Methodical evaluation and improvement of matrix compatible PDMS-overcoated coating for direct immersion solid phase microextraction gas chromatography (DI-SPME-GC)-based applications

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Abstract

The main quest for the implementation of direct SPME to complex matrices has been the development of matrix compatible coatings that provide sufficient sensitivity towards the target analytes. In this context, we present here a thorough evaluation of PDMS-overcoated fibers suitable for simultaneous extraction of different polarities analytes, while maintaining adequate matrix compatibility. For this, eleven analytes were selected, from various application classes (pesticides, industrial chemicals and pharmaceuticals) and with a wide range of log P values (ranging from 1.43 to 6).The model matrix chosen was commercial Concord grape juice, which is rich in pigments such as anthocyanins, and contains approximately 20 % of sugar (w/w). Two types of PDMS, as well as other intrinsic factors associated with the PDMS-overcoated fiber fabrication are studied. The evaluation showed that the PDMS-overcoated fibers considerably slowed down the coating fouling process during direct immersion in complex matrices of high sugar content. Longevity differences could be seen between the two types of PDMS tested, with a proprietary Sylgard ® giving superior performance because of lesser amount of reactive groups and enhanced hydrophobicity. Conversely, the thickness of the outer layer did not seem to have a significant effect on the fiber lifetime. We also demonstrate that the uniformity of the overcoated PDMS layer is paramount to the achievement of reliable data and extended fiber lifetime. Employing the optimum overcoated fiber, limits of detection (LOD) in the range of 0.2 - 1.3 ng/g could be achieved. Additional improvement is attainable by introducing washing of the coatings after desorption, so that any carbon build-up (fouling) left on the coating surface after thermal desorption can be removed.

Keywords: Solid-Phase Microextraction (SPME), Coatings, Gas Chromatography (GC), Food analysis, Pesticides Residues, Matrix-compatible.

1. Introduction

Since its introduction in early 1990s, SPME has been expanding to different areas such as biological, clinical, pharmaceutical, environmental and food studies [1–11]. Successful and reliable utilization of SPME for different applications relies on addressing the requirements for various and specific cases of study. Given the rising interest in the development of more environmentally friendly, yet very sensitive methods in the area of food analysis, SPME can be a perfect fit-for-purpose green sample preparation tool. Nonetheless, the drawbacks associated with commercially-available extraction phases in the context of insufficient matrix-compatibility still persist when such coatings are employed in the analysis of complex matrices such as food in direct immersion mode. Unquestionably, the main quest for the implementation of SPME to complex matrices has been the development of competent coatings that present compatibility towards the matrices being investigated while providing enough sensitivity towards the analytes being targeted [12–19]. This limitation propels research in the field of design and development of novel extractive phases focusing on high quality, efficiency, and long-term reusability. In this sense, we have reporteed previously the fabrication of a matrix-compatible fiber for direct immersion analysis in complex food matrix utilizyng GC-based platform [20]. The matrix-compatible coating was realized by incorporating a thin and smooth PDMS layer onto the surface of a solid commercially available SPME coating (PDMS/DVB). The results reported in our previous studies showed that the PDMS-modified coating exhibited enhanced compatibility towards complex matrix such as grape pulp, as well as satisfactory extraction capabilities towards triazole pesticides for extended series of extraction [20,21].

Nothwithstanding the rewarding results obtained for triazole pesticides, some additional challenges may emerge during SPME method development, not only due to the complexity of the matrix to be investigated, but also when the study requires the analysis of compounds from different classes bearing a wide diversity of physicochemical properties.

So far, Sylgard® has been used for the in-house fabrication of PDMS-overcoated fibers. Sylgard® is one amongst the plethora of commercial blends of PDMS available. In fact, a different type of PDMS is used to fabricate the commercially available PDMS fibers. Therefore, it is important to study the anti-fouling and sorption properties of these two PDMS formulations independently rather than assuming that they would perform similarly.

Therefore, the focus of this study is the thorough evaluation of PDMS-overcoated fibers capability to simultaneously provide adequate matrix compatibility while extracting analytes bearing different polarities. Two different types of PDMS, as well as other intrinsic factors associated with the PDMS-overcoated fiber fabrication are herein evaluated. For this, a mixture of analytes comprising a broad range of polarities and molecular weight is used. The model matrix chosen for this investigation, Concord grape juice, represent one of the most challenging classes of food commodities to analyze by DI-SPME coupled to GC-based platforms because of the high content of pigments such as anthocyanins, and sugars (approximately 20 % w/w), factors that can lead to a precocious deterioration of the coating surface and consequent alteration of its extraction capability.

2. Experimental

2.1. Chemicals and Materials

All contaminants standards used in this study were Pestanal®grade and kindly provided by Supelco (Bellefonte, PA, U.S.A.). PDMS/DVB Stableflex® fibers were purchased from Supelco. Sylgard (PDMS pre-polymer and curing agent) was purchased from Dow Corning (Midland, MI, USA). Deionized water used was from a Barnstead/Thermodyne NANO-pure ultra-water system (Dubuque, IA, U.S.A.).

2.2. Standards and Samples Preparation

Individual solutions of standards were prepared in methanol at 1 or 2 mg/mL, with the exception of chlorothalonil, which was prepared in dichloromethane. A working standard mixture was prepared containing each contaminant in the range of 2.5 to 150 µg/mL. The concentration of each analyte was carefully chosen in order to guarantee enough sensitivity for all analytes with all coatings tested. A detailed list of chemical structures, log P values, concentrations, and structures for analytes in the working mixture is presented on Supplementary Information (Table S1 and Figure S1). To evaluate the amounts extracted for each analyte, a stock standard mixture was prepared at 100 ng/µL in methanol. This stock solution was used for successive dilutions in order to obtain calibration solutions ranging from 0.5 to 80 ng/µL (8 levels). Liquid injections of calibration solutions were carried out in quadruplicates.

2.3. Preparation of PDMS-modified coating

Home-made PDMS-modified coatings were prepared as described elsewhere [20]. The only difference from the previous procedure is that the Sylgard 184 mixture was left to stand for 1h to start the cross-linking, allowing it to gain more viscosity before the coating procedure started.

This modification allowed for thinner and more homogenous coatings to be attained with only one immersion into the Sylgard solution. PDMS-modified coatings were prepared by coating once (~ 10 µm PDMS layer) or twice (~ 30 µm PDMS layer). All coatings were prepared at least in triplicate. Prior to their usage, each coating was conditioned at 250 ºC for one hour, and visually evaluated for uniformity and smooth surface coverage. If any defects were noted, coatings were discarded and new coatings were prepared.

Also, PDMS-modified coated PDMS-DVB fibers were produced by Supelco as prototype fibers using a highly crossed-linked PDMS phase (GC-PDMS). The process used in this report did not coat the ends of the fibers. The Supelco PDMS-modifed fibers shown in this paper were prototype fibers and do not reflect the SPME overcoated fibers that are now commercially produced by Supelco.

2.4. *SPME Procedure*

2.4.1. Analysis of Grape Juice

The different types of PDMS-modified DVB/PDMS coatings were evaluated using DI-SPME mode. An aliquot (200 g) of Concord grape juice was weighed into a 250 mL jar and spiked with 200 µL of working standard mixture. Proper seal of the jar was ensured, and the spiked matrix was pre-incubated at room temperature for 60 min prior to extraction to allow for the binding analytes-matrix to occur. Subsequently, aliquots of 7 g of spiked grape juice were weighed into 10-mL amber glass vials for SPME procedure. A 1 min incubation of the sample was performed in the agitation unit at 300 rpm and at 35 °C, followed by a 40 min extraction at 35 ºC, while stirring at 300 rpm. Following extraction, fibers were rinsed in water for 30 s, followed by desorption for 2 min at 270 ºC.

During the entire duration of this study the analysis of instrumental quality control (QC) samples, which were used to correct for any inter-day instrumental drift, was accomplished by extracting water samples spiked with the analyte of interest were extrated (n=3) using a PDMS/DVB at the beginning of each working day. This one fiber was exclusively dedicated for daily instrument QCs. Figure S2, in Supplementary Information, shows a typical chromatogram obtained during daily QC checks.

The coating longevity experiment sets were divided in batches of 20 grape juice extractions. At the beginning and at the end of each batch of grape juice extractions the fiber being assessed was submited to extractions in spiked water in order to evaluate the effect on the coating fouling on its extraction capabilities. Following each batch of 20 extractions, the fiber was taken out of the autosampler and microscope pictures were taken in order to track the build up of fouling onto the coating surface.

2.4.2. Analysis of Water Samples

The performance of each coating type was evaluated through extractions from spiked grape juice and water samples. Three water samples were extracted followed by 20 grape juice samples, and then another triplicate of water samples. The water samples at the beginning and at the end worked as fiber QCs in order to evaluate any variations in extraction capabilities due to coating degradation by fouling. An aliquot of 7 mL nanopure water was transferred into a 10-mL amber vial, spiked with 7 µL of working standard mixture, and vortexed to ensure the homogeneous distribution of analytes in the solution. The same SPME procedure was employed for the grape juice matrix, except that the pre-desorption rinsing step was omitted. All extraction time points were performed in triplicate.

2.5. Instrumentation

2.5.2. SPME-GC-IT/MS

Analyses of water and grape juice samples were performed using a Varian 3800 GC/4000 IT-MS system equipped with a SLB-5MS column (30 m, 0.25 mm I.D., 0.25 μm film thickness). Helium as the carrier gas was set to 1.5 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, then ramped at 20ºC/min to 300ºC and held for 5 min, for a total run time of 25 min. The ion trap analyzer was operated in full scan mode: electron ionization (EI) at 70eV; temperatures of 200, 50 and 280 °C for the trap, manifold and transfer line respectively; a mass range of 70-340 *m/z* was scanned; a minimum of three ions were chosen for identification of each analyte. Automatic gain control (AGC) was turned on with an AGC target value of 25000 counts; the emission current was 10 μA. Automated analysis was performed using a CTC CombiPal autosampler (Zwingen, Switzerland) using the associated Cycle Composer software (Version 1.4.0). The CombiPal autosampler was equipped with a SPME fiber holder, a temperature controlled six-vial agitator tray, and a fiber-conditioning device.

2.5.3. Micropictures and scanning electron microscopy (SEM)

After curing and throughout the longevity experiments, PDMS-overcoated coatings were inspected using an optical stereomicroscope to ensure that a thin layer of smooth surface was achieved.

Prior to examination under a SEM microscope, coatings were sputtered with ~10 nm of gold. SEM images of the coating were acquired using a LEO 1530 field emission SEM (Carl Zeiss NTS GmbH, Germany).

3. Results and discussion

3.1. Effect of overcoating thickness & PDMS type on extraction efficiency and coating reusability

Initially, the extraction efficiency of the tested coatings was investigated by evaluating the influence of matrix modification, namely pH and ionic strength (NaCl, % (*w:w*)). As presented in Supplementary Information Figure S3 and Figure S4, the effect of pH was insignificant for most analytes in both water and grape juice matrices, with the exception of those analytes that exhibit their ionized forms, or might undergo degradation such as chlorothalonil. The addition of NaCl caused a decrease in the amounts extracted from grape juice samples for most of the analytes. A possible explanation for this phenomenon is that a change in ionic strength, as a result of *salting-out*, enhanced binding of the analytes to the hydrophobic matrix components. Conversely, for the water matrix, the extraction efficiency for the most polar analytes such as nitrobenzene, 1,3-dinitrobenzene, 2,6-dinitrotoluene, 4-phenylphenol and diazepam was improved by adding NaCl.

Based on the results, further experiments were conducted without any matrix modification. Hence, the grape juice was analyzed at its natural pH (~3.5), and no salt was added. It is important to emphasize that the aim of this work is to evaluate coating reproducibility, longevity and robustness, rather than a complete optimization of the SPME method. For this reason, all factors within the experimental design, from the concentration of the analytes to SPME parameters, were set as to ensure appropriate sensitivity in order to guarantee a meaningful comparison between coatings.

The first batch of fibers analyzed comprised of (i) unmodified PDMS/DVB Stableflex, (ii) PDMS-modified PDMS/DVB using Sylgard (~ 30 µm PDMS layer), (iii and iv) Supelco's prototype GC-PDMS modified fibers (10 and 30 µm PDMS layers).

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Results of a preliminary investigation, presented in Figure 1 A-D, clearly demonstrate the necessity of investigating the effect of the PDMS outer layer, in terms of type and thickness, on the extraction efficiency of compounds with a wide range of polarities and diverse chemical functionalities. Indeed, considering the first set extractions (Figure 1 A-D), , a decrease in the extracted amounts of some more polar analytes in water is noticed for the PDMS-modified coatings as compared to the amounts extracted by the unmodified PDMS/DVB coating. This is due to presence of the outer PDMS layer, constituting an additional barrier that slows down the kinetics of extraction. For example, in the first set of extractions from water samples, compared to the unmodified PDMS/DVB for 1,3-dinitrobenzene, the amounts extracted were decreased by 14.6% (GC-PDMS 10µm), 23.4 % (GC- PDMS 30 µm), and 17.5 % (Sylgard - PDMS 30 µm). Similar trends were also observed for diazepam and 4-phenylphenol, except that the amount extracted by the GC-PDMS 10µm overcoated coating was not statistically different from the amount extracted by the PDMS/DVB coating. It is important to emphasize, however, that extractions were performed in pre-equilibrium regimen and therefore do not reflect the actual coating capacity at equilibrium conditions.

However, when investigating the effect of the PDMS layer on extractions performed in grape juices samples, a decrease in extraction efficiency was only observed for diazepam for GC-PDMS 30 µm (28.2%) and the Sylgard PDMS 30 µm (20%). Once again, the amount of diazepam extracted from grape juice by GC- PDMS 10µm overcoated coating was not

statistically different from the amount extracted by the PDMS/DVB coating. In the overcoated configuration, the polar analytes must first diffuse through the PDMS interface prior to adsorption in the solid DVB coating. Since this additional phase is a liquid polymer and the analytes have low diffusion coefficients in it, the mass transfer is slowed down and the extraction process is kinetically delayed. Moreover, owing to the hydrophobicity of this outer film, the concentration of polar analytes on the interface sample/PDMS is diminished, thus, also partitioning of analytes between sample and coating is decreased. As regarding the effect of PDMS layer thickness, the comparison of both GC-PDMS modified coatings with different outer layer thicknesses, namely 10 µm and 30 µm, clearly demonstrates that, within the pre-equilibrium extraction time of 40 min, a thicker PDMS outer layer leads to a more prominent decrease in the amount extracted for these polar analytes as compared to unmodified PDMS/DVB.

In agreement to these findings, Kloskoeski *et al*. presented a system comprised of a polyethylene glycol (PEG) coating restricted within a PDMS outer layer. The authors referred to the system as a membrane-SPME, where an external layer of PDMS of 25µm significantly slowed down the diffusion of the polar phenol analytes across the PDMS membrane, which could serve as a physical barrier as well as a concentrating medium, analogous to the extraction phase [22].

It is also worth noting the opposite effect of the PDMS overcoating on the extraction efficiency of more hydrophobic compounds, such as trifluralin, pendimethalin, and p,p'-DDE. For instance, the amount of trifluralin extracted from water samples increased in comparison to the amount extracted by commercial PDMS/DVB coating by 37 % for GC- PDMS 10µm, 19.1 % for GC-PDMS 30 µm, and 48.4 % for Sylgard PDMS 30 µm. A similar trend was also observed for grape juice sample extractions. Differently from what seen for polar analytes, these hydrophobic

compounds have a higher affinity for the PDMS polymer, and even though their diffusion is also impaired by this additional barrier, their accumulation on the interface sample/PDMS is greatly enhanced as compared to their accumulation on the sample/DVB interface when employing unmodified PDMS/DVB fibers. As a result, the liquid matrix boundary layer, rather than the PDMS layer, is the major contributor as the rate limiting step for the diffusion of these analytes from the matrix to the overall coating. Additionally, for these hydrophobic compounds, the PDMS layer acts also as a concentrating media, adding capacity to the overall sorbent, as also demostrated in recent studies on coating saturation thresholds [23]; thus, increased amounts extracted are obtained in comparison to amounts obtained by the unmodified PDMS/DVB coating, even in pre-equilibrium conditions (Figure 1).

Besides extraction efficiency, the most sought after feature of this new type of matrix-compatible coating is its ability to perform upwards to dozens of extractions from complex matrices, not only overcoming typical drawbacks of commercial coatings (e.g. precocious deterioration by fouling and limited reproducibility), but also enhancing method throughput in an automated fashion. In the context of food analysis, solid SPME coatings are the most commonly used; PDMS/DVB for pesticides, and DVB/Car/PDMS for a wider range of compounds. Giving the need to overcome the limitations associated with these coatings in regards to DI-SPME extractions from complex matrices, a compromise between extraction efficiency and matrix-compatibility would still be advantageous for the area of food analysis.

To investigate fiber reusability, each coating was subjected to 40 extractions of Concord grape juice samples, divided into 2 sets of 20 extractions. After each set of extractions, the fiber was submitted to an extraction in water (QC), and microscope pictures of the coatings were taken to assess the extent of fouling. As can be seen in Figure 2 A-C, for three analytes representing

different polarities, regardless of PDMS type or overcoating thickness, the addition of PDMS clearly improved the response obtained in the 40th extraction in grape juice, as compared to the 1st extraction. The lower and upper lines in the plots denote a ± 20% error interval. In terms of extracted amounts, the curve obtained for the GC- PDMS 10 µm outerlayer is the one that mostly resembles the profile exhibited by the non-modified fiber. The curves obtained for all other analytes are presented in Supplementary Information Figures S8 to S12.

The pronounced decrease in extracted amounts exhibited by Diazinon for all fibers was closely investigated. Spiked juice samples were allowed to stand overnight, and the extraction results showed the same decreasing trend. For this reason, one possible explanation would be that given the relatively high Henry's constant of diazinon and the headspace volume in the vial of approximately 3 mL, diazinon could have been transferred to the headspace, which would account for the decrease on the amount extracted over time (time of vial sitting on the autosampler tray prior to extraction). To evaluate this behaviour, 10 water samples were analyzed. Six samples were spiked and run immediately after spiking, two samples were run 12h after spiking, and another two samples were extracted 24 h after spiking. The results can be visualized in Supplementary Information Figure S13; as denoted by the graph, no decreased extraction amounts were observed, even after 24 h of samples sitting on the autosampler tray. Therefore, the observed behaviour may be attributed to degradation and/or interaction of diazinon with ascorbic acid present in grape juice, rather than a binding effect. In fact, previous data acquired for diazinon in pure grape pulp did not show such effect. As no meaningful data regarding coating longevity and reproducibility could be obtained with such data, the results from diazinon will be excluded from further discussion.

The results summarized in Figure 3 allow for a better visualization of the effect of the PDMS overcoating on the quality of data obtained from a batch of 40 grape juice samples. Based on a comparison between the amounts of analyte extracted in the 40th sample, as compared to the first sample, the extraction efficiencies also changed according to the type of PDMS used for overcoating, although it is evident that an improvement in reusability by the PDMS overcoating took place for all analytes. As presented by the experimental data, the Sylgard was shown to outperform the GC- PDMS when comparing both coatings with the same overcoating layer of 30 µm. Sylgard - also offered the best reproducibility, by means of RSD (%) for *n=40* samples; it provided RSDs < 20% for nearly all analytes, except for chlorothalonil, which yielded an RSD of 20.8 % (Supplementary Information Figure S14).

Matrix effect in DI-SPME-GC analysis of fruits and some vegetables may lead to a decrease in extraction capabilities, this can be mainly attributed to the attachment of sugars onto the coating surface; the attached sugars caramelize once brought into the hot injector for desorption, forming a layer of fouling. The extent of this fouling is a critical parameter in the acquisition of reliable analytical data and in the determination of the coating lifetime.

In order to test the longevity of the coatings, all coating types were further studied with another set of fibers throughout 100 extractions (except PDMS/DVB, n=60) in grape juice, divided in sets of 20 extractions. After each set of extractions, the fiber was submitted to an extraction in water (QCs), and coating pictures were taken. In agreement with the previously discussed results, the longevity studies have shown that the introduction of a PDMS outer layer slowed down the damaging process in the coating compared to the non-modified fiber (Figure 4 A-D). In fact, the unmodified PDMS/DVB coating could not be used beyond 60 extractions due to

extensive fouling of the coating that irreversibly damaged the coating making unusable for further extractions.

The behaviour exhibited by the Sylgard-overcoated fibers is worthy of note; after each fiber QC, the coating presented a higher response than the one obtained for the last extractions from the previous set of samples. For instance, after the 21st extraction, the extraction efficiency of this coating steadily decreased until the end of that set of samples (40th extraction). After extraction in water (QC), it once again recovered its extraction capability for the 41st extraction. The same declining behaviour was observed from the 41st to the 60th extraction, with extraction efficiency restored again at the 61st extraction. One of the possible explanations to this behaviour is that the water QC extraction (40 min) acted as a cleaning step, hence, restoring the extraction capabilities of the fiber. The effect of this water extraction was not as pronounced in the GC-PDMS 10 µm and the GC-PDMS 30 µm overcoatings because both extremities of these fibers had an exposed DVB phase (not coated with PDMS – see coating pictures in Supplementary Information Figure S7), that allowed irreversible fouling accumulation. Conversely, in the case of the Sylgard fibers, both extremities were overcoated with the polymer. This difference in fiber configuration is mainly due to the coating process that implemented for each fiber: The GC-PDMS fibers were prepared by overcoating DVB/PDMS fibers strands of ~ 60 cm, which was then cut into 1 cm segments that were subsequently assembled into the commercial SPME assembly. On the other hand, the Sylgard overcoat was performed by dipping commercial PDMS/DVB fibers (with their assembly) into the liquid polymer, thus assuring a complete seal of fiber's extremities. The abovementioned data, including the water QC results and coating pictures, add valuable information to the longevity studies, as well as also help to corroborate the aforementioned hypothesis.

3.2. Statistical Analyses

A statistical evaluation by means of Student's t-test (at 95% confidence level) was performed for each pair of fibers, as follows: Table S2 shows intra-fiber paired two samples for means (for each fiber, comparing the first 20 and last 20 extractions), and Table S3 shows inter-fiber: two-sample assuming unequal or equal variances (comparing each pair of fibers throughout the entire longevity study).

Statistically, the Sylgard-overcoated fiber extracted the same amount (at 95% confidence level) between the first 20 and last 20 extractions (averages) for nearly half of the analytes studied. Interestingly, the best performance was shown towards the early eluting, more polar analytes.

In terms of inter-fiber performance, it can be seen that the GC-PDMS 10µm and 30µm were statistically equal throughout the 100 extractions performed. However, none of these GC-PDMS overcoat fibers performed statistically the same as Sylgard-ovecoated fibers. Therefore, given the same type of PDMS, the thickness of the overcoat did not display a significant effect on the fiber longevity. Conversely, the type of polymer used may have had a significant influence on the fiber lifetime; an inspection of the results obtained for different types of PDMS overcoated at the same layer thickness (30 µm), demonstrated that Sylgard 184® and The GC-PDMS fibers behaved differently towards the investigated analytes. Indeed, considering the observed differences between the commercial blends of PDMS, it becomes impossible to blanket the sorption properties of these materials under just one name (i.e., PDMS); rather, these results corroborate previous findings, which indicate that each formulation needs to be independently assessed [24].

To better understand the performance differences observed within this work between these two distinct PDMS coatings, the specific attributes of each need to be further examined. While a

thorough examination of the particulars of these fibers may fall beyond the scope of the current work, a cursory overview of their distinctive fabrication processes can help shed some light into possible reasons for the observed discrepancies in performance.

In the present study, for the preparation of home-made coatings, after degassing, the polymer was used for overcoating PDMS/DVB fibers in their original commercial assembly (as detailed elsewhere [20]). Here, curing of the Sylgard® was carried out in a vacuum oven at 50 ºC for 12 h. It has been reported in the literature that both temperature and time of curing affects the properties of the final polymer [25,26]. Indeed, Johnston *et al.* reported that the hardness of the Sylgard increased linearly as curing temperature was increased [26]. Another feature of the Sylgard worth mentioning is the presence of fumed silica (SiO_2), which functions as a filler to increase its mechanical strength. However, the effect of SiO_2 nanoparticles in the polymer's properties towards analytes, such as permeability, has been a source of debate. One school of thought believes that SiO_2 nanoparticles somewhat induce the disruption of polymer chains, by means of less crosslinking, which increases the size of the free volume within this membrane through which molecular transport can occur, thus enhancing polymer permeability {FormattingCitation}[24,27,28]. Regardless, the mechanical properties of the polymer are not compromised, since SiO_2 glassy nanoparticles add significant stiffness to the polymer. Furthermore, it has also been reported that the fumed silica nanoparticles in the Sylgard® do not alter the surface morphology, and as such, a smooth and uniform coverage can still be attained [29].

In a retrospective fashion, taking into account the above-mentioned characteristics of both types of PDMS used for overcoating and the results obtained, it is evident that Sylgard provides better results compared to a high density GC-PDMS. The exact mechanism that leads to this

improvement is not fully understood; however, one can hypothesize that the enhanced hydrophobicity displayed by the Sylgard® PDMS as compared to the high density GC- could render the final polymer less prone to superficial attachment of polar sugars onto its surface resulting in an enhanced inertness towards matrix constituents and an easier cleaning of the cleaning of the coating surface. (See Supplementary Information Figure S17 for contact water angles obtained for both types of PDMS).

3.3. The importance of sealed extremities

As previously mentioned, one possible explanation for Sylgard's enhanced performance could be attributed to the sealed extremities obtained during the Sylgard overcoating process. Sealed extremities would ensure that no porous surface (e.g., DVB) is exposed to the matrix, thus, avoiding easier attachment of matrix constituents that induce irreversible fouling.

To continue this investigation towards the most robust coating, Sylgard overcoated fibers were fabricated with sealed and unsealed ends. To prepare the sealed fibers, after assembly of the overcoated segments, each fiber was dipped into a diluted Sylgard solution. It should be noted that for this investigation, a post-desorption fiber-washing step was added throughout the experiment for a lifetime evaluation, similarly to the step implemented in our previous studies [20,21]. After desorption, fibers were washed for 5 minutes in a water:methanol (50:50, v/v) solution.

The results obtained for the overcoated fibers prepared at Supelco's facilities (unsealed = open ends, and sealed= closed ends), as well as the results obtained with the Sylgard-overcoated fiber are shown in Figure 5 and in Supplementary Information (Figure S17). Significant differences can be observed between sealed and unsealed fibers. For instance, RSD%,(n=60) obtained for 1,3- dinitrobenzene were 43%, 16% and 9% for open ends, closed ends prepared with GC-

PDMS, and Sylgard, respectively. Indeed, the highest values for RSD% obtained with the unsealed fibers were observed for the most polar analytes.

Sealed fibers yielded acceptable results with all compounds, having RSD% (n=60) < 20% (except for 4-phenylphenol, RSD = 25%). Even though minimal, some differences were observed between sealed prepared with GC-PDMS and Sylgard, most likely related to the finishing used during the sealing of the fiber. By using the diluted Sylgard solution, DVB particles were only superficially covered by the PDMS film. In this scenario, the rough surface at the tip of the coating still allowed for the attachment of matrix particulate that would be eventually brought into the hot injector, thus, leading to fouling. The SEM pictures, reported in Supplementary Information Figure S18, evidences that such overcoating would not deter matrix particulates from entering the void volume between the coating and the fused-silica core, as is the case for the unsealed fibers. In brief, it can be concluded that ensuring total coverage of DVB particles and effectively sealing the fibers can lessen the extent of fouling, and as such, increase the longevity of the fiber.

In summary, it is of utmost importance for the realization of proper matrix-compatible PDMS-overcoated fibers that the PDMS layer have a uniform and smooth coverage throughout the coating. Likewise, the proper sealing of both coating's extremities are paramount to the achievement matrix compatibility extremities. It can be seen that fiber lifetime is prolonged if both ends are also coated with PDMS, so there is no porosity (DVB particles) exposed and susceptible to fouling occurrence (see Supplementary Information Figures S19 depicting an optimal PDMS overcoating sealing). Additionally, it is also important to mention that despite the best performance in matrix compatibility, the implementation of a washing step after desorption is needed when aiming to prolong the coating lifetime. This step has the purpose of washing

away any matrix macromolecule or particulates that might be superficially attached to the coating surface. Therefore, one can expect that the same washing procedure utilized in the present work (water:methanol, 50:50, %), which has been successful towards matrix characterized by high sugar content, may not be effective for other food matrices dealing with different challenges, such as high fat. In such cases, it is advisable to experiment with the most appropriate washing step for each case, keeping in mind the limited compatibility of PDMS regarding non-polar solvents.

3.4. Figures of Merit

The performance of the optimum fiber resulting from the study presented above, i.e. Sylgard® 184 overcoated PDMS/DVB fiber with both extremities sealed by the PDMS layer, was evaluated for the extraction of the target analytes in order to obtain the main analytical figures of merit. Sample preparation and experimental conditions are described in section 4.2.1. To construct the calibration curve, Concord grape juice was spiked at nine concentration levels ranging from 0.5 to 250 ng/g. The analytical parameters including the linear range, correlation coefficient (R^2) , precision (RSD, %), and limits of detection (LOD) and quantitation (LOQ) are listed in Table 1. LOD obtained ranged between 0.2 and 1.3 ng/g. Despite the fact that the objective of the present study is not the development of an analytical method through fine optimization of SPME parameters, the results presented in Table 1 clearly demonstrates the good reliability and potential of the PDMS overcoated coating as a powerful tool for determination of contaminants of various functionalities from food matrices *via* DI-SPME. In addition of being an environmentally-friendly option to other solvent-based extraction methods, the entire sample

preparation protocol, including fiber rinsing, can be performed by an autosampler which offers automated high-throughput analysis, ideal for routine analysis.

Table 1 Figures of merit for Concord grape juice analysis using Sylgard 184® overcoated PDMS/DVB fiber.

Analyte	Linear Range (ng/g)	R^2	LOD (ng/g)	LOQ (ng/g)	RSD (%)[a]	RSD (%)[b]	RSD (%)[c]
Nitrobenzene	5-250	0.9987	1.1	3.2	2.1	2.8	4.4
1,3-Dinitrobenzene	5-250	0.998	1.3	4.0	10.2	1.2	1.4
2,6-Dinitrotoluene	5-100	0.9975	0.5	1.4	4.4	5.9	6.1
Trifluralin	1-100	0.9978	0.3	0.9	11.9	5.2	2.4
4-Phenylphenol	1-100	0.9952	0.2	0.6	5.6	1.5	5.1
Chlorothalonil	0.5-100	0.9957	0.2	0.5	9.7	8.0	5.3
Parathion	0.5-250	0.9991	0.2	0.5	10.9	2.3	0.9
Pendimethalin	1-100	0.9937	0.2	0.7	11.3	2.9	7.2
p,p'-DDE	1-100	0.9998	0.3	0.8	6.6	4.2	5.5
Diazepam	5-250	0.9992	0.5	1.4	15.7	9.7	7.0
LOD calculated as 3.3 x (s/S), where s is the standard deviation of 10 replicates (spiked at lowest concentration), and S is the slope of the calibration curve.							
LOQ calculated as 10 x (s/S), where s is the standard deviation of 10 replicates (spiked at lowest concentration), and S is the slope of the calibration curve.							
a - concentration 5 ng/g (n=4)							
b- concentration 50 ng/g (n=4)							
c- concentration 250 ng/g (n=4)							

4. Conclusion

In this work we presented a critical evaluation of the PDMS-overcoated fiber for food-matrix analysis employing a wide range of analyte polarities, molecular weights, and functionalities. Although the evaluation was performed in one matrix, Concord grape juice, due to its high sugar content (~ 20%), this matrix can be assumed to be an appropriate model for high water and high sugar content fruits and vegetables. The evaluation showed that the PDMS-overcoated fibers considerably inhibited the damaging process onto the coating surface during direct immersion in complex matrices of high sugar content. The thickness of the outer layer did not seem to have a

significant effect on the fiber lifetime. However, the results outline significant differences in the rate of uptake (sorption mechanism) associated with the addition of the PDMS outer layer, especially towards the more polar analytes. Moreover, it has been shown that the uniformity of the overcoated PDMS layer, as well as proper sealing of both extremities, are paramount to the achievement of reliable data and extended fiber lifetime. It can be concluded that fiber lifetime can be prolonged if both ends are also coated with PDMS to avoid exposure to porosity (DVB particles), which decreases the likelihood of fouling. Additional improvement might be attainable by introducing washing of the coatings after desorption in addition to the pre-desorption rinsing step, so that any carbon build-up (fouling) left on the coating surface after desorption can be removed. The use of metal core for the fibers, as well as the development and implementation of a cleaning station to be added to commercial autosamplers would offer enhanced robustness, allowing for prolonged fiber use and cleaning.

The research presented herein supports the use of SPME for GC-based platforms in food analysis as a sensitive and cost-effective method. In future, this protocol can also be evaluated for the analysis of different food matrices posing different challenges, such as spinach, tomatoes and carrots (high pigment content), avocado (high fat content), legumes (low water content), and dairy products (high fat and protein content).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version.

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Figure Captions

Figure 1 - Comparison of analyte response from grape juice (1st and 20th extractions) and water (before and after grape juice extractions) using commercial PDMS/DVB fiber (A); using PDMS/DVB with Sylgard ® 30 µm overcoat (B); using PDMS/DVB with a 10µm GC-PDMS overcoat (C); and using PDMS/DVB with a 30µm GC-PDMS overcoat (D).

Figure 2 - Reusability profile of coatings subjected to 40 DI-SPME in Concord grape juice for1,3-Dinitrobenzene (A); Parathion (B); and Pendimethalin (C). Relative response has been calculated as the ratio between amounts extracted at each extraction and first extraction in grape juice.

Figure 3 - Comparison of changes in extracted amounts between 1st and 40th extractions in grape juice.

Figure 4 - Comparison of analytes responses from extractions in water (fiber QCs) obtained with PDMS/DVB (A); with the GC-PDMS 10 µm overcoat (B); with the GC-PDMS 30 µm overcoat (C); and with Sylgard 30 µm overcoat (D).

Figure 5 - Comparison of R.S.D, % obtained from 60 consecutive extraction cycles in grape juice with different PDMS-overcoated fibers.