As the extraction efficiency of SPME is strongly dependent on the distribution constant of the target chemical between the fiber coating and sample matrix, the fiber coating is the most important and key factor in SPME (Yang et al., 2013; Ghaemi et al., 2014). Up to now, several coating fibers are commercially available for SPME, including polydimethylsiloxane (PDMS), polycrylate (PA), divinylbenzene (DVB), carboxen (CAR), carbowax (CW), and their copolymers (Luo et al., 2012). The different fiber materials offer a range of polarities to extract volatile and semi-volatile compounds. The materials have also been combined to create fibers able to sample compounds with a wider range of properties than if a single material had been used (Heaven et al., 2012). Four coating materials with different polarity (PA, PDMS, PDMS-DVB, DVB-CAR-PDMS and CAR-PDMS) were evaluated at the SPME extraction of 14 pesticides in mango (Heaven et al., 2012). Up to now, several coating fibers are commercially available for SPME, including polydimethylsiloxane (PDMS), polycrylate (PA), divinylbenzene (DVB), carboxen (CAR), carbowax (CW), and their copolymers (Luo et al., 2012). The different fiber materials offer a range of polarities to extract volatile and semi-volatile compounds. The materials have also been combined to create fibers able to sample compounds with a wider range of properties than if a single material had been used (Heaven et al., 2012). Four coating materials with different polarity (PA, PDMS, PDMS-DVB, DVB-CAR-PDMS and CAR-PDMS) were evaluated at the SPME extraction of 14 pesticides in mango (Heaven et al., 2012). PA coating was able to extract great number of pesticides and also capable to concentrate larger amounts of each extracted pesticide.

Among the commercial fibres, PA, PDMS/DVB and PDMS were evaluated (Song et al., 2014). PA was well suited for the diphenylamine determination when coupled with gas chromatography-mass spectrometry (GC-MS) and provided good sensitivity and dynamic range. Extraction performance of four different SPME coating, such as PDMS, PDMS/DVB, CW/templated resin (CW/TPR) studied and PA for pesticides with log P (partition coefficient) ranged from 0.8 to 4.12 (Melo et al., 2012). CW/TPR showed the best extraction performance for pesticides with higher value of log P (higher then two). Although these commercial fibers have been successfully applied in many fields, they still have some drawbacks such as expensive, thermal, mechanical and/or chemical instability, insufficient selectivity, and fragility of the fused-silica substrate, limited range of polarity and reuse times, which largely restricts the application of SPME (Luo et al., 2012). Their relatively low recommended operating temperature (generally in the range 200–270 °C) causes incomplete sample desorption and memory effect problems. In addition, swelling in organic solvent, relatively high thickness and difficult preparation in a routine laboratory are the further disadvantages of the studied fibers (Feng et al., 2013; Saraji et al., 2013). Numerous efforts have been made to produce novel SPME fibers with improved durability and satisfactory extraction capacity (Feng et al., 2013). Studies have been focused on fabricating low cost, simple, robust and long-life fiber for the target analytes. A variety of methods for the production of fibers have been developed such as sol-gel, and physical and electrochemical polymerization (Korba et al., 2013). Sol-gel technology can provide efficient incorporation of organic components into the polymeric structures in solution under the relatively mild conditions. Furthermore, strong chemical bonding between the polymer coating and the surface of fused-silica fiber improves the properties of SPME fibers (thermal stability, solvent stability, and lifetime). The coating porosity introduced by the sol-gel method enlarges the surface area of the fiber and allows for the utilization of thinner coatings to obtain higher enrichment during the extraction process (Wang et al., 2008; Ghaemi et al., 2014). Another advantageous possibility is to trap compounds containing different functional groups into the film by simply adding them into the sol-gel solution to prepare special SPME coating (Saraji et al., 2013).

Sol-gel technology has been applied in SPME as fibre coating for the extraction of various pesticides from food and environmental matrices (Cai et al., 2006; Wang et al., 2008; Ibrahim et al., 2010; Saraji et al., 2013). Three kinds of vinyl crown ether polar fibers, 3'-allyl benzo-15-crown-5 (B15C5), 4'-allyl dibenzo-18-crown-6 (DB18C6) and allyloxy ethoxymethyl-18-crown-6 (PSO18C6) were prepared by a sol-gel method and compared with commercial PA and PDMS-DVB fibers (Cai et al., 2006). New coatings showed higher extraction efficiency and sensitivity for organophosphorus pesticides (OPPs). Specifically, the B15C5 coating with larger polarity and selectivity than other studied fibers was the most effective for the target analytes. The higher extraction efficiency co-poly(hydroxy-terminated silicone divinylbenzene) (OH-TSO/DVB) fiber prepared by sol-gel technology in comparison to commercial available fibers was reported by Wang et al., 2008. Higher extraction efficiency of sol-gel derived OH-TSO/DVB fiber is attributed to the three dimensional porous network in the coating structure and also the polarizable phenyl groups in this fiber. The prepared fiber was applied to determine OPPs in pakchoi samples.

Despite advantages of the sol-gel technology, its SPME application has been limited due to the fragility of the silica fiber applied as the base of sol-gel technology (Saraji et al., 2013). The electrochemical method for preparing SPME fibers is simple, low cost, reproducible and convenient. It is especially suitable for fibers with metal wires as supporting substrate, because the metallic support can be directly used as one of the electrodes (Feng et al., 2013).
Polypyrrole (Ppy)/sol-gel coating fiber was prepared through electrochemical decomposition on stainless steel wire for the determination of OPPs in vegetable samples such as cucumber and lettuce (Saraji et al., 2013). Extraction efficiency of the Ppy/sol-gel composite fiber on a stainless steel wire was compared to Ppy fiber without sol-gel and two commercial fibers (PDMS, PDMS/DVB). The Ppy/sol-gel composite provides better efficiency than other studied fibers. The affinity of the Ppy/sol-gel coating for the analytes is due to the phenyl and hydrophilic groups in the coating that enhanced the π—π interaction, hydrogen bonding, and dipole–dipole interactions with the selected pesticides. Moreover, the sol-gel coating technique provides a porous structure, which increases the surface area of the fiber and affords a fast mass transfer rate in the film (Saraji et al., 2013). Ppy fiber for HS-SPME was fabricated by electrochemical polymerization onto a steel wire and in-situ produced fiber coating was proven to have high extraction efficiency for endocrine disruptor pesticides (Korba et al., 2013). Even after 250 usages, the fiber can still be used sensitively with a reasonable precision. Recently, new laboratory-made coating materials have been developed for SPME, such as molecularly imprinted polymers (MIPs), ionic liquids (ILs) and carbon nano tubes (CNTs). The vast majority of MIP-coated fibers, by far, are synthesized by the co-polymerization method, using methacrylic acid and its derivatives as monomers (Wang et al., 2013). Monolithic SPME fiber on the base of MIP for the selective extraction of triazine herbicides in rice and onion was prepared by copolymerization of methacrylic acid-ethylene glycol dimethacrylate imprinted with atrazine (Djozan and Ebrahimii, 2008).

A sol-gel technique was applied for the preparation of water-compatible MIP for SPME using diazinon as template and polyethylene glycol as functional monomer (Wang et al., 2013). The MIP-coated fibers had a life-time of more than 100 uses for monomer (Wang et al., 2013). The MIP-coated fibers had a life-time of more than 100 uses for monomer (Wang et al., 2013). The MIP-coated fibers had a life-time of more than 100 uses for monomer (Wang et al., 2013). The MIP-coated fibers had a life-time of more than 100 uses for monomer (Wang et al., 2013).

**Sampling mode**

In the last several years, SPME has been widely applied to the extraction of different classes of pesticide residues in a wide variety of food matrices (Table 1, 2). Direct immersion SPME (DI-SPME), where the extraction phase is placed directly in contact with the sample, is most frequently used for isolation of pesticide residues from fruit, vegetable, honey, milk and wine samples. Therefore, a pre-treatment step is essential prior to DI-SPME. As the fiber coating is in direct contact with the sample phase, it has high extraction efficiency, and it is suitable for semivolatile compounds (Yang et al., 2013). Many types of pesticides have been determined in food matrices using the DI-SPME method such as organochlorine (Moliner-Martinez et al., 2010), OPPs (Capobianco et al., 2005; Saraji et al., 2013), carbamates (Song et al., 2013), triazoles (Souza-Silva et al., 2008), triazines (Djozan et al., 2008; Tian et al., 2014) and strobilurines (Viñas et al., 2009). Alternatively to DI, in headspace-SPME (HS-SPME) mode, the extraction phase is placed in the headspace above the sample rather than immersed into the sample. HS is the preferred method for extraction of complex matrices, since no direct contact with sample protects the fiber coating from being damaged by high molecular mass and other non-volatile interferences present in the sample matrix. Albeit, HS-SPME is not suitable for all the cases; major limitations include low rates of extraction.
for poorly volatile or polar analytes (Souza-Silva et al., 2013). HS-SPME has been predominantly used for the determination of OCPs (Dong et al., 2005; Cai et al., 2006; Chai et al., 2009) and OPPs (Cai et al., 2006; Chai et al., 2009; Ibrahim et al., 2010; Wang et al., 2013; Sang et al., 2013) in different food matrices. The majority of food applications were performed in the DI mode (Table 1), while the HS mode (Table 2) is more frequently used in combination with fibers prepared by sol-gel techniques. SPME extraction by DI mode was predominantly used to extract pesticide residues from fruit samples using PDMS fibre. HS mode was used for determination of OPPs in cow milk without previous cleaning step (Rodrigues et al., 2011). Determination of OPPs in milk in DI mode has been preformed after adjusting pH with buffer and centrifugation (Cardeal et al., 2006). Filho et al., 2008 compared both, DI and HS extraction modes, using PA fiber for determination of pesticides residues in mangoes. HS extraction was efficient in extracting the more volatile compounds, such as OPPs and pyrethroids. In DI mode were successfully extracted all studied pesticides with lower sensitivity than HS mode. Extraction of OPPs in water and vegetable samples using fiber coated with polypyrrole/sol gel composite was performed in DI mode (Saraji et al., 2013). HS mode was used for extraction of organochlorine pesticides in wine using dodecylsulfate-doped polypyrrole (Ppy-DS) fiber (Korba et al., 2013). Selection of operating in HS or in DI mode depends on the volatility of the analyte and the matrix characteristics (Martins et al., 2013).

**Magnetic stirring**

Agitation of sample can accelerate mass transfer of the analytes between the aqueous sample and the SPME fiber and reduces the time required to reach equilibrium (Wu et al., 2010). At higher stirring rate (>800 rpm) in DI air bubbles can occur on the fiber surface resulting in lower extraction (Saraji et al., 2013). Low stirring rate (250 rpm) can be used in DI provided that the signal for pesticides was decreased at higher stirring rate (Filho et al., 2008). The most frequently used stirring rate for HS mode was 600 rpm (Rodrigues et al., 2011; Korba et al., 2013; Wang et al., 2013), nevertheless some studies were performed using lower (120 rpm) (Ibrahim et al., 2010) or higher stirring rate (800 rpm) (Chai et al., 2009). For HS mode stirring rate 600 rpm was used for determination of OCPs pesticides in radish (Dong et al., 2005). In DI mode stirring rate was often higher than 500 rpm up to 1700 rpm (Chai et al., 2009).

**Ionic strength and pH adjustment**

In many cases, an addition of NaCl is used to increase ionic strength of sample solution. The addition of salt may lead to a decrease of the extraction efficiency (Campillo et al., 2006). The decrease of extraction efficiency for OCPs and OPPs when the portion of NaCl exceeds 10 % because of predominant salt interaction with the analyte in the solution could be observed (Chai et al., 2009).

Modification of the ionic strength prior the solvent extraction or the SPME extraction did not affect significantly the instrumental response obtained (Cortés-Aguado et al., 2008). It was observed that for some compounds the sensitivity was increased by a salting-out effect during the solvent extraction (lindane, malathion, iprodione) but for others (bromopropylate, buprofezin, chlorpyrifos-methyl) their instrumental response was reduced especially when the addition of salt was done just before the SPME extraction. It was attributed to a negative influence of the deposition of salt on the coating material. Higher extraction efficiency in HS mode adding 1 g of potassium sulphate into the radish sample instead of NaCl was observed (Dong et al., 2005).

In the DI determinations, pH adjustment was also evaluated. Sodium hydroxide (Song et al., 2013; Munitz et al., 2013) and phosphate buffer (Campillo et al., 2006; Viñas et al., 2009) were predominantly used for the pH adjustment. Some determinations were performed at pH 7 (Djozan et al., 2008; Munitz et al., 2013) and lower (Cardeal et al., 2006; Viñas et al., 2009; Wu et al., 2010; Song et al., 2013). SPME of pesticides residues in fruit and vegetable (Tian et al., 2014) at pH 8 was performed. Nevertheless, pH 9.5 was used for the SPME extraction of pesticide residues in wine and subsequently for the determination by micellar electrokinetic chromatography (MEKC). Maximum selectivity at pH 5.5 for the determination of pesticides residues in tea was obtained by Wu et al., 2010. pH adjustment in SPME for the determination of pesticide residues in apples showed no significant influence on the extraction efficiency (Hu et al., 2008).

**Extraction temperature**

It is known that sample temperature has a double influence; high temperature increases the diffusion coefficient of analytes in aqueous medium and shortens the extraction time (Campillo et al., 2006). On the other side, high extraction temperature normally improves extraction rate, but simultaneously reduces the distribution coefficient and extraction sensitivity (Wu et al., 2010). Extraction temperature for HS-SPME determination of pesticide residues in fruits and vegetable should be optimized (Chai...
The extraction time of 60 °C was found to be the optimum. This is due to the fact that the extraction efficiency decreases when the extraction temperature exceeds 60 °C as a result of the degradation of the pesticides with increasing water vapor pressure in a gas tight vial and the formation of air bubbles. Also, SPME in HS mode was performed at room temperature (Ravelo-Peréz et al., 2007). Extraction efficiency HS-SPME for OCPs in radish was increasing up to 70 °C but decreased above 70 °C (Dong et al., 2005). Extraction efficiency of SPME in DI mode for multiclass pesticides in tea reached maximum at 50 °C (Wu et al., 2010). Extraction temperature is less important when working by DI of the fiber than HS mode. In general, higher extraction temperatures are used in HS mode in comparison to DI mode maintaining the same fiber and thickness. The most frequently used temperature in HS mode was 70 °C and in DI mode was 50 °C and most often at room temperature.

**Extraction time**

Extraction time is an important parameter for the extraction performance of analytes since SPME is an equilibrium-based technique. Generally, extraction efficiency is improved with the increase of extraction time (Wu et al., 2010). Extraction time has a direct relationship with the thickness and type of fiber coating, and the amount analyte extracted (Saraji et al., 2013).

**Desorption conditions (desorption temperature, time and solvent)**

The aim of thermal desorption is to ensure the vaporization of each pesticide and to avoid the thermal degradations and also to protect the fiber from degradation (Cortés-Aguardo et al., 2008). The analyte can be desorbed effectively under a higher temperature in a shorter time, but the stability and the lifetime of the fiber will be affected and the analyte may be decomposed if the desorption temperature is too high (Dong et al., 2005).

Therefore, thorough optimization of conditions is essential. Overall, 2 min were sufficient to allow the complete desorption of the analytes at 270 °C using laboratory made calix[4]arene fiber to determine OPPs in radish (Dong et al., 2005). Desorption time 3 min at 280 °C was sufficient to complete desorption of 3 OPPs from the fiber, but 4 min were chosen due to better reproducibility (Saraji et al., 2013). The desorption of 6 OCPs at desorption temperature of 200 °C and desorption time of 5 min was obtained by Korba et al., 2013. The complete desorption of analytes from the fiber enhances the detector response and eliminates memory effect (Filho et al., 2008).

Liquid desorption of analytes was performed using methanol (Ravelo-Peréz et al., 2007; Song et al., 2013), methanol in combination with water (Sagratini et al., 2007; Hu et al., 2008; Melo et al., 2012), and acetonitrile (Bagheri et al., 2012). Hu et al., 2008 performed liquid desorption using mobile phase (methanol:water, 50 : 50, v/v) as the desorption solvent for 5 min. Liquid desorption using mixture methanol:water (70 : 30) for 15 min was performed by work Blasco et al., 2011. The most frequently used desorptive technique in SPME for pesticide residues analysis is thermal desorption, especially in a combination with GC. Liquid desorption was used in the combination with HPLC (Sagratini et al., 2007; Hu et al., 2008; Blasco et al., 2011; Melo et al., 2012; Song et al., 2013), capillary electrophoresis (Hernández-Borges et al., 2005) and MEKC (Ravelo-Pérez et al., 2007). Despite, the use of liquid desorption with subsequent GC-MS analysis was reported (Bagheri et al., 2012).

To obtain the satisfactory extraction efficiency all mentioned parameters must be optimized.

**Sample pretreatment**

The pretreatment of the sample prior to SPME is the first step in a method development. Sample pretreatment is necessary to protect the fiber coating and avoid the fouling of the extraction phase by irreversible adsorption of macromolecules from the sample matrix, which could not only lead to a substantial decrease in the fiber lifetime, but also could possibly change the coating extraction properties (Souza-Silva et al., 2013). The simplest possible pretreatment was centrifugation and subsequently dilution (Sagratini et al., 2007) or filtration applied for juice samples (Hernández-Borges et al., 2005). Fruit and vegetable samples were homogenized, extracted by liquid-liquid extraction, diluted and centrifuged. However, some complex matrices like milk adversely affect DI-SPME performance in such circumstances. Fouling the SPME fiber reduces reproducibility of results due to large molecular weight compounds within the matrix either attaching to the fiber or hindering smaller compounds reaching the fiber. Fouling of the SPME fiber can be lessened by removing matrix components (e.g., saponifying milk fats), dilution of the sample, the adjustment of pH or increasing the solution salt content (Saraji et al., 2013).

**Interface to analytical instrumentation**

SPME can be conveniently interfaced to various analytical instruments such as GC (Wu et al., 2010; Tranchida et al., 2013), LC (Sagratini et al., 2007),
HPLC (Hu et al., 2008; Song et al., 2013), MEKC (Ravelo-Pérez et al., 2007) and electrophoresis (Hernández-Borges et al., 2005) with different detectors. The choice of the separation/detection system will largely depend on the goals of the analysis (Kudlejova et al., 2012b). Analysis of pesticides in vegetables has for many years been performed by use of GC especially coupled with nitrogen phosphorus detector (NPD), electron capture detector (ECD) and mass spectrometry (MS) or MS/MS. At present, MS is the most applicable due to the identification power. However, HPLC coupled to other less powerful detectors, that are easier to acquire and use, is preferable for polar, low volatile and/or thermolabile compounds that are not directly determinable by GC (Melo et al., 2012).

To date, SPME coupled with GC has been widely investigated for the determination of pesticide residues in different fruit matrices (Table 1, 2). SPME was more frequently used in connection with GC in comparison with LC or HPLC because of its solvent-less nature. When coupled with GC, SPME can integrate sampling, isolation, enrichment and injection steps, which makes the analysis much more convenient (Luo et al., 2012), in addition, it provides higher sensitivity, selectivity and better detection limits than LC in pesticide analysis, while CE provides a faster alternative to the chromatographic techniques.

LODs for SPME in HS mode obtained by GC-MS varied from 0.01 μg/kg (OPPs in fruit) to 2.88 μg/kg (OPPs in vegetable). Values of LODs from 0.005 μg/kg (strobulines in baby food) to 88 μg/kg (triazine herbicides in rice and onion) were obtained using SPME in DI mode.

GC coupled with ECD was used in a combination with SPME in HS mode for the determination of OPPs or OCPs in fruit and vegetable (Dong et al., 2005; Chai et al., 2009; Ibrahim et al., 2010), also, in DI mode for the determination of OCPs in biota samples (Korba et al., 2013) or for determination of bosalid in blueberries (Munitz et al., 2013). LODs varied from 0.0014 μg/kg (OCPs in radish) to 4.8 μg/kg (OPPs in fruit samples) utilizing SPME in HS mode as sample preparation method. In DI mode, LODs varied from 0.3 to 7.1 μg/kg (OCPs in biota samples). FPD (flame photometric detector) for determination of eight OPPs in fruit and vegetable samples with LODs in the range of 0.003—0.076 μg/kg was used by Cai et al., 2006.

The MS detector in combination with LC after SPME in the DI mode was used for the determination of carbamates and phenylurea in fruit juice (Sagratini et al., 2007) and OPPs and carbamates in honey samples (Blasco et al., 2011) with LOD 1—60 μg/kg.

HPLC was the most often used technique with the diode array detector in combination with SPME in the DI mode (Melo et al., 2012; Song et al., 2013). Limits of detection varied from 0.09 μg/kg (carbamate in apples) to 1540 μg/kg (multiclass pesticides in lettuce). The diode array detection was used with MEKC for the determination of multiclass pesticides in wine with LOD 54—113 μg/kg. SPME in combination with MEKC and DAD was studied for determination of 12 multiclass pesticides in wine with LOD from 54 μg/kg to 113 μg/kg.

Tab. 1. Applicability of solid phase microextraction (SPME) method in direct immersion mode (DI) for the determination of pesticide residues in different food matrices.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pesticides</th>
<th>Pretreatment</th>
<th>Fiber</th>
<th>Extraction conditions</th>
<th>Analytical technique</th>
<th>Real samples positive findings</th>
<th>LOD (LOQ) [μg/L]</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>Tea</td>
<td>13 multiclass pesticides</td>
<td>grinding, sieving, 1 g homogenized sample extracted with 10 mL acetone, centrifugation, 1 mL supernatant diluted to 20 mL with NaCl solution (15 % NaCl, w/v, pH 5.5)</td>
<td>SWCNTs</td>
<td>600 rpm, 50 °C, 40 min</td>
<td>TD-GC-MS</td>
<td>1500 μg/kg (chlorfenapyr) 17—54 μg/kg (l-cyhalothrin)</td>
<td>0.027—0.23</td>
<td>Fu et al., 2010</td>
</tr>
<tr>
<td>Brewed tea</td>
<td>30 pesticides</td>
<td>2 mL tea sample</td>
<td>PDMS</td>
<td>750 rpm, 60 °C, 5 min</td>
<td>TD-GC-QqQ MS –</td>
<td>–</td>
<td>0.004—1.47 (0.014—15.87)</td>
<td>Tranchida et al., 2013</td>
</tr>
<tr>
<td>Apple and orange juice</td>
<td>5 multiclass pesticides</td>
<td>filtration, centrifugation, 6 mL sample + 1.8 g NaCl (31 %, w/v), pH 6 adjusted with 0.1 mol/L NaOH</td>
<td>60 μm PDMS/DVB</td>
<td>800 rpm, ambient temperature, 150 min</td>
<td>LD (200 μL methanol, 200 μL 0.4 M HAc) CE-UV</td>
<td>15—25.5 μg/L 3.1—47</td>
<td>Hernández-Borges et al., 2005</td>
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</tr>
<tr>
<td>Fruit juice (orange, apple, cherry, strawberry)</td>
<td>6 carbamates, 3 phenylureas</td>
<td>centrifugation, 0.5 mL supernatant diluted with 0.5 mL water + 0.3 g NaCl</td>
<td>50 µm CW/ TPR, 60 µm PDMS/DVB, 85 µm PA</td>
<td>1000 rpm, 15 min</td>
<td>LD (methanol: water, 70:30, v/v) LC-MS</td>
<td>-</td>
<td>1–10</td>
<td>Sagratini et al., 2007</td>
</tr>
<tr>
<td>Fruit juice (orange, pineapple, peach)</td>
<td>54 multiclass pesticides</td>
<td>1 mL sample + 1 mL ethyl acetate, shaken, 0.5 mL ethylacetate extraction evaporation, 1 mL water:acetone (9:1, v/v) addition</td>
<td>100 µm PDMS, 65 µm PDMS/DVB</td>
<td>ambient temperature, 10 min</td>
<td>TD-GC-MS</td>
<td>48 µg/L (chlorpyrifos)</td>
<td>0.4–19.6</td>
<td>Córtes-Aguardo et al., 2008</td>
</tr>
<tr>
<td>Apples</td>
<td>Carbendazim, thiabendazole</td>
<td>centrifugation, resultant supernatant + 10 mL water, aliquot 4 mL for SPME</td>
<td>25 g mashed apples + 20 mL water, extraction with 0.5 mL water + 0.3 g NaCl</td>
<td>1100 rpm, 20 °C, 35 min</td>
<td>LD (methanol: water, 50:50, v/v) HPLC-FD</td>
<td>33–520 µg/kg (chlorpyrifos)</td>
<td>0.4–11</td>
<td>Hu et al., 2008</td>
</tr>
<tr>
<td>Apples</td>
<td>5 carbamate pesticides</td>
<td>2 g of pulp homogenized sample, ultrapure water addition with salt concentration of 16 % (w/v), pH adjusted to 5.5</td>
<td>60 µm PDMS/DVB</td>
<td>1000 rpm, 30 min</td>
<td>GC-μECD</td>
<td>-</td>
<td>1.33 (4.42)</td>
<td>Munitz et al., 2013</td>
</tr>
<tr>
<td>Blueberries</td>
<td>Boscalid</td>
<td>1 mL sample + 1 mL ethyl acetate, shaken, 0.5 mL ethylacetate extraction evaporation, 1 mL water:acetone (9:1, v/v) addition</td>
<td>25 g mashed apples + 20 mL water, centrifugation, resultant supernatant + 10 mL water, aliquot 4 mL for SPME</td>
<td>1100 rpm, 20 °C, 35 min</td>
<td>LD — 25 µl methanol HPLC-DAD</td>
<td>methiocarb</td>
<td>0.09–6 (0.4–11)</td>
<td>Song et al., 2013</td>
</tr>
<tr>
<td>Grapes and wine</td>
<td>Azoxystrobin, kresoxymethyl, trifloxystrobin</td>
<td>grapes crushed and filtered, 3 mL sample + 300 mg Na2SO4</td>
<td>100 µm PDMS</td>
<td>1500 rpm, 15 min</td>
<td>GC-μECD</td>
<td>-</td>
<td>73–181</td>
<td>Abdulráuf et al., 2013</td>
</tr>
<tr>
<td>Grapes and strawberries pulps</td>
<td>10 triazole fungicides</td>
<td>homogenization, 9 g fruit pulp</td>
<td>65 µm PDMS/DVB</td>
<td>500 rpm, 50 °C, 15 min</td>
<td>GC-ToFMS</td>
<td>9–500 µg/kg (grapes) 0.25–5 (strawberries) 0.5–5 (strawberries)</td>
<td>0.25–5 (strawberries) 0.5–5 (strawberries)</td>
<td>Zhao et al., 2011</td>
</tr>
<tr>
<td>Mango</td>
<td>14 multiclass pesticides</td>
<td>homogenization, 3 g sample + 10 mL isopropyl alcohol:water (20:80, v/v) with 5 % NaCl and pH 3, stirring at 1000 rpm for 10 min, centrifugation, upper layer completed with alcohol/water mixture to 10 mL</td>
<td>85 µm PA</td>
<td>250 rpm in alternate cycles, 50 °C, 30 min</td>
<td>TD-GC-MS</td>
<td>12.67–95.36 µg/kg</td>
<td>1–10</td>
<td>Filho et al., 2008</td>
</tr>
<tr>
<td>Cucumber</td>
<td>7 multiclass pesticides</td>
<td>chopped, homogenized, diluted with 25 mL water and shaken, mixture was transferred into a 50 mL volumetric flask made up with water, centrifugation, 1 mL for SPME</td>
<td>stainless steel wire coated by PDMS</td>
<td>150 rpm, 30 min</td>
<td>LD (600 µL acetonitrile) GC-MS</td>
<td>55–82 µg/kg (diazinone) 114 µg/kg (malathion) 90 µg/kg (penconazole) 187 µg/kg (phosalone)</td>
<td>8–60</td>
<td>Bagheri et al., 2012</td>
</tr>
<tr>
<td>Cherry tomato and strawberry, cucumber, garlic sprout, cole, cabbage, and tomato</td>
<td>4 triazines</td>
<td>2 g crushed sample×20 mL acetonitrile, extraction in water bath, ultrasonic bath, centrifugation, extraction with supernatant obtained by centrifugation repeated, concentration, dissolution with 1 mL methanol, reconstitution to 10 mL with NaH2PO4 buffer (pH 8.0), centrifugation, filtration</td>
<td>IL-calixarene-coated fibres</td>
<td>40 °C, 20 min</td>
<td>GC-FID</td>
<td>-</td>
<td>3.3–13</td>
<td>Tian et al., 2014</td>
</tr>
<tr>
<td>Sample</td>
<td>Pesticides</td>
<td>Pretreatment</td>
<td>Fiber</td>
<td>Extraction conditions</td>
<td>Analytical technique</td>
<td>Real samples positive findings</td>
<td>LOD (LOQ) [µg/L] [µg/kg]</td>
<td>Ref.</td>
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<tr>
<td>Tomato</td>
<td>25 multiclass pesticides</td>
<td>200 g sample + 240 mL acetonitrile:water (50 : 50, v/v) (for pyrethroids), 200 g sample + 240 mL water (for other pesticides), microwave heating, irradiation, cooling to room temperature, centrifugation, 9 mL aliquots of supernatant for SPME</td>
<td>60 µm PDMS/DVB (for pesticide soluble in water), 100 µm PDMS (for pyrethroids)</td>
<td>500 rpm, ambient temperature, 30 min (pyrethroids), 45 min (other pesticides)</td>
<td>TD-GC-MS</td>
<td>1—860 µg/kg</td>
<td>0.01—7.62</td>
<td>Guillet et al., 2009</td>
</tr>
<tr>
<td>Tomato</td>
<td>19 chlorinated pesticides</td>
<td>4 g homogenized sample + 18 mL ultrapure water, sonication</td>
<td>100 µm PDMS</td>
<td>400 rpm, 28 °C, 30 min</td>
<td>TD-GC- ECD</td>
<td>without positive findings</td>
<td>0.5—8 (5—30)</td>
<td>Mariani et al., 2013</td>
</tr>
<tr>
<td>Lettuce</td>
<td>10 multiclass pesticides</td>
<td></td>
<td>50 µm CW/TPR</td>
<td>1000 rpm, room temperature, 30 min</td>
<td>TD-GC- NPD</td>
<td>were not detected</td>
<td>280—1540</td>
<td>Melo et al., 2012</td>
</tr>
<tr>
<td>Cucumber, lettuce</td>
<td>3 OPPs</td>
<td>2 g homogenized sample + 2 mL water, ultrasonication for 10 min</td>
<td>stainless steel fiber coated with Ppy/sol gel composite film</td>
<td>800 rpm, 30 min</td>
<td>TD-GC- NPD</td>
<td>without positive findings</td>
<td>0.0015—0.01</td>
<td>Saraji et al., 2013</td>
</tr>
<tr>
<td>Cabbage, kale, mustard</td>
<td>11 OPPs</td>
<td>0.5 g chopped and homogenized sample + 2 mL methanol/acetone (1 : 1, v/v)+1 mL NaCl solution (10 %, w/v, dilution to 10 mL with water)</td>
<td>85 µm PA</td>
<td>1275 rpm, room temperature, 30 min</td>
<td>TD-GC- FPD</td>
<td>mustard (chlorpyrifos)</td>
<td>0.01—0.14 (0.03—0.42)</td>
<td>Sapahin et al., 2015</td>
</tr>
<tr>
<td>Onion, rice, bran</td>
<td>12 triazine herbicides</td>
<td>onion smashed to produce juice, ultrasonic bath, centrifuged, 3 mL supernatant for SPME, rice and bran dried and powdered + 12.5 mL methanol, ultrasonic water bath, filtration, evaporation, dissolved with 3 mL methanol, pH 7 adjusted by phosphate buffer</td>
<td>atrazine-MIP fiber</td>
<td>500 rpm, room temperature, 25 min</td>
<td>TD-GC-MS</td>
<td>onion 91.1—485 µg/L</td>
<td>20—88</td>
<td>Djozan et al., 2008</td>
</tr>
<tr>
<td>Onion, maize, onion</td>
<td>12 triazine herbicides</td>
<td>54 g crushed onion and maize stirred in a ultrasonic bath and centrifugated, 3 mL supernatant solution filtered 10 g dried and powdered rice seeds + 12.5 mL methanol dilution up to 25 mL with ultrapure water, ultrasonication, filtration, 5 mL filtered solution dried under N2 + 3 mL methanol to dissolve residues and diluted up to 10 mL with ultrapure water</td>
<td>ametryn-MIP fiber</td>
<td>room temperature, 20 min</td>
<td>TD-GC-MS</td>
<td>onion and maize 10—90 rice 30—230</td>
<td>10—90</td>
<td>Djozan et al., 2009</td>
</tr>
<tr>
<td>Baby food</td>
<td>7 strobilurin fungicides</td>
<td>25 g baby food dilution up to 10 mL water, sonication, filtration, 8 mL sample solution + 1 mL 0.15 M phosphate buffer pH 5 + water to adjust to final volume 14 mL</td>
<td>60 µm PDMS/DVB</td>
<td>1700 rpm, 60 °C, 40 min</td>
<td>TD-GC-MS</td>
<td></td>
<td>5—30</td>
<td>Viñas et al., 2009</td>
</tr>
</tbody>
</table>

**Future trends**

In the last years, there has been an increase of interest in microextraction techniques in the field of pesticide residues. Future trends within sample preparation for pesticide residue analysis go through miniaturization and automation and the use of solvent-free techniques, in order to reduce the time required and also decrease the possibility of introducing contaminants (Masiá et al., 2014). SPME can be considered as a well established and widely accepted technique and current research in this field focuses mainly on the development of new coatings and novel analytical strategies that contribute to improvement of sensitivity of the technique (Ramos, 2012). In recent years, additional efforts have been made to improve the SPME techniques through the development of new fiber coatings and materials through chemical bonding, physical and electrochemical deposition or other methods and introducing ILs, MIPs and CNTs as coating material.

We expect that future research in the testing of new coatings materials will continue. A development of fibers with new extraction phases extend the possibilities of SPME application for selective purposes or may result in the spreading SPME utilization in routine analytical laboratories.

**Conclusion**

In the last years, there has been an increase in the number of microextraction sample preparation method in the field of pesticide residues in food...
Tab. 2. Applicability of solid phase microextraction (SPME) method in head space mode (HS) for the determination of pesticide residues in different food matrices.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pesticides</th>
<th>Pretreatment</th>
<th>Fiber</th>
<th>Extraction conditions</th>
<th>Analytical technique</th>
<th>Real samples positive findings</th>
<th>LOD (LOQ) [µg/l] [µg/kg]</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit juice (peach, orange, pineapple)</td>
<td>46 multiclass pesticides</td>
<td>1 mL juice</td>
<td>65 µm PDMS/DVB</td>
<td>40 °C, 30 min</td>
<td>TD-MDGC-MS</td>
<td>-</td>
<td>-</td>
<td>Ruiz del Castillo et al., 2012</td>
</tr>
<tr>
<td>Apple juice, apples, tomato</td>
<td>8 OPPs</td>
<td>juice: dilution in water (1 : 50, w/v), 15 mL diluted 5 g NaCl, tomato-water dilution (1 : 50)</td>
<td>sol-gel fibre 80 µm B15C5</td>
<td>70 °C, 45 min</td>
<td>TD-GC- FPD</td>
<td>tomato</td>
<td>6.35 µg/kg (phorate) 0.003–0.075</td>
<td></td>
</tr>
<tr>
<td>Apple</td>
<td>4 pesticides</td>
<td>5 g homogenized sample, dilution in 10 % NaCl, ultrasoundication for 10 min</td>
<td>100 µm PDMS</td>
<td>60 °C, 30 min</td>
<td>TD-GC-MS</td>
<td>0.2 µg/kg (chlorpyrifos) 0.01–0.2</td>
<td></td>
<td>Abdulrauf et al., 2013</td>
</tr>
<tr>
<td>Apple Diphenylamine</td>
<td>cca 2 kg apples</td>
<td>placed in three 4.0 L glass jars, sealed and equilibrated for 180 min</td>
<td>100 µm PDMS</td>
<td>800 rpm, 60 °C, 30 min</td>
<td>TD-GC-ECD</td>
<td>6.2–56.8 µg/L 0.01–1</td>
<td></td>
<td>Chai et al., 2009</td>
</tr>
<tr>
<td>Fruit and vegetable samples</td>
<td>11 OPPs</td>
<td>1 g sample, addition 100 µL methanol/acetone (1 : 1) + 10 % NaCl</td>
<td>100 µm sol-gel hybrid PDMS-2-OHMe-18C6</td>
<td>120 rpm, 75 °C, 10 min</td>
<td>TD-GC-ECD</td>
<td>-</td>
<td>4.5–4.8</td>
<td>Ibrahim et al., 2010</td>
</tr>
<tr>
<td>Green apple, strawberries, grape</td>
<td>3 OPPs</td>
<td>30 g sample + 60 mL water, homogenization, sonication, dilution with 1 µL for SPME</td>
<td>100 µm PDMS</td>
<td>70 °C, 10 min</td>
<td>GC-MS</td>
<td>lettuce 49 µg/kg (chlorpyrifos) fruit 0.01–2.7 vegetable 0.17–2.88</td>
<td></td>
<td>Sang et al., 2013</td>
</tr>
<tr>
<td>Cucumber, green pepper, Chinese cabbage, eggplant and lettuce</td>
<td>5 OPPs</td>
<td>2 g homogenized sample + 1.2 g NaCl, dilution with 2 mL of distilled water</td>
<td>sol-gel MIP-coated fiber</td>
<td>600 rpm, 70 °C, 30 min</td>
<td>TD-GC-NPD</td>
<td>without positive findings 0.017–0.77</td>
<td></td>
<td>Wang et al., 2013</td>
</tr>
<tr>
<td>Radish</td>
<td>12 OCPs</td>
<td>100 g sample homogenized with 100 mL water, 25 g pyre diluted to 100 mL with water, 4 mL radish matrix solution + 1 g K₂SO₄</td>
<td>100 µm laboratory made fiber with C[4]/OH-TSO</td>
<td>600 rpm, 70 °C, 30 min</td>
<td>TD-GC-ECD</td>
<td>-</td>
<td>0.00148–0.174</td>
<td>Dong et al., 2005</td>
</tr>
<tr>
<td>Pakchoi</td>
<td>5 OPPs</td>
<td>20 g sample + 60 mL water, homogenization, dilution with water (1 : 20, w/v), 5 mL for SPME</td>
<td>65 µm sol-gel derived OH-TSO/DVB</td>
<td>75 °C, 5 min</td>
<td>TD-GC-NPD</td>
<td>-</td>
<td>7–70</td>
<td>Wang et al., 2008</td>
</tr>
</tbody>
</table>

matrices. One of them, SPME, have been driven by the move towards the low or none solvent consumption, automatization, sample throughput. In this paper, different fibers, supporting materials, SPME modes and applications of SPME in food analysis were discussed. The most frequently used are commercially available fibres consist of fused-silica fiber as supporting substrate. Moreover, some metallic materials are used as support materials/substrate for SPME fiber. Also, the new laboratory-made fibers prepared on the basis of MIPs, ILs and CNTs have found application in pesticide residue analysis. Higher extraction efficiency for fibers with laboratory-made coating in comparison to commercially available fibers was reported. HS mode was primarily used for determination of OPPs or OCPs in food matrices. Wider application of DI mode in food matrices has been recorded. The pretreatment of the sample prior to SPME is necessary to protect the fiber coating. SPME has been widely used in the analysis of pesticide residues, due to its simple operation and easy coupling to separation techniques (GC, LC) and detection techniques, mainly mass spectrometry. SPME coupled with GC utilizing thermal desorption was the most frequently alternative used. The further development and widespread application of SPME is expected in the future with the advance mainly in the area of new more selective coatings development.

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References


