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Optimization of the HS-SPME–GC–IT/MS method using a central composite design for volatile carbonyl compounds determination in beers

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ABSTRACT

An automated headspace solid-phase microextraction (HS-SPME) combined with gas chromatography and ion trap mass spectrometry detection (GC–IT/MS) was developed in order to quantify a large number of carbonyl compounds in beers. Carbonyl compounds were previously derivatized with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA).

Volatile carbonyl compounds associated with staling beer aroma includes alkanals, alkenals, alkadienals, dicarbonyl compounds, Strecker aldehydes, ketones and furans. The HS-SPME was performed using a polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber. The procedures were optimized for HS-SPME pre-incubation temperature and time, extraction temperature and time, and PFBHA addition. A central composite design was used in the optimization of extraction conditions and PFBHA addition. The volatile compounds showed optimal extraction incubating 5 ml of beer with 700 mg l^{-1} of PFBHA for 7 min and extracted for more 20 min at 45 °C. The method was validated with regard to the linearity, repeatability, inter and intra-day precision and accuracy. The method achieved detection limits ranging from 0.003 to 0.510 μg l^{-1}, except for furans (1.54–3.44 μg l^{-1}). The quantification limits varied from 0.010 to 1.55 μg l^{-1}, except for 2-furfural (4.68 μg l^{-1}), 5-methyl-2-furfural (5.82 μg l^{-1}) and 5-hyfroxymethylfurfural (10.4 μg l^{-1}). Repeatability values of all compounds were lower than 17%. The method accuracy was satisfactory with recoveries ranging from 88% to 114%. The validated method showed to be suitable for a fast and reliable determination of main carbonyl compounds in beers.

1. Introduction

One of the most appreciated sensory characteristics of beer is its fresh flavor which is lost during storage due to chemical modifications [1,2]. Carbonyl compounds, particularly aldehydes, are considered to play an important role in the deterioration of flavor and aroma of beers [3–5]. These compounds can be originated from raw materials, alcoholic fermentation or from a wide range of chemical reactions, such as oxidation of unsaturated fatty acids, Maillard reactions, Strecker degradation, degradation of bitter acids, aldol condensation and autoxidation of aldehydes during beer processing and/or storage [1,3,6–8]. The carbonyl compound concentrations in beer are generally very low; however, when presents they are responsible for mostly undesirables off-flavors because of their particular sensory descriptors (Table 1) and low perception thresholds.

Gas chromatograpy coupled to mass spectrometry (GC–MS) has been extensively employed to identifiy and quantify aroma/flavor components in several foodstuffs, including beer [9]. In some cases, derivatized reagents are required in order to detect and quantifiy minor aldehydes at trace levels [10]. The most commonly used derivatisation reagent is *O*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA). The oximes formed with PFBHA showed relatively specific mass spectra and high sentivity in different detection systems [10–20].

Diferent extraction techniques, solid-phase extraction (SPE), headspace solid-phase microextraction (SPME), stir bar sorptive extraction (SBSE) and headspace in-tube extraction (ITEX) were apllied in the analysis of aldehydes in alcoholic beverages [10,13,14,20–22]. In beer, the mostly often used extraction technique is the SPME–GC–MS using different strategies of derizatization, directly on fiber and in solution

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Table 1
Compounds identified in beers: odor characteristics, retention time (RT) and selected ions used as *m/z* quantifiers.

Compound	Odor	RT (min)	Ions (m/z)
Alkanals			
Ethanal	Green leaves, apple skin, green apple, fruity, paint, sweat, pungent [1,5–8]	14.61	236/253
Propanal	Oxidized apple, green, fruity [7]	15.21/15.40	236/253
Butanal	Melon, green malt, varnish [6,7]	17.62/17.80	239
Pentanal	Grass, apple, cheese, almond, malt, pungent [5,7]	20.04/20.20	239
Hexanal	Vinous, aldehydic, bitter, fat, grass, tallow, winery [1,5–8]	22.42/22.6	239
Heptanal	Aldehyde, bitter, fat, citrus, rancid [5,7]	24.70/24.79	239
Octanal	Orange peel, aldehydic, bitter, fat, soap, lemon, green [6,7]	26.87/26.94	239
Nonanal	Astringent, bitter, fat, citrus, green [5,7]	28.90	239
Decanal	Orange peel, aldehydic, bitter, soap, tallow [5–7]	31.07	239
Undecanal	–	33.03	239
Dodecanal	Caprylic, aldehydic [6]	34.90	239
Tridecanal	–	36.70	239
Tretadecanal	–	38.43	239
Pentadecanal	–	40.10	239
Alkenals			
2-propenal	–	15.26/15.67	250
(*E*)-2-butenal	Apple, almond [7]	19.47	250
(*E*)-2-pentenal	–	21.72	250
(*E*)-2-hexenal	Bitter, astringent, apple, green [5,7]	24.11	250
(*E*)-2-heptenal	Fatty, green	26.30/26.46	250
(*E*)-2-octenal	Green, nut, fat [5]	28.41/28.53[a]	250
(*E*)-2-nonenal	Papery, cardboard, oxidized, stale, plastic-like, cucumber, orris, fat [1,5–8]	30.42/30.72	250
(*E*)-2-decenal	–	32.37/32.70	250
Alkadienals			
(*E*,*E*)-2,4-hexadienal	–	25.75/25.84	276
(*E*,*E*)-2,4-nonadienal	Cucumber, green leaves [6]	31.81/32.27	276
(*E*,*E*)-2,4-decadienal	Oily, aldehydic, deep-fried, papery [6–8]	33.58/34.08	276
Strecker aldehydes			
2-Methyl-1-propanal	Banana, melon, fruity, green malt, malty, wine, grainy, varnish, solvent [1,5–8]	16.30	250
2-Methyl-1-butanal	Green grass, fruity, apple-like, almond, malty, cocoa [1,5,6,8]	18.68	239
3-Methyl-1-butanal	Unripe banana, apple, cherry, cheesy, malty, chocolate, almond [1,5,6,8]	19.0/19.19	239
Benzaldehyde	Almond, cherry stone, burnt sugar [1,5,8]	27.46/27.67	271+301
Phenyl acetaldehyde	Hyacinth, flowery, roses [1,8]	28.62/28.80	91
Methional	Cooked potatoes, worty [1,8]	25.59[a]/25.69	61
Dicarbonyl			
Diacetyl	Buttery, butterscotch [5,6,8]	30.72[a]/32.22/34.07	279
Glyoxal	–	32.80/32.94	182
Methylglyoxal	–	33.05/33.24/33.53	182
Ketones			
2-Butanone	–	16.68/16.77	250
2-Pentanone	–	18.59/18.79	253
2-Hexanone	–	19.58/19.78	253
2-Heptanone	Varnish, hops, sulfur, pungent, green [5,7]	22.49/23.06	253
2-Octanone	Varnish, walnut [7]	25.03/25.40	253
2-Nonanone	Ketonic, varnishy [7]	27.14/27.53	253
2-Decanone	Ketone, varnish [7]	29.17/29.59	253
2-Undecanone	Ketone, flowery [7]	31.14/31.58	253
Furans			
2-Furfural	Caramel, bready, cooked meat, papery, husky [1,7,8]	23.32/23.86	291
5-Methyl-2-furfural	Almond, marzipan, spicy [1,7,8]	25.88/26.10	305
5-Hydroxymethylfurfural	Bready, caramel, aldehyde, stale [1,7,8]	31.57/31.76	123+321
Internal standard			
p-Fluorobenzaldehyde	–	27.28/27.47	319

[a] Peak co-eluated with an unidentified compound.

[12,16,20]. SPME techniques provides many advantages, such as easy automatization, simple management, inextensive work-up or manual labor, and the absence of organic solvents.

In most of the published studies, SPME procedures are optimized taken in account one single variable at a time, keeping all the others constant during experiments [23]. Hence, the interactions among the factors involved in the extraction procedure are thus overlooked [24]. The use of experimental design enables the study of the effects of several variables estimated simultaneously. In particular, factorial designs coupled with a central composite design (CCD) are an effective tool for optimizing a process involving several parameters at the same time [23].

The aim of the present study was to optimize a method to analyze carbonyl compounds in beers using a derivatization procedure with PBFHA followed by an automated HS-SPME combined with GC–IT/MS. An experimental design was used to investigate the influence of the principal parameters affecting the HS-SPME extraction of volatile carbonyl compounds associated to beer aroma deterioration. Thus, for determining the best experimental conditions the central composite design (CCD) was applied.

Validation was carried out in terms of limit of detection and quantification, linearity, method precision and accuracy. Applicability of the method to commercial larger beers was also assessed. To our knowledge, the application of CCD to optimize

the derivatization and extraction conditions for the analysis of volatile carbonyl compounds in beer was never used before.

2. Materials and methods

2.1. Materials

PFBHA (≥98%), propanal (≥97%), hexanal (≥98%), heptanal (≥92%), octanal (≥98%), nonanal (≥95%), decanal (≥95%), undecanal (≥96%), 2-propenal (≥99%), (*E*)-2-pentenal (≥95%), (*E*)-2-hexenal (≥98%), (*E*)-2-heptenal (≥95%), (*E*)-2-octenal (≥95%), (*E*)-2-nonenal (≥93%), (*E*)-2-octenal (≥94%), (*E*)-2-decenal (≥92%), (*E*,*E*)-2,4-hexadienal (≥95%), (*E*,*E*)-2,4-nonadienal (≥85%), (*E*,*E*)-2,4-decadienal (95%), 2-methyl-1-butanal (≥90%), 2-methyl-1-propanal (≥98%), 3-methyl-1-butanal (≥97%), benzaldehyde (≥99.5%), phenyl acetaldehyde (≥90%), 3-(methylthio)propional (methional, ≥98%), diacetyl (≥97%), methylglyoxal (40%), 2-butanone (≥99%), 2-hexanone (≥98%), 2-pentanone (≥99.5%), 2-heptanone (≥98%), 2-octanone (≥98%), 2-nonanone (≥97%), 2-decanone (≥98%), 2-undecanone (≥97%), 2-furfural (≥99%), 5-methyl-2-furfural (≥99%), 5-hydroxymethylfurfural (≥99%), and *p*-fluorobenzaldehyde (internal standard, ≥98%) were purchased from Sigma-Aldrich (Madrid, Spain). Acetaldehyde (≥99.5%), butanal (≥99%) and glyoxal (40%) were purchased from Fluka (Madrid, Spain). Ethanol (≥99.8%) was also supplied by Sigma-Aldrich. Ultrapure water was obtained from a Milli-Q water Millipore purification system (Millipore, Bedford, MA, USA). PDMS/DVB (65 μm) SPME fibers were purchased from Supelco (Madrid, Spain).

In this study 10 commercial lager beers were used to prove the applicability of the method.

2.2. Standard solutions

For each carbonyl compounds, standard solutions were prepared in ethanol at 1 g l^{-1} and stored at 4 °C. These standard solutions were subsequently diluted in beer for the optimization experiments and for the method validation. Each carbonyl compound was diluted in ethanol solution (5%) for determining retention time and mass spectra characteristics.

The PFBHA solution was daily prepared in ultrapure water at 20 g l^{-1}. *p*-Fluorobenzaldehyde was selected as internal standard (IS) taking into account a previous work reported by Saison et al. [16,20]. The IS was also prepared in ethanol at 1 g l^{-1} and further diluted at 500 μg l^{-1}.

2.3. Optimization of the derivatization and HS-SPME procedure

The HS-SPME conditions were optimized using a CCD (with α=2.000), based on a 2^4 factorial design plus eight axial points plus five replicates in the center of the design. The variables chosen for HS-SPME optimization were the extraction time (t_{ex}, min), the incubation time (t_{inc}, min), the extraction temperature (T_{ex}, °C) and the amount of PFBHA in the solution/beer. The factor levels and experimental domain are shown in Table 2. Twenty-nine experiments were generated by CCD and executed in randomized order. The effect of salting out in the HS-SPME procedure was also assessed at the optimum conditions, by the addition of 0.5, 1 and 1.5 g of NaCl to beer. All the experiments were performed with a beer spiked with 10 μg l^{-1} of alkanals, alkenals alkadienals, dicarbonyl compounds and ketones and 500 μg l^{-1} of 5-hydroxymethylfurfural. The 65 μm PDMS/DVB fiber coating was selected for its affinity to retain the oxime derivatives as previously investigated [10,12,16,18,20]. Five milliliters of beer containing 100 μl of IS and different amounts of the PFBHA solution were added to a 20 ml vial. The vials were equilibrated at least for 20 min at room temperature

Table 2
Factor levels and experimental domain applied to optimize the HS-SPME experimental conditions.

Factor	Experimental domain				
	$-\alpha$[a]	−1	0	+1	$+\alpha$[a]
PFBHA (mg l^{-1})	200	400	600	800	1000
Incubation time (t_{inc} − min)	5	10	15	20	25
Extraction time (t_{ex} − min)	20	30	40	50	60
Extraction temperature (T_{ex} − °C)	30	40	50	60	70

[a] α=2.000.

previously to HS-SPME–GC–IT/MS analysis. The HS-SPME procedures were performed using a Combi-PAL autosampler (Varian Pal Autosampler, Switzerland) and the Cycle Composer software (CTC Analytics System Software, Switzerland). The vials were equilibrated at the extraction temperature with a stirring speed of 250 rpm. Afterwards, the fiber was exposed to the HS under the same stirring speed. Adsorption time of the fiber into GC injector was 2 min.

2.4. GC–MS analysis

GC–MS analysis was performed with a Varian CP-3800 gas chromatograph coupled to a Varian Saturn 4000 ion trap mass selective detector and a Saturn GC/MS workstation software version 6.8 equipped with a VF-5 ms (30 m × 0.25 mm × 0.25 μm) column (VARIAN). A CombiPAL automatic autosampler (Varian, Palo Alto, CA) was used for all experiments. The injector port was heated to 250 °C. Injections were performed in splitless mode. The carrier gas was helium C-60 (Gasin, Portugal), at a constant flow of 1 ml min^{-1}. The oven temperature program was from 40 °C (1 min) to 250 °C (5 min) at 5 °C/min. The ion trap detector was set as follow: the transfer line, manifold and trap temperatures were 280 °C, 50 °C and 180 °C, respectively. All mass spectra were acquired in the electron impact (EI). The mass range was 35–600 *m*/*z*, with a scan rate of 6 scan/s. The emission current was 50 μA, and the electron multiplier was set in relative mode to auto-tune procedure. The maximum ionization time was 25,000 μs, with an ionization storage level of 35 *m*/*z*. The analysis was performed in Full Scan mode. The selected ions used for qualitative analysis are presented in Table 1. A chromatogram of a beer is presented in Fig. 1A and B. As expected, for many carbonyl compounds, two or three isomers oximes appear separated in the chromatogram and the sum of peaks was considered in the different steps of method development. When co-elution was observed, it was only considered one peak.

2.5. Method validation

2.5.1. Calibration and detection limits

Calibration curves were created for quantification of volatile compounds using the optimized HS-SPME conditions. The linear ranges of the method were analyzed by performing calibration curves using different concentration levels in beer. All analyzes were performed in triplicate. The method linearity was determined by evaluation of the regression curves (ratio of the standard peaks areas by IS peak area against the concentration) and expressed by the squared determination coefficient (r^2).

The limits of detection (LOD) and quantification (LOQ) were determined from calibration curves data, following European Medicines Agency (EMEA) criteria. The LOQ was defined as the lowest concentration of the calibration curve based on a signal-to-noise ratio of 10. To define the LOD, it was used a model synthetic solution containing small known concentrations of the standards aroma until reach the signal-to-noise ratio of 3. All the analyses were performed in triplicate.

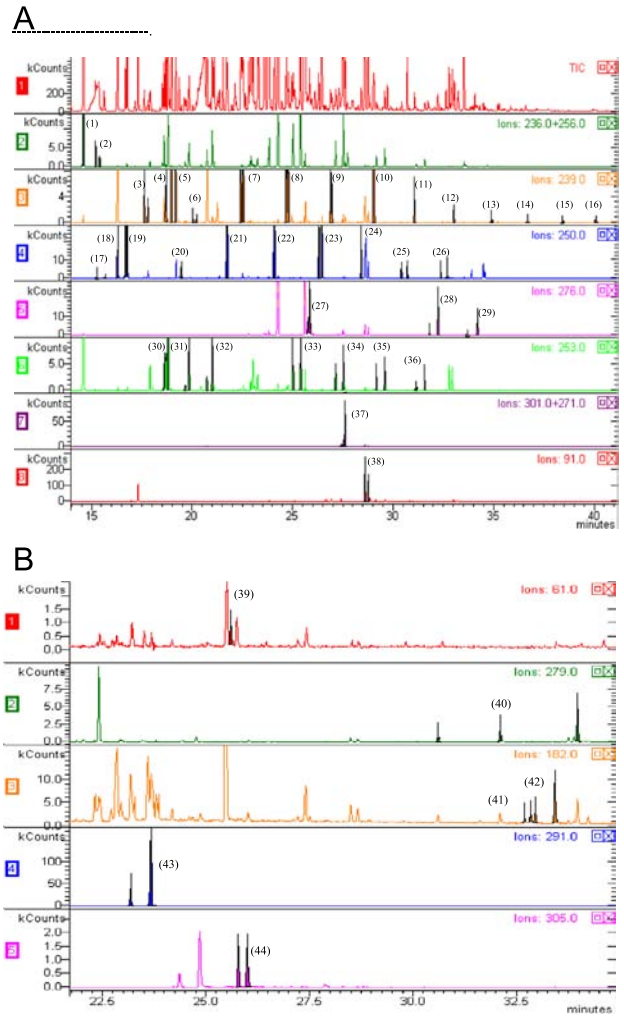

Fig. 1. Chromatogram of carbonyl compounds in beer. (A) ethanal (1), propanal (2), butanal (3), 2-methyl-1-propanal (4), 3-methyl-1-butanal (5), pentanal (6), hexanal (7), heptanal (8), octanal (9), nonanal (10), decanal (11), undecanal (12), dodecanal (13), tridecanal (14), tretadecanal (15), pentadecanal (16), 2-propenal (17), 2-methyl-1-propanal (18), 2-butanone (19), (*E*)-2-butenal (20), (*E*)-2-pentenal (21), (*E*)-2-hexenal (22), (*E*)-2-heptenal (23), (*E*)-2-octenal (24), (*E*)-2-nonenal (25), (*E*)-2-decenal (26), (*E*,*E*)-2,4-hexadienal (27), (*E*,*E*)-2,4-nonadienal (28), (*E*,*E*)-2,4-decadienal (29), 2-pentanone (30), 2-hexanone (31), 2-heptanone (32), 2-octanone (33), 2-nonanone (34), 2-decanone (35), 2-undecanone (36), benzaldehyde (37) and phenylacetaldehyde (38). (B) methional (39), diacetyl (40), glyoxal (41), methylglyoxal (42), 2-furfural (43), and 5-methyl-2-furfural (44).

2.5.2. Precision and accuracy

The intraday precision was evaluated after an analysis by GC–IT/MS, on the same day, of three different concentrations of the standard compounds in the same beer. The interday precision was determined by repeating the intraday precision study during 3 different days. All the analyses were performed in triplicate. Precision was assessed as the relative standard deviation of these values. The accuracy of the method was determined through the calculation of the percent deviation between the calculated value and the nominal value.

2.6. Qualitative and quantitative analyses

The identification of carbonyl compounds in samples was achieved by comparing the retention time and mass spectra obtained by comparison with the standard compounds injected at the same conditions and also comparing the MS fragmentation present with the mass spectra of the National Institute of Standards and Technology (NIST) MS 05 spectral database.

2.7. Statistical analyses

An analysis of variance (ANOVA) was applied to the experimental data; results were considered significant if the associated p value was below 0.05. The significant differences were determined by Tukey tests. The statistical analyses and the CCD were performed using Statistica, Version 7.0 (Statsoft Inc., Tulsa, OK, USA).

3. Results and discussion

3.1. Derivatization and optimization of HS-SPME extraction

The use of the most appropriate SPME fiber depends on the target compounds and the matrix studied. The selection of the best type of fiber coating in the HS-SPME extraction of carbonyl compounds, after derivatization with PFBHA, has previously been studied in beer [12,16,19,20], wine [17] and spirits [10]. These authors concluded that the most effective fiber is composed of PDMS and DVB components. Thus, the present study was performed using PDMS/DVB fiber, and the optimization of sampling conditions affecting the microextraction processes were evaluated using a CCD. The factors (variables) selected were the concentration of PBFHA in beer, the incubation and the extraction time and temperature. The CCD is used not only to evaluate the variables significance, but also to analyze the interaction among them.

The design involved 29 runs, which were performed in random order (Table 3). The response was based on the sum of the peak areas of all the volatile compounds identified in the sample. Peak areas were obtained using m/z characteristic ions (diagnosis ions)

Table 3
Experimental conditions and response values (total area) of the CCD used to optimize the extraction and derivatization conditions of volatile compounds in beer.

Run	PFBHA (mg l^{-1})	t_{in} (min)[a]	t_{ex} (min)[b]	T (°C)[c]	Response value (total area)[d]
1	400	10	40	30	28073874
2	400	10	40	50	36438143
3	400	10	60	30	33582901
4	400	10	60	50	44412875
5	400	20	40	30	30386553
6	400	20	40	50	34935525
7	400	20	60	30	31710030
8	400	20	60	50	37922457
9	800	10	40	30	30802921
10	800	10	40	50	34932881
11	800	10	60	30	33210367
12	800	10	60	50	41225822
13	800	20	40	30	27589728
14	800	20	40	50	31438977
15	800	20	60	30	28819370
16	800	20	60	50	33074405
17	200	15	50	40	33537809
18	1000	15	50	40	31333392
19	600	5	50	40	40761483
20	600	25	50	40	34137284
21	600	15	30	40	26221159
22	600	15	70	40	33950061
23	600	15	50	20	27484182
24	600	15	50	60	41126511
25	600	15	50	40	35995180
26	600	15	50	40	37250693
27	600	15	50	40	36505862
28	600	15	50	40	36812579
29	600	15	50	40	36636468

[a] t_{ext} – extraction time.
[b] t_{inc} – incubation time.
[c] T – extraction and incubation temperature.
[d] Total area is expressed in arbitrary units.

(Table 1). The sum of the peak areas is one of the most employed parameters for the optimization of the SPME conditions and gives information on the intensity of the volatile compounds extracted [23,24]. In the mass spectra of the pentafluorobenzyloxime derivatives, molecular ions are often missing and spectra are characterized by the pentafluorobenzyl ion of m/z 181, which is used in many applications for screening the total ion current (TIC) chromatograms for such derivatives [17,18]. However, a better separation of derivatives is obtained selecting diagnosis m/z ions (Table 1). Mass spectra of several carbonyl compounds are presented in Fig. 2.

To evaluate the significance of each factor and interaction terms, analysis of variance (ANOVA) was used. The quality of fit of the model equation was represented by the coefficient of determination (R^2 and adjusted-R^2). R^2 of 0.989 and adjusted-$R^2$of 0.979 showed a good relationship between experimental data and fitted model, as well as the high potential of model in prediction of response. The experimental error was calculated using the replication values of the central point; the RSD was 1.2%, showing the good reproducibility of the method.

The main effects and their interactions are presented in the Pareto chart shown in Fig. 3. The extraction time, extraction temperature and concentration of PFBHA were the most significant variables. The incubation time was not significant in the experimental domain and was fixed at 7 min.

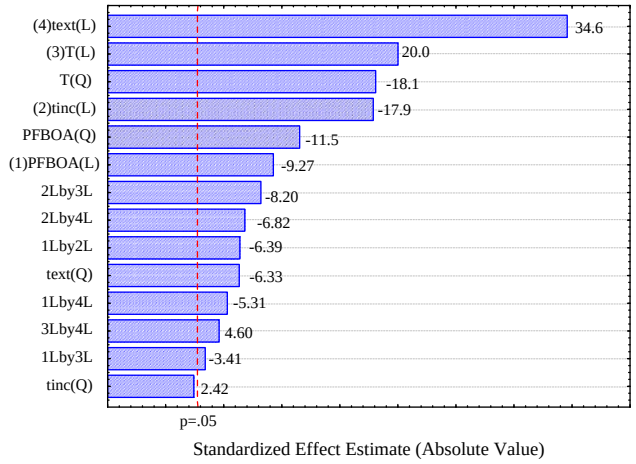

Fig. 3. Pareto chart for the total area of all volatile carbonyl compounds of the GC–IT/MS analysis of the HS-SPME of beer.

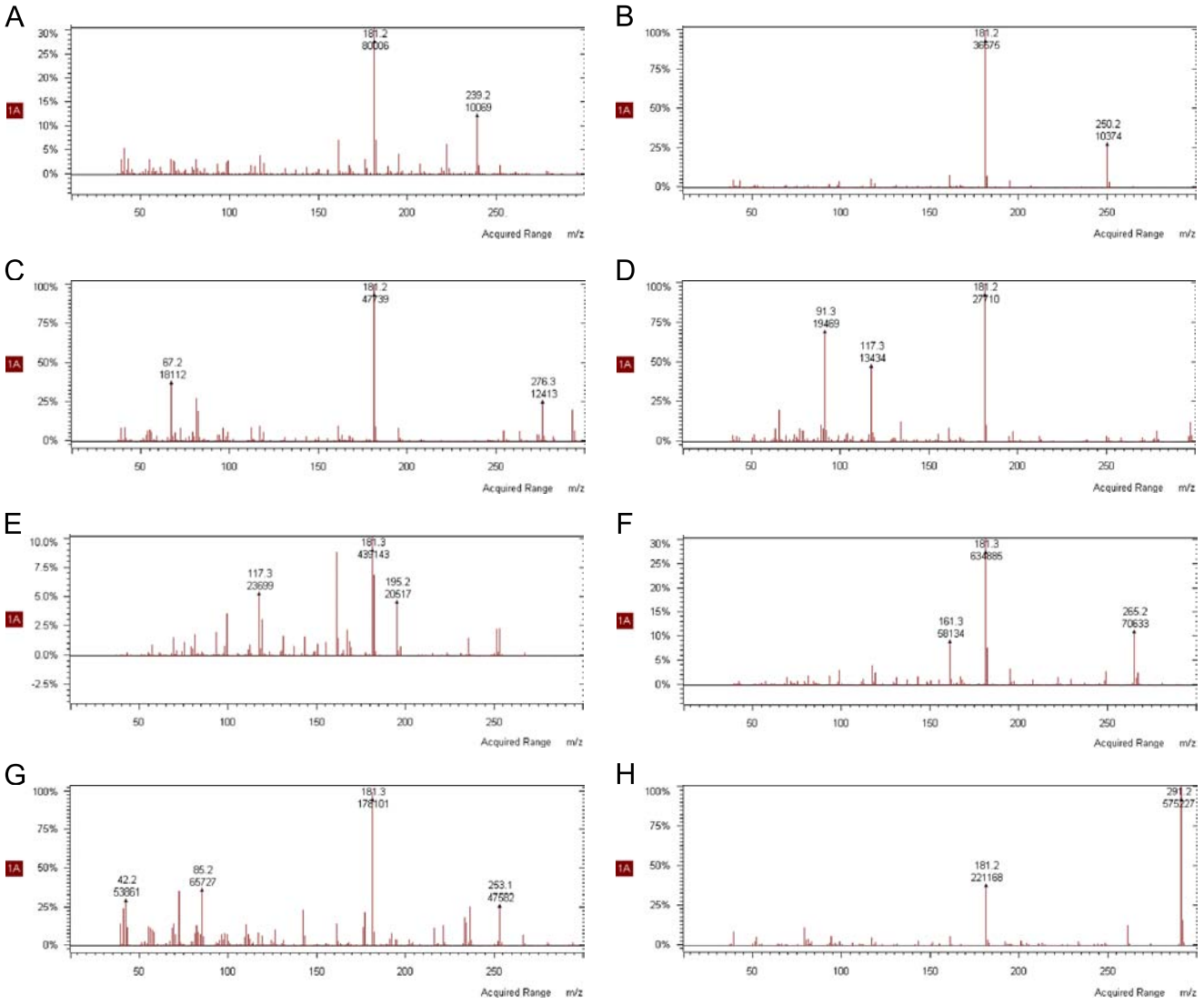

Fig. 2. Mass spectra of the PBFHA derivative of (A) hexenal, (B) (*E*)-2-butenal, (C) (*E,E*)-2,4-hexadienal, (D) phenylacetaldehyde, (E) glyoxal, (F) methylglyoxal, (G) 2-octanone and (H) 2-furfural.

The extraction time and temperature had a strong positive influence, and the interaction between these variables was also significant. The effects of extraction time and temperature, with a PBFHA content of 600 mg l^{-1} and an incubation temperature equal to 15 min, on the total area of volatile compounds can be visualized in the surface model (Fig. 4A). The extraction temperature influences the partition coefficients of the volatile compounds both between the sample and the HS and between the HS and the fiber, as well as the vapor pressure of the compounds in the sample [23,25,26]. In this way, an increase in sampling temperature increases the HS concentration of volatile compounds, favoring their extraction. However, the PFBHA content in the solution had a negative effect, as well as its interaction with extraction time and temperature. The influence of PFBHA and extraction time, and PFBHA and extraction temperature fixed in their central points on the total area of volatile compounds can be visualized in the surface model presented in Fig. 4B and C. High temperatures and long extraction times increase the levels of PFBHA molecule in the HS, resulting in a saturation of fiber with PFBHA and consequently a low adsorption of pentafluorobenzyloxime derivatives into the fiber causing a decrease of the total of volatile carbonyl compounds analyzed.

The optimum conditions obtained for beer matrix were 700 mg l^{-1} of PFBHA, a temperature of extraction of 45 °C and 20 min of extraction time and 7 min of incubation time at 45 °C.

Under these conditions, the effect of ionic strength on the extraction efficiency was also examined through the addition of 0.5, 1 and 1.5 g of NaCl. Results obtained showed that the addition of NaCl had a negative effect on the extraction efficiency, reducing the response value (data not shown). Moreover, no significant differences were found respecting the response value of each compound extracted from beer with and without the addition of 0.5 g of NaCl. Taking into account these results, it was decided not include NaCl in the HS-SPME procedure. Similar results were obtained in previous works [19,20].

3.2. Method validation

Linear range, determination coefficients, limits of quantification (LOQ) and limits of detection (LOD) of the proposed method are presented in Table 4. The determination coefficients indicated a good linearity for most compounds; only four compounds showed linearity less than 0.99. In the proposed method, ethanal and 2-butanone were not validated as their initial contents in beer were very high, so calibrations curves obtained by the addition of

Table 4
Linear range, determination coefficients, limits of quantification (LOQ) and limits of detection (LOD) of the proposed method.

Compounds	Linear range (μg l^{-1})	Determination coeficiente (r^2)	LOQ (μg l^{-1})	LOD (μg l^{-1})
Alkanals				
Propanal	0.770–19.2	0.988	0.107	0.035
Butanal	1.13–28.3	0.991	0.113	0.037
Hexanal	0.915–22.9	0.990	0.132	0.043
Heptanal	1.01–25.0	0.999	0.164	0.054
Octanal	1.12–22.4	0.995	0.105	0.035
Nonanal	0.963–24.08	0.997	0.096	0.032
Decanal	1.07–26.8	0.996	0.107	0.035
Undecanal	0.103–25.8	0.990	0.011	0.003
Alkenals				
2-Propenal	2.15–28.8	0.993	1.55	0.510
(*E*)-2-pentenal	1.13–28.2	0.996	0.186	0.061
(*E*)-2-hexenal	0.021–25.9	0.993	0.012	0.004
(*E*)-2-heptenal	0.024–29.8	0.999	0.010	0.003
(*E*)-2-octenal	0.111–27.9	0.998	0.041	0.013
(*E*)-2-nonenal	0.104–25.9	0.998	0.027	0.009
(*E*)-2-decenal	0.104–33.9	0.989	0.089	0.030
Alkadienals				
(*E*,*E*)-2,4-hexadienal	1.80–18.0	0.993	0.015	0.005
(*E*,*E*)-2,4-nonadienal	0.826–26.4	0.997	0.010	0.031
(*E*,*E*)-2,4-decadienal	1.81–18.1	0.997	0.025	0.074
Strecker aldehydes				
2-Methyl-1-propanal	0.464–65.9	0.987	0.116	0.038
2-Methyl-1-butanal	0.194–30.2	0.995	0.150	0.050
3-Methyl-1-butanal	1.17–117	0.988	0.094	0.031
Benzaldehyde	1.24–30.9	0.996	0.180	0.059
Phenyl acetaldehyde	2.17–32.6	0.992	0.087	0.029
Methional	2.18–32.7	0.993	0.139	0.046
Dialdehydes				
Diacetyl	3.86–38.6	0.996	0.124	0.041
Glyoxal	3.50–140	0.994	0.140	0.046
Methylglyoxal	9.55–191	0.991	0.061	0.020
Ketones				
2-Hexanone	1.42–35.4	0.994	0.028	0.009
2-Octanone	1.36–34.1	0.996	0.061	0.021
2-Nonanone	1.42–22.7	0.992	0.026	0.009
2-Decanone	1.04–26.1	0.992	0.076	0.025
2-Undecanone	1.48–23.7	0.994	0.058	0.019
Furans				
2-Furfural	58.2–582	0.991	4.68	1.54
5-Methyl-2-furfural	25.1–251	0.998	5.82	1.92
5-Hydroxymethylfurfural	325–10,280	0.990	10.4	3.44

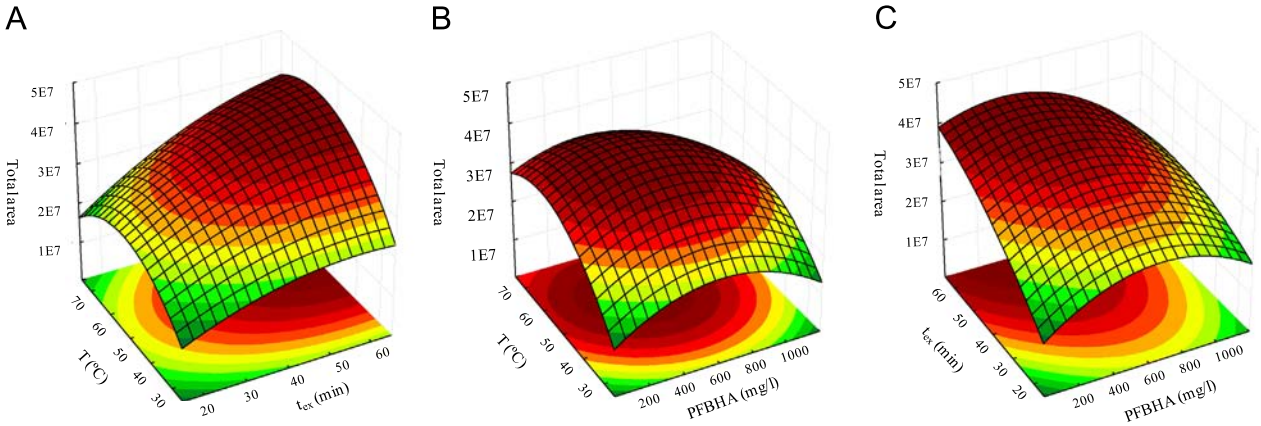

Fig. 4. Response surface model for total area of all volatile compounds vs. (A) temperature (T) and extraction time (t_{ex}; incubation time = 15 min, PFBHA = 600 mg l^{-1}), (B) temperature (T) and PFBHA content (incubation time = 15 min, extraction time = 40 min), and (C) extraction time (t_{ex}) and PFBHA content (incubation time = 15 min, temperature = 50 °C).

Table 5
Precision and accuracy of the proposed method.

Compounds	Concentration ($\mu g\ l^{-1}$)	Intraday precision (%)	Interday precision (%)	Accuracy (%)
Propanal	0.770	7.7	8.3	94
	3.08	5.8	5.4	106
	7.70	3.9	11	104
Butanal	1.13	10	7.0	111
	4.53	3.4	9.6	109
	11.3	3.9	7.3	99
Hexanal	0.915	7.5	7.8	94
	3.66	7.2	7.3	100
	9.15	3.6	5.6	99
Heptanal	1.01	2.6	3.0	105
	4.03	4.5	6.7	105
	10.1	1.5	4.0	98
Nonanal	0.963	8.5	8.9	100
	3.85	4.8	8.9	99
	9.63	4.3	7.5	96
Decanal	1.07	6.3	11	101
	4.29	3.1	5.7	105
	10.7	5.6	6.2	98
Undecanal	1.03	12	13	95
	4.13	7.4	8.1	99
	13.3	4.6	4.7	100
2-Propenal	2.15	6.3	6.2	102
	4.61	2.7	4.9	98
	11.5	2.4	4.4	100
(*E*)-2-pentenal	1.13	5.9	8.7	96
	4.50	2.9	9.5	103
	11.3	2.4	4.6	102
(*E*)-2-hexenal	1.04	5.4	9.9	99
	4.15	4.4	9.5	100
	10.4	2.9	4.1	104
(*E*)-2-heptenal	1.19	1.8	3.7	92
	4.76	3.7	4.3	102
	11.9	1.9	2.9	102
(*E*)-2-octenal	1.11	4.4	6.6	109
	4.46	5.5	6.2	96
	11.1	0.8	4.4	98
(*E*)-2-nonenal	1.04	2.9	3.6	97
	4.14	8.1	7.8	101
	10.4	1.2	5.5	100
(*E*)-2-decenal	1.35	4.1	4.3	92
	5.42	6.8	6.9	100
	13.5	2.7	4.8	99
(*E*,*E*)-2,4-hexadienal	1.80	4.0	15	96
	7.19	0.3	5.4	100
	18.0	2.5	1.8	98
(*E*,*E*)-2,4-nonadienal	0.826	12	8.1	99
	3.30	1.1	2.3	100
	8.26	4.5	13	105
(*E*,*E*)-2,4-decadienal	1.80	12	17	101
	7.21	8.5	4.9	98
	18.0	1.2	1.3	100
2-Methyl-1-propanal	6.59	6.6	11	95
	26.4	3.9	6.4	97
	65.9	7.2	6.5	99
2-Methyl-1-butanal	3.02	6.2	11	101
	12.1	6.6	10	97
	30.2	3.0	3.1	99
3-Methyl-1-butanal	11.7	1.2	7.6	105
	46.9	1.7	5.1	98
	117	4.0	9.0	98
Benzaldehyde	1.24	1.1	2.8	93
	4.95	6.1	7.2	101
	12.4	2.9	3.7	99
Phenyl acetaldehyde	5.43	4.3	13	102
	21.7	1.4	4.6	105
	54.3	2.3	3.1	99
Methional	2.18	14	16	99
	13.1	3.0	7.6	96
	21.8	2.6	4.2	99
Diacetyl	3.86	9	10	93
	15.5	0.1	3.5	99
	38.6	0.3	2.3	100
Glyoxal	5.46	0.4	15	88
	21.9	1.0	15	99
	54.6	3.6	10	98
Methylglyoxal	14.3	1.2	9.7	88
	38.2	5.0	4.4	95
	95.5	4.0	5.6	94
2-Hexanone	1.42	3.4	5.1	98
	5.66	3.6	4.5	97
	14.2	1.9	3.8	100
2-Octanone	1.36	4.2	6.1	104
	5.46	5.2	6.2	102
	13.7	3.3	3.3	99
2-Nonanone	1.42	1.6	16	95
	3.64	1.5	4.0	103
	9.09	3.2	6.4	99
2-Decanone	1.04	4.8	7.0	98
	4.18	6.5	5.6	98
	10.4	1.9	1.7	100
2-Undecanone	1.48	2.7	10	99
	5.93	1.5	5.1	105
	14.8	3.7	3.8	100
2-Furfural	58.2	2.8	1.5	103
	349	5.6	4.8	99
	582	0.5	1.3	99
5-Methyl-2-furfural	25.1	5.6	13	89
	116	3.7	9.2	101
	251	2.0	11	98
5-Hydroxymethylfurfural	428	11	6.4	114
	857	5.5	12	97
	1714	0.8	3.9	101

known contents of carbonyl compounds in beer did not result properly.

LOQ obtained for the compounds analyzed varied between 0.010 and 1.55 $\mu g\ l^{-1}$; however, higher LOQ values were found for furans presenting a LOQ of 4.68 $\mu g\ l^{-1}$ for 2-furfural, 5.82 $\mu g\ l^{-1}$ for 5-methyl-2-furfural and 10.4 $\mu g\ l^{-1}$ for 5-hydroxymethylfurfural. Similarly, the LOD for furans (1.54–3.44 $\mu g\ l^{-1}$) were higher than for the others compounds (0.003–0.510 $\mu g\ l^{-1}$).

Precision and accuracy results are presented in Table 5. The developed method is precise as the RSD values calculated for intra and interday precision did not exceed 15%. Moreover, the recovery values calculated varied from 88% to 114%.

3.3. Proof applicability

Proof applicability of the method was performed to quantify carbonyl compounds composition of commercial lager beers (Table 6). Within alkanals, nonanal (3.94–10.6 $\mu g\ l^{-1}$), hexanal (0.458–3.53 $\mu g\ l^{-1}$) and propanal (0.322–1.87 $\mu g\ l^{-1}$) were found at the highest amounts. When detected in beers, 2-propenal was found with concentrations up to 4.61 $\mu g\ l^{-1}$ and (*E*)-2-nonenal up to 0.304 $\mu g\ l^{-1}$. (*E*)-2-Nonenal, when present at levels superior to its olfactory perception threshold (0.035 $\mu g\ l^{-1}$), is responsible for the papery/cardboard character developed in aged beers [3,4].

Table 6
Concentration of carbonyl compounds in beers.

Compound (μg/l)	A	B	C	D	E	F	G	H	I	J
Propanal	0.399 (0.022)^{ab}	0.322 (0.043)^{a}	0.488 (0.147)^{ab}	1.38 (0.29)^{c}	1.57 (0.00)^{cd}	1.33 (0.18)^{c}	0.506 (0.005)^{ab}	1.87 (0.31)^{d}	0.841 (0.129)^{b}	0.648 (0.137)^{ab}
Butanal	nq^{ab}	nd^{a}	0.192 (0.051)^{bc}	0.336 (0.049)^{de}	0.364 (0.017)^{de}	0.333 (0.042)^{cde}	0.228 (0.006)^{cd}	0.463 (0.056)^{e}	0.347 (0.093)^{de}	0.318 (0.074)^{cd}
Pentanal[i]	0.182 (0.022)^{ab}	0.083 (0.014)^{a}	0.496 (0.219)^{d}	0.348 (0.062)^{bcd}	0.483 (0.043)^{cd}	0.406 (0.029)^{bcd}	0.313 (0.029)^{abcd}	0.808 (0.078)^{e}	0.354 (0.040)^{bcd}	0.252 (0.048)^{abc}
Hexanal	0.949 (0.074)^{ab}	0.458 (0.095)^{a}	2.54 (1.23)^{cd}	1.39 (0.21)^{abc}	1.61 (0.18)^{abc}	1.98 (0.83)^{bc}	1.07 (0.13)^{ab}	3.53 (0.22)^{d}	1.07 (0.11)^{ab}	0.939 (0.183)^{ab}
Heptanal	0.369 (0.050)^{cde}	nq^{a}	0.186 (0.051)^{ab}	0.492 (0.082)^{def}	0.457 (0.031)	0.380 (0.022)^{cde}	0.327 (0.017)^{bcd}	0.582 (0.092)^{f}	0.272 (0.048)^{bc}	0.549 (0.028)^{f}
Octanal	0.553 (0.036)^{ab}	0.422 (0.067)^{a}	0.575 (0.159)^{bc}	0.963 (0.224)^{bc}	1.02 (0.32)^{c}	0.709 (0.009)^{abc}	0.614 (0.063)^{abc}	0.765 (0.142)^{abc}	0.668 (0.116)^{abc}	0.755 (0.016)^{abc}
Nonanal	6.94 (0.46)^{abc}	3.94 (0.34)^{a}	5.19 (0.68)^{ab}	10.0 (0.4)^{bc}	10.6 (4.9)^{c}	6.32 (0.19)^{abc}	7.17 (0.83)^{abc}	6.19 (1.03)^{abc}	7.00 (1.63)^{abc}	7.35 (1.11)^{abc}
Decanal	0.957 (0.104)^{a}	0.718 (0.151)^{a}	0.663 (0.088)^{a}	1.21 (0.22)^{a}	1.22 (0.55)^{a}	0.743 (0.052)^{a}	1.15 (0.25)^{a}	0.936 (0.174)^{a}	2.27 (0.21)^{b}	0.717 (0.124)^{a}
Undecanal	nd^{a}	0.137 (0.001)^{b}	nd^{a}	nd^{a}	nd^{a}	0.167 (0.013)^{b}	nd^{a}	0.260 (0.056)^{c}	0.331 (0.007)^{d}	0.269 (0.031)^{c}
2-Propenal	nq^{ab}	nd^{a}	nd^{a}	2.06 (0.43)^{d}	4.61 (0.69)^{e}	1.75 (0.23)^{cd}	2.57 (0.21)^{d}	1.83 (0.10)^{cd}	0.976 (0.238)^{bc}	nd^{a}
(*E*)-2-Butenal[j]	1.79 (0.24)^{bc}	0.284 (0.006)^{a}	1.09 (0.10)^{b}	1.57 (0.15)^{b}	4.45 (0.31)^{f}	1.23 (0.13)^{b}	3.47 (0.33)^{e}	2.33 (0.17)^{cd}	1.22 (0.17)^{b}	2.62 (0.50)^{d}
(*E*)-2-pentenal	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
(*E*)-2-hexenal	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
(*E*)-2-heptenal	0.010 (0.001)^{b}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.014 (0.001)	nd^{a}	nd^{a}
(*E*)-2-octenal	0.254 (0.012)^{a}	0.552 (0.015)^{f}	0.408 (0.025)^{bcde}	0.377 (0.006)^{bcd}	0.312 (0.097)^{abc}	0.411 (0.008)^{cde}	0.796 (0.001)^{g}	0.455 (0.023)^{def}	0.516 (0.082)^{ef}	0.286 (0.014)^{ab}
(*E*)-2-nonenal	0.304 (0.002)^{b}	0.282 (0.006)^{b}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.195 (0.169)^{b}	0.277 (0.005)^{b}	0.274 (0.003)^{b}
(*E*)-2-decenal	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
(*E*,*E*)-2,4-hexadienal	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
(*E*,*E*)-2,4-nonadienal	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
(*E*,*E*)-2,4-decadienal	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2-Methyl-1-propanal	13.0 (1.3)^{ab}	5.11 (1.30)^{a}	7.96 (1.86)^{ab}	26.6 (4.47)^{bcd}	42.5 (3.8)^{cd}	26.2 (3.3)^{bc}	69.7 (8.3)^{e}	68.2 (11.8)^{e}	46.2 (11.9)^{d}	33.9 (8.3)^{cd}
2-Methyl-1-butanal	5.35 (0.25)^{ab}	4.56 (0.46)^{a}	5.38 (0.15)^{ab}	8.89 (1.03)^{d}	14.6 (0.7)^{f}	9.11 (0.67)^{d}	12.4 (0.8)^{e}	11.5 (0.7)^{e}	7.37 (0.53)^{cd}	6.48 (0.64)^{bc}
3-Methyl-1-butanal	28.9 (1.1)^{a}	25.6 (4.2)^{a}	35.7 (5.7)^{ab}	60.7 (7.3)^{c}	92.3 (0.1)^{d}	68.7 (6.2)^{c}	62.0 (5.4)^{c}	66.3 (3.4)^{c}	44.8 (2.6)^{b}	39.0 (5.3)^{ab}
Benzaldehyde	1.17 (0.01)^{e}	0.627 (0.071)^{ab}	0.704 (0.004)^{b}	0.608 (0.019)^{ab}	0.691 (0.003)^{ab}	0.636 (0.040)^{ab}	1.01 (0.02)^{d}	0.853 (0.022)^{c}	1.06 (0.05)^{d}	0.592 (0.025)^{a}
Phenyl acetaldehyde	12.8 (0.3)^{e}	7.33 (0.13)^{d}	6.58 (1.53)^{cd}	4.60 (0.54)^{bc}	5.29 (0.20)^{bcd}	3.99 (0.47)^{ab}	21.6 (0.9)^{f}	7.36 (0.43)^{d}	12.2 (1.2)^{e}	2.18 (0.03)^{a}
Methional	24.2 (4.9)^{c}	nd^{a}	nd^{a}	nd^{a}	21.8 (1.6)^{c}	nd	nd^{a}	15.7 (0.2)^{b}	nd^{a}	nd^{a}
Diacetyl	23.4 (0.8)^{d}	14.5 (1.4)^{bc}	29.6 (0.6)^{e}	13.9 (1.1)^{bc}	12.9 (2.7)^{bc}	10.3 (1.8)^{ab}	14.4 (0.1)^{bc}	30.1 (2.2)^{e}	7.40 (0.64)^{a}	12.8 (0.2)^{bc}
Glyoxal	30.5 (2.5)^{d}	38.4 (1.4)^{c}	11.0 (0.7)^{bc}	2.12 (0.80)^{a}	8.24 (1.24)^{b}	5.28 (0.84)^{ab}	29.6 (5.2)^{d}	7.28 (1.04)^{ab}	15.1 (1.0)^{c}	5.72 (0.44)^{ab}
Methylglyoxal	25.5 (2.4)^{e}	15.4 (1.3)^{abc}	18.6 (1.9)^{d}	13.4 (0.52)^{abc}	6.30 (2.52)^{ab}	15.4 (0.2)^{cd}	38.2 (1.2)^{f}	14.3 (1.6)^{bcd}	16.4 (2.3)^{cd}	10.6 (0.7)^{ab}
2-Pentanone	0.104 (0.005)^{a}	0.148 (0.014)^{ab}	0.130 (0.002)^{ab}	0.390 (0.039)^{d}	0.423 (0.012)^{de}	0.461 (0.033)^{e}	0.249 (0.010)^{c}	0.225 (0.002)^{c}	0.159 (0.003)^{b}	0.253 (0.012)^{c}
2-Hexanone	nd^{a}	0.083 (0.009)^{ab}	0.191 (0.012)^{bc}	0.786 (0.096)^{f}	0.851 (0.005)^{ef}	0.931 (0.090)^{f}	0.171 (0.013)^{bc}	0.304 (0.032)^{cd}	0.067 (0.005)^{ab}	0.356 (0.069)^{d}
2-Heptanone	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}
2-Octanone	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.033 (0.002)^{b}	nd^{a}	0.107 (0.011)^{c}	nd^{a}	0.028 (0.001)^{b}
2-Nonanone	nd^{a}	0.024 (0.002)^{b}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.036 (0.003)^{c}	nd^{a}	0.056 (0.012)^{d}
2-Decanone	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}
2-Undecanone	0.022 (0.002)^{b}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.167 (0.014)^{c}	nd	nd
2-Furfural	1.68 (0.04)^{a}	27.3 (0.01)^{g}	16.1 (2.0)^{e}	12.8 (0.3)^{d}	3.03 (0.33)^{ab}	11.8 (0.4)^{d}	18.6 (0.2)^{f}	7.55 (0.18)^{c}	32.7 (1.8)^{h}	4.36 (0.22)^{b}
5-Methyl-2-furfural	6.55 (0.02)^{e}	5.90 (0.02)^{c}	5.88 (0.03)^{abc}	5.84 (0.01)^{ab}	5.87 (0.00)^{abc}	5.86 (0.00)^{abc}	5.96 (0.00)^{abc}	6.55 (0.04)^{e}	5.89 (0.01)^{bc}	5.83 (0.01)^{a}
5-Hydroxymethylfurfural	1878 (2 3 8)^{c}	2109 (3 3 3)^{c}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	814 (57)^{b}

Values in parenthesis are standard deviation from three determinations.
Values not sharing the same superscript letters (a–h) within the horizontal line are different according to the Tukey test ($p < 0.05$).
nd: not detected.
nq: not quantified.

[i] Concentration calculated using the calibration curve of hexanal.
[j] Concentration determined using the calibration curve of (*E*)-2-pentenal.

Other alkenals quantified were (*E*)-2-butenal (0.284–4.45 μg l^{-1}), (*E*)-2-heptenal (0–0.014 μg l^{-1}) and (*E*)-octenal (0–0.254 μg l^{-1}). Alkadienals were not detected in any beer sample. The Strecker aldehydes have also an important role in the deterioration of flavor and aroma of beers. In beers, the concentration of 2-methyl-1-propanal varied between 5.11 and 69.7 μg l^{-1}, while 2-methyl-1-butanal varied from 4.56 to 14.6 μg l^{-1} and 3-methyl-1-butanal from 28.9 to 92.3 μg l^{-1}. Benzaldehyde was present in levels ranged from 0.608 to 1.17 μg l^{-1} and phenyl acetaldehyde from 2.18 to 21.6 μg l^{-1}. Methional was not detected in beers B–D; however, it was found at concentrations up to 24.2 μg l^{-1} in beer A. The levels of diacetyl varied between 7.40 and 29.6 μg l^{-1}, glyoxal from 2.12 to 38.4 μg l^{-1} and methyl glyoxal from 6.30 to 38.2 μg l^{-1}. In general, few ketones were detected in beers; the major contributors were 2-pentanone (0.104–0.461 μg l^{-1}) and 2-hexanone (0–0.931 μg l^{-1}). However, furans were presented in higher levels than the other families of carbonyl compounds. 2-Furfural was found at contents varying between 1.68 and 27.3 μg l^{-1} and 5-methyl-2-furfural from 5.83 to 6.55 μg l^{-1}, while 5-hydroxymethylfurfural, when detected, was present at concentrations up to 2109 μg l^{-1}.

4. Conclusion

The HS-SPME coupled with GC–IT/MS is a rapid, simple and solventless method to determine volatile carbonyl compounds in beers. The central composite design was used to optimize the derivatization and extraction conditions. Optimal extraction conditions were obtained using 5 ml of beer added with 700 mg l^{-1} of PFBHA and incubated for 7 min and extracted for 20 min at 45 °C with a PDMS/DVB fiber. Afterwards, calibration and validation was performed for all volatile compounds considered as important aromatic contributors to the staling aroma of beers. The proposed method showed to be linear, precise, accurate and sensitive. The present study showed that short extraction times and low extraction temperatures are sufficient to allow a precise, accurate and sensitive analysis of carbonyl compounds in beers.

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