



Effectiveness of high-throughput miniaturized sorbent- and solid phase microextraction techniques combined with gas chromatography–mass spectrometry analysis for a rapid screening of volatile and semi-volatile composition of wines—A comparative study

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ABSTRACT

In this study the feasibility of different extraction procedures was evaluated in order to test their potential for the extraction of the volatile (VOCs) and semi-volatile constituents (SVOCs) from wines. In this sense, and before they could be analysed by gas chromatography–quadrupole first stage mass spectrometry (GC–qMS), three different high-throughput miniaturized (ad)sorptive extraction techniques, based on solid phase extraction (SPE), microextraction by packed sorbents (MEPS) and solid phase microextraction (SPME), were studied for the first time together, for the extraction step. To achieve the most complete volatile and semi-volatile signature, distinct SPE (LiChrolut EN, Poropak Q, Styrene-Divinylbenzene and Amberlite XAD-2) and MEPS (C_2 , C_8 , C_{18} , Silica and M1 (mixed C_8 -SCX)) sorbent materials, and different SPME fibre coatings (PA, PDMS, PEG, DVB/CAR/PDMS, PDMS/DVB, and CAR/PDMS), were tested and compared. All the extraction techniques were followed by GC–qMS analysis, which allowed the identification of up to 103 VOCs and SVOCs, distributed by distinct chemical families: higher alcohols, esters, fatty acids, carbonyl compounds and furan compounds. Mass spectra, standard compounds and *retention index* were used for identification purposes.

SPE technique, using LiChrolut EN as sorbent (SPE_{LiChrolut EN}), was the most efficient method allowing for the identification of 78 VOCs and SVOCs, 63 and 19 more than MEPS and SPME techniques, respectively. In MEPS technique the best results in terms of number of extractable/identified compounds and total peak areas of volatile and semi-volatile fraction, were obtained by using C_8 resin whereas DVB/CAR/PDMS was revealed the most efficient SPME coating to extract VOCs and SVOCs from Bual wine. Diethyl malate ($18.8 \pm 3.2\%$) was the main component found in wine SPE_{LiChrolut EN} extracts followed by ethyl succinate ($13.5 \pm 5.3\%$), 3-methyl-1-butanol ($13.2 \pm 1.7\%$), and 2-phenylethanol ($11.2 \pm 9.9\%$), while in SPME_{DVB/CAR/PDMS} technique 3-methyl-1-butanol ($43.3 \pm 0.6\%$) followed by diethyl succinate ($18.9 \pm 1.6\%$), and 2-furfural ($10.4 \pm 0.4\%$), are the major compounds. The major VOCs and SVOCs isolated by MEPS _{C_8} were 3-methyl-1-butanol ($26.8 \pm 0.6\%$, from wine total volatile fraction), diethyl succinate ($24.9 \pm 0.8\%$), and diethyl malate ($16.3 \pm 0.9\%$). Regardless of the extraction technique, the highest extraction efficiency corresponds to esters and higher alcohols and the lowest to fatty acids.

Despite some drawbacks associated with the SPE procedure such as the use of organic solvents, the time-consuming and tedious sampling procedure, it was observed that SPE_{LiChrolut EN}, revealed to be the most effective technique allowing the extraction of a higher number of compounds (78) rather than the other extraction techniques studied.

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1. Introduction

The volatile fraction of wine plays a prominent role in its organoleptic characteristics. It determines their aroma, which is the major contributor to overall flavor perception and one

of the most important parameters influencing the wine quality and consumer acceptance [1,2]. Their chemical composition contains numerous small molecules (over 1000 volatile compounds) belonging to distinct chemical families, including monoterpenoids, C_{13} -norisoprenoids, sesquiterpenoids, higher alcohols, ethyl esters, fatty acids, acetates, isoamyl esters, carbonyls, sulphurs, furan compounds, and volatile phenols. Moreover, these compounds have different physicochemical properties regarding concentration (ranging from several mg L^{-1} (e.g., ethyl acetate) to less than a

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Table 1
Basics of the considered extraction techniques.

Sorbent materials	SPE			
	Type	Interactions	Coating stability	Retention mechanism
LiChrolut EN (40–120 μm)	Porous polymer	Non polar	Hypercrosslinked	Sorption and partition
Porapak Q (50–80 mesh)	Porous polymer	Non polar	Partially crosslinked	Sorption and partition
Styrene-DVB (18–100 mesh)	Macroporous polymer	Polar	Partially crosslinked	Sorption and partition
Amberlite XAD-2 (20–60 mesh)	Porous polymer	Polar	Partially crosslinked	Sorption and partition
Sorbent materials	MEPS			
	Type	Interactions	Coating stability	Retention mechanism
Octadecyl (C_{18}) ^{a,b}	Polymer	Non polar	Partially crosslinked	Sorption and partition
Octyl (C_8) ^{a,c}	Polymer	Non polar	Partially crosslinked	Sorption and partition
Ethyl (C_2) ^{a,d}	Polymer	Non polar	Partially crosslinked	Sorption and partition
Mixed (C_8 -SCX) M_1 ^{a,e,f}	Polymer	Bipolar	Partially crosslinked	Sorption and partition
Silica (SIL) ^{a,f}	Porous particle	Polar	Partially crosslinked	Sorption and partition
Coating ^g	SPME			
	Type	Interactions	Coating stability	Retention mechanism
PDMS (100 μm)	Homogeneous polymer	Nonpolar	Nonbonded	Absorption
PDMS/DVB (65 μm)	Porous particle/polymer	Bipolar	Partially crosslinked	Adsorption
PA (85 μm)	Homogeneous polymer	Polar	Bonded crosslinked	Absorption
PEG (60 μm)	Homogeneous polymer	Polar	Highly crosslinked	Absorption
CAR/PDMS (75 μm)	Porous particle/polymer	Bipolar	Partially crosslinked	Adsorption
DVB/CAR/PDMS (50/30 μm)	Porous particle/polymer	Polar	Highly crosslinked	Adsorption

^a The base material is silica with mean particle and pore size of 50 μm and 60 Å, respectively.

^b Low carbon load C_{18} ; general purpose phase.

^c Less hydrophobic than C_{18} ; less retention of highly hydrophobic compounds; used when C_{18} is too retentive.

^d Short chain functional group is less hydrophobic than C_8 ; less retention of hydrophobic compounds; used when C_8 is too retentive.

^e Mixed-mode sorbents constituted by C_8 and SCX copolymer.

^f Highly polar surface; most common polar phase.

^g PDMS, polydimethylsiloxane; PDMS/DVB, polydimethylsiloxane/divinylbenzene; PA, polyacrylate; PEG, polyethylene glycol; CAR/PDMS, CARboxenTM/Polydimethylsiloxane; DVB/CAR/PDMS, divinylbenzene/carboxen/polydimethylsiloxane.

few ng L^{-1} (e.g., 3-isobutyl-2-methoxypyrazine, IBMP)), polarity, volatility and odour impact [3,4]. They are largely derived from four different sources: (i) the grape berry; (ii) processing of the grapes (namely crushing, pressing, etc.) by chemical, enzymatic-chemical and thermal reaction in grape must; (iii) the yeast strain used for fermentation; (iv) from containers used for wine making (wood, commonly oak) and chemical reactions during maturation wine storage [1].

Owing to the complex nature of the wine matrix, there is a consensus on that efficient sample preparation, trace-level detection and identification are important aspects of analytical methods to determine VOCs and SVOCs in wines. Many of the reported methods relies on extraction with organic solvents, including conventional techniques such as liquid–liquid extraction (LLE) [5], static or dynamic headspace extraction (SHS, DHS) [6], supercritical fluid extraction (SFE) [7], soxhlet extraction [8], and ultrasound extraction (USE) [9]. The broad polarity range of solvents and its general applicability made these techniques very popular [10]. However, most of these approaches present important drawbacks, typically are time-consuming and labour-intensive, uses of significant amounts of environmentally unfriendly solvents, and involves multi-step procedures, which can lead to analyte losses and a reduction in sensitivity. Finally, but also of importance, is the fact that many aroma compounds are chemically very unstable and can be easily oxidized or thermo degraded [4]. So, the search and development of adequate extraction techniques, that minimize the use of harmful organic solvents and/or even solvent-free procedures, and therefore more sustainable and easily implemented, has attracted the attention of many scientists. Therefore, in recent years miniaturized analytical techniques [11,12] had gained attention due to its many special features over conventional approaches [5–8]. Among many advantages, usage of little or no solvent, the low volumes of sample required, the greater sensitivity in sample preparation




than do the exhaustive extraction procedures, increasing of sensitivity of analysis and user-friendly system, should be pointed out (Table 1). So, relatively new miniaturized extraction techniques, such as microextraction by packed sorbents (MEPS), solid phase microextraction (SPME), and stir bar sorptive extraction (SBSE), have been gradually replacing conventional analytical methods.

The technique most frequently used for wine VOCs and SVOCs extraction or clean-up is SPE, which involves a liquid–solid partitioning, and the analytes are bound to active sites on the surface of solid sorbent. The possibility of using different sorbents for trapping analytes over a wide range of polarities, such as highly cross-linked copolymers, functionalized copolymers, graphitized carbons or some specific *n*-alkyl silicas, and eluents makes SPE a very selective technique (Table 1). The fact that only minor amounts of organic solvents are used compared to LLE, is why SPE has been extensively used for the analysis of volatile aroma compounds [8,13–16] and off-flavors [17,18] in wines.

More recently the conventional SPE (mL bed volumes) has been adopted for microextraction through a syringe packed with suitable solid phase material, a novel method for sample preparation and sample handling – microextraction by packed sorbent (MEPS) (μL volumes). Is a miniaturization of the conventional SPE in which the sample volume and volumes for extraction and washing solvents are reduced compared to SPE technique [19] (Table 2). A wide range of sorbents are available including C_2 , C_8 and C_{18} bonded phases on silica, polymeric resins (polystyrene/divinylbenzene copolymer), molecular imprinted polymers (MIPs), polar sorbents such as silica and ion-exchange sorbents, and mixed-mode sorbents. These different phases enable interactions based on adsorption, H-bonding, polar and nonpolar interactions, cation, anion exchange or size exclusion. This new technique is very promising because it is fast, simple, requires very small volume of samples (few μL) to produce comparable results to conventional SPE technique, and the cost of

Table 2

Schematic diagrams and comparison of a range of factors of the extraction techniques used in the study for liquid samples.

SPE	MEPS	SPME
 <p>Automation available Difficult to put on line No emulsions Parallel operation gives high throughput Polar and charged analytes may be extracted High sample size and moderate solvent volume Depleted sample may be recovered uncontaminated 15–30 min of extraction time Semi-volatile to non-volatile, nonpolar to polar, and ionisable analytes Excellent repeatability</p> <p>Few extractions Quantitative extraction A large range of sorbents are available, from nonpolar to ion-exchange materials Off-line</p>	 <p>Automation possible Designed for on-line (GC–MS, LC–MS) No emulsions Low volumes gives a fast method Polar and charged analytes may be extracted Low sample size and solvent volume Depleted sample may be recovered uncontaminated</p> <p>2–10 min Semi-volatile to non-volatile, nonpolar to polar, and ionisable analytes Good repeatability Suitable for on-site sampling 80–100^a extractions Quantitative extraction A large range of sorbents are available, from nonpolar to ion-exchange materials Can be used off-line or connected on-line with LC, GC, LC–MS, GC–MS and CE</p>	 <p>Automation possible Designed for on-line (GC–MS, LC–MS) No emulsions Fast and reproducible method Charged analytes are normally not efficiently extracted Solvent-free Depleted sample may be recovered uncontaminated</p> <p>15–60 min Suited for very volatile to semi-volatile, nonpolar and polar analytes Good repeatability Suitable for <i>on-site</i> and <i>in vivo</i> sampling Fibre used in 80–100^a extractions Not quantitative extraction Limited range of stationary phases</p> <p>Can be used off-line or connected on-line with LC, GC, LC–MS, GC–MS and CE</p>

^a Depends on the nature and purity of the matrix. According to Nerin et al. [19] the packed syringe can be used more than 400 times for water sample.

analysis is minimal compared to SPE (Table 2). Furthermore, this technique can be easily interfaced to LC–MS and GC–MS to provide a completely automated MEPS/LC–MS or MEPS/GC–MS system [20].

The MEPS technique has been used to extract various analytes from biological samples. Several drugs such as local anaesthetics and their metabolites [21]; the anticancer drugs roscovitine [22], olomoucine [23], and busulfan [24]; the β -blocker drugs acebutolol and metoprolol [25]; the anti-depressant drugs dopamine and serotonin [26] as well as anti-addictive methadone [27] have been successfully extracted by MEPS from biological samples such as plasma, urine or blood.

Solid-phase microextraction (SPME) emerged as a versatile alternative method of analyte extraction and pre-concentration, which requires little or no organic solvents, is easily automated, and can also improve the limits of detection [28]. SPME encompasses sampling, extraction, pre-concentration and introduction of the sample into the system of analyses in a single uninterrupted process, thus avoiding contamination of the matrix [28]. Generally accepted drawbacks are a relatively poor reproducibility, lot-to-lot variations, lack of selectivity, sensitivity against organic solvents, and the limited range of stationary phases which are commercially available, only roughly covering the scale of polarity [28]. Fibres are available coated with pure liquid polymeric phases or with porous solid phases (more accurately, porous solids dispersed in liquid polymer matrixes), in different coating combinations (Table 1), blends or copolymers, film thickness, and fibre assemblies enlarging to a certain extent the field of possible applications. The theoretical foundations of SPME have been extensively addressed in the literature [29–32]. Since its development, this technique has become very popular and gained growing acceptance and increasing use in routine laboratories applications mainly to the sampling and analysis of environmental [33], food [34–36], forensic [37] and pharmaceutical samples [38,39]. Typically followed by GC in combination with mass spectrometric detection (MS) [40–42] or its multidimensional alternative, comprehensive two-dimensional GC (GC \times GC–ToFMS) [43–45], SPME technique has been successfully used for wine samples.

Based on the volatile and semi-volatile global composition of Bual wines, we aimed to evaluate and compare the extraction potential of three different miniaturized (ad)sorptive extraction techniques, based on solid phase extraction (SPE), microextraction in packed syringe (MEPS), and solid phase microextraction (SPME), followed by gas chromatography–mass spectrometry (GC–qMS). A comparative study of several SPE sorbent materials (the polymeric sorbents LiChrolut EN, Poropak Q, styrene-divinylbenzene (Sty-DVB) and Amberlite XAD-2), different MEPS adsorbents (the classical bonded hydrocarbon phase RP-C₁₈, -C₈, -C₂, silica (SIL) and mixed-mode C₈-SCX sorbent: M1), using dichloromethane (DCM) as solvent, and all commercially available SPME fibre coatings (PA, PDMS, PEG, DVB/CAR/PDMS, PDMS/DVB, and CAR/PDMS), were evaluated, in order to obtain an in-depth characterization of the volatile and semi-volatile signature of Bual wine, and to select the best sorbent/fibre for further investigations.

Although many studies reporting the comparison between different extraction methodologies for the analysis of wine VOCs and SVOCs compounds have been published, as far we know, this is the first work aimed to test the effectiveness of miniaturized (ad)sorptive extraction techniques, SPE, MEPS and SPME, for the first time together. Moreover, MEPS applied to the analysis of wine volatile and semi-volatile constituents has been poorly studied [46]. The study presented herein represents a first approach of the analysis of wine volatile profile using MEPS.

2. Experimental

2.1. Reagents and materials

All chemicals were of analytical grade and were used without further purification. Ethanol absolute (EtOH) (99.5%), sodium chloride (NaCl) (AR Grade) and sodium sulphate anhydrous (Na₂SO₄) (Panreac, Barcelona, Spain). were obtained from Labodidáctica – Equipamentos de Laboratório e Didáticos, Lda (Funchal, Portugal). Dichloromethane (DCM) (99.99%) and methanol (MeOH) (HPLC gradient grade) were purchased from Fisher Scientific (Leicestershire, UK), ethyl acetate (99.8%) and diethyl ether (99.5%) from LAB

SCAN analytical sciences (Gliwice, Poland) and octan-3-ol (99%) from Sigma–Aldrich (Madrid, Spain). Ultra-pure water was prepared using a Milli-Q Plus water purification system to a resistivity of 18 M Ω cm (Millipore Corporation (Bedford, MA, USA). Ultra-high purity (UHP) gases for chromatography were obtained from Air Liquide (Portugal).

The SPE sorbents, Porapak Q 50/80 mesh, Dow Styrene-DVB 18-100 and Amberlite XAD-2 (Sigma) and Lichrolut EN 40–120 μ m (Merck, Darmstadt, Germany). A VisiprepTM SPE vacuum manifold (12-port model, Supelco), the manifold accessories, and the KNF-Laboport[®] vacuum pump for vacuum manifold were from Sigma–Aldrich (Bellefonte, PA, USA). Solid phases used in MEPS procedure (C₂, C₈, C₁₈, SIL, and M1 (mix-mode C₈-SCX)), the MEPS BIN and syringes, from SGE Analytical Science (SGE Europe Ltd., United Kingdom), were purchased from SGE Analytical Science (SGE Europe Ltd., United Kingdom).

The SPME fibres (Supelco, Bellefonte, PA, USA) coated with the following polymers: polydimethylsiloxane (PDMS, 100 μ m), polyacrylate (PA, 85 μ m), divinylbenzene/carboxen on polydimethylsiloxane (DBV/CAR/PDMS; StableFlex, 50/30 μ m), carboxen/polydimethylsiloxane (CAR/PDMS, 75 μ m) and polydimethylsiloxane/divinylbenzene (PDMS/DVB, 65 μ m), and polyethyleneglicol (PEG, 60 μ m), the SPME holder for manual sampling, clear glass screw cap vials for SPME with PTFE/silica (film thickness 1.3 mm) septa from Supelco (Bellefonte, PA, USA). Prior to initial use, all new fibres were conditioned as per the manufacturer's recommendations by heating in the injection port of the GC. Before the initial application, blank runs were completed before each sampling to ensure no carry-over of analytes from the previous extraction.

2.2. Wine samples

A monovarietal Madeira wine (medium sweet) with 5 years old (vintage 2005) with alcoholic degree of 16.0% (v/v) produced from *Vitis vinifera* L. Bual grape varieties, and a pH value of 3.4, was used in this study. The samples were kindly provided by Madeira Wine Company (MWC), Madeira Island, Portugal. All samples were kept at –20 °C until analysis. All analysis were carried out in triplicate.

2.3. Extraction procedures

Three different miniaturized extraction techniques, the conventional SPE, and the recent MEPS (using different sorbents and DCM as extracting solvent), and SPME in headspace mode, were tested and compared in order to evaluate their ability to extract VOCs and SVOCs from wines. Each technique has its own advantages and disadvantages, some of which are summarised in Table 2. To select a suitable sample pre-treatment technique, several factors such as the nature of analytes, the type of sample, the simplicity and ruggedness of the technique, the time required for the sample treatment, and the subsequent analytical technique are the key factors [10].

2.3.1. Solid phase extraction (SPE)

Four distinct hydrophilic reversed-phase SPE sorbents: LiChrolut EN (styrene-divinylbenzene copolymer), Porapak Q (ethylvinylbenzene-divinylbenzene copolymer), styrene-divinylbenzene copolymer (Sty-DVB), and Amberlite XAD-2 (styrene-divinylbenzene copolymer), were evaluated and compared. Styrene-DVB resin has been poorly studied, as opposed to other sorbents [47,48].

Each cartridge, containing 200 mg of sorbent, was processed manually with a SPE VisiprepTM 12-port vacuum manifold (Sigma–Aldrich). LiChrolut EN resin was activated with 4 mL of DCM, 2 mL of MeOH and, finally with 4 mL water–EtOH solution

(13%, v/v). For Sty-DVB and Porapak Q sorbents, the conditioning was carried out using 4 mL of MeOH and 2 mL of water. Amberlite XAD-2 resin, previously washed according to Edwards and Beelman procedure [49] to remove impurities, and conditioned with 2 M of HCl in MeOH. The extractions were performed according to the methodology proposed by Zapata et al. [50]. Briefly, 5 mL of wine, containing 200 μ L of internal standard (octan-3-ol, 3 mg L^{–1}), were passed through the SPE cartridge at around 1.5 mL min^{–1}. After this, the sorbent was dried by letting air pass through it. The adsorbents were washed with 4 mL of Milli-Q ultra-pure water to remove interferents. The analytes were recovered by elution with 1 mL of DCM. The organic extracts were dehydrated with anhydrous sodium sulphate and concentrated under N₂ atmosphere to a volume of 200 μ L. Then 1 μ L of extract were injected in GC–qMS system. The analyses were performed in triplicate.

2.3.2. Microextraction by packed sorbent (MEPS)

Using the MEPS procedure, the sample pre-treatment takes place on the packed bed, available in a variety of sorbents and inserted inside a syringe between the barrel and the needle (Barrel Insert and Needle Assembly, BIN). The MEPS BIN is easily installed into the syringe housing and then secured by a locking nut.

In the present work, MEPS was carried out using different BINs each containing 1 mg of based solid-phase material silica-C₂, C₈, C₁₈, SIL and M1 (mixed-mode C₈ + SCX sorbent; particle size 50 μ m) inserted into a 250 μ L gas-tight syringe. Before being used for the first time, the sorbents were manually conditioned firstly with 50 μ L of MeOH and then with 50 μ L of 0.1% formic acid solution. After that, an aliquot of 100 μ L of sample was drawn through the syringe up and down 10 times (multiple extraction cycles, increases the extraction recovery) from different aliquots (extract-discard). The sorbent was washed once with 100 μ L of 0.1% formic acid to remove interferences. The analytes were then eluted with 250 μ L DCM directly into the autosampler vials. After extraction, and in order to minimize/eliminate carry-over, the stationary phase was washed with 250 μ L of MeOH and 250 μ L 0.1% formic acid solution. To reuse MEPS cartridge the sorbent was washed 3–4 times with water and 3–4 with solvent (elution solution), to remove unwanted weakly retained interferences.

2.3.3. Solid phase microextraction (SPME)

To test the potentialities of SPME extraction technique and to compared them with the SPE and MEPS techniques, extractions were performed in the headspace mode using six distinct SPME fibres commercially available, namely a tripolar phase (divinylbenzene/carboxen/polydimethylsiloxane, DVB/CAR/PDMS 50/30 μ m), two bipolar phases (carboxen/polydimethylsiloxane, CAR/PDMS 75 μ m; and polydimethylsiloxane/divinylbenzene, PDMS/DVB 65 μ m); two polar phases (polyethylene glycol, PEG 60 μ m, and polyacrylate PA 85 μ m); and a non-polar phase (polydimethylsiloxane, PDMS 100 μ m). The fibres were conditioned before use as recommended by the manufacturer. Before the first daily analysis, the fibres were conditioned for 10 min at 250 °C in the GC injection port to eliminate any possible carryover on the coated fibre.

The HS-SPME experimental parameters were previously established by Câmara et al. [2]. Briefly, aliquots of 4 mL of Bual wine were placed into 8-mL glass vial, which correspond to a ratio of the volume of the liquid phase to the headspace volume (1/ β) of 0.5. After the addition of 0.5 g of NaCl (added in order to improve the extraction efficiency by decreasing the solubility of hydrophilic compounds in the aqueous phase) and a micro stirring bar (0.5 mm \times 0.1 mm; Supelco), the vial was capped with a PTFE septum and an aluminium cap (Chromacol, Hertfordshire, UK) and placed in a thermostat bath. All samples were incubated at 40.0 \pm 0.1 °C with agitation at 800 rpm for 5 min prior to extraction. Then the SPME fibre was manually inserted into the sample

vial headspace for 60 min at $40.0 \pm 0.1^\circ\text{C}$. As stirring of the solution usually improves the extraction, since the static layer resistant to mass transfer is destroyed (facilitate mass transport between the bulk of the aqueous sample and the fibre), all the experiments were performed under constant stirring velocity (800 rpm). Afterwards the SPME fibre was withdrawn into the needle, removed from the vial and inserted into the injection port of the GC–qMS system for 6 min where the analytes were thermally desorbed and transferred directly to the analytical column. Desorption temperatures, depending on the fibre used, ranging from 250°C for PDMS/DVB and PDMS to 300°C for CAR/PDMS. All measurements were performed in triplicate. Blanks, corresponding to the analysis of the coating fibre not submitted to any extraction procedure, were run between sets of five analyses.

2.4. GC–qMS analysis

The SPE and MEPS extracts and the SPME coating fibres containing VOCs and SVOCs of Bual wine were manually introduced into the GC injection port at 250°C (equipped with a glass liner, 0.75 mm i.d.). In SPME the fibres were kept for 5 min for VOCs and SVOCs thermal desorption. The desorbed VOCs and SVOCs were separated on a Agilent Technologies (Palo Alto, CA, USA) 6890N Network gas chromatograph equipped with a BP-20 (polyethylene glycol) fused silica capillary column (30 m \times 0.25 mm i.d.; film thickness, 0.25 μm) from Scientific Glass Engineering (Milton Keynes, UK) and interfaced with an Agilent 5975 quadrupole inert mass selective detector. The injection volume for liquid extracts (SPE and MEPS) was 1 μL . Ultra-high purity (UHP) helium (Air Liquide, Portugal) was used as the carrier gas at a constant flow of 1.1 mL min^{-1} (column head pressure of 13 psi). For the analysis of the SPE and SPME extracts, the temperature program was 40°C for 3 min, ramped at $1.7^\circ\text{C min}^{-1}$ to 200°C (3 min) then to 220°C at 3°C min^{-1} and held isothermally for a further 5 min. For MEPS extracts, and being in consideration that the extracts are much more simple than the obtained by SPE and SPME techniques, it was used a GC oven temperature program slightly different with a shorter run time, 40°C (1 min) to 220°C (2 min) ramped at 3°C min^{-1} , so the retention time of the analogues compounds is quite different. The rest of conditions were similar to the ones described.

The mass spectrometer was operated in the electron-impact (EI) ionisation mode at 70 eV. For the MS system the temperatures of the transfer line, quadrupole and ionisation source were 220, 180 and 230°C , respectively. The ionisation was maintained off during the first 4 min to avoid solvent overloading with a source temperature of 230°C . The mass spectra were recorded in range of 35–300 m/z and the acquisition was made in Full Scan mode with a scan rate of 6 scans/s. The electron multiplier voltage was set in the relative mode to auto tune procedure. MS system was operated under identical conditions in all analysis (SPE, MEPS, and SPME).

Identification of VOCs and SVOCs was achieved (1) comparing the GC retention times and mass spectra, with those, when available, of the pure standard compounds, (2) all mass spectra were also compared with the data system library (NIST, 2005 software, Mass Spectral Search Program V.2.0d; NIST 2005, Washington, DC), and (3) Kovats retention index (RI) values calculated through injection of SPE_{LiChrolut EN} and HS-SPME_{DVB/CAR/PDMS} extracts of a series of C_8 – C_{20} straight-chain *n*-alkanes (concentration of 40 mg L^{-1} in *n*-hexane) according to the following expression:

$$\text{RI}(x) = 100 \times z + 100 \frac{\text{RT}(x) - \text{RT}(z)}{\text{RT}(z+1) - \text{RT}(z)}$$

where $\text{RI}(x)$ is the retention index of the unknown compound x , z is the number of carbon atoms of the *n*-alkane eluted before the unknown compound x , $z+1$ is the number of carbon atoms of the *n*-alkane eluted after the unknown compound x , $\text{RT}(x)$ is the retention

time of the unknown compound x , $\text{RT}(z)$ is the retention time of the *n*-alkane eluted before the unknown compound x , $\text{RT}(z+1)$ is the *n*-alkane eluted after the unknown compound x . These values were compared, when available, with values reported in the literature for similar chromatographic columns [47].

The GC peak area data obtained were used as an indirect approach to estimate the relative content of each volatile and semi-volatile compound.

2.5. Statistical analysis

Significant differences among the three extraction techniques were assessed with a one-way analysis of variance (ANOVA) using a SPSS Program, version 19.0 (SPSS Inc. Headquarters, Chicago IL, USA). The Least Square Difference (LSD) test (p -value < 0.05) was used to compare the means. Principal component analysis (PCA) was used in order to establish an objective comparison among the extraction techniques. PCA is a tool for data exploration which allows the reduction of the dimensionality of data facilitating the analysis of inter-technique relationships. New variables, so called principal components, are obtained to explain the greater part of total variance with a minimum of information loss. Figures and Tables were generated using Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA, USA).

3. Results and discussion

Three extraction techniques, SPE, MEPS and SPME, were used to test their ability to extract VOCs and SVOCs from an aged (5 years old) medium sweet Madeira wine. The compounds are listed in Tables 3–5 following elution order, and including their chemical name, retention index for polar columns, the identification method used, and the corresponding odour descriptor. Differences in the chromatographic profiles were observed by using different sorbents. The identified compounds were organized in distinct chemical groups according to their chemical structure as higher alcohols (HA), esters (EST), volatile fatty acids (VFA), carbonyl compounds (CC) and furan compounds (FC), while compounds of differentiated structure were considered together in the class “miscellaneous” (others). The peak numbers of the chromatograms match the numbers of Tables 3–5.

3.1. SPE analysis

A comparison of different sorbents for the extraction of VOCs and SVOCs from Bual wines was carried by using hydrophobic sorbents. The sorbents selected were the classical polymeric sorbents (LiChrolut EN, Poropak Q; Sty-DVB, and Amberlite XAD-2). Fig. 1 shows a typical total ion chromatogram (TIC) of VOCs and SVOCs from a Bual wine obtained by SPE extraction technique with distinct sorbents.

Table 3 displays the compounds identified in the four SPE extracts derived from triplicate extractions, along with their retention indices (RI), odour description and peak area [51–61].

Effectiveness of the sorbents for wine volatile and semi-volatile constituents was evaluated by total peak area of the volatile fraction, reproducibility and number of the volatiles extracted. Among the four SPE sorbents evaluated, LiChrolut-EN clearly showed the highest extraction capacity as evidenced by previous studies [8,62] suggesting that its retention ability for VOCs and SVOCs is much stronger than the remaining three sorbents. In this approach, and for comparison of the extraction capacity, the maximum analytical signal obtained from the LiChrolut EN extract was taken as 100%, and the relative results for other SPE sorbent materials are shown in Fig. 2.

Table 3

Volatile and semi-volatile constituents identified in Bual Madeira wine samples (5 years old) obtained by SPE followed by GC–qMS, the corresponding retention times, retention indices and odour descriptors.

Peak no.	RT ^a (min)	RI _{lit} ^b	RI _{cal} ^c	Compound	ID ^d	Odour description	SPE [Peak area × 10 ⁶ ± SD(%)] (n = 3)			
							LiChrolut EN	Poropak Q	Sty-DVB ^e	Amberlite XAD-2
1	5.080	1046	1044	1-Propanol	S, MS	Alcohol, pungent	4.9 ± 1.9	0.8 ± 6.7	n.d ^f	n.d
2	5.352	1034	1049	Ethyl butyrate	MS	Strawberry, fruity	9.0 ± 8.4	1.5 ± 6.6	n.d	n.d
3	5.773	1049	1057	Ethyl 2-methylbutyrate	MS	Strawberry, berry	1.6 ± 8.3	n.d	n.d	n.d
4	5.966	1051	1061	1,1-Diethoxybutane	MS	Floral	9.1 ± 1.5	0.9 ± 4.5	n.d	n.d
5	6.267	1069	1066	Ethyl 3-methylbutyrate	MS	Exotic fruits	7.1 ± 0.8	1.8 ± 2.8	n.d	n.d
6	6.796	1099	1110	2-Methyl-1-propanol	S, MS	Fusel, bitter	146.7 ± 2.3	14.8 ± 8.0	n.d	2.0 ± 1.0
7	8.107	1125	1121	Isoamyl acetate	MS	Banana, glue-like	7.2 ± 1.4	2.0 ± 1.9	n.d	n.d
8	8.715	1159	1153	1-Butanol ^m	S, MS	Fruity, medicine	2.4 ± 0.2	n.d	n.d	n.d
9	12.101	1220	1210	3-Methyl-1-butanol	S, MS	Fusel, bitter, harsh	1317.3 ± 1.7	170.5 ± 0.9	n.d	20.4 ± 3.3
10	13.491	1239	1229	Ethyl hexanoate	S, MS	Apple, fruity, anise	4.4 ± 0.6	1.6 ± 4.3	n.d	n.d
12	14.772		1220	3-Ethyl-3-methylheptane	MS		n.d	n.d	n.d	0.4 ± 3.6
13	17.024		1260	Ethyl ethoxyacetate ^m	MS		3.6 ± 7.1	n.d	n.d	n.d
14	19.806		1304	Ethyl 3-ethoxypropanoate	MS		n.d	0.7 ± 2.4	n.d	n.d
15	20.230	1348	1345	Ethyl lactate	S, MS	Fruity	546.1 ± 0.8	73.4 ± 2.7	n.d	11.8 ± 6.1
16	21.005	1351	1352	1-Hexanol	S, MS	Green, grass	43.8 ± 1.5	4.9 ± 1.2	n.d	n.d
17	22.875	1367	1364	(Z)-3-hexen-1-ol ^m	MS	Grass, cypress, piney	5.7 ± 3.5	n.d	n.d	n.d
18	24.189	1387	1384	Ethyl 2-hydroxy butyrate	MS	Caramel	2.0 ± 8.7	n.d	n.d	n.d
19	25.235		1389	Ethyl 2-hydroxy acetate	MS		7.2 ± 1.6	0.6 ± 1.7	n.d	n.d
20	25.630	1383	1394	EHMB ^{g,m}	MS	Ripened pineapple, fruity	14.8 ± 3.7	n.d	n.d	n.d
21	26.477	1423	1428	cis-linalooloxide ^m	MS	Sweet, floral, creamy	4.7 ± 4.6	n.d	n.d	n.d
22	27.500	1452	1447	Acetic acid	S, MS	Vinegar, pungent	8.1 ± 1.9	3.7 ± 1.7	n.d	0.9 ± 1.1
23	28.114		1454	Isobutyl lactate ^m	MS		5.1 ± 1.0	n.d	n.d	n.d
24	28.431	1476	1468	2-Furfural	S, MS	Sweet, wood	77.7 ± 2.1	7.1 ± 4.1	n.d	n.d
26	31.089	1490	1482	2-Acetyl furan	MS	Balsamic, caramel, sweet	7.9 ± 0.7	n.d	n.d	n.d
27	31.841	1495	1493	Benzaldehyde	S, MS	Almond, burnt sugar	58.5 ± 4.0	8.5 ± 2.2	13.2 ± 4.0	n.d
28	33.799	1556	1523	2,3-Butanediol	S, MS	Fruity, onion	7.7 ± 5.1	1.5 ± 3.9	n.d	n.d
29	34.031	1551	1542	Ethyl di-2-hydroxyhexanoate	MS	Green, fruity, fresh	16.9 ± 5.5	1.7 ± 4.7	n.d	n.d
30	35.296	1550	1563	1-Octanol	S, MS	Pleasant, sweet	n.d	1.6 ± 3.3	n.d	1.0 ± 6.1
31	35.698	1558	1568	Isoamyl lactate	MS	Creamy, nutty	12.2 ± 6.2	2.0 ± 1.3	n.d	n.d
32	35.841	1572	1574	5-Methyl-2-furfural	S, MS	Caramel, burnt sugar	16.6 ± 5.8	1.1 ± 1.4	n.d	n.d
33	38.613		1595	Ethyl levulinate	MS	Sweet, fruity, cherry,	89.9 ± 4.4	5.9 ± 2.6	n.d	0.3 ± 3.3
34	39.424	1596	1608	Ethyl 2-furoate	MS	Woody, oily	10.5 ± 3.2	1.0 ± 2.8	n.d	n.d
35	39.725	1605	1614	Butyric acid ^m	S, MS	Cheese, rancid, sweat	7.7 ± 1.7	n.d	n.d	n.d
36	40.218	1635	1623	Phenylacetaldehyde	S, MS	Honey, floral, sweet	n.d	n.d	1.0 ± 7.8	n.d
37	40.532	1645	1629	Acetophenone	S, MS	Jasmine, almond	n.d	n.d	2.5 ± 1.9	n.d
38	41.097		1639	Diethyl methyl succinate ^m	S, MS	Fruity, wine-like	18.7 ± 4.8	n.d	n.d	n.d
39	41.741	1648	1650	Ethyl benzoate	S, MS	Camomile, celery	10.6 ± 5.0	1.7 ± 3.1	n.d	n.d
40	43.827	1684	1686	Diethyl succinate	MS	Wine, fruity, watermelon	1349.4 ± 5.3	213.9 ± 4.2	6.0 ± 3.7	12.7 ± 1.0
41	44.291		1694	3-Ethyl benzaldehyde	S, MS	Almond, sweet	n.d	n.d	1.0 ± 0.7	n.d
42	44.307		1695	4-Ethyl benzaldehyde	MS		n.d	4.6 ± 2.7	n.d	n.d
43	45.582	1665	1719	γ-Ethoxybutyrolactone ^m	MS		6.9 ± 2.9	n.d	n.d	n.d
44	48.347	1789	1772	Ethyl propyl succinate ^m	MS		4.9 ± 6.3	n.d	n.d	n.d
45	49.942	1782	1801	Ethyl phenylacetate	MS	Sweet, flowery, rose	7.7 ± 5.0	1.0 ± 0.9	n.d	n.d
46	50.093		1804	Diethyl glutarate	MS		10.5 ± 5.1	1.2 ± 7.8	n.d	n.d
47	50.393		1810	1-(2-Butoxyethoxy)-ethanol ^m	MS		7.7 ± 3.5	n.d	n.d	n.d
48	52.188		1845	m-Ethylacetophenone	MS	Strong, acacia	n.d	7.7 ± 4.1	n.d	n.d
49	53.986	1867	1879	Hexanoic acid	S, MS	Cheese, fatty	16.1 ± 1.8	0.04 ± 3.5	n.d	n.d
50	54.291		1885	p-Ethylacetophenone	MS	Hawthorn, fresh, floral	n.d	13.5 ± 4.7	n.d	n.d
51	54.489		1888	DEHMS ^{h,m}	MS		46.8 ± 1.1	n.d	n.d	n.d
52	55.584	1881	1909	Benzyl alcohol	S, MS	Floral, raspberry	36.8 ± 3.8	2.0 ± 1.9	n.d	n.d
53	57.794	1931	1951	2-Phenylethanol	S, MS	Roses, honey, pollen	1116.5 ± 9.9	114.2 ± 2.3	2.5 ± 1.7	12.2 ± 4.7
54	59.784	1977	1987	(E)-whiskey lactone	MS	Coconut, flowery	41.0 ± 5.8	5.2 ± 3.3	n.d	n.d
55	62.102	1944	2028	2,5-Furandicarboxaldehyde ^m	S, MS		31.5 ± 7.8	n.d	n.d	n.d

Table 3 (Continued)

Peak no.	RT ^a (min)	RI _{lit} ^b	RI _{cal} ^c	Compound	ID ^d	Odour description	SPE [Peak area × 10 ⁶ ± SD(%)] (n = 3)			
							LiChrolut EN	Poropak Q	Sty-DVB ^e	Amberlite XAD-2
56	66.116	2053	2094	diethyl malate	MS	Caramel	1877.6 ± 3.2	137.4 ± 1.5	2.7 ± 2.4	12.5 ± 2.7
57	66.884	2083	2087	Octanoic acid	S, MS	Sweat, cheese	24.6 ± 6.1	4.4 ± 0.50	n.d	n.d
58	71.571		2179	Sorbic acid ^m	S, MS	Bland	9.8 ± 6.4	n.d	n.d	n.d
59	72.411		2192	Ethyl 4-ethoxybenzoate	MS		211.2 ± 2.7	4.2 ± 0.6	1.1 ± 6.3	n.d
60	73.104	2202	2202	4-Ethyl-phenol	S, MS	Animal, stall	14.5 ± 5.3	0.8 ± 3.1	n.d	n.d
61	75.827		2242	EOTH2F ^f	MS		48.4 ± 5.9	5.2 ± 4.5	n.d	n.d
62	77.196		2261	EHMP ^g . ^m	MS	Fruity, sweet, musty	21.5 ± 5.8	n.d	n.d	n.d
63	77.938		2271	4-Hydroxybenzene ethanol	MS		n.d	18.2 ± 2.2	n.d	n.d
64	81.243		2330	bDMEP ^h	MS	Sweet	n.d	n.d	n.d	0.5 ± 4.1
65	81.432	2358	2351	Diethyl tartrate	S, MS	Earth, must	242.7 ± 2.4	17.1 ± 0.5	n.d	n.d
66	84.067	2440	2358	Ethyl succinate	S, MS		1032.1 ± 2.6	41.9 ± 5.6	n.d	n.d
67	85.845	2457	2389	Benzoic acid	S, MS	Aromatic, flowery	84.7 ± 6.3	5.9 ± 2.0	5.2 ± 1.9	n.d
68	88.519	2476	2436	Ethyl citrate	S, MS		249.0 ± 8.3	21.8 ± 0.7	n.d	0.4 ± 1.8
69	90.045	2518	2485	5-(Hydroxymethyl)-2-furfural	S, MS	Butter, caramel, musty	648.8 ± 1.7	43.4 ± 4.8	n.d	7.7 ± 3.5
70	92.373	2555	2538	Vanillin	S, MS	Vanilla, spicy	67.2 ± 7.4	3.0 ± 2.3	n.d	n.d
71	94.386	2598	2556	Methyl vanillate ^m	MS	Vanilla, dry herbs	17.7 ± 0.7	n.d	n.d	n.d
72	94.638		2558	Ethyl pyrrole-2-carboxylate	MS		63.0 ± 2.1	n.d	n.d	n.d
73	95.831	2650	2564	Ethyl vanillate	MS	Vanilla, pollen, flowery	25.3 ± 9.2	2.4 ± 0.5	n.d	n.d
74	106.671		2754	Ethyl <i>p</i> -hydroxyl cinnamate	MS		n.d	n.d	n.d	9.8 ± 1.2
75	108.265		2782	2-Phenylbutyrophenone	MS	Cherry, vanilla	n.d	n.d	2.4 ± 3.1	n.d
76	108.562		2787	Ethyl 4-ethoxy benzoate ^m	MS		14.1 ± 2.8	n.d	n.d	n.d
77	109.775	2904	2809	Syringaldehyde	MS	Chocolate, grape, woody	164.3 ± 2.6	8.3 ± 2.2	n.d	n.d
78	110.765		2826	Isovanillic acid	MS		n.d	n.d	8.3 ± 0.6	n.d
Total peak area (× 10⁶)							9995.4	986.8	46.0	92.6
Average SD (%)							4.1	3.0	3.1	3.1
No. compounds/sorbent							63	47	11	14

^a Retention time (min).^b Retention indices reported in the literature for BP-20 capillary column or equivalents [51–61].^c Retention indices calculated from C₈ to C₂₀ *n*-linear alkanes with BP-20 capillary column.^d Identification method: S, identification based on retention time and electro impact mass spectrum of standards; MS, identification based on examination of electron impact mass spectrum and comparison to NIST05 library.^e Styrene-DVB.^f Not detected.^g Ethyl 2-hydroxy-3-methyl-butylate.^h Diethyl 2-hydroxy-3-methylsuccinate.ⁱ Ethyl 5-oxotetrahydro-2-furancarboxylate.^j Ethyl 2-hydroxy-4-methyl-pentanoate.^l 2,5-Bis(1,1-dimethylethyl)-phenol.^m VOCs and SVOCs extracted only by LiChrolut EN sorbent.

Table 4

Volatile and semi-volatile composition of Bual Madeira wine obtained by MEPS/GC–qMS methodology, the corresponding retention times, retention indices, and odour description.

Peak no.	RT ^a (min)	RI _{lit} ^b	RI _{cal} ^c	Compound	ID ^d	Odour description	MEPS [Peak area × 10 ⁶ ± SD(%)] (n = 3)				
							C ₁₈	C ₈	C ₂	SIL	M1
1	5.337	1099 ^g	1118	2-Methyl-1-propanol	S, MS	Fusel, bitter	1.3 ± 4.0	1.3 ± 1.1	n.d. ^e	n.d	1.0 ± 6.8
2	8.696	1220 ^g	1202	3-Methyl-1-butanol	S, MS	Fusel, bitter, harsh	27.3 ± 2.2	26.2 ± 0.06	1.8 ± 1.0	0.9 ± 4.3	19.2 ± 1.9
3	13.674	1348	1340	Ethyl lactate	S, MS	Fruity	6.2 ± 2.7	9.0 ± 0.6	1.8 ± 0.6	1.4 ± 4.1	6.5 ± 2.2
4	14.079	1351	1336	1-Hexanol	S, MS	Green, grass	1.1 ± 2.6	0.7 ± 9.5	n.d	n.d	0.6 ± 0.8
5	18.165	1452 ^g	1469	Acetic acid	S, MS	Vinegar, pungent	0.8 ± 3.7	0.3 ± 2.2	n.d	n.d	0.2 ± 1.3
6	24.503		1579	Ethyllevulate	MS	Sweet, fruity, cherry	0.3 ± 5.8	0.1 ± 2.7	n.d	n.d	0.2 ± 0.4
7	27.219		1621	Diethyl succinate	S, MS	Fruity, wine-like	25.4 ± 1.8	25.5 ± 0.8	1.1 ± 0.3	0.1 ± 1.6	20.0 ± 1.1
8	35.698	1931	1953	2-Phenylethanol	S, MS	Roses, honey, pollen	12.3 ± 2.4	11.7 ± 0.9	0.3 ± 5.1	n.d	9.1 ± 0.9
9	40.343	2053 ^h	2075	Diethyl malate	S, MS	Caramel	16.6 ± 1.6	20.0 ± 0.9	2.2 ± 0.4	0.9 ± 4.7	16.1 ± 0.9
10	44.363		2196	Ethyl 4-ethoxybenzoate	MS		1.6 ± 3.5	0.7 ± 2.6	n.d	n.d	0.7 ± 4.5
11	46.288		2233	HMBL ^j	S, MS		0.50 ± 0.7	0.6 ± 9.3	n.d	n.d	0.5 ± 6.9
12	48.972		2330	bDMEP ^l	MS	Sweet	0.2 ± 1.2	n.d	n.d	0.6 ± 0.1	0.1 ± 0.4
13	50.943	2440	2388	Ethyl succinate	S, MS		0.8 ± 4.1	0.6 ± 1.1	n.d	n.d	n.d
14	53.404	2476 ^h	2438	Ethyl citrate	S, MS		3.9 ± 1.8	4.0 ± 0.2	0.2 ± 8.7	0.4 ± 1.9	3.2 ± 0.6
15	54.293	2518 ^h	2489	5-(Hydroxymethyl)-2-furfural	S, MS	Butter, caramel, musty	3.3 ± 3.0	5.9 ± 1.6	1.8 ± 2.1	7.1 ± 0.9	4.6 ± 0.1
Total peak area (× 10⁶)							101.7	106.7	9.8	11.3	81.9
Average SD (%)							2.8	2.4	2.6	2.5	2.1
No. compounds/sorbent							15	14	8	7	14

^a Retention time (min).^b Retention indices reported in the literature for BP-20 capillary column or equivalents [51–61].^c Retention indices calculated from C₈ to C₂₀ *n*-linear alkanes.^d Identification method: S, identification based on retention time and electron impact mass spectrum of standards; MS, identification based on examination of electron impact mass spectrum and comparison to NIST05 library.^e Not detected.^g Reference [53].^h Reference [60,61].^j 5-Hydroxymethyl γ -butyrolactone.^l 2,5-Bis(1,1-dimethyl)-phenol.

Table 5
Volatile and semi-volatile components present in the wine headspace of a Bual medium sweet Madeira wine (extraction temperature 40.0 °C for 60 min) followed by a GC–qMS analysis, using GC conditions that allow an adequate chromatographic resolution, the corresponding retention times, retention indices, and odour description.

Peak no.	RT ^a (min)	RI _{lit} ^b	RI _{cal} ^c	Compound	ID ^d	Odour description	SPME (Peak area × 10 ⁶ ± RSD(%))(n = 3)					
							DVB/CAR/PDMS	CAR/PDMS	PDMS/DVB	PDMS	PA	PEG
1	7.365	1099	1110	2-Methyl-1-propanol	S, MS	Fusel, bitter,	34.0 ± 1.1	13.7 ± 1.0	13.7 ± 1.5	n.d	24.8 ± 1.3	22.4 ± 3.2
2	9.735	1201	1126	Limonene	S, MS	Citrus-like, fresh	37.3 ± 2.1	12.6 ± 3.5	10.8 ± 3.4	7.0 ± 4.8	1.8 ± 2.5	0.6 ± 0.86
3	10.637		1145	(E)-6-dodecene	MS		2.0 ± 0.4	n.d ^e	n.d	n.d	n.d	n.d
4	11.853		1168	1-Dodecene	MS		n.d	n.d	n.d	10.7 ± 0.8	n.d	n.d
5	12.644	1220	1210	3-Methyl-1-butanol	S, MS	Fusel, bitter, harsh	354.3 ± 0.6	420.6 ± 0.7	236.8 ± 0.9	195.2 ± 1.3	242. ± 0.84	294.4 ± 1.6
6	12.942	1239	1229	Ethyl hexanoate	S, MS	Apple, fruity, anise	n.d	15.3 ± 4.3	n.d	n.d	n.d	n.d
7	13.347		1232	(E)-3-dodecene	MS		2.1 ± 0.3	n.d	n.d	n.d	n.d	n.d
8	14.007		1240	(Z)-2-dodecene	MS		5.1 ± 0.4	n.d	2.6 ± 0.4	3.1 ± 0.9	n.d	n.d
9	14.501	1263	1245	<i>p</i> -Cymene	MS	Spicy, herbaceous, citrus-like	n.d	7.7 ± 0.73	n.d	n.d	n.d	n.d
10	19.567		1304	Ethyl 3-ethoxypropanoate	MS		3.0 ± 6.7	3.3 ± 1.5	1.7 ± 1.7	n.d	n.d	n.d
11	20.368	1348	1345	Ethyl lactate	MS	Fruity	53.9 ± 3.7	58.1 ± 1.0	49.8 ± 1.7	53.9 ± 4.2	44.7 ± 0.8	72.0 ± 0.8
12	21.061	1351	1352	1-Hexanol	S, MS	Green, grass	15.8 ± 1.4	39.9 ± 1.5	8.0 ± 7.4	8.7 ± 1.3	8.1 ± 0.6	7.7 ± 1.0
13	22.908	1367	1364	(Z)-3-Hexen-1-ol	S, MS	Grass, cypress, piney	n.d	1.4 ± 1.02	n.d	n.d	n.d	n.d
14	23.002	1385	1373	Nonanal	MS	Citrus, fatty	3.4 ± 1.42	n.d	2.0 ± 2.71	n.d	n.d	n.d
15	23.471	1400	1379	Tetradecane	MS		n.d	n.d	n.d	5.5 ± 4.3	n.d	n.d
16	25.634	1383	1395	EHMB ^f	MS	Ripened pineapple, fruity	1.9 ± 2.38	1.4 ± 1.00	n.d	n.d	n.d	n.d
17	26.111	1436	1402	Ethyl octanoate	S, MS	Fruity, fat	5.5 ± 3.8	n.d	4.6 ± 4.9	7.4 ± 1.4	1.0 ± 2.4	n.d
18	26.683		1412	1-Tetradecene	MS		5.3 ± 0.6	n.d	8.6 ± 2.51	11.7 ± 6.0	n.d	n.d
20	27.729	1452	1447	Acetic acid	S, MS	Vinegar, pungent	5.6 ± 1.4	3.6 ± 1.1	5.7 ± 2.8	15.9 ± 5.0	11.8 ± 1.5	35.8 ± 0.6
21	28.468	1476	1468	2-Furfural	S, MS	Sweet, wood	62.2 ± 0.4	100.7 ± 0.7	6.7 ± 3.3	12.7 ± 6.8	6.1 ± 0.85	7.8 ± 1.4
22	30.399	1484	1471	Decanal	MS	Tallow, soap, orange	n.d	n.d	10.5 ± 3.9	16.6 ± 3.2	n.d	n.d
23	30.504		1473	Isooctanol	MS		9.1 ± 0.1	2.8 ± 3.4	n.d	n.d	7.2 ± 0.4	1.1 ± 0.4
24	31.096	1490	1482	2-Acetylfuran	MS	Balsamic, caramel	n.d	2.7 ± 0.4	n.d	n.d	n.d	n.d
25	31.243		1484	Ethyl sorbate	MS	Fruity, sweet, pineapple	3.3 ± 0.14	n.d	2.2 ± 3.3	n.d	n.d	n.d
26	31.828	1495	1493	Benzaldehyde	S, MS	Almond, burnt sugar	91.1 ± 0.4	86.7 ± 1.1	9.1 ± 0.2	8.7 ± 2.2	6.6 ± 0.2	5.6 ± 4.2
27	33.952		1526	2,6-Dimethyl-4-heptanol	MS		3.7 ± 0.1	2.2 ± 0.6	3.7 ± 1.3	n.d	n.d	n.d
28	34.047	1556	1523	2,3-Butanediol	MS	Fruity, onion	n.d	n.d	n.d	n.d	n.d	4.8 ± 0.9
29	35.838	1572	1574	5-Methyl-2-furfural	MS	Caramel, burnt sugar	13.9 ± 0.7	7.5 ± 0.2	n.d	n.d	n.d	n.d
30	38.568		1595	Ethyllevulate	MS	Sweet, fruity, cherry	3.2 ± 0.6	3.7 ± 5.3	3.5 ± 0.6	5.3 ± 1.2	2.3 ± 2.9	2.0 ± 0.8
31	38.741		1596	γ-Butyrolactone	MS	Cheese, burnt sugar	n.d	n.d	n.d	n.d	1.0 ± 0.8	n.d
32	38.983		1600	Carbitol	MS		n.d	n.d	n.d	n.d	3.6 ± 0.6	2.7 ± 3.3
33	39.346	1596	1608	Ethyl 2-furoate	MS	Woody, oily	9.0 ± 0.2	6.4 ± 4.4	3.2 ± 0.9	4.2 ± 1.5	1.6 ± 2.4	1.4 ± 2.5
34	41.003		1639	Diethyl methylsuccinate	S, MS	Fruity, wine-like	3.0 ± 0.4	n.d	3.0 ± 1.4	n.d	0.7 ± 0.7	n.d
35	41.614	1648	1650	Ethyl benzoate	S, MS	Camomile, celery	21.1 ± 0.5	6.4 ± 2.4	12.4 ± 3.9	n.d	4.5 ± 1.0	2.5 ± 1.6
36	43.425	1684	1686	diethyl succinate	S, MS	Wine, fruity, watermelon	203.1 ± 0.1	124.3 ± 0.5	266.3 ± 1.3	129.8 ± 2.4	120 ± 2.4	78.6 ± 0.8
37	49.828	1782	1801	Ethyl phenylacetate	MS	Sweet, flowery, rose	10.6 ± 3.1	1.7 ± 0.7	10.7 ± 6.3	9.3 ± 0.3	2.8 ± 1.5	1.4 ± 0.4
38	50.894		1820	3,4-Dimethyl-benzaldehyde	MS		8.8 ± 0.9	1.2 ± 0.5	9.9 ± 0.9	n.d	2.3 ± 1.0	n.d
39	51.724		1836	2-Phenylethyl acetate	MS	roses	n.d	n.d	n.d	n.d	0.9 ± 0.75	n.d
40	54.456		1888	DEHMS ^g	MS		n.d	n.d	7.5 ± 3.23	21.8 ± 2.75	n.d	n.d
41	55.646	1881	1909	Benzyl alcohol	S, MS	Floral, raspberry	4.7 ± 0.2	0.7 ± 4.4	6.2 ± 0.7	n.d	1.6 ± 0.4	1.6 ± 1.9
42	55.669		1911	TMPOPMP ^h	MS		n.d	n.d	n.d	18.6 ± 2.7	n.d	n.d
43	57.16		1939	α-Ionol	MS		14.4 ± 0.3	0.7 ± 0.2	12.8 ± 0.9	19.2 ± 0.9	6.5 ± 0.7	4.3 ± 1.6

Table 5 (Continued)

Peak no.	RT ^a (min)	RI _{lit} ^b	RI _{cal} ^c	Compound	ID ^d	Odour description	SPME (Peak area $\times 10^6 \pm$ RSD(%))(n = 3)					
							DVB/CAR/PDMS	CAR/PDMS	PDMS/DVB	PDMS	PA	PEG
44	57.611	1931	1951	2-Phenylethanol	S, MS	Roses, honey, pollen	58.4 \pm 0.3	41.4 \pm 5.7	67.8 \pm 1.5	38.1 \pm 3.5	78.2 \pm 0.7	79.7 \pm 1.6
45	65.881	2053	2094	Diethylmalate	MS	Caramel	4.9 \pm 0.8	1.6 \pm 2.5	7.3 \pm 0.83	21.9 \pm 5.2	13.7 \pm 0.8	11.3 \pm 1.1
46	67.109	2083	2087	Octanoic acid	S, MS	Sweat, cheese	1.9 \pm 0.7	n.d.	n.d.	22.5 \pm 1.6	4.1 \pm 2.6	4.2 \pm 4.6
47	72.454		2192	Ethyl 4-oxobenzozoate	MS		5.4 \pm 0.2	0.7 \pm 0.2	25.4 \pm 4.7	32.5 \pm 1.4	16.2 \pm 2.5	14.5 \pm 4.4
48	73.186	2202	2202	4-Ethyl-phenol	MS	Animal, stall	n.d.	n.d.	0.6 \pm 6.6	n.d.	0.9 \pm 7.5	0.8 \pm 1.3
49	81.178		2330	bDMEP ^f	MS	Sweet	14.8 \pm 2.4	1.1 \pm 0.3	11.2 \pm 2.1	26.0 \pm 1.5	10.0 \pm 0.7	12.8 \pm 0.3
50	87.602		2345	Benzophenone	MS	Balsamic	n.d.	n.d.	n.d.	n.d.	0.6 \pm 1.2	n.d.
51	89.961	2518	2485	5-(Hydroxymethyl)-2-furfural	S, MS	Butter, caramel, musty	n.d.	n.d.	n.d.	n.d.	1.8 \pm 0.7	n.d.
52	99.761		2716	Tetradecanoic acid	MS		n.d.	n.d.	n.d.	44.5 \pm 1.3	n.d.	4.8 \pm 3.2
53	109.041		2818	Hexadecanoic acid	MS		n.d.	n.d.	n.d.	57.4 \pm 0.6	7.7 \pm 1.2	8.9 \pm 2.2
Total peak area ($\times 10^6$)							1076.0	970.0	824.5	863.3	636.2	721.9
Average RSD (%)							1.2	1.8	2.5	2.5	1.4	1.8
No. compounds by fibre							34	29	31	27	32	26

^a Retention time (min).^b Retention indices reported in the literature for BP-20 capillary column or equivalents [51–61].^c Retention indices calculated from C₈ to C₂₀ n-linear alkanes with BP-20 capillary column.^d Identification method: S, identification based on retention time and electro impact mass spectrum of standards; MS, identification based on examination of electron impact mass spectrum and comparison to NIST05 library.^e Not detected.^f Ethyl 2-hydroxy-3-methyl-butyrate.^g Diethyl ester 2-hydroxy-3-methyl-succinic acid.^h [2,4,4-trimethyl-1-(2-methylpropanoyloxy)pentan-3-yl] 2-methylpropanoate.ⁱ Moon and Shibamoto [55]; bDMEP: 2,5-bis(1,1-Dimethylethyl)-phenol.

As can be observed, LiChrolut EN was, by far, the sorbent that provided the most globally favourable analytical response, whereas for the other sorbents the sorption capacity was variable ranging from less than 0.5% (Sty-DVB) to ca. 10% (Poropak Q). The higher sorption capacity of polymeric LiChrolut EN for Bual wine VOCs and SVOCs, with regards to other sorbents, can be ascribed to specific interactions of this sorbent with relatively polar solutes, in addition to the typical hydrophobic mechanisms. According to Table 3, Poropak Q sorbent present lower potential to extract Bual wine VOCs and SVOCs (47 compounds) than LiChrolut EN, while Amberlite XAD-2 and Sty-DVB sorbent present the lowest extraction efficiency, being able to extract only 14 and 11 compounds, respectively.

Considering the aroma compounds by groups (Fig. 3), the esters and higher alcohols were the predominant volatiles, followed by furan and carbonyl compounds, the two first groups, produced during the alcoholic fermentation, playing an important role on the flavor of wines. C-6 alcohol fraction is easily recognizable by their strong and pungent smell and taste, related to herbaceous notes [63,64]. As for the esters, they are regarded as very important in the aroma of young wines due to their fruity flavors [63]. Other minor groups of aroma compounds detected were volatile fatty acids, carbonyl compounds and furan compounds. According to Fig. 3, the nature of the sorbent/coating is an important aspect for the pre-concentration of the considered chemical groups since each shows a different extraction profile.

Considering individual compounds, diethyl malate (**56**; $1877.6 \times 10^6 \pm 3.2\%$) was the main component found in the SPE_{LiChrolut EN} extracts followed by diethyl succinate (**40**; $1349.4 \times 10^6 \pm 5.3\%$), 3-methyl-1-butanol (**9**; $1317.3 \times 10^6 \pm 1.7\%$) and 2-phenylethanol (**53**; $1116.5 \times 10^6 \pm 9.9\%$). Moreover 20 VOCs and SVOCs (coded as “m” in Table 3) were extracted only by this sorbent. Poropak Q seems able to extract diethyl succinate (**40**; $213.9 \times 10^6 \pm 4.2\%$), 4-hydroxybenzene ethanol (**63**; $18.2 \times 10^6 \pm 2.2\%$), 1-hexanol (**16**; $4.9 \times 10^6 \pm 1.2\%$), and ethyl hexanoate (**10**; $1.6 \times 10^6 \pm 4.3\%$), better than the other sorbents used. Benzaldehyde (**27**; $13.2 \times 10^6 \pm 4.0\%$), isovanillic acid (**78**; $8.3 \times 10^6 \pm 0.6\%$), diethyl malate (**56**; $2.7 \times 10^6 \pm 2.4\%$), and benzoic acid (**67**; $5.2 \times 10^6 \pm 1.9\%$), had high affinity for Sty-DVB sorbent, while Amberlite XAD-2 exhibit a strong sorption capacity for 3-methyl-1-butanol (**9**; $20.4 \times 10^6 \pm 3.3\%$), diethyl succinate (**40**; $12.7 \times 10^6 \pm 1.0\%$), diethyl malate (**56**; $12.5 \times 10^6 \pm 2.7\%$), 2-phenylethanol (**53**; $12.2 \times 10^6 \pm 4.7\%$), and ethyl lactate (**15**; $11.8 \times 10^6 \pm 6.1\%$). Ethyl p-hydroxycinnamate (**74**; $9.8 \times 10^6 \pm 1.2\%$) was extracted only by using Amberlite XAD-2 as sorbent.

3.2. MEPS analysis

Until the present there are no previous studies reporting the use of MEPS to extract VOCs and SVOCs from wine samples. To fulfil this requirement, and although the most common sorbent is C₁₈, different types of sorbents, namely C₁₈, C₈, C₂, M1 and SIL (Table 4 and Fig. 4) were evaluated and compared [51–61].

Fig. 4 shows typical chromatograms of VOCs and SVOCs from a Bual wine sample obtained by MEPS using the sorbents under consideration. Table 4 shows the compounds identified in the above five sorbents, along with their retention indices (RI), odour descriptors and peak area. The results shows, that the C₁₈, C₈ and M₁ sorbents extracted almost the same VOCs and SVOCs, 15, 14 and 14, respectively, C₈ sorbent was able to concentrate larger amount of extracted compounds (Fig. 2). C₂ and SIL sorbents were found to be the least efficient to extract VOCs and SVOCs from wine samples. Fig. 2 shows the number of VOCs and SVOCs extracted as a function of the sorbent material and also compares the relative average

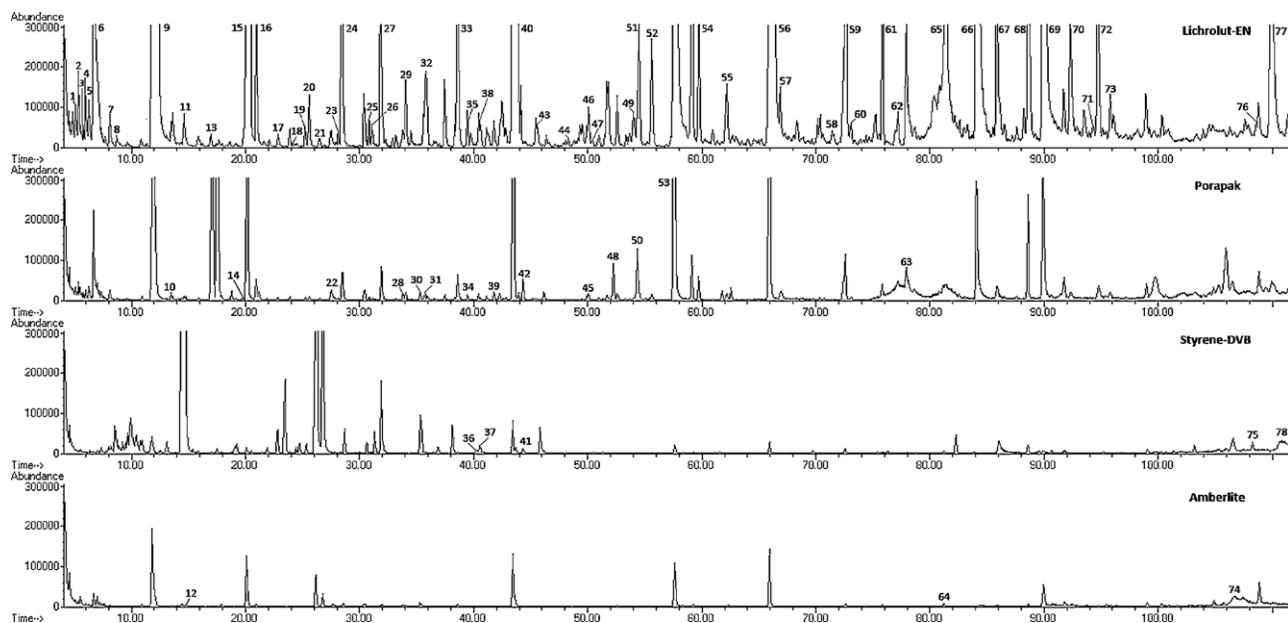


Fig. 1. Comparative profile of VOCs and SVOCs composition of Bual Madeira wine sample obtained by SPE-GC-qMS through four different sorbents, namely LiChrolut EN, Poropak Q, styrene-DVB and Amberlite XAD-2. Peak assignments and identification appear in Table 3.

percent peak areas obtained for each sorbent, relatively to C₈ (taken as 100%).

Considering the identified compounds, the predominant ones isolated through MEPS were 3-methyl-1-butanol (**2**; $24.2 \pm 3.8\%$ on average), diethyl succinate (**7**; $23.2 \pm 5.4\%$), diethyl malate (**9**; $17.9 \pm 6.2\%$), 2-phenylethanol (**8**; $10.7 \pm 9.8\%$), and ethyl lactate (**3**; $8.0 \pm 11.2\%$), for all sorbents.

As determined by SPE, esters and higher alcohols were the main chemical groups identified by MEPS/GC-qMS. The C₁₈ sorbent revealed higher extraction efficiency to esters and fatty acids, whereas C₈ is more efficient to extract ethyl esters and furan compounds. For carbonyl compounds and miscellaneous (Mis) SIL present better results (Fig. 3b). Considering the individual VOCs

and SVOCs, 5-(hydroxymethyl)-2-furfural and ethyl lactate, were efficiently extracted by SIL, 63 ± 2.1 and $12 \pm 4.1\%$, respectively, of the total fraction extracted by this sorbent. C₁₈, C₈ and M1 sorbents showed higher potential to extract 3-methyl-1-butanol ($27.3 \times 10^6 \pm 2.2\%$; $26.2 \times 10^6 \pm 0.06\%$, and $19.2 \times 10^6 \pm 1.9\%$, respectively, of the total Bual wine VOCs and SVOCs extracted by the corresponding sorbent), diethyl succinate ($25.4 \times 10^6 \pm 1.8\%$, $25.5 \times 10^6 \pm 0.8\%$ and $20.0 \times 10^6 \pm 1.1\%$, respectively), diethyl malate ($16.6 \times 10^6 \pm 1.6\%$; $20.0 \times 10^6 \pm 0.9\%$, and $16.1 \times 10^6 \pm 0.9\%$, respectively), and 2-phenylethanol ($12.3 \times 10^6 \pm 2.4\%$; $11.7 \times 10^6 \pm 0.9\%$, and $9.1 \times 10^6 \pm 0.9\%$, respectively), than C₂ and SIL materials. C₂ showed highest efficiency to diethyl malate ($2.2 \times 10^6 \pm 0.4\%$) and ethyl lactate ($1.8 \times 10^6 \pm 0.6\%$).

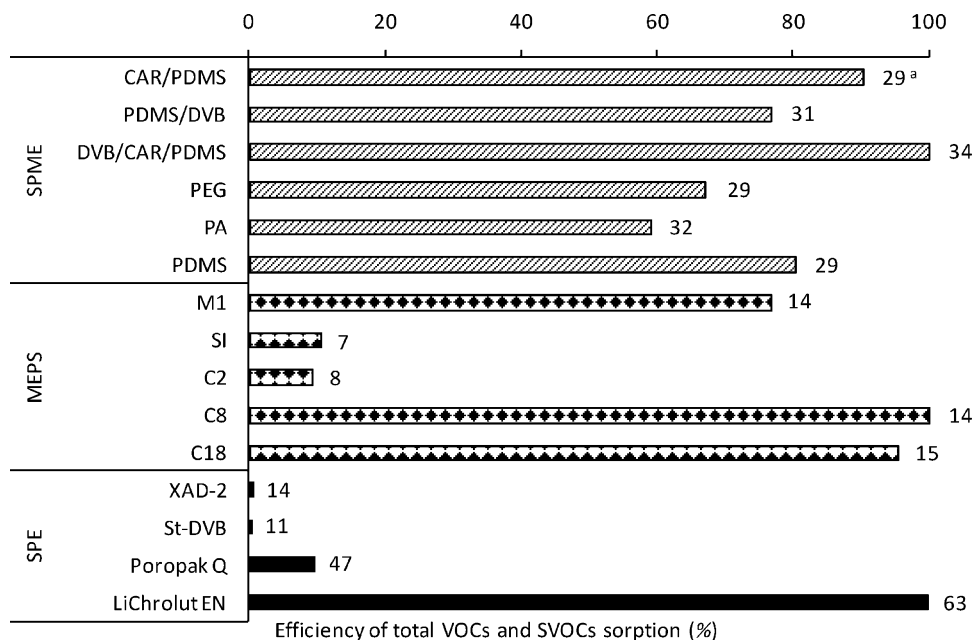


Fig. 2. Efficiency of different sorbent materials and fibre coatings in the extraction of volatile and semi-volatile constituents from Bual Madeira wine sample, by the three techniques under study. ^a Number of VOCs and SVOCs identified as a function of the type of sorbent.

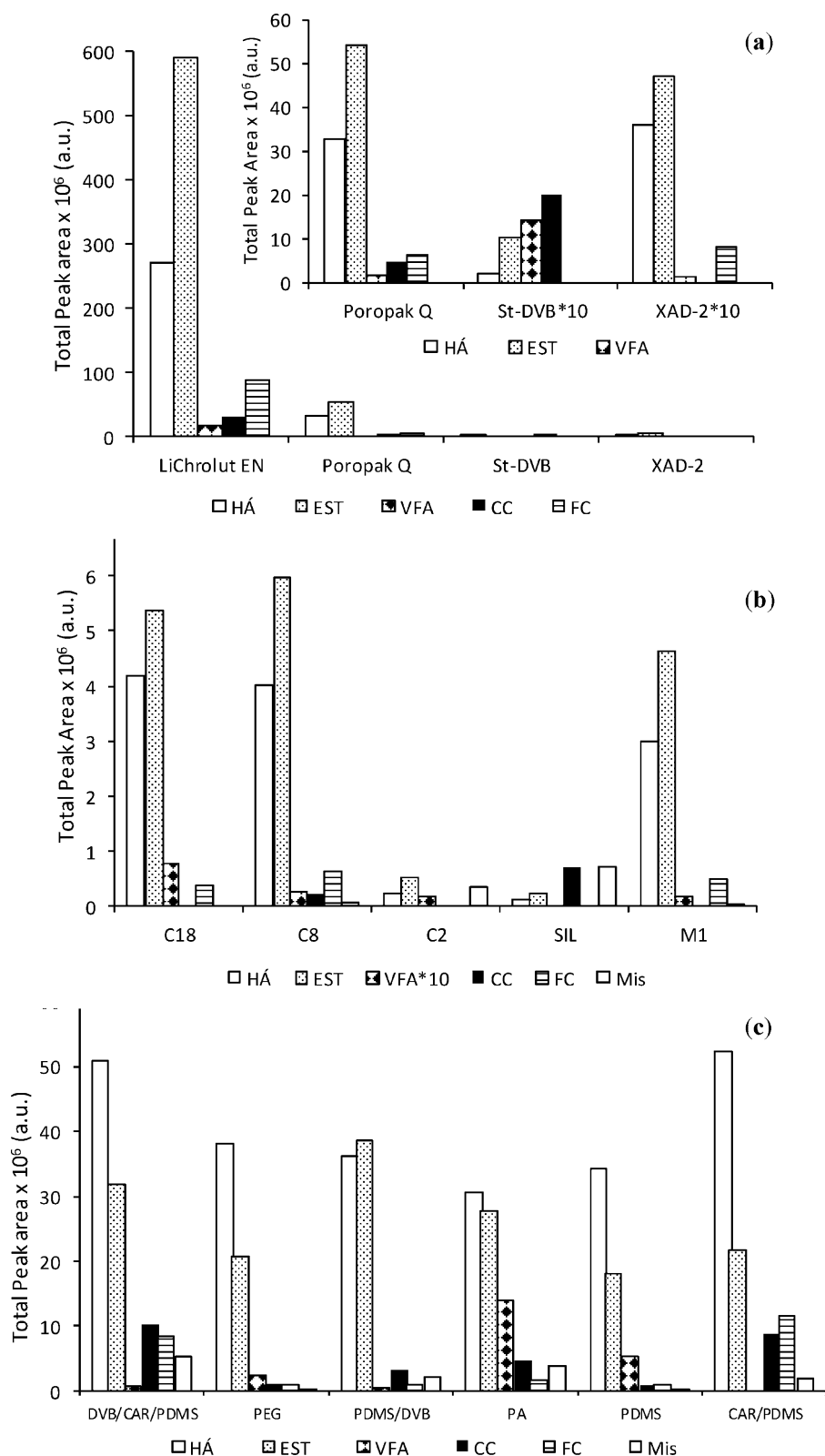


Fig. 3. Comparison of the efficiency of SPE, MEPS and SPME procedures to extract distinct chemical groups found in Bual wine samples.

3.3. HS-SPME analysis

The sensitivity of SPME extraction methodology depends greatly on the value of the distribution constant of analytes partitioned between the sample and fibre coating material. To ensure the determination of as many compounds as possible, extraction were

performed using six SPME fibres, among the most routinely used for assaying volatiles and semi-volatiles, differing in the polarity and thickness of the stationary phase. They were tested and compared individually in order to find such that the coating having highest affinity towards Bual wine VOCs and SVOCs. The SPME-influencing experimental factors namely extraction time and extraction

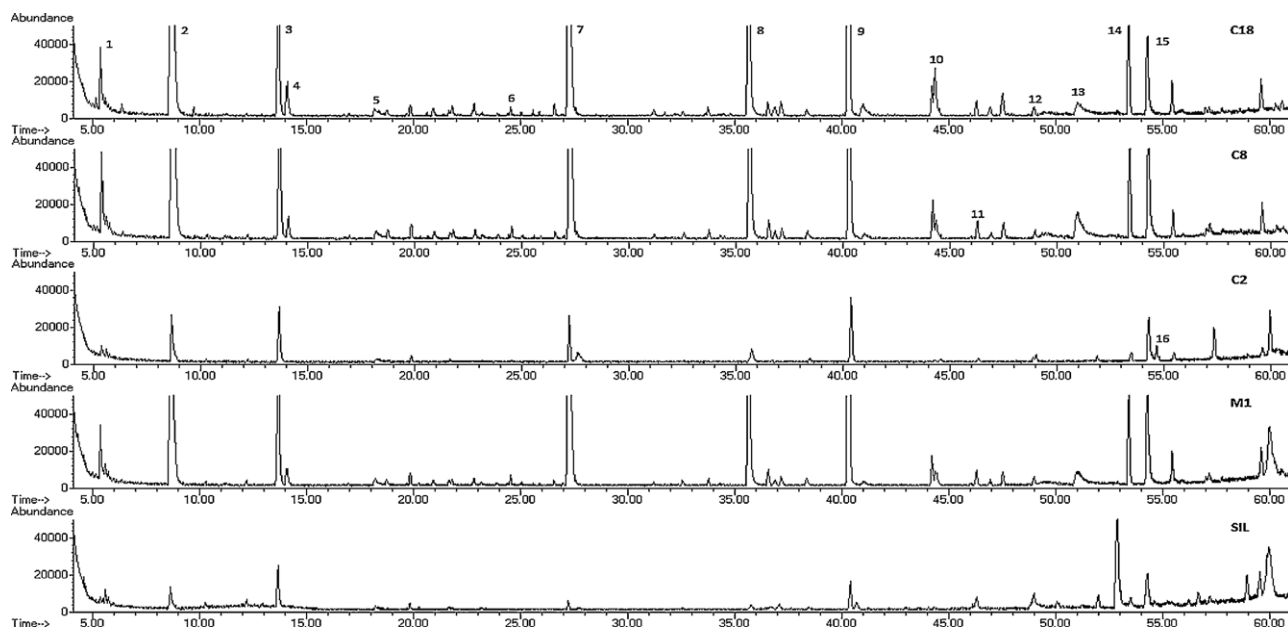


Fig. 4. Typical chromatograms (GC–qMS) of VOCs and SVOCs from a Bual Madeira wine sample, obtained by MEPS using different sorbents, C₁₈, C₈, C₂, M1 (mixed C₈–SCX), and SIL (silica). Peak assignments and identification appear in Table 4.

temperature, previously evaluated and optimized by Câmara et al. [65], were applied in this work. Wine samples were extracted at a fixed extraction time (60 min) and temperature (40 °C).

The comparison of the SPME fibre performance was performed in terms of total peak areas, number of identifiable compounds in the extract and reproducibility. The chromatographic profiles presented in Fig. 5, obtained for Bual wine using different coatings in same experimental conditions, shows different extraction efficiency of the fibres for the chemical groups identified in wines. The results obtained in rigorously reproduced temperature and exposure time conditions, are reported in Table 5 and Fig. 5 [51–61].

The SPME fibre coated with DVB/CAR/PDMS afforded the highest extraction sensitivity (highest peak areas, highest number of

detected compounds and high reproducibility). In contrast and under the same experimental conditions PA fibre present the lowest sorption capacity (Fig. 2; Table 5), although extracts a larger number of VOCs and SVOCs. Fig. 2 also shows the number of VOCs and SVOCs extracted as a function of the type of fibre, and the relative sorption efficiency of the different SPME coatings, taking as 100% the maximum analytical signal obtained from DVB/CAR/PDMS extract. This procedure allowed the identification of 56 VOCs and SVOCs: 34 with DVB/CAR/PDMS coating, 32 with PA, and 31 with PDMS/DVB. CAR/PDMS, PDMS and PEG extracts a fewer number of volatiles and semi-volatiles, 29, 27 and 26 compounds, respectively (Fig. 2). However the total peak area obtained using CAR/PDMS is significantly higher in comparison with those

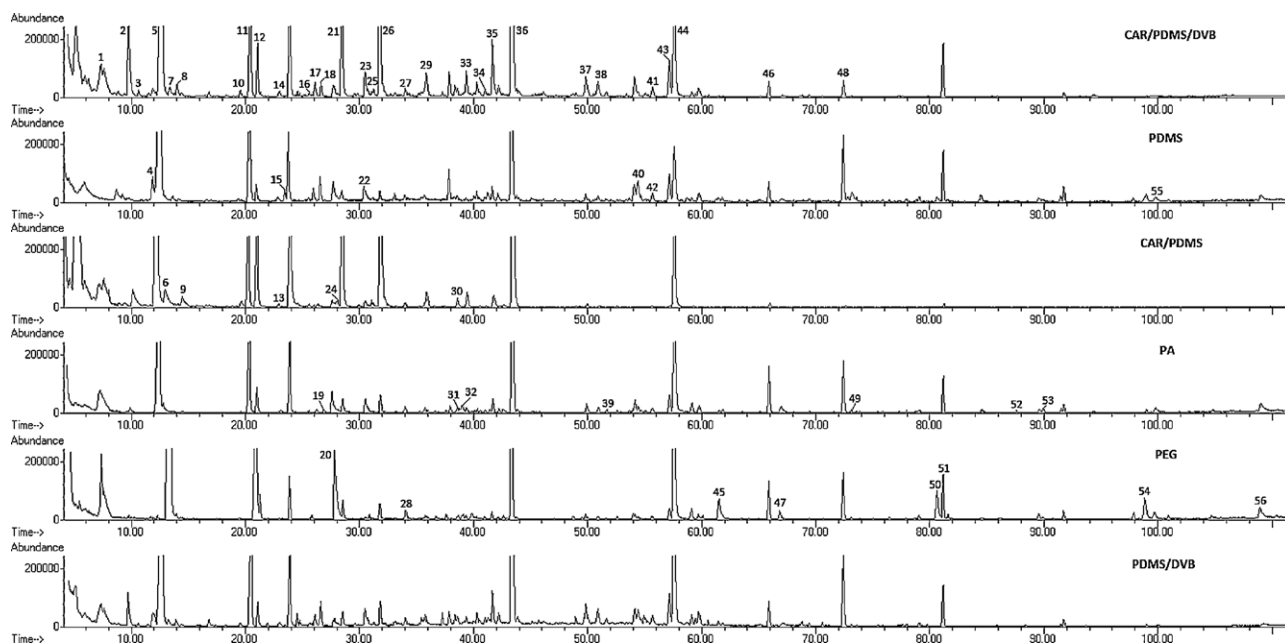


Fig. 5. Influence of SPME coating on the VOCs and SVOCs profile (TIC) of Bual Madeira wine obtained by HS-SPME/GC–qMS (60 min of extraction time at 40 °C with constant magnetic stirring (800 rpm)). Peak assignments and identification appear in Table 5.

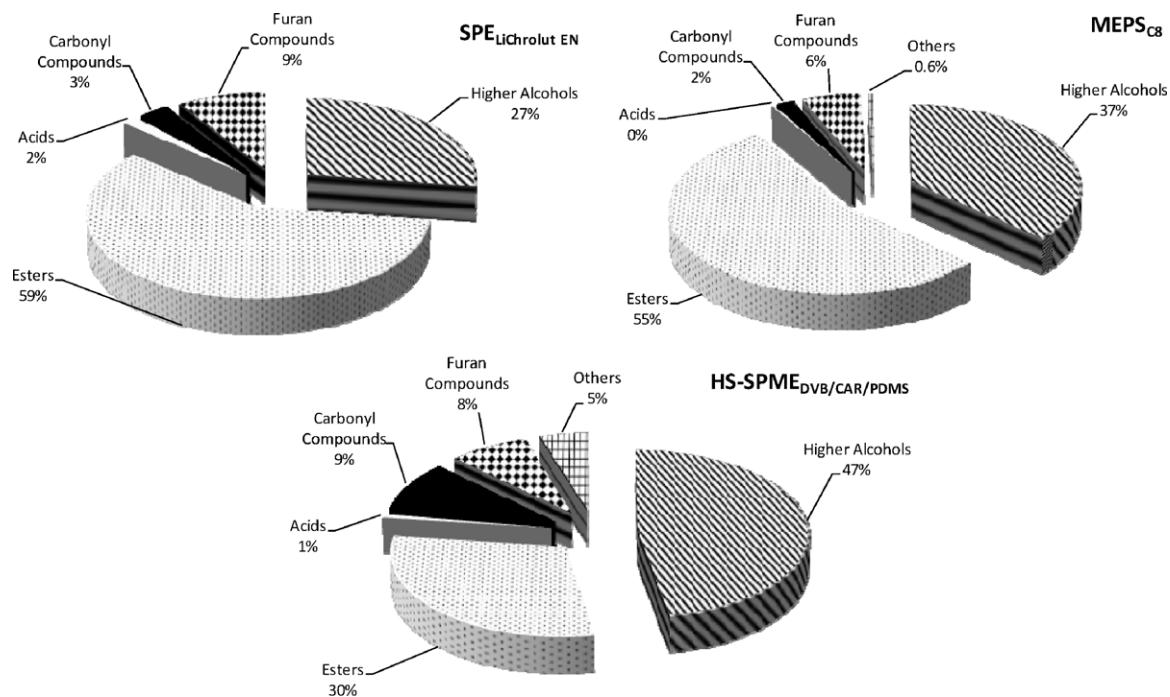


Fig. 6. Comparison of the main chemical classes of VOCs and SVOCs found in Madeira Bual wine, obtained with the best sorbents, LiChrolut EN (for SPE extraction technique); C₈ (for MEPS extraction technique), and DVB/CAR/PDMS fibre (for SPME extraction technique).

obtained by using PDMS/DVB, PEG or PA fibres. CAR/PDMS fibre coating extracted 90.1% of DVB/CAR/PDMS while PA coating extracted the lowest amount (about 59.0% of DVB/CAR/PDMS). Similar amounts of volatiles (70.9–80.2% of DVB/CAR/PDMS) were extracted using, either PDMS, PEG, or PDMS/DVB coatings (Fig. 3c). According to Fig. 3, independent of the employed fibre, higher alcohols and ethyl esters showed the most intense signals, which suggests that both chemical groups might be constituted by VOCs and SVOCs with higher concentrations.

Higher alcohols had higher affinity for 85 μ m PA fibre. A great affinity for CAR/PDMS and DVB/CAR/PDMS coatings was also observed for this group of compounds. These coatings also present better sensitivity for furan compounds, and carbonyl compounds. PA coating showed a strong extraction capacity for volatile fatty acids while esters are more efficiently isolated by PDMS/DVB coating (Fig. 3c). In any case, fibres containing carbon showed the best results, as obtained by other authors in the analysis of VOCs and SVOCs [59–61]. Apparently the desorption process on porous carbon particles maximises the capture of polar compounds.

Considering the HS-SPME extraction conditions, 3-methyl-1-butanol (**5**; $36.6 \pm 1.7\%$, on average) was the major component found in Bual wines (Table 5). The diethyl succinate (**36**; $18.4 \pm 3.3\%$) was the second largest compound extracted with this coating, followed by 2-phenylethanol (**44**; $7.7 \pm 0.2\%$), ethyl lactate (**11**; $6.7 \pm 3.7\%$), and 2-furfural (**21**; $4.6 \pm 0.4\%$).

For 2-methyl-1-propanol (**1**), α -limonene (**2**), ethyl octanoate (**17**), benzaldehyde (**26**), 5-methyl-2-furfural (**29**), ethyl 2-furoate (**33**), ethyl benzoate (**35**), and benzyl alcohol (**41**) better results were observed for DVB/CAR/PDMS, while PDMS/DVB fibre exhibited good potential to extract diethyl succinate (**36**), 3-methyl-1-butanol (**5**), 2-phenylethanol (**44**), ethyl lactate (**11**), and ethyl 4-ethoxy benzoate (**48**). CAR/PDMS extracts with high efficiency ethyl hexanoate (**6**), *p*-cymene (**9**), (Z)-3-hexen-1-ol (**13**), 2-acetylfuran (**24**), whereas PDMS is more indicated to extract 1-dodecene (**19**), decanal (**22**), α -ionol (**43**), diethyl malate (**46**), and octanoic (**47**), tetradecanoic (**55**), and hexadecanoic acids (**56**). PA

presents better results for γ -butyrolactone (**31**), ethyl phenylacetate (**39**), benzophenone (**52**), 5-(hydroxymethyl)-2-furfural (**53**) while PEG showed high extraction efficiency for ethyl lactate (**45**), acetic acid (**20**), 2,3-butanediol (**28**), and 2-phenylethanol (**44**).

3.4. Comparison of miniaturized sorbent and solid phase microextraction techniques

In order to compare SPE, MEPS, and SPME extraction techniques, the data obtained by analysing Bual wine samples, were compared (Tables 3–5). A total of 103 VOCs and SVOCs obtained by the three different extractive methods were classified into six different groups: (i) higher alcohols; (ii) ethyl esters; (iii) fatty acids; (iv) carbonyl compounds; (v) furan compounds; (vi) miscellaneous (Fig. 3). They were identified with an average mass spectral match of 864 with an upper and lower 95% of the mean at 923 and 815, respectively, identified based on comparison of their mass spectra to reference database (MS), and by comparison of the RIs calculated (RI_{calc}) with the values reported in the literature (RI_{lit}) for equivalent column (Tables 3–5). An average difference ($|RI_{calc} - RI_{lit}|$) for RI_{calc} compared to the RI_{lit} reported in the literature for similar chromatographic columns of 13.4 units with an upper and lower 95% of the mean at 84.0 (2,5-furandicarboxaldehyde) and 0 (4-ethyl phenol), respectively, was obtained. This difference in RI is considered reasonable (<5%) if one takes into account that the literature data is obtained from a large range of GC stationary phases. Bianchi et al. [66] commented that differences in retention indices for aroma compounds on comparable stationary phases may vary between 5 and 20 units, however, larger differences have been observed [67]. Babushok et al. [68] also noted that in the development of the NIST database of retention indices, 80,427 retention indices representing 9722 species analysed on dimethylpolysiloxane stationary phases had an average deviation of 10 units but a 99th percentile deviation of 91 units [67]. The differences in calculated and reported retention indices reported in this study fall well within these values. Similar results were observed by Robinson et al. [67] on the analysis of wine

volatiles by comprehensive two-dimensional gas chromatography (GC \times GC) time of flight mass spectrometry (ToFMS).

As was expected, qualitative and quantitative differences were definitely observed in the chromatographic profiles obtained by the three approaches (Figs. 1–3). Tables 3–5 display the results of the VOCs and SVOCs derived from triplicate extractions. As can be seen from the data, a total of 76 VOCs and SVOCs were isolated and identified by SPE/GC–qMS methodology, whereas only 15 and 56 compounds were positively identified by MEPS/GC–qMS and HS-SPME/GC–qMS methodologies, respectively. In addition SPE technique is able to concentrate larger amounts than MEPS and HS-SPME. This suggests that SPE is generally a more sensitive technique than SPME and MEPS for the extraction of VOCs and SVOCs from wines, although in practice the SPE and SPME may complement each other quite well.

Fig. 6 compares the main chemical class of compounds identified in volatile and semi-volatile fraction of Bual wines obtained by the three techniques studied, using the best sorbents/fibre. That figure shows, without ambiguity, that the products preferentially obtained are ethyl esters for SPE and MEPS and higher alcohols for SPME.

Regarding SPE, Lichrolut-EN proved higher potential for extraction ethyl esters (59%), furan compounds (9%), and volatile fatty acids (2%) than MEPS/GC–qMS and HS-SPME/GC–qMS whereas, higher alcohols (47.0%) and carbonyl compounds (9.0%) showed higher affinity for HS-SPMS_{DVB/CAR/PDMS} than others fibre coatings and sorbents. MEPS technique using C₈ as sorbent material, exhibit high extraction capacity for esters and higher alcohols. Comparing all methods, LiChrolut EN sorbent (SPE) obtained a good response on the number of extracted compounds and total peak area. The physico-chemical processes which occurs in every extraction method, could explain the variation observed in the chemical composition, according to technique used.

The results showed that Bual wines are characterized by high peak areas, and probably high concentrations, of diethyl malate, diethyl succinate, 3-methyl-1-butanol, 2-phenylethanol and 5-(hydroxymethyl)-2-furfural as determined by the three techniques utilized. The other major compounds include ethyl lactate, diethyl tartrate, ethyl citrate, and syringaldehyde.

One-way ANOVA with p values <0.05 was achieved for the three techniques using the chemical groups constituted, higher alcohols

(HA) ethyl esters (EST), fatty acids (FA), carbonyl compounds (CC), furan compounds (FC) and miscellaneous compounds coded as OTHERS (SPSS, version 19.0). All chemical groups were statistically significant (ANOVA, $p < 0.05$) between the three techniques studied (Supplementary Tables 6 and 7).

To study the principal sources of variation among the results, detect intrinsic clustering and possible outliers, and differentiate between the three techniques evaluated, exploratory PCA was applied to the GC–qMS peak areas that were obtained for the volatile and semi-volatile fraction. PCA is an unsupervised projection method that is used to visualise the dataset and display similarities and differences. PCA of the data showed that the variables selected (chemical groups) were enough to describe subsets with similar characteristics and relate to the extraction techniques. Although this set of variables only explained 79.15% of the variability between the first two PCs, it was enough to deconvolute the set of cases in three subsets according to the extraction technique (Fig. 7).

4. Conclusions

A total of 103 VOCs and SVOCs belonging to several chemical classes were identified in Bual Madeira wines using three different high-throughput miniaturized (ad)sorptive extraction techniques – SPE, MEPS, and SPME, and different sorbents designed to extract a broad range of analytes. The extracts resulting from distinct methodologies showed different global volatile and semi-volatile signature. Based on the extraction potential of the three methodologies and under the experimental condition employed, it can be concluded that:

- the more traditional technique, SPE, using as sorbent the LiChrolut EN copolymer (SPE_{LiChrolut EN}) was the most suitable procedure for the extraction of wine VOCs and SVOCs allowing for the identification of up to 78 components, 19 and 63 more than SPME and MEPS, respectively;
- HS-SPME extracted more than half of VOCs and SVOCs compared with SPE technique;
- MEPS revealed to be the less efficient extraction technique regarding for wine VOCs and SVOCs.

Results showed that the three extraction methods studied both of them, SPE and SPME, can be used for the qualitative and quantitative determination of VOCs and SVOCs from wines. Nevertheless, when wishing to choose one of these isolation techniques, it would be convenient to consider other factors such as the amount of solvent required or the time consumption. Despite the fact that extracts obtained by means of SPE displayed a higher qualitative and quantitative composition in comparison with the other techniques tested, SPME can also be used to extract VOCs and SVOCs from wines. However, SPME is solvent-free, required smaller amount of sample and shorter extractions times. On the other hand, extraction procedure by means of MEPS was the fastest and it used minor amount of sample and solvent than SPE, nevertheless the results obtained are generally poor in comparison with SPE and SPME techniques.

Acknowledgements

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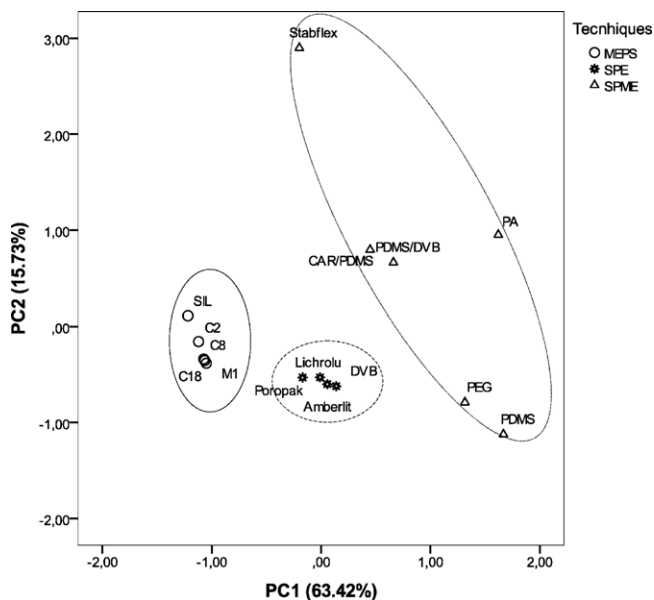


Fig. 7. Separation of SPE, SPME and MEPS extraction techniques based on PCA factor scores.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.talanta.2011.10.010](https://doi.org/10.1016/j.talanta.2011.10.010).

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