Evaluation of beer deterioration by gas chromatography–mass spectrometry/multivariate analysis: A rapid tool for assessing beer composition

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A B S T R A C T

Beer stability is a major concern for the brewing industry, as beer characteristics may be subject to significant changes during storage. This paper describes a novel non-targeted methodology for monitoring the chemical changes occurring in a lager beer exposed to accelerated aging (induced by thermal treatment: 18 days at 45 °C), using gas chromatography–mass spectrometry in tandem with multivariate analysis (GC–MS/MVA). Optimization of the chromatographic run was performed, achieving a threefold reduction of the chromatographic time. Although losing optimum resolution, rapid GC runs showed similar chromatographic profiles and semi-quantitative ability to characterize volatile compounds. To evaluate the variations on the global volatile signature (chromatographic profile and *m*/*z* pattern of fragmentation in each scan) of beer during thermal deterioration, a non-supervised multivariate analysis method, Principal Component Analysis (PCA), was applied to the GC–MS data. This methodology allowed not only the rapid identification of the degree of deterioration affecting beer, but also the identification of specific compounds of relevance to the thermal deterioration process of beer, both well established markers such as 5-hydroxymethylfufural (5-HMF), furfural and diethyl succinate, as well as other compounds, to our knowledge, newly correlated to beer aging.

1. Introduction

The organoleptic stability of beer during storage has been a major concern in the brewing industry [1–3]. In fact, consumer acceptability of a specific type of beer depends on a complex network of chemical reactions occurring during storage, which may result in significant flavour deterioration. Indeed, flavour deterioration rate is affected by several external factors, namely packaging [4], temperature [5,6], light [7,8], as well as internal ones such as oxygen content [4,8–10], pH [11,12–16], antioxidants content [17–19] and precursor concentrations of key odorants [11,20–23]. In order to study the impact of these parameters, forced aging protocols have been largely used, with thermal deterioration being widely employed to mimic the natural aging process [11,21,24–27]. Nevertheless, it is important to underline that thermal treatment of beer may originate a disproportionate development of staling compounds when compared with natural aging [10].

Several different chemical mechanisms are known to contribute to the generation of powerful sensory active compounds in beer [2]. Interestingly, the same mechanism may impart simultaneously positive and negative aromas notes. For example, Strecker aldehydes 3-methylbutanal, 2-methylbutanal and 3-methylpropanal could have a positive contribution to beer aroma quality, whereas methional and phenylacetaldehyde are well related to undesirable flavours such as cooked potato and honey-like, respectively [6]. Acetaldehyde [28], (*E*)-2-nonenal [15,25,28], (*E*)-β-damascenone [21,25,28], dimethyl trisulfide (DMTS) [15,20], 3-methylbutanal [28], ethyl 2-methylbutyrate [29], ethyl 3-methylbutyrate [29], 5-hydroymethylfurfural (5-HMF) [28] and furfuryl ethyl ether [11,26,28], are other examples of compounds related to off-flavours characteristic of beer aging. Their presence is usually identified/detected by sensory analysis. Typically, results provided by sensory panels constitute the backbone of quality control of flavour stability at the industrial level. However, the quest for objective analytical methods for on-line assessment of beer stability justifies the need for a high-throughput methodology.

Gas chromatography coupled with mass spectrometry (GC–MS) has been extensively employed to identify and quantify aroma/flavour components in several foodstuffs, including

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beer [5,6,11,27]. However, the process of detection and identification of substances is time-consuming, not only due to the chromatographic deconvolution process but also to data contextualisation [30]. Recent advances on the field of metabolomics enable the application of high throughput methodologies for the characterization of small molecules in biological matrices. Recent work showed that rapid analysis of complex foodstuffs by mass spectrometry, without optimum chromatographic separation, produces spectral fingerprints containing relevant information that could be extracted using multivariate analysis (MVA) [31]. This is, however, limited by the quantitative requirements of a minimum acceptable chromatographic resolution, $R = 1.5$ [32]. MVA allows the extraction of information from complex datasets (e.g. GC–MS datasets) in an untargeted manner, considering the system as multi-dimensional and taking into account potential chemical and physical interactions between the different constituents present in the sample [33]. In fact, GC–MS/MVA has been used with great success in several areas, such as environmental, clinical and pharmaceutical investigation, as well as food and nutrition research [34–37].

In this work, a faster method for identifying/measuring the compositional changes occurring on the complex matrix of a lager beer during thermal deterioration is developed. Faster chromatographic runs were developed, leading to decreased chromatographic resolution, but without losing relevant spectral information. Comparison of rapid runs with longer/conventional GC–MS runs was performed in order to validate the findings. Principal Component Analysis (PCA) of GC–MS data (GC–MS/PCA) enabled the assessment of the main profile changes accompanying beer deterioration, allowing specific relevant compounds to be identified and deeper knowledge of the beer aging process to be obtained.

2. Experimental

2.1. Beer samples

All beer samples were of the same brand (lager beer) produced on the same site and date, and were kindly donated by UNICER, Bebidas de Portugal. To induce beer thermal deterioration, twenty-seven lager beer samples (330 mL bottles with crown cork sealing caps) were stored at a temperature of 45 (±1) °C, in the dark, during 18 consecutive days. Beer bottles were removed on days 0, 1, 2, 3, 5, 7, 10, 13 and 18 for analysis.

2.2. Chemicals

All chemicals employed were of analytical grade: dichloromethane and sodium sulphate anhydrous (Panreac), ethanol and methanol (Merck), n-pentane (Lab-Scan), 3-octanol (Sigma–Aldrich).

2.3. Sample preparation

Beer samples were degassed in an ultrasonic bath for 10 min. Extraction was performed by solid phase extraction (SPE) technique and using previously reported conditions [38]. After optimization of the method, the following conditions were employed: LiChrolut EN (500 mg) cartridges were conditioned by passing through 10 mL of pentane–dichloromethane (20:1), 5 mL of methanol and 5 mL of an aqueous solution containing 5% (v/v) of ethanol. 50 mL of beer sample (after filtration), containing 50 μL of internal standard 3-octanol (concentration of 460 mg L^{-1}), was passed through the SPE cartridge bed at a speed lower than 2 mL min^{-1}. The analytes were then eluted with 6 mL of dichloromethane. The extract was dried by adding anhydrous sodium sulphate.

2.4. GC–MS analysis

A gas chromatograph, Varian 450 GC, equipped with a mass detector, Varian 240-MS, and an ion trap analyzer was used. The injection port was lined with 0.75 mm I.D. splitless glass liner, and worked at 220 °C. The split valve was opened during 0.5 min after injection. Volatile were separated in a VF-WAX (15 m × 0.15 mm × 0.15 μm) capillary column from Varian. Two different oven temperatures were used: (1) conventional runs (optimum chromatographic separation) with the oven temperature held at 40 °C for 1 min, and then increased at a rate of 5 °C min^{-1} to a final temperature of 230 °C, which was held for 2.5 min and (2) rapid runs consisting in holding the oven temperature at 70 °C for 1 min and then increasing at a rate of 25 °C min^{-1} to a final temperature of 240 °C (held for 5 min).

The mass spectrometer operated in the electron impact (E.I) mode at 70 eV, scanning the range m/z 33–350. The electron ionization parameters, ionization time of 200 μs and a maximum ion time of 5000 μs, were fixed from 6.0 to 41.5 min and from 2.0 to 12.8 min for conventional and rapid runs, respectively.

2.5. Multivariate analysis

PCA is a non-supervised method widely used for screening, extracting and compressing multivariate data [39]. Two types of GC–MS data were acquired and processed for chemometric studies:

(i) Conventional runs: the chromatographic domain used was set between 7.00 and 33.5 min (1332 scans). The range of m/z values used was from 39 to 250, as no significant information was detected off that m/z range; m/z values related to chemical noise characteristic of column and solvent, namely 73 and 221 m/z, were excluded giving a total of 278,4k (1332 × 209) points per analysis. As stated above, for each day of analysis, 3 beer (bottled) samples were analyzed, without replicates, giving a total of 27 GC–MS analyses. Therefore, the size of GC–MS data matrix was 7516,5k (278,4k × 27) points.

(ii) Rapid runs: the chromatographic domain used was from 2.25 to 8.00 min (275 scans). The m/z range used was the same as above, excluding m/z values 40, 73 and 221 (chemical noise characteristic of column and solvent) and giving a total of 57,2k (275 × 208) points per analysis. As two replicates were analyzed per sample, a total of 54 analyses were performed and the matrix data size for rapid GC–MS had 3088,8k points (57,2k × 54). It is noted that, although the rapid GC–MS data had two replicates for each sample, the corresponding matrix had less than half the number of points of the conventional one.

Each GC–MS sample data is inherently a matrix, with one dimension related to the chromatographic information (retention time), and the other related to the mass spectra (m/z pattern fragmentation). Therefore, each sample consisted of $\mathbf{S}_{i(1332\times209)}$ and $\mathbf{S}_{i(275\times208)}$ matrices for conventional and rapid runs, respectively, where i corresponds to the number of samples (27 for conventional and 54 for rapid runs). For multivariate analysis, each matrix $\mathbf{S}_i$ was unfolded to give a vector $\mathbf{S}^{T}_{i(1,j)}$, where j corresponds to the number of variables of each analysis. Then, all vectors were row concatenated to give a $\mathbf{Q}_{(i,j)}$ matrix. This $\mathbf{Q}$ matrix was then decomposed by PCA into: $\mathbf{Q}_{(i,j)} = \mathbf{T}_{(i,k)} \cdot \mathbf{P}^{T}_{(k,j)}$, where $\mathbf{T}$ represents the scores matrix, $\mathbf{P}$ represents the loadings matrix and k corresponds to the extracted number of principal components. The scores matrix ($\mathbf{T}$) gives the relationships between samples, whereas the loadings matrix ($\mathbf{P}$) gives the importance of each variable (i.e. retention times and their correspondent mass spectra) to the scores distribution. Finally, and in order to ease the interpretation of the loadings ($\mathbf{P}$), each one of

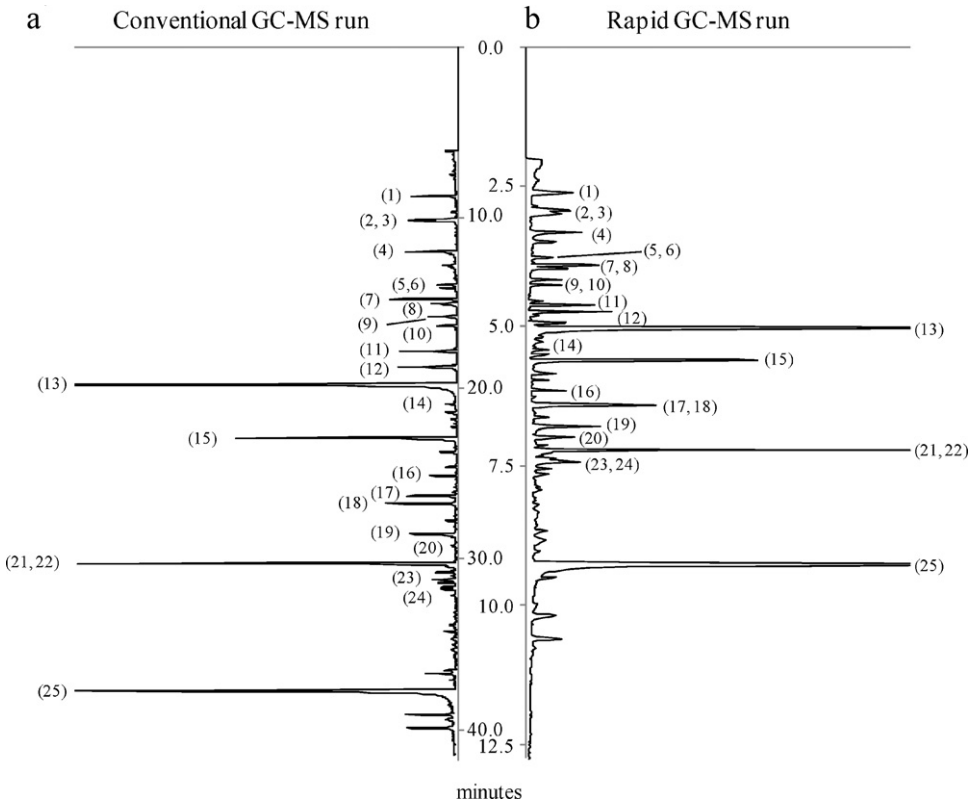

Fig. 1. Chromatograms obtained for thermally deteriorated lager beer at day 18 by (a) conventional GC–MS and (b) rapid GC–MS runs. The main signals were assigned as: (1) 3-octanol, internal standard, (2) acetic acid, (3) furfural, (4) 2,3-butanediol, (5) butyric acid, (6) phenylacetaldehyde, (7) furfuryl alcohol, (8) valeric acid, (9) methionol, (10) unknown peak 1 (characteristic *m*/*z* 43), (11) 2-phenylethyl acetate, (12) caproic acid, (13) β-phenylethanol, (14) 2-acetylpyrrole, (15) caprylic acid, (16) vinylguaiacol, (17) 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (DDMP), (18) capric acid, (19) diethyl succinate, (20) benzoic acid, (21) 5-hydroxymethylfurfural (5-HMF), (22) unknown peak 2 (characteristic *m*/*z* values 150 and 179), (23) tetrahydro-4-hydroxy-4-methyl-2(H)-pyran-2-one, (24) benzeneacetic acid and (25) tyrosol.

loadings columns *k* (a vector) were folded back to give a matrix, which was depicted as a 2D map: $\mathbf{p}_{k(j,1)} \rightarrow \mathbf{P}_{k(l,m)}$, where $j = l \cdot m$ such as *l* and *m* are, respectively, the number of scans (expressed as retention time) and the *m*/*z* values correspondent to each sample.

PCA was performed using MATLAB 7.8.0 and an in-house application co-developed by the University of Aveiro and the AgroParisTech, France.

3. Results and discussion

3.1. Chemical characterization of thermally deteriorated beer – comparison of conventional and rapid GC–MS runs

In this work, the chromatographic time for beer analysis by conventional GC (41.5 min) was shown to be reduced by a threefold factor when rapid GC runs are employed (12.8 min). To confirm that no relevant information loss occurs when rapid GC–MS is performed, chemical characterization of thermally deteriorated beer, with emphasis on the identification of known aging markers, was studied by both GC methodologies. Fig. 1 shows typical chromatograms obtained for a lager beer using (a) conventional and (b) rapid GC runs. Overall, the same 25 compounds were identified for both methodologies, the two chromatograms showing similar profiles, although a slight loss of chromatographic separation on the chromatogram corresponding to rapid runs (Fig. 1b) was observed, with the convolution of peaks 7 and 8 (furfuryl alcohol and valeric acid), 17 and 18 (2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (DDMP) and capric acid) and 23 and 24 (tetrahydro-4-hydroxy-4-methyl-2(H)-pyran-2-one and benzeneacetic acid).

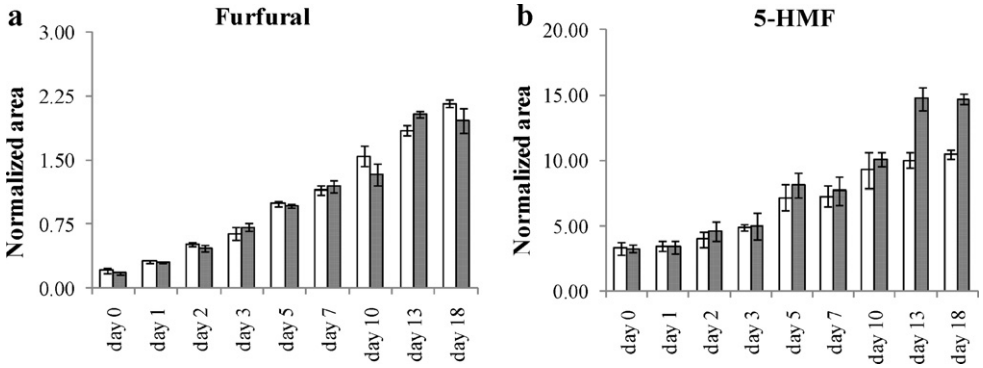

Fig. 2. Example of normalized areas plots corresponding to the characteristic peaks of (a) furfural (extraction of *m*/*z* value 95) and (b) 5-HMF (extraction of *m*/*z* value 97), as a function of deterioration time, obtained for conventional (dark bars) and rapid GC–MS runs (blank bars).

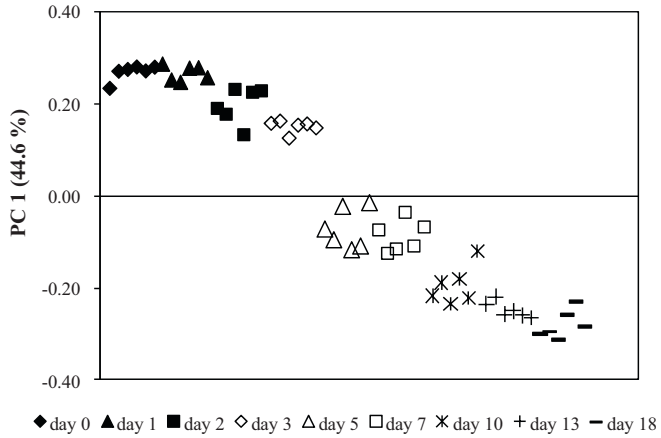

Fig. 3. PC1 scores plot of rapid GC–MS data obtained for the monitoring of the thermal deterioration process.

To ensure that the rapid GC methodology also maintained the (semi-) quantitative ability to characterize the detected compounds, comparison of the normalized areas obtained by rapid and conventional GC–MS data for the relevant compounds in beer deterioration process was performed, using the signal of the internal standard – 3-octanol as intensity reference. The compound peaks choice was based on their intensity, contribution to the deteriorated process and the occurrence of convolution in their peaks on rapid GC runs. Accordingly, the following compounds were chosen: acetic acid, furfural, 2,3-butanediol, butyric acid, phenylacetaldehyde, furfuryl alcohol, valeric acid, 2-phenylethyl acetate, caproic acid, caprylic acid, vinylguaiacol, DDMP, capric acid, diethyl succinate, 5-hydroxymethyl furfural (5-HMF), tetrahydro-4-hydroxy-4-methyl-2(H)-pyran-2-one, benzeneacetic acid and two unknown compounds with peaks corresponding to *m*/*z* values of 43 and 179. Comparing the integral response of the selected peaks as a function of the thermal deterioration period, for both conventional and rapid GC runs, it became apparent that the response was similar for the majority of the studied compounds, with variations lower than 20%. An example of this comparison

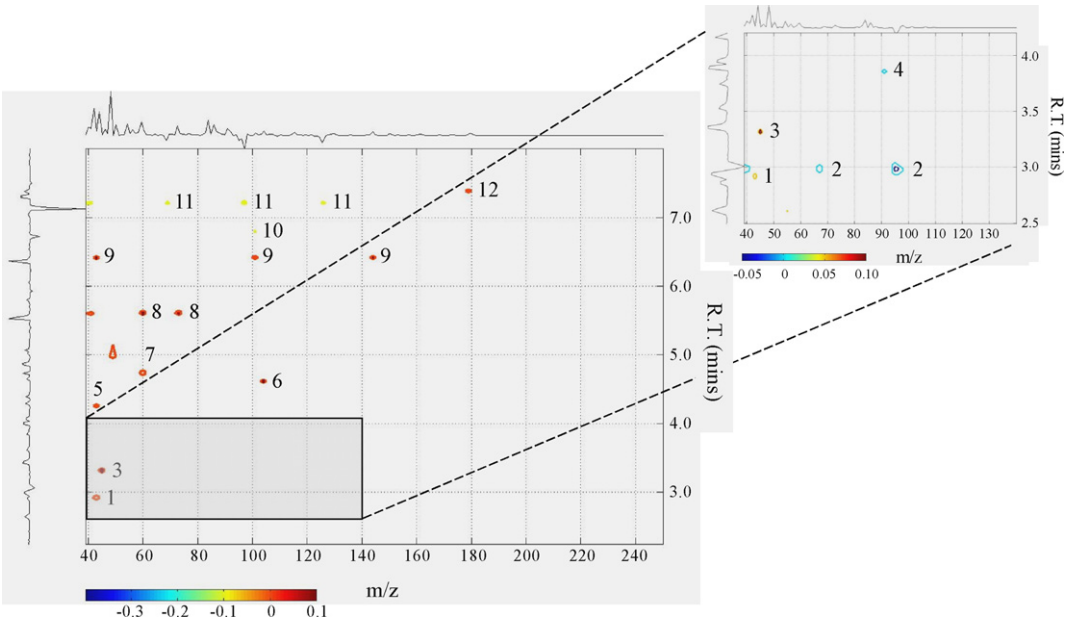

Fig. 4. PC1 contour loadings plot obtained for rapid GC–MS data, combining chromatographic data and *m*/*z* fragmentation data corresponding to each scan. The inset shows the expansion of the region between 2.5 and 4.2 min and *m*/*z* fragments range between 39 and 140. Compounds identified as varying are (1) acetic acid, (2) furfural, (3) 2,3-butanediol, (4) phenylacetaldehyde (5) unknown compound 1 (*m*/*z* 43), (6) 2-phenylethyl acetate, (7) caproic acid, (8) caprylic acid, (9), DDMP, (10) diethyl succinate, (11) 5-HMF, (12) unknown compound 2 (*m*/*z* 179). In blue and yellow (negative values): spots increasing with deterioration process; in red (positive values): spots illustrated as decreasing with deterioration. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 1
Structural information corresponding to the spots identified on the PC1 contour loadings plot. The underlined values represent the *m*/*z* values used for peak integration and the variation percentage, % variation, refers to compounds variation between day 0 (fresh beer) and day 18 of aging (last day of deterioration).

Spot number	Retention time (min)	Main *m*/*z* fragments	Compounds	Variation trend	% Variation
	2.9	43	Acetic acid[a]	–	–
2	3.0	67/95	Furfural	Increase	946
3	3.3	45	2,3-butanediol[a]	–	–
4	3.9	91	Phenylacetaldehyde	Increase	28
5	4.3	43	Unknown 1	Decrease	11
6	4.6	104	2-Phenylethyl acetate[a]	–	–
7	4.8	60	Caproic acid[a]	–	–
8	5.6	60	Caprylic acid[a]	–	–
9	6.4	43/101/144	DDMP	Decrease	42
10	6.8	101	Diethyl succinate	Increase	353
11	7.2	69/97/126	5-HMF	Increase	218
12	7.3	179	Unknown 2	Decrease	91

[a] Compounds for which no clear trend was identified by analysis of their normalized areas as a function of thermal deterioration.

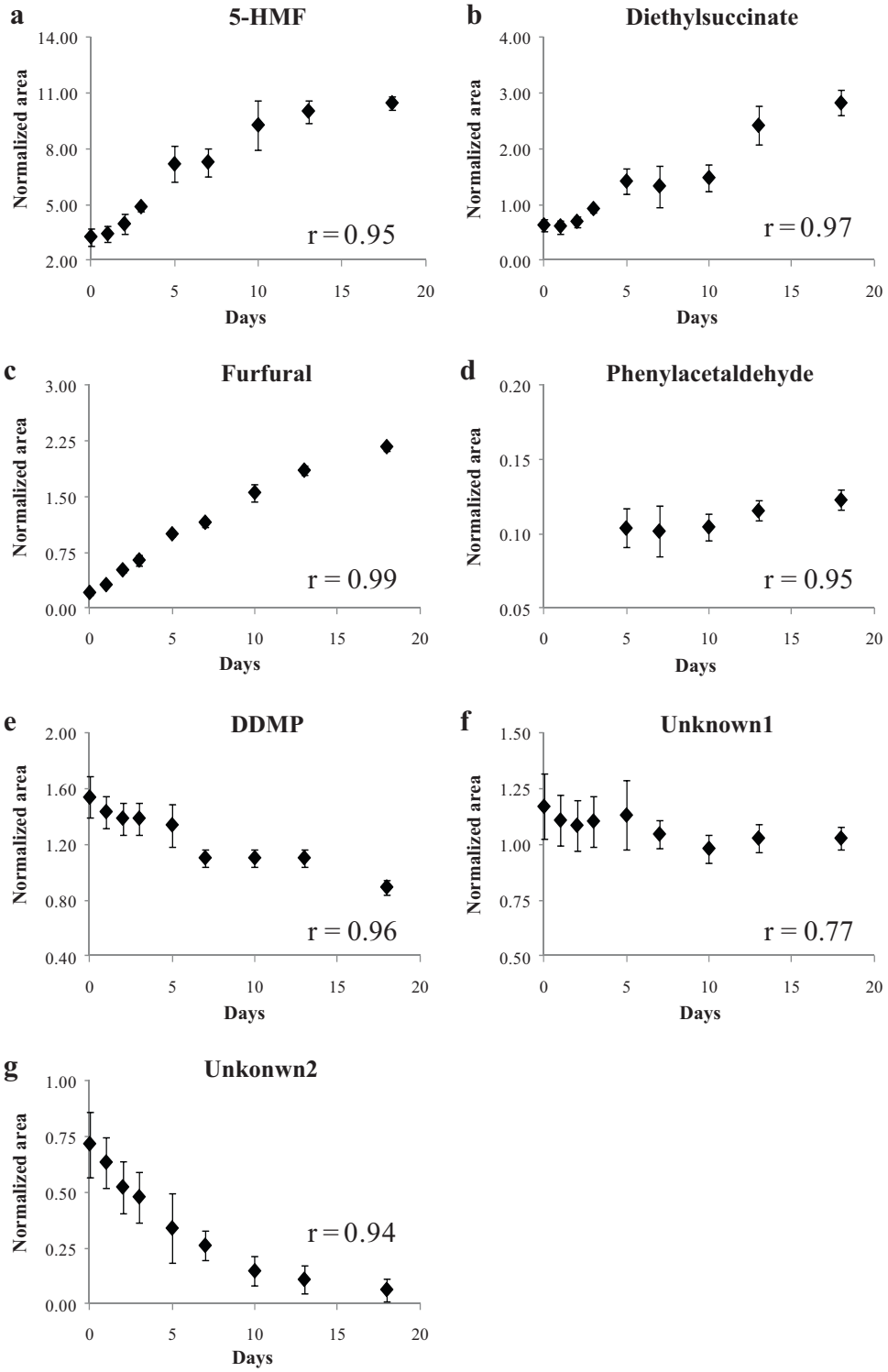

Fig. 5. Plots of normalized areas as a function of days of deterioration obtained for some of the compounds identified on PC1 as responsible for deterioration, namely (a) 5-HMF, (b) diethyl succinate, (c) furfural, (d) phenylacetaldehyde, (e) DDMP, (f) unknown 1, and (g) unknown 2. Correlation coefficients, *r*, are shown in each plot.

is shown for furfural (Fig. 2a). One exception was noted involving 5-HMF, which showed variations of approximately 30% at days 13 and 18 of thermal deterioration (Fig. 2b), with conventional runs giving higher values, a fact for which no explanation could be advanced at this stage. In any case, apart from 5-HMF at later deterioration stages, it became apparent that no relevant information is lost when the rapid GC–MS methodology approach is employed.

3.2. Study of thermal deterioration of beer by GC–MS/MVA

Principal Component Analysis (PCA) models were built based on rapid and conventional GC–MS data to aid the identification of the compositional variations occurring during beer thermal deterioration. Fig. 3 shows the results obtained for rapid GC–MS data, indicating that a clear trend exists, as a function of deterioration extent, with an explained variability in principal component 1 (PC1)

of 44.6%. PC1 was the only component that captured the variability related to the deterioration extension, the remaining components showed no meaningful patterns. A similar result was obtained for conventional GC runs (not shown), with an explained variability in PC1 of 37.8%. Plotting both PC1 scores results (obtained for conventional and rapid GC methods) against each other, a correlation with r 0.92 was obtained (not shown), thus demonstrating the similarity between the two datasets.

In Fig. 3, fresher beer samples may be identified as located in positive PC1, whereas more deteriorated samples are positioned in negative PC1. To interpret this trend, the corresponding contour PC1 loadings plot was obtained (Fig. 4). This plot combines the chromatographic data corresponding to each beer sample with the m/z fragmentation data of each scan, allowing the identification of the main m/z fragments responsible for the trend observed in Fig. 3. Negative spots (in blue and yellow in Fig. 4) are related to m/z fragments which increase in concentration during deterioration. On the other hand, positive spots (in red) correspond to m/z fragments diminishing with the deterioration process. Overall, 12 spots were identified by rapid GC–MS/MVA as varying with beer deterioration. The interpretation of such spots is shown in Table 1, where two still unidentified relevant compounds (named as unknowns 1 and 2) are noted. PCA results were validated by integration of the identified peaks and examples of the compounds variations, as a function of days of thermal deterioration, are shown in Fig. 5 for (a) 5-HMF, (b) diethyl succinate, (c) furfural, (d) phenylacetaldehyde, (e) DDMP, (f) unknown compound 1, and (g) unknown compound 2.

The spots corresponding to 5-HMF (m/z 69, 97 and 126), diethyl succinate (m/z 101), furfural (m/z 67 and 95) and phenylacetaldehyde (m/z 91) showed negative intensity, confirming their expected increasing tendency with thermal deterioration process. Accordingly, approximate linear increases were detected for 5-HMF, diethyl succinate and furfural, with correlation values $r > 0.95$ and variation percentages between fresh (day 0) and deteriorated beer in day 18 of 218%, 946% and 353%, respectively. Indeed, 5-HMF and furfural are well known products of sugar degradation, being expected to increase with time at an approximately linear rate and vary logarithmically with the storage temperature [2]. Diethyl succinate, a product of the esterification of ethanol with succinic acid, has also been shown to increase linearly, its formation rate being dependent on storage temperature [5]. An increasing trend was also noted for phenylacetaldehyde, although this compound was found present in trace amounts. This compound is a Strecker aldehyde, derived from phenylalanine degradation, and is a known aging marker, its formation being greatly affected by temperature and level of dissolved oxygen [6]. Due to its low content, peak integration was only possible after day 5 of thermal deterioration (Fig. 5d).

The positive intensity spots identified comprised, amongst others, DDMP (m/z 43, 101 and 144), and unknown compounds 1 and 2, with characteristic fragments (m/z 43) and (m/z 179), respectively, meaning that they are more abundant in fresh beer and, therefore, decreasing with deterioration. In fact, those compounds show a clear decrease, with variation percentages between fresh beer (day 0) and deteriorated beer at day 18 of 42%, 11% and 91% for DDMP and unknown 1 and 2, respectively. For DDMP and unknown compound 2, an approximately linear decrease with days of aging was observed, with r values > 0.94. DDMP, originating from hexoses degradation, has been observed as an important intermediate of Maillard reactions, being sensitive to higher temperatures [40,41]. Although a trend was observed for unknown 1, with r 0.77, variations were relatively small compared to the previous compounds.

In addition, 2-phenylethyl acetate (m/z 104), 2,3-butanediol (m/z 45), acetic (m/z 43), caproic (m/z 60) and caprylic acids (m/z 60) were also identified as positive intensity spots on the PC1 contour loadings plot (decreasing with aging). However, by analysis of their integrals as a function of the deterioration period, no statistically relevant trends were identified for these compounds, thus indicating that these compounds may not be adequate markers of beer deterioration (at least under the specific experimental conditions used in this work). The importance and involvement of these compounds in beer aging has, however, been reported [2,41,42]. For instance, 2-phenylethyl acetate seems to tend to hydrolyze with aging, especially at higher temperatures [41]. In addition, 2,3-butanediol can react with aldehydes (acetalization of aldehydes) producing cyclic acetals [2] and acetic acid can be involved in various reactions during storage, such as the chemical hydrolysis of acetate esters [41] and the oxidation of ethanol [2]. Finally, caproic and caprylic acids are medium straight-chain fatty acids that, during beer storage, can react with alcohols producing the corresponding esters [42].

4. Conclusions

In this work we propose a reliable and fast GC–MS/MVA methodology to monitor beer deterioration phenomena, not only by reducing chromatographic separation time, without losing relevant spectral information and the (semi-) quantitative ability to characterize the identified compounds, but also by improving data interpretation by using a MVA approach to analyze GC–MS data.

The time needed for chromatographic deconvolution was reduced by threefold compared to conventional GC runs and, although some loss of resolution was detected, equivalent compositional information was observed for both rapid and the conventional GC–MS runs. The subsequent GC–MS/MVA methodology enabled the identification of the degree of deterioration of beer and the identification of the specific compounds related to the process. In fact, the thermal deterioration process of beer was clearly correlated to the increase of the well known aging compounds 5-HMF, furfural, diethyl succinate and phenylacetaldehyde. Furthermore, a decrease in DDMP was observed, together with two still unknown compounds (1 and 2). To our knowledge, this variation in DDMP was here observed, for the first time, as correlating with beer deterioration, while the remaining two unassigned compounds may, in time, unveil possible new aging markers.

We believe that the strategy employed in this work was, hereby, demonstrated to be a suitable and rapid screening approach for the evaluation of beer deterioration and pinpointing of the main chemical players in the process. As a consequence, interesting potential applications of this method for the rapid quality control of beer may be envisaged.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.12.088.

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