Semi quantitative and comparative analysis of 2 matrixes by SBSE-LD-GC-MS

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Introduction

Gas-Chromatography Mass Spectrometry is a powerful tool to study impact of various factors on the aromatic profile in food and especially in the beverage industry. The extraction method is considered as the key point to analyse such complex matrix which can have 40 to 1000 volatile compounds.

In order to allow comparison between samples, the extraction technique has to be highly reproducible but also the least time-consuming as possible. Stir Bar Sorptive Extraction (SBSE) has proven to be one of the best techniques to do such work [1], [2].

Here we propose to compare two Whiskies and two coffees thanks to Stir Bar Sorptive Extraction followed by Liquid Desorption and Gas Chromatography Mass Spectrometry. It is then possible to easily identify specific compounds which are different between samples.

Material

Samples

Whisky samples were given by Serge Valentin (http://www.whiskyfun.com/).
Coffee samples were bought in a supermarket and were prepared by immersing 10g of coffee in distilled water at 90°C for 2 min.

Volatile compounds analysis

Stir Bar Sorptive Extraction method was done according to Coehlo et al [3] and adapted to our laboratory conditions, with a 1 µL injection volume. Each sample (20mL) was analyzed in triplicate by stiring during 120min stir bar in 3x20mL of sample at 20°C .Stir Bars (length = 20 mm) were coated with 47 µL of polydimethylsiloxane (Twister; Gerstel, Mülheim a/d Ruhr, Germany).

The GC-MS analyses was performed with an Agilent 6890N gas chromatography equipped with an Agilent 7683 automatic liquid sampler coupled to an Agilent 5975B inert Mass Spectrometer Detector (Agilent Technologies). The gas chromatography was fitted with a DB-Wax capillary column (60 m × 0.32 mm i.d. × 0.50 µm film thickness, J&W Scientific) and helium was used as carrier gas (1 mL/min, constant flow). Agilent MSD ChemStation software (G1701DA, Rev D.03.00) was used for instrument control and data processing. The mass spectra were compared with the Wiley's library reference spectral bank and confirmed by Retention Index (RI) from the in-house database and macro developed on Excel (Microsoft office 2013 ®). All compounds were semi-quantified using the ratio of their Total Ion Current peak to that of the 3-octanol (final concentration of 2000 µg/L corrected by their respective log Kow

estimated by Epi Suite software (EPA's Office of Pollution Prevention Toxics and the Syracuse Research Corporation (SRC)). Olfactive descriptions of each volatile compounds were based on our in-house database.

Statistical analysis

Statistical analysis was performed with Excel (Microsoft office 2013 ®). The paired t-test was applied to find compounds that have significant differences in the concentrations between two samples. P-values < 0,05 were considered to be significant.

Result

Whisky analysis

Figure 1 shows the difference between the aromatic profiles of whiskies. Whisky 2 is highly concentrated in styrene (more than 10x in comparison to Whisky 1). It is also richer in various esters like farnesyl acetate, isoamyl decanoate, propyl decanoate, isoamyl octanoate, Isoamyl acetate, isobutyl dodecanoate, decyl acetate, isoamyl hexanoate, ethyl tetradecanoate, Isoamyl dodecanoate, ethyl dodecanoate (Table 1) which contributes to fruity-note of the Whisky [4]–[6]. Whisky 1 is richer in diacetal (1-(1-ethoxyethhoxy)-pentane, hexanal- and heptanal- diacetal) which have been reported to increase during ageing [7] and various esters like diethyl succinate (also reported to increase with ageing [8] , ethyl 9 decenoate, and ethyl hexadecanoate. All of these compounds have been already reported in Whisky [4]–[6] and have positive descriptions. Most of them are reported to be odour active compounds in Whisky [4].

Among the three compounds present in Whisky 2 and absent in Whisky 1, b-damascenone can be considered as a quality marker because of its very low perception threshold and sweet odour [9]. Farnesol and mesitylene are also characteristic of the volatile profile of Whisky 2 (Table 1). On the other hand, 4 compounds namely propyl octanoate, isobutyl isohexanoate, benzaldehyde and dodecanoic acid seem to be characteristic of the volatile profile of Whisky 1 in comparison to Whisky 2.

Table 1 :Volatile compounds detected by SBSE-LD-GC-MS in two Whisky samples

Compounds (olfactive description)	RT (min)	Concentration (µg/L eq 3-octanol)						p-value
		mean		error	mean		error	
1-(1-ethoxyethoxy)-pentane (unknown)	9,67	747,31	±	11%	20,87	±	11%	**0,008**
Isoamyl acetate (banana, fruit, sweet)	10,08	8 799,95	±	7%	12 001,00	±	2%	0,018
Ethylbenzene (unknown)	10,47	416,84	±	1%	386,51	±	5%	0,156
Xylene (unknown)	10,73	1 861,61	±	5%	1 645,04	±	2%	0,088
2-Heptanone (fruit, bluecheese, sweet)	11,68	142,73	±	8%	25,15	±	11%	0,047
Isoamyl alcool (alcoholic, malty, fusel)	12,02	40 122,01	±	7%	14 867,62	±	1%	0,006
hexanal diethyl acetal (unknown)	12,80	477,36	±	7%	329,27	±	1%	0,024
Ethyl hexanoate (fruit, green apple, sweet)	13,00	18 020,68	±	7%	6 111,03	±	2%	0,006
triethylorthoformate (unknown)	13,56	290,39	±	4%	67,37	±	0%	0,001
Styrene (sweet)	14,02	69,97	±	11%	914,63	±	1%	**>0,001**
Hexyl acetate (fruit, floral, pear)	14,23	283,41	±	7%	322,84	±	1%	0,109
1,1,3-triethoxypropane (mushroom, vegetal)	15,25	122,44	±	11%	74,87	±	12%	0,051
heptanal diethyl acetal (unknown)	16,08	137,47	±	8%	78,12	±	6%	0,020
Ethyl heptanoate (fruit, wine)	16,39	27 779,19	±	1%	27 530,54	±	1%	0,483
N,N-Dimethylformamide (unknown)	16,69	391,51	±	4%	251,17	±	2%	0,006
Mesitylene (distinctive aromatic odor)	**16,99**	**ND**	**±**	**NC**	**71,67**	**±**	**2%**	**>0,001**
Isobutyl Isohexanoate (sweet, wood)	**17,10**	**63,96**	**±**	**16%**	**ND**	**±**	**NC**	**0,013**
Ethyl octanoate (fruit)	20,35	208 770,96	±	8%	89 024,44	±	3%	0,01
isoamyl hexanoate (fruit, green, pine apple)	21,31	368,88	±	1%	475,94	±	2%	0,007
Octylacetate (fruit, pear)	21,94	216,75	±	13%	183,25	±	1%	0,243
Propyl octanoate (fruit)	**23,79**	**383,44**	**±**	**8%**	**ND**	**±**	**NC**	**0,003**
Benzaldehyde (almond, nutty, wood)	**24,27**	**175,49**	**±**	**6%**	**ND**	**±**	**NC**	**0,002**
Ethyl nonanoate (fruit, rose, floral)	24,46	2 627,27	±	7%	1 155,60	±	2%	0,007
isobutyl octanoate (unknown)	25,13	911,29	±	4%	618,10	±	2%	0,01
Methyl decanoate (unknown, wine)	26,96	104,60	±	9%	ND	±	NC	0,004
Ethyl decanoate (fruit, grape fruit, pleasant)	28,89	412 380,86	±	7%	344 944,15	±	3%	0,101
Isoamyl octanoate (fruit, sweet)	29,61	4 481,62	±	4%	6 170,72	±	1%	0,006
Diethyl succinate (fruit, wine, wet)	30,18	1 217,57	±	3%	213,39	±	3%	**>0,001**
decyl acetate (unknown)	30,45	519,33	±	2%	670,98	±	0%	0,002
Ethyl-9-decenoate (fruit, unknown)	30,90	6 553,82	±	6%	622,52	±	1%	0,002
N-Methyl-2-Pyrrolidone (NMP) (unknown)	31,09	1 280,23	±	2%	1 156,57	±	0%	0,017
Propyl decanoate (unknown)	32,24	654,68	±	6%	902,55	±	8%	0,05
Ethyl undecanoate (unknown, cognac)	32,93	861,90	±	4%	806,88	±	2%	0,178
2-(2-Butoxyethoxy)ethanol (minty)	35,21	422,67	±	1%	372,98	±	2%	0,012
2-phenylethyl acetate (floral, rose, honey)	36,10	2 945,64	±	5%	2 973,02	±	2%	0,81
b-Damascenone (apple, honey)	**36,56**	**ND**	**±**	**NC**	**137,85**	**±**	**17%**	**0,014**
Ethyl dodecanoate (fruit, sweet, floral)	37,14	230 850,33	±	6%	287 272,56	±	2%	0,04
isoamyl decanoate (unknown)	37,80	8 429,07	±	5%	16 485,41	±	0%	0,001
2-Phenylethanol (rose, floral, honey)	39,74	1 962,93	±	7%	494,74	±	0%	0,005
propyl dodecanoate (unknown)	40,32	459,68	±	12%	377,24	±	1%	0,171
isobutyl dodecanoate (unknown)	41,46	538,30	±	6%	698,46	±	0%	0,017
Dodecanol (unpleasantin higher concentration, wax)	41,68	1 129,15	±	2%	662,63	±	8%	0,007
Pentadecan-2-one (unknown)	43,85	301,00	±	13%	239,18	±	9%	0,178
trans-nerolidol (rose, wax)	44,32	618,20	±	7%	737,24	±	1%	0,054
Ethyl tetradecanoate (wax)	45,28	27 027,88	±	6%	34 612,99	±	1%	0,023
Isoamyl dodecanoate (unknown)	45,45	2 319,01	±	6%	2 933,11	±	2%	0,028
Ethyl pentadecanoate (unknown)	48,42	674,80	±	5%	477,27	±	10%	0,036
Phenethyl hexanoate (fruit, unknown)	49,33	883,98	±	0%	579,51	±	5%	0,005
Ethylhexadecanoate (fatty, fruit, wax)	51,95	52 846,48	±	6%	37 345,64	±	1%	0,019
farnesyl acetate (unknown)	52,21	1 537,75	±	2%	4 792,10	±	3%	**>0,001**
Ethyl9-hexadecenoate (powder, unknown)	52,98	70 092,81	±	6%	59 227,58	±	0%	0,063
Decanoic acid (rancid, soapy)	53,60	18 055,82	±	3%	2 375,23	±	39%	0,002
Farnesol, isomer- (floral)	**55,17**	**ND**	**±**	**NC**	**486,61**	**±**	**2%**	**>0,001**
Pentadecanol (unknown)	55,92	48 039,29	±	6%	33 542,60	±	1%	0,017
Phenethyl octanoate (unknown)	56,38	3 933,08	±	3%	4 144,42	±	5%	0,353
dodecanoic acid (metallic, wax)	**60,41**	**8 215,78**	**±**	**8%**	**ND**	**±**	**NC**	**0,003**

ND : not detected ; NC : not calculated; Significant differences (p-value<0,05) are indicated in bold

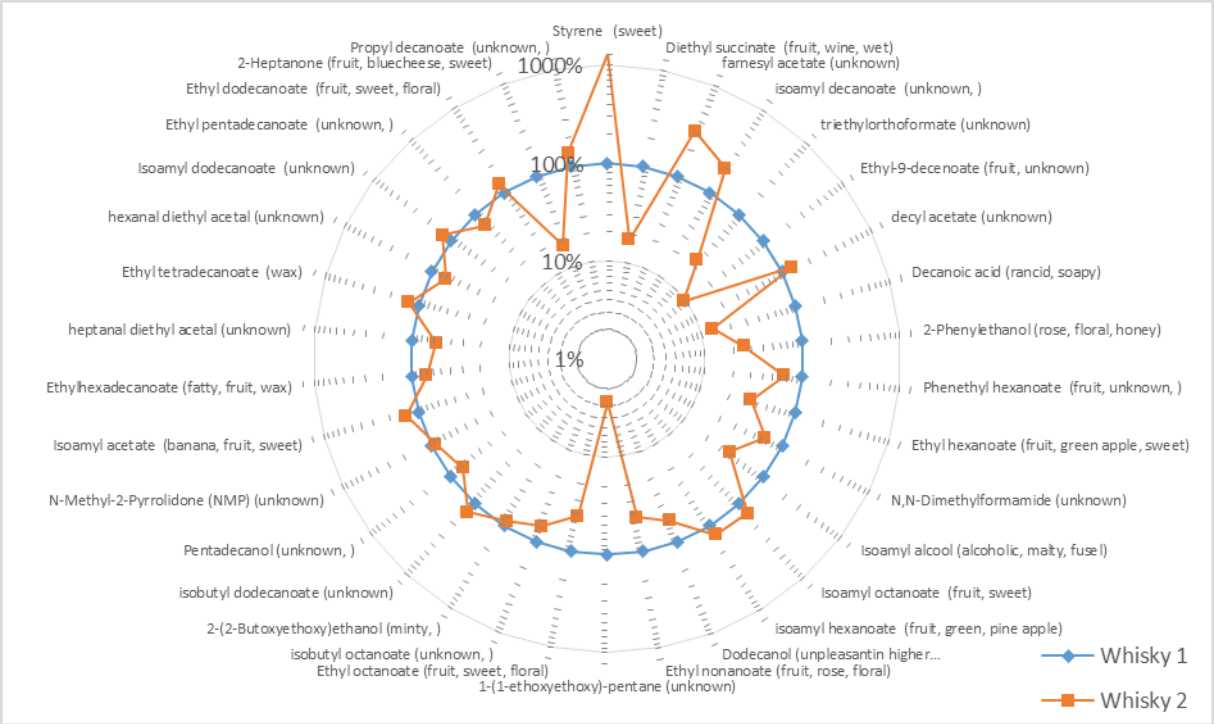

Figure 1 : Volatile compounds statistically different between Whisky 1 (100%) and Whisky 2

Coffee analysis

Figure 2 shows the difference between the aromatic profiles of coffee 1 and 2. Most of these compounds have been reported to be odour active compounds in coffee [10]–[14]. Volatile profile of coffee 2 is characterized by higher concentrations of 2 compounds: 2-phenylethanol (rose, floral, honey) and 4-(2-furyl)but-3-en-2-one (unknown) but is less concentrated in the other compounds. Two compounds are found only in the coffee 2: ethyl benzene and 2-ethyl-1H-pyrrole (Table 2). To our knowledge, there are no data about thresholds and odour of these 2 specific compounds even if they have been already reported in coffee [15], [16].

Table 2 : Volatile compounds detected by SBSE-LD-GC-MS in two coffee samples

Compounds (olfactive description)	RT (min)	Concentration (µg/L eq 3-octanol)						p-value
		mean		error	mean		error	
trans-2-methyl-2-butenal (green)	9,83	215,17	±	27%	98,87	±	4%	0,105
Pent-3-en-2-one (unknown)	10,41	680,45	±	22%	457,46	±	14%	0,196
Ethylbenzene (unknown)	**10,57**	**3,95**	**±**	**1%**	**0,00**	**±**	**NC**	**>0,001**
N-methylpyrrole (unknown)	10,72	801,80	±	8%	228,60	±	39%	0,133
2-ethyl-1H-pyrrole (unknown)	**11,84**	**27,09**	**±**	**2%**	**0,00**	**±**	**NC**	**>0,001**
Pyridine (burnt)	12,04	17 273,76	±	13%	11 227,26	±	9%	0,069
trimethyloxazole (unknown)	12,21	79,24	±	141%	80,69	±	7%	0,987
pyrazine (coffee)	12,74	9 331,02	±	12%	6 199,16	±	15%	0,090
Furfuryl methylether (Herbal)	13,22	1 277,88	±	6%	626,34	±	10%	**0,010**
3-methylbut-3-enol (herbaceous, unpleasant)	13,51	174,13	±	6%	136,17	±	16%	0,162
2-methyltetrahydrofuran-3-one (nutty)	14,31	61 507,16	±	6%	58 734,32	±	12%	0,683
Methyl pyrazine (green, toasted)	14,52	28 405,46	±	14%	25 771,88	±	16%	0,585
Acetoin (butter, creamy, wood)	15,05	8 483,66	±	19%	9 670,83	±	5%	0,429
Acetol (unknown, nutty)	15,53	12 145,89	±	9%	16 832,65	±	24%	0,258
2,5-Dimethylpyrazine (roasted, toasted)	16,54	8 302,16	±	17%	7 515,26	±	12%	0,568
2,6-dimethyl Pyazine- (roasted, roasted nut)	16,75	8 401,55	±	14%	7 695,68	±	16%	0,613
Ethylpyrazine (roasted, wood)	16,95	6 449,73	±	13%	5 046,10	±	12%	0,189
2,3-Dimethyl-pyrazine (nutty, toasted)	17,46	1 706,05	±	14%	1 399,52	±	15%	0,304
2-methyl-2-Cyclopenten-1-one (toasted,)	18,43	666,68	±	11%	454,38	±	11%	0,079
2-ethyl-6-methylpyrazine (toasted)	18,91	2 801,33	±	10%	2 072,79	±	8%	0,087
2-Ethyl-5-methylpyrazine (roasted, toasted)	19,20	1 645,77	±	11%	1 289,46	±	9%	0,143
Trimethylpyrazine (roasted, potato)	19,69	1 609,28	±	11%	1 308,82	±	11%	0,204
Propylpyrazine (Herbal)	20,22	247,45	±	12%	142,92	±	9%	**0,043**
acetol acetate (unpleasant)	21,31	166 564,65	±	8%	143 816,60	±	10%	0,256
Furfural (alkane, sweet, floral)	21,63	7 983,53	±	8%	8 233,86	±	9%	0,755
trans-linalool oxide furanoid (floral, wood)	21,72	185,84	±	44%	68,04	±	4%	0,178
3-Ethyl-2,5-dimethylpyrazine (potato, roasted)	21,96	258,44	±	0%	193,23	±	11%	**0,048**
2-Furfuryl methylsulfide (coffee)	22,77	297,97	±	3%	83,51	±	5%	**0,001**
2-methyl-6-vinylpyrazine (roasted, smoky)	23,02	741,04	±	22%	544,18	±	11%	0,254
2-vinyl 5-methylpyrazine (unknown)	23,31	648,55	±	6%	457,43	±	9%	0,043
acetyl furan (sweet)	23,48	23 334,41	±	8%	18 823,42	±	11%	0,152
2-furyl acetone (pleasant,)	23,92	6 030,72	±	7%	4 239,57	±	11%	0,052
1-Acetyloxy-2-butanone (unknown)	24,24	22 816,63	±	8%	21 083,96	±	12%	0,514
2-Furfuryl acetate (nutty)	24,47	35 512,41	±	4%	15 689,37	±	4%	**0,003**
Dihydro-2-methyl-3(2H)- thiophenone (wet)	24,76	10 754,92	±	5%	6 331,51	±	11%	**0,021**
2,3-Dimethylcyclopent-2-en-1-one (unknown)	25,46	425,05	±	11%	295,33	±	12%	0,084
5-methylfurfural (caramel, spicy)	26,32	12 215,24	±	8%	11 658,97	±	9%	0,645
furfuryl propionate (spicy)	27,04	457,64	±	2%	187,48	±	5%	**0,001**
2-Acetylpyridine (roasted)	27,82	4 364,69	±	49%	3 857,18	±	10%	0,772
2-Acetyl-5-methylfuran (nutty, strong)	28,15	2 315,80	±	7%	1 747,84	±	8%	0,062
2-Formyl-1-methylpyrrole (butter)	28,54	5 645,55	±	6%	4 896,11	±	8%	0,181
g-Butyrolactone (sweet, caramel, fruit)	28,90	256 896,55	±	8%	207 832,59	±	9%	0,131
furfuryl acetone (unknown)	29,20	2 631,44	±	27%	1 354,57	±	3%	0,125
Furfuryl Alcohol (burnt, sweet)	29,58	96 511,38	±	8%	86 698,34	±	11%	0,372
2-Acetyl-1-methylpyrrole (unknown)	29,92	5 749,18	±	7%	4 278,52	±	7%	**0,054**
methyl nicotinate (unknown)	35,00	3 773,93	±	5%	2 424,01	±	8%	**0,021**
2-METHYLBENZYL ALCOHOL (unknown)	35,22	597,23	±	4%	441,71	±	9%	**0,039**
furfuryl pyrrole (roasted, green)	36,66	144,27	±	1%	87,64	±	0%	**>0,001**
Guaiacol (burnt, smoky)	37,89	3 294,52	±	5%	3 516,43	±	5%	0,323
4-(2-Furyl)but-3-en-2-one (unknown)	39,75	1 177,31	±	4%	5 227,05	±	22%	**0,037**
2-Phenylethanol (rose, floral, honey)	39,98	351,44	±	13%	2 573,59	±	6%	**0,002**
2-Acetylpyrrole (walnut)	42,20	19 140,06	±	5%	17 629,66	±	12%	0,449
difurfuryl ether (unpleasant)	42,56	523,43	±	8%	266,72	±	0%	**0,012**
maltol (caramel, burnt sugar)	42,75	49 273,27	±	38%	83 344,78	±	21%	0,201
o-Cresol (phenolic, wood)	43,11	112,41	±	78%	133,05	±	16%	0,775
2-Formylpyrrole (unpleasant)	44,20	5 419,33	±	7%	6 340,67	±	13%	0,288
4-Ethylguaiacol (spicy, clove, smoky)	44,34	387,36	±	3%	228,10	±	5%	**0,005**
4-Vinylguaiacol (spicy, clove, smoky)	50,16	797,96	±	9%	655,53	±	17%	0,271
Indole (mothball, nutty)	58,54	133,66	±	41%	39,02	±	11%	0,227

ND : not detected ; NC : not calculated; Significant differences (p-value<0,05) are indicated in bold

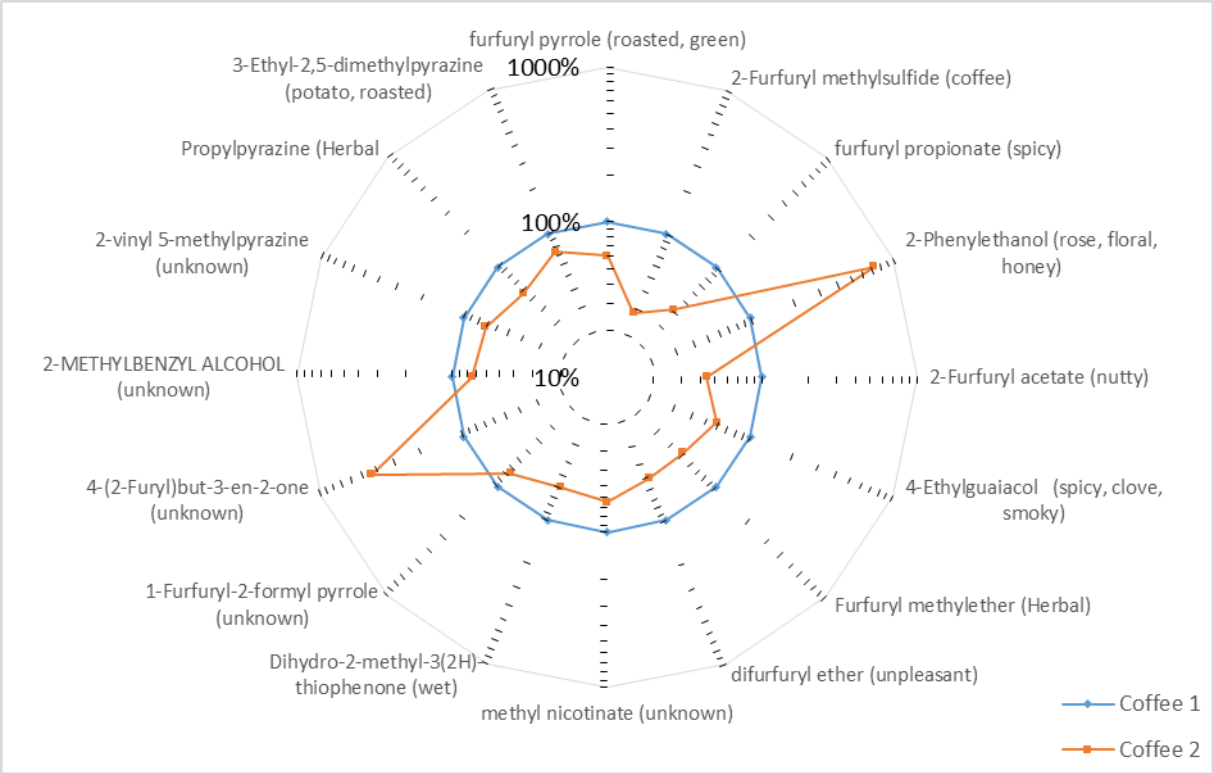

Figure 2 : Volatile compounds statistically different between coffee 1 (100%) and coffee 2

Conclusion

SBSE-LD-GC-MS allows detection and identification ofy 57 and 59 volatile compounds in whisky and coffee respectively. Standard error of the mean of the concentration of all the compounds detected in this work varies from less than 1% to 44% which confirms that SBSE-LD-GC-MS is a highly-reproducible technique. Thanks to statistical analysis, this approach allows us to compare easily and quickly two matrices and identify specific compounds.

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