Matrix compatible solid phase microextraction coating, a greener approach to sample preparation in vegetable matrices

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Abstract

This work proposes the novel PDMS/DVB/PDMS fiber as a greener strategy for analysis by direct immersion solid phase microextraction (SPME) in vegetables. SPME is an established sample preparation approach that has not yet been adequately explored for food analysis in direct immersion mode due to the limitations of the available commercial coatings. The robustness and endurance of this new coating were investigated by direct immersion extractions in raw blended vegetables without any further sample preparation steps. The PDMS/DVB/PDMS coating exhibited superior features related to the capability of the external PDMS layer to protect the commercial coating, and showed improvements in terms of extraction capability and in the cleanability of the coating surface. In addition to having contributed to the recognition of the superior features of this new fiber concept before commercialization, the outcomes of this work serve to confirm advancements in the matrix compatibility of the PDMS-modified fiber, and open new prospects for the development of greener high-throughput analytical methods in food analysis using solid phase microextraction in the near future.

Keywords: solid phase microextraction (SPME); PDMS-modified coating; analysis in vegetables; tomato; carrot; spinach; gas chromatography-mass spectrometry

1. Introduction

As advances in technology have brought forward innovative new methods in analytical chemistry, their applicability towards monitoring of organic residues in food has gathered increasing interest from both the scientific field and the food industry. Accurate detection of residues in food products imparts necessary information needed to implement safety measures in the protection of human health, in addition to yielding information that allows for a more rational use of these compounds. While vegetables and fruits constitute an essential part of a balanced diet, they are often among the principal causes of human exposure to harmful chemicals. In this context, analysis of organic residues in food occupies a significant role in both academic and industrial research (Fenik, Tankiewicz, & Biziuk, 2011).

In analytical chemistry, in order to obtain accurate analytical information regarding the system under study, proper sample preparation is essential. Generally, sample preparation consists of three steps: extraction, clean-up, and concentration. These make use of different techniques and suitable organic solvents. QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) is among the most recent and widespread analytical approaches for multiresidue sample preparation (Anastassiades, Lehotay, Štajnbaher, & Schenck, 2003). This approach is characterized by good flexibility, manifested in its many possible modifications and adaptions to different analytical applications (Bruzzoniti, Checchini, De Carlo, Orlandini, Rivoira, & Del Bubba, 2014). Although this technique is often introduced as a greener, faster, and cheaper approach to conventional methods, it is still characterized by the use of organic solvents and multiple stages, which affect its applicability towards full automation (Bruzzoniti, Checchini, De Carlo, Orlandini, Rivoira, & Del Bubba, 2014).

Solid-phase microextraction (SPME) is an established solvent-free analytical technique that integrates sampling, extraction, and concentration in a single step. SPME is currently counted among the most eco-friendly analytical techniques available today, and is often presented as a greener alternative to traditional approaches (Pena-Pereira, Kloskowski, & Namiesnik, 2015; Tobiszewski, Tsakovski, Simeonov, & Namieśnik, 2013). While SPME can be used in many modes, direct immersion (DI-SPME) and headspace (HS-SPME) extraction are the two approaches most extensively reported in the literature (Beltran, Peruga, Pitarch, Lopez, & Hernandez, 2003). SPME is a user-friendly technique that can be fully automated and combined with analytical instrumentation such as the gas chromatograph. Due to its advantages over traditional analytical techniques, SPME is becoming widely recognized in many analytical fields, and has been extensively used in environmental and clinical investigations

(Bojko, Cudjoe, Gómez-Ríos, Gorynski, Jiang, Reyes-Garcés, et al., 2012; Monteleone, Naccarato, Sindona, & Tagarelli, 2012; Naccarato, Gionfriddo, Sindona, & Tagarelli, 2014).

Regarding food analysis, SPME has yet to realize its full potential due to the complexity of the studied matrices, which have in the past limited SPME methodology in this field to mostly headspace approaches. Indeed, for analysis of complex matrices, SPME is generally preceded by an extraction step and suitable reconstruction of the extract in an aqueous medium (Fenik, Tankiewicz, & Biziuk, 2011; Gionfriddo, Naccarato, Sindona, & Tagarelli, 2012; Yang, Wang, & Li, 2013). These steps aim to reduce fouling of the fiber coating caused by irreversible adsorption of macromolecules on the extraction phase, which can result in changes in fiber properties and in the decreased extraction reliability of the fiber upon subsequent use (Ridgway, Lalljie, & Smith, 2007).

Recently, a new modification for the commercial PDMS/DVB fiber was proposed by Souza Silva and co-workers (Souza Silva & Pawliszyn, 2012). In this new configuration, a thin layer of PDMS is used to overcoat the extraction phase of a commercial fiber. The overcoating step was shown to yield better matrix compatibility between the fiber and complex matrices, allowing for direct immersion SPME sampling of grapes and strawberries (Silva, Lopez-Avila, & Pawliszyn, 2013; Souza-Silva & Pawliszyn, 2015).

In the present work, the new PDMS-modified fiber (i.e., overcoated fiber, OF) is proposed as a tool for analysis by direct immersion in vegetables. Tomato, spinach, and carrot were selected as target matrices in order to test this PDMS/DVB/PDMS fiber in new challenging analytical scenarios. The performance of the fiber was assessed by extractions in raw blended matrices that exposed the coating to samples comprised of varying pigmentations, water contents, interfering matrix compounds, and vegetable textures.

The robustness of the new matrix-compatible coating was compared with the commercial PDMS/DVB to evaluate potential enhancements provided by the new configuration. The most relevant SPME extraction parameters were optimized using both univariate and multivariate approaches. Fiber lifetime was evaluated by submitting the fiber to one hundred extractions and by stereomicroscope inspection of the coating surface.

The features exhibited by this matrix-compatible fiber open new paths toward greener analytical approaches for direct investigations in food matrices.

2. Experimental

2.1 Materials and Reagents

All pesticides standards (nitrobenzene, 1,3-dinitrobenzene, 2,6-dinitrotoluene, trifluralin, 4-phenylphenol, diazinon, chlorothalonil, parathion, pendimethalin, p,p'-DDE and diazepam) were of standard grade, and purchased from Sigma Aldrich (Oakville, ON, Canada). Tomato, spinach, and carrot were purchased at a local store.

2.2 Instrumentations

Analyses were performed on a Varian Saturn 3800 GC/2000 ITMS system and an Agilent 6890 gas chromatograph coupled to a 5973 MSD quadrupole mass spectrometer (Agilent Technologies, Mississauga, ON, Canada). Both instruments were equipped with a HP-5MS column (30 m, 0.25 mm i.d., 0.25 μm film thickness) (Hewlett-Packard, Avondale, PA). Helium was used as the carrier gas, and set to 1.0 mL/min. The 1079 injector was set at a temperature of 270 °C (unless otherwise specified). The column temperature program was initially set at 40 °C for 2 min, ramped at 10 °C/min to 180 °C, and then ramped at 20 °C/min to 300 °C and held for 5 min, for a total run time of 27 min. The ion trap was operated in full scan mode (MS). The MS operational conditions were as follows: electron ionization (EI) was maintained at 70 eV. Ion trap mass spectrometer temperatures were set at 180, 50, and 260 °C for the trap, manifold, and transfer line, respectively. For the single quadrupole mass spectrometer, the transfer line, MS Quad, and MS source temperatures were set at 280°C, 150°C, and 230°C, respectively. The multiplier voltage (1×10^5 gain) was 1600 V with a multiplier offset of +200 V. Automated analysis was performed using a CTC CombiPal autosampler (Zwingen, Switzerland) and the associated Cycle Composer software (version 1.4.0). The PAL was equipped with an SPME fiber holder, a temperature controlled six-vial agitator tray, and a fiber conditioning device.

The structure of the fibers was inspected using an optical stereomicroscope (Olympus SZX10, Olympus, Japan).

2.3 Preparation of overcoated SPME fiber

Overcoated fibers were prepared in-house according to the procedure reported by Souza Silva and co-workers (Souza Silva & Pawliszyn, 2012). Briefly, 5g of Sylgard 184 PDMS and 0.5g of curing agent were mixed into a polypropylene centrifuge tube. The mixture was centrifuged for approximately 3 min at 4000 rpm for degassing. The commercial PDMS/DVB fiber was immersed into the prepared PDMS mixture. Next, it was pulled out at a slow rate, passing through a micropipette tip with an aperture of

approximately 350 μm in diameter to ensure that a thin layer (approximately 25-30 μm) was formed, with the excess polymer being removed. The PDMS layer was cured by placing the coated fiber in a vacuum oven at 50 °C under N_2 for 12 h. The overcoating procedure was repeated twice to ensure complete and uniform coverage of the coating. Prior to use, each fiber was conditioned at 250°C for 1 h and visually inspected for uniformity and smooth surface coverage. If any defect was noted, coatings were discarded and new coatings prepared.

2.4 Sample preparation and analytical procedure

All analysed vegetables were first washed with deionised water, and then rinsed with nanopure water. Following, vegetables were dried, then crushed with the use of a blender. For tomatoes and carrots, stems were removed after the washing step. In the blending step, a proper amount of water (30%, w/w) was added to carrot and spinach samples in order to obtain better homogenization. For each sample, 7 g of blended matrix was weighted in a 10 mL vial and spiked with 7 μL of standard mix. Individual analyte concentrations (Table S1) were determined in order to ensure enough sensitivity for all analytes. Next, each sample was stirred for 30 seconds using a vortex and kept under agitation for 18 hours. This step allowed for proper equilibration and binding of analytes with the matrix components. All samples were submitted to direct immersion analysis by solid phase microextraction. For all the investigated matrices, extractions were performed with both a commercial 65 μm PDMS/DVB fiber and a PDMS/DVB/PDMS fiber in direct immersion mode for 40 min at 35 C°. Before each extraction, samples were incubated for 1 min in the agitation module of the autosampler, with stirring set at 300 rpm. Following extraction, the fiber was rinsed for 30 seconds in ultrapure water. The adsorbed analytes were thermally desorbed by introducing the fiber for 5 min into the GC injector, which was set at 270 C°. Following desorption, the fiber was washed for 2.5 min in a water/methanol mixture (50:50, v/v). After each batch of analysis, a cotton swab soaked with methanol was utilized to gently clean the fiber surface of any attached debris. Extractions of the target analytes in water (quality control, QC) were performed before and after fiber cleaning to assess coating performance. Analytes extractions in QC samples were carried out under the same sorption and desorption conditions used for vegetable matrices.

3. Results and discussion

Performance evaluations of the new PDMS-modified fiber versus the commercial PDMS/DVB fiber were carried out with spinach, tomato, and carrot as target food matrices, with different strategies for optimization of analytical parameters. Analyses were performed by direct immersion of the OF in blended vegetable samples without prior extraction, clean-up steps, or use of organic solvents. Eleven analytes (Table 1) with different physicochemical properties and belonging to various categories of possible food contaminants were selected as target analytes for this study. To address common issues that arise in analyses of complex matrices, a fiber-rinsing step and a cleaning step were integrated in the SPME routine before and after analyte desorption, respectively. Preliminary tests revealed that no significant analyte losses occurred as a result of rapid fiber rinsing in deionized water (30 seconds). Fibers were then submitted to a cleaning step of 2.5 minutes by immersion of fibers in a water:methanol (50:50, v/v) solution. The gas chromatographic method was optimized so as to achieve separation of all analytes with good peak shape within the shortest run time.

3.1 Spinach

Spinach is a green leafy vegetable characterized by a large surface area, high iron content, and an average content percentage of 91% water and 2% total dietary fiber (w/w). Spinach was selected as a sample matrix in this work to compare the performances of the PDMS/DVB fiber and PDMS-modified fiber in regards to direct immersion analysis of complex vegetable matrices characterized by a high content of chlorophyll and fibrous texture.

Preliminary extractions in samples of blended spinach spiked with the target analytes were carried out to assess optimum analyte concentrations to be used during fiber evaluations, as well as determine the optimum analyte-matrix binding equilibration time. Each sample was prepared by fortifying 7 g of blended spinach with 7 µL of spiking standard mixture. In order to obtain better matrix homogenization, ultrapure water was added to the blended leaves (50%, w/w). The individual concentrations of analytes are reported in Table S1. Optimization of the binding equilibration time was performed to accelerate the whole procedure while ensuring that any fluctuations in analyte response were related only to fiber performance, and not to chemical interactions occurring between matrix components. A batch of spiked samples was kept under vortexing to promote analyte-matrix equilibration, and analyzed after 1, 18, 24, and 36 hours. An equilibration time of 1 hour was chosen as a reference point in order to ascertain whether any possible binding effect took place. After 18 hours, the signals for all target compounds were observed to decrease and reach a steady value. Analyses were carried out after 24 and 36 hours to

ensure that sample analyte concentrations remained constant throughout the automated sequencing process. A particularly strong binding effect was observed for 1,3-dinitrobenzene and 2,6-dinitrotoluene; these two troublesome compounds could not be detected properly after just one hour of sample incubation. Consequently, they were excluded from fiber lifetime evaluations, and monitored only in quality control samples.

Despite the addition of a rinsing step, significant amounts of matrix compounds were detected in chromatograms. Consequently, using multivariate optimization of matrix dilution and stirring rate, SPME extraction parameters were further investigated so as to improve analyte signals and minimize the extraction of endogenous compounds. In particular, a central composite design (CCD), consisting of a 2^2 factorial design with four star points positioned at $\pm\alpha$ from the center of the experimental domain, was carried out. An axial distance α with a value of 2.0 was designated in order to fulfill the rotatability condition i.e., the design generates information uniformly in all directions. The design was completed with 2 experiments in the central point, and replicated once. Therefore, the complete design consisted of 20 experiments (i.e., $4+ (2 \times 2) + 2$, replicated one time). Dilution ratio (w/w) was evaluated in the range 30 – 80 %, whereas stirring rate values were surveyed between 250 – 600 rpm. An investigation of the obtained Pareto charts and desirability plots revealed that the two factors were not statistically significant for the majority of the analytes; only for the most polar analytes ($\log P \leqslant 3$) was the linear term of stirring rate noted to be statistically significant, yielding a negative coefficient. This result implies that low agitation values lead to an increase in analyte signals. An investigation of the response surface plots (see Fig. S1) showed that the behavior of p,p'-DDE, the most lipophilic of the studied analytes, differed significantly from the others: the signal of this compound increased at both higher matrix percentages with low stirring levels, and in more diluted samples with higher stirring levels. For this hydrophobic analyte, water dilution did not improve the partitioning between the sample and the coating; instead, it drove this analyte into matrix binding. In more diluted samples, higher agitation was shown to be necessary to promote the release of the matrix-bonded analyte and its mass transfer through the boundary layer. Optima

working conditions in terms of desirability scores were achieved at the minimum values for both considered variables (30%, w/w and 300 rpm) (Fig. 1).

Further optimization of SPME parameters was undertaken by obtaining extraction time profiles for the PDMS-modified fiber within a range of 5 to 60 minutes. An extraction time of 40 minutes was selected as a good compromise between sensitivity of the method for the analytes and extraction of matrix components.

The robustness and endurance of the new PDMS/DVB/PDMS fiber was evaluated throughout 100 DI-SPME extractions followed by GC-MS analysis. Analyses were performed in random sequence batches of 15 and 20 samples. A similar evaluation was carried out with a commercial PDMS/DVB fiber in order to compare the performances of the two coatings. Spiked spinach samples were prepared in accordance with the procedure reported in the "Sample preparation and analytical procedure" section. The PDMS-modified fiber allowed for completion of the whole sequence of analysis (100 extractions), whereas only 40 sequential extractions could be performed with the commercial fiber. A stereomicroscope was used to inspect both coatings after extractions. (Fig. S2 and Fig. S3). In comparison to the PDMS/DVB fiber, the overcoated fiber was shown to provide improved mechanical strength and an easier cleaning surface. Indeed, as illustrated in Fig. 2 and Fig. S2, although small unblended parts of the spinach leaves clung to the fiber during extraction, the coating was easily cleaned.

The smooth PDMS layer prevented irreversible sticking of matrix components. Contrariwise, the solid porous surface of the commercial PDMS/DVB could not be scrubbed properly (Fig. S3). The complexity of the matrix led to gradual fouling of the fiber coating and its consequent cracking. A comparison of the obtained results (Fig. S4 and Fig. S5) revealed a significant decrease in the extraction performance of the commercial fiber, along with poor repeatability.

After each batch of analysis, both the PDMS-modified fiber and the commercial fiber were submitted to an extraction in water (QC) before and after the cleaning step (Fig. S6 and Fig. S7). The obtained profiles confirm that the overcoated fibers retain their extraction efficiency for a longer number of runs in comparison to the commercial fiber. It is worth noting that the PDMS/DVB fiber showed an increase in extraction efficiency after manual cleaning; this confirms the important role of matrix components in limiting adsorption of analytes by the porous solid coating.

3.2 Tomatoes

Tomatoes are vegetables used globally for human consumption. They are counted among the most important foods in the Mediterranean diet, and have been shown to have healing properties associated with the presence of antioxidants such as carotenoids, polyphenols, and vitamins. Tomatoes are fruit vegetables with an approximate weight percent consisting of 93–95% water and 5–7% total solids. The total solid composition is comprised of roughly 80–90% soluble and 10–20% insoluble solids. The latter, which are derived from cell walls, are the greatest contributor to the texture of tomato products (Barrett, Beaulieu, & Shewfelt, 2010).

Although tomatoes have high water content, past SPME investigations in tomatoes have been mainly performed in headspace mode (Lo Feudo, Macchione, Naccarato, Sindona, & Tagarelli, 2011). Extractions in direct immersion have been carried out either following dilution of the matrix, or with the use of organic solvents, evaporation, and reconstruction of extracts in water (Mariani, Giannetti, Testani, & Ceccarelli, 2013; Ravelo-Perez, Hernandez-Borges, Borges-Miquel, & Rodriguez-Delgado, 2008; Yang, Wang, & Li, 2013). In light of this, the lifetime evaluation of the PDMS-modified fiber was performed by direct immersion in raw blended tomato samples so as to expose the coating to difficult extraction conditions; that is, analysis in an undiluted matrix. Preliminary analyses were carried out to assess the stability of the selected analytes in the matrix and determine the optimum binding equilibrium time.

Similarly to the procedure used for spinach, 7 grams of blended tomato were spiked with 7 µL of a standard mixture of analytes, then vortexed 1 hour before analysis. Individual analyte concentrations are reported in Table S1. Another batch of fortified samples kept under stirring was analysed after 18, 24, and 36 hours. All analyses were carried out in triplicate. After 18 hours, an overall decrease in the free available concentrations of the analytes was observed. The binding equilibrium was reached and kept in a steady state.

The amount of standard solution spiked to the sample necessary to obtain an adequate instrumental response was also investigated as a parameter. 7 µL, 14 µL, and 30 µL fortified samples were analysed by GC-MS after 18 hours of binding equilibration. An increase in the amount of spiked standard solution led to improvements in analyte signals, along with a decrease in the precision of the analyses. The higher presence of organic solvent, due to the larger amount of standard solution added to the sample, was observed to affect the binding equilibration, and consequently the free concentration of analytes. In light of these results, 7 µL was selected as the spiked amount for the lifetime evaluation.

A very strong binding effect for 2,6-dinitrotoluene and 1,3-dinitrobenzene was also observed for tomato samples. As these compounds could not be detected properly due to the low availability of their free form in the aqueous phase of the matrix, they were excluded from lifetime extractions but still monitored in the quality control (QC) samples carried out between batches.

A lifetime evaluation of the PDMS/DVB/PDMS fiber was performed throughout 100 DI-SPME extractions, followed by GC-MS analysis. The results were compared with those obtained using a commercial PDMS/DVB fiber. Spiked tomato samples were prepared according to the procedure reported in the "Sample preparation and analytical procedure" section. The one hundred successive cycles were divided in 6 sets of 15 or 20 analyses each. Both the PDMS-modified and the commercial fiber allowed for performance of the whole sequence of analyses. However, a gradual flaking of the PDMS/DVB coating was observed during the cleaning step, along with a progressive reduction in extraction efficiency, in particular for trifluralin and p.p'-DDE (Fig. S9, S11). This fouling of the polymer can be attributed to the combined effect of matrix components and the unsmooth surface of the coating (Fig. 3). Coating deterioration was not noticed for the PDMS-modified fiber due to the protective external layer that confers upon the fiber improved mechanical strength (Fig. S8, S10).

For both fibers, extractions of the investigated analytes in water (QC) were performed before and after each coating cleaning step. Fig. S12 and Fig. S13 illustrate that both coatings retained satisfactory and similar extraction performances throughout the analytical sequence. These results can be attributed to the endogenous high water content of the tomato, along with the rinsing and cleaning steps performed by the autosampler after each run. Implementation of these procedures is thus recommended in order to partially reduce the sticking of matrix components and improve fiber lifetime.

A comparison between quality control analyses and extractions in tomato outlined that the PDMS/DVB fiber showed a lower extraction capability in the tomato matrix for lipophilic analytes such as p.p'-DDE with respect to the same analyses in water. In this regard, although the PDMS/DVB coating appeared damaged, it still worked well in a less complex analytical environment.

During the lifetime evaluation, an inner colour change was observed for the PDMS/DVB/PDMS coating. This can be attributed to the irreversible absorption of tomato pigments. Inspection of the obtained chromatograms led to the identification of 2-penthylfuran (2-Pf) and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), which are compounds derived from the thermal degradation of matrix compounds (Maillard reaction) (Cosmai, Summo, Caponio, Paradiso, & Gomes, 2013; Kim & Baltes, 1996). Their presence can be reasonably related to the amount of matrix extracted

by the coating. Both compounds were more abundant in the analyses performed by the commercial PDMS/DVB fiber. This confirms the protective role of the PDMS external layer, since the porosity of the DVB particles was not clogged by tomato macromolecules, allowing the fiber to be better cleaned out. This trend was reversed in the second half of the lifetime sequence, probably due to the damage incurred in the external layer of the PDMS/DVB coating and the slight carry-over of matrix compounds by the PDMS/DVB/PDMS fiber (Fig. S20).

3.3 Carrots

Carrot is a root vegetable rich in bioactive compounds such as carotenoids and dietary fibers, as well as appreciable levels of several other health-promoting components. Its water content varies from 86 to 89% (w/w), whereas the total crude fiber content is approximately 1.2% (w/w) (Sharma, Karki, Thakur, & Attri, 2012). Carrots are an important source of phytonutrients such as β-carotene, which contribute along with other pigments to their natural color.

The use of solid phase microextraction towards investigations in carrots has been noted in few published papers, and mainly for volatile compound investigations. Only Berrada et al. reported the use of a polyacrylate fiber in direct immersion mode, and only after proper dilution of the carrot juice (Berrada, Font, & Molto, 2004).

Preliminary analyses on blended carrot samples fortified with the standard mix were carried out to assess the optimum analyte concentration to be used during fiber evaluation (see Table S1).

Similarly to the approach used for spinach, the addition of a proper amount of water was necessary to obtain satisfactory sample homogenization. The minimum suitable amount was optimized along with the binding equilibrium time. A matrix percentage over 30% (w/w) was observed to confer onto the samples a high viscosity that may easily cause fiber breakage during stirring. Furthermore, higher matrix amounts led to stronger binding of analytes and a consequent signal reduction. For determination of binding equilibrium time, spiked samples were kept under stirring and analyzed after 1, 18, 24, and 36 hours. Analyte concentrations were observed to reach steady values after 18 hours.

Lifetime evaluations of the new PDMS/DVB/PDMS fiber and the commercial PDMS/DVB fiber were performed throughout 100 DI-SPME-GC-MS analyses for each fiber, carried out in batches of 20 each. The obtained results for both fibers were then compared and contrasted. Carrot samples were prepared according to the optimized conditions reported in the "Sample preparation and analytical procedure" section. The PDMS overcoated fiber allowed for successful performance of the whole sequence of

analysis, whereas the commercial fiber permitted 97 analyses before incurring breakage at the site where the coating connects with the metal plunger. A stereomicroscope inspection of the PDMS/DVB/PDMS fiber confirmed the improved mechanical strength and surface cleanability of the new coating (Fig. 4, S14 and S15).

Similarly to the fibers used in the tomato study, stereomicroscope inspection revealed progressive flaking of the PDMS/DVB coating, associated with a decrease in extraction efficiency, in particular for pendimethalin and parathion (Fig. S16-S17). No coating fouling was observed for the PDMS-modified fiber, just a browning due to adsorption of matrix pigments; however, this irreversible extraction did not affect the performance of the overcoated fiber. Indeed, the PDMS-modified fiber kept satisfactory results compared to the commercial fiber, as demonstrated by quality control analyses.

An examination of the chromatograms of fortified carrot samples revealed the predominant presence of many lipophilic compounds such as terpenes, whereas, in agreement with findings previously reported in the literature, Maillard reaction products could not be identified (Wellner, Huettl, & Henle, 2009).

Analyses conducted with the PDMS/DVB/PDMS fiber yielded chromatographic profiles with higher signal intensities. This improved extraction capability, according to the SPME fundamentals, could be attributed to the protective action of the PDMS external layer for compounds that have a high diffusion coefficient through the thin PDMS layer. For compounds with distribution coefficients higher in the PDMS compared to the solid DVB coating, the mass transfer is promoted by the presence of the external PDMS layer. In this scenario, the overcoating layer is not only a protection, but actively contributes to enhance the extraction efficiency of the fiber. Different behaviors could be observed depending on the matrix-compound-coating system examined; that is, the thickness of the PDMS layer, the physicochemical properties of the analytes, and their interactions with the matrix constituents.

4. Conclusions

In the presented work, the new PDMS/DVB/PDMS fiber is proposed as a tool for analysis by direct immersion in vegetables. To date, DI-SPME has not yet been extensively explored in food analysis. However, due to the advantages afforded by the technique, such as its greener approach and full automation capabilities, new applications can be expected in the near future.

The robustness and endurance of this new coating were evaluated for analyses of vegetables (i.e. spinach, tomato, and carrot) that present different analytical challenges such as pigmentation, water content, interfering matrix compounds, and vegetable texture.

The new matrix-compatible coating exhibited very promising features attributed to the capability of the external PDMS layer to protect the commercial coating. The PDMS-modified fiber allowed for execution of whole sequences of analyses in all the surveyed matrices. Conversely, the commercial coating was unable to complete all the lifetime sequences, and exhibited greater fragility, especially for extraction in fibrous matrices. These differences are confirmed by the superior extractive performance of the overcoated fiber in the quality control samples, i.e., the overcoated fiber allowed for steadier analyte response trends compared to the commercial fiber.

The PDMS/DVB/PDMS fiber was shown to be suitable for use for more than one hundred extractions in raw blended vegetable samples. The overcoated fiber was revealed to provide advancements not only in terms of robustness and durability, but also in cleanability and sensitivity in respect to the commercial coating. The results of this work have contributed to the recognition of the superior features of the PDMS/DVB/PDMS fiber by Supelco, which has started to commercialize the overcoated fiber.

The outcomes of this work open new prospects toward greener analytical approaches in which DI-SPME is utilized for direct investigations in food matrices. Additional changes in the PDMS layer and coating may result in further development of the SPME technique.

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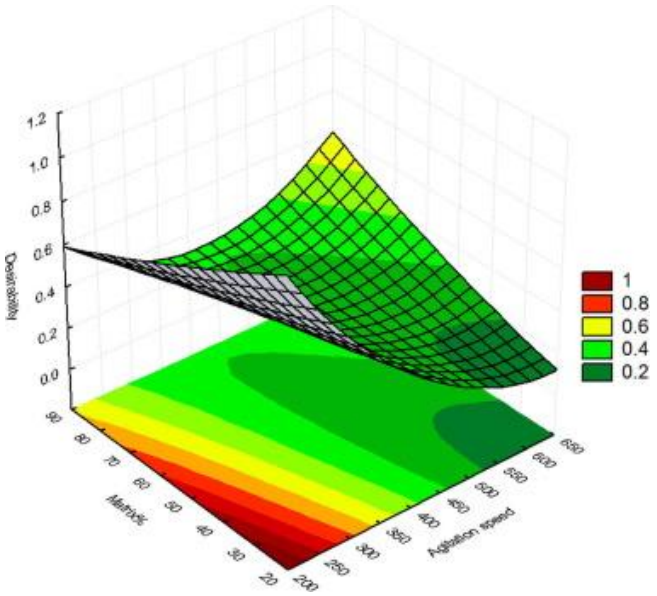

Fig. 1. Evaluation in spinach: desirability surface for matrix % (w/w) and agitation speed (rpm) optimization.

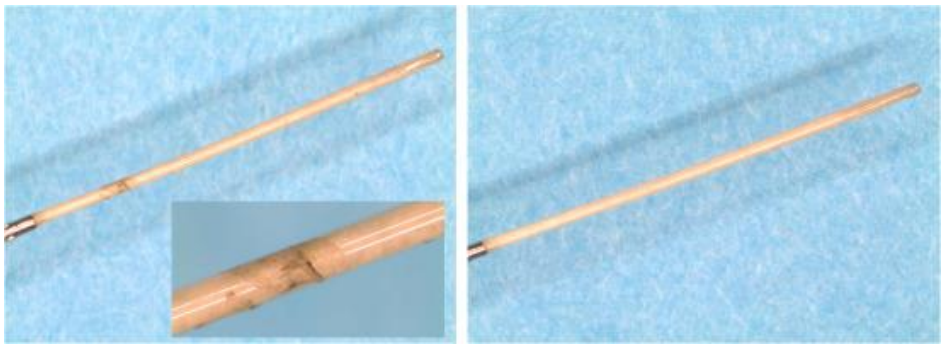

Fig. 2. PDMS/DVB/PDMS fiber lifetime evaluation in spinach: stereomicroscope images of the fiber before (left) and after (right) manual cleaning of the coating.

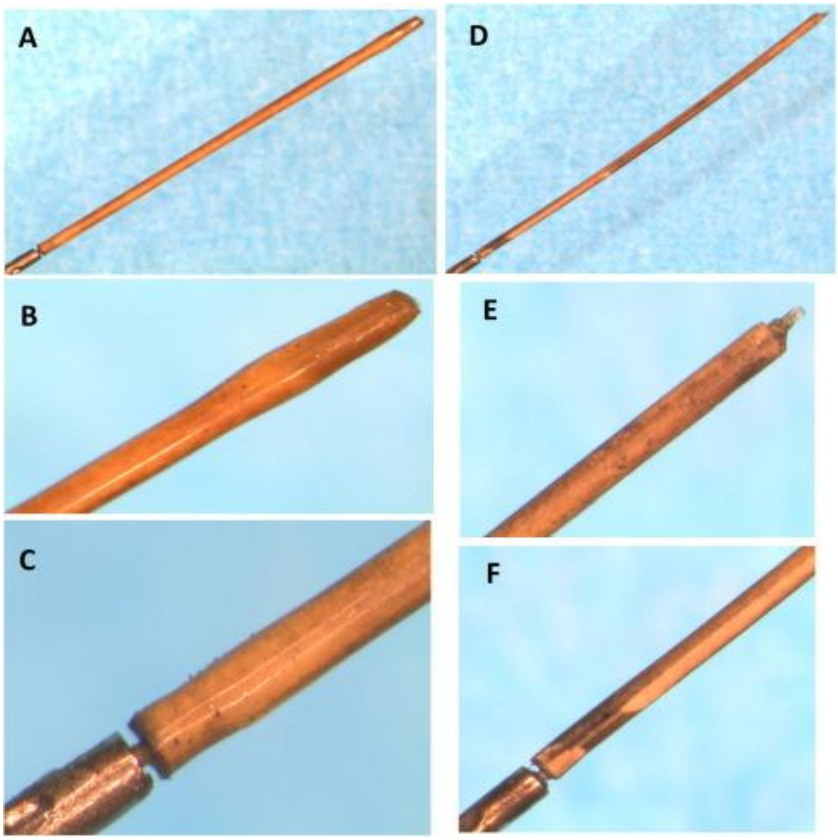

Fig. 3. Evaluation of coating lifetime in tomato: stereomicroscope images of the PDMS/DVB/PDMS (a, b, and c) and PDMS/DVB fibers (d, e, and f) after the penultimate batch of analyses.

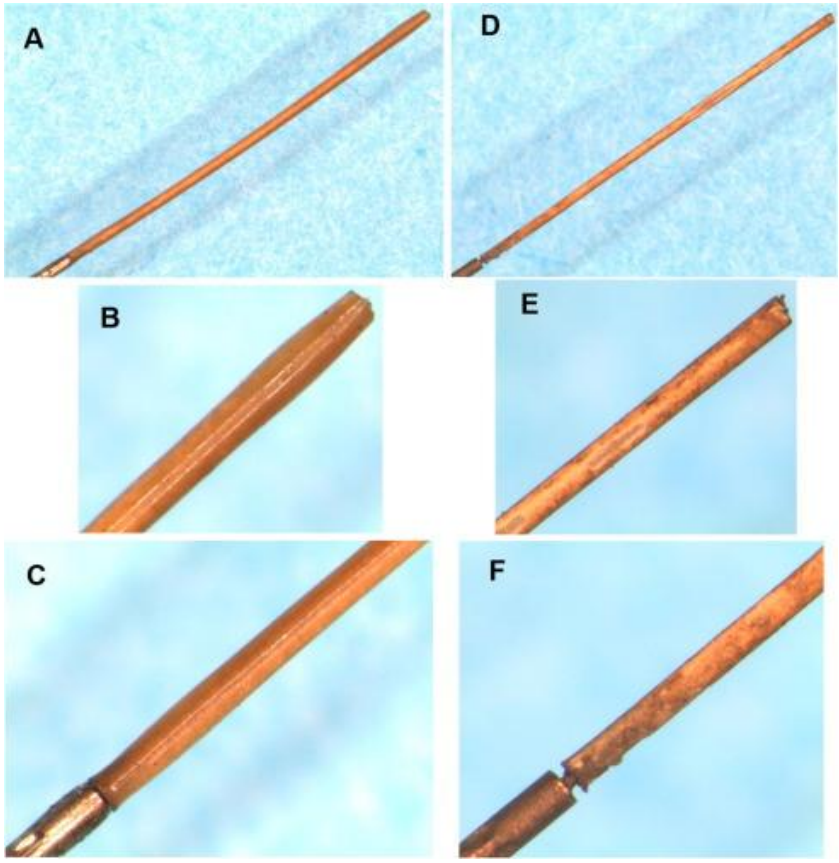

Fig. 4. Evaluation of coating lifetime in carrot: stereomicroscope images of the PDMS/DVB/PDMS (a, b and c) and PDMS/DVB fibers (d, e and f) after 80 analyses.

Table 1. Components in standard mixture, physicochemical properties and selected ions for quantification (data obtained from ChemSpider, http://www.chemspider.com)

Analyte	LogP	MW (Da)	Quantifier (m/z)
Nitrobenzene	1.9	123	77
1,3-Dinitrobenzene	1.43	168	168
2,6-Dinitrotoluene	2.42	182	165
Trifluralin	5.07	325	306
4-Phenylphenol	3.20	170	170
Diazinon	3.40	304	304
Chlorothalonil	2.94	266	266
Parathion	3.83	291	291
Pendimethalin	5.18	252	252
p,p'-DDE	6.00	318	318
Diazepam	2.80	256	256