ANALYSIS OF FIVE BOTTLE-REFERMENTED HANDMADE BEERS BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY TIME-OF-FLIGHT MASS SPECTROMETRY, A COMPARATIVE STUDY

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Key points

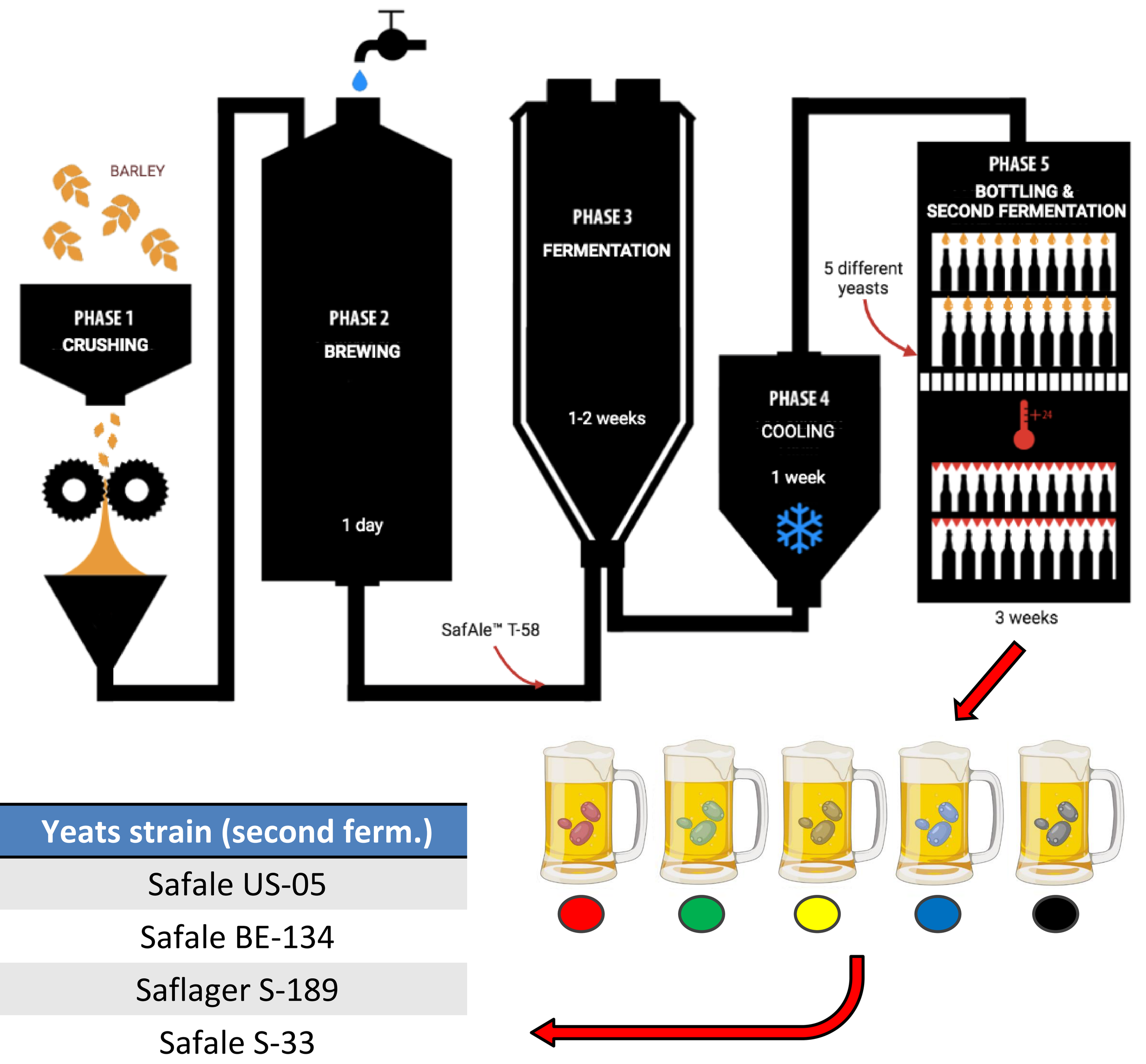
- The combination of uni- and multivariate statistic tools allowed us to highlight volatiles giving the specific smell of the beers. As a result, the yeast used for the bottle fermentation has an impact on the beer's volatolome.
- The specific smell of the 5 beers is essentially due to a complex combination of different esters.

Introduction

Fermented beverages, such as beer, are considered as complex mixtures by flavor and food chemists. The beer volatolome contains hundreds of compounds, which affect the taste and the mouthfeel. In this highly competitive market, it is crucial for brewers to master each step of the process to understand and control the taste and to guarantee aroma quality and stability. Aroma compounds are essentially derived from the raw ingredients and the conditions used to produce beers, including the fermenting yeast strain. This study aims to determine the impact of the yeast on beer's volatolome. More specifically, the impact of the yeast used for bottle fermentation. Due to the complexity of the sample, a comprehensive two-dimensional gas chromatography (GC×GC-TOFMS) system has been used.

Beers production

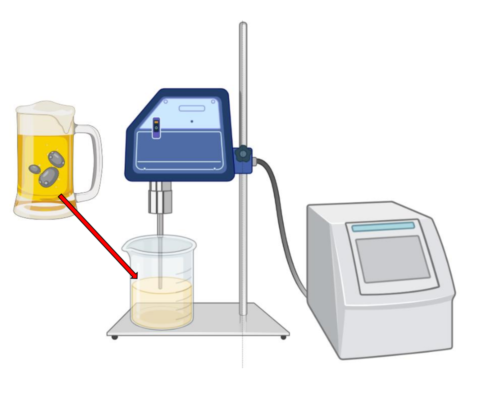
Five beers were produced from the same feedstock. First, barley malt (Pilsen) was crushed **(Phase 1)** and mixed with water. The mixture was heated up to 62 °C for 30 min to trigger β-amylase activity, 72 °C for a further 30 min to initiate α-amylase activity and finally at 78°C for 10 min. Mash was then filtrated, washed with water and brought to a boil for 1h15; hops were then added to the mixture **(Phase 2)**. A cooling step was performed, bringing the temperature from 100 °C to 24 °C. The first fermentation **(Phase 3)** was carried out with the same dry yeast strain; SafAle™ T-58 was added. The mixture was then stored at low temperature **(Phase 4)**. Finally, 5 different yeasts (table 1) were pitched in their respective bottle containing the precursor to activate the second fermentation **(Phase 5)**.



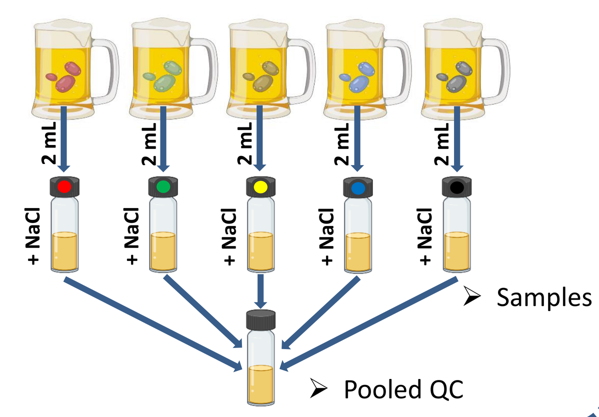
	Beer identification	Yeats strain (second ferm.)
(red)	Red Beer	Safale US-05
(green)	Green Beer	Safale BE-134
(yellow)	Yellow Beer	Saflager S-189
(blue)	Blue Beer	Safale S-33
(black)	Black Beer	Safale S-04

Samples analysis

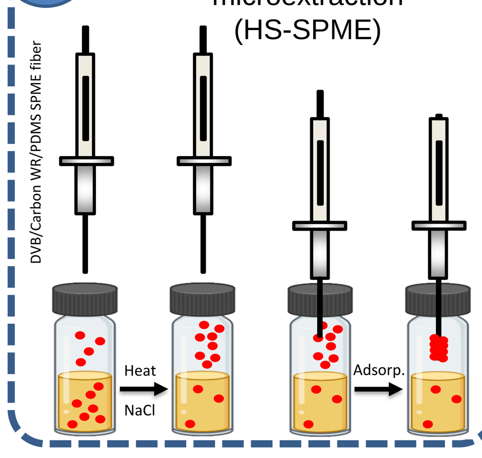
1 Gas elimination using a sonicator



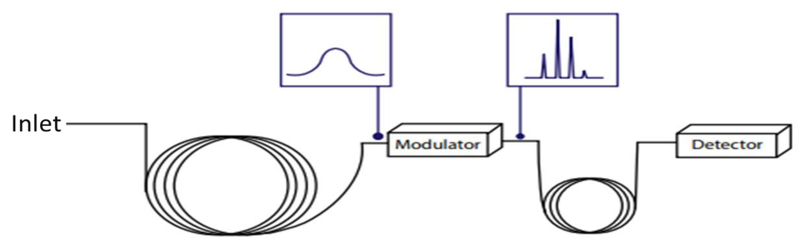
2 Samples preparation (5 replicates for each beer)



3 Headspace solid phase microextraction (HS-SPME)



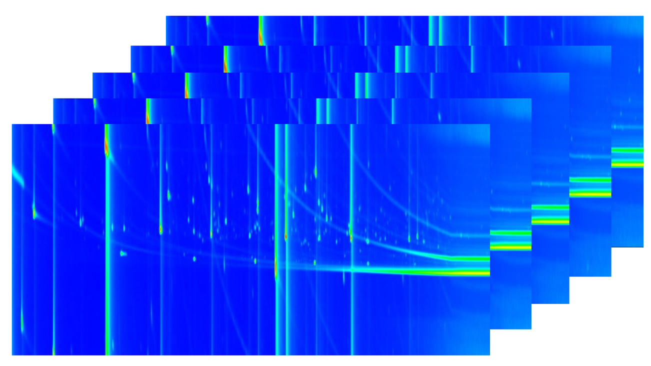
4 Analysis by GC×GC-TOFMS (Pegasus BT 4D)



GC×GC :
Columns : ^{1}D Rxi-624Sil MS (30m x 0.25 mm x 1.4 µm d_f)
^{2}D Stabilwax (2m x 0.25 mm x 0.5 µm d_f)
Temperature prog. : 40°C (5 min), ramped 5°C/min to 235°C (3 min)
Modulator : Quad jet dual-stage, P_M= 3.5 s, hot pulse time : 1.05 s

TOFMS :
Acquisition delay : 310 s
Acquisition frequency : 200 Hz
Electron ionization energy : 70 eV
Mass range : 33 - 450 mu

5 Data exploration



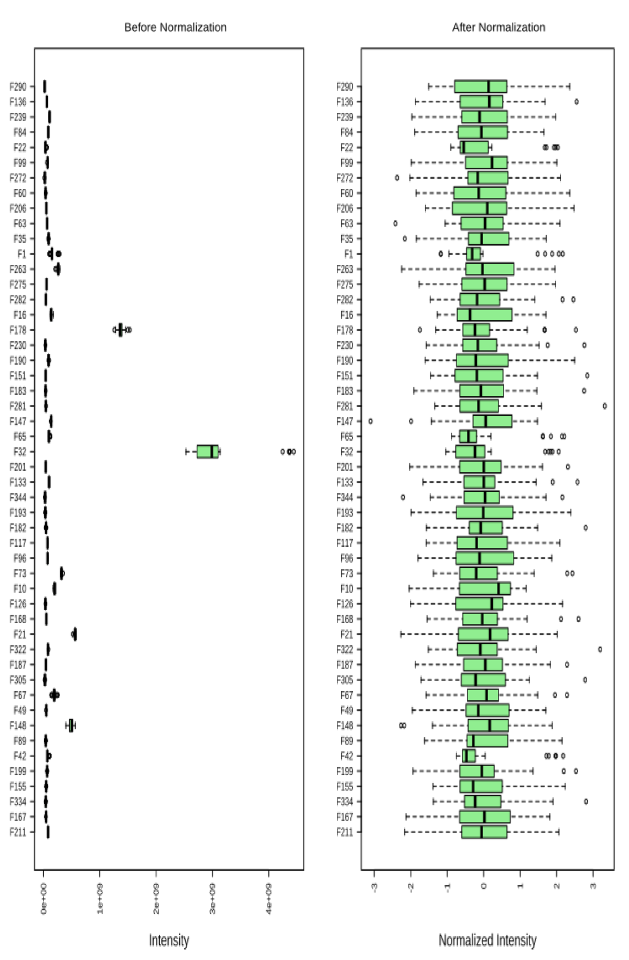
25 chromatograms were acquired in addition to blanks and QCs. ChromaTOF® 5.0 has been used to visualize the data.

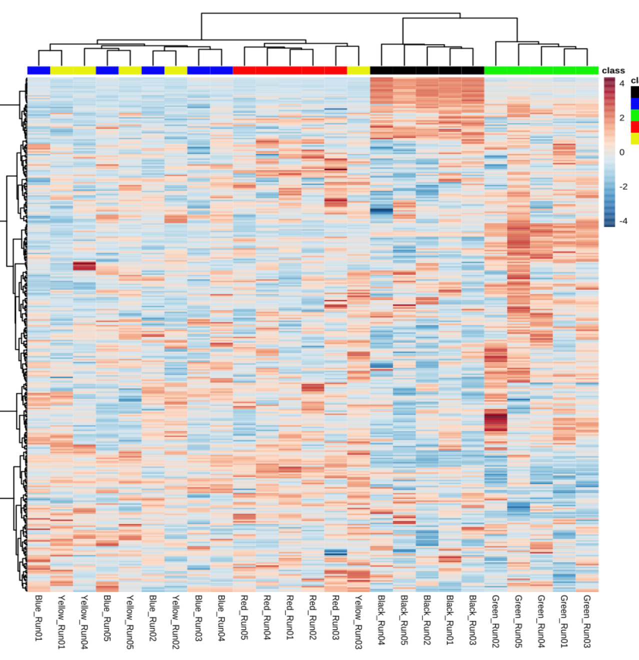
Data treatment/ Results

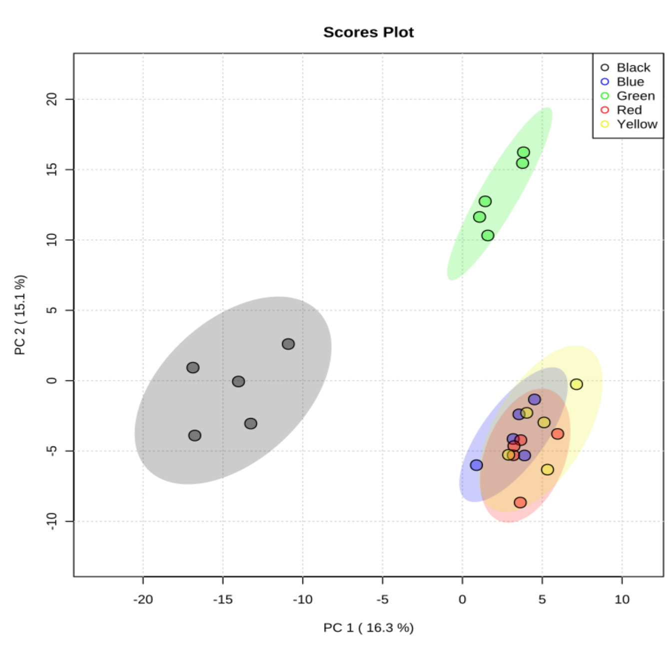
MetaboAnalyst 5.0

Data curation

- Sample normalization by median
- Auto scaling of the data





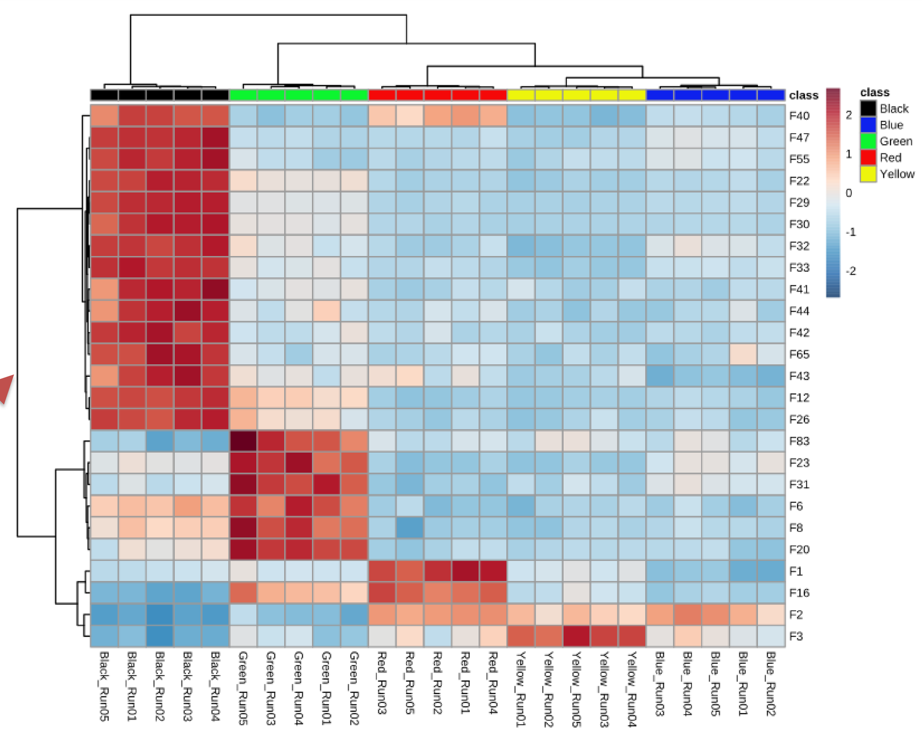


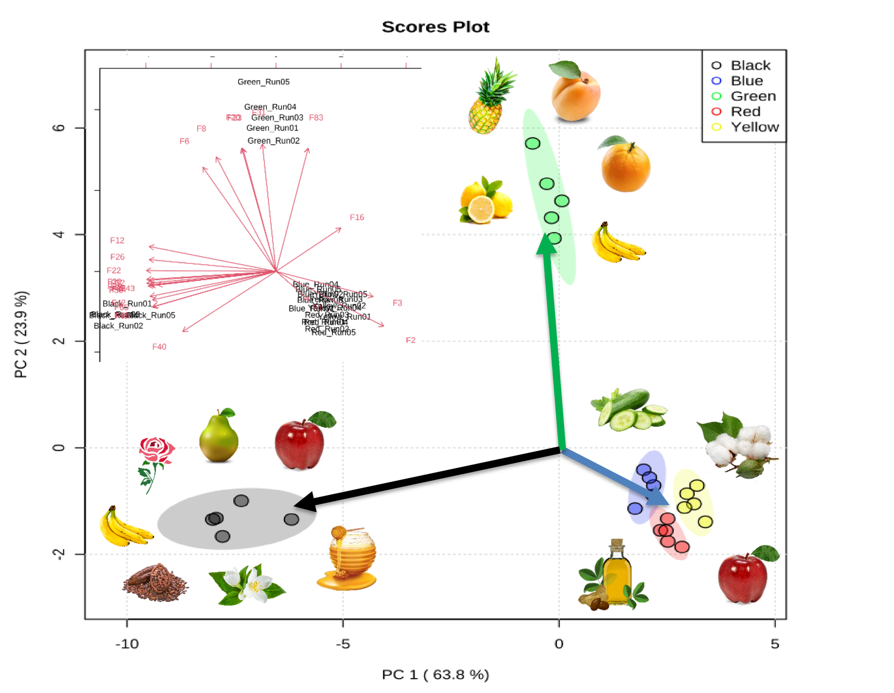
Features selection

One-way ANOVA analysis :

Top 25 features selection based on the f.values

ID	f.values	Identification	Odor type
F29	1135	6-Octen-1-ol, 3,7-dimethyl-, acetate	Fruity, citrus
F33	644.55	Acetic acid, 2-phenylethyl ester	Rose, Honey
F22	535.02	Acetic acid, heptyl ester	Pear, Apricot
F47	490.77	Acetic acid, hexyl ester	Apple, banana peel
F30	336.71	Acetic acid, octyl ester	Jasmine, Fruity
F12	330.38	Hexanoic acid, ethyl ester	Pineapple, Banana
F32	229.25	1-Butanol, 3-methyl-, acetate	Banana, Pear
F42	218.03	Hexanoic acid, 2-phenylethyl ester	Fruity
F1	197.34	Ethyl 9-decanoate	Fruity
F55	191.33	Isobutyl acetate	Banana, Tropical
F40	167.84	Dodecanoic acid, ethyl ester	Fruity, Floral
F23	145.99	Propanoic acid, pentyl ester	Apricot, pineapple
F20	135.03	Octanoic acid, ethyl ester	Apricot, Floral
F31	128.2	1-Decene	/
F16	117.37	1-Heptanol	Apple, cucumber
F26	113.23	2-Nonen-1-ol, (E)-	Green
F2	95.278	2-Undecanol	Waxy
F6	89.793	1,1-diethoxyethane	Fruity, Nutty
F8	84.873	Butanoic acid, ethyl ester	Fruity, Pineapple
F41	80.281	2-Decanol	Waxy
F65	74.546	Octanoic acid, 2-phenylethyl ester	Cocoa, fruity
F3	65.64	3-Heptanone, 5-methyl-	Herbal, Oily
F43	63.327	10-Undecen-1-al, 2-methyl-	Orange-like floral
F44	59.886	2-Nonanol, acetate	/
F83	49.388	1-Octanol	Citrus, Orange





Conclusion

The beer aroma profile is impacted by the yeast strain used for the bottle fermentation. Specific esters were produced during this second fermentation resulting in beer-type classification on principle component analysis and hierarchical clustering analysis. While the black and green beers are characterized by a "fruity" fragrance, the scent of the three other is considered as more "herbal and oily". Moreover, we have demonstrated that the combination of uni- and multivariate statistical tools allowed to highlight molecules driving a better classification of the beers regarding their volatolome.

[1] F. A. Franchina, D. Zanella, E. Lazzari, P. H. Stefanuto, and J. F. Focant, "Investigating aroma diversity combining purge-and-trap, comprehensive two-dimensional gas chromatography, and mass spectrometry," *J. Sep. Sci.*, vol. 43, no. 9–10, pp. 1790–1799, May 2020.

[2] P. Stefanuto, K. Perrault, L. D.-… of C. A, and undefined 2017, "Advanced method optimization for volatile aroma profiling of beer using two-dimensional gas chromatography time-of-flight mass spectrometry," *Elsevier*.

[3] T. Massenet, H. Muller, L. Dubois, P.-H. Stefanuto, and J.-F. Focant, "Profiling the ester aroma of fruity beers using comprehensive two-dimensional gas chromatography: a comparison of flow and cryo-genic modulators."

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