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Rapid quantification and comparison of major volatile compounds of ciders from France (Normandy and Brittany) using Microextraction by Packed Sorbent (MEPS)

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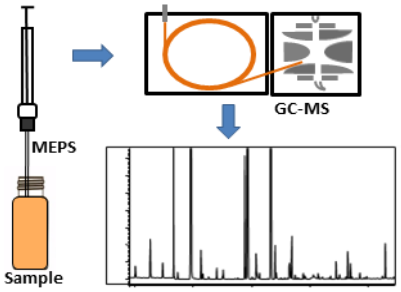
The volatile composition of French ciders from two different regions was statistically compared by using Microextraction by packed sorbent followed by GC-MS and GC-FID analyses.



Abstract

Microextraction by packed sorbent (MEPS) was used for the determination of volatile composition of 29 French ciders samples from two regions: Normandy and Brittany. Extractions using a C18 sorbent were followed by GC-MS analyses for the identification of major volatile organic compounds (VOCs), and GC-FID analyses for the quantification of 19 selected major compounds. The method was found to be rapid and linear up to 300 mg/L for quite all compounds with an average relative standard deviation of 8.5% for tests of repeatability at low concentrations. The limit of detection (LOD) is below 0.1 mg/L except for three VOCs which exhibited much higher concentrations in samples. No significant difference in concentrations of higher alcohols were observed in the cider samples from two regions, however their concentrations were higher in hard ciders than sweet ciders. Acetates were found to be more present in sweet ciders from Normandy than those from Brittany reflecting important differences in the yeasts acting during the fermentation.

Keywords

Cider, MEPS, Volatile compounds, quantification, GC-FID, GC-MS

1. Introduction

Cider is a traditional beverage elaborated mainly in the United Kingdom, France, Ireland and Spain.[1] Ciders produced in Brittany and Normandy are dominating the French market and are representing a whole production of more than 100 million litres each year. These beverages are sold quite exclusively under the labels of protection of geographical indication (IGP): "IGP Cidre de Bretagne" and "IGP Cidre de Normandie" which were introduced in 2000. In these, two particular products obtained the French label "Appellation d'Origine Contrôlée" (AOC) in 1996: "Cidre AOC Pays d'Auge" is produced in a specific region of Normandy and "Cidre AOC Cornouaille" in a specific region of Brittany. French ciders are produced by natural fermentation with indigenous yeasts and bacteria present in the apple and are commercialized as "Cidre Doux" (Sweet Cider) with an ethanolic content which may vary from 2 to 3% and "Cidre Brut" (Hard Cider) with an ethanolic content of more than 3% (typically from 4.5 to 5%).

Volatile compounds of ciders from different origins were widely studied in the past by many authors.[2-9] They showed that the volatile composition of cider is dominated by the presence of higher alcohols such as propan-1-ol, butan-1-ol, isobutan-1-ol and isopentan-1-ols.[4] High concentrations of esters (mainly ethyl lactate and short chain ethyl esters) and aromatic compounds such as 2-phenylethanol and derivated esters, 4-ethylphenol and 4-ethylguaiacol were also recorded.[2] The presence/absence of these compounds and their respective quantities could have a great influence on the organoleptic quality of the product. For instance, high concentrations of volatile phenols give rise to a typical phenolic off-flavor,[10] but on the contrary, high concentrations of ethyl 2-methylbutanoate contribute to a highly "fruity" cider,[2] and 2-phenylethanol is known to bring a "rose-like" aromatic note to beverages.

Gas Chromatography (GC) coupled with a variety of detectors is considered to be the best and most sensitive technique for the analysis of volatile organic compounds (VOCs). However, due to the complexity of the cider matrixes, numerous sample preparation techniques have been developed over the years. Liquid-liquid extraction (LLE) is one of the traditional techniques that is used for the extraction of aroma compounds in beverages before Gas Chromatography-Olfactometry (GC-O), GC-FID and GC-MS analysis. However, this technique can only be employed for the distillates of ciders[11-14] which contain a low amount of macromolecules such as sugars, polyphenols or proteins. The problem of formation of emulsion using LLE may be resolved by using Solid Phase Extraction (SPE) for the determination of VOCs in cider.[3] Due to the opportunity of obtaining

a very concentrated extract after SPE, a lower limit of detection and quantification can be obtained. However, a large quantity of solvent is required and this technique may be rather time-consuming.

Solid-Phase Microextraction (SPME), which was developed by Pawliszyn and co-workers,[15] was successfully applied for the quantification of volatile compounds of fermented beverages. In cider, Pizarro *et al.* [16] studied specifically the volatile phenols using the headspace (HS) of sample. This method was more widely applied to the determination of major volatile compounds of different cider samples.[5,17] The recovery of more polar compounds like acids and alcohols could be increased by using a Solvent-Assisted Flavour Evaporation.[7] Volatile compounds could also be well recovered by using Stir Bar Sorptive Extraction technique before analysis by Gas Chromatography.[18]

Dynamic Headspace Extraction (DHE) was also tested for the quantification of minor volatile compounds and notably esters in ciders using the Purge and Trap method.[8,19] However, this technique which may propose good repeatabilities and give an access to the determination of low concentrated compounds needs a specific apparatus and is rather time-consuming.

Microextraction by Packed sorbent (MEPS) is rather similar to SPE and is relatively handy, cheap, rapid and needs a little amount of solvent. MEPS consists of a syringe of 100-250µL with a needle packed with approximately 1-4 mg of solid sorbent called BIN (Barrel insert and needle assembly). Due to availability of a variety of sorbents (Silica, C2, C8, C18, SAX, SCX), it is potentially applicable for a wide range of compounds in a variety of matrices. A single BIN can be used for more than 200 extractions and each extraction is a double pass of sample through BIN by pulling and pushing of plunger. Nevertheless, it is rather limited to the quantification of major compounds due to the adsorption capacity of the sorbents. It was notably applied in the past few years for the determination of drugs in biological samples,[20-22] Polycyclic Aromatic Hydrocarbons in water,[23] and was also used for the evaluation of compounds such as Ochratoxin A, 2,4,6-Trichloroanisole and 2,4,6-Tribromoanisole in wine.[24] Due to the potentiality of this technique to achieve a rapid quantification of volatile compounds without a sophisticated apparatus, we propose in this work, the validation of a new method of quantification of the major volatile compounds of ciders using MEPS. Extractions of volatiles will be performed on a C18 MEPS sorbent followed by separations and quantifications in GC-FID. This method which is never described before, will be then applied to the characterization of volatile composition of sweet and hard ciders originating from Brittany and Normandy. The concentration of VOCs will be correlated with ethanol content of cider samples. The quantitative data of compounds will also be statically analysed by Partial least

squares discriminant analysis (PLS-DA) and analysis of variance (ANOVA) in order to compare the sweet and hard ciders from two regions.

2. Material and Methods

2.1. Chemicals

Solvents used were dichloromethane (Sigma aldrich, Steinheim, Germany), ethanol (VWR, Fontenay-sous-Bois, France), methanol (Sigma aldrich, Steinheim, Germany) and acetone (Carlo Erba, Val de Reuil, France). They were all of analytical grade. Standards include, 2-methylpropan-1-ol, 2-phenylethyl acetate, benzyl alcohol (Fluka, Buchs, Switzerland), 3-methylbutyl acetate, acetoin, 4-ethylguaiacol (Aldrich, Steinheim, Germany), butan-1-ol, 3-methylbutan-1-ol (Carlo Erba, Val de Reuil, France), heptan-2-one, ethyl hexanoate, ethyl lactate, hexan-1-ol, nonan-2-one, 2-phenylethanol (Acros Organics, Geel, Belgium), pentan-1-ol (Prolabo, Paris, France), hexyl acetate, ethyl octanoate, methionol (Aldrich, Schnelldorf, Germany), 4-ethylphenol (Aldrich, Milwaukee, WI). All standards had a minimum purity of 98% except for 3-methylbutan-1-ol and ethyl lactate (97%). 4-Methylpentan-2-ol (Merck, Schuchardt, Germany) was used as internal standard.

2.2. Samples

Twenty-nine ciders of two different regions of France “Normandy” and “Brittany” with varying alcoholic content were bought from a local market. They were all belonging to French labels “Appellation d’Origine Contrôlée” (AOC): AOC “Cidre Pays d’Auge”, AOC “Cidre de Cornouailles” or “Indications Géographiques Protégées” (IGP): IGP “Cidre de Normandie”, IGP “Cidre de Bretagne”. Fifteen were produced in Normandy with 6 “sweet ciders” (ethanolic content between 2 and 3%) labelled from NS1 to NS6 and 9 “hard ciders” (ethanolic content between 4.5 and 8%) labelled from NH1 to NH9. Fourteen were produced in Brittany with 7 “sweet ciders” (ethanolic content ranging from 2 to 3%) labelled from BS1 to BS7 and 7 “hard ciders” (ethanolic content ranging from 4.5 to 5.5%) labelled from BH1 to BH7.

2.3. Preparation of solutions

50 mL stock solution of the 19 standard VOCs was prepared in ethanol at a concentration of 6 g/L. This stock solution was used to build 50 mL diluted solutions of VOCs with concentrations ranging from 0.018 to 300 mg/L in a water/ethanol (95/5:v/v) medium. Tested concentrations were 0.018, 0.037, 0.073, 0.146, 0.293, 0.586, 1.172, 2.344, 4.688, 9.375, 18.750, 37.500, 75, 150 and 300 mg/L. These solutions were used to check the linearity, limits of detection and quantification of the method. 500 µL of ethanol (volume further used to perform standard additions) and then 50 µL of a solution of 4-methylpentan-2-ol (10g/L in ethanol) were added to each

diluted solutions before extraction using MEPS. Ultra-pure water was used for all dilutions and sample preparations. The stock solution was stored at 4°C in a refrigerator for a maximum of one month.

2.4. Preparation of samples

For each sample, 50 mL cider was taken, added with 500 µL of ethanol and 50 µL of a solution of 4-methylpentan-2-ol (10 g/L in ethanol) and were then extracted using MEPS.

To perform standard additions, microvolumes of the stock solution were diluted to 500 µL with ethanol. They were added to 50 mL of two different samples of hard ciders from Normandy (NH1 and NH8). Added concentrations were 0, 1.172, 2.344, 4.688, 9.375, 18.750, 37.500 and 60 mg/L. 50 µL of a solution of 4-methylpentan-2-ol (10 g/L) were finally added before extraction using MEPS.

2.5. MEPS Operation

A 250 µL gas-tight MEPS syringe and Barrel Insert and Needle assembly (SGE, Ringwood, Australia) was used. The BIN was packed with approximately 4 mg of C18 as a sorbent. The volume of packing was 8 µL with particle size diameter of 45 µm. The sorbent had a pore size of 60Å and was tightened by polyethylene filters from both sides.

Firstly the sorbent was conditioned with 50 µL of of ultra-pure water. After this, the extraction was performed by taking 100 µL of sample through the sorbent by a manual up and down movement of plunger. To increase the recovery of volatile compounds, another 4x100 µL of the sample were taken slowly (about 15 seconds for each). Finally the analytes were eluted with 25 µL of dichloromethane in a 1 mL vial equipped with a 100 µL conical insert. The extracts were stored in a freezer at – 20°C prior to analyses in GC-MS and GC-FID. The BIN was properly cleaned and washed with 5x100 µL of elution solvent followed by 5x100 µL of a methanol/acetone (50/50:v/v) mixture before another utilization. The same sorbent was used for a maximum of 200 extractions.

2.6. Gas chromatographic analyses

A 1µL extract was injected for each sample twice in GC-MS with Electron Impact and Chemical Ionization modes and 1.5 µL duplicates in GC-FID. The split ratios were 1:5 and 1:2 for GC/MS and GC-FID respectively. GC-MS analyses were carried out on Varian 3800 Gas Chromatograph coupled with a Varian™ Saturn 2000R Mass Spectrometer equipped with an ion trap analyzer. Separations were made by using a 50m×0.25mm (I.D.) capillary column coated with a 0.25µm film of BP-20 stationary phase (100% polyethyleneglycol from SGE). Helium was used as a carrier gas with a flow rate of 1 mL/min. Injector

temperature was 240°C. The oven temperature program used was 35–240°C at a rate of 5°C/min, with an initial temperature hold for 10 min and a final temperature hold for 15 min resulting in a total run of about 66 min. MS detection was started after 10 minutes to avoid a large solvent peak. Therefore a lower initial oven temperature (35°C) was used to obtain the analyte peaks after this time. Analyses were made through a transfer line heated at 270°C and detections were performed at 150°C in the Ion Trap both in electron impact (EI with m/z between 40 and 400) and chemical ionization mode (CI with m/z between 65 and 400). Chemical ionization was operated with acetonitrile as reagent.

GC-FID analyses were carried out on a Varian 3900 Gas Chromatograph. Separations were led using a capillary column BP-20 of same dimensions and stationary phase as for GC-MS. Hydrogen was used as a carrier gas with a flow rate of 1 mL/min. Injector temperature was 240°C. The oven temperature program used was 40–240°C at a rate of 5°C/min, with an initial temperature hold for 10 min and a final temperature hold for 15 min resulting in a total run of about 65 min. Temperature of the detector was 250°C. Peak integration was realised using the Saturn WS (version 5.3) or Varian Star softwares.

2.7. Identification of volatile compounds

Peak identifications of the volatile compounds were achieved by comparison of mass spectra with those of the NIST 98MS database and of an in-house database containing more than 300 compounds previously identified in distilled beverages like Calvados and Cognac.[11,14,25] Retention index was calculated for each peak from an injection of alkane mixture (C8 to C32 from Sigma, Saint-Louis, MO, USA) by using the same chromatographic conditions as for cider samples according to the Van Den Dool approach.[26] The calculated retention indices were then compared to those reported in literature. The CI mass spectra were observed to confirm the molecular weight of target compounds. The presence of peaks was also confirmed by injections of pure and synthesized compounds as previously described.[25] Results of peak identifications are shown in **Table 1**. The retention indices were also calculated as explained above for the peaks obtained by GC-FID. These retention indices were compared to those obtained by GC-MS in order to identify the peaks in GC-FID chromatograms.

2.8. Quantification of volatile compounds

Nineteen selected volatiles of ciders were quantified. A relative area was calculated for each of the selected peaks on GC-FID chromatograms by dividing the area of the peaks, attributed to compounds as given above, by that of the internal standard (4-methylpentan-2-ol).

After dilution of the prepared stock solutions in water/ethanol mixture followed by extraction using MEPS, calibration curves for each compound were built by plotting relative area *versus* concentration of standard VOCs in order to check the linearity of the method. Range, slope and correlation coefficient of the curves are given in **Table 2** for the 19 VOCs to be quantified.

Repeatability was determined for ten replicated experiments realized on two different samples of hard ciders from Normandy and Brittany (labelled NH9 and BH6). For each compound it was expressed as the relative standard deviation (RSD). The limits of detection and quantification were determined, using calibration curve of each VOC, by successive dilution of the stock solution until signal to noise ratios of 3 and 9 respectively. Standard additions were also performed into two different samples of hard ciders from Normandy (NH1 and NH8) prepared as given above. The slopes of the curves obtained for cider samples were compared with the ones obtained for the model solutions in water/ethanol mixture.

2.9. Statistical Analyses

One-way ANOVA was used to observer the significant differences between hard and sweet ciders, and ciders from two regions (Normandy and Brittany) on the basis of VOCs. Both ANOVA and correlation studies were performed by Microsoft Excel® spreadsheet. PLS-DA was used to develop models to discriminate samples according to their volatile composition. The objective of PLS-DA is to find a model that separates classes of observations on the basis of their X variables. The X matrix consists of the volatile composition data of the observations. The Y matrix contains dummy variables, which describe the class membership of each observation. PLS-DA finds a discriminant plane in which the projected observations on the components are well separated according to class. The PLS weight plot of composition variables enables an understanding of which variables contribute to the separation. Compounds that are close to the dummy variables of class membership contribute strongly to the separation of classes.[27]

PLS-DA was carried out with SIMCA-P software (UMETRICS). SIMCA software uses the NIPALS algorithm (nonlinear iterative partial least squares) for the PLS regression. The number of components is determined by cross-validation. In this study, all composition variables were centered and scaled to unit variance (UV scaling).

3. Results and Discussion

3.1. Identification of volatile compounds

35 major volatiles belonging to different families of compound (alcohols, carboxylic acids, ketones, esters, hydrocarbons) were identified using MEPS followed by GC-MS analyses in 29 samples of ciders. The compounds were first identified by comparing the Mass spectra of these compounds with mass spectra databases. Identification was further confirmed by the comparison of retention indices with those found in literature. CI-MS spectra helped to determine the molecular weights of the identified compounds. A dilute standard solution of most of the identified compounds was directly injected in GC-MS to confirm the presence of peaks. Finally some of previously synthesized compounds[25] were also injected to confirm the identification. These results are presented in **Table 1** and an example of GC/MS chromatogram obtained from a hard cider (NH1) is given in **Figure 1**. This step enabled to characterize the most important peaks observed in GC-FID analyses of the same samples (examples of chromatograms given in **Figure 2**). GC-FID and GC-MS chromatograms were compared using the calculated retention indices. The presence of the VOCs to be quantified in GC-FID was then confirmed by standard additions of the stock solution in samples.

3.2. Method Performance

The method was first optimized by testing different elution solvents: pentane, diethyl ether and dichloromethane. The best overall recoveries of all volatiles (data not shown) were obtained for dichloromethane. 100 µL of sample was firstly tested followed by an elution with 50 µL of dichloromethane but the concentration of VOCs in the final extract was too low to obtain a sufficient sensibility in GC-FID. The concentrations of the VOCs were gradually increased by taking 2x100, 3x100 and 4x100 µL of the sample in different experiments and then elution by 25 µL of the dichloromethane. Finally a sufficient sensibility was obtained by extracting a whole 500 µL of sample in 5 successive extractions of 100 µL followed by a single elution with 25 µL of dichloromethane. These volumes were then systematically used for the validation of method and for the determination of concentrations in the tested samples.

Linearity of the method was studied in a cider type model solution (water/ethanol (95/5:v/v)) spiked with VOCs at 15 different concentration levels between 0.018 mg/L and 300 mg/L. The linearity of curves obtained with this method was sufficient as compare to others methods[5,19] for envisaging a correct quantification with correlation coefficient varying between 0.9907 and 0.9995 (see **Table 2**). The linear range varies a lot with class of compounds due to differences of adsorption of polar and less polar compounds. Most of the higher

alcohols presented a linear range up to 300 mg/L. In case of esters like ethyl octanoate, the linearity began to be less interesting over a concentration of 37.5 mg/L. Nevertheless, it should be noted that the concentrations further measured in samples were never exceeding 1 mg/L (see **Table 3** and **Table 4**).

The lowest limits of detection (LOD) and limits of quantification (LOQ) (see **Table 2**) were obtained for compounds with the highest values of linearity slopes. These were recorded for the most hydrophobic compounds (e.g. esters, aromatic compounds and ketones) which are better retained by the C18 sorbent. These compounds were detected in samples for concentrations over 20 µg/L and quantified over 40 µg/L. These limits are approximately the same as those found by Rodriguez Madrera *et al.*[19] with a Purge and Trap method. The smallest alcohols like 2-methylpropan-1-ol, butan-1-ol were exhibiting much higher LOD and LOQ. However, samples of cider are rich in 2-methylpropan-1-ol and butan-1-ol (concentrations systematically over 1.76 mg/L) and as a consequence their amount could be given in all samples. In comparison with these two compounds, heavier higher alcohols (pentan-1-ol, 3-methylbutan-1-ol and hexan-1-ol) which are more hydrophobic, are presenting lower LOD and LOQ (LOD≤0.04 mg/L) and the calculated linearity slopes are much higher. The worst LOD and LOQ (2.34 and 4.69 mg/L respectively) were recorded for acetoin. We may think that this compound which is rather hydrophilic, is poorly adsorbed on the C18 fiber of MEPS. Nevertheless, this compound was under the limit of quantification for only 4 samples out of 29 studied ciders. These limits could be further improved by the use of GC-MS which presents higher selectivities and sensibilities than GC-FID as this was performed for the determination of volatile phenols.[16] However, LOD and LOQ were sufficient to follow almost all the selected volatile compounds in every sample and due to its cheapness, GC-FID is still the most common encountered apparatus found in laboratories.

For standard additions two different hard ciders from Normandy (NH1 and NH8) were selected. The cider samples were spiked with stock solution at 8 different concentrations between 0 and 60 mg/L. The obtained curves were compared to those obtained from cider type model solution. The values of calculated slopes in cider type model solution were close to the values found for spiked cider samples (see **Table 2**). Therefore, no significant effect of matrix was observed.

Repeatability of the method was checked for two different hard ciders samples from Normandy and Brittany (samples labelled NH9, BH6). The experiments (from the original sample to the analysis) were repeated 10 times for both ciders. A reasonable repeatability was obtained with an average relative standard deviation (RSD) of 8.5% (see **Table 2**). It should be noted that the two samples contain very low concentrations of a few

selected analytes; this explains why high values of RSD (between 10 and 15%) were observed for benzyl alcohol, ethyl octanoate, heptan-2-one and nonan-2-one. All these compounds were indeed under the limit of quantification in the two samples. The best repeatabilities were recorded for alcohols because these compounds were exhibiting the highest concentrations in samples.

As a conclusion, this method worked quite well for the quantification of a variety of major volatile compounds having different functional groups. This method is fast, economical, reliable, and was applied to the study of 29 samples of French ciders.

3.3. Analysis of cider samples

Apart from highly volatile compounds, such as ethyl acetate and ethanol which were not studied in this work, isopentan-1-ols, acetoin, 2-phenylethanol and ethyl lactate are the main volatile components of French ciders. The 19 quantified compounds could be classified into 3 principal categories: higher alcohols, aromatic compounds, esters and are accompanied with a few miscellaneous compounds such as heptan-2-one, nonan-2-one, acetoin, ethyl lactate and methionol. The selected classes (Hard and sweet ciders from Normandy, Hard and sweet ciders from Brittany, sweet ciders from Normandy and Brittany) were discriminated by PLS-DA according to the concentrations of quantified volatile compounds. Distribution of samples was studied on the first and second component of the statistical model (**Fig.** 3A, 4A, 5A). The contribution of each volatile compound for the discrimination of classes was presented in two dimensions on a PLS-DA loading weight scatter plot. The compounds close to a particular class barycentre have the highest discriminatory power between the classes (**Fig.** 3B, 4B, 5B). One-way ANOVA was also applied on the quantitative data of VOCs in order to observe the considerable differences of volatile compounds with respect to ethanol content (sweet and hard) and region (**Table 5**).

Concentrations found for higher alcohols are directly correlated to the ethanolic content of the samples (see **Table 5**). In the case of the major higher alcohol, isopentanols; sweet ciders (with ethanolic content between 2 and 3%) have an average concentration of about 35 mg/L, whereas hard ciders (with ethanolic content between 4.5 and 5.5%) have an average concentration of about 55 mg/L. Sample NH9 with a concentration of 138 mg/L has the most important content of ethanol (8%), is the only sample to reach 100 mg/L. Such a high concentration of isopentan-1-ols was already observed in ciders with the same ethanolic content[17] and concentrations over 200 mg/L could also be encountered in highly ethanolic ciders[5] and in Spanish sparkling ciders[8]. Average values obtained for 2-methylpropan-1-ol and pentan-1-ol are also generally higher in hard ciders than in sweet ciders.

Thus, majority of higher alcohols greatly contributes to the discrimination of sweet and hard ciders (see **Table 3, 4** and **Fig. 3, 4**). The localization of the projections of butan-1-ol and hexan-1-ol (compounds n° 2 and 5) on the statistical models (**Fig.** 3A, 3B) tends to prove that these two higher alcohols seem not to be specific of either sweet ciders or hard ciders. Similarly one-way ANOVA has shown no significant difference ($P > 0.05$) of these compounds in hard and sweet ciders (see ANOVA 1 in **Table 5**). No major differences were observed for higher alcohols between ciders from Normandy and those from Brittany (see ANOVA 2 & 3in **Table 5**), therefore hard ciders from Normandy and Brittany could not be well discriminated. The appearance of higher alcohols in fermented beverages is thought to be directly linked to the action of yeasts on either sugars or amino-acids[28] and consequently directly linked to the presence of ethanol; this was verified in this work for ciders from Normandy and Brittany.

The overall quantity of the aromatic compounds is also generally more significant in hard ciders than in sweet ciders (see ANOVA 1 in **Table 5**). However, big differences may be found between samples even if they present a similar ethanolic content. In these, 2-phenylethanol exhibits the highest concentrations and is known to be produced by the metabolism of yeast which can degrade phenylalanine.[28] As a consequence, the formation of 2-phenylethanol is dependent not only on ethanolic content but also on the availability of aminoacids. This can explain why for example two samples of sweet ciders from Normandy with the same alcoholic proof have a concentration of 7.6 mg/L for one sample (NS4) and 51.7 mg/L for the other one (NS5). 2-Phenylethylacetate is supposed to be issued from 2-phenylethanol in fermented beverages.[29] However, yeasts may have really different abilities to convert alcohols into acetates. Xu *et al.*[30] showed, for instance, that an alcoholic fermentation performed with *Hanseniaspora valbyensis* could better produce 2-phenylethyl acetate than one performed with *Saccharomyces cerevisiae*. Moreover, the concentrations of this compound could be higher in sweet ciders than in hard ciders from Normandy (**Fig.** 3B, compound n° 7), this is possibly due to esterase activities of yeasts. Indeed, decreases of acetates were already observed in ciders during the step of alcoholic fermentation.[8] Anyhow this compound is not significantly different in sweet and hard ciders (see ANOVA 1in **Table 5**).

The presence of volatile phenols may be an important problem for the organoleptic quality of ciders and more generally of fermented beverages. They may give an “animal” or “spicy” aromatic note to cider derived products.[14] In a previous work, we develop a specific HPLC with a Diode Array Detection (HPLC-DAD) method for the determination of some volatile phenols in ciders.[10] A part of the selected samples were also from Normandy and Brittany (samples different from this work), and the range of concentration found with MEPS-

GC-FID for 4-ethylphenol and 4-ethylguaiacol was convenient with that found with HPLC-DAD. Spoilage of ciders *via* the production of volatile phenols in high concentrations seems to be linked to the presence of either yeasts belonging to the genus *Brettanomyces* or lactic acid bacteria like *Lactobacillus collinoides*[31] along with ethanol content of ciders (see ANOVA 1 and correlation in table 5). As a consequence, volatile phenols may be present in high proportions either in sweet ciders (cider labelled BS7 is containing 5.05 mg/L of 4-ethylphenol and 0.87 mg/L of 4-ethylguaiacol) or in hard ciders (cider labelled NH9 is containing 6.04 mg/L of 4-ethylphenol and 0.77 mg/L of 4-ethylguaiacol). It should also be noted that highest concentrations of 4-ethylphenol (over 5 mg/L) are systematically accompanied by high amounts of 4-ethylguaiacol (over 0.5 mg/L).

Low concentrations of benzyl alcohol (under 1 mg/L) were recorded in the tested samples. However, sample NH1 (hard cider from Normandy) was really different from the other ones (**Fig.** 3A) and exhibits a concentration of benzyl alcohol of 3.91 mg/L. This sample was also the one containing the highest content of 4-ethylphenol (6.25 mg/L) and we may think that this sample was spoiled during the process by undesired microorganisms.

Esters are known to bring interesting "fruity" aromatic notes to ciders even at low concentrations.[7] In these isoamyl acetate (3-methylbutyl acetate) is researched because of its "banana, pear" odor-like. It is considered to be produced by yeasts from the corresponding alcohol (3-methylbutan-1-ol) using an acetyltransferase.[29] However, its formation will depend upon the type of yeast leading the alcoholic fermentation. Big differences for this compound were recorded in the studied samples. Highest concentrations of isoamyl acetate were surprisingly found for sweet ciders produced in Normandy (concentrations systematically over 0.18 mg/L). On the contrary, sweet ciders produced in Brittany were never containing more than 0.16 mg/L (sample BS4). This reflects that the yeasts involved in the alcoholic fermentations of ciders of Normandy and Brittany should be rather different. This characteristic is also found for hexyl acetate which also presents highest concentrations in sweet ciders from Normandy. Therefore the projections of these compounds were located close to the center of sweet ciders from Normandy class in the PLS-DA plot (see **Table 4** and **Fig. 5B**). The concentration of hexyl acetate is negatively correlated to the ethanol content of cider samples. The much higher quantity of this compound in sweet ciders from Normandy can be attributed to esterase activities of yeasts.

The level of ethyl octanoate (around 0.3 mg/L in sweet ciders and 0.35 mg/L in hard ciders) is generally over that of ethyl hexanoate (around 0.07 mg/L in sweet ciders and 0.10 mg/L in hard ciders). However, no clear

difference could be established between ciders from Normandy and those from Brittany. Moreover, the extent of the alcoholic fermentation seems not to have a great influence on their concentration.

The concentrations of heptan-2-one and nonan-2-one in ciders are generally very low and consequently nonan-2-one could not be quantified in many samples. However, it should be noted that sample NH1 was really different from the others with concentrations of these two ketones which were at least 4 fold that of the others (1.51 mg/L for heptan-2-one and 0.37 mg/L for nonan-2-one) and that the highest quantities were observed in ciders from Normandy (samples NS6, NH1, NH2, NH4).

Acetoin can be formed in fermented beverages by lactic acid bacteria and yeasts. However, high concentrations of this compound at the early stage of the alcoholic fermentation may be attributed to the action of low fermentative yeasts belonging to the genus *Kloeckera* or *Hanseniaspora*.[32] Great differences were observed between samples and the highest levels (84.9 and 50.7 mg/L) were recorded for two ciders of Normandy possessing the lowest ethanolic content (samples NS1 and NS2). Moreover, sweet ciders from Normandy have generally high concentrations of acetoin (negative correlation with ethanol) except for the sample NS5 which is particular due to its high concentrations of isopentan-1-ols and 2-phenylethanol (highest concentrations in sweet ciders). This may be due to a difference in the yeasts involved in the fermentation process; indeed, *Saccharomyces* strains are able to produce large amounts of higher alcohols and can metabolize acetoin produced by apiculate yeasts.[32] Sweets ciders from Brittany have significant lower content of acetoin than those from Normandy (table 4). In this selection, the sample labelled BS4 seems to be different from the others with a high content of acetoin (38.1 mg/L) but also with quite high concentrations of 3-methylbutyl acetate and hexyl acetate. The concentrations of acetoin could be very different in each hard cider; it is however noticeable that this compound could not be detected in the sample containing the highest ethanolic content (8% for sample NH9) and that it should have been completely metabolized during the process.

Ethyl lactate is one of the major volatile compounds that can be formed during the elaboration of cider. It is formed from ethanol and lactic acid, acid which may be produced from many substrates and by many microorganisms. Yeasts are able to transform pyruvic acid obtained from the degradation of sugars into D-lactic acid or L-lactic acid.[33] Lactic acid bacteria may also metabolize sugars in glycerol which can undergo a production of lactic acid.[34] Nevertheless, the major part of lactic acid present in ciders seems to be issued from the degradation of malic acid by the action of lactic acid bacteria.[35] As a consequence, the level of ethyl lactate is thought to be due to the extent of malolactic conversion in ciders which may be simultaneous or sequential

towards alcoholic fermentation (strong positive correlation).[36] Sweet ciders from Normandy exhibit very low concentrations of ethyl lactate (from 3.99 mg/L to 18.42 mg/L) while sweet ciders of Brittany have an average concentrations of ethyl lactate about 26 mg/L (see **Table 4** and **Fig. 5**). As a conclusion, malolactic conversion seems to take place in general more rapidly in ciders from Brittany. This behaviour is however not systematic in all ciders from this region as some samples may contain low quantities of ethyl lactate (2.18 mg/L for sample BS2). Average concentrations of ethyl lactate are quite the same for hard ciders (50 to 60 mg/L) but this value is masking huge differences between samples. For instance, samples NH2, NH3, NH6, BH2, BH3 and BH4 have concentrations of ethyl lactate under 25 mg/L; values which tend to prove that malolactic conversion was not accomplished or not achieved for these samples. Malolactic conversion which is important to obtain a wine with interesting organoleptic characteristics is not systematically obtained for ciders.[35] Ethyl lactate may have concentrations around 100 mg/L in a few samples of ciders from Normandy and Brittany (Samples NH1, NH9, BH5).

The last quantified compound in ciders was methionol. This compound is issued from the degradation of methionine by the action of yeasts.[28] The maximum concentration for this compound was 3.8 mg/L and this was found in the sample containing the most important level of ethanol. Methionol has a concentration between 1 mg/L and 2.5 mg/L in quite all hard ciders (13 samples out of 15) whereas the concentration of 1 mg/L was reached only for 7 samples out of 13 for sweet ciders. Thus, the quantity of methionol could depend upon the extent of the alcoholic fermentation (see **Table 5**) and availability of methionine for yeasts which also justify the significant difference of this compound in sweet ciders from two regions.

4. Conclusion

In this study, a quantification method was developed using MEPS with a C18 sorbent followed by GC-FID analyses for the determination of volatile organic compounds (VOCs) of ciders from Normandy and Brittany. The method was found to be quick and efficient for the quantification of a variety of major VOCs with a good linearity. A better LOD was observed for hydrophobic compounds than hydrophilic; however most of higher alcohols were quantified with a good repeatability due to their higher concentrations in ciders.

Hard ciders have generally higher concentrations of higher alcohols than sweet ciders however no significant difference was observed among the ciders samples from two regions. Sweet ciders from Normandy had higher concentrations of esters than sweet ciders of Brittany. The concentrations of aromatic compounds were generally

higher in hard ciders however different quantities were observed due to the involvement of multiple factors including extent of fermentation, type of yeast and substrate.

Abbreviations used

MEPS, microextraction by packed sorbent; VOCs, volatile organic compounds; LOD, limit of detection; LOQ, limit of quantification; PLS-DA, Partial least squares discriminant analysis; ANOVA, analysis of variance.

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Table 1 Major compounds detected in GC-MS analyses of MEPS extracts of cider with retention indices calculated on a 50m×0.25mm× 0.25µm BP-20 stationary phase.

		Retention index			
Entry	**Compound**	**calculated**	**From literature**	**EI major peaks**[a]	**CI major peaks**
1	2-Methylpropan-1-ol	1096	1099[b]	41, 43 (54), 57 (29)	83[c], 98[c] ,82[c]
2	3-Methylbutyl acetate	1125	1127[b]	43, 55 (59), 70 (56)	71, 131, 130
3	Butan-1-ol	1150	1151[b]	41, 56 (82), 43 (31)	98[c], 83[c]
IS	4-Methylpentan-2-ol	1172		45, 69 (48), 85 (39)	85
4	Heptan-2-one	1181	1192[d]	43, 58 (48), 71 (18)	115
5	Isopentan-1-ols	1212	1214[b]	41, 56 (64), 70 (50)	71
6	Ethyl hexanoate	1235	1239[b]	88, 43 (78), 55 (53)	145
7	1,2,4-Trimethyl benzene	1240		105, 120 (57), 77 (16)	121
8	Pentan-1-ol	1255	1255[b]	41, 55 (99), 70 (42)	71
9	Hexyl acetate	1275	1278[b]	43, 56 (54), 69 (34)	85, 145
10	Acetoin	1291	1292[b]	45, 43 (70), 88 (5)	89
11	Ethyl lactate	1351	1351[b]	45, 91 (11)	119
12	Hexan-1-ol	1357	1357[b]	56, 41 (83), 69 (54)	85
13	Nonan-2-one	1391	1386[e]	43, 58 (72), 71 (20)	143
14	Unknown (terpenic structure)	1426		69, 43 (77), 111 (57)	173, 129
15	Ethyl octanoate	1438	1440[b]	88, 55 (84), 101 (57)	173
16	Acetic acid	1481	1475[b]	43, 45 (82), 60 (33)	84[c], 83[c]
17	Unknown	1490		101, 57 (46), 45 (43)	171, 127, 109
18	2-Methylpropanoic acid	1593	1590[b]	41, 73 (94), 43 (90)	71
19	Ethyl decanoate	1642	1644[b]	88, 55 (55), 73 (52)	201
20	Butanoic acid	1654	1651[b]	60, 42 (57), 73 (41)	71
21	Diethyl succinate	1684	1684[b]	101, 129 (65), 55 (26)	129, 175
22	2-Methylbutanoic acid	1694	1691[b]	74, 41 (70), 60 (49)	85
23	Methionol (3-methylthiopropanol)	1729	1742[f]	106, 45 (62), 57 (56)	89, 107
24	2-Phenylethyl acetate	1828	1830[b]	104, 43 (37), 91 (20)	105
25	Hexanoic acid	1869	1867[b]	60, 73 (55), 42 (46)	99
26	Benzyl alcohol	1892	1890[b]	79, 108 (55), 51 (32)	91
27	2-Phenylethanol	1927	1925[b]	91, 122 (49), 65 (42)	105
28	Ethyl 3-hydroxyoctenoate	1955	1953[b]	117, 71 (84), 43 (78)	169, 187
29	4-Ethylguaiacol	2047	2046[b]	137, 152 (45), 122 (15)	153
30	Unknown	2064		43, 57 (53), 88 (23)	111, 171
31	Octanoic acid	2083	2079[b]	60, 73 (71), 41 (69)	127
32	Unknown	2146		57, 75 (69), 43 (57)	147, 111, 129
33	Unknown	2176		57, 75 (90), 55 (89)	83, 109, 127
34	4-Ethylphenol	2195	2191[b]	107, 122 (43), 77 (27)	123
35	Decanoic acid	2295	2292[b]	129, 73 (84), 60 (77)	155

[a] EI major peaks with the base peak underlined and other major peaks with their respective intensity in brackets, [b] reference from [25], [c] Peaks corresponding to acetonitrile adducts formed in ion trap, [d] reference from [37], [e] reference from [38], [f] reference from [39]

Table 2 Method performance in term of linear range, slopes of standard curves, LOD & LOQ and repeatability.

Entry	Compound	Linearity slopes					Limits		Repeatability	
		Range mg/L	Model Slope (m)	R^2	Cider A[a] Slop (m)	Cider B[a] Slop (m)	Detection mg/L	Quantification mg/L	Concentration mg/L	RSD %
	Higher alcohols									
1	2-Methylpropan-1-ol	1.76 - 300	0.021	0.9977	0.024	0.020	0.88	1.76	29.40	7.1
2	Butan-1-ol	1.76 - 300	0.026	0.9995	0.027	0.024	0.59	1.76	11.79	5.1
3	Pentan-1-ol	0.15 - 300	0.081	0.9992	0.085	0.074	0.04	0.15	0.34	8.0
4	3-Methylbutan-1-ol	0.29 - 300	0.092	0.9994	0.098	0.100	0.04	0.29	43.11	1.9
5	Hexan-1-ol	0.04 - 150	0.237	0.9991	0.212	0.229	0.02	0.04	4.61	5.2
	Aromatic compounds									
6	2-Phenylethanol	0.04 - 150	0.169	0.9984	0.182	0.141	0.02	0.04	17.59	8.2
7	2-Phenylethyl acetate	0.04 - 150	0.267	0.9981	0.259	0.306	0.02	0.04	0.49	8.7
8	4-Ethylguaiacol	0.04 - 150	0.245	0.9975	0.278	0.276	0.02	0.04	0.13	7.3
9	4-Ethylphenol	0.04 - 150	0.347	0.9985	0.374	0.352	0.02	0.04	0.72	7.2
10	Benzyl alcohol	0.15 - 150	0.116	0.9981	0.115	0.094	0.04	0.15	0.12[b]	11.3
	Esters									
11	3-Methylbutyl acetate	0.15 - 150	0.149	0.9995	0.149	0.144	0.04	0.15	0.16	10.3
12	Ethyl hexanoate	0.04 - 150	0.223	0.9907	0.215	0.198	0.02	0.04	0.10	7.5
13	Hexyl acetate	0.04 - 75	0.231	0.9963	0.226	0.203	0.02	0.04	0.01[c]	5.7
14	Ethyl octanoate	0.04 - 37.5	0.243	0.9974	0.266	0.224	0.02	0.04	0.03[b]	14.0
	Miscellaneous									
15	Heptan-2-one	0.04 - 300	0.231	0.9980	0.207	0.220	0.02	0.04	0.02[b]	15.9
16	Nonan-2-one	0.04 - 150	0.405	0.9980	0.419	0.394	0.02	0.04	0.01[c]	14.3
17	Acetoin	4.69 - 300	0.002	0.9921	0.002	0.002	2.34	4.69	39.75	7.8
18	Ethyl lactate	0.29 - 300	0.007	0.9987	0.005	0.006	0.07	0.29	63.84	5.4
19	Methionol	0.59 - 150	0.013	0.9984	0.012	0.014	0.07	0.59	2.42	11.2

[a] linearity slopes (relative area x L/mg) obtained with standards additions in cider A (NH1) and cider B (NH8), [b] below the limit of quantification, [c] below the limit of detection

Table 3 Volatile composition of hard ciders from Normandy and Brittany (concentrations in mg/L).

		Hard ciders from Normandy											Hard ciders from Brittany						
	Sample	NH1	NH2	NH3	NH4	NH5	NH6	NH7	NH8	NH9	NH	BH	BH1	BH2	BH3	BH4	BH5	BH6	BH7
	% Ethanolic content[a]	4.5	4.5	4.5	5	5	5	5	5	8	mean value	mean value	4.5	4.5	4.5	5	5.5	5.5	5.5
Entry	Compound																		
	Higher alcohols																		
1	2-Methylpropan-1-ol	8.98	6.82	17.67	11.07	13.85	9.64	10.90	10.40	29.40	13.19	17.62	12.97	9.80	11.40	27.06	17.19	14.89	30.05
2	Butan-1-ol	7.12	5.13	8.21	4.93	2.67	5.46	2.50	5.35	11.79	5.91	5.66	6.03	3.72	3.46	7.61	7.85	7.89	3.07
3	Pentan-1-ol	0.37	0.47	0.20	0.65	*0.10*	0.28	0.20	0.16	0.34	0.31	0.18	*0.12*	*0.13*	0.21	*0.12*	0.38	0.19	*0.10*
4	Isopentan-1-ols	61.63	62.67	50.08	43.68	43.16	41.19	47.34	62.54	138.30	61.18	58.10	66.83	58.07	55.02	54.61	48.90	43.11	80.13
5	Hexan-1-ol	2.72	2.74	3.71	2.62	3.13	3.79	2.43	3.81	7.98	3.66	3.54	4.10	2.79	3.10	3.73	3.65	4.61	2.82
	Aromatic compounds																		
6	2-Phenylethanol	44.75	55.56	11.38	39.54	8.25	13.99	24.05	48.25	53.50	33.25	21.32	23.58	32.66	26.14	13.10	16.18	17.59	20.01
7	2-Phenylethyl acetate	0.15	0.51	0.23	0.21	0.19	0.25	0.32	0.76	0.11	0.30	0.41	0.09	1.34	0.09	0.48	0.23	0.49	0.16
8	4-Ethylguaiacol	0.60	0.16	0.95	0.37	0.06	0.94	0.04	0.11	0.77	0.44	0.53	0.76	0.22	0.98	0.69	0.78	0.13	0.13
9	4-Ethylphenol	6.25	0.19	3.94	1.83	0.61	3.15	0.46	0.82	6.04	2.59	1.83	1.30	0.81	2.25	4.21	2.56	0.72	0.94
10	Benzyl alcohol	3.91	< LOD	*0.10*	0.36	0.23	*0.08*	*0.13*	0.23	0.97	0.67	0.13	0.22	< LOD	0.25	*0.10*	*0.08*	*0.12*	*0.11*
	Esters																		
11	3-Methylbutyl acetate	*0.12*	0.21	*0.12*	< LOD	0.22	< LOD	0.38	0.26	0.25	0.17	0.15	0.16	0.21	< LOD	0.19	< LOD	0.16	0.33
12	Ethyl hexanoate	0.04	0.21	*0.03*	0.12	0.04	0.09	0.10	0.15	0.10	0.10	0.11	0.13	0.10	0.04	0.10	0.07	0.14	0.17
13	Hexyl acetate	0.13	0.08	0.04	0.12	0.13	0.08	0.16	0.11	< LOD	0.09	0.03	< LOD	0.09	< LOD	*0.03*	0.04	0.04	0.04
14	Ethyl octanoate	0.20	0.51	0.48	0.92	0.08	0.35	0.27	0.46	0.06	0.37	0.31	0.40	0.50	0.17	0.32	0.59	*0.03*	0.14
	Miscellaneous																		
15	Heptan-2-one	1.51	0.30	0.30	0.45	0.08	0.16	0.09	0.16	*0.02*	0.34	0.14	0.15	0.26	0.06	0.20	0.29	< LOD	0.05
16	Nonan-2-one	0.37	0.16	< LOD	< LOD	< LOD	*0.02*	< LOD	< LOD	< LOD	0.06	0.01	< LOD	< LOD	*0.02*	< LOD	*0.03*	< LOD	< LOD
17	Acetoin	25.79	17.06	7.15	13.10	16.34	*3.36*	25.09	21.94	< LOD	14.42	13.78	15.91	5.48	*2.84*	5.30	*3.17*	39.75	23.99
18	Ethyl lactate	101.48	9.20	12.29	46.23	83.68	19.93	29.46	92.85	128.04	58.13	50.52	82.62	17.08	20.08	11.89	99.51	63.84	58.63
19	Methionol	1.26	1.07	1.35	1.84	*0.51*	1.57	*0.57*	1.21	3.80	1.46	1.75	2.02	1.28	1.21	1.95	2.26	2.42	1.15

In italics: calculated concentrations between the LOD and LOQ, [a] Ethanolic content provided by the producers of cider

Table 4 Volatile composition of sweet ciders from Normandy and Brittany (concentrations in mg/L).

		Sweet ciders from Normandy								Sweet ciders from Brittany						
	Sample	NS1	NS2	NS3	NS4	NS5	NS6	NS	BS	BS1	BS2	BS3	BS4	BS5	BS6	BS7
	% Ethanolic content[a]	2	2.5	2.5	2.5	2.5	3	mean value	mean value	2	2	2	2	2.5	2.5	3
Entry	Compound															
	Higher alcohols															
1	2-Methylpropan-1-ol	8.19	6.47	6.18	6.67	12.84	12.53	8.81	8.68	5.09	4.58	6.89	8.73	16.20	10.31	8.97
2	Butan-1-ol	4.71	4.74	2.65	4.63	4.18	8.31	4.87	5.72	5.74	3.41	6.81	4.61	5.30	4.59	9.57
3	Pentan-1-ol	*0.10*	0.35	< LOD	< LOD	< LOD	0.18	0.11	0.12	0.17	*0.14*	0.17	< LOD	*0.13*	< LOD	0.26
4	Isopentan-1-ols	29.87	27.76	31.15	29.41	68.69	39.37	37.71	32.91	20.89	13.09	34.86	41.15	52.43	40.95	26.98
5	Hexan-1-ol	3.38	2.58	2.25	3.30	1.85	3.16	2.75	3.11	2.57	2.03	2.80	3.80	3.07	2.74	4.74
	Aromatic compounds															
6	2-Phenylethanol	7.81	22.71	16.80	7.59	51.75	10.41	19.51	9.84	8.13	4.87	12.07	6.46	15.24	16.21	5.91
7	2-Phenylethyl acetate	0.15	0.37	0.37	0.15	0.74	0.74	0.42	0.18	0.06	0.15	0.19	0.08	0.07	0.58	0.13
8	4-Ethylguaiacol	0.24	0.25	< LOD	0.19	0.24	0.13	0.18	0.24	0.14	0.17	0.16	0.12	0.11	0.10	0.87
9	4-Ethylphenol	0.19	*0.02*	0.13	0.23	0.30	0.70	0.26	1.10	0.34	0.06	1.13	0.41	0.44	0.30	5.05
10	Benzyl alcohol	*0.07*	< LOD	< LOD	0.15	0.18	0.20	0.10	0.05	*0.06*	*0.08*	< LOD	*0.07*	*0.08*	*0.05*	< LOD
	Esters															
11	3-Methylbutyl acetate	0.21	0.29	0.18	0.22	0.55	0.20	0.27	0.07	*0.10*	*0.12*	< LOD	0.16	< LOD	*0.10*	< LOD
12	Ethyl hexanoate	0.07	0.06	0.10	0.13	*0.03*	*0.03*	0.07	0.07	0.08	0.05	0.04	0.17	0.05	0.06	0.06
13	Hexyl acetate	0.28	0.23	0.18	0.35	0.13	0.04	0.20	0.08	0.15	0.13	0.03	0.15	0.04	0.07	< LOD
14	Ethyl octanoate	0.29	0.18	0.15	0.36	0.20	0.69	0.31	0.29	0.41	0.42	0.32	0.10	0.26	0.18	0.33
	Miscellaneous															
15	Heptan-2-one	0.16	0.17	0.13	0.21	0.15	0.53	0.23	0.17	0.25	0.29	0.13	0.04	0.16	0.14	0.15
16	Nonan-2-one	< LOD	< LOD	< LOD	< LOD	*0.02*	< LOD	0.00	0.00	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
17	Acetoin	50.69	84.92	35.67	23.83	8.34	32.59	39.34	12.03	11.48	6.45	*4.30*	38.06	11.12	7.77	5.06
18	Ethyl lactate	8.77	6.37	18.42	7.75	3.99	6.47	8.63	26.14	17.24	2.18	11.12	24.03	63.40	25.07	39.91
19	Methionol	< LOD	0.71	0.66	< LOD	1.42	1.22	0.67	1.23	1.09	1.63	1.14	0.89	1.63	0.97	1.27

In italics: calculated concentrations between the LOD and LOQ, [a] Ethanolic content provided by the producers of cider

Table 5 Summary of one-way ANOVA applied on volatile compounds present in ciders from two regions, along with correlation of these VOCs with ethanol content of cider samples

	Compounds	ANOVA 1[a] Hard with sweet ciders		ANOVA 2[b] Hard ciders		ANOVA 3[c] Sweet ciders		Correlation with ethanol
		F value	*P value*	*F value*	*P value*	*F value*	*P value*	
	Higher alcohols							
1	2-Methylpropan-1-ol	8.22	0.01[d]	1.45	0.25	0.00	0.95	0.68
2	Butan-1-ol	0.31	0.58	0.04	0.85	0.62	0.45	0.34
3	Pentan-1-ol	6.96	0.01[d]	3.13	0.10	0.09	0.77	0.46
4	Isopentan-1-ols	11.12	0.00[d]	0.06	0.80	0.35	0.56	0.74
5	Hexan-1-ol	2.56	0.12	0.03	0.87	0.66	0.44	0.58
	Aromatic compounds							
6	2-Phenylethanol	6.59	0.02[d]	2.51	0.14	2.15	0.17	0.48
7	2-Phenylethyl acetate	0.30	0.59	0.42	0.53	3.68	0.08	0.06
8	4-Ethylguaiacol	5.81	0.02[d]	0.20	0.66	0.28	0.61	0.43
9	4-Ethylphenol	5.83	0.02[d]	0.58	0.46	1.32	0.27	0.53
10	Benzyl alcohol	1.81	0.19	1.29	0.27	2.00	0.19	0.23
	Esters							
11	3-Methylbutyl acetate	0.00	0.99	0.14	0.71	12.13	0.01[d]	0.07
12	Ethyl hexanoate	3.03	0.09	0.12	0.73	0.01	0.91	0.29
13	Hexyl acetate	5.39	0.03[d]	7.79	0.01[d]	6.15	0.03[d]	-0.46
14	Ethyl octanoate	0.33	0.57	0.27	0.61	0.07	0.80	-0.01
	Miscellaneous							
15	Heptan-2-one	0.36	0.55	1.22	0.29	0.80	0.39	0.00
16	Nonan-2-one	1.76	0.20	1.23	0.29	1.18	0.30	0.12
17	Acetoin	2.49	0.13	0.01	0.91	6.16	0.03[d]	-0.29
18	Ethyl lactate	9.84	0.00[d]	0.14	0.71	4.21	0.06	0.67
19	Methionol	5.76	0.02[d]	0.50	0.49	4.91	0.05[d]	0.63

[a]ANOVA 1: Comparison of hard ciders from Normandy and Brittany with sweet ciders from both regions (Significance based on ethanol content); [b]ANOVA 2: Comparison of hard ciders from Normandy with hard ciders from Brittany (Significance based on region); [c]ANOVA 3: Comparison of sweet ciders from Normandy with sweet ciders from Brittany (Significance based on region); [d]Significant difference, $p \leq 0.05$

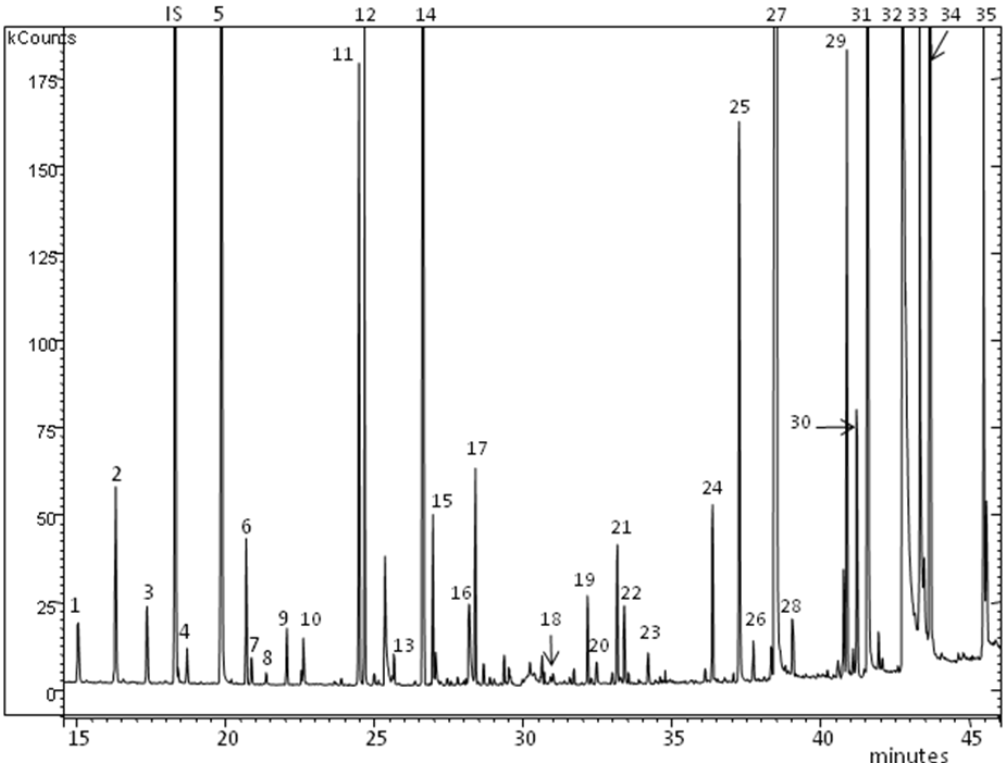


Fig. 1 GC/MS Chromatogram of one MEPS extract of hard cider from Normandy (NH1) showing major peaks for volatile compounds mentioned in **Table 1**. Separation on a 50m×0.25mm×0.25µm BP-20 stationary phase.

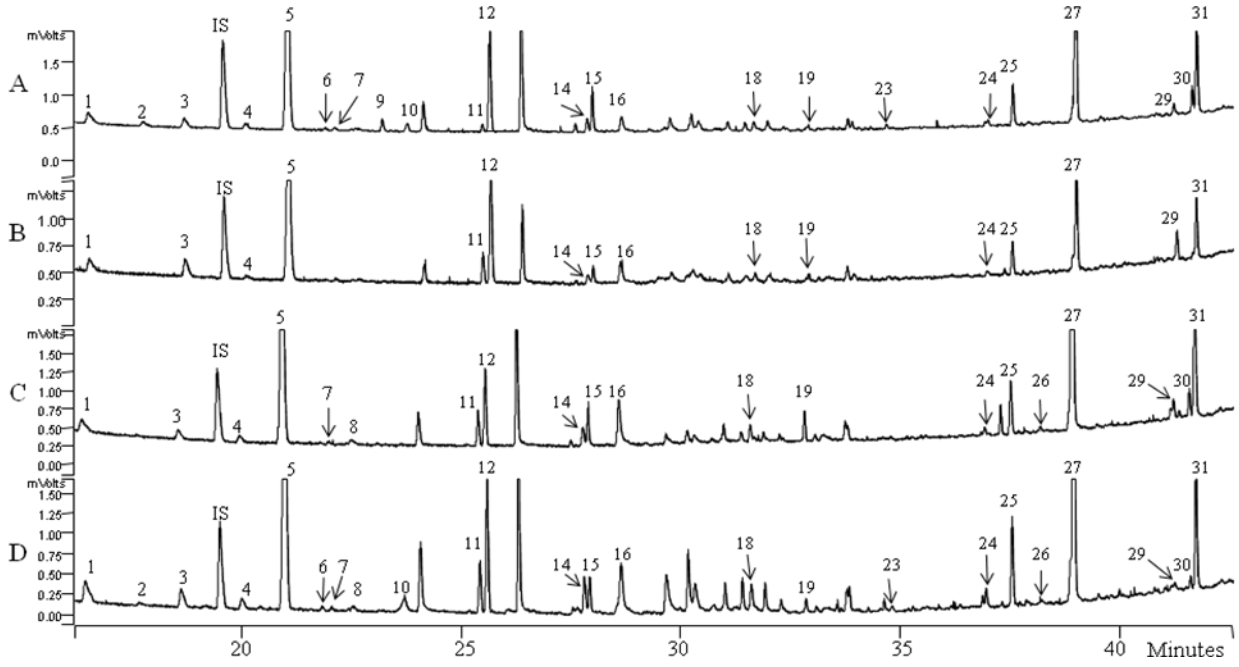
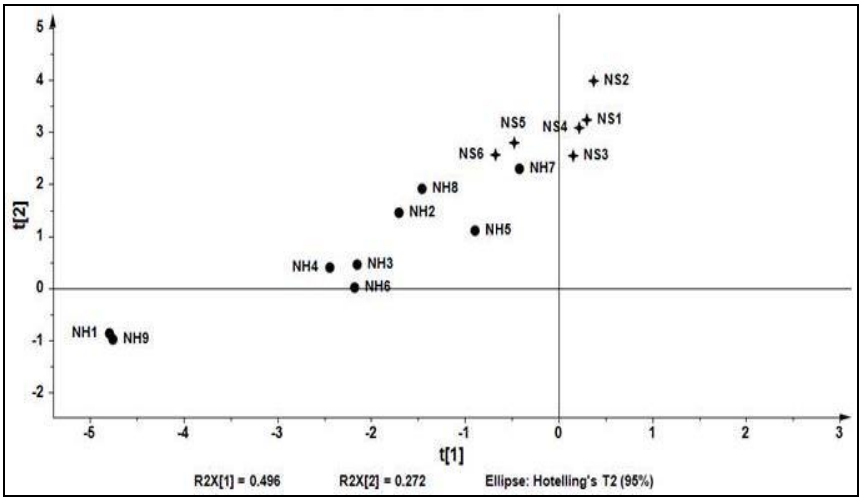


Fig. 2 GC/FID Chromatograms of MEPS extracts of sweet and hard ciders from Normandy and Brittany showing major peaks for volatile compounds mentioned in **Table 1.** Whereas A, B, C and D corresponds to samples NS4, BS7, NH4 and BH6 respectively. Separation on a 50m×0.25mm×0.25µm BP-20 stationary phase.

A



B

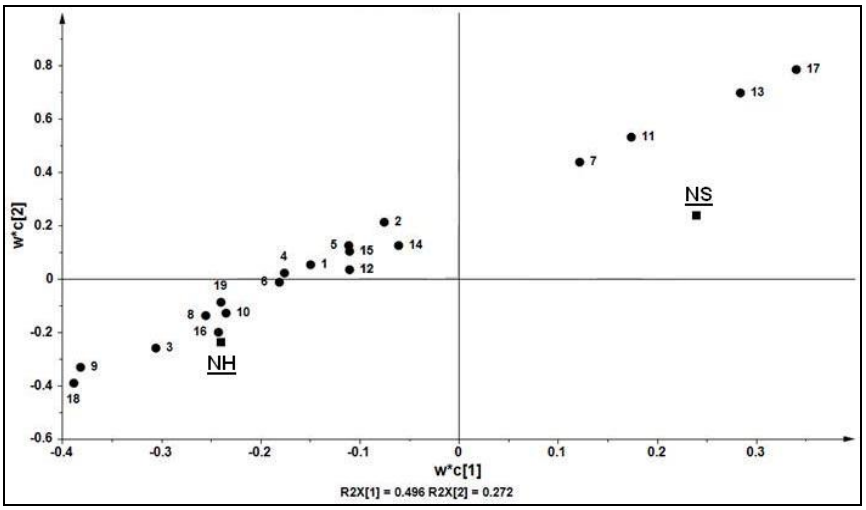
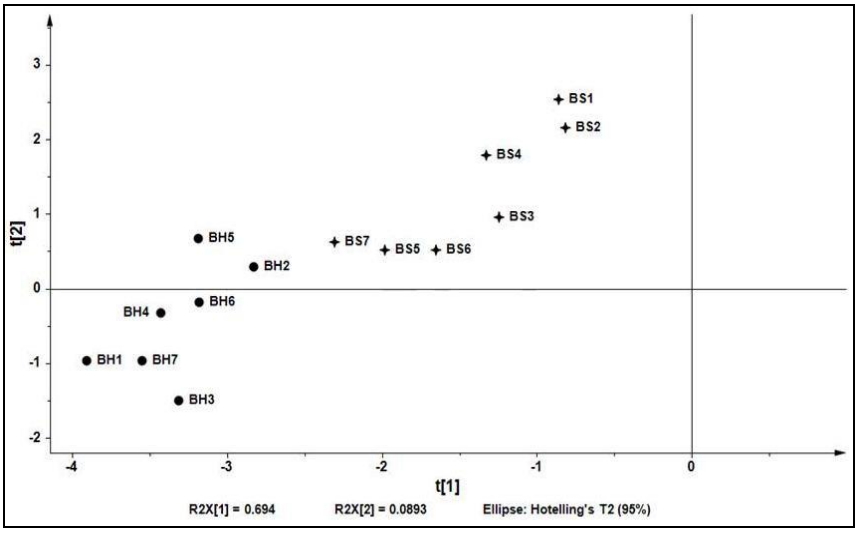


Fig. 3 A. PLS-DA of the volatile composition of hard and sweet ciders from Normandy given as a two-dimensional representation of the scores (t[1] and t[2]) on the first [1] and second [2] PLS components. The first PLS component (R2X [1]) explains 50% and the second PLS component (R2X [2]) 27% of the variation of the X data. Circles and stars indicate hard and sweet cider samples respectively from Normandy.

B. PLS-DA weight plot of composition variables, w*c[1] and w*c[2], for studied hard and sweet ciders from Normandy, on the first [1] and second [2] components respectively. Compounds (listed in Table 3 and 4) are represented by a circle along with numbers for visualization purposes. NH and NS stand for hard and sweet ciders respectively from Normandy.

A



B

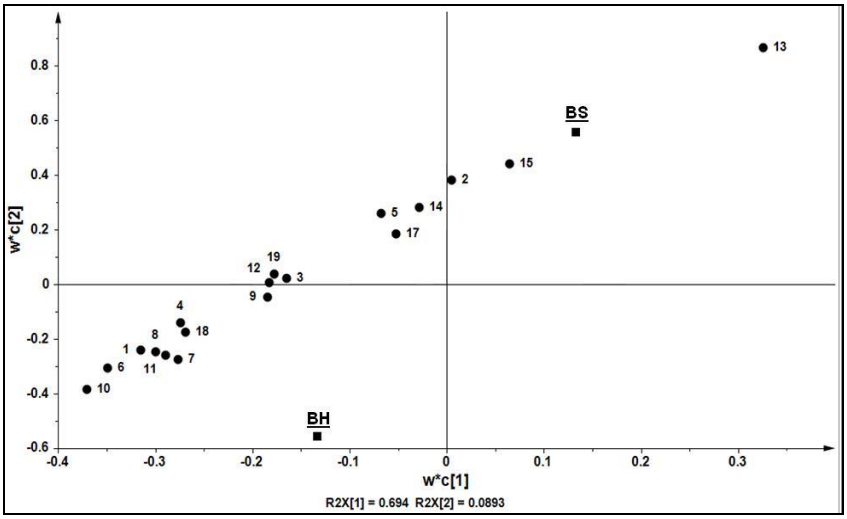
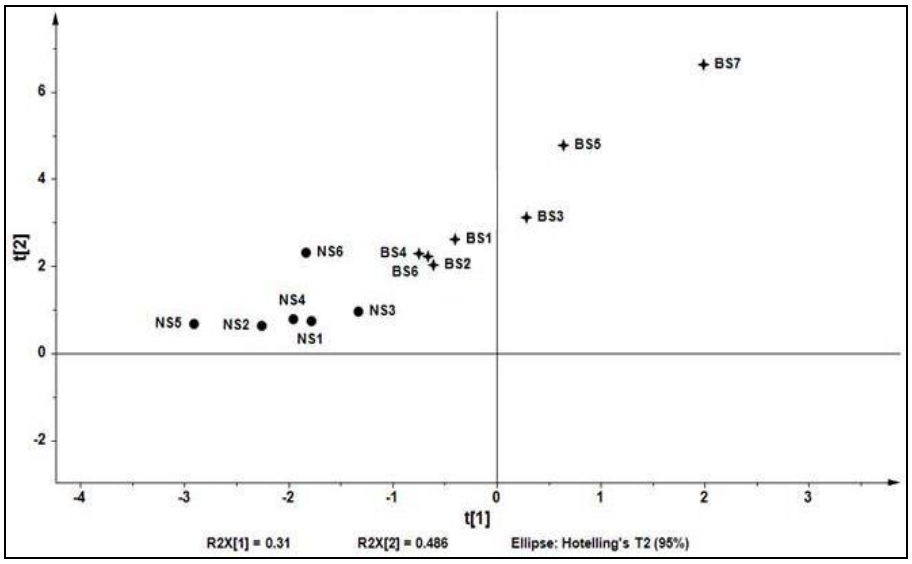


Fig. 4 A. PLS-DA of the volatile composition of hard and sweet ciders from Brittany given as a two-dimensional representation of the scores (t[1] and t[2]) on the first [1] and second [2] PLS components. The first PLS component (R2X [1]) explains 69% and the second PLS component (R2X [2]) 9% of the variation of the X data. Circles and stars indicate hard and sweet cider samples respectively from Brittany.

B. PLS-DA weight plot of composition variables, w*c[1] and w*c[2], for studied hard and sweet ciders from Brittany, on the first [1] and second [2] components respectively. Compounds (listed in Table 3 and 4) are represented by a circle along with numbers for visualization purposes. BH and BS stand for hard and sweet ciders respectively from Brittany.

A



B

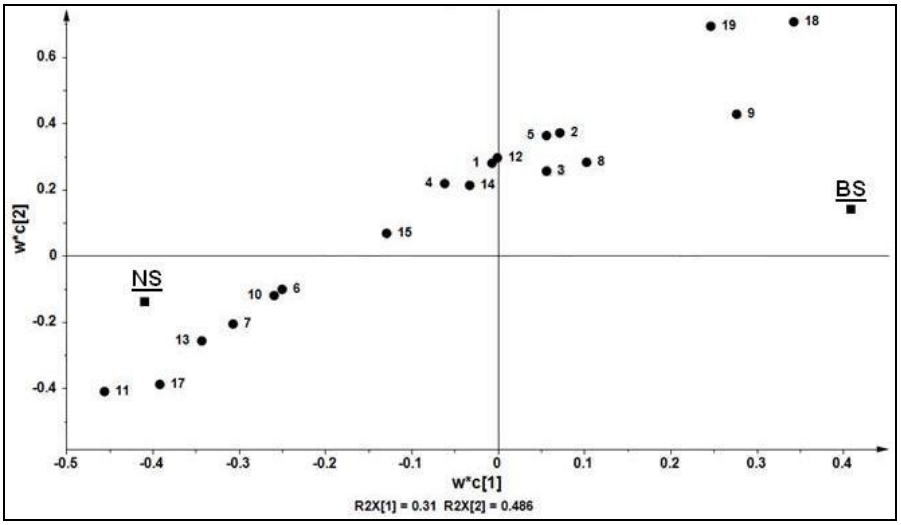


Fig. 5 A. PLS-DA of the volatile composition of sweet ciders from Normandy and Brittany given as a two-dimensional representation of the scores (t[1] and t[2]) on the first [1] and second [2] PLS components. The first PLS component (R2X [1]) explains 31% and the second PLS component (R2X [2]) 49% of the variation of the X data. Circles and stars indicate sweet ciders samples from Normandy and Brittany respectively.

B. PLS-DA weight plot of composition variables, w*c[1] and w*c[2], for studied sweet ciders from Normandy and Brittany, on the first [1] and second [2] components respectively. Compounds (listed in Table 4) are represented by a circle along with numbers for visualization purposes. NS and BS stand for sweet ciders from Normandy and Brittany respectively.