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Comparative study of the whisky aroma profile based on headspace solid phase microextraction using different fibre coatings

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Abstract

A dynamic headspace solid-phase microextraction (HS-SPME) and gas chromatography coupled to ion trap mass spectrometry ($GC–_{IT}MS$) method was developed and applied for the qualitative determination of the volatile compounds present in commercial whisky samples which alcoholic content was previously adjusted to 13% (v/v). Headspace SPME experimental conditions, such as fibre coating, extraction temperature and extraction time, were optimized in order to improve the extraction process. Five different SPME fibres were used in this study, namely, poly(dimethylsiloxane) (PDMS), poly(acrylate) (PA), Carboxen-poly(dimethylsiloxane) (CAR/PDMS), Carbowax-divinylbenzene (CW/DVB) and Carboxen-poly(dimethylsiloxane)-divinylbenzene (CAR/PDMS/DVB). The best results were obtained using a 75 μm CAR/PDMS fibre during headspace extraction at 40 °C with stirring at 750 rpm for 60 min, after saturating the samples with salt. The optimised methodology was then applied to investigate the volatile composition profile of three Scotch whisky samples—Black Label, Ballantines and Highland Clan. Approximately seventy volatile compounds were identified in the these samples, pertaining at several chemical groups, mainly fatty acids ethyl esters, higher alcohols, fatty acids, carbonyl compounds, monoterpenols, C_{13} norisoprenoids and some volatile phenols. The ethyl esters form an essential group of aroma components in whisky, to which they confer a pleasant aroma, with "fruity" odours. Qualitatively, the isoamyl acetate, with "banana" aroma, was the most interesting. Quantitatively, significant components are ethyl esters of caprilic, capric and lauric acids. The highest concentration of fatty acids, were observed for caprilic and capric acids. From the higher alcohols the fusel oils (3-methylbutan-1-ol and 2.phenyletanol) are the most important ones.

Keywords: Whisky analysis; Headspace-solid phase microextraction (HS-SPME); Volatile compounds; Gas chromatography–Mass spectrometry (GC–MS)

1. Introduction

Whisky samples contain a great variety of flavour compounds belonging to different chemical families such as: higher alcohols, ethyl and isoamyl esters, acetates, fatty acids, ketones, monoteropenes, C_{13} norisoprenoids and phenols. These compounds can be present in a wide range of concentrations (from ng/l to several g/l) and vary widely in volatility and polarity which consequently affects their extraction and chromatographic profile. Some of these originate from the raw materials and the subsequent processes of mashing, fermentation, distillation and ageing, while others are oak derived. Many of these compounds are common to different whisky samples but differ analytically in terms of the relative amount. The qualitative and quantitative study with some whisky available commercially, is an important data base for ensuring process continuity and product authenticity.

Several extraction-concentration methods have been used for analysis of volatile compounds in whisky samples, such as LLE, simultaneous extraction, distillation, solid phase extraction and supercritical fluid extraction. These classical analytical methods have some drawbacks such as the relatively low reproducibility, possibility of contamination with solvents, the length of time required and insufficient selectivity. In the beginning of 90 decade, a new variation of adsorption technique called solid phase micro-extraction (SPME) has been developed by J. Pawlizyn and co-workers [1–3]. Compared to traditional techniques this new technique offers many advantages such as high sensitivity and reproducibility, does not require solvent and combines extraction and pre-concentration in a single step without pre-

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Table 1
Composition and properties description of SPME fibres appplied in the study

Fibre coating	Type	Polarity	Coating stability	Extraction mechanism
PDMS (100 μm)	Homogeneous polymer	Nonpolar	Nonbonded	Absorption
PA (85 μm)	Homogeneous polymer	Polar	Bonded crosslinked	Absorption
CAR/PDMS (75 μm)	Porous particle/polymer	Bipolar	Partially crosslinked	Adsorption
CW/DVB (65 μm)	Porous particle/polymer	Polar	Partially crosslinked	Adsorption
DVB/CAR/PDMS (50/30 μm)	Porous particle/polymer	Polar	Highly crosslinked	Adsorption

treatment of samples. Moreover it is fast, inexpensive, requires low sample volumes and can be easily automated. This technique is based on sorption–absorption and/or adsorption, depending on the fibre coating, which is useful for extraction and concentration analysis either by submersion into a liquid phase or by exposure to a gaseous phase. The sorbed analytes are desorbed into a suitable instrument for separation and quantification. The most important stage of this two-stage process is the adsorption of analyte onto a suitably coated-silica fibre or stationary phase. The choice of sorbent is essential, in that it must have a strong affinity for the target organic compounds so that pre-concentration can occur from either dilute aqueous samples or the gas phase.

Several kinds of coatings, with various polymeric phases, have become commercially available, namely poly(dimethylsiloxane) (PDMS), poly(acrylate) (PA), Carboxen/poly(dimethylsiloxane) (CAR/PDMS), Carbowax/divinylbenzene (CW/DVB) and divinylbenzene/Carboxen on poly(dimethylsiloxane) (DVB/CAR/PDMS). Among those, PDMS and PA are the most well-studied and characterized coatings. For a specific application, the coating is chosen based on the polarity of the target analytes. There is no single fibre coating that will extract the analytes to the same extent. Polar fibres are effective for extracting polar analytes and nonpolar fibres are effective for extracting the nonpolar ones, from different matrices. At present, the types of coatings available can be classified as nonpolar, polar (Table 1) and semipolar (PDMS/DVB) coatings. Fibres with different polarity, provides high extraction selectivity and reduce the possibility of extracting interferences. Both PDMS (high-viscosity rubbery liquid) and PA (solid crystalline) extract analytes *via* absorption. The other coatings extract the analytes *via* adsorption (Table 1). In this mechanism, the molecules can be associated with surfaces *via* van der Waals, dipole–dipole and other weak intermolecular forces.

The application of SPME to different areas in analytical chemistry has been steadily increasing. In recent years, this methodology has been widely adopted in many fields including pharmaceutical, clinical, forensic, food, environmental, physicochemical and flavour, fragence and pheromone applications. This technique has been successfully applied in wine samples [4–6] to characterise a wide range of aroma compounds, including monoterpenes and C_{13} norisoprenoids [7], esters [8], volatile and low volatile sulphides and disulphides [9–11], oak lactones in barrel aged wines [12], organochlorine insecticides in Portuguese red and white wines [13] and 3-alkyl-2-methoxypyrazines in Cabernet-Sauvignon and Merlot wines [14]. SPME has also been applied for the analysis of Portuguese muscatel wines [15], for the classification of Nebbiolo based wines from Piedmont [16] and for varietal characterisation of Madeira wines [7]. More recently was reported the application of SPME to the characterisation of varietal wines, using PDMS as stationary phase [17]. The determination of esters [8] and major compounds in dry and sweet wines [18] were also performed by headspace solid-phase microextraction (HS-SPME) in commercial wines from the Canary Islands. More recently Deng et al. [19] developed a SPME methodology for investigation of long cancer volatile biomarkers. The same authors applied HS-SPME with on-fibre derivatization for the determination of hexanal and heptanal in normal blood and lung cancer blood [20].

Câmara et al. [21] studied the opimization of headspace SPME for the analysis of wine aroma compounds, in which the influence of various parameters, such as sampling time, temperature and alcohol content, on the extraction efficiency of terpenoids was investigated. Rocha et al. [22] decribes a novel methodology for the rapid distinction in wines based on the global volatile signature obtained by HS-SPME coupled to gas chromatography–mass spectrometry followed by principal component analysis of the data. The free and pre-fermentative related volatile compounds, mainly monoteroenoids, norisoprenoids, aromatic alcohols, as well as, sesquiterpenoids that arise after crushing the grapes were followed by HS-SPME [23]. HS-SPME has also been applied to the determination of specific trace components, such as diacetyl [24], oak lactones in aged wines [25], the cork taint compound, 2,4,6-trichloroanisole [26], fungicides in Spanish wines [27] and even organophosphorous insecticides in honey [28].

Although the SPME analysis of volatiles and semi-volatiles in a wide range of matrices has been described, to date very few papers are available to the analysis of whisky volatiles. In this study a HS-SPME-GC-$_{IT}$MS method for the analysis, identification and evaluation, of the volatile constituents in Scotch whisky samples is proposed. Three SPME parameters with influence in the extraction process were selected for optimisation: fibre coating, extraction time and extraction temperature. A comparison between five SPME different fibres is made.

2. Experimental

2.1. Samples

Commercial Scotch whisky samples (40%, v/v, alcohol), Black Label (BL), Ballantines (Bal) and Highland Clan (HC), were purchased from a local store (Funchal, Madeira Island),

and frozen at −28 °C until their analysis. Before extraction the volatile compounds, the samples were unfrozen at 3–4 °C. All samples were adjusted to 13% (v/v) alcohol by dilution with distilled water prior extraction.

2.2. Reagents and standards

All reagents used were of analytical quality. Absolute ethanol and sodium chloride were supplied from Panreac (Barcelone, Spain). The chemical standards used as internal standards, 3-octanol and 4-methyl-2-pentanol, were purchased from Sigma–Aldrich (Madrid, Spain). Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA).

2.3. Optimization of HS-SPME procedure

HS-SPME is an equilibrium technique that requires a previous optimization of the extraction parameters that can affect extraction efficiencies, in order to obtain high recoveries of volatiles. Some of these sampling conditions are fibre sorbent (absorvent/adsorvent) phase, extraction temperature and extraction time.

2.3.1. SPME fibre coatings

The SPME fibres tested in this work were: 100 μm poly(dimethylsiloxane) layer (PDMS), recommemded for nonpolar volatiles; 85 μm poly(acrylate) (PA), with high selectivity for polar semivolatile compounds; 75 μm Carboxen/poly(dimethylsiloxane) (CAR/PDMS), recommended for gases and low molecular weight compounds; 65 μm Carbowax/divinylbenzene (CW/DVB), adequate for alcohols and polar compounds; and 50/30 μm divinylbenzene/Carboxen on poly(dimethylsiloxane) (DVB/CAR/PDMS) on a 1 cm StableFlex fibre, recommended for flavours (volatiles and semivolatiles), and the SPME holder for manual sampling, were purchased from Supelco (Bellefonte, PA, USA). The fibres were conditioned prior to use according to the manufacturer's instructions by inserting them into the GC injector port. Before the first daily analysis the fibres were conditioned for 6 min at 240 °C. A blank test was performed to check possible carry-over.

2.3.2. Extraction temperature and time

Both the temperature and time of HS-SPME influence compound extraction, such that have an effect on the equilibrium during extraction. Samples heated at higher temperatures prove to be extracted more successfully. Operating conditions were optimized realising SPME extractions of real samples at different adsorption temperature (25, 40, 50 and 60 °C) and times 5, 15, 30, 45 and 60 min.

2.4. Dynamic HS-SPME of volatile compounds

Three SPME parameters with influence in the extraction process were selected for optimisation: fibre coating, extraction time and extraction temperature. The methodology developed by Câmara et al. [21,29] for the volatile compounds extraction using HS-SPME was used with minor modifications.

An equilibration study was performed (5, 15, 30, 45 and 60 min) to determine the most suitable extraction time for whisky volatiles. The high ethanol concentration (40%, v/v) of the whiskys required sample dilution. After adjust to 13% (v/v) alcohol by dilution with distilled water, whisky samples were adjusted to pH 3.3 and the ionic strength was increased to improve the extraction efficiency using NaCl (30%). A 60 ml vial containing 35 ml of sample, spiked with 50 μl of octan-3-ol at 422 mg/l used as internal standard 0.422 μg/l of octan-3-ol (Sigma–Aldrich), which was used as internal standard (50 μl of alcoholic solution at 422 mg/l), was placed in a thermostatic block on a stirrer. The fibre was then exposed to the gaseous phase for an appropriate time period at temperature of 30 ± 1 °C. As stirring usually improves the extraction, because 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 (750 rpm). After extraction, the SPME fibre was withdrawn into the needle, removed from the vial and inserted into the hot injector port (240 °C) of the GC–MS system for 6 min where the extracted chemicals were desorbed thermally and transferred directly to the analytical column.

2.5. Analysis of volatile compounds by GC–MS

The whisky extracts were analyzed by GC–MS using a Varian STAR 3400Cx series II gas chromatograph, equipped with a 30 m × 0.5 mm I.D., with a 0.25 μm film thickness, *DBWax-ter* fused silica capillary column, connected to a Varian Saturn III mass selective detector, according to the method described by Câmara et al. (2006). Splitless injections were used. The initial oven temperature was set to 40 °C (for 1 min), then increased in three steps: 40 to 120 °C, at 1 °C/min; 120 to 180 °C at 1.7 °C/min and 180 to 220 °C, at 25 °C/min. Each step was preceded by a small period at constant temperature for 2, 1 and 10 min, respectively. The injector temperature was 260 °C and the transfer line was held at 220 °C. The carrier gas was Helium N60 (Air Liquid, Portugal) with a column-head pressure of 13 psi (1 psi = 6894.76 Pa). The detection was performed by a Saturn III mass spectrometer in the electronic impact (EI) mode (ionization energy, 70 eV; source temperature, 180 °C). The electron multiplier was set to the auto tune procedure. The acquisition was made in full scan mode (the mass-to-charge ratio range used was 30–300 *m/z*; 1.9 spectra s^{-1}).

The compounds were identified by comparison of mass spectra data obtained from the sample with that taken from pure commercially available standards injected in the same conditions. The Kováts indexes and the mass spectra were compared with those from the NIST library.

3. Results and discussion

The influence of the main parameters that can affect the HS-SPME process from headspace, i.e. fibre coating, extraction temperature and extraction time were evaluated. Headspace

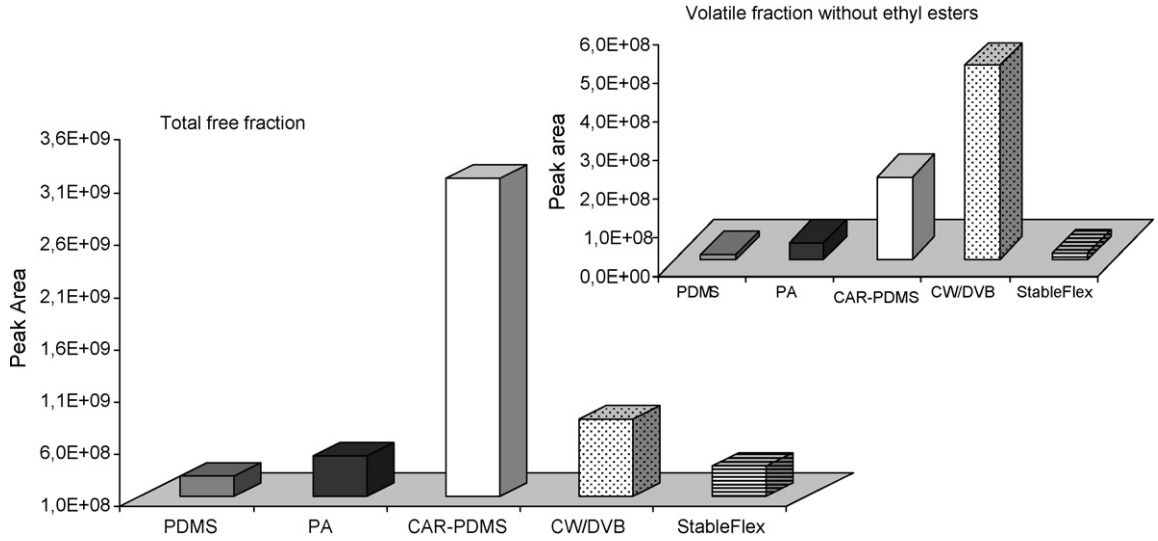

Fig. 1. Influence of the type of HS-SPME fibre coatings on the GC–MS peak area for the total fraction of the volatile compounds in BL whisky sample, using an extraction temperature of 30 °C and an extraction time of 60 min.

SPME mode was used instead of direct sampling mode because, for volatile analytes, in the former mode the equilibrium times are shorter compared to direct extraction. The headspace mode also protect the fibre from adverse effects caused by non-volatile, high molecular weight substances present in the sample matrix, and allows matrix modifications, including pH adjustments, without affecting the fibre. Temperature has a significant effect on the extraction kinetics, since it determines the vapour pressure of the analytes, and for that their influence in the extraction process was also investigated.

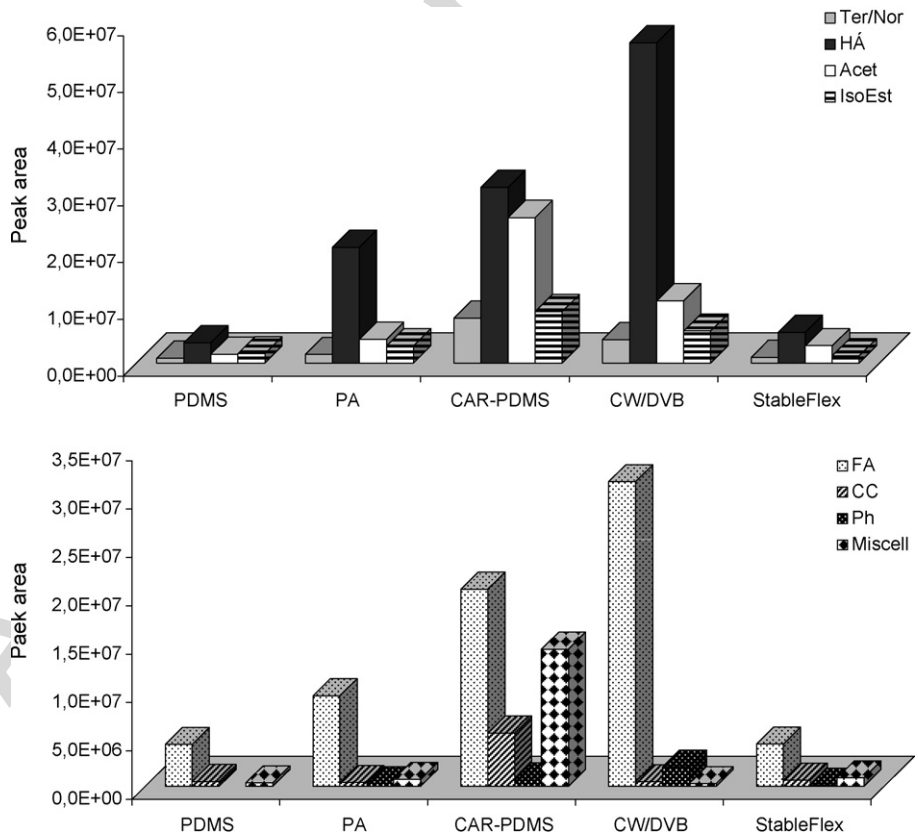

Fig. 2. Comparison sorption capacity of five selected coatings for the extraction of the main chemical groups of BL volatile compounds, during dynamic headspace SPME extraction (60 min, 40 °C), after salt saturation expressed as peak area ($n = 3$). (Ter/Nor – monoterpenes, terpenols and C_{13} norisoprenoids; HA – higher Alcohols; Acet – acetates; IsoEst – isoamyl esters; FA – fatty acids; CC – carbonyl compounds; Ph – phenols; Miscell – miscellaneous).

Table 2
Influence of the extraction temperature on adsorption of different BL whisky volatile compounds, classified according to functional group, during dynamic headspace SPME extraction using a 75 μm CAR/PDMS fibre (extraction of salt saturated sample—30% (w/v) NaCl at 40 °C, for 60 min), expressed as peak area ($n = 3$)

Class of compounds	Extraction temperature (°C)			
	25	40	50	60
Terpenes/norisoprenoids	5.74E+06	7.58E+06	4.15E+06	4.59E+06
Higher alcohols	2.89E+07	2.48E+08	3.05E+08	4.63E+08
Acetates	2.15E+07	3.09E+07	1.87E+07	1.78E+07
Isoamyl esters	9.14E+06	1.10E+07	1.25E+07	1.27E+07
Ethyl esters	5.23E+07	5.71E+07	5.78E+07	6.05E+07
Fatty acids	2.78E+07	5.41E+07	2.28E+07	7.90E+07
Carbonyl compounds	9.14E+05	4.14E+05	4.12E+05	3.14E+05
Phenols	6.08E+05	6.65E+05	4.72E+05	4.35E+05
Miscellaneous	9.25E+06	7.17E+06	9.54E+06	6.24E+06

3.1. Selection of SPME fibre coating

The chemical nature of the target analyte determines the type of coating used. This is based primarily on the polarity and volatility characteristics of the analyte. According to Table 1 PDMS (non-polar liquid phase) is the most useful coating for non-polar analytes. To investigate the extraction yields of the whisky volatiles components, five fibre coatings: PDMS, PA, DVB/CAR/PDMS, CW/DVB and CAR/PDMS, were checked. To select the best coating the headspace extraction of whisky volatile constituents was carried out using a temperature of 40 °C for 60 min, after saturating the samples with NaCl. For reasons of comparability all tests were carried out with the same whisky sample (Black Label). The peak areas of the total free fraction present in the Black Label whisky were used for the evaluation of the optimal fibre. The results were shown in Fig. 1., indicating that the 75 μm CAR/PDMS fibre provided the highest extraction efficiency for the volatiles. Therefore this fibre was chosen for the remaining studies: extraction temperature, extraction time and whisky volatiles.

PDMS is less polar than PA, thus it is widely used for the extraction of non-polar compounds. For polar compounds like ketones and alcohols polar coatings like PA and Carbowax work better. From Fig. 2., it can be observed the fibres show different selectivity to different groups of compounds. The ethyl esters from fatty acids have a larger affinity for CAR/PDMS fibre. Volatiles higher alcohols, fatty acids and phenols were effectively extracted by CW/DVB. CAR/PDMS show great ability to extract terpenes, C_{13} norisoprenoids, acetates from higher alcohols, isoamyl esters and carbonyl compounds. It is shown that with the 65 μm CW/DVB fibre, 2.9 higher extraction yields were found compared to the PDMS fibre, whereas using the 75 μm CAR/PDMS fibre the extraction yields increase to a factor of 6.5.

3.2. Effect of extraction temperature

Temperature is an important parameter for the SPME process. It controls the diffusion rate of the analytes into the coating. Since extraction by SPME is an exothermic process, a temperature increase will increase the partial vapour pressure of analytes in the headspace but simultaneously the sorption onto the fibre will decrease with increasing temperature, mainly for highly volatile components. However for the less volatile compounds, an increase in the signal was found when the sample temperature increase. The influence of the extraction temperature was investigated by sampling a BL whisky at different temperatures—25, 40, 50 and 60 °C, with a constant extraction time of 60 min. The results are summarised in Table 2. As can be observed an increase in extraction temperature generally improves the mobility of volatile compounds through liquid and gas phases and better extraction efficiencies were obtained.

3.3. Effect of extraction time

A time profile of the adsorption of the different class of compounds onto the 75 μm CAR/PDMS was determined in order to

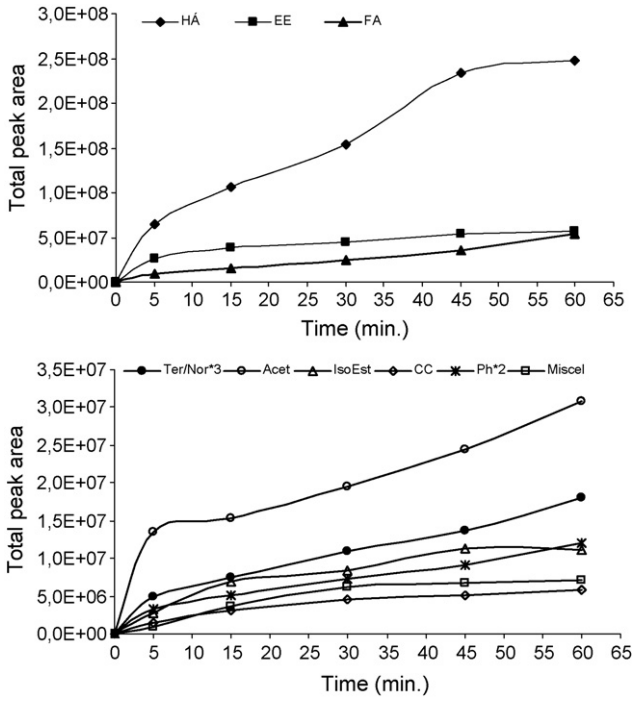

Fig. 3. Influence of the sampling time on the extraction efficiency for volatile compounds, during dynamic headspace SPME extraction using a 75 μm CAR/PDMS fibre (extraction of salt saturated sample—30% (w/v) NaCl at 40 °C), expressed as peak area.

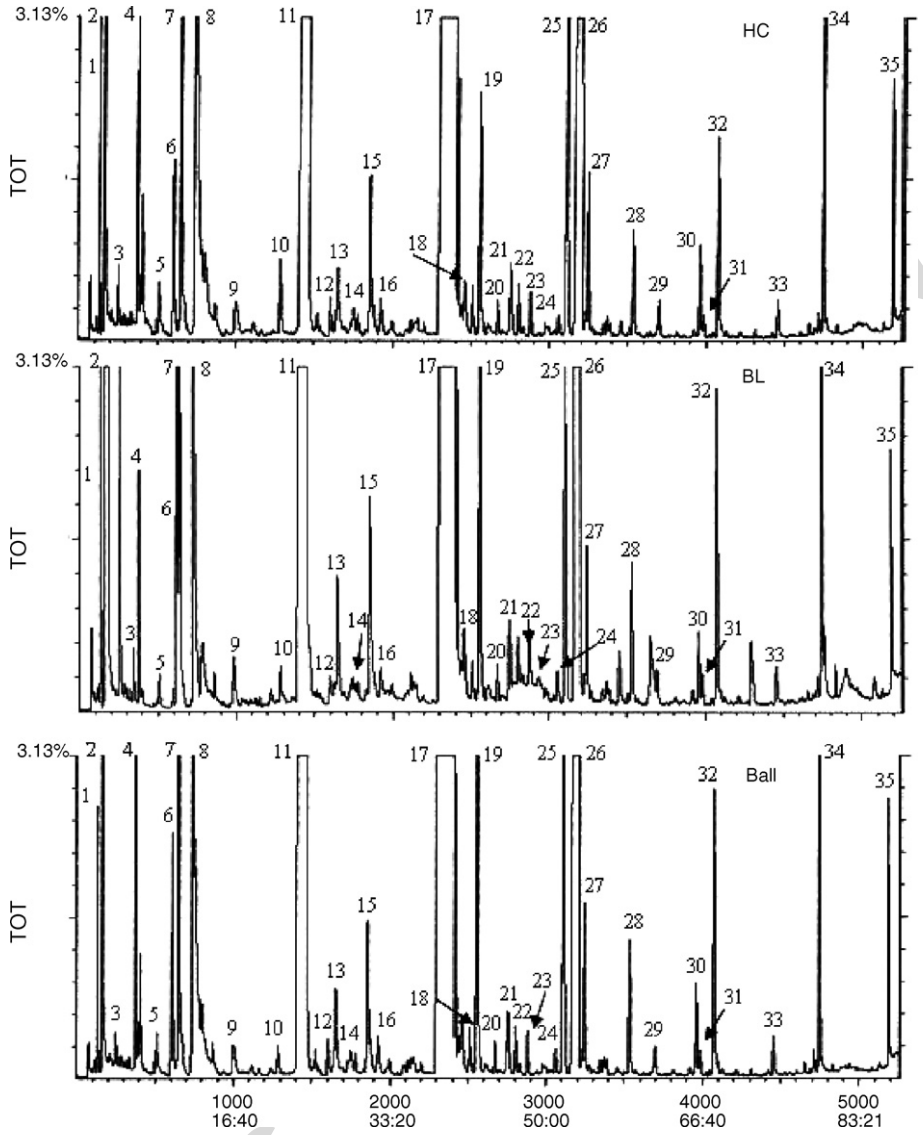

Fig. 4. Typical total ion chromatogram of volatile constituents from HC, BL and Ball s samples obtained by SPME using a CAR/PDMS coating in the headspace sampling mode. Extraction conditions: extraction temperature: 40 °C; extraction time: 60 min; stirring: 750 rpm; sample volume: 30 ml; headspace volume: 30 ml; desorption was performed at 220 °C for 6 min. Peak Identification: (1) ethyl acetate; (2) ethanol; (3) butan-1-ol; (4) isoamyl acetate; (5) 4-methylpentan-2-ol (IS); (6) 3-methylbutan-1ol; (7) ethyl hexanoate; (8) estirene; (9) ethyl heptanoate; (10) octan-3-ol (IS); (11) ethyl decanoate; (12) 1,15-pentadecanediol; (13) furfural; (14) VitisI + VitisII; (15) propyl octanoate; (16) butyl caprylate; (17) isoamyl octanoate; (18) cyclodecanemethanol; (19) ethyl 9-decanoate; (20) propyl decanoate; (21) azulene; (22) buthyl decanoate; (23) dodecan-1-ol; (24) β-Damascenone; (25) 2-phenylethanol acetate; (26) ethyl decanoate; (27) isoamyl decanoate; (28) phenylethanol; (29) 1,14-tetradecanediol; (30) ethyltetradecanoate; (31) nerolidol; (32) octanoic acid; (33) 1,12 dodecanediol; (34) decanoic acid; (35) dodecanoic acid.

assess the optimum SPME sampling period. A graph of the MS response against the SPME sampling period, for CAR/PDMS fibre desorption after different extraction times (5–60 min), for the chemical groups considered in this study, is displayed in Fig. 3. It is observed that a typical extraction profile consist of an initial rapid portioning followed by a slower prolonged uptake and finally a steady-state equilibrium between the fibre and the vapour phase of the analyte. It is also apparent that the extraction time profile depends on the chemical group, but in particular on the polymeric phase. While for the ethyl esters, higher alcohols and isoamyl esters, the equilibrium was reached after 15 and 45 min, respectively, much longer equilibration times are needed for acetates, terpenes/C_{13} norisoprenoids and volatile phenols.

3.4. Study of the volatile compounds in BL, Bal, and HC whisky

After optimisation the experimental parameters with influence in the extraction process, the volatile constituents present in the headspace of BL, Ball and HC whiskys were analysed. A typical total ion chromatogram obtained from studied whisky samples using the experimental conditions discussed above are shown in Fig. 4. More than seventy compounds were identified

Table 3
Average peak area (APA) and relative peak areas (RPA) of volatile components present in the whisky headspace of Black Label (BL), Ballantines (Ball) and Highland Clan (HC) whiskeys obtained by dynamic HS-SPME using a 75 μm CAR/PDMS fibre ($n = 3$) followed by GC–MS analysis

RT (min)	KI	Compound	HC		Ball		BL	
			APA	RPA (%)	APA	RPA (%)	APA	RPA (%)
					Monoterpens/C_{13} norisoprenoids			
8.5	1211	TDN isomer	nd		nd		314149	0.06
28.33	1588	Vitispirane isomer 1	152640	0.03	172170	0.03	89851	0.02
28.48	1590	Vitispirane isomer 2	1082948	0.21	835882	0.16	834535	0.15
32.12	1758	Linalool	700580	0.14	125003	0.02	nd	
44.46	2001	TDN	1210091	0.24	1227597	0.24	1051415	0.19
45.56	2022	Azulene	1513278	0.30	1381490	0.27	1568942	0.28
51.26	2129	β-Damascenone	878146	0.17	1208118	0.24	1181703	0.21
57.8	2272	α-Ionol	563541	0.11	349245	0.07	1867754	0.35
66.69	2453	Nerolidol	689357	0.14	786580	0.15	948474	0.17
		Subtotal	7074695	1.40	6086085	1.19	7917572	1.44
					Higher alcohols			
2.96	984	Ethanol	30366778	6.01	2386881	0.47	92024139	16.44
5.52	1121	Butan-1-ol	322381	0.06	331396	0.06	1455166	0.26
10.26	1255	3-Methylbutan-1-ol	6501755	1.29	8271359	1.61	14489281	2.57
18.43	1413	Hexan-1-ol	762860	0.15	666093	0.13	134741	0.02
19.13	1426	(*Z*)-3-Hexen-1-ol	nd		nd		215204	0.04
25.21	1535	2-Ethyl hexan-1-ol	131354	0.03	209871	0.04	162755	0.03
26.26	1553	1,15-Pentadecanediol	1599659	0.32	1419060	0.28	830715	0.15
28.08	1584	1,10-Decanediol	169228	0.03	153033	0.03	198835	0.04
33.04	1774	(*E*)-3-Decan-1-ol	nd		348223	0.07	473543	0.09
41.39	1933	Cyclohexanemethanol	1697142	0.34	1640360	0.32	1303615	0.24
48.01	2062	1-Dodecanol	1814244	0.36	1802735	0.35	2215855	0.40
55.33	2218	(*Z*)-11-Hexadecan-1-ol	478383	0.09	545994	0.11	411968	0.07
56.33	2240	Cyclodecanemethanol	243357	0.05	370459	0.07		
58.5	2287	2-Phenyletanol	4273292	0.85	5608902	1.09	5611257	1.00
61.35	2346	1,14-Tetradecanediol	1069930	0.21	1416511	0.28	1737289	0.31
68.44	2488	Hexadecan-1-ol	230343	0.05	195513	0.04	nd	
74.18	2603	1,12-Dodecanediol	1734325	0.34	1686238	0.33	1558140	0.28
82.29	2772	4-Tetradecanol	184386	0.04	142957	0.03	139313	0.03
		Subtotal	51579417	10.21	29938149	5.84	122961819	21.97
					Acetates from higher alcohols			
2.09	907	Ethyl acetate	4853712	0.96	3881041	0.76	5330557	0.14
6.18	1144	Isoamyl acetate	7002145	1.39	7783517	1.52	3656051	0.10
51.45	2128	Phenylethyl acetate	18603608	3.68	19293719	3.76	16636199	0.43
		Subtotal	30459465	6.03	30958277	6.04	25622807	0.67
					Isoamyl acetates			
24.58	1523	Isoamyl hexanoate	503263	0.10	569897	0.11	178072	0.03
40.05	1906	Isoamyl octanoate	5688679	1.13	5744730	1.12	4960114	0.89
54.01	2189	Isoamyl decanoate	4869801	0.96	4987422	0.97	3852118	0.70
67.01	2460	Isoamyl laurate	96115	0.02	112544	0.02	66627	0.01
		Subtotal	11157858	2.21	11414593	2.23	9056931	1.63
					Ethyl esters			
10.37	1257	Ethyl hexanoate	15937009	3.16	21187505	4.13	9961764	1.81
16.17	1373	Ethyl heptanoate	3052675	0.60	2372160	0.46	1963781	0.36
23.07	1496	Ethyl octanoate	87185237	17.27	109424623	21.34	78375066	14.16
29.25	1703	Propyl octanoate	622242	0.12	630437	0.12	391270	0.07
32.04	1756	Butyl caprylate	1817005	0.36	1965765	0.38	1054649	0.20
35.11	1812	Metyl decanoate	465743	0.09	405041	0.08	588337	0.11
37.57	1860	Ethyl decanoate	191024703	37.83	204760692	39.93	189039650	34.06
40.5	1915	Ethyl benzoate	2334566	0.46	2278338	0.44	3297376	0.59
42.22	1949	Ethyl 9-decanoate	9034418	1.79	16621365	3.24	19034685	3.45
45.33	2009	Propyl decanoate	236865	0.05	233246	0.05	218044	0.04
46.36	2030	Butyl decanoate	1916752	0.38	1867906	0.36	1481765	0.27
52.49	2156	Ethyl dodecanoate	27133286	5.37	2735913	0.53	20691102	3.74

Table 3 (*Continued*)

RT (min)	KI	Compound	HC		Ball		BL	
			APA	RPA (%)	APA	RPA (%)	APA	RPA (%)
56.1	2235	Ethyl benzenepropanoate	523452	0.10	425250	0.08	451311	0.08
59.3	2304	Ethyl nonanoate	94469	0.02	88053	0.02	nd	
60.2	2323	Butyl dodecanoate	85641	0.02	92715	0.02	nd	
66.02	2440	Ethyl tretadecanoate	3151723	0.62	3313409	0.65	2290969	0.41
68.17	2483	Ethyl 3-hydroxyhexanoate	155614	0.03	173791	0.03	195526	0.04
77.32	2667	Ethyl hexadecanoate	358337	0.07	382170	0.07	267886	0.05
78.1	2683	Ethyl 9-hexadecanoate	598310	0.12	677898	0.13	166528	0.03
		Subtotal	345728047	68.47	369636277	72.09	329469712	59.46
					Fatty acids			
26.43	1556	Acetic acid	158016	0.03	121001	0.02	nd	
67.45	2468	Octanoic acid	7619209	1.51	11448859	2.23	12512476	2.22
79.14	2704	Decanoic acid	20211957	4.00	22773692	4.44	22800880	4.08
85.3	2828	Benzoic acid	239485	0.05	197546	0.04	217216	0.04
86.2	2846	Dodecanoic acid	5721837	1.13	6498841	1.27	5237130	0.93
		Subtotal	33950504	6.72	41039939	8.00	40767703	7.27
					Carbonyl compouds			
14.04	1333	Furan 2-methyl	nd		755304	0.15	nd	
20.22	1447	2-Methylundecanal	336024	0.07	580743	0.11	478466	0.09
27.24	1570	2-Furfural	2375322	0.47	3186814	0.62	5012574	0.90
60.47	2328	Undecanone	191464	0.04	125027	0.02	nd	
		Subtotal	2902810	0.57	4647888	0.91	5491041	0.99
					Miscellaneous			
6.44	1152	1,2-Dimethylbenzene	4303484	0.85	3591078	0.70	231877	0.04
8.17	1202	1,3-Dimethylbenzene	326016	0.06	207277	0.04	113424	0.02
12.03	1292	Stryene	14880323	2.95	12653919	2.47	12996851	2.32
15.32	1358	1,2,4-Trimethylbenzene	288225	0.06	310413	0.06	nd	
17.27	1391	EDMB1	379161	0.08	232419	0.05	194289	0.04
27.09	1567	EDMB2	869300	0.17	638103	0.12	nd	
34.52	1800	Decahydronaptalene	371100	0.07	616553	0.12	294951	0.05
54.32	2195	DPTMB	217400	0.04	227835	0.04	281204	0.05
62.31	2365	Biphenyl	71744	0.01	78129	0.02	66084	0.01
		Subtotal	21706753	4.30	18555726	3.62	14178681	2.53
					Phenol Compounds			
62.1	2361	2-Methoxy-4-methylphenol	30963	0.01	31677	0.01	nd	
65.19	2423	2-Methoxyphenol	364612	0.07	427898	0.08	657368	0.12
		Subtotal	395575	0.08	459575	0.09	657368	0.12
		TOTAL	504955124		488094698		432504448	
		RSD (%)	10.2	7.1	9.5	4.5	10.3	6.3

For each compound is indicated the chromatographic area and the respective *m/z* fragment obtained in full scan mode. RSD (%) – coefficient of variation (%); DPTMB: 4-bis(2,2-dimethylpropyl)-2,3,5,6-tetramethylbenzene; EDMB1: 1-Ethyl-2,3-dimethylbenzene; EDMB2: 1-Ethyl-2,4-dimethyl benzene; TDN isomer: 1,2-dihydro-3,6,8-trimethylnaphthalene.

in BL and sixty in Ball and HC whisky samples (Table 3), including ethyl esters, higher alcohols, acetates, isoamyl esters, fatty acids, terpenes/C_{13} norisoprenoids, carbonyl compounds and phenols. The differences observed according to the whisky sample were mainly quantitative. The abundances of the different volatile components (i) extracted by HS-SPME were calculated as relative peak areas (RPA_i), defined as the ratio between the component peak area (A_i) and the internal standard peak area (A_{is}): $RPA_i = A_i/A_{is}$. The relative standard deviation (RSD %) for the different RPA_i values were 4.5, 7.1 and 6.3%, for BL, Ball and HC whiskys, respectively. The total free fraction, in terms of RPA, of BL whisky (3883.5) was 1.7 times higher than HC (2267.2) and 1.5 times than Ball (2482.5). Typical HS-SPME-GC-$_{IT}$MS profiles of studied whiskys are shown in Fig. 5.

Quantitatively, the ethyl esters are the largest group of the studied whisky volatile constituents: 72.1% in Ball, 68.5% in HC and 59.5% in BL. These compounds are produced from ethanolysis of acylCoA that is formed during fatty acids synthesis or degradation. These compounds make a positive contribution to the general quality of whisky being responsible for their "fruity"

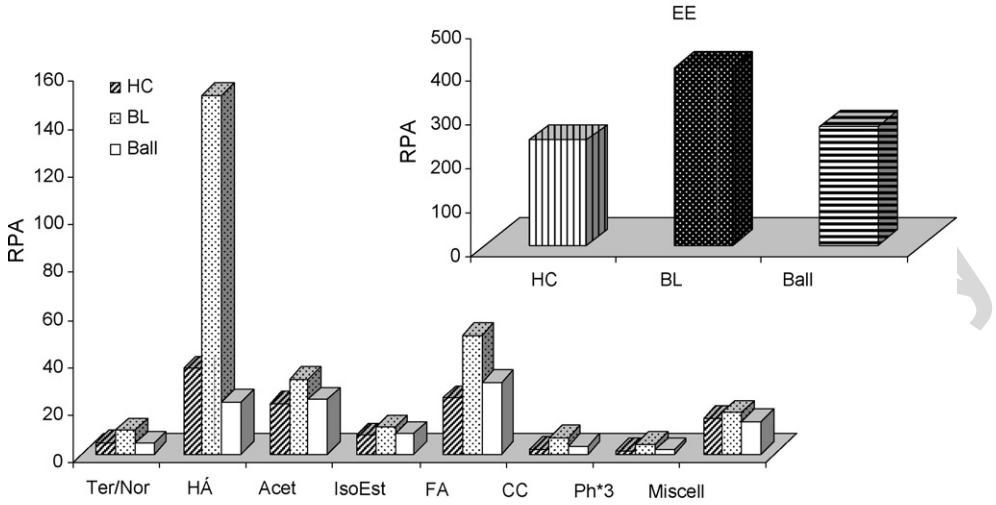

Fig. 5. Relative composition of the highland clan (HC), black label (BL) and ballantines (Ball) – ethyl esters – EE; monoterpenes, terpenols and C_{13} norisoprenoids – Ter/Nor; higher alcohols – HA; acetates – Acet; isoamyl esters – IsoEst; fatty acids – FA; carbonyl compounds – CC; phenols – Ph; miscellaneous – Miscell.

and "floral" sensory properties. The ethyl esters from C8, C10 and C12 fatty acids, which contributed with sweet and fruity notes, and isoamyl alcohols, represent the major compounds in either of the analysed whiskys. The relative amount of ethyl esters in the different types of studied whiskys was reasonably constant and the differences were not significant. From the ethyl esters of diprotic acids, the relative amount of diethyl succinate is much higher than that found for ethyl lactate. Similar contents of diethyl succinate were observed in all whisky samples studied.

Acetates are the result of the reaction of acetylCoA with higher alcohols that are formed from degradation of amino acids or carbohydrates. Isoamyl acetate with a characteristic odour of "banana", was found at similar values in different studied whiskys. The content of 2-phenylethyl acetate determined in Ball, which give "roses, flowery, honey" nuances to the whisky, was significantly different at the 95% level, from the determined for BL and HC samples.

Higher alcohols fraction is composed mainly by *n*-alcohols of C_6 chain length and aromatic compounds such as 2-phenylethanol. The presence of these compounds may cause a "flowery" and "sweet" notes which could be considered as a positive characteristic for whisky. The alcohol fraction of BL (RPA = 150.3) is significantly different at the 95% level from the other studied whiskys, which present RPA values of 35.9 and 22.1 for HC and Ball whisky, respectively. 3-Methylbutan-1-ol and 2-phenylethanol were markedly the most abundant higher alcohols in BL whisky, with an RPA of 17.6 for the first and 6.9 for the second. The contents of isoamyl alcohol were notably higher in the BL whisky (17.6) contrary to HC (4.5) that present the lowest content. The percentage of relative peak area is much higher in BL (21.9%) and HC (10.2%) than Ball (5.8%) whisky.

The third class of compounds in terms of quantitative volatile composition are the fatty acids The most important fatty acids present in the whisky samples studied were C8, C10 and C12. Fatty acids content in the analysed whisky samples, not differ significantly at the 95% level: 8.0% for Ball, 7.3% for BL, and 6.7% for HC samples. The carbonyl compounds include aldehydes and ketones. Only few aldehydes have been detected among the whisky volatile constituents, probably because they can be reduced to the corresponding alcohols. The carbonyl compounds content in Ball (RPA = 3.4) and HC (RPA = 2.0) whiskys is similar but in BL samples this values is much higher (RPA = 6.8). Terpenoids, which may have an important contribution on the "floral" and "fruity" aromas of the whisky, and C_{13} norisoprenoids (derived from carotenoids degradation) that contributed with "camphor", "honey-like" or "cassis" notes, are most abundant in BL samples. Contrary the lowest level of these compounds were found in Ball whisky samples. The three analysed whisky samples have in common the *trans*-β-damascenone, 1,2-dihydro-1,1,6-trimethylnaphthalene and the two vitispirane isomers.

4. Conclusions

Headspace solid-phase microextraction sampling followed by GC–MS analysis provides a clean and selective way to characterize the volatile compounds in whiskies. Five SPME fibres were compared in this study—PDMS, PA, CW/DVB, CAR/PDMS and DVB/CAR/PDMS. The highest enrichment of volatiles and the highest repoducibility of the peak areas were CAR/PDMS and CW/DVB. This coating showed the best extraction performance for the most polar analytes, higher alcohols, fatty acids and phenols, whilst the CAR/PDMS coating gave the best results for nonpolar and medium-polarity compounds. The best conditions were 60 min extraction at 40 °C. More than seventy compounds, ethyl esters, higher alcohols, isoamyl acetates, fatty acids, mainly, were identified. Their relative contents were found to be different which might lead to the difference in volatiles profile. Quantitatively, ethyl esters (mainly ethyl octanoate, ethyl decanoate and ethyl dodecanoate) are the largest group of the volatile composition found in studied whisky samples. The higher alcohols (aliphatic and aromatic) and fatty acids constitute important groups of aroma compounds that contribute with "fruity" and "cheese/fatty" notes to whisky sensory properties. The dominating esters are the ethyl esters of

fatty acids and acetates of higher alcohols. Ethyl octanoate and ethyl decanoate predominated in Ball whisky samples analysed.

These results show that HS-SPME/GC–$_{IT}$MS is a simple procedure of extraction with a great capacity of concentration and combines extraction and concentration in one step, rapid, sensitive and solvent-free method suitable for determination of volatiles and semivolatiles constituents in whisky.

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