Investigation of the similarities and differences of two beer aroma profiles using comprehensive gas chromatography and mass spectrometry

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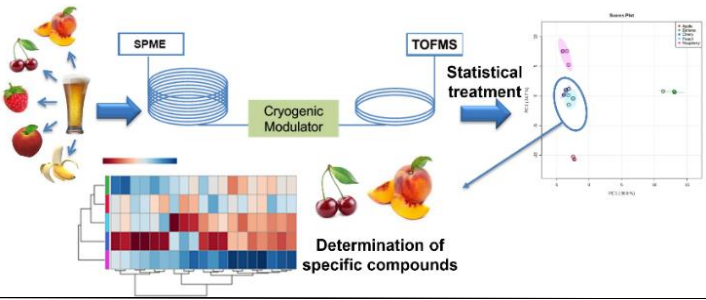
ABSTRACT: This benchmarking study of fruity beers volatile profiles, analyzed by SPME-GC×GC-MS, highlights the similarities and differences between two beers' profiles (cherry and peach flavored) based on a PCA, an ANOVA test and heat map. The common volatile organic compounds (VOCs) of the two beers were related to the similar brewing process compared to the three other beers whereas the different VOCs define the aroma and the taste of the beer.



1. INTRODUCTION

Nowadays, a great variety of beers are on the market, including the fruity beers. They may be flavored like apple, peach, banana, or another fruity flavor. Several beers exist for the same fruity flavor, but their taste differs due to process variations. These process variations can be highlighted by the VOCs profile of the beers. A way to analyse the beer's VOCs is SPME-GC×GC-MS (a solid phase microextraction unit linked to a comprehensive two-dimensional gas chromatography coupled with a mass spectrometer). In this study, five different fruity beers were analyzed by SPME-GC×GC-MS: raspberries flavored (Leffe Rubby®), cherry-flavored (Lindemans Kriek®), banana-flavored (Mongozo Banana®), apple-flavored (Lindemans Apple®) and peach-flavored (Lindemans Pecheresse®).

A quick PCA analysis of the results (*Figure 1*, on top of page 2), shows that the profiles of the cherry-flavored beer and the peach-flavored beer are close to each other. The aim of this study will be to identify, based on an ANOVA test and a heat map and researches, the VOCs responsible for the similarities between the cherry and peach beer profiles that other beers do not share and to highlight the specific VOCs of the cherry or peach beer.

2. MATERIALS AND METHODS

2.1. Sample protocol

After 5 minutes of being degassed by sonification, 10 mL of the beer samples were transferred into a 20 mL headspace (HS) vial with 1 g of NaCl. The vial was sealed and ready for the extraction and injection. This protocol was repeated for each beer in five replicates.

2.2. SPME-GC×GC-MS

The HS-SPME part collects and injects the sample in the GC columns. Its principle is based on the extraction of volatile components, based on equilibrium between HS gas and liquid or solid sample, thanks to a sorbent fibre. The coating used for this study is a mixed phase of DVB, CAR and PDMS (Divinylbenzene, carboxen and polydimethylsiloxane). The volatile components, contained in the sample, diffuse into the gas phase until reaching an equilibrium between the two phases. The fibre is introduced in the HS vial containing the sample by the pierced septum and during a defined time. During this time, the sorbents of the fibre trap the volatile components contained in the HS gas. Subsequently, the trapped compounds are injected in the GC by thermal desorption and a split of 50. The extraction conditions are optimized, and the fully automated process limits the variations. [1]

GC×GC unit aims to separate the VOCs of the sample. It is the association of two different types of capillary column; in our study, a non-polar followed by a polar column. The association of columns with different properties lets us separate the compounds according to their physical and chemical affinities. This double separation favors the separation of the coeluting compounds after crossing the first column. The two columns are connected by a modulator which conveys the eluted compounds of the first column in the second one. The modulator used in this study was a cryogenic modulator which works as follow. First, a cold jet of cryogenic liquids traps the eluted compounds of the first column. Then, a hot jet of nitrogen delivers the compounds in the delay tube. When the delay time is over, a second cryogenic jet colds and refocuses the compounds before a final hot jet of nitrogen sends them in the second column. [2]

The detector is a mass spectrometer (MS). The eluted compounds are fragmented by electron ionization and the fragments are charged and separated by a TOF according to their m/z ratio. The MS detects the compounds and provides mass spectra for each eluted compound which is needed for their identification. [3]

Thanks to all these specificities, the SPME-GC×GC-MS is an appropriate instrument for these analyses. All the experimental parameters optimized are the *table S1*. The resource used to treat the data statistically is Metaboanalyst.

3. RESULTS AND DISCUSSION

After a data treatment and normalization, a first PrincipalComponent Analysis (PCA) was done to visualize the five analyzed beers' VOC profile (*Figure 1*, on top of page 2). An ANOVA test was realized to identify the significant disparities between the samples and to eliminate background noises. The selected VOCs were treated with MetaboAnalyst and displayed on a heat map *(Figure S1)*. At this step of the study, the VOCs of interest were chosen according to their intensity.

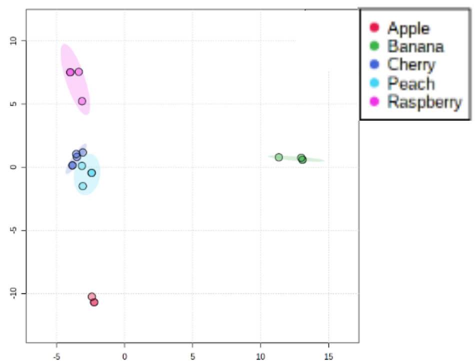


Figure 1: PCA of the 5 analyzed beer.

The VOCs with a high intensity in cherry flavored and/or peach flavored sample but weak signal in the other three were selected. The 19 VOCs corresponding to these criteria are represented in the simplified heat map in *Figure 2.*

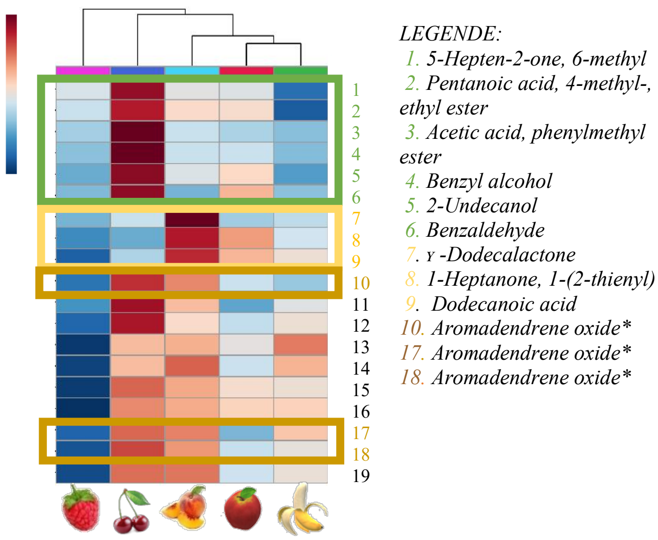


*Figure 2: Heatmap of the 19 VOCs of interest with averaged replicates (full heatmap in Figure S2 and detailed heat map in Figure S3). * The VOCs are proposed identities based on mass spectral comparison with the NIST library. Further investigations to identify these VOCs could be done based on additional injections with standards.*

Based on this heat map, many VOCs were highlighted. Among the specific VOCs of the cherry (green rectangle), only the benzyl alcohol and the benzaldehyde will be developed in this work whereas for the peach (yellow rectangle) it will be the γ-Dodecalactone.

Several VOCs seem to be specific to the peach and the cherry beers (brown rectangles). The analysis of the origin of these compounds could explain the similarities between the two profiles. The aromadendrene oxid (10) was selected because it is the only one that is only intense in the peach and the cherry and not in the other beers.

Benzaldehyde (6) and benzyl alcohol (4) are two characteristic flavors of cherry. In fact, benzaldehyde is one of the main aromatic compounds in cherry. It has a high aroma value and is responsible for the almond odor whereas benzyl alcohol is responsible for the floral odor. In addition, benzaldehyde and benzyl alcohol are closely linked. Indeed, benzaldehyde is enzymatically produced from amygdalin or prunasin that are found in cherries whereas benzyl alcohol can be formed by reduction of benzaldehyde. Thus, both compounds are due to the cherries found in cherry beers. [4-5] However, amygdalin is a compound that is also found in other fruits including apple. The presence of benzaldehyde in apple beer and not just in cherry beer could therefore be explained by the presence of amygdalin which would have formed benzaldehyde. [6]

The γ-Dodecalactone (7) is only present in the peach sample. This compound is a natural aroma of the peach. They can be synthetized by the *Sporobolomyces odorus (So)* yeast starting from oleic acid. The *So* yeast could be used for the fermentation yeast of the beer and to produce this flavor. However, this hypothesis cannot be confirmed because the official recipe of the Peach beer is not yet published. According to Lindemans' website, the beer is produced by a mix of fresh peaches' juice and Lambic. The γ-Dodecalactone comes from either these fresh peaches and/or from *So* yeast added in the mixture. [7-8]

The aromadendrene oxid (10) is a compound found in the hops, but that disappears after the boiling step to get the beer wort. Our hypothesis of this shared compounds for peach and cherry is the same brewing process. Indeed, the cherry, peach and apple beers are produced in Lindemans brewery unlike banana beer (Hyghe) and the raspberry (AB InBev). Moreover, in the brewing process of the peach and cherry beers, the fruit macerates in the Lambic whereas it does not in the apple brewing process. This maceration could modify the composition of the wort and the oxide is less degraded or even not degraded at all. In addition, the variety of Lambic used for apple is different than the one use for peach and cherry beers. Those hypotheses are only suppositions and cannot be proved because of the secret recipe. [8-9]

4. CONCLUSION

The analytical method used, SPME-GCxGC-MS, lets us highlight the specific VOCs of cherry and/or peach beers. The VOCs, specific to cherry or peach, come from the aromas of the fruit used. Benzaldehyde and benzyl alcohol are mainly related to cherry, and γ-Dodecalactone to peach. The similar profile of beers probably comes from the beer brewing process. In fact, the aromadendrene oxid, present in both beers, is found in hops. Cherry, peach, and apple beers are produced at the Lindemans brewery unlike raspberry and banana beers, but the brewing process of the apple beer seems slightly different from the two others. Apples do not macerate in the Lambic unlike cherries and peaches for their respective beers. This could therefore have an impact on the final composition of the beer. However, these are only hypotheses given the trade secrets.

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SUPPORTING INFORMATION

Table S1: Experimental parameters of the study

HS-SPME	
Incubation parameters	
Equilibrium temperature	*60°C*
Extraction time	*20 minutes*
Vector gas flow	1mL/min
Injector temperature	250°C
Split	50
GC×GC	
Vector gas flow	1mL/min
Carried gas	Helium
First column	Non polar - 30×0.25×0.25 - Rxi 5
Temperature program	*Initial temperature: 40°C* *Duration: 5 minutes* *Ramp temperature: 5°C/min* *Duration: 47minutes* *Final temperature 240°C* *Duration: 5 minutes*
Second column	Semi polar - 1×0.25×0.25 - Rxi17
Temperature program	*Initial temperature: 55°C* *Duration: 5 minutes* *Ramp temperature: 5°C/min* *Duration: 47minutes* *Final temperature 255°C* *Duration: 5 minutes*
Modulator type	Cryogenic modulator
Modulation time	3s
Make up gas	Nitrogen
DATA	
Data acquisition	Chroma TOF by LECO
Data treatment	GC Image and Excel
Data processing	MetaboAnalyst and Excel
STATISTICAL TREATMENT	
Data normalisation	By median
Data transformation	Log transformation
Data scaling	Mean center scalling
ANOVA test	Excel
PCA	MetaboAnalyst
Heatmap	MetaboAnalyst

Figure S1a: First part of the heatmap of the VOCs present in the five analyzed beer. Each beer is represented by one column which is the average of the replicates (five replicates for cherry; four replicates for raspberry; four replicates for peach; three replicates for apple and four replicates for banana)

*VOCs **in bold** are the compounds of interest and the orange are those developed*

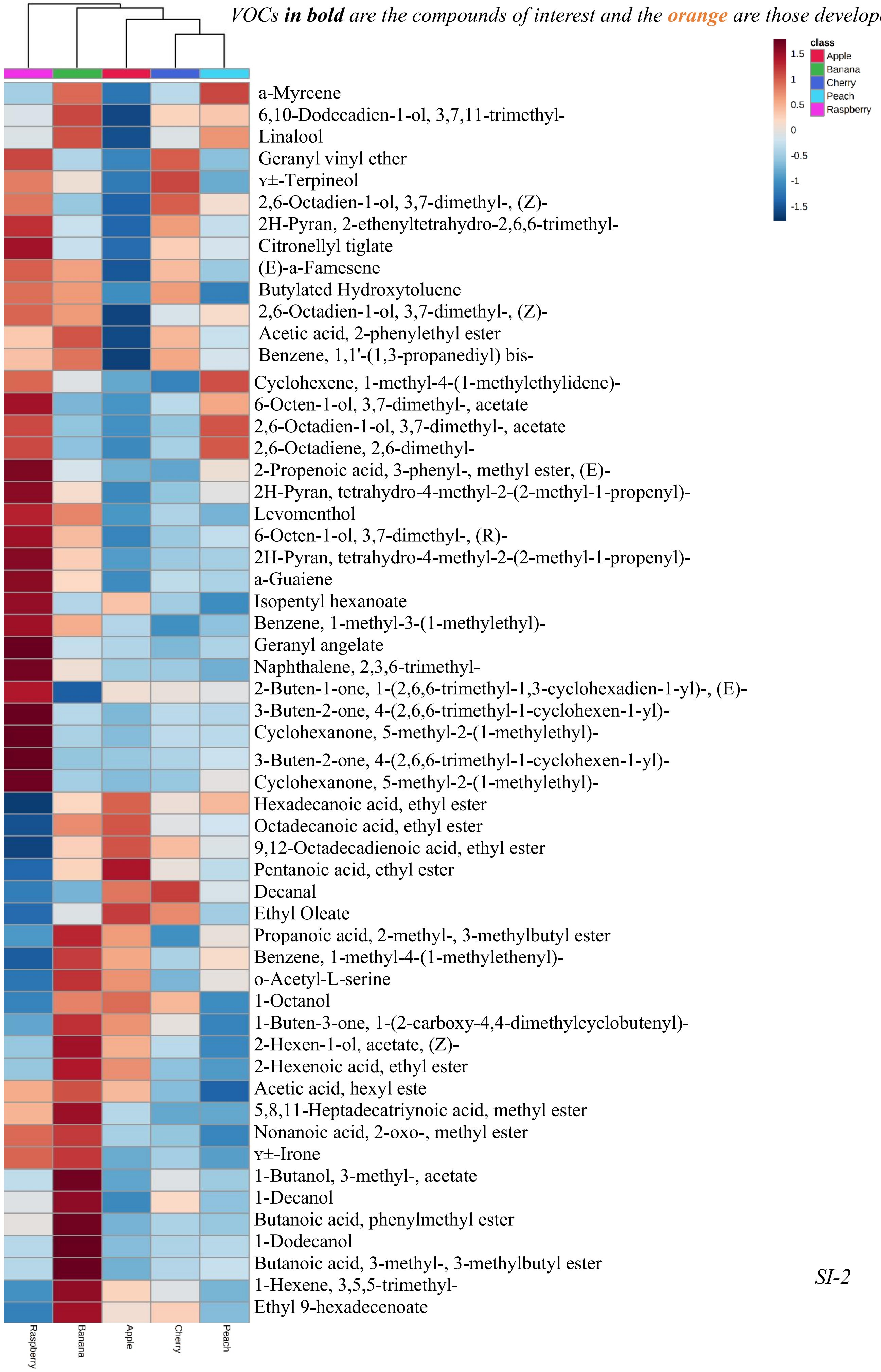

Figure S1b: Second part of the heatmap of the VOCs present in the five analyzed beer. Each beer is represented by one column which is the average of the replicates (five replicates for cherry; four replicates for raspberry; four replicates for peach; three replicates for apple and four replicates for banana)

*VOCs **in bold** are the compounds of interest and the orange are those developed*

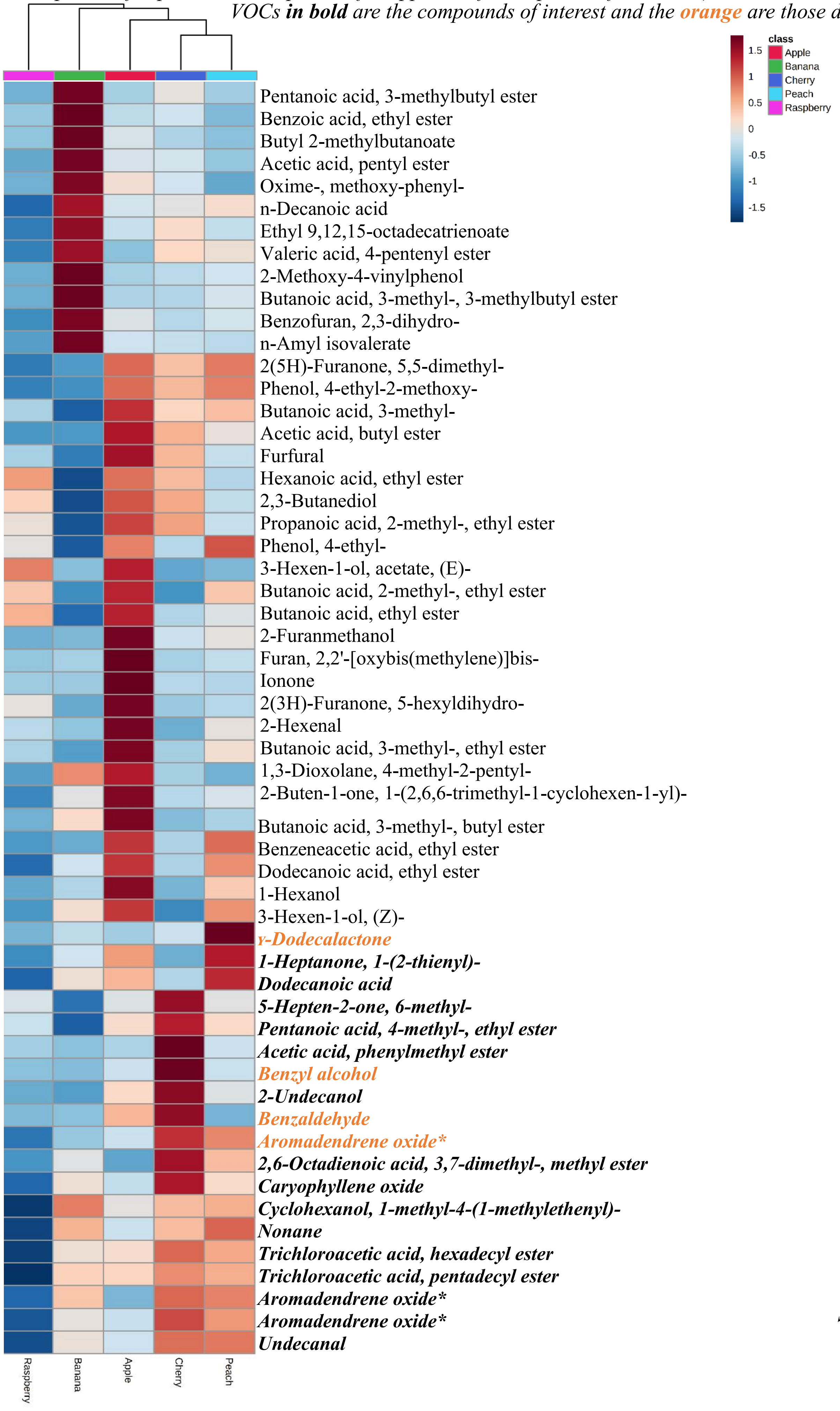

Figure S2: Heatmap of the 19 VOCs of interest. Only the average value of the replicates of each beer are represented (five replicates for cherry; *four replicates for* raspberry; *four replicates for peach; three replicates for apple and four replicates for banana. Compounds* ***in orange*** *are the VOCs developed in the article.*

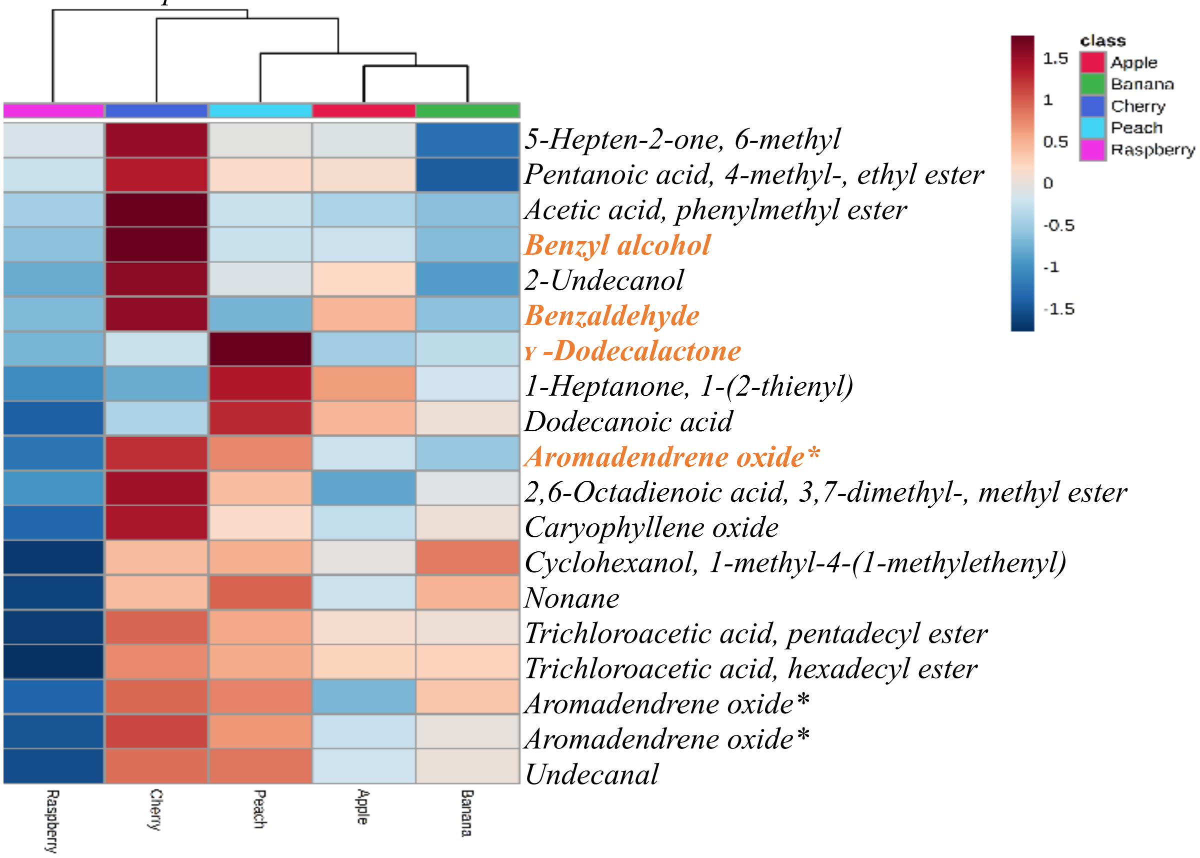

Figure S3: Detailed heatmap of the 19 VOCs of interest with all the replicated. Compounds ***in orange*** *are the VOCs developed in the article.*