Volatile compounds screening of 14 commercial Alsatian beers by SBSE-LD-GC-MS

www.twistaroma.fr

Nathalie Brignier[a], Céline Clayeux[a] , Behnam Taïdi, Damien Steyer[a]

Damien.steyer@twistaroma.fr

[a]*TWISTAROMA 28 rue de Herrlisheim 68021 Colmar, FRANCE*

[b]*Centre of Excellence for White Biotechnology, LGPM, Ecole Centrale Paris, Grande voie des Vignes, 92295 Châtenay-Malabry, FRANCE*

Introduction : Volatile compounds of 14 different Alsatian beers (6 lagers, 3 white; 3 special (named "other") and 2 stouts were investigated using Stir-Bar-Sorptive extraction followed by Gas Chromatography-Mass spectrometry (SBS-LD-GC-MS) to characterize each beer. Statistical analysis were performed to compare each type of beer.

Material and methods : Beer aroma compounds were analyzed by the Stir Bar Sorptive Extraction method adapted to our laboratory conditions, with a 1 µL injection volume. The analyses were performed with an Agilent 6890N gas chromatograph equipped with an Agilent 7683 automatic liquid sampler coupled to an Agilent 5975B inert MSD (Agilent Technologies). The gas chromatograph 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^{-1} constant flow). The GC oven temperature was programmed without initial hold time at a rate of 2.7°C min^{-1} from 70°C to 235°C (hold 10 min). The injector was set to 250°C and used in pulsed splitless mode (25 psi for 0.50 min). The temperatures of the interface, MS ion source and quadrupole were 270°C, 230°C and 150°C, respectively. The mass spectrometer was operated in electron impact ionization mode (EI, 70 eV) and the masses were scanned over a m/z range of 29 – 300 amu. Agilent MSD chemStation software (G1701DA, Rev D.03.00) was used for instrument control and data processing (with XCMS) . The mass spectra were compared with the Wiley's library reference spectral bank, Retention Index and Standard when available. All extractions were done in duplicate. Statistical analysis were performed with Minitab 16.0 and R15.1 (FactomineR package[1] and XCMS[2]).

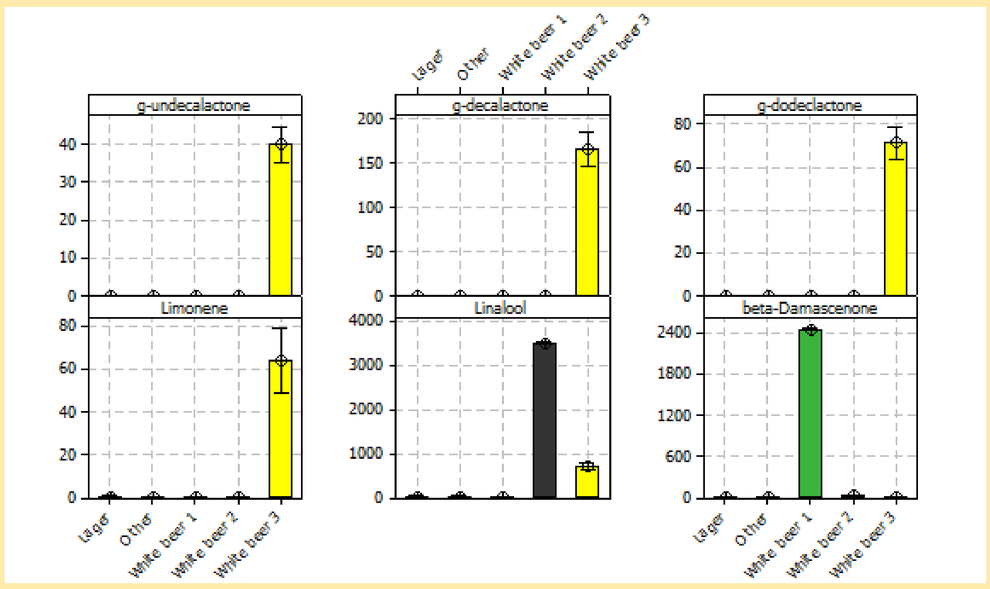

Figure 3: Specific compounds of white beers (µg/L rel to Internal Standard)

White beers : They can be easily differentiated. Each white beer have specific compounds : high amount of linalool for white beer 1, high amount of β-damascenone for white beer 2 and lactones for white beer 3 (fig 1). Theses beers were removed of the following statistical analyses due to their volatile composition.

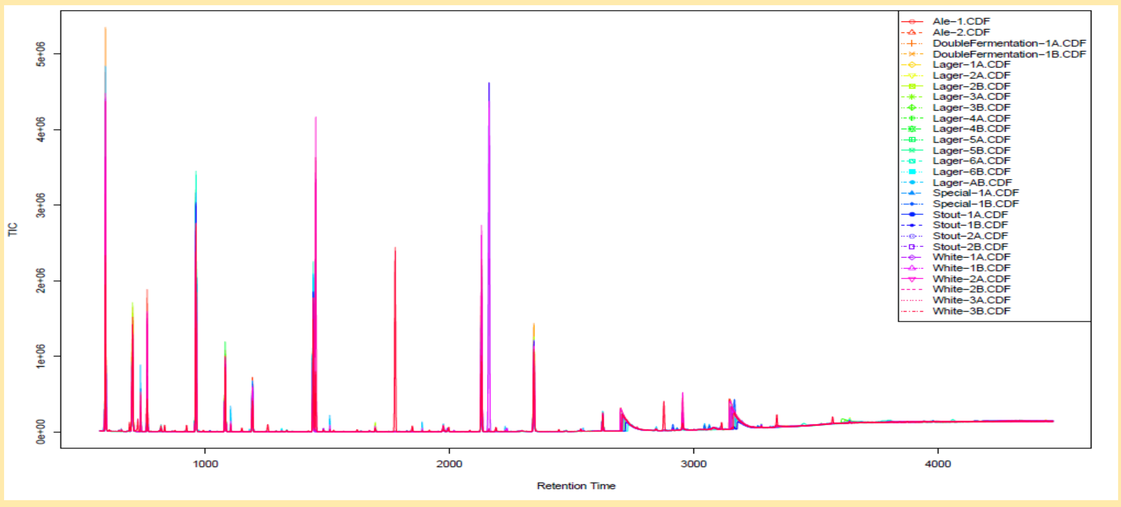

Figure 1 : Chromatogram of the 14 alsatian beers

Results: A total of 40 compounds were analysed in the 14 beers (Table 1 and Fig 1). Statistical analysis of the aroma for each compounds were used to classify the beers..

Lager vs Stout vs "Other": ": It was possible to explain more than 50% of the variability of each beer from its volatile composition (fig 2). Three different groups could be distinguished: lager, stout and "other". The first axis is positively linked with the 2-phenylethylacetate, 2-phenylethanol, Isoamyl acetate, Isoamylalcool and ethyl octanoate (correlation between 66% and 90%, data not shown). In other words this first axis is linked with yeast compounds. The second axis is inversely linked with Methyl-4-methyl-2-hexenoate, α and β-eudesmol and linalool (correlation between -68% and -77%, data no shown). This second axis is related to hop volatile compounds.

"Hoppy" beers : Beers that are most concentrated in hop volatile compounds are beer Lager 1 (1) and "other" 2 and 3 (8 and 9). All these beers are known to be rich in hoppy character (fig 2).

Fruity beers : The group "other" is largely composed of beers high in fruity esters like 2-PHA, IsoAc and ethyl esters. One exception is lager 2 which the beer with the highest concentration of ethyl decanoate out of all the beers analysed (fig 2).

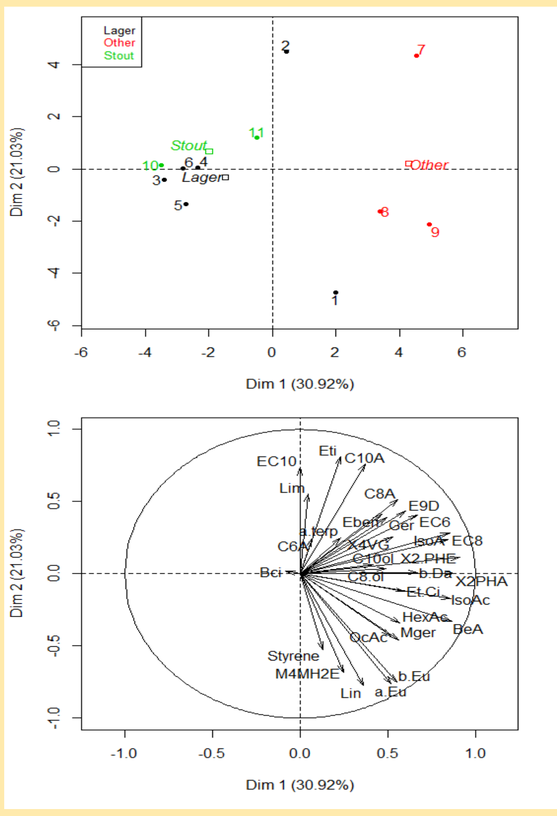

Figure 2: PCA of volatile compounds for Stout, Lager and "Other" beers.

Table 1 : Volatile compounds of 14 alsatian beers determined by SBSE-GC-MS

Relative concentration (µg/L rel SI)	Identification	Type of beer				p-value ANOVA testing Type of beer	Odour	Threshold (µg/L)
		White	Lager	Stout	Other			
Isoamylalcetate[1] (IsoAc)	**MS,RI,ST**	**1131,69± 170,35**	**666,04± 83,56**	**570,16± 86,24**	**1243,82± 30,42**	**0,000**	**banana**	**1 200**
ethyl pentanoate [1]	MS,RI,ST	3,88± 1,13	0,76± 0,28	1,73± 0,15	1,65± 0,53	0,123	green,mint	900
Isoamyl alcool[2](IsoA)	**MS,RI,ST**	**1202,60± 69,14**	**986,29± 66,39**	**819,00± 42,30**	**1371,84± 40,34**	**0,000**	**Fusel alcohol**	**70 000**
Limonene [1]	MS,RI,ST	21,24± 14,00	0,29± 0,20	0,00± 0,00	0,00± 0,00	0,441	Citruslike	210*
Ethyl hexanoate[1] (EC6)	**MS,RI,ST**	**277,75± 88,55**	**131,37± 9,55**	**132,07± 13,92**	**291,51± 70,09**	**0,007**	**Sweet,fruity,anise**	**210**
Ethyl tiglate[1]	MS,RI	0,67± 0,43	0,26± 0,18	0,25± 0,25	0,38± 0,38	0,929		
Styrene[1]	MS,RI	15,71± 2,79	6,93± 3,18	7,02± 4,05	6,61± 1,28	0,997	balsamic, gasoline	
Hexylacetate [1]	MS,RI,ST	11,99± 5,10	3,33± 0,45	2,29± 0,53	4,50± 0,37	0,053	Pleasantfruity,pear	3 500,00
Methyl 4-methyl hexa-2-enoate[1]	MS,RI	0,00± 0,00	1,51± 0,84	0,00± 0,00	0,55± 0,55	0,474		
ethyl octanoate[1] (EC8)	**MS,RI,ST**	**174,86± 19,01**	**126,50± 10,87**	**168,91± 28,38**	**219,27± 13,25**	**0,001**	**Pineapple,pear,floral**	**900**
1-Heptanol[2] (C7ol)	**MS,RI,ST**	**0,49± 0,49**	**0,00± 0,00**	**2,77± 1,62**	**0,00± 0,00**	**0,004**		
Octyl acetate [1]	MS,RI	1,67± 0,80	1,41± 0,43	1,47± 0,86	2,23± 0,74	0,588	Orangefloral,jasmine,pear	500
Benzaldehyde[1] (BenzA)	**MS,RI**	**1,96± 1,28**	**0,17± 0,13**	**0,00± 0,00**	**0,95± 0,44**	**0,045**	**Burnt sugar, Almond**	**2 000,00**
Linalool[2]	MS,RI,ST	1407,80± 680,50	17,07± 4,46	12,27± 3,85	19,86± 3,74	0,670	Citrus,floral,sweet,grape-like	27
1-Octanol[2] (C8ol)	**MS,RI,ST**	**19,07± 1,45**	**15,21± 1,47**	**27,42± 9,03**	**27,84± 3,92**	**0,023**	**fresh,sunflower,seeds,**	**900**
Ethyl decanoate[1]	MS,RI,ST	17,91± 3,49	13,76± 3,12	13,97± 0,89	11,43± 1,53	0,842	Sweet,fruity	1 500,00
Ethyl benzoate[1] (EB)	**MS,RI**	**1,22± 0,78**	**1,01± 0,38**	**3,37± 0,06**	**2,54± 0,47**	**0,004**	**herbal,fruity**	**6**
Ethyl 9-decenoate[1] (E9D)	**MS,RI**	**1,50± 0,68**	**1,62± 0,38**	**3,12± 0,19**	**4,26± 0,59**	**0,002**	**?**	**100****
Methyl geranate[1]	MS,RI	0,00± 0,00	0,22± 0,22	0,00± 0,00	1,24± 0,79	0,170	greenery	
α-terpineol[2]	MS,RI,ST	37,82± 13,74	3,61± 1,35	1,06± 1,06	1,98± 0,90	0,463	Lilac,floral,sweet	2 000,00
1-Decanol[2] (C10ol)	**MS,RI,ST**	**8,54± 1,86**	**11,98± 1,92**	**36,58± 12,48**	**29,25± 6,06**	**0,007**	**Orange, flowery**	**400**
Citronellol[2]	MS,RI,ST	22,28± 11,69	3,64± 1,39	0,00± 0,00	6,78± 3,31	0,190	Green,citrus,Fresh,citrus,	5
Nerol[2]	MS,RI,ST	11,32± 3,64	0,00± 0,00	0,00± 0,00	1,70± 1,70	0,275	Rose,apple,green,citrus	80
Ethyl nicotinate[1]	MS,RI	0,57± 0,57	0,25± 0,18	0,77± 0,44	0,00± 0,00	0,162	honey, cinnamon	4 555
2-phenylethylacetate[1] (2-PHA)	**MS,RI,ST**	**861,94± 158,20**	**366,03± 49,33**	**231,98± 67,98**	**748,24± 77,30**	**0,000**	**Apple, honey, roses**	**250****
β-Damascenone[1] (b-dam)	**MS,RI,ST**	**828,19± 516,69**	**2,63± 0,13**	**2,03± 0,22**	**3,49± 0,44**	**0,010**	**honey,sweetfloral**	**0,05***
Geraniol[2]	MS,RI,ST	36,58± 12,38	8,59± 1,82	4,74± 2,82	15,19± 3,70	0,077	Roselike	36
2-phenylethanol[2] (2-PHE)	MS,RI,ST	**1 057,50± 56,19**	**763,94± 57,05**	**581,51± 75,82**	**1 207,60± 48,53**	**0,000**	**honey,rose**	**125 000**
α-Caryophyllene alcohol	MS	0,00± 0,00	0,73± 0,73	5,62± 3,25	4,81± 4,81	0,338	moss, earth, spice	
Octanoic Acid[1]	MS,RI,ST	596,73± 120,70	311,80± 36,35	266,24± 80,63	470,08± 76,00	0,077	sweat, cheese	50
Ethyl cinnamate[1] (Ecinn)	**MS,RI**	**0,00± 0,00**	**0,24± 0,24**	**1,33± 0,78**	**2,70± 0,89**	**0,010**	**honey, cinnamon**	**67***
γ-decalactone[1]	MS,RI,ST	55,39± 35,36	0,00± 0,00	0,00± 0,00	0,00± 0,00		Fruity, lactonelike, cocos	50
4-Vinylguaiacol[2]	MS,RI,ST	334,96± 74,69	70,57± 10,83	63,83± 2,70	109,55± 22,90	0,135	clove, curry	100
α-selinene[1]	MS,RI	0,00± 0,00	19,25± 19,25	0,00± 0,00	0,00± 0,00	0,681	Weak spicy, balsamic, mild	
α-Eudesmol[2]	MS,RI	0,00± 0,00	19,70± 8,78	0,00± 0,00	38,50± 13,58	0,139	wood, green	
β-Eudesmol[2]	MS,RI	0,00± 0,00	18,52± 8,43	0,00± 0,00	40,40± 14,47	0,110	wood, green	
γ-undecalactone[1]	MS,RI	13,30± 8,50	0,00± 0,00	0,00± 0,00	0,00± 0,00		apricot	
Decanoic acid[1]	MS,RI,ST	805,65± 123,10	321,88± 73,23	362,48± 209,86	527,37± 214,54	0,547	rancid, fat	1 000**
γ-dodeclactone[1]	MS,RI	23,84± 15,21	0,00± 0,00	0,00± 0,00	0,00± 0,00		sweet, fruit, flower	7
Ethyl dihydrocinnamate[1]	MS,RI	0,00± 0,00	2,15± 0,89	0,00± 0,00	3,58± 2,28	0,344	flower	1,6*

Threshold determined in beer except * in water; ** in wine model; MS :tentitavely identified by Mass spectrometry (NIST/WILEY07); RI : identified by Retention index ; ST : Identified with standard ;
[1] : ethyl heptanoate equivalent : [2]: 3-octanol equivalent; Data from TWISTDatabase ©

Conclusion:

This study allowed the comparison of the volatiles profile of different beer types.

The analysis can be performed to precisely determine which compounds are specific for a given beer.

1 :Husson, F., Josse, J. & Lê, S. (2008). FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software*. **25(1)**. pp. 1-18.

2 : Smith, C.A. and Want, E.J. and O'Maille, G. and Abagyan,R. and Siuzdak, G.: XCMS: Processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching and identification, Analytical Chemistry, 78:779-787 (2006)