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ARTICLE

Multivariate Optimization of a HS-SPME+GC×GC-MS Procedure to Determine Fingerprints of the Volatile Fraction of Lager Beers

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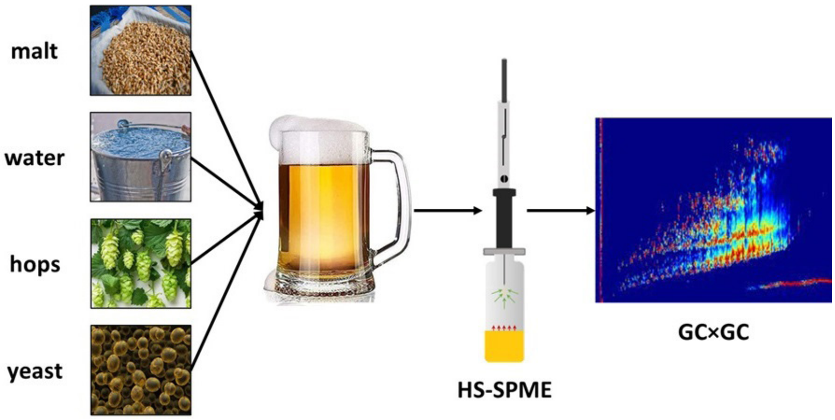

In this work we describe the optimization and application of a HS-SPME-GC×GC-MS method to assess the volatile fraction of Lager beers. The method development was performed using a combination of univariate and simultaneous multivariate approaches. Before use, canned beer samples were decarbonated by a freeze+taw procedure designed to minimize loss of volatile compounds and ensure complete elimination of CO_2 before extraction. The GC×GC cryogenic modulation conditions were optimized considering overall peak resolution and widths, where for the HS-SPME step parameters a multivariate approach using a Doehlert matrix was adopted, targetting the maximization of the total integrated peak area. The results of the multivariate optimization pointed that the extraction efficiency was maximum with 40 °C and 30 min as extraction temperature and time respectively. Using these optimized extraction conditions, it was possible to detect from 240 to 270 chromatographic peaks on the volatile fraction of the samples; among them 85 were identified. These results point out that careful optimization of operational parameters render HS-SPME combined to GC×GC-MS as a powerful tool to retrieve qualitative and quantitative information regarding the composition of beers.

Keywords: beer, optimization, volatile fraction, HS-SPME-GC×GC-MS

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INTRODUCTION

Alcoholic beverages have been present in human traditions since the early eras, among them beers, whose consumption dates back more than six millennia. Beer is an alcoholic beverage obtained by fermentative processes, and in its production are mainly used as ingredients: malt, hops, yeast and water. However, in some cases some adjuncts can be added to beer to replace and/or complement barley grain such as rice, corn and wheat. The taste of beer is determined by the raw material that is related to quality, by the yeast used that classifies beer into two types: Ale and Lager, and by the fermentation and maturation stages with the production of compounds that exert the greatest influence on its sensory characteristics. From the fermentation process two classes of this drink are originated,[1–3] lager (beers produced in low fermentation) and ale (beers produced in high fermentation).[4]

The increase in the diversity of beer styles generates the need for studies that address quality control, from the raw material to the sensory evaluation of the consumed. Quality control can be evaluated by means of its profile of volatile organic compounds (VOC). This approach is the best choice when applied to samples with matrices consisting of sugars, amino acids, proteins, carbohydrates and non-volatile organic compounds, due to the resulting simplification of analytical procedures. In the context for the characterization of volatile fingerprints, it is indispensable to use a more sensitive analytical instrumentation to identify VOC such as acids, acetone, alkanes, alcohols, esters, phenols and terpenes, which bring useful information in the characterization of beer quality and organoleptic properties.[5]

The analysis of VOC in beers requires an initial stage of sample preparation, and headspace extraction techniques are advantageous because they enable the exclusion of nonvolatile compounds, such as sugars, capable of influencing the characterization by masking the compounds of interest and, moreover, can damage instrumental analysis equipment. Among the most explored sample preparation techniques, solid phase microextraction through headspace (HS-SPME) is currently considered one of the most appropriate for the analysis of VOC due to its ability to integrate the sampling, extraction and preconcentration steps in a single step, besides enabling the automation of processes using automatic samplers and dispensing the use of organic solvents harmful to the environment. Compared to conventional extraction methods, HS-SPME is an extraction technique that can accurately reflect the basic composition of volatile components in the analyzed samples.[6,7]

Currently, most studies to investigate food and beverage fingerprinting are focused on analyses of VOC isolated by HS-SPME by conventional one-dimensional gas chromatography.[2,8–21] However, the use of comprehensive two-dimensional gas chromatography (GC×GC) has been shown to be a powerful alternative for characterizing the profile of volatiles from food samples, since it provides higher chromatographic resolution and increased sensitivity.[15,21–25] GC×GC enables the separation of several volatile and semivolatile components of a sample and when associated with the study where the objective is the search and identification of the profile of volatiles responsible for the aroma and flavor of beers, GC×GC coupled to mass spectrometry (GC×GC-MS) is an extremely powerful technique in the elucidation of this multicomponent matrix sample.[26–30]

The aim of this study is to optimize a HS-SPME-GC×GC-MS procedure to identify the fingerprints of volatile compounds characteristic of Lager beers using samples with different styles, to enable the association of the composition of the volatile fraction with organoleptic properties of the samples. In this stage, multivariate experimental design experiments where selected to evaluate how HS-SPME operational variables influence the quality of results. In this work we selected the use of Doehlert experimental planning for this task. This approach has the advantage of reducing the number of experiments needed to optimize HS-SPME methods.[31–33]

MATERIALS AND METHODS

Materials

Twenty-two samples of beers labeled by the manufacturers as *Lagers* (the most popular in Brazil) and commercially available in the local market were used in the optimization and validation of the optimized method. Table I presents information on the composition declared on the label of the beers analyzed.

Table I. Identification and qualification of the beer samples analyzed

#	Style[1]	Ingredients[2]
1	Pilsen	MHWY
2	Pilsen	MHWY + corn
3	Pilsen	MHWY
4	Standard American Lager	MHWY + orange peel
5	Standard American Lager	MHWY
6	Pilsen	MHWY + cassava
7	Premium American Lager	MHWY
8	Lager	MHWY
9	Premium American Lager	MHWY
10	Pilsen	MHWY
11	Premium American Lager	MHWY
12	American Lager	MHWY
13	Premium Amber Lager	MHWY
14	Premium American Lager	MHWY
15	Premium American Lager	MHWY
16	Pilsen	MHWY + corn
17	Standard American Lager	MHWY
18	Premium American Lager	MHWY
19	American Lager	MHWY
20	Lager	MHWY
21	American Lager	MHWY + corn
22	American Lager	MHWY

[1]Style of beer as declared by the manufacturer on the label; [2]MHWY: barley malt, hops, water and yeast.

For HS-SPME, 22 mL vials were used with appropriate caps and septa, 7×20 mm magnetic bars and SPME fiber with 65 mm Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) and (Divinylbenzene/Carboxen/Polydimethylsiloxane) and 30/50 mm PDMS/CAR/DVB (Supelco, Bellefonte, CT, USA) coatings. Saturated solution of NaCl (LABSYNTH, Diadema-SP), prepared in deionized water using a Milli-Q device (Merck-Millipore, Darmstadt - Germany), was also employed. The linear temperature programming retention indexes (LTPRI) for chromatographic peaks were determined using chromatograms of selected samples spiked with C_8-C_{22} n-alkane mixture (Sigma-Aldrich, St. Louis, MO, USA). The analyses were conducted in GC×GC-MS prototype based on a QP2010 Ultra GC equipped with a TQ8030 mass detector (Shimadzu Corporation, Kyoto Japan) fitted with a cryogenic loop modulator designed and assembled in the laboratory.[34] The column set used consisted of a 30 m × 0.25 mm × 0.25 µm Rxi-5SIL-ms column (Restek, Bellefonte, PA) as first dimension (^{1}D) connected to a 1 m × 0.1 mm × 0. 1 µm DB-Wax column

(Supelco, Bellefonte, CT, USA) as second dimension (^{2}D) through a SilTite zero-volume FS union (SGE Analytical, Ringwood, Australia). The modulation loop between columns consisted of a 0.8 m segment of capillary column (same phase and internal dimensions as ^{2}D).

All data obtained by GC×GC-MS was processed using GCImage software (Zoex, Houston, TX, USA). Doelhlert experimental design planning and assessment was made using proper tools implemented on Matlab platform, version R2014a (The Matworks, Inc., Natick, MA, USA).

Preparation of beer samples

For decarbonation, the beer cans were cooled to -180 ºC on liquid nitrogen before opening; the frozen beer slush was immediately transferred to a glass container also chilled under LN_2 and kept there for at least 5 min. The samples were then thawed to room temperature, immediately transferred to a previously refrigerated glass container and analyzed. This procedure was designed to minimize loss of volatile compounds during the manipulation of the samples.

Optimization of GC×GC-MS operational conditions

Modulation conditions

The conditions of the GC×GC-MS method were optimized using extractions from a randomly selected sample from the batch. The operational conditions for the GC×GC cryogenic modulation evaluated were the modulation period (between 4 and 6 s), period of cold jet activation (from 3.0 to 4.5 s) and of hot jet activation (from 1.0 to 1.5 s). The temperature of the modulator block (200 °C), hot jet temperature (250 °C) and cold jet pressure (10 psi) were previously defined after preliminary tests and kept constant for all experiments.

Chromatographic conditions

The general chromatographic conditions for the analysis of beer VOC were based on previous studies.[2,9,35] Hydrogen was used as the carrier gas at a constant flow rate of 1.2 mL/min. The column oven temperature program started at 40 °C and was increased at a rate of 3 °C min^{-1} until reaching 200 °C, then increased to 250 °C at a rate of 20 °C min^{-1} and held at 250 °C for 2 min. The injector temperature was maintained at 230 °C, and both ion source and transfer line to the MS was kept at 250 °C. Data acquisition was performed at a rate of 25 Hz, using full-scan mode with a mass-to-charge ratio (*m/z*) range from 40 to 500 *m/z*.

HS-SPME optimization procedures

Before optimization of extraction parameters, the SPME fiber coating was selected between PDMS/DVB/CAR or PDMS/DVB, using a randomly selected sample from the set. An aliquot of 8 mL decarbonated beer was mixed to 2 mL under magnetic stirring on a septum-sealed sample vial (conditions based on previous reports);[2,3] the vial was thermostated at 50 °C and a SPME fiber exposed to the sample headspace for 30 min. After extraction, the fiber was exposed to the GC×GC injector and the eluates were analysed immediately.

After selection of the SPME fiber for the following experiments, multivariate experimental planning was used to find the best operational conditions for the procedure. Two independent variables – extraction temperature and time – were optimized using a response surface methodology. A total of 11 experiments using different combinations of experimental conditions, arranged according to a Doehlert matrix[36,37] were carried out (Table II).

Table II. Doehlert planning for HS-SPME optimization

Variable	Variable levels				
Extraction temperature (°C)	**(-1,00)**	**(-0,50)**	**(0,00)**	**(0,50)**	**(1,00)**
	25,0	28,7	32,5	36,2	40,0
Extraction time (min)		**(-0,866)**	**(0,00)**	**(0,866)**	
		15,0	22,5	30,0	

RESULT AND DISCUSSION

Optimization of the GC×GC-MS system

Modulation

The modulation process allows the isolation of eluent segments of the first column and its reinjection into the second column continuously and sequentially throughout the analysis.[27] An adequate modulation of the sample promotes good extra sample variability with good intersample repeatability in addition to eliminating the chances of a sub-sampling in separation ^{1}D, which could cause loss of analytical information.[38] In the present work, cryogenic modulation was used, which consists of a type of thermal modulator with high transfer efficiency of the eluate fraction of ^{1}D to ^{2}D during the modulation period.[38] The modulation period is defined as the amount of time between modulations, that is, the sum of the collection time of the separate eluent in ^{1}D (cold jet) with the reinjection time at ^{2}D (hot jet). This parameter will have great influence on the width of the peaks and, therefore, on the chromatographic resolution capacity, being a variable that depends on the sample under study. The other parameters that influence modulation had their conditions already determined in previous studies and remained fixed in this study. Thus, the modulation period was optimized specifically for the study of beer samples, and the width of the peaks was used as a response. Table III presents the optimized conditions.[2,9]

Table III. Optimized conditions for cryogenic modulation

Modulation	
Period	6 s
Hot jet	4.5 s
Cold Jet	1.5 s

Best results were obtained for modulation period of 6.0 s (1.5 s with hot jet active and 4.5 s with cold jet active).[38] Figure 1 compares chromatograms obtained with modulation periods of 4.0 s and 6.0 s: the latter provided narrower peaks and higher peak capacity.

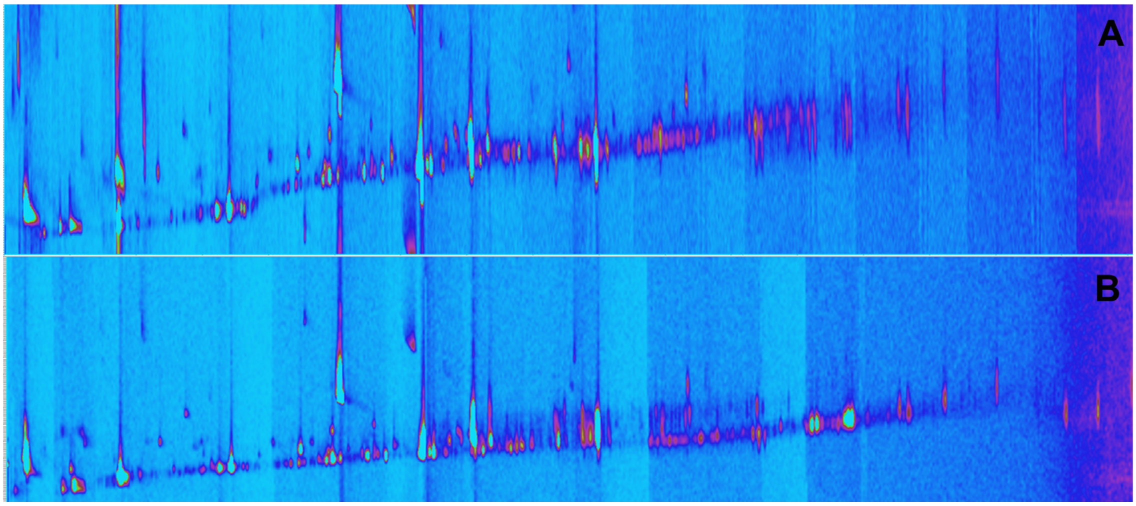

Figure 1. GCxGC-MS chromatograms obtained during the optimization of the modulation period for (A) 4 s (3 s of cold jet and 1 s hot jet) and (B) 6 s (4.5 s of cold jet and 1.5 s of hot jet).

HS-SPME

In HS-SPME several variables impact extraction efficiency,[39–42] but the more significant parameters are extraction time and temperature, as well as the nature of the fiber coating. For the selection of the SPME fiber coating, PDMS/DVB/CAR and PDMS/DVB fibers were qualitatively compared using extractions from the same sample. Figure 2 shows the chromatograms obtained.

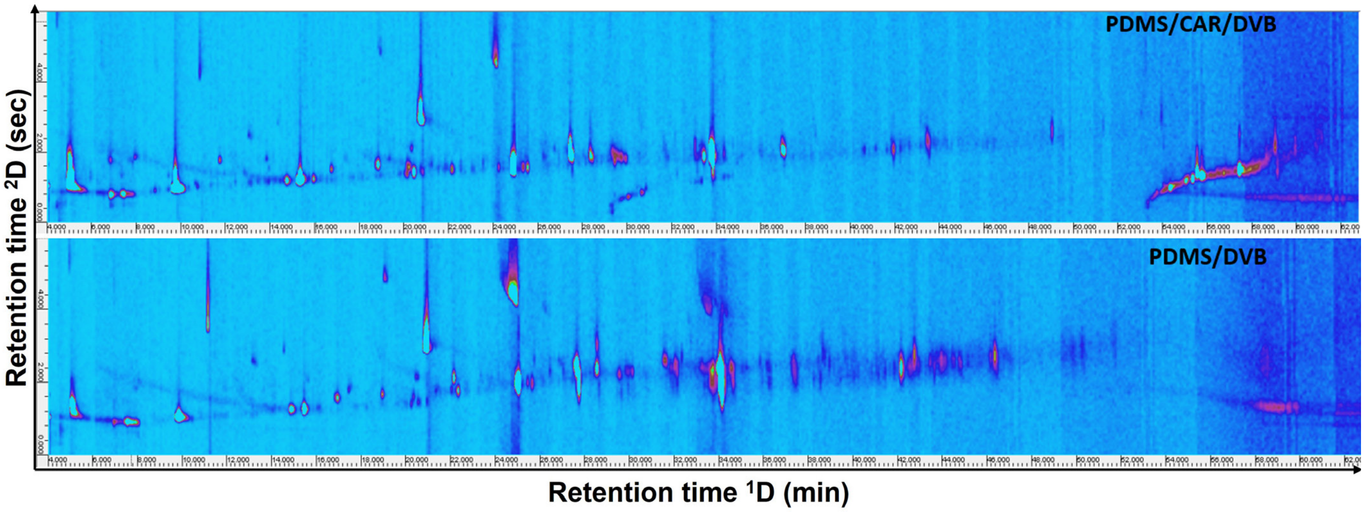

Figure 2. VOC chromatograms from a representative sample obtained after HS-SPME isolation using DVB/CAR/PDMS and PDMS/DVB fibers.

Visual comparison of chromatograms shows that the PDMS/DVB fiber provided higher efficiency for extraction for analytes more retained on the 1st dimension column – i.e., with high molecular masses (in the same range of C_{18} to C_{24} alkanes).[43,44] PDMS/DVB/CAR fibers allowed better extraction for species with low volatility and polarity (retentions roughly corresponding to alkane C_{16}). Compounds of higher molecular mass are the main markers of sensory attributes and better describe the differences between lager beer samples.[45] Also, ethanol is a major volatile component on beers and due to its volatility is present on its *headspace* on very high concentrations and likely to be sorbed in large amounts on PDMS/DVB/CAR fibers along with water, which is undesirable since it would result in the introduction of major amounts of vapour on the MS ionization sector. Therefore, PDMS/DVB fibers were selected for the remaining experiments.

After fiber selection, the extraction time and temperature parameters were optimized using Doehlert multivariate experimental planning to obtain the conditions providing higher extraction efficiency. The optimized response was defined as the total number of chromatographic peaks detected for each experiment; the peak detection threshold was adjusted on preliminary experiments considering typical signal/noise ratios. Table IV presents decoded values of the variables used in each experiment and the corresponding responses (detected peak count).

Table IV. Levels for optimized variables for each optimization experiment and corresponding responses (total of detected chromatographic peaks)

#	T (°C)	t_{ext} (min)	Response
1	32.50	22.5	281
2	40.00	22.5	268
3	28.75	30.0	285
4	25.00	22.5	215
5	28.75	15.0	230
6	36.25	15.0	235
7	28.75	30.0	253
8	32.50	22.5	281
9	32.50	22.5	287
10	32.50	22.5	293
11	32.50	22.5	326

The results could be properly fitted to a quadratic response surface (Figure 3). Analysis of the results showed that the quadratic model was statistically significant (F = 6.00 $p < 0.0272$). The quality of the model was confirmed by the corresponding value of F test (*p-value* = 0.0272), as well as the correlation coefficient for the model ($r^2 = 0.800$). Additional insight on the validity of the model can be obtained by inspection of Figure 4, which shows a plot correlating standardized residuals for each predictor value used to build the model. This figure shows that the fitting residuals are randomly scattered around zero and that there are no outliers, since all residuals were within the accepted range (-2 to +2) for model validation.

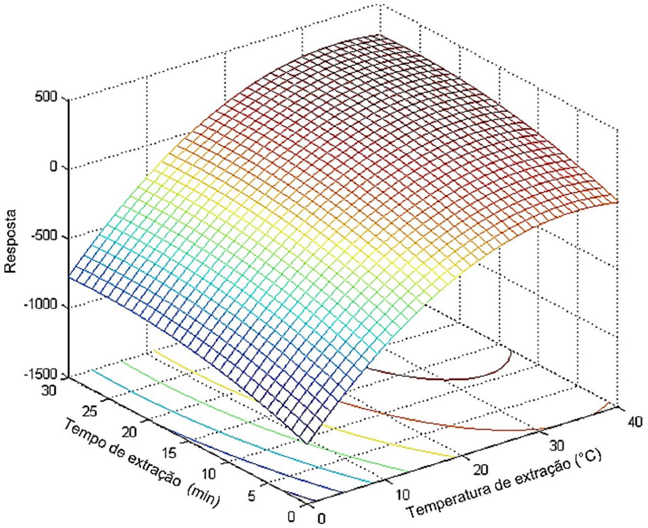

Figure 3. Response surface diagram showing the effects of significant variables of HS-SPME-GC×GC-MS.

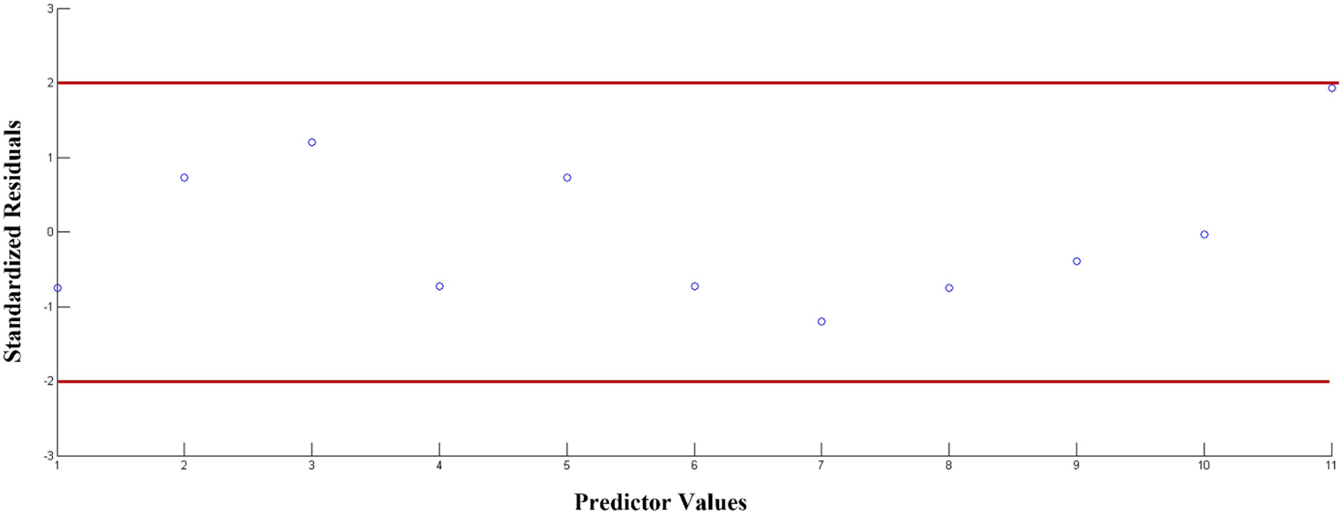

Figure 4. Residual graph *versus* the values predicted for the validation of the Response Surface Methodology model.

According to the quadratic model obtained after multivariate experiments, the number of detectable peaks is maximized for extractions with T = 40 ºC and t = 30 min. Figure 5 compares chromatograms for a random sample, obtained under optimized and non-optimized HS-SPME conditions.

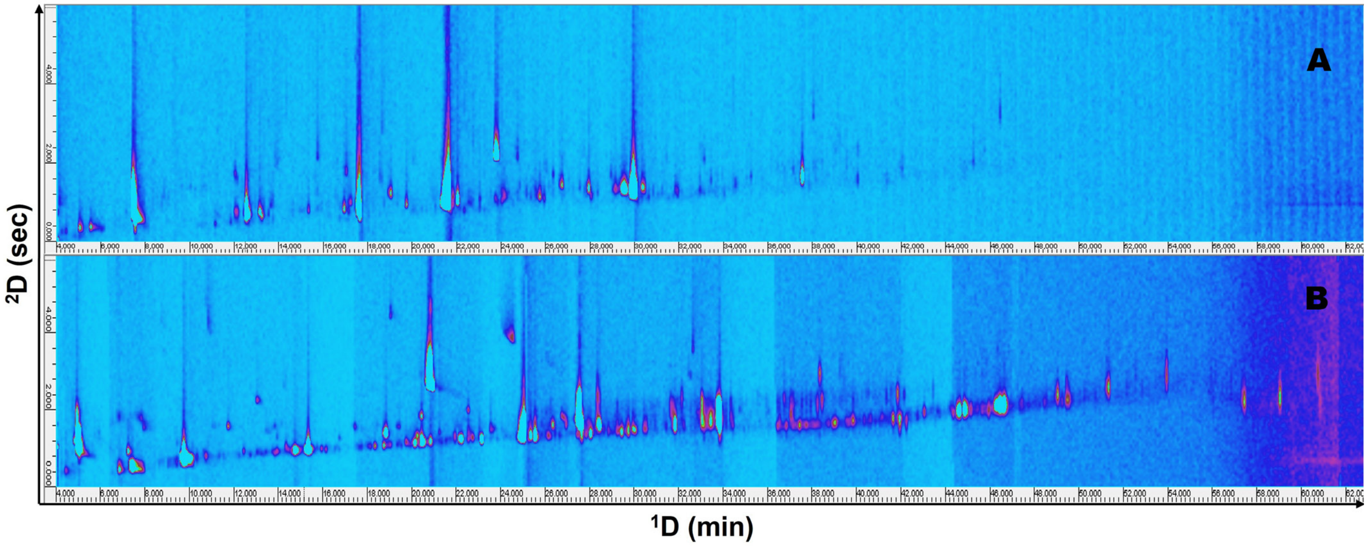

Figure 5. Two-dimensional chromatogram of the analysis of a beer sample obtained by HS-SPME-GC×GC-MS: (A) non-optimized method (T = 32 ºC and t = 22 min); (B) optimized method (T = 40 ºC and t = 30 min).

Under the optimized conditions, the number of detectable peaks increases considerably for optimized experiments. Also, it can be seen that the range of 1D retention times also increases, along with analyte mass. Although the extraction efficiency in terms of total mass of isolated VOC may not be enlarged, the resulting chromatograms are certainly more adequate for non-target analysis and sample profiling. It was possible to identify 85 compounds in the volatile fraction of the samples (see Supplementary Material).

Characterization of volatile compounds from beer Lagers

The optimized method HS-SPME-GC×GC-MS was applied to the screening of the volatile fraction of 22 samples of Lager beers. Figure 6 shows chromatograms obtained for representative samples for each beer style on the sample set. Chromatograms for the remainder samples can be found on the Supplementary Material of this paper.

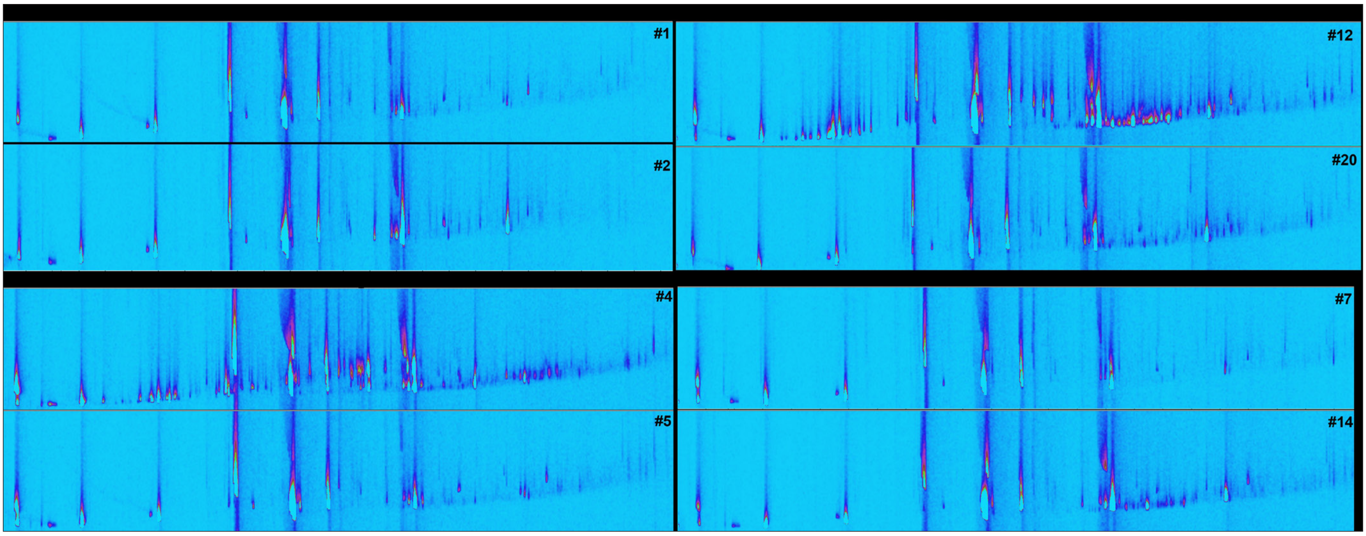

Figure 6. Two-dimensional fingerprints chromatograms of lager beer samples obtained by the optimized HS-SPME-GCxGC-MS method. (# = sample codes)

With the optimized method it was possible to assess the main differences between the fingerprints of samples according to the respective style, presented in Table I. According to the Beer Jugde Certification Program (BJCP) Style Guidelines,[46] Lite American Lager and Standard American Lager beers are low-aroma malt beers, low hop aroma, low bitterness and with the addition of up to 40% adjuncts such as rice and corn. In Brazil, beers labeled as Pilsen are actually more similar to American Lagers. Premium American Lager beers are described as more perceptive malt aroma beers than the styles mentioned above, the aroma of medium intensity hops, also characterizing a mid-level bitterness and with addition of up to 25% adjuncts such as rice and corn. Many of the beers labeled Premium American Lager in Brazil are popularly known as pure malt.

Within this context, the differences in fingerprints of samples of the same style can be correlated with the quality of the raw material used and in what form it is added, as an example, Hops can be added in the shape of pallets, flower and essential oil. As for the adjunct, different types can be added, for the pilsen-style beer group the sample #2 uses corn and sample #1 only informs on the label that it has the presence of unmaled cereals, with the fingerprints of these samples it is possible to verify that there is a difference in the VOC and this may indicate that sample #1 there was no addition of corn as an adjunct. The same style can also possess in its additive ingredients that complement the aromatic notes. In the Standard American Lager style beer group, sample #4 has the addition of orange, which generated a fingerprint with more VOC than sample #5. Additives can also be factors that influence VOC profile and have purposes of complementing the flavor and aroma of beers, that is, the sensory notes responsible for classification are not altered.

The other American Lager-style beer groups and Premium American Lager had no identification of anything different in the ingredients, meaning the labels contained the same information. However, it is known that the quality and origin of the ingredients used generate different fingerprints, which justifies the presence of different VOC in the characterization profiles of samples #12 and #20 belonging to the American Lager style and samples #7 and #14 belonging to the Premium American Lager style.

When approaching the difference between styles, the most influenced ingredients are: hops and malt. Hops can be a classification factor, as different types can be added for two purposes: types of hops that assign swine notes and types of Hops that assign aromatic notes, and in some beers you have the addition of the two types of Hops. Malt is present in the malting process and is different for each beer style, generating aromas and flavors specific to each style, for example, Pilsen Malt are characterized by producing beers of mild and refreshing flavor.

Therefore, the optimized method HS-SPME-GC×GC-MS allowed the obtaining of fingerprints of lager beer samples that can be used to characterize and obtain important information about the ingredients used in its production.

CONCLUSIONS

The optimized method HS-SPME-GC×GC-MS was successfully applied to the analysis of the profile of volatile compounds in 22 samples of industrialized Lager beers in Brazil, with affordable cost and high standardization. The study allowed the identification of differences in fingerprints of different samples belonging to the same style and between the different beer styles, correlated with the quality and origin of the ingredients used, as well as the presence of additives that complement the aromatic notes. The study provides valuable information for the brewing industry in improving quality control and producing beers with specific sensory profiles.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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SUPPLEMENTARY MATERIAL

GC×GC chromatograms for studied samples as listed on Table I. Style of beer as declared by the manufacturer on the label; MHWY = ingrediens (barley malt, hops, water and yeast).

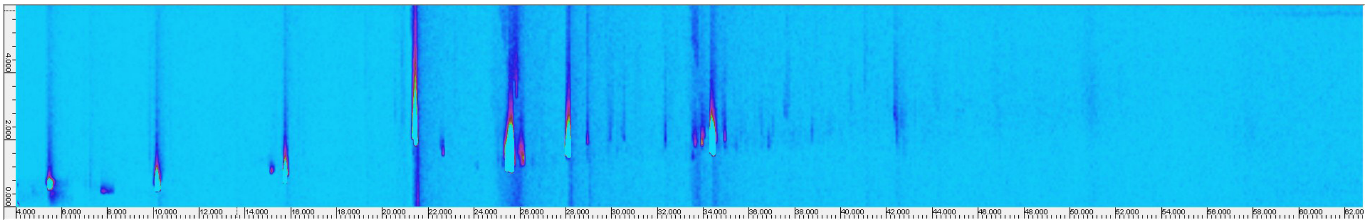

Figure S1. Sample #1 (Pilsen / MHWY).

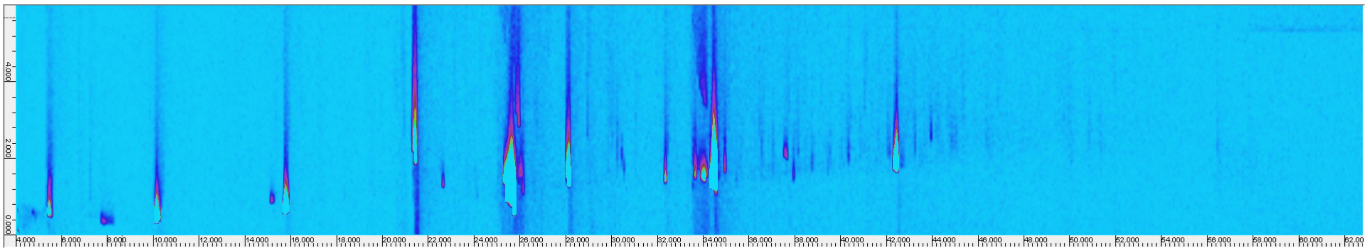

Figure S2. Sample #1 (Pilsen / MHWY + corn).

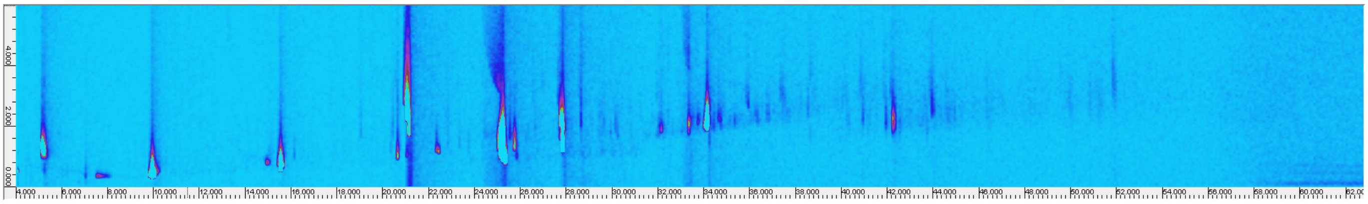

Figure S3. Sample #3 (Pilsen / MHWY).

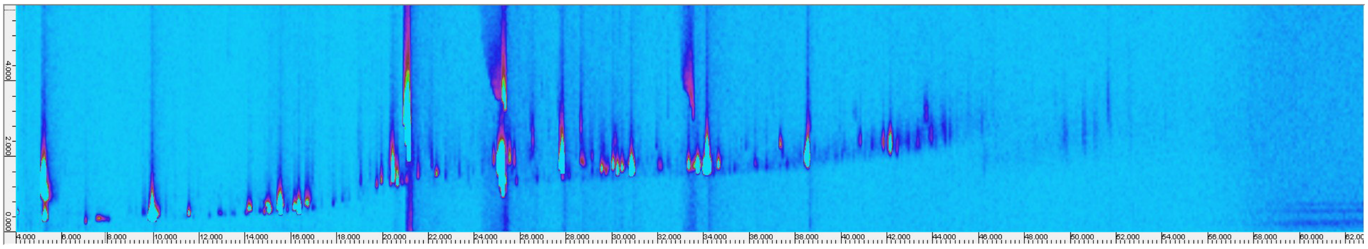

Figure S4. Sample #4 (Standard American Lager / MHWY + orange peel).

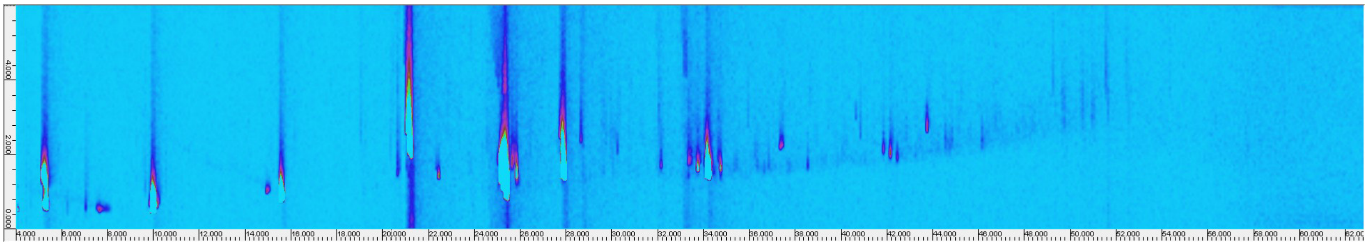

Figure S5. Sample #5 (Standard American Lager / MHWY).

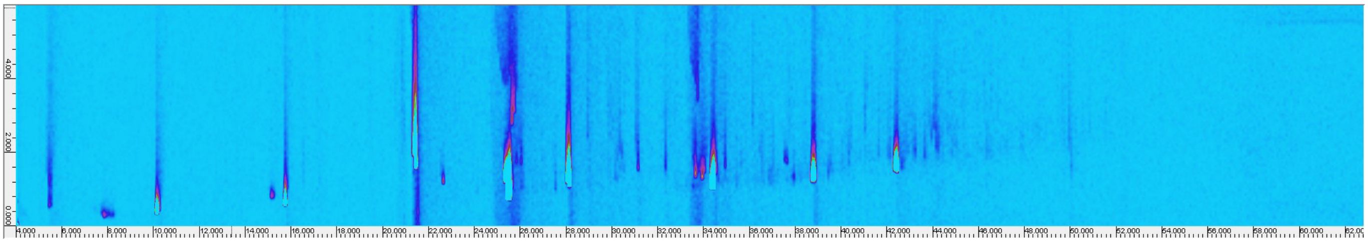

Figure S6. Sample #6 (Pilsen / MHWY + cassava).

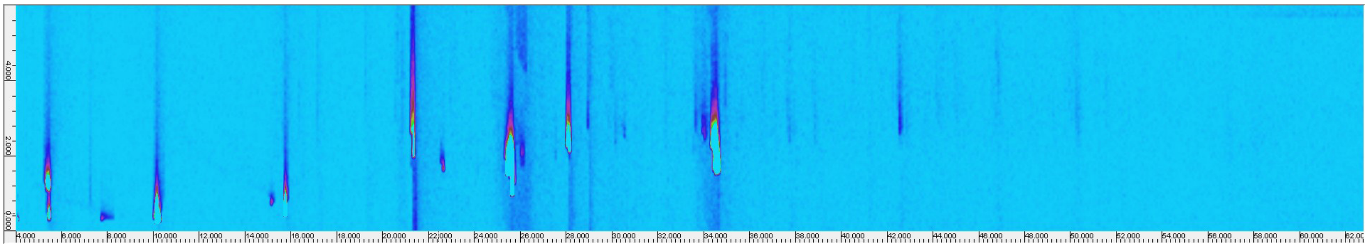

Figure S7. Sample #7 (Premium American Lager / MHWY).

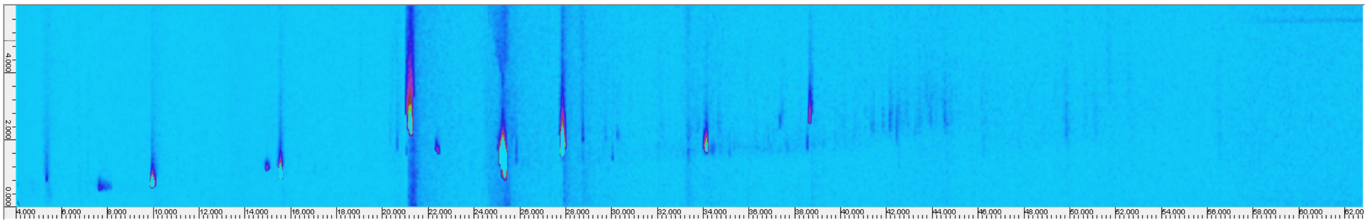

Figure S8. Sample #8 (Lager / MHWY).

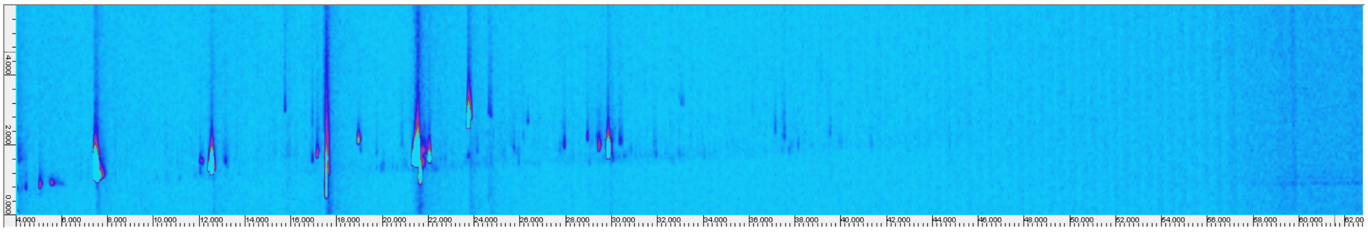

Figure S9. Sample #9 (Premium American Lager / MHWY).

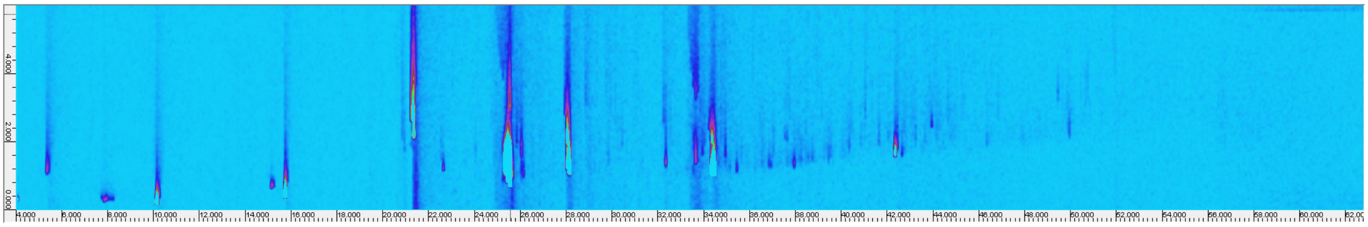

Figure S10. Sample #10 (Pilsen / MHWY).

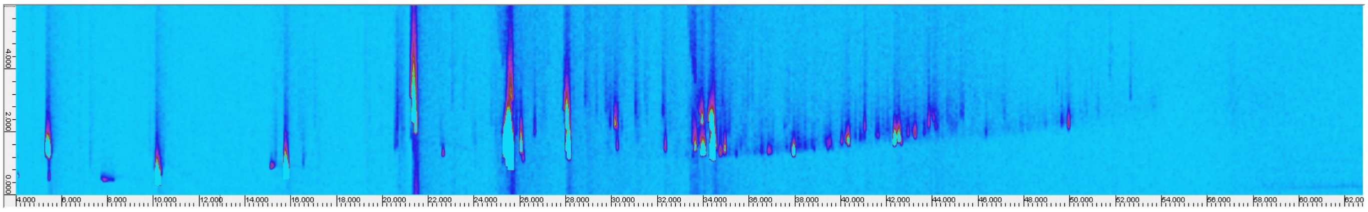

Figure S11. Sample #11 (Premium American Lager / MHWY).

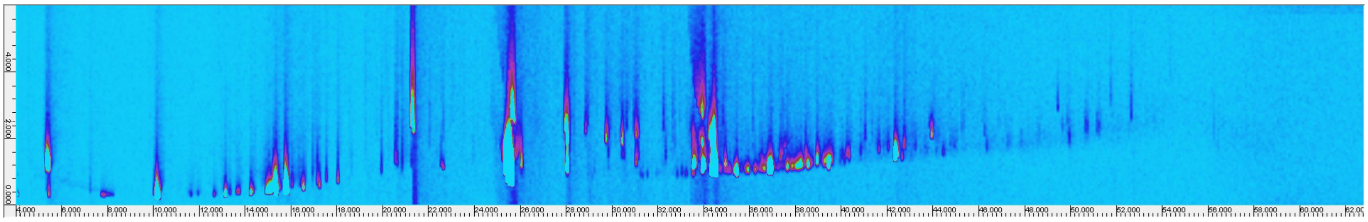

Figure S12. Sample #12 (American Lager / MHWY).

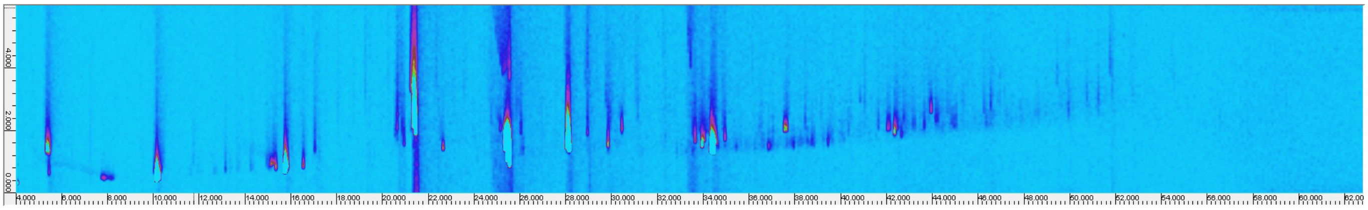

Figure S13. Sample #13 (Premium Amber Lager / MHWY).

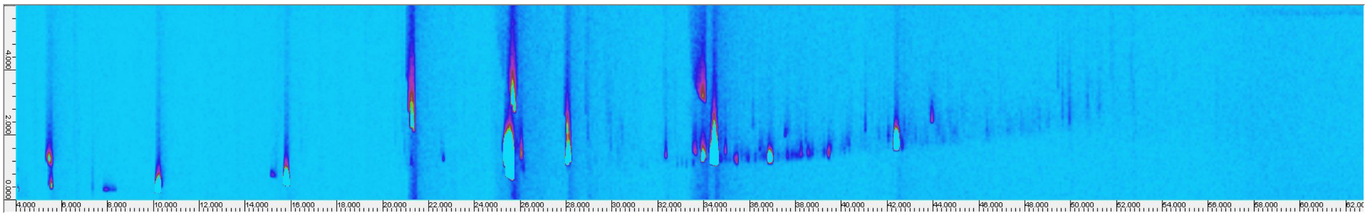

Figure S14. Sample #14 (Premium American Lager / MHWY).

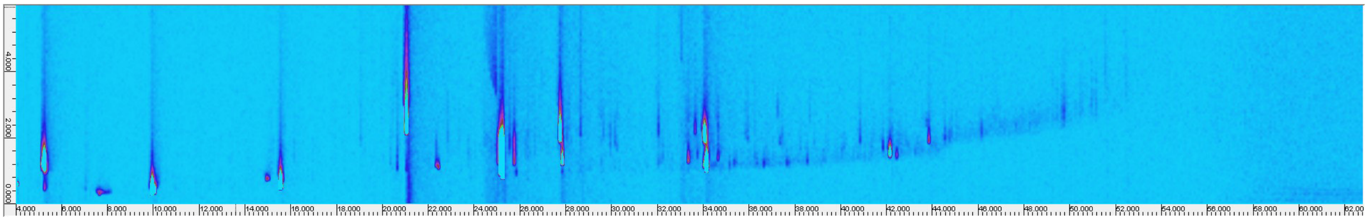

Figure S15. Sample #15 (Premium American Lager / MHWY).

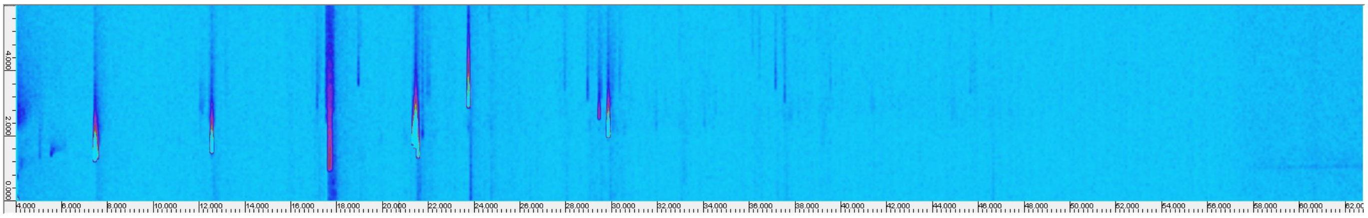

Figure S16. Sample #16 (Pilsen / MHWY + corn).

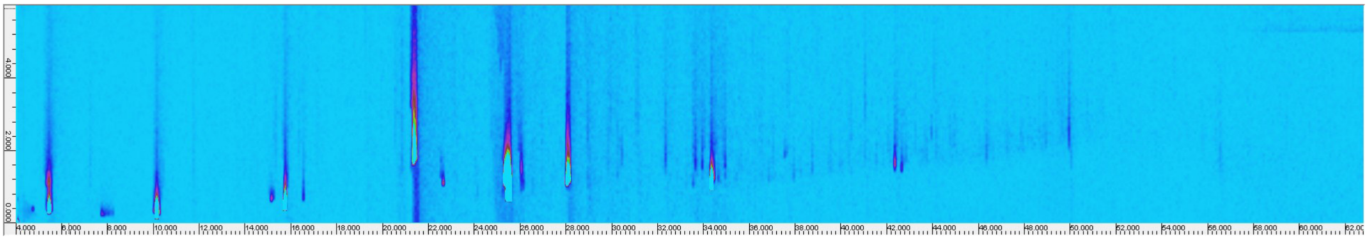

Figure S17. Sample #17 (Standard American Lager / MHWY).

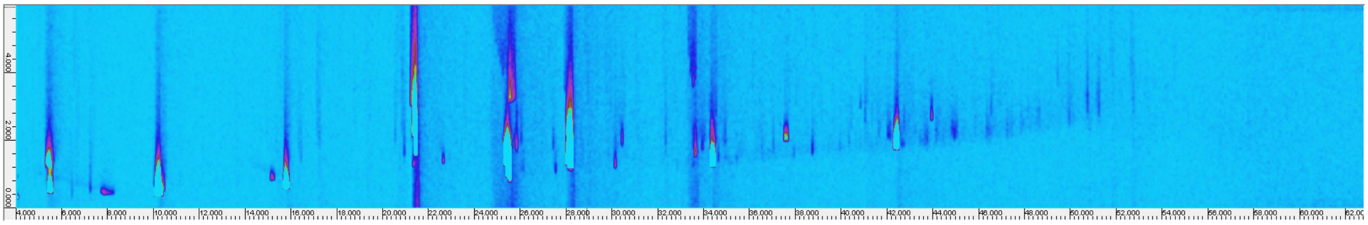

Figure S18. Sample #18 (Premium American Lager / MHWY).

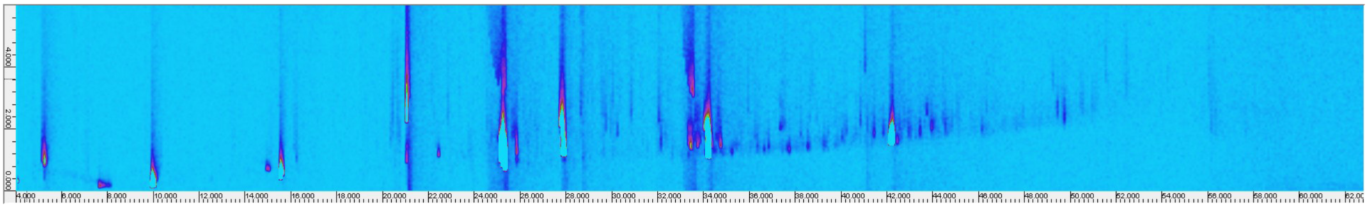

Figure S19. Sample #19 (American Lager / MHWY).

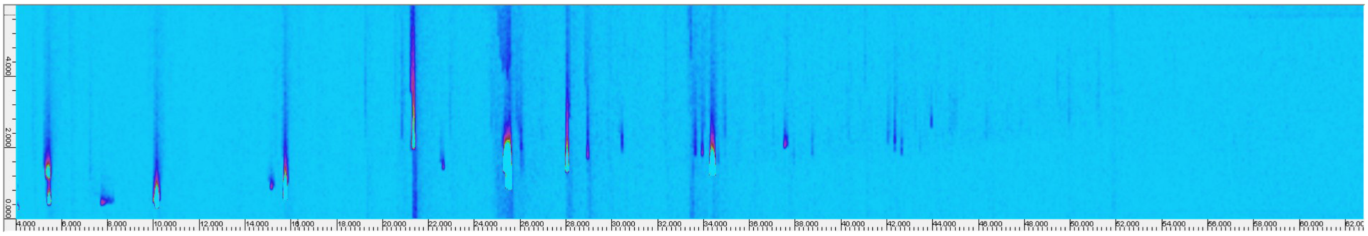

Figure S20. Sample #20 (Lager / MHWY).

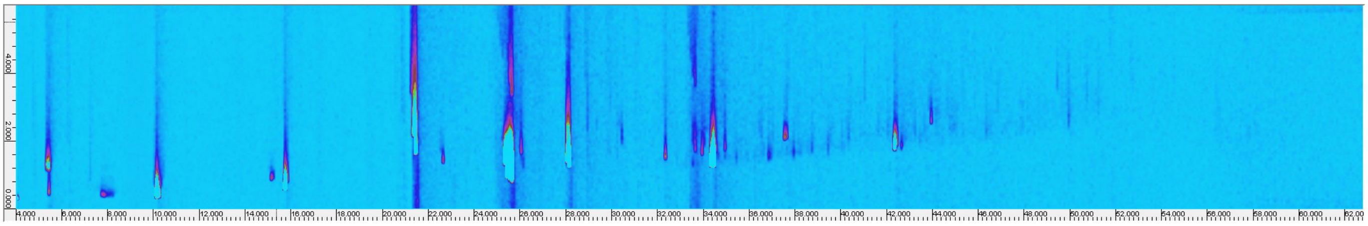

Figure S21. Sample #21 (American Lager / MHWY + corn).

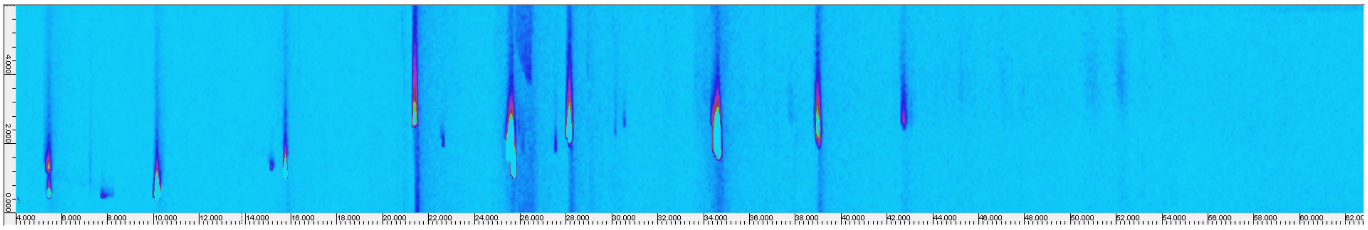

Figure S22. Sample #22 (American Lager / MHWY).

Table SI. Putative identification of beer samples VOC detected on HS-SPME-GC×GC-MS chromatograms. CAS = Chemical Abstracts Registry No.; Match = LTPRI = Linear Temperature Programming Retention Index.

		t_R		LTPRI		
Compound	**CAS**	**^{1}D (min)**	**^{2}D (s)**	**Exp.**	**Lit.**	**Samples**
1-propanol	71-23-8	9,13	4,82	585	568	3
n-hexane	110-54-3	10,13	3,57	610	623	3,5,6,7,10,12,17,21,22
ethyl acetate	141-78-6	10,23	5,45	613	606	All samples
3-methylexane	589-34-4	12,23	4,10	664	672	3,5,12,17,21,22
2-methylexane	591-76-4	12,23	4,17	664	663	3,5,12,22
4-methyl-1-hexene	3769-23-1	12,43	4,62	669	665	2
iso-octane	540-84-1	13,03	4,27	685	683	3, 5,22
ethyl propionate	105-37-3	13,53	1,07	698	706	3
3-methyl-1-heptene	4810-09-7	14,43	4,10	721	747	10
amyl alcohol	71-41-0	14,53	1,02	723	760	All samples
2-methyl-1-butanol	137-32-6	14,53	3,92	723	740	2,3,4,8,9,12,15,16,18,22
isoamyl alcohol	123-51-3	14,63	5,02	726	732	9,12,20
toluene	108-88-3	15,83	2,10	757	750	17
isobuthyl acetate	110-19-0	15,93	1,30	759	755	1,2,4,6,7,11,13,15,18,19,22
ethyl butyrate	105-54-4	17,03	1,57	787	794	All samples
2,4-dimethyleptane	2213-23-2	18,93	4,82	836	823	5
m-xylene	108-38-3	19,93	2,85	862	848	5,17
ethylbenzene	100-41-4	19,93	2,95	862	839	3,12,21,22
acetate de isoamila	123-92-2	20,43	1,70	875	856	1,2,3,4,5,6,7,8,9,10,12,13,14,15,16,17,18,19,20,21,22
isoamyl acetate	628-63-7	20,43	4,65	875	926	1,8,9,10,12,15,16,17,20,21
styrene	100-42-5	21,23	4,12	895	895	15
p-xylene	106-42-3	21,33	3,47	898	872	3,5,12,22

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Table SI. Putative identification of beer samples VOC detected on HS-SPME-GC×GC-MS chromatograms. CAS = Chemical Abstracts Registry No.; Match = LTPRI = Linear Temperature Programming Retention Index. (continuation)

		t_R		LTPRI		
Compound	**CAS**	**^{1}D (min)**	**^{2}D (s)**	**Exp.**	**Lit.**	**Samples**
o-xylene	95-47-6	21,33	3,35	898	871	17
isobuthyl isobutirate	97-85-8	22,23	1,02	921	899	4,13
cumene	98-82-8	22,83	2,72	936	930	18
α-thujene	2867-05-2	23,33	0,60	949	932	5,12
α-pinene	80-56-8	23,63	0,87	957	942	3,5,12,17,21,22
m-ethyltoluene	620-14-4	24,43	3,07	977	968	17
amyl propionate	624-54-4	24,63	1,72	982	952	13
pseudocumene	95-63-6	24,73	3,22	985	987	3,5,12,13,17,22
mesitilene	108-67-8	24,73	3,30	985	997	22
limetol	7392-19-0	25,13	1,22	995	971	4,12
methyl *iso*-heptanoate	2177-83-5	25,33	2,07	1000	963	4
β-pinene	18172-67-3	25,43	1,80	1003	974	1,2,3,5,6,7,11,12,15,17,22
ethyl caproate	123-66-0	25,73	1,77	1010	996	All samples
β-mircene	123-35-3	25,83	0,87	1013	992	4,12,13
pseudopinene	127-91-3	25,83	0,95	1013	984	20
hexyl acetate	142-92-7	26,23	1,67	1023	987	11
isoamyl butyrate	106-27-4	26,63	1,40	1034	1061	12
alyl 2-ethylbutirate	7493-69-8	26,63	1,35	1034	995	6
methyl 4-methylenoexanoate	73805-48-8	26,83	3,17	1039	1005	11,13
methyl enanthate	106-73-0	26,83	2,20	1039	1008	4
α-terpinene	99-86-5	27,03	1,82	1044	1020	4
o-cymene	527-84-4	27,13	3,00	1046	1030	3,4,5,12,13,17,22
cis-sabinene hydrate	15537-55-0	27,53	2,35	1057	1060	4
limonene	5989-27-5	27,53	2,02	1057	1024	6,7,8,10
undecane	1120-21-4	28,03	5,17	1069	1061	10
α-ocimene	502-99-8	28,13	1,42	1072	1057	4
2,6,7-trimethyldecane	62108-25-2	28,93	5,22	1093	1058	21
2-methyldecane	6975-98-0	29,13	5,10	1098	1061	10, 12, 21

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Table SI. Putative identification of beer samples VOC detected on HS-SPME-GC×GC-MS chromatograms. CAS = Chemical Abstracts Registry No.; Match = LTPRI = Linear Temperature Programming Retention Index. (continuation)

		t_R		LTPRI		
Compound	**CAS**	**^{1}D (min)**	**^{2}D (s)**	**Exp.**	**Lit.**	**Samples**
methyl 6-methyleptanoate	2519-37-1	29,43	2,00	1105	1068	4
2-nonanone	821-55-6	29,53	2,92	1108	1089	4
ethyl enanthate	106-30-9	29,83	1,92	1116	1083	6,14
linalool	78-70-6	30,13	2,52	1123	1101	2,3,4,5,6,7,8,13,14,15,17,19,21,22
nona-2,6-dien-1-ol	7786-44-9	30,13	3,67	1123	1156	19
4-methylundecane	2980-69-0	30,23	5,45	1126	1160	10, 12, 21
myrtenyl methyl ether	202527-57-9	30,43	1,97	1131	1160	13
β-phenylethyl alcohol	60-12-8	30,43	1,72	1131	1117	1,2,3,4,5,6,7,8,9,12,13,14,15,16,17,18,19,20,21,22
cis-2-nonenal	60784-31-8	30,43	2,97	1131	1125	15
2-methylundecane	7045-71-8	31,13	5,05	1149	1160	21
2,3-dimethyldecane	17312-44-6	31,43	5,32	1157	1155	21
caprylic acid	124-07-2	33,23	1,72	1203	1183	9,12,13,14,15,19,22
4-etildecane	1636-44-8	33,53	5,55	1211	1200	12
ethyl caprylate	106-32-1	33,73	1,37	1216	1178	All samples
capryl acetate	112-14-1	34,23	2,02	1229	1211	1,9,15
α-ioneno	475-03-6	34,93	3,20	1247	1266	5,9,15,19
β-phenylethyl acetate	103-45-7	35,73	3,27	1267	1258	1,2,3,4,5,6,7,8,9,11,12,13,14,15,16,17,18,19,21,22
deidro-ar-ioneno	30364-38-6	39,73	5,77	1370	1349	19
ethyl 9-decenoate	67233-91-4	40,33	2,77	1385	1389	1,2,6,7,8,11,12,14,21
β-damascenone	23726-93-4	40,53	4,87	1390	1382	1,2,3,5,6,7,8,9,13,14,17,18,19,20,21,22
ethyl 5-methylnonanoate	116530-40-6	40,83	3,87	1398	1395	2,5,10
ethyl caprate	110-38-3	40,83	2,07	1398	1397	1,2,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22
β-caryophyllene	87-44-5	42,53	3,17	1441	1420	11,12
trans-α-bergamotene	13474-59-4	42,83	1,92	1449	1441	12
cis-β-farnesene	28973-97-9	43,13	1,57	1457	1440	5,12,15,19
humulene	6753-98-6	43,63	3,72	1470	1456	10,11,12,13,15,17,21
cis-α-bisabolene	29837-07-8	43,63	3,72	1470	1494	1,2,6,9

(continues on next page)

Table SI. Putative identification of beer samples VOC detected on HS-SPME-GC×GC-MS chromatograms. CAS = Chemical Abstracts Registry No.; Match = LTPRI = Linear Temperature Programming Retention Index. (continuation)

		t_R		LTPRI		
Compound	**CAS**	**^{1}D (min)**	**^{2}D (s)**	**Exp.**	**Lit.**	**Samples**
γ-muurolene	30021-74-0	44,13	3,32	1483	1477	12
α-selinene	473-13-2	44,63	4,15	1495	1494	12
butilidroxitoluene (BHT)	128-37-0	44,73	3,42	1498	1505	4,7,8,22
α-muurolene	31983-22-9	44,83	3,35	1500	1500	12
aloaromadendrene	25246-27-9	44,93	3,80	1503	1462	12
trans-calamenene	73209-42-4	45,43	4,60	1516	1532	1,12
β-cadinene	523-47-7	45,53	3,15	1518	1518	12
ethyl laurate	106-33-2	47,13	2,16	1559	1590	1,2,4,6,7,12,14,15,16,18,19,22