Fabric Phase Sorptive Extraction as a Reliable Tool for Rapid Screening and Detection of Freshness Markers in Oranges

M. Aznar[1], S. Úbeda[1], C. Nerin[1,*], A. Kabir[2,**], K. G. Furton[2]

[1]Analytical Chemistry Department, GUIA Group, I3A, EINA, University of Zaragoza, Mª de Luna 3, 50018 Zaragoza, Spain.

[2]International Forensic Research Institute, Department of Chemistry and Biochemistry, Florida International University, 11200 SW 8th Street, Miami, FL 33199, USA

*Corresponding author. Tel: +34976761873, E-mail address: cnerin@unizar.es (C. Nerin)

**Corresponding author. Tel: +3053482396, E-mail address: akabir@fiu.edu (A. Kabir)

ABSTRACT

A simple, fast and sensitive analyte extraction method based on fabric phase sorptive extraction (FPSE) followed by gas chromatography-mass spectrometry (GC-MS) and ultra-performance liquid chromatography-quadrupole time of flight mass spectrometry (UPLC-QTOF-MS) analysis was developed for the analysis of 12 volatile compounds that represent most of the principal chemical families possessing different polarities and volatilities. Five FPSE media coated with different sol-gel sorbent chemistries having different polarities and selectivities were studied: long chain poly(dimethylsiloxane) (PDMS), short chain poly(tetrahydrofuran) (PTHF), Carbowax 20M (CW20M), short chain poly(dimethyl siloxane) (SC PDMS) and polyethylene glycol-polypropylene glycol-polyethylene glycol triblock copolymer (PEG-PPG-PEG). CW20M coated FPSE media was found to be the most efficient extraction media for the analytes of interest in the intended study. The developed methodology was applied to the analysis of orange juice obtained from fresh oranges and oranges after storing at 5ºC for two months in order to identify the best chemical markers, both volatiles and non-volatiles, attributed to the freshness of orange. For this purpose, aliquots of the same juice extracts were analysed by GC-MS as well as by UPLC-QTOF-MS. Monoterpenes and terpenoids, such as terpinene, citronellal or estragole were among the volatile compounds that endured the biggest decrease after the extended storage period. Three non-volatile compounds including one amide (subaphyllin) and two flavanoids (tangeretin and nobiletin) also showed a clear decrease in signal intensity (>70%) after orange stored for two months.

Keywords: Fabric phase sorptive extraction, oranges, freshness markers, screening, GC-MS, UPLC-QTOF-MS

1. Introduction

A new sample preparation technique, fabric phase sorptive extraction (FPSE), has been recently developed by Kabir and Furton based on the innovations in sol-gel microextraction phases [1,2]. This technique eloquently addresses the main shortcomings of conventional sorbent based sample preparation techniques. FPSE uses a natural or synthetic fabric as the substrates, where a sorbent is chemically immobilized through sol-gel coating technology in the form of ultra-thin coating uniformly distributed on the fabric substrate. The permeability of the substrate is retained even after the sol-gel sorbent coating, which favors the flow of the sample solution through the extraction system during the extraction process and provides fast extraction equilibrium. The strong covalent bond between the fabric substrate and the sol-gel sorbent provides an efficient extraction medium and allows exposing the FPSE media to any organic solvent or harsh chemical environment (pH 1-13) without compromising the chemical/structural integrity of the microextraction device. Other important advantages of FPSE include high primary contact surface area (1000 mm^2 for a 2.5 cm x 2.0 cm unit) for rapid sorbent-analyte interactions and the availability of a large number of sorbents with unique polarity and selectivity. Several research works using FPSE for the analysis of a wide variety of analytes such as non-steroidal anti-inflammatory drugs [3], triazine herbicides [4] or emerging contaminants [5], amphenicols [6], androgens and progestogens [7], residual sulphonamides [8], benzodiazepines [9], alkyl phenols [10], UV stabilizers or plastic additives [11] present in different sample matrices including environmental water, biological fluids, milk have been recently published. In all the cases mentioned above, liquid chromatography was used for the analysis of the extracted analytes. Since, FPSE allows utilizing any organic solvent of choice for solvent mediated analyte back-extraction, a solvent equally compatible with gas chromatography as well as liquid chromatography can be chosen. Subsequently, an aliquot of the same sample can be analysed by both gas chromatography and liquid chromatography to obtain a holistic chromatographic information comprised of highly volatile as well as semi-volatile and non-volatile target analytes.

Fresh orange juice possesses a very pleasant aroma due to the complex mixture of volatiles present in it that belong to different chemical families, such as esters, aldehydes, terpenes or alcohols [13]. Multiple studies related to orange juice aroma have been performed [14-17] using gas chromatography-mass spectrometry (GC-MS) and

other techniques, such as olfactometry, in order to evaluate the role of each compound in the global aroma profile of orange juice. The changes in the volatile composition of orange juices during different processes such as thermal processing, pasteurization, freezing or even during harvest have also been studied by several authors [18-21].

The objective of the current research work was to study the evolution of chemical changes occur in oranges during a prolonged period of storage at 5ºC. In order to determine the main markers of orange freshness, FPSE technology was applied simultaneously to extract both volatile and non-volatile compounds from orange juices followed by analyses simultaneously using GC-MS and UPLC-QTOF-MS.

Results from this experiment would allow selecting markers related to orange freshness. The acquired knowledge of the chemical changes occur in oranges during storage can be applied to future studies on the effectiveness of new active packaging, that protect the food thanks to the incorporation of antioxidant and/or antimicrobial substances, in preserving freshness of oranges and other fruits till their consumption.

2. Materials and methods

2.1. Reagents, solvents and FPSE media

Volatile compounds selected for the optimization of FPSE process (Table 1) and volatiles used in the analyses of oranges (p-Cymene, β-citronellal, camphor, 1-octanol, α-Terpineol, estragole, 5-hydroxy methyl furfural, phellandrene, and α-pinene) were of analytical quality and were purchased from Sigma-Aldrich (Barcelona, Spain). Purified water was obtained from a Milli-Q 185 Plus system (Millipore, Bedford, MA, USA), and methanol and acetonitrile (LC-MS quality) were purchased from Scharlau Chemie S.A (Sentmenat, Spain).

Substrates used in creating sol-gel sorbent coated FPSE media, unbleached Muslin cotton (100% cellulose) was purchased from Jo-Ann Fabric (Miami, FL, USA). Organic polymers: long chain poly(dimethylsiloxane), average molecular weight 36,000 Da (PDMS); short chain poly(tetrahydrofuran), average molecular weight 250 Da (PTHF); and short chain poly(dimethylsiloxane), average molecular weight 400-700 Da (SC PDMS) were purchased from Gelest Inc. (Morrisville, PA, USA). Organic polymers Carbowax 20M, average molecular weight 20,000 Da; polyethylene glycol-polypropylene glycol- polyethylene glycol triblock copolymer, average molecular

weight 1100 Da (PEG-PPG-PEG); sol-gel precursors methyltrimethoxysilane (MTMS), organic solvents acetone and methylene chloride; sodium hydroxide, hydrochloric acid were purchased from Sigma-Aldrich (St. Louis, MO, USA).

A Barnstead NANOPure Diamond (Model D11911) deionized water system (Thermofisher Scientific, Waltham, MA, USA) was used to obtain ultra-pure deionized water (18.2 MΩ) for sol-gel synthesis. Centrifugation of different solutions to obtain particle free sol solution for the sol-gel coating was performed in an Eppendorf Centrifuge Model 5415 R (Eppendorf North America, Hauppauge, NY, USA). Scrupulous mixing of all solutions were achieved by a Fisher Scientific Digital Vortex Mixture (Fisher Scientific, Waltham, MA, USA). A 2510 Branson Ultrasonic Cleaner (Branson Ultrasonics Inc., Danbury, CT, USA) was employed to obtain bubble free sol solution.

For fabric phase sorptive extraction (FPSE) method development, the following 5 FPSE media coated with different sol-gel sorbents possessing different polarities and selectivities were studied (describing by the organic polymers used and the organic ligand connected to sol-gel precursor (Methyl) in the sorbent synthesis: long chain poly(dimethylsiloxane) (PDMS), short chain poly(tetrahydrofuran) (PTHF), Carbowax 20M (CW20M), short chain poly(dimethylsiloxane) (SC PDMS) and polyethylene glycol-polypropylene glycol- polyethylene glycol triblock copolymer) (PEG-PPG-PEG). Dimensions of FPSE media were 2 cm x 2.5 cm.

2.2. Creation of sol-gel coated fabric phase sorptive extraction media

Substrate used in creating sol-gel sorbent coated fabric phase sorptive extraction media, commercial Muslin 100% cotton cellulose fabric often contains residual finishing chemicals, dust and other debris on its surface accumulated over the period of its self-life and needs thorough cleaning. In addition, the surface hydroxyl functional groups of cellulose fabric requires activation to obtain maximum loading of sol-gel sorbents during the chemical sorbent coating process. This was accomplished by carrying out a rigorous cleaning process developed in our laboratory and described elsewhere [22]. Briefly, a 150 cm^2 (15 cm x 10 cm) piece of the fabric was soaked and cleaned with water, followed by treating with 1.0 M NaOH for 1 h and 0.1 M HCl for 1 h under sonication, respectively. The chemically treated and cleaned fabric was then

dried in an inert atmosphere overnight and stored in an air-tight container until coated with sol-gel sorbent.

The design of the sol solution to create sol-gel sorbent coating on the substrate surface primarily depends on the polarity/functionality of the target analytes. Taking the polarity/functional makeup and other physico-chemical characteristics of the target analytes into consideration, a number of sol-gel sorbents were synthesized which include: sol-gel long chain poly(dimethyl siloxane) (PDMS), short chain poly(dimethyl siloxane (SC PDMS), short chain poly(tetrahydrofuran) (PTHF), short chain polyethylene glycol-polypropylene glycol-polyethylene glycol block copolymer (PEG-PPG-PEG) and Carbowax 20M (CW20M). Sol solutions were prepared using an organic polymer, a sol-gel precursor, a solvent system, a catalyst and water. All sol solutions in the current study were prepared using methyltrimethoxysilane (MTMS) as the sol-gel precursor, trifluoroacetic acid (TFA) as the acid catalyst, mixture of acetone and methylene chloride (50:50 v/v) as the solvent system and water for hydrolysis. The molar ratio between sol-gel precursor, organic polymer, acetone, methylene chloride, TFA and water was optimized and maintained at ($1:4.0x10^{-3}$:1.94:2.3:0.75:3) for PDMS; (1:0.36:1.94:2.3:0.75:3) for SC PDMS; (1:0.57:1.94:2.3:0.75:3) for PTHF; (1:0.13:1.94:2.3:0.75:3) for PEG-PPG-PEG and ($1:7.1x10^{-3}$:1.94:2.3:0.75:3) for CW20M, respectively.

A detail description of the sol solution preparation for sol-gel coating is described elsewhere [1, 2]. The fabric substrates during the sol-gel dip coating were kept inside the sol solution for 4 h. At the end of the residence time in the sol solution, the coated fabric was removed from the solution and was kept in the desiccator overnight for solvent evaporation and conditioning the sol-gel coating. The sol-gel coated FPSE media was then rinsed with methylene chloride: acetone (50:50 v/v) mixture under sonication to remove unreacted and unbonded residual coating ingredients from the fabric surface. The cleaned FPSE media coated with sol-gel sorbents were then stored in an airtight container so that it does not accumulate unwanted analytes from the environment.

2.3. Instrumental analysis

2.3.1. Analysis by gas chromatography-mass spectrometry (GC-MS)

Gas chromatographic analyses were carried out with a HP 6890N gas chromatograph coupled to a mass spectrometer MS 5975B detector, both from Agilent Technologies (Madrid, Spain). The capillary column used was a BP20 (Wax) (30 m x 0.25mm x 0.25 µm) purchased from SGE Analytical Science (Milton Keynes, United Kingdom). Temperature program in the GC oven was as follows: initial 40 ºC held for 4 min, then rose at 10 ºC·min^{-1} up to 160 ºC and at 15 ºC·min^{-1} up to 220 ºC, temperature was held at 220 ºC for 8 minutes. One µL aliquot of the sample was injected in splitless mode. The mass detector was set at SCAN mode (in the range m/z 50-400) for the identification of the compounds. For confirmation purposes, the linear retention indexes (LRI) of compounds identified were calculated and compared with those obtained in the literature. LRIs were calculated using a mixture of alkanes from C8 to C23 injected using the same experimental conditions than the samples and using the following equation:

$$LRI = 100\,x\,(\frac{t - tx}{ty - tx} + n)$$

Where t was the retention time of component, t_x the retention time of the preceding n-alkane and t_y the retention time of subsequent n-alkane. Bibliographic LRIs were obtained from Pherobase (www.pherobase.com)____and Chemspider (www.chemspider.com) databases. For quantification purposes, acquisition was performed in single ion monitoring (SIM) mode. Quantification and confirmation ions are shown in Table 2.

2.3.2. Analysis by ultra-performance liquid chromatography-quadrupole time of flight mass spectrometry (UPLC-QTOF-MS)

In order to obtain a complementary and holistic chromatographic profile consisting of semi-volatile and non-volatile analytes from orange juices, aliquots of the samples prepared from orange juices were also analysed by UPLC-QTOF-MS. Chromatography was carried out using an Acquity system supplied by Waters (Milford, MA, USA). A UPLC BEH C18 column of 1.7 µm particle size (2.1 x 100 mm) from Waters (Milford, MA, USA) was used. Injection volume was 10 µL. Chromatography was carried out at 0.4 mL min^{-1} column flow and 40 ºC column temperature. The mobile phase was water with 0.1 % formic acid (phase A) and methanol with 0.1 % formic acid (phase B). Chromatography started at 98:2 phase A: phase B (1 minute), changed to 0:100 in 6 minutes and stays at 0:100 for an additional 2 minutes. The UPLC was connected with

an ESI probe to the mass spectrometer. A Xevo G2 QTOF mass spectrometer supplied by Waters (Milford, MA, USA) was used for the identification of orange non-volatile compounds. Instrumental parameters were as follows: positive ionization, sensitivity mode, capillary at 2.5 kV, sampling cone at 30 V, extraction cone at 4 V, source temperature at 120 ºC, desolvation temperature at 450 ºC, cone gas flow at 20L h^{-1}, desolvation gas flow at 650L h^{-1}. Acquisition was carried out in MS^E mode, as this mode allows both low and high collision energies (CE) in the collision cell during the same run, and thus provides two kinds of mass spectra of the compounds. The low energy (CE at 4 V) spectra provide information about the precursor ion and high energy (CE ramp: from 15 to 30 V) spectra provide information about fragment ions. Data were recorded using MassLynx v4.1 software. For the identification of the compounds detected, the following methodology was used. First, the elemental composition of the precursor ion was determined using the low energy spectrum. For this purpose, the exact mass and the isotopic pattern of the precursor ion and the elemental compositions proposed by Masslynx were compared. Those elemental compositions with a low mass error and a good isotopic fit were selected. Afterwards, the elemental composition was linked to a chemical structure using different chemical database websites such as Chemspider [www.chemspider.com] or Scifinder [www.scifinder.com]. The selection of candidates from the chemical database was made according to the chemical criteria and background knowledge of the analyst. Finally, the selection of the best candidate was carried out using the high energy spectrum of the compounds detected. For this purpose, the MassFragment tool from MassLynx was used. This tool allowed the comparison of high energy mass spectra of the unknown peaks and the candidate. For each fragment ion detected in the spectrum, MassFragment provides a fragment structure and a score (S) with values between 1 and 14, the lower the value, the more plausible is the structure proposed. When a candidate structure explained at least two main fragment ions of the spectrum with scores values below 3, the candidate was considered *confirmed by MassFragment*.

2.4. Sample preparation

For FPSE analysis, oranges were squeezed individually and an aliquot of 75 mL from the orange juice was mixed with 25 mL of milliQ water.

2.5. Optimized FPSE protocol

Through a rigorous optimization workout, FPSE protocol was established as follows:

I. FPSE media cleaning step: FPSE media were placed in a vial with 5 mL of a mixture of methanol/acetonitrile (50:50 v/v) for 30 minutes in an ultrasound bath. Subsequently, the FPSE media were removed from the solvent, rinsed with water rinsed and dried in air.

II. Sample extraction step: Sample (100 mL) was placed in a 100mL vial closed hermetically with a magnetic stir bar and a FPSE medium (2.5 cm x 2.0 cm) at 900 rpm for 60 minutes. Afterwards, the FPSE media was removed, rinsed with milliQ water and dried in air.

III. Solvent mediated back-extraction step: The FPSE medium was placed in a 2mL vial and 1 mL of methanol was added. The vial was placed in an ultrasound bath for 10 minutes. Before and after sonication, FPSE medium was squeezed with a glass rod 20 times. Afterwards the FPSE medium was removed, and extraction solution was filtered through a 20 μm PET filter. The final extracts were analysed by GC-MS or UPLC-QTOF-MS.

After these steps, FPSE media were cleaned following the *FPSE media cleaning step* (residence time in the solvent mixture was kept at 5 min) and stored in a Petri dish until next analysis.

2.6. Evolution of oranges composition over time

Orange samples were purchased from a supermarket in Zaragoza, Spain and stored for two months at 5 ºC. Oranges were weighted at the beginning and at the end of the experiment and pH was also measured using a pH meter CRISON 5053T (pH 2-14).

A first set of 8 oranges was analyzed the same day of the acquisition and a second set of 8 oranges was analyzed after 2 months, each orange was analyzed individually. Samples were prepared according to the procedure mentioned in Section 2.4 and extracted according to the procedure described in Section 2.5.

For the study of volatiles composition, FPSE extracts were analyzed by GC-MS in SCAN mode following the conditions defined in Section 2.3.1. The differences between chromatographic profiles obtained from GC-MS before and after the storage experiment were carefully studied. Compounds detected were quantified by external calibration using their standards or compounds with a similar structure.

For the study of non-volatiles composition, FPSE extracts were analyzed by UPLC-QTOF-MS following the conditions described in Section 2.3.2. Identification of the compounds was performed following the methodology described in this section.

3. Results and discussion

Volatile analytes were chosen to represent aroma compounds with different polarities and volatilities, as demonstrated in Table 1 where the hydrophobicity values (log P) ranged from 0.099 (furfuryl alcohol) to 3.604 (limonene). Most of the important chemical families were represented by the 12 volatile analytes selected for the study and these analytes allowed obtaining a representative extract of volatile compounds. Table 2 shows the calibration curves for the compounds studied, calibration range and its determination coefficient. Values for determination coefficient (R^2) were between 0.997 and 0.999. The limit of detection in juice was at ng mL^{-1} level, with values from 1 ng mL^{-1} to 30 ng mL^{-1}, except for furfuryl alcohol that showed higher values. Nine out of the 12 compounds showed LODs below 10 ng mL^{-1}.

3.1. Optimization of fabric phase sorptive extraction parameters

In order to maximize the extraction efficiency while keeping the overall sample preparation time as low as possible, different parameters were optimized including FPSE sorbent chemistry, sample volume, extraction time, back-extraction solvent or influence of ionic strength modification.

3.1.1. Selection of the most efficient FPSE sorbent chemistry

Due to the extremely high to moderate polarity of the selected analytes, extraction sorbents characterized with high polarity to medium polarity would be the best for the current study. As mentioned above, five different sorbents were selected: SC PDMS, PTHF, PDMS, PEG-PPG-PEG, and CW20M. Table 3 demonstrates the chemical structures, polarity of the monomers/polymer blocks, polarity of the sol-gel composite

sorbents and tentative intermolecular interactions that may be exerted by different sol-gel sorbents in order to carry out efficient extraction of the target analytes. Although, the same type of interactions may appear in different sorbents, the magnitude of individual interactions differs widely and consequently, different sorbent demonstrate distinctly different selectivity towards the target analytes. All sol-gel sorbents used in the current study were synthesized using methyltrimethoxysilane (MTMS) as the network building sol-gel precursor. The methyl group connected to silica backbone is not subjected to hydrolysis during the sol-gel reaction and therefore becomes the integral part of the network during the sol-gel synthesis. Sol-gel synthesis allows incorporation of one/more functional groups in the gel to fine-tune the overall polarity/selectivity of the composite material. As such, the selectivity of the sol-gel sorbent is partly determined by the organic modification of the sol-gel precursor(s) and partly by the characteristics of the organic polymer. All sol-gel sorbents coatings were created on 100% cotton cellulose. Strong hydrophilic property of the cellulose fabric substrate also made substantial contribution to the overall selectivity of the FPSE media. The extraction efficiency of five different sol-gel sorbents were evaluated using 100 mL aqueous solutions containing all selected volatile compounds at 200 ng g^{-1}. Extractions were carried out for 1 h. Subsequently, the FPSE media were back extracted following step II of FPSE optimized protocol (Section 2.5). Three replicates were performed for each FPSE media. The extracts were injected into the GC-MS. It is worth noting that the selectivity and extraction sensitivity of fabric phase sorptive extraction media depend on (1) the organic polymer; (2) inorganic precursor; (3) hydrophobicity or hydrophilicity of the fabric substrate. Among all the phases studied, CW20M was found to be the optimum sorbent chemistry for the current study. Therefore, all other extraction parameters were optimized using CW20M. It is important to note that the selectivity of pristine polyethylene glycol (CW20M) is totally different than that of sol-gel CW20M. The incorporation of CW20M polymer into a three dimensional network of organically (methyl functional group) modified silica provides a completely different composite material and chemical environment, leading to high selectivity towards analytes possessing wide range of polarity. Figure 1a shows the enrichment factors of the volatile compounds studied with the different FPSE media. CW20M showed good results for seven out of the ten compounds compared to the other media studied. For this reason, it was the first candidate for the analysis of orange juice samples as it would allow obtaining a representative profile of volatile compounds. Only ethyl octanoate,

343 octanal and limonene showed lower EFs than in other media such as SC PDMS. Thus,
344 SC PDMS was also selected for performing analysis on real samples. The results
345 obtained from both media would allow checking which one offered the best results.
346 Vanillin and furfuryl alcohol were not displayed since they showed similar values for all
347 the fabrics. For these two compounds, EF values were always below 1, but even though
348 no concentration enrichment was observed, this technology allowed the transference of
349 the compounds from aqueous to organic solvent, and subsequently, its injection into the
350 GC-MS system.

351 Chromatograms obtained from the analysis of orange juices with CW20M and SC
352 PDMS FPSE media (3 replicates) showed that all the peaks detected in extracts from SC
353 PDMS coated media were also present in the extracts from CW20M coated media, even
354 with higher intensities. For this reason, CW20M coated FPSE media was selected for
355 the analysis of orange juice samples

356 It can be pointed out that those compounds with high vapor pressure values, such as
357 ethyl butyrate and ethyl isovalerate (13.9 and 7.9 respectively), showed low EFs values
358 in all FPSE media (Figure 1a). A Pearson correlation test was performed using
359 enrichment factors data obtained for all the FPSE media and compounds physico-
360 chemical parameters, such as molecular weight or hydrophobicity (log P). Results
361 showed that high EF results were mostly correlated with high log P. Molecular weight
362 showed also a positive correlation with EF values for most of the FPSE fabrics,
363 especially for SC PDMS (0.708) and PEG-PPG-PEG (0.733) (Figure 1b). The test also
364 showed a positive correlation between EFs values obtained with different media, such
365 as for SC PDMS and PEG-PPG-PEG (0.979); or CW20M and PTHF (0.844).

366 **3.1.2. Characterization of sol-gel CW20M coated FPSE media**

367 One of the major shortcomings of conventional sorbent based sample preparation
368 techniques is their poor batch-to-batch coating reproducibility. Fabric phase sorptive
369 extraction has articulately addressed this issue by incorporating sol-gel coating
370 technology to create the extraction sorbent, chemically bonded to the fabric substrates.
371 Due to the superior control over the chemical reactions used in sol-gel coating process,
372 it ensures high degree of reproducibility. To verify the reproducibility in sol-gel coating,
373 4 independent batches of CW20M coated FPSE media were created using identical
374 coating formulation and the mass of the CW20M sorbent loading per unit area (mg/cm^2)

were calculated gravimetrically. The average sorbent loading was calculated as 8.63 mg/cm^2 with a relative standard deviation (%RSD) at 7.19. The data clearly demonstrate the highly reproducibility of sol-gel coating process.

Another important feature of FPSE media is its surface porosity that mimics a permeable solid phase extraction bed. The inherent porosity of the fabric is well preserved even after the sol-gel sorbent coating. Although, analyte extraction in FPSE is an equilibrium driven process (as in solid phase microextraction), the through-pores in FPSE media allows rapid permeation of the sample matrix through its surface (as in solid phase extraction) and consequently, expedites the analyte extraction kinetic, resulting in a shorter extraction equilibrium time. In addition, due to these through-pores, FPSE is capable of extracting target analyte in a near-exhaustive manner (similar to solid phase extraction) even under equilibrium driven extraction settings and consequently, improves the overall sensitivity of the analytical method. Figure 2 demonstrates scanning electron microscopy (SEM) images of an FPSE media before and after CW20M coating at different magnifications and a photographic image of a coated FPSE medium.

3.1.3. Optimization of fabric phase sorptive extraction protocol

Two solvents were verified in order to select the best back-extraction solvent that allowed maximum recoveries: methanol, a polar protic solvent, and acetonitrile, a polar aprotic solvent. For the selection of extraction solvent, FPSE media were first spiked with 20 μL of a solution at 20 μg g^{-1} of the selected compounds prepared in methanol. Afterwards, the FPSE media were back extracted following step II of FPSE optimized protocol (Section 2.5). The extracts were analyzed by GC-MS. Blanks were performed by extracting FPSE media spiked with 20 μL of methanol. Each experiment was performed in triplicate.

Table 4 shows the recovery percentage for both of them. Except for vanillin, the values obtained for methanol were higher than those obtained for acetonitrile, reaching recoveries above 80% except for butyric acid (70.1%). For this reason, methanol was selected as the back-extraction solvent.

Afterwards, sample volume and extraction time were optimized in parallel, since they could have cross effects. The aim was to check if similar results could be obtained

406 with lowest sample extraction times or volumes. For this experiment, three sample
407 volumes were checked, 15, 50 and 100 mL and three different sample extraction times
408 were tested for each sample volume: 15, 30 and 60 minutes.. Afterwards, the FPSE
409 media were back extracted following step II of FPSE optimized protocol (Section 2.5).
410 Extracts were analyzed by GC-MS. Blanks were performed by extracting water samples
411 in the same conditions. Each experiment was performed in triplicate.

412 All the compounds showed a similar pattern, where the effect of increasing sample
413 extraction time from 15 to 60 minutes had relevance at high sample volumes. This
414 observation made sense since high sample volumes required more time to pass through
415 the FPSE media and be retained on it (Figure 3a). According to the results, 100 mL and
416 60 minutes were found as the optimum conditions for sample volume and extraction
417 time, respectively. It was also observed that for compounds such as limonene and ethyl
418 butyrate, long sample times could have a negative effect and may produce a decrease on
419 the signal (Figure 3b), probably because even the flasks had been hermetically closed,
420 some volatiles could be lost when extraction time is too long. For this reason, no longer
421 periods were explored.

422 For testing the effect of ionic strength, NaCl (10% w/v) was added to an aliquot of
423 the volatiles aqueous solution A t-student test performed on results did not show any
424 significant differences with or without the salt ($p>0.05$). Due to the differences among
425 the compounds studied, they were not expected to behave in the same way when pH
426 was modified. For this reason, this parameter was not evaluated.

427 **3.2. Optimization of FPSE for orange samples**

428 **3.2.1. Study of matrix effect**

429 The first aim of this study was to evaluate the presence of matrix effects in the
430 analysis of orange juice extracted by FPSE. For this study, orange juice (OJ) was
431 diluted with water (W) in different proportions and afterwards extracted following the
432 FPSE extraction protocol using CW20M coated medium. Dilutions studied were: 25/75
433 (OJ/W), 50/50 (OJ/W), 75/25 (OJ/W), 100/0 (OJ/W). Subsequently, a 600 μL aliquot of
434 each extract was spiked with 20 μL of a mix solution containing all the volatiles (15 μg
435 g^{-1}) (*sample*). This solution was also used to spike 600 μL of pure methanol (*reference*).
436 Blanks were prepared adding 20 μL of methanol to a 600 μL aliquot of the different

extracts (*blank*). Extracts were analyzed and the matrix effect was calculated following next equation where A_{sample}, A_{blank} and $A_{reference}$ were the areas of the analytes in the sample, in the blank and in the reference, respectively:

$$Matrix\ effect\ (\%) = 100\ x\ \frac{Asample - Ablank}{A\ reference}$$

Results obtained were very similar for all the dilutions studied, which implied that no differences in matrix effect were expected if orange juice was or not diluted with water. Matrix effect values from 80-120% are considered suitable values since they indicate minor matrix effects in this experiment. Results showed that matrix effects values were between 85 and 115% for most of the compounds. Only linalool and vanillin were out of this range and showed values above 120% (136.0 ± 11.6 % and 227.0 ± 5.5 % respectively) which meant that there was an enhancement of the signal for these due to the extract composition.

3.2.2. Optimization of orange juice/water proportion

Finally, it was studied if the orange juice/water proportion could affect the FPSE sample extraction process due to the variations in parameters such as sample density. Orange juice was spiked with a mix solution containing all the volatiles (15 μg g^{-1}) and afterwards, it was diluted in different proportions: orange juice (OJ)/water (W): 25/75 OJ/W, 50/50 OJ/W, 75/25 OJ/W and 100/0 OJ/W. These solutions were extracted following the FPSE final protocol and analyzed by GC-MS

Results are shown in Figure 4. It was observed that the concentration of the compounds in the extract increased at high orange juice/water proportions, which makes sense since the samples had higher initial concentrations than when they were highly diluted. But, when samples were not diluted at all, the final concentration showed a slight decrease in most cases, probably due to a higher viscosity of the initial sample that hindered the rapid diffusion of the analytes through the sample matrix as well as to freely interact with the FPSE media, a prerequisite for successful extraction. For this reason, a dilution 75/25 (OJ/W) was finally selected for orange juice analysis.

3.4. Study of chemical compositional differences between fresh oranges and oranges sored at 5ºC for 2 months

The medium orange weight decreased 25.4% (± 5%) after 2 months of storage. The pH was measured overtime (average of 6 oranges) and the results showed that the pH values didn't change significantly over its storage. Therefore, it appeared that oranges are naturally maintained buffered systems.

Table 5 shows the volatile compounds that display differences in concentration between orange juices obtained at initial time and after two months of orange storage. Twelve compounds were identified, all of them except methyl methoxyphenyl acetate and estragole had been previously detected in citrus fruits. Most of the compounds were monoterpenes, such as myrcene or terpinene and terpenoids, such as eucalyptol, camphor, linalool or terpineol. This kind of compounds have been described in the bibliography as key compounds in citrus fruits. For eucalyptol, terpinene, cymene, β-citronellal, camphor and estragole, the final concentration was below 10% of the initial concentration, what makes them good markers of orange freshness.

Three non-volatile compounds showed a clear decrease in signal intensity when comparing juice analysis of fresh oranges (initial time) and oranges after 2 months of storage at 5ºC: subaphyllin ($C_{14}H_{20}N_2O_3$, CAS: 501-13-3), tangeretin ($C_{20}H_{20}O_7$, CAS: 481-53-8) and nobiletin ($C_{21}H_{22}O_8$, CAS: 478-01-3). All of them were *confirmed by MassFragment*, since at least two of the main fragments obtained scores below 2. Subaphyllin is an amide of ferulic acid and putrescine and it had been previously detected in citrus fruits and in grapefruit [26] [27] [28]. Tangeretin and nobiletin are flavonoids and they also had been previously detected in citrus fruits [29] [28]. Figure 5 shows the variation on signal intensity of these compounds after two months, relative decrease (%) was always above 70%, 80.6% for subaphyllin, 86.1% for tangeretin and 71.8 % for nobiletin. Therefore, these compounds could be used as nonvolatile markers of orange freshness and quality, together with other typical analysis such as sugar and acids content [28].

Conclusions

Fabric phase sorptive extraction has been proved to be a useful tool for the simultaneous extraction of both volatile and nonvolatile compounds from a very complex sample matrix such as orange juice. Among five different sol-gel based sorbent chemistries possessing different polarities and selectivities, CW20M coated FPSE media performed the best in extracting highest numbers of representative volatile

analytes that may distinctly differentiate between fresh and stored oranges. The application of this technique to the study of the evolution of the chemical make-up of oranges, both in terms of volatiles and non-volatiles, provided interesting results about the global chemical changes in oranges over time and offers a simpler and greener strategy to monitor the quality markers of oranges. These results will also allow the application of FPSE to the study of evolution of oranges in different packaging systems. The effectiveness of active packaging, that protect the food thanks to the incorporation of antioxidant and/or antimicrobial substances integrated to the packaging materials, could be evaluated through the study on the evolution of orange quality during its storage. Although, the current study was limited to only orange, the underlying principle and strategy can be conveniently applied to a number of unique and novel applications such as progressive chemical changes in fruits during their different developmental stages, chemical changes that take place during ripening before and after the harvesting, impact of different storage conditions on the fruits' global chemical profile, correlations between the rate of the chemical changes with the storage conditions, etc.

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612 **Figure captions:**

613 **Figure 1.** Enrichment factors of the volatile compounds studied using 5 different FPSE
614 media (PDMS, PTHF, CW20M, SC PDMS and PEG-PPG-PEG) (**1a)** and its plot versus
615 compounds molecular weight values **(1b)**.

616 **Figure 2.** (a) SEM image of cotton (100% cellulose) fabric surface at 100x
617 magnifications before sorbent coating; (b) SEM image of an uncoated fabric at 1000x
618 magnifications, demonstrating individual microfibril; (c) photographic image of a
619 CW20M coated FPSE medium; (d) SEM image of CW20M coated FPSE medium at
620 1000x magnifications, demonstrating uniform coating around the individual microfibril.

621 **Figure 3.** Signal intensity of ethyl octanoate (**3a**) and limonene (**3b**) after FPSE of a
622 sample solution using 3 different sample volumes (15, 50 and 100 mL) and 3 sample
623 extraction times (15, 30 and 60 minutes)

624 **Figure 4.** Concentration (mg/Kg) of volatile compounds after FPSE extraction from
625 different spiked orange juice (OJ) diluted with water (W) at different rates: 25/75,
626 50/50, 75/25 and 100/0

627 **Figure 5**. Non-volatile compounds detected in juice from oranges at initial time (■)
628 and after 2 months of storage time (■)

629

Table 1. Selected volatile and semi-volatile compounds analysed, CAS number, molecular weight (MW), hydrophobicity (log P) and vapour pressure at 25ºC (Vp)

Compound	**CAS nº**	**MW**	**Log P**	**Vp (mm Hg)**
Furfuryl alcohol	98-00-0	98.30	0,099	1.0
Butyric acid	107-92-6	88.11	0,838	1.4
cis-3-hexen-1-ol	928-96-1	122.34	0,929	1.0
Ethyl butyrate	105-54-4	130.78	1,443	13.9
Vanillin	121-33-5	152.15	1,516	0.0
Ethyl isovalerate	108-64-5	145.97	1,801	7.9
Linalool	78-70-6	186.89	2,130	0.1
1-Octen-3-one	4312-99-6	152.30	2,434	1.1
Eugenol	97-53-0	164.20	2,511	0.0
Octanal	124-13-0	157.36	2,856	2.1
Ethyl octanoate	106-32-1	199.97	3,211	0.2
Limonene	138-86-3	169.17	3,604	1.5

Table 2: Target **c**ompounds analysed by FPSE-GC-MS, equation, determination coefficient (R^2), calibration range, instrumental limit of detection (LOD), quantification (Qt) and qualifier (Ql) ion and its relative abundance (RA%), and limit of detection in juice (LOD_{juice})

Target compound	**equation**	**R^2**	**Calibration range**	**LOD (ng g^{-1})**	**Qt Ion**	**Ql Ion (RA%)**	**LOD_{juice} (ng mL^{-1})**
Furfuryl alcohol	y = 17.55x – 673.1	0.999	37.8-4860	12.5	98	81 (56.8)	90
Butyric acid	y = 2.76x + 583.52	0.998	445-4670	150	60	73 (72.7)	20
cis-3-hexen-1-ol	y = 30.61x – 1153.4	0.998	37.0-4760	12.5	67	82 (60.8)	3.0
Ethyl butyrate	y=50.25x-1668.3	0.999	93.4-5150	31.1	71	88 (68.5)	10
Vanillin	y = 19.38x +1980	0.997	93.0-5140	31.0	151	108 (33.5)	10
Ethyl isovalerate	y=36.52x + 856.5	0.999	42.5-5470	10.0	85	88 (73.6)	10
Linalool	y = 21.46x – 1394.4	0.998	40.7-5230	10.0	93	121 (20.7)	1.0
1-Octen-3-one	y = 41.33x – 628.23	0.997	37.6-4830	12.5	70	97 (34.4)	1.0
Eugenol	y = 24.24x + 425.2	0.999	37.8-4850	12.5	164	149 (40.2)	1.0
Octanal	y = 12.62x + 894.3	0.998	33.4-4290	11.1	84	69 (78.8)	2.0
Ethyl octanoate	y = 43.65x + 388.8	0.998	43.6-5610	15.0	88	101 (42.0)	0.5
Limonene	y = 49.20x + 2094.3	0.999	40.8 - 5240	10.0	93	136 (27.9)	30

Table 3. Selected fabric phase sorptive extraction sorbents, structure of the organic polymer, polarity and tentative interaction mechanisms

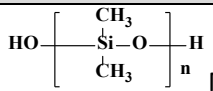
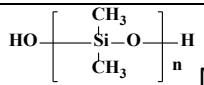
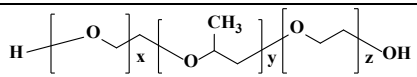
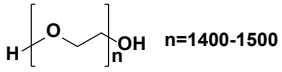
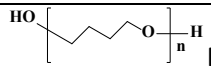
Sorbent	Organic Polymer	Polarity of the building block (monomer/block) (logKow)	Polarity of the composite sol-gel sorbent	Predominant Interactions
Sol-gel Long Chain PDMS	 MW: 36,000 Da	5.20	Nonpolar	London dispersion,
Sol-gel Short Chain PDMS	 MW: 400-700 Da	5.20	Nonpolar	London dispersion
Sol-gel PEG-PPG-PEG	 MW: 1100 Da	EG: -1.93 PG: -0.92	Medium polar	London dispersion, hydrogen bonding, dipole-dipole interaction
Sol-gel CW20M	 n=1400-1500 MW: 20,000 Da	-1.93	Highly polar	Hydrogen bonding, dipole-dipole interaction, hydrogen bonding
Sol-gel PTHF	 MW: 250 Da	0.5	Medium polar	London dispersion, hydrogen bonding

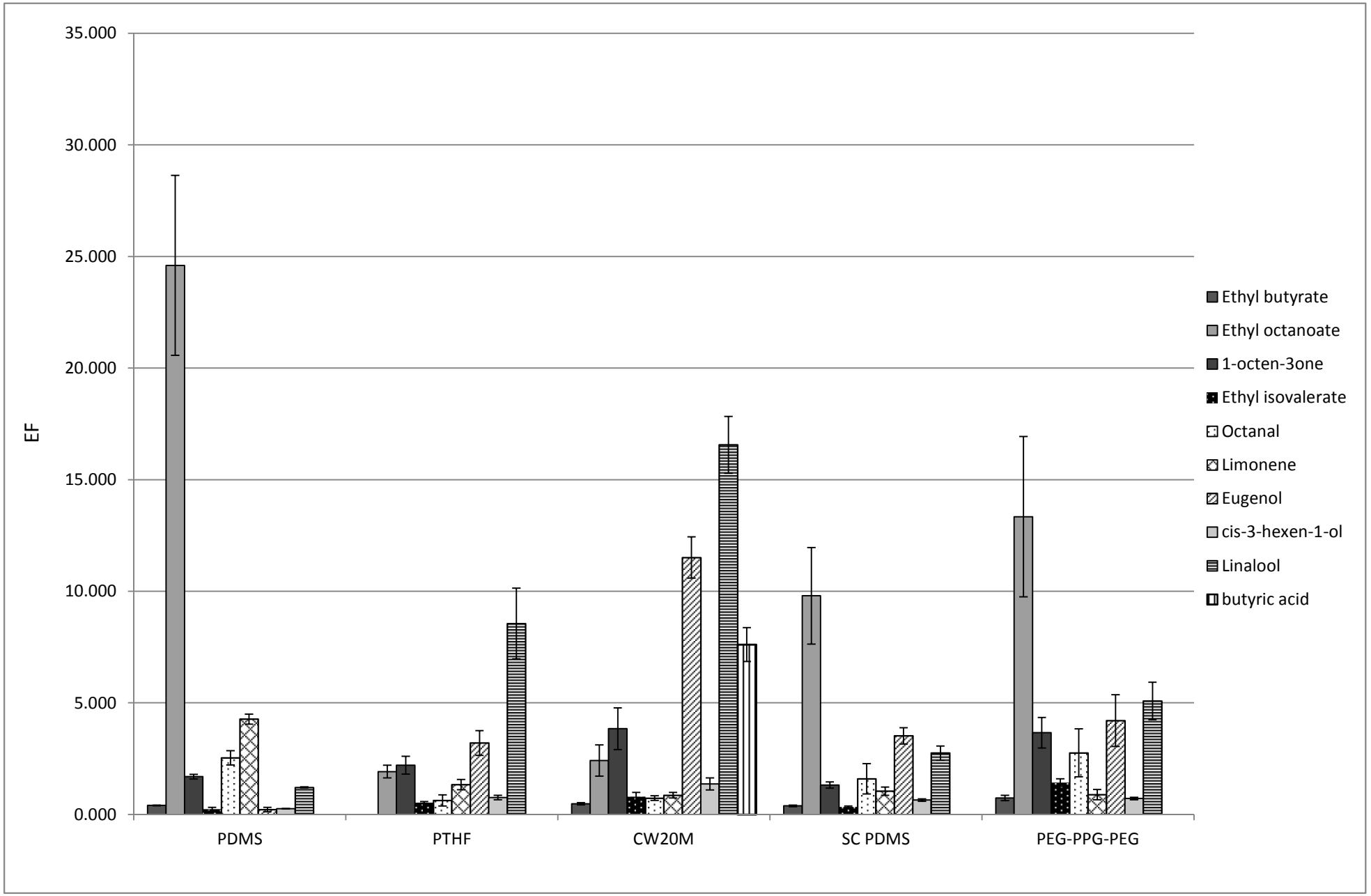
Table 4: Recovery percentage (%) using methanol (MetOH) or acetonitrile (ACN) as desorption solvents during back-extraction step of FPSE.

Compound	**MetOH (%)**	**ACN (%)**
Furfuryl alcohol	**98.9**	83.4
Butyric acid	**70.1**	60.3
cis-3-hexen-1-ol	**93.7**	35.7
Ethyl butyrate	**70.5**	44.9
Vanillin	89.4	**91.6**
Ethyl isovalerate	**80.8**	54.5
Linalool	**102.1**	81.6
1-Octen-3-one	**104.1**	38.6
Eugenol	86.7	70.6
Octanal	**106.5**	42.7
Ethyl octanoate	**102.6**	38.5
Limonene	**85.4**	47.8
Average	**90.1**	57.5
Total	**1171.4**	690.2

Table 5: Concentration of volatile compounds detected in juice obtained from fresh oranges and oranges stored for 2 months at 5ºC, experimental and bibliographic linear retention indexes (LRI) and references where these compounds have been detected in citrus fruits.

rt (min)	LRI (exp)	Compound	LRI (biblio)	CAS Nº	Family	Aroma	Fresh oranges ng mL^{-1}	Old oranges ng mL^{-1}	Reference
8.66	1150	a-myrcene[1]	1168-1187	123-36-3	Monoterpene	Musty, Geranium, Fruity, Lemon, Spicy	119	21.6	[16] [20] [21]
9.67	1211	Eucalyptol[2]	1214-1224	470-82-6	Monoterpenoid	Spicy, eucalyptus, sweet, pine	1800	59.4	[15] [22]
10.0	1232	α-Terpinene[3]	1178	99-86-5	Monoterpene	Fruity, Lemon	885	68.2	[15] [21]
10.50	1264	p-Cymene*	1261-1282	99-87-6	Alkylbenzene	Fresh, citrus, terpenic, spicy, lemon	5600	15.6	[15] [21]
13.67	1473	β-Citronellal*	1425-1488	106-23-0	Aldehyde/ monoterpenoid	floral, green, citrus, green	524	<10	[15] [22]
14.34	1525	Camphor*	1498	76-22-2	Terpenoid	Green, dry, leafy	126	<10	[23]
14.49	1538	Linalool*	1484-1570	78-70-6	Terpenoid	Floral, spicy	147	90.8	[15] [16] [20] [21]
14.55	1543	Methyl methoxyphenyl acetate[4]		56143-21-6	Ester		30.5	<10	
14.62	1548	1-Octanol*	1553	111-87-5	Alcohol	Green, citrus, fruity, orange	54.9	16.1	[15] [16]
15.32	1606	α-Terpineol*	1669-1720	98-55-5	Terpenoid	Terpenic, lilac, citrus, floral	22.8	<10	[15] [16] [20] [21]
16.16	1676	Estragole*	1661-1676	140-67-0	Phenylpropene	Phenolic, anise, green, herbal, minty	542	31.8	
24.09	2534	5-Hydroxy methylfurfural*	2592	620-02-0	Furan	Fatty, caramel	236	188	[24]

Compounds quantified with: * its internal standard; [1]phellandrene, [2]camphor, [3]α-pinene; [4]estragole
Bibliographic LRI were obtained from Pherobase (www.pherobase.com) and Chemspider (www.chemspider.com) databases.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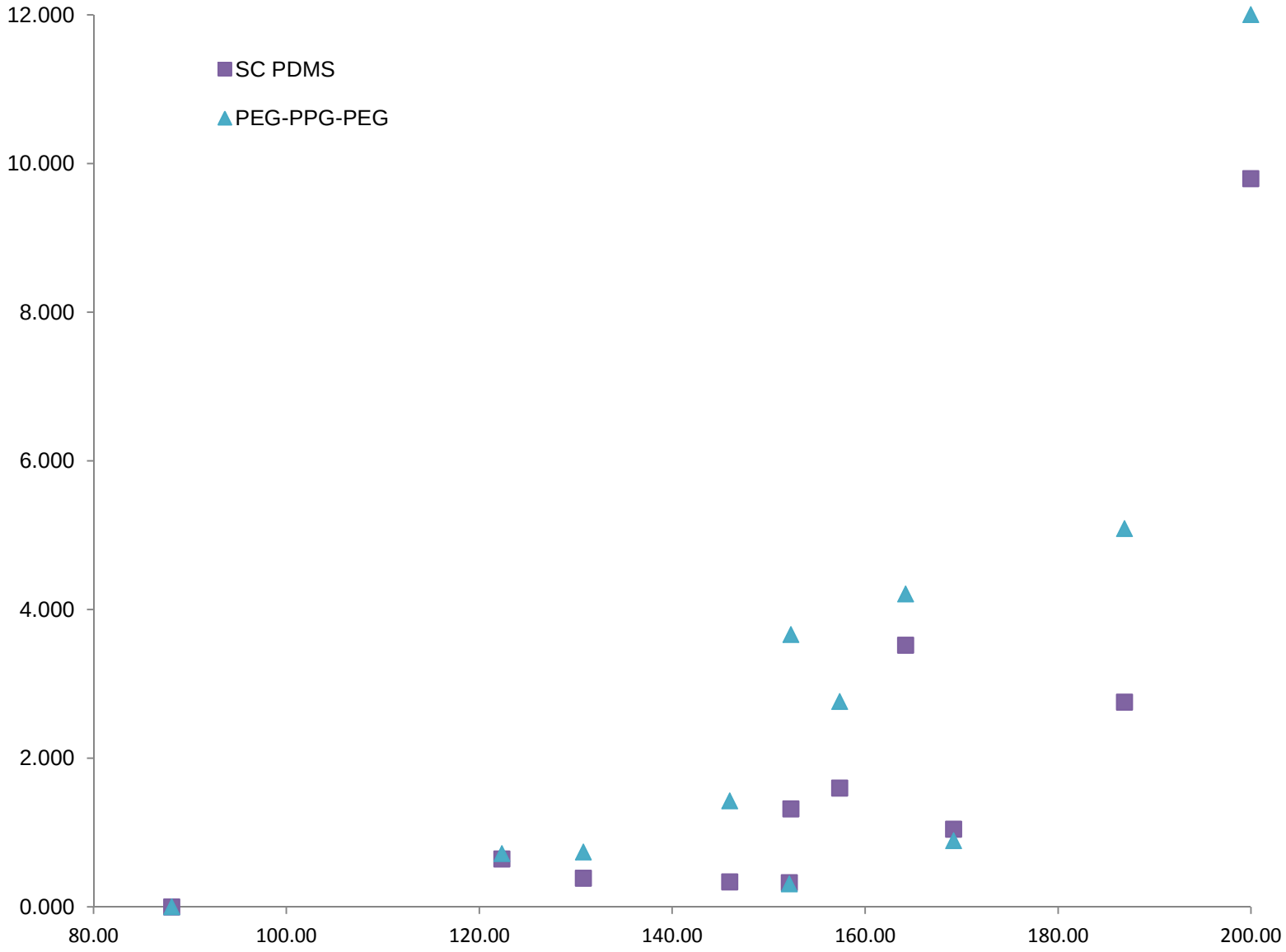
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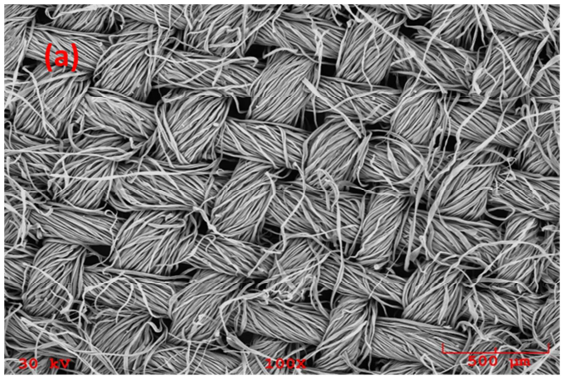
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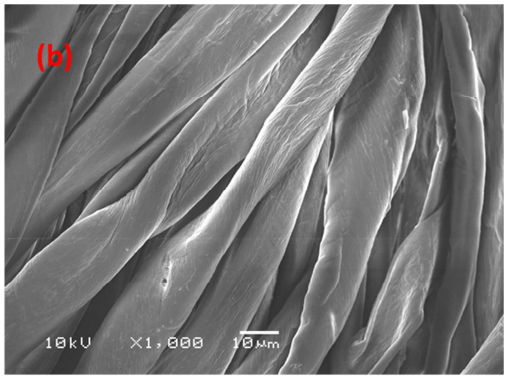
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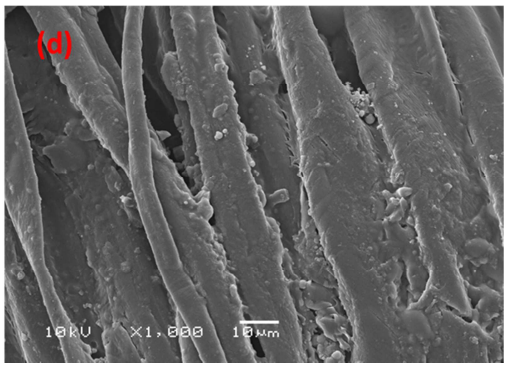
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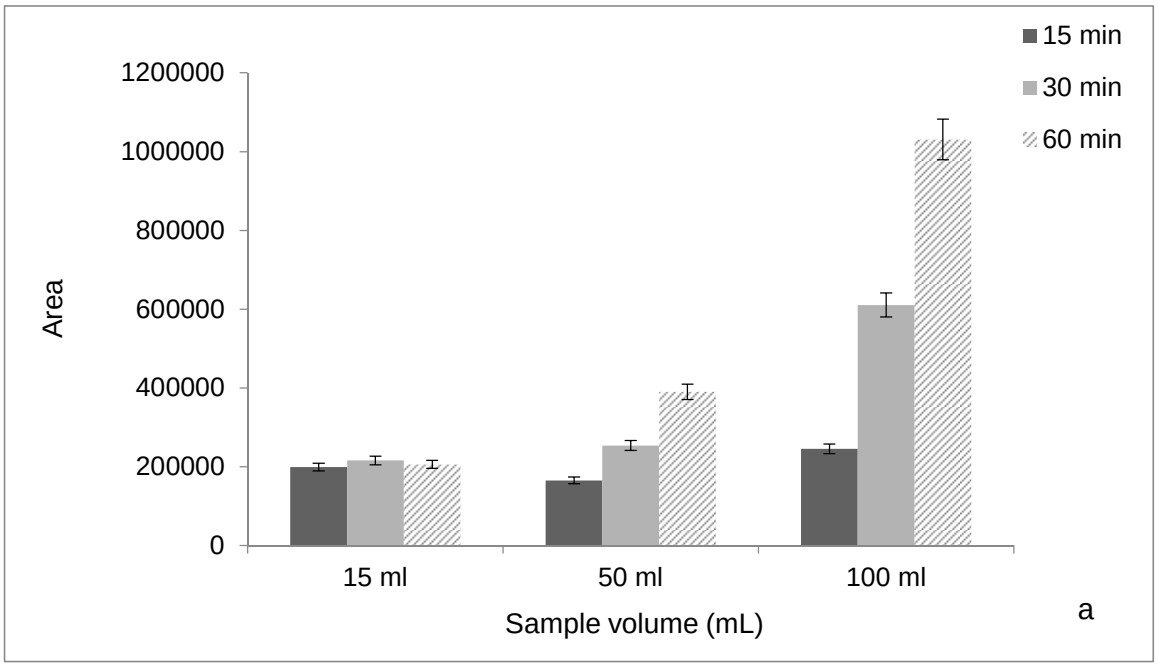
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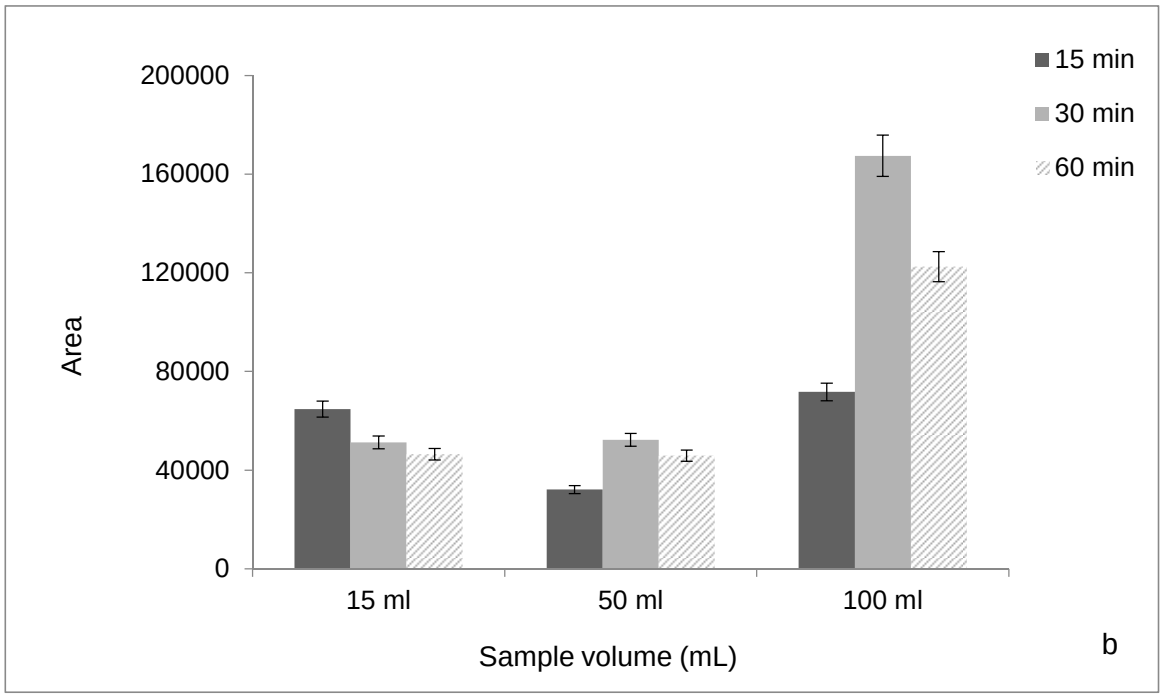
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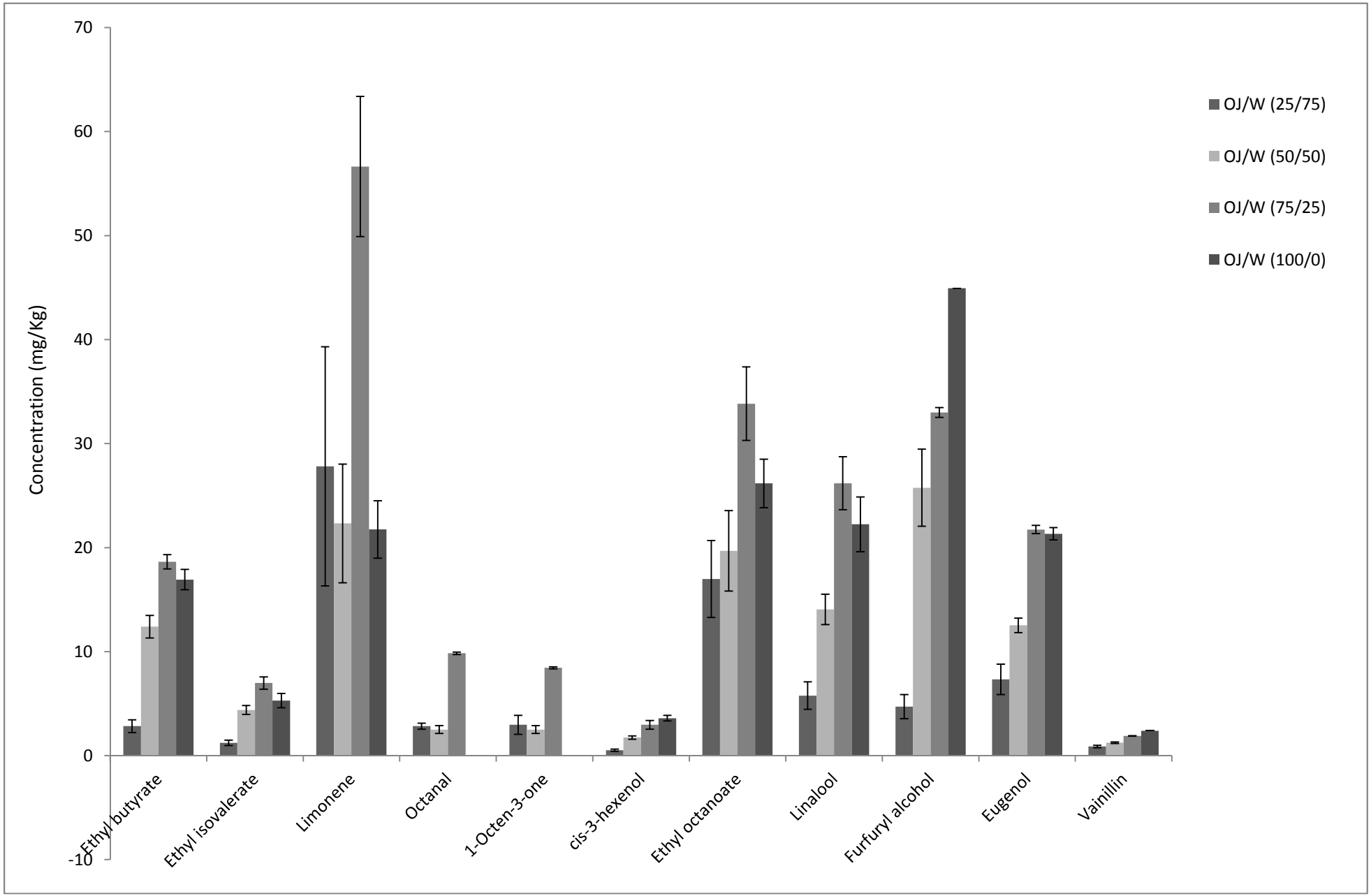
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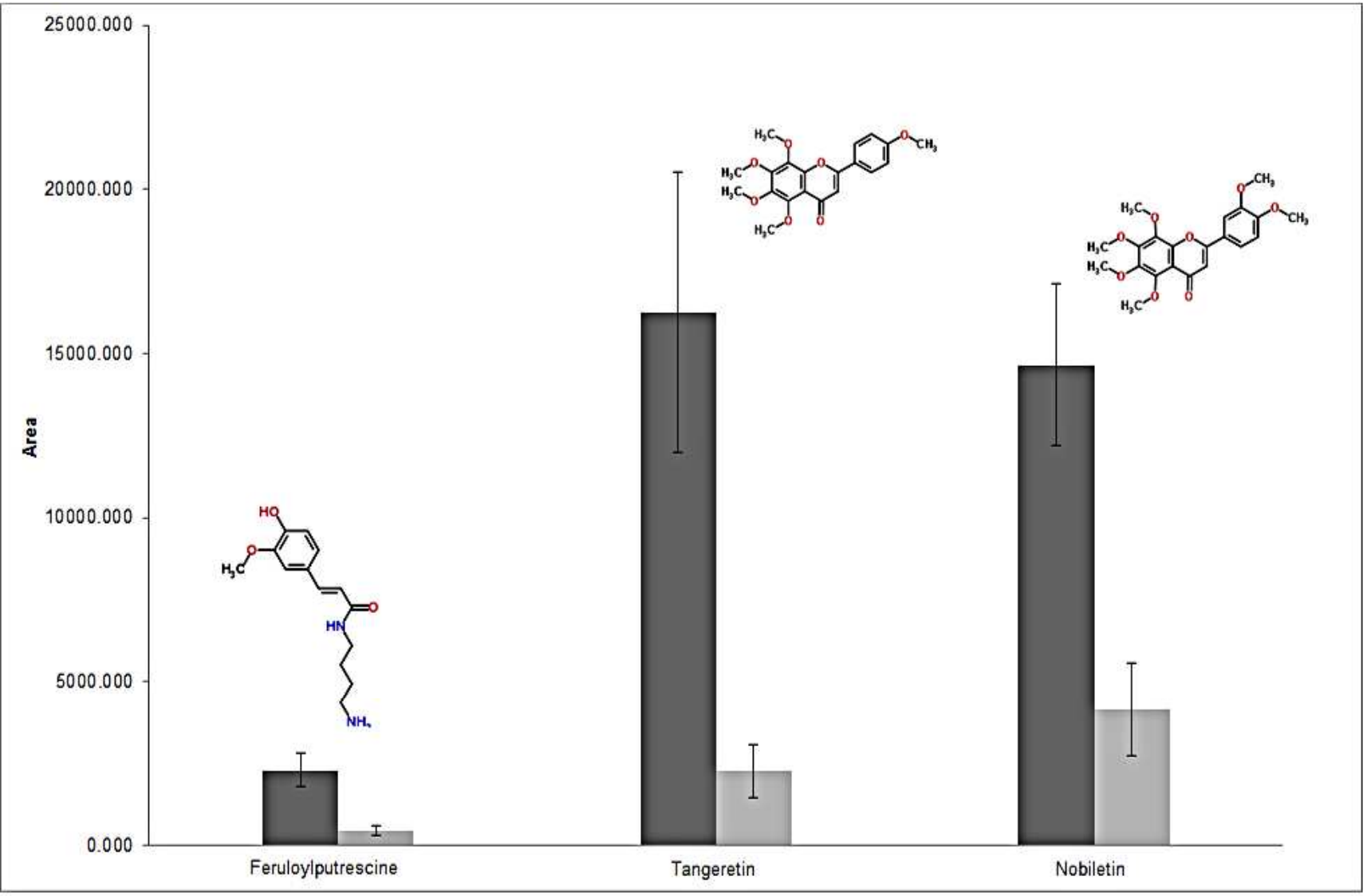
OJ/W (50/50)
OJ/W (75/25)
OJ/W (100/0)
Ethyl butyrate
Ethyl isovalerate
Limonene
Octanal
1-Octen-3-one
cis-3-hexenol
Ethyl octanoate
Linalool
Furfuryl alcohol
Eugenol
Vainillin


25000.000
20000.000
15000.000
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5000.000
0.000
Area
Feruloylputrescine
Tangeretin
Nobiletin