Food Chemistry 326 (2020) 126856

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Beer volatile fingerprinting at different brewing steps

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ARTICLE INFO

Keywords:
Beers
Brewing process
Volatile fingerprint
VOMs
HS-SPME/GC–MS

ABSTRACT

Volatile fingerprints of a lager beer were carried out throughout five brewing steps to characterize the changes encompassing this process. Overall, 60 volatile organic metabolites (VOMs) were identified by headspace solid-phase microextraction followed by gas chromatography mass spectrometry (HS-SPME/GC–MS). Specific profiles were observed at different brewing steps - aldehydes and furans dominate in wort, whereas the aliphatic esters and alcohols predominate in the following steps. Such variations can be assigned to specific VOMs, as 3-methylbutanal (wort), ethyl alcohol and ethyl octanoate (fermentation, maturation and filtration), or ethyl alcohol and isoamyl acetate (final product). These VOMs can influence the beer final flavour. Ethyl alcohol contributes to its strong and pungent smell and taste, while isoamyl acetate adds intense 'fruity' and 'banana' odours. These beer volatile fingerprints constitute a valuable tool to obtain insights on the impact of each brewing step on the final product, being also very useful for certification purposes.

1. Introduction

Beer is one of the most popular alcoholic beverages, being consumed in large amounts worldwide (with an annual production of almost two billion hectolitres in 2018, according to the data available in statista.com). Is a complex mixture containing numerous flavour-active volatile organic metabolites (VOMs) belonging to a diversity of chemical families over a wide range of concentrations, polarities and volatilities. These VOMs reflect the brewing process and have a strong influence on the quality and character of the beer. Consequently, they are also important for beer characterization. Overall, beer flavour results from a complex combination of different aspects that give each brew distinctive profiles. Such interferences result mainly from the ingredients composition, the roasting malt and boiling wort conditions, the metabolites produced by yeast during fermentation as well as the ones produced by contaminant microorganisms and also the effects of oxygen and sunlight during product storage (Bettenhausen et al., 2018; Dong et al., 2015; Figueira et al., 2012; Olaniran, Hiralal, Mokoena, & Pillay, 2017; Preedy, 2009).

In generic terms, the brewing process involves four main steps, wort preparation, fermentation, maturation, and filtration and/or stabilization (detailed in the Supplementary Fig. 2). Before starting the brewing process, barley is artificially induced to germinate and dried. This process, known as malting, allows the maturation of enzymes that digest complex starches in the grain into simple fermentable sugars. These sugars will be used later during mashing (Briggs, Boulton, Brookes, & Stevens, 2004; Eßlinger, 2009). Although malted barley is the most important cereal, wheat, wheat malt, corn, rice and millet are often used (Briggs et al., 2004; Eßlinger, 2009). The transition of malted barley to wort begins by grinding the grains. At this stage the grist is diluted, filtered and brought to boiling for one to two hours (Briggs et al., 2004). In this process, hops or hop-derived products, such as hop pellets or hop essential oils are added. Hops possess a characteristic flavour and aroma that exert a strong influence on the final product and so its dosage needs to be adjusted to the profile of the desired beer and integrated harmonically in the matrix during the beer maturation (Eßlinger, 2009). At the end of the boil, the resulting wort contains coagulated proteins or 'trub' and suspended fragments of hops

Abbreviations: DBV/CAR/PDMS, divinylbenzene/carboxen on polydimethylsiloxane; DMSO, dimethyl sulfoxide; DMS, dimethyl sulphide; ECM, Empresa de Cervejas da Madeira; GC/MS, gas chromatography – mass spectrometry; GC-qMS, gas chromatography – quadrupole mass spectrometry; HS-SPMCE, headspace solid-phase microcolumn extraction; IEC, ion extraction chromatogram; LLE, liquid–liquid extraction; RI, retention index; RSD, relative standard deviation; SBSE, stir-bar sorptive extraction; SDE, simultaneous extraction and distillation; SPE, solid-phase extraction; SPME, solid-phase microextraction; SVOMs, semi-volatile organic metabolites; VOMs, volatile organic metabolites

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https://doi.org/10.1016/j.foodchem.2020.126856
Received 18 November 2019; Received in revised form 28 March 2020; Accepted 17 April 2020
Available online 25 April 2020
0308-8146/

that must be removed, often using a 'whirlpool tank' or 'settling tank'. After cooled and aerated, the clear wort is then pumped to the fermentation tanks, where yeasts are added. During fermentation, yeasts take up amino acids and sugars from the wort. The sugars are metabolized, under anaerobic conditions, and converted mainly in ethanol and carbon dioxide. Additionally, this carbohydrates fermentation generates a typical fingerprint of volatile metabolites at relatively low levels, like aldehydes, ketones, higher alcohols, organic acids, and esters, which are called 'fermentation by-products' or 'congeners'(Supplementary Fig. 2) (Christoph & Bauer-Christoph, 2007; Olaniran et al., 2017). The temperature and time of fermentation are the key factors in the quality of the beer. Fermentation takes place at 7–14 °C, for lager beers, or 16–18 °C for ale beers (Briggs et al., 2004; Eßlinger, 2009). In the particular case of lambic type, the wort is contaminated by yeasts present in the atmosphere and fermentation occurs spontaneously during 1–2 years at room temperature (Goldammer, 2008).

When all the fermentable sugar is exhausted, fermentation slows and the yeast begins to fall out of suspension (flocculate) (Goldammer, 2008). The bulk of the yeast can then be separated from the product, now recognizably beer, in a process known as 'racking' (Briggs et al., 2004; Eßlinger, 2009). This freshly produces or 'green' beer is now ready for the final stages of processing including maturation, filtration and packaging. The green beer still contains undesirable levels of acetaldehyde and diacetyl generated by partial oxidation. These oxidized by-products can be reduced by the few remaining yeast cells during a period of secondary fermentation. During this period, oxygen is completely excluded, and the temperature is maintained about 0 °C or lower (Goldammer, 2008).

After the maturing stage, the beer is piped to filtration room, where it passes through special filters coated with diatomaceous earth, to remove suspended particles and to unhinge potential turbidity formers (stabilization). This step is important to preserve the beer so that no visible changes occur in the long run and the beer keeps its original appearance (Eßlinger, 2009).

Tetra-hydro isomerized hop extract, commonly referred as tetrahop, is normally used after the final filtration. This aqueous alkaline solution of the potassium salts of tetrahydro-iso-α-acids enhances beer foam and protects it from the formation of light-struck flavours. Finally, beer is packaged in bottles, cans or kegs and pasteurised in order to preserve the character of the product until it is consumed.

Beer aroma compounds are very important as they make a major contribution to the quality of the final product. A variety of flavour compounds may arise depending on the brewing process and storage conditions. Therefore, there is a large demand for fast and reliable methods to evaluate organoleptic characteristics of beer. Thus, sensitive analytical methodologies are required for the extraction and analysis of a great number of beer volatile compounds.

Several extraction methods, such as liquid–liquid extraction (LLE), simultaneous extraction and distillation (SDE), static or dynamic headspace analysis and the purge-and-trap technique, stir-bar sorptive extraction (SBSE), solid-phase extraction (SPE) and headspace solid-phase microcolumn extraction (HS-SPMCE) have been employed for the analysis of volatile compounds in beer (Horák et al., 2009; Hrivňák, Šmogrovičová, Nádaský, & Lakatošová, 2010; Tian, 2010). Nevertheless, these techniques present certain non-negligible drawbacks such as the use of solvents, the time required and the use of expensive devices with a limited lifetime which may entail carryover or cross-contamination problems. Consequently, in order to overcome these drawbacks, solid phase microextraction (SPME) has emerged as an efficient extraction-preconcentration method. When combined with appropriate detection modes, SPME is a reliable alternative to traditional sample preparation techniques, exhibiting important features as simplicity, low cost, selectivity and sensitivity (Câmara et al., 2007; Mendes, Gonçalves, & Câmara, 2012). This method developed by Pawliszyn and co-workers (Lord & Pawliszyn, 1997), eliminates the use of organic solvents, and substantially shortens the time of analysis. SPME can integrate sampling, extraction, concentration and sample introduction into a single uninterrupted process, resulting in high sample throughput. It can be also used as a solvent-free sample preparation method with GC–MS analysis, to isolate and identify the main constituents of the volatile metabolome present in the different steps of the brewing process. There are, nevertheless, some limitations affecting SPME, particularly in what concerns to the stationary phase commercially available that are not suitable to all type of volatiles, particularly very volatile, polar, or thermal unstable analytes (Pereira et al., 2014).

In this work, the establishment of the HS-SPME/GC–MS volatile fingerprint of a lager beer throughout five brewing steps – wort, fermentation, maturation, filtration and final product, is presented as a powerful methodology to highlight differences during the production process. This approach allowed the identification of the VOMs that can influence the beer aroma and flavour both individually and in a synergistic or antagonistic sense. Accordingly, abundant aldehydes and furans in wort are gradually converted in aliphatic esters and alcohols during the following steps. Such variations can be assigned to specific VOMs, as 3-methylbutanal (wort), ethyl alcohol and ethyl octanoate (fermentation, maturation and filtration), or ethyl alcohol and isoamyl acetate (final product). These VOMs have a strong influence on beer final flavour, contributing ethyl alcohol for its strong and pungent smell and taste, while isoamyl acetate adds intense 'fruity' and 'banana' aromas.

2. Material and methods

2.1. Reagents and materials

The SPME fibre coated with divinylbenzene/carboxen on polydimethylsiloxane (DBV/CAR/PDMS; StableFlex, 50/30 μm) and the SPME holder for manual sampling, clear glass screw cap vials for SPME with PTFE/silica (film thickness 1.3 mm) septa were purchased from Supelco (Bellefonte, PA, USA). Prior to initial use, the new fibre was conditioned as per the manufacturer's recommendations by heating in the injection port of the GC. Sodium chloride (99.5%) was supplied by Panreac (Barcelona, Spain). The series of C_8 to C_{20} straight-chain *n*-alkanes (concentration of 40 mg L^{-1} in *n*-hexane) was supplied from Fluka (Buchs, Switzerland).

2.2. Samples

Samples from the different brewing steps (described in Fig. 1) of a lager beer (kindly provided by Empresa de Cervejas da Madeira (ECM, Madeira Island, Portugal), were refrigerated (ca. 2–5 °C), transported to the laboratory and stored at −20 °C until analysis.

2.3. HS-SPME extraction conditions

To obtain high recoveries of volatile organic metabolites (VOMs) and semi-volatile organic metabolites (SVOMs), several SPME parameters with influence in the extraction process were previously optimized (Gonçalves, Figueira, Rodrigues, & Camara, 2012; Gonçalves et al., 2014). Accordingly, the best conditions were obtained using two mL of sample placed into a 4 mL glass vial, which correspond to a ratio of the volume of the liquid phase to the headspace volume ($1/\beta$) of 0.5. After the addition of 0.2 ± 0.001 g of NaCl (to improve the extraction efficiency by decreasing the solubility of hydrophilic compounds in the aqueous phase) and a micro stir bar (0.5 mm × 0.1 mm; Supelco), the vial was closed and placed in a thermostat bath adjusted to 40.0 ± 0.1 °C. Then, the SPME fibre was exposed to the sample vial headspace during 30 min.

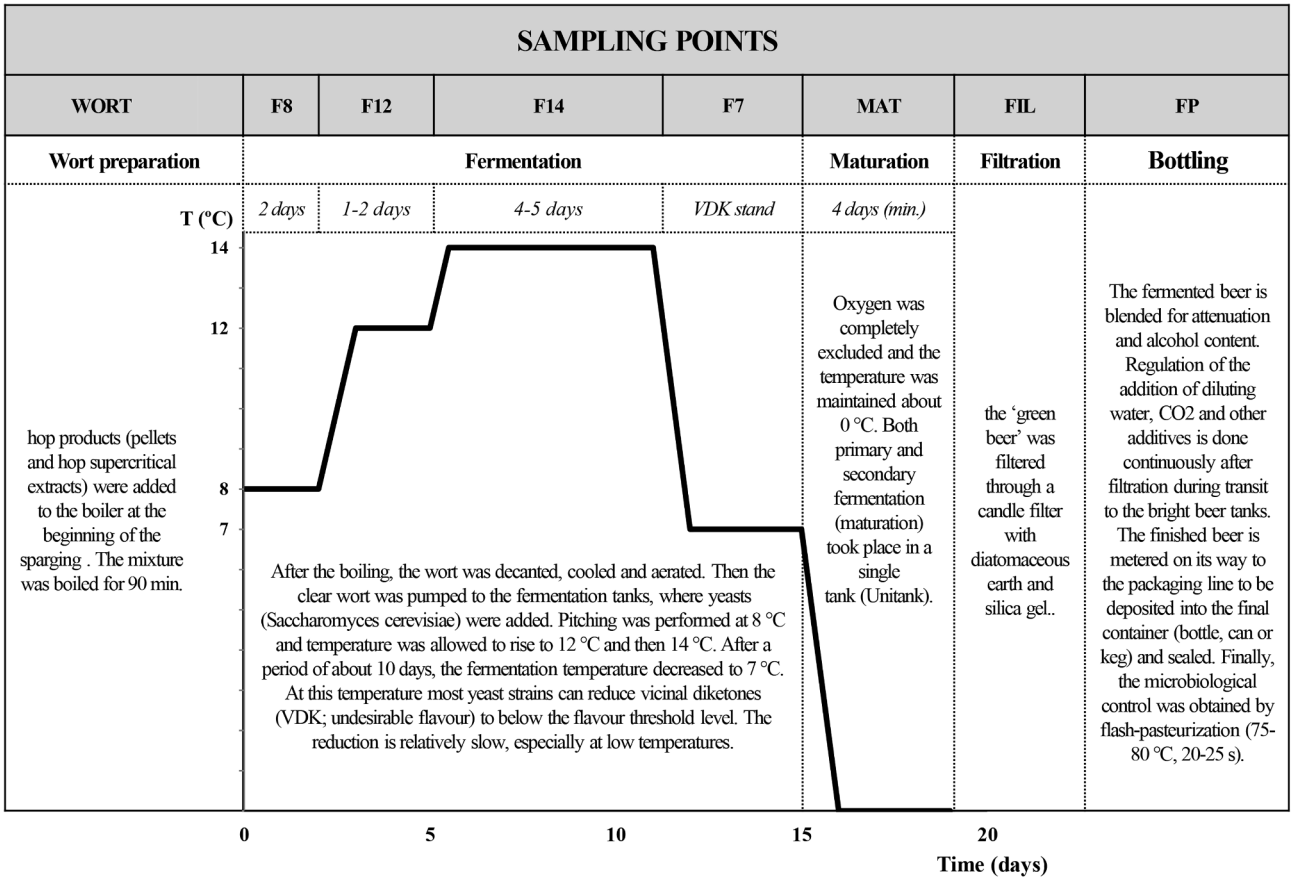


Fig. 1. Characterization of the sampling points studied in this work, wort, fermentation at 8 °C (F 8 °C), 12 °C (F 12 °C), 14 °C (F 14 °C) and 7 °C (F 7 °C), maturation, filtration and final product, with a brief description of the main operations and reactions that occur in each step, as well as their typical time length, in days.

2.4. GC-qMS conditions

The SPME fibre containing the trapped volatiles was manually introduced into the GC injection port at 250 °C (equipped with a glass liner, 0.75 mm I.D.) and kept for six min for desorption. The desorbed metabolites were separated in an Agilent Technologies 6890N Network gas chromatography equipped with a BP-20 fused silica capillary column (30 m × 0.25 mm I.D. × 0.25 μm film thickness) supplied by SGE (Darmstadt, Germany) and connected to an Agilent 5973N quadrupole mass selective detector. Helium (Air Liquid, Portugal) was used as the carrier gas at a flow rate of 1.1 mL min^{-1} (column-head pressure: 12 psi) and injections were performed in the splitless mode (5 min). The GC oven temperature was programmed as follows: 40 °C for 1 min, ramped at 1.7 °C min^{-1} to 180 °C (1 min) then to 220 °C at 30 °C min^{-1} and held isothermally for a further 1 min. For the MS system, the temperatures of the transfer line, quadrupole and ionization source were 250, 180 and 230 °C, respectively; electron impact mass spectra were recorded at 70 eV and the ionization current was about 30 μA. The acquisitions were performed in full scan mode (30–300 *m/z*) and the GC peak area of each compound was obtained from the ion extraction chromatogram (IEC) by selecting target ions for each one. Signal acquisition and data processing were performed using the HP Chemstation (Agilent Technologies). All measurements were made with, at least, three replicates and blank runs were completed before each sampling to ensure no carry-over from the previous extraction. The retention index (RI) was calculated through injection of a series of C_8 to C_{20} straight-chain *n*-alkanes (concentration of 40 mg L^{-1} in *n*-hexane).

Identification of volatile and semi-volatile metabolites was achieved (1) by comparing the GC retention times and mass spectra, with those, when available, of the pure standard compounds; (2) all mass spectra were also compared with the data system library (NIST, 2005 software, Mass Spectral Search Program V.2.0d; NIST 2005, Washington, DC); and (3) Kovat's retention indices (RI) value were determined according to the Van den Dool and Kratz (van Den Dool & Kratz, 1963). For the determination of the RI, a C_8-C_{20} *n*-alkanes series was used, and the values were compared, when available, with values reported in the literature for similar chromatographic columns.

2.5. Statistical analysis

Analysis of variance and Tukey's test was performed to determine statistically significant differences in volatile composition between brewing stages, for a 95% confidence interval.

3. Results and discussion

The combination of SPME with gas chromatography-mass spectrometry is a powerful tool to establish volatile metabolomics fingerprints of different samples with great sensitivity, namely food samples and beverages (Figueira, Gonçalves, Rodrigues, Ornelas, Branco, Silva, & Câmara, 2012; Gonçalves et al., 2012; Gonçalves et al., 2014; Rodrigues, Caldeira, & Camara, 2008; Saison, De Schutter, Delvaux, & Delvaux, 2009; Tian, 2010). However, the efficiency of SPME extraction methodology depends greatly on the value of the distribution constant of the analytes partitioned between the sample and fibre coating material. For this reason, the fibre selected for this work was the SPME fibre DBV/CAR/PDMS (also known as StableFlex, 50/30 μm), previously reported as the most efficient for this type of applications (Gonçalves et al., 2012; Gonçalves et al., 2014). In fact, this fibre and

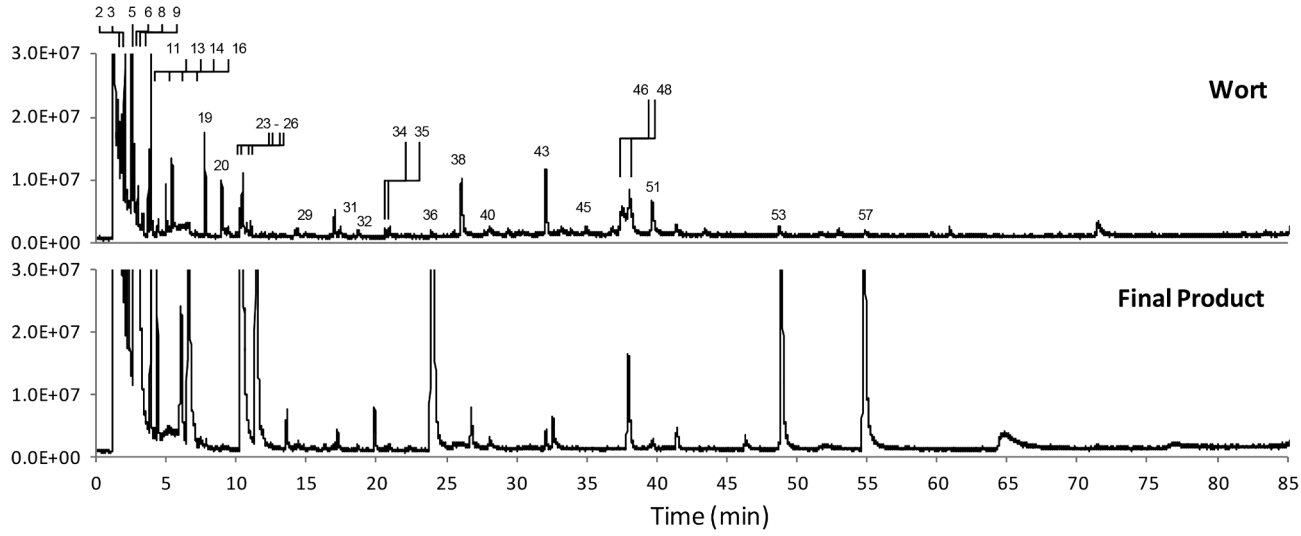


Fig. 2. Representative chromatograms (GC-qMS) of the first and last brewing steps studied in this work, wort and the final product, obtained by HS-SPME-GC/MS. Typical chromatograms for remaining steps, fermentation at 8 °C (F 8 °C), 12 °C (F 12 °C), 14 °C (F 14 °C) and 7 °C (F 7 °C), maturation and filtration, are available in the Supplementary Fig. 4. Peak assignments and identification are available in Table 2.

methodology has been already used to profile the volatile metabolomic pattern of different beer raw materials (Gonçalves et al., 2014).

3.1. Chromatographic profiles

The chromatographic profiles of the volatile metabolites of different brewing steps obtained by HS-SPME/GC-qMS are presented in Fig. 2 (for simplification, only wort and final product are shown. The collection of all brewing steps is available in the Supplementary Fig. 4).

A total of 60 metabolites were identified based on the comparison of their mass spectra to the reference database (MS), as well as the calculated RIs (RI_{calc}) with the values reported in the literature (RI_{lit}) for the polyethylene glycol (or equivalent) capillary column (Table 1). The retention indices of the experimental data were in good accordance with those of the literature. A range between 0 and 105 was obtained for $|RI_{calc}\text{-}RI_{lit}|$, resulting from the comparison between RI_{cal} and the RI_{lit} reported in the literature with the polyethylene glycol GC capillary column or equivalent (Supplementary Fig. 2). The difference in the determined RI is considered reasonable (< 5%, on average) attending to the fact that data reported in the literature is obtained from a large range of GC stationary phases (several commercial GC columns are composed of polyethylene glycol or equivalent stationary phases). Up to a retention time of about 65 min, a linear correlation of retention index and retention time could be obtained. The average relative standard deviations (RSD, %) of the retention indices calculated ranged from 0.6 to 4.3%.

The 60 volatile metabolites identified in this work are listed in Table 1 according to their elution order on a BP-20 capillary column, and including their Kovat's retention indices (RI), odour descriptor, molecular formula, chemical class, and the corresponding average peak areas (n = 3).

The metabolites identified were grouped into different chemical classes and include aliphatic esters (17), ketones (9), alcohols (8), aldehyde (8), furan compounds (6), monoterpenes (4), fatty acids (4), sesquiterpenes (1), sulphur compounds (1), nitrogen compounds (1) and halogen compounds (1). Among these VOMs, 13 were identified in only one of the brewing processes analysed and mostly in wort. In contrast, nine VOMs were detected in all the all sample points considered, including the most abundant VOMs, as isoamyl acetate (**16**), 3-Methylbutan-1-ol (**23**) or ethyl octanoate (**36**), as well as some low abundant VOMs with a great impact in beer flavour. This is the case of furfuryl alcohol (**51**), for instance, which is the main precursor of furfuryl ethyl ether, an important marker of beer ageing (Vanderhaegen et al., 2004). This volatomic data was processed by ANOVA and 41 VOMs were found statistically relevant for the discrimination between the brewing points analysed (indicated in bold in Table 1). To compare the results obtained with the literature, an exhaustive data survey was performed. However, it was not possible to find reports studying the variation of the volatile composition throughout the brewing process in the same experiment. Most studies were focused in the final product (Gonzalez Viejo, Fuentes, Torrico, Godbole, & Dunshea, 2019; Martins, Brandao, Almeida, & Rocha, 2018; Ocvirk, Mlinaric, & Kosir, 2018) or in specific parameters, as the influence of the yeast or the raw material in the final product (Gonçalves et al., 2012; Gonçalves et al., 2014; Kishimoto, Wanikawa, Kono, & Shibata, 2006). The comparison of the results obtained was therefore restricted to the volatomic composition of the final product. In this regard, Ocvirk et al. (2018), for instance, performed an SPME-GC/MS of lager beers using a PDMS/DVB fibre and reported 33 VOMs in their composition, which compares with the 24 VOMs here reported in the final product (Table 1). Interestingly, five of six VOMs identified by Ocvirk et al. (2018) as being the most relevant for the aroma profile (ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate and phenylethyl alcohol), were also identified in this work as being statistically relevant for the descrimination of the brewing steps analysed (in bold in Table 1).

3.2. Analysis of the volatile profile through the brewing process

Fig. 3 shows a global vision of the volatile composition variation during brewing taking as reference the selected sampling points. For simplification, this overview only included the most abundant VOMs (Fig. 3A) and to the least abundant VOMs with significant variations along the brewing process and previously described as having the ability to potentially influence the organoleptic characteristic of the final product (Fig. 3B). The structure of these metabolites, as well as their characteristic aroma, are available in Table 2.

Overall, the complexity of the volatile composition increases concomitantly with the progression of the brewing step, being the wort significantly poorer than remain steps. This observation is obviously related with the fact that fermentation of the wort sugars results in a richer volatile profile. Accordingly, almost half (29 out of 60) of the VOMs identified in this work were not detected in the wort, while only 11 VOMs were exclusively detected in the raw material. The aliphatic esters and higher alcohols, for instance, which are the most abundant

Table 1
Identification of volatile compounds in different brewing steps, expressed as total peak areas along with the corresponding retention indexes (RI).

N°	RT[a] (min)	RI_{Cal}[b]	Volatile metabolites	MF[c]	Family	Total Peak Area ($\times 10^6 \pm \sigma$)						
						Wort	Fermentation				Maturation	Filtration	Final Product
							F 8 °C	F 12 °C	F 14 °C	F 7 °C			
1	1.490	913	Acetaldehyde[e]	C_2H_4O	Aldehyde	-[d]	0.10 ± 0.002	2.78 ± 0.2	4.72 ± 0.7	2.03 ± 0.5	2.41 ± 0.2	–	–
2	1.602	921	Dimethyl sulfide[e]	C_2H_6S	Sulfur compound	1.59 ± 0.1	–	–	–	–	–	–	–
3	1.797	933	Furan[e]	C_4H_4O	Furan compound	2.22 ± 0.2	–	–	–	–	–	–	–
4[f]	2.286	959	Ethyl acetate[e]	$C_4H_8O_2$	Aliphatic ester	–	2.87 ± 0.3	10.36 ± 0.8	16.92 ± 2.1	18.06 ± 2.2	16.00 ± 0.9	15.78 ± 1.5	13.97 ± 1.6
5	2.533	970	3-Methyl butanal[e]	$C_5H_{10}O$	Aldehyde	6.96 ± 0.8	5.98 ± 0.8	1.33 ± 0.1	–	–	–	–	–
6	2.690	976	Methylene chloride	CH_2Cl_2	Halogen compound	1.10 ± 0.05	–	–	–	–	–	–	–
7	2.708	977	Ethyl alcohol[e]	C_2H_6O	Alcohol	–	107.8 ± 21	329.32 ± 60	465.60 ± 28	474.89 ± 82	354.72 ± 39	352.60 ± 32	272.43 ± 30
8	2.956	986	2-Ethyl furan	C_6H_8O	Furan compound	0.67 ± 0.1	–	–	–	–	–	–	–
9	3.747	1011	4-Methylpentan-2-one[e]	$C_6H_{12}O$	Ketone	0.58 ± 0.06	0.092 ± 0.01	–	–	–	–	–	–
10	4.354	1027	Ethyl butanoate[e]	$C_6H_{12}O_2$	Aliphatic ester	–	0.68 ± 0.1	1.01 ± 0.1	3.24 ± 0.7	2.18 ± 0.2	1.59 ± 0.1	2.07 ± 0.3	1.32 ± 0.3
11	4.422	1029	2-Methyl-3-buten-2-ol[e]	$C_5H_{10}O$	Higher alcohol	0.21 ± 0.02	–	–	–	–	–	–	–
12	4.474	1030	Propan-1-ol[e]	C_3H_8O	Higher alcohol	–	0.50 ± 0.1	0.31 ± 0.05	0.33 ± 0.04	0.43 ± 0.09	0.32 ± 0.03	0.20 ± 0.03	0.26 ± 0.02
13	4.938	1041	Hexan-3-one[e]	$C_6H_{12}O$	Ketone	0.32 ± 0.03	–	–	–	–	–	–	–
14	5.414	1051	Hexanal[e]	$C_6H_{12}O$	Aldehyde	0.72 ± 0.2	0.095 ± 0.01	–	–	–	–	–	–
15	5.923	1060	2-Methylpropan-1-ol[e]	$C_4H_{10}O$	Higher alcohol	–	1.32 ± 0.07	2.85 ± 0.2	4.02 ± 0.3	4.05 ± 0.4	2.38 ± 0.3	3.15 ± 0.2	2.12 ± 0.3
16	6.637	1072	Isoamyl acetate[e]	$C_7H_{14}O_2$	Aliphatic ester	0.29 ± 0.04	6.82 ± 0.8	42.52 ± 7.3	78.59 ± 6.7	64.43 ± 6.9	65.35 ± 3.1	63.57 ± 13	49.39 ± 2.9
17	6.982	1078	Ethyl pentanoate	$C_7H_{14}O_2$	Aliphatic ester	–	0.25 ± 0.05	–	–	–	–	–	–
18	7.462	1085	2-Methylheptan-4-one[e]	$C_8H_{16}O$	Ketone	–	0.14 ± 0.01	–	–	–	–	–	–
19	7.701	1088	β-Myrcene[e]	$C_{10}H_{16}$	Monoterpene	0.76 ± 0.01	7.76 ± 0.2	1.63 ± 0.3	0.35 ± 0.04	0.42 ± 0.03	0.31 ± 0.04	0.16 ± 0.02	–
20	8.921	1108	Heptanal[e]	$C_7H_{14}O$	Aldehyde	0.63 ± 0.1	–	–	–	–	–	–	–
21	8.945	1108	Heptan-2-one[e]	$C_7H_{14}O$	Ketone	–	0.077 ± 0.02	–	–	–	–	–	–
22	9.021	1110	D-Limonene[e]	$C_{10}H_{16}$	Monoterpene	–	0.80 ± 0.04	0.23 ± 0.01	–	–	0.13 ± 0.007	–	–
23	10.469	1142	3-Methylbutan-1-ol[e]	$C_5H_{12}O$	Higher alcohol	0.75 ± 0.2	30.83 ± 6.2	49.53 ± 7.0	74.60 ± 11	59.68 ± 6.1	47.86 ± 3.9	49.75 ± 4.7	39.34 ± 6.1
24	10.715	1147	Methallyl cyanide	C_5H_7N	Nitrogen compound	0.20 ± 0.01	–	–	–	–	–	–	–
25	10.974	1152	2-Pentyl furan	$C_9H_{14}O$	Furan compound	0.27 ± 0.02	0.70 ± 0.03	0.46 ± 0.08	–	–	–	–	–
26	11.493	1162	Ethyl hexanoate[e]	$C_8H_{16}O_2$	Aliphatic ester	0.23 ± 0.02	20.53 ± 0.6	31.97 ± 6.6	83.66 ± 1.9	35.92 ± 5.1	29.09 ± 1.8	24.92 ± 3.0	18.08 ± 3.2
27	13.580	1197	Hexyl acetate[e]	$C_8H_{16}O_2$	Aliphatic ester	–	0.22 ± 0.02	0.53 ± 0.03	0.94 ± 0.05	0.71 ± 0.09	0.53 ± 0.03	0.57 ± 0.1	0.35 ± 0.07
28	14.213	1209	3-hydroxybutan-2-one[e]	$C_4H_8O_2$	Ketone	–	–	0.91 ± 0.08	–	–	–	–	–
29	14.273	1210	Octanal	$C_8H_{16}O$	Aldehyde	0.17 ± 0.03	–	–	–	–	–	–	–
30	17.220	1264	Ethyl heptanoate[e]	$C_9H_{18}O_2$	Aliphatic ester	–	3.10 ± 0.07	2.41 ± 0.2	0.41 ± 0.2	0.64 ± 0.05	0.52 ± 0.03	0.34 ± 0.01	0.23 ± 0.04
31	17.333	1266	6-Methyl-5-hepten-2-one[e]	$C_8H_{14}O$	Ketone	0.14 ± 0.02	0.36 ± 0.08	0.23 ± 0.03	0.11 ± 0.01	–	–	–	–
32	18.673	1287	Hexan-1-ol[e]	$C_6H_{14}O$	Higher alcohol	0.13 ± 0.02	0.22 ± 0.05	0.17 ± 0.02	0.17 ± 0.03	0.14 ± 0.01	0.13 ± 0.01	0.069 ± 0.004	0.087 ± 0.02
33	19.861	1305	Heptyl acetate[e]	$C_9H_{18}O_2$	Aliphatic ester	–	0.45 ± 0.03	0.80 ± 0.09	1.65 ± 0.2	1.25 ± 0.2	0.97 ± 0.09	0.76 ± 0.06	0.46 ± 0.09
34	20.605	1318	Nonan-2-one[e]	$C_9H_{18}O$	Ketone	0.12 ± 0.01	–	–	–	–	–	–	–
35	20.884	1323	Nonanal[e]	$C_9H_{18}O$	Aldehyde	0.45 ± 0.09	–	0.62 ± 0.07	–	0.68 ± 0.2	0.092 ± 0.005	0.11 ± 0.01	0.11 ± 0.02
36	23.996	1372	Ethyl octanoate[e]	$C_{10}H_{20}O_2$	Aliphatic ester	0.12 ± 0.03	80.52 ± 3.7	177.91 ± 30	533.17 ± 48	166.85 ± 40	157.79 ± 24	91.08 ± 4.1	31.08 ± 4.8
37	25.470	1393	Acetic acid[e]	$C_2H_4O_2$	Fatty acid	–	–	2.21 ± 0.4	2.15 ± 0.1	1.56 ± 0.3	0.77 ± 0.1	0.51 ± 0.06	0.36 ± 0.08
38	26.023	1400	Furfural[e]	$C_5H_4O_2$	Furan compound	0.98 ± 0.1	–	–	–	–	–	–	–
39	26.717	1412	Octyl acetate	$C_{10}H_{20}O_2$	Aliphatic ester	–	0.25 ± 0.09	1.31 ± 0.2	2.62 ± 0.09	1.55 ± 0.04	1.43 ± 0.2	0.86 ± 0.1	0.43 ± 0.06
40	28.001	1434	Decanal[e]	$C_{10}H_{20}O$	Aldehyde	0.56 ± 0.08	–	1.27 ± 0.1	–	–	–	–	–
41	30.926	1479	Ethyl nonanoate[e]	$C_{11}H_{22}O_2$	Aliphatic ester	–	1.15 ± 0.08	0.75 ± 0.1	3.08 ± 0.3	1.24 ± 0.3	0.73 ± 0.05	0.37 ± 0.01	–
42	31.276	1484	Furfuryl acetate[e]	$C_7H_8O_3$	Furan compound	–	–	0.14 ± 0.03	0.23 ± 0.02	–	–	0.14 ± 0.03	–
43	32.059	1496	(Z)-β-Ocimene[e]	$C_{10}H_{16}$	Monoterpene	0.88 ± 0.1	0.47 ± 0.06	0.58 ± 0.3	0.75 ± 0.06	0.62 ± 0.2	0.45 ± 0.04	0.42 ± 0.1	0.25 ± 0.03
44	32.581	1504	Octan-1-ol[e]	$C_8H_{18}O$	Higher alcohol	–	0.20 ± 0.07	0.31 ± 0.04	1.18 ± 0.2	0.85 ± 0.09	0.80 ± 0.1	0.90 ± 0.07	0.52 ± 0.1
45	34.849	1540	Undecan-2-one[e]	$C_{11}H_{22}O$	Ketone	0.21 ± 0.03	0.14 ± 0.04	–	0.39 ± 0.09	–	–	–	–
46	37.536	1580	Benzeneacetaldehyde[e]	C_8H_8O	Aldehyde	0.93 ± 0.1	1.82 ± 0.3	0.18 ± 0.04	–	–	–	–	–
47	37.946	1585	Ethyl decanoate[e]	$C_{12}H_{24}O_2$	Aliphatic ester	–	10.96 ± 0.6	0.49 ± 0.03	166.75 ± 23	60.68 ± 10	31.35 ± 3.8	12.11 ± 0.2	2.03 ± 0.2
48	38.011	1586	α-Caryophyllene[e]	$C_{15}H_{24}$	Sesquiterpene	1.34 ± 0.2	–	–	–	–	–	–	–
49	39.242	1605	Isoamyl octanoate[e]	$C_{13}H_{26}O_2$	Aliphatic ester	–	–	0.24 ± 0.03	0.96 ± 0.006	0.40 ± 0.1	0.42 ± 0.06	0.19 ± 0.08	–
50	39.375	1607	2,6-Dimethylocta-2,6-diene[e]	$C_{10}H_{18}$	Monoterpene	–	–	–	0.55 ± 0.1	0.42 ± 0.05	0.27 ± 0.04	0.19 ± 0.05	–

(*continued on next page*)

Table 1 (*continued*)

N°	RT[a] (min)	RI_{Cal}[b]	Volatile metabolites	MF[c]	Family	Total Peak Area ($\times 10^6 \pm \sigma$)						
						Wort	Fermentation			Maturation	Filtration	Final Product
							F 8 °C	F 12 °C	F 14 °C	F 7 °C		
51	39.660	1613	Furfuryl alcohol[e]	$C_5H_6O_2$	Furan compound	0.86 ± 0.2	0.74 ± 0.2	24.73 ± 3.0	0.23 ± 0.1	0.63 ± 0.1	0.57 ± 0.09	0.46 ± 0.1	0.23 ± 0.03
52	41.421	1645	Ethyl 9-decenoate[e]	$C_{12}H_{22}O_2$	Aliphatic ester	–	9.03 ± 3.7	12.84 ± 0.6	29.51 ± 4.2	4.77 ± 0.1	3.17 ± 0.4	3.38 ± 0.1	–
53	48.669	1778	β-Damascenone[e]	$C_{13}H_{18}O$	Ketone	0.28 ± 0.04	–	–	–	–	–	–	–
54	48.913	1782	Phenylethyl acetate[e]	$C_{10}H_{12}O_2$	Aliphatic ester	–	0.53 ± 0.1	4.97 ± 0.4	23.38 ± 1.2	13.62 ± 3.2	9.80 ± 1.9	8.98 ± 0.4	5.08 ± 0.9
55	51.343	1829	Ethyl dodecanoate[e]	$C_{14}H_{28}O_2$	Aliphatic ester	–	–	0.45 ± 0.07	4.08 ± 1.3	1.86 ± 0.6	0.76 ± 0.03	0.17 ± 0.06	–
56	51.60	1834	Hexanoic acid[e]	$C_6H_{12}O_2$	Fatty acid	–	–	1.12 ± 0.06	–	1.34 ± 0.3	0.85 ± 0.3	0.72 ± 0.2	0.60 ± 0.1
57	54.876	1895	Phenylethyl alcohol[e]	$C_8H_{10}O$	Higher alcohol	0.20 ± 0.01	1.63 ± 0.3	14.97 ± 0.8	27.03 ± 2.8	32.17 ± 1.5	27.32 ± 4.5	22.75 ± 1.4	13.43 ± 2.4
58	64.390	2181	Octanoic acid[e]	$C_8H_{16}O_2$	Fatty acid	–	–	4.24 ± 0.6	9.18 ± 1.5	11.30 ± 1.0	3.71 ± 0.4	4.88 ± 0.2	3.05 ± 0.6
59	76.521	2318	*n*-Decanoic acid[e]	$C_{10}H_{20}O_2$	Fatty acid	–	–	1.45 ± 0.3	1.28 ± 0.02	1.36 ± 0.5	–	–	–
60	80.796	2329	Phenylethyl octanoate	$C_{16}H_{24}O_2$	Aliphatic ester	–	–	0.53 ± 0.2	–	–	–	–	–
Total volatile metabolites						31	35	40	33	32	32	31	24
Total Peak Area ($\times 10^7$)						2.49	29.92	73.07	154.19	96.67	76.26	66.22	45.52
SD (%) (n = 3)						8.9	11.4	12.8	4.8	12.5	8.3	7.5	9.9

N – peak number; [a] RT: Retention time; [b] Kovat's retention index relative *n*-alkanes (C_8-C_{20}) on a BP-20 capillary column; [c] MF: Molecular formula; empty boxes mean that the respective VOMs was not identified in that condition; [d] – not detected; [e]VOMs identified using pure standards in addition to NIST library mass spectra. All other volatiles were identified by NIST library and pattern fragmentation; [f]Bold – represents VOMs with statistically significant differences among the selected brewing steps (ANOVA test ($p < 0.05$).

VOMs throughout the brewing process, are almost inexistent in the wort. In contrast, aldehydes are more abundant in the wort and their level decrease progressively till they become vestigial in the final product. This is the case, for instance, of 3-methylbutanal (**5**) which was the main component found in wort samples. As referred above, the fermentation step is crucial for the quality of the beer. During this process numerous by-products that have a considerable effect on the taste, aroma, and other properties that define the beer style are formed. In fact, the fermentation flavour of the lager beer here analysed is totally dominated by aliphatic esters and alcohols, which account for 97% of the volatile metabolite fraction. Among these volatiles, ethyl alcohol (**7**) and ethyl octanoate (**36**) are the most relevant, representing 66% of the volatile metabolite fraction (see Table 1 and Fig. 3). Throughout the fermentation process, significant differences in the volatile profiles were observed, particularly the pronounced peak of aliphatic esters during the fermentation step at 14 °C (F14°C, Fig. 3A), being ethyl octanoate (**36**) the most abundant of the aliphatic esters and the compound with the most distinctive variation, followed by phenylethyl acetate (**54**), ethyl hexanoate (**26**) and isoamyl acetate (**16**). The variation of the alcohols is very similar to the aliphatic esters, with exception of the peak observed in F 14 °C. Among this family of compounds, ethyl alcohol is the most relevant, followed by phenylethyl alcohol (**57**) and 3-methylbutan-1-ol (**23**, also known as isoamyl alcohol). A detailed analysis of the lower abundant compounds identified aldehydes, fatty acids and furans with very specific variations, being present only in certain brewing steps. Among the most abundant aldehydes, acetaldehyde (**1**) was only detected from the second fermentation step (F 12 °C) to the maturation. In contrast, 3-methyl butanal (**23**) decreases from the wort to the F 12 °C. Nonanal (**35**), often reported above odour threshold levels in wort (Dong et al., 2015), have been detected in vestigial levels in almost all the selected sampling points of the brewing process (with exception of F 8 °C and F 14 °C). Furan compounds, particularly furfuryl alcohol (**51**), have been detected in wort, exhibit a notable increase during the F 12 °C fermentation step, decreasing to vestigial levels throughout remain brewing process till the final product. This results agrees with different reports in the literature, indicating furans as a major class of compounds formed during the Maillard reactions that occur during food processing (Vranová & Ciesarová, 2009). Among such Maillard compounds, furfural (**38**) can be further reduced to furfuryl alcohol (**51**) during fermentation (Christoph & Bauer-Christoph, 2007; Hernandes, Souza-Silva, Assumpcao, Zini, & Welke, 2020), therefore contributing to the peak in the abundance of this compound observed in F 12 °C. Remarkably, fatty acids presence increases from this same F 12 °C step, reaching a maximum in the end of the fermentation process and then decreasing. Such trend agrees with previous reports in the literature (Preedy, 2009). Among fatty acids, octanoic acid (**58**) was observed as the most abundant, followed by acetic acid (**37**) and hexanoic acid (**56**) (see Fig. 4).

As the name suggest, maturation is the brewing step in which green beer is treated to develop its finished flavour and aroma (Goldammer, 2008). This allows some personalization between breweries and in the case of the traditional lager beers, as the one here analysed, beer is subjected to a low temperature with bottom fermenting yeasts followed by a lengthy low-temperature maturation phase. This results in a beer with a cleaner flavour profile which is reflected in the decrease in the number of volatile metabolites observed during maturation (32 vs the 40 identified during fermentation). A major change in the volatile profile is that ethyl alcohol (**7**) becomes more abundant than other VOMs that dominate the volatile profile of the previous stages, as ethyl octanoate (**36**), isoamyl acetate (**16**) and 3-methylbutan-1-ol (**23**). Beer filtration is an important step during brewing, contributing significantly to the appearance of the final product that is obtained in the end of this process. The major change observed in the volatile composition of the lager beer upon the filtration process was the decrease in the aliphatic esters, which contrast with the alcohols that maintain their abundance

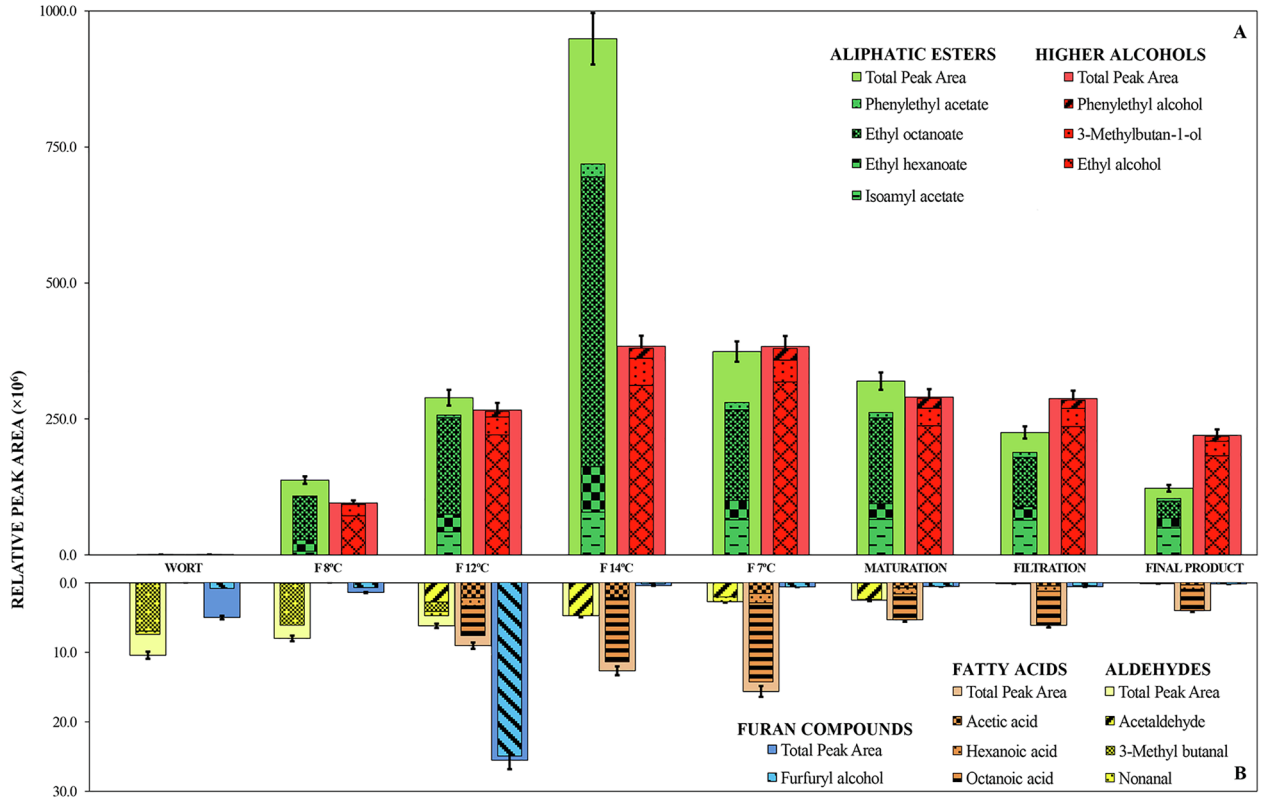


Fig. 3. Evolution of the volatile composition of beer during brewing as assessed by the VOMs composition (average relative area) in the selected samples (wort; fermentation at 8, 12, 14 and 7 °C, respectively F 8 °C, F 12 °C, F14 °C; maturation, filtration and final product). This analysis included the most abundant VOMs (A) and the lower abundant VOMs with relevant variations during the brewing process (B).

almost unaltered from the previous step. Also, the low abundant aldehydes become even less abundant, almost vestigial. This variation in the volatile composition occurs upon the use of candle filters with diatomaceous earth and silica gel were employed to filtrate the lager beers here analysed. Following this filtration, the tetrahop is added to the beer, carbonated and stored in bright beer tanks beer. Then the final product is pumped to the packaging area, where is bottled, canned or kegged. According, the final product flavour comprises two major fractions: the first includes alcohols and aliphatic esters which together account for about 99% of the total peak area from volatile compounds fraction; the remaining 1% includes fatty acids, monoterpenes, furan compounds and aldehydes. The major constituents identified in the final product were the ethyl alcohol (**7**) followed by isoamyl acetate (**16**), 3-methylbutan-1-ol (**23**), ethyl octanoate (**36**) and ethyl hexanoate (**26**). This result agrees with similar reports in the literature that were restricted to the analysis of the volatile profiles of the final product (da Silva et al., 2015; Gonzalez Viejo et al., 2019; Martins et al., 2018; Ocvirk et al., 2018; Stefanuto et al., 2017).

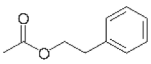
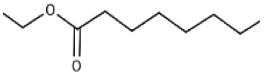
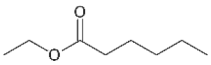
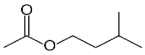
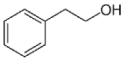
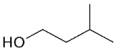
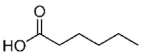
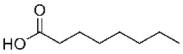
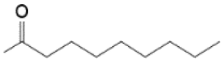
3.3. Major volatomic changes during brewing

The presence and interaction of the VOMs produced and consumed during the brewing process originates a final product (beer) with different organoleptic characteristics and consequently specific identities and characters. Ultimately, it is this combination that makes the consumers decision and so it is crucial to know which VOMs are more important in the definition of each beer flavour.

Upon wort fermentation, the more flavour-active aliphatic esters and their immediate precursors higher alcohols become highly abundant, constituting the most important classes in beer flavour. Yeast and hops are the main sources for these compounds that deliver most of the fruity and flowery aroma compounds present in perceived beer aroma (Christoph & Bauer-Christoph, 2007; Holt, Miks, de Carvalho, Foulquie-Moreno, & Thevelein, 2019). In the lager beer analysed in this work, the most important alcohols observed were propan-1-ol (**12**), 2-methylpropan-1-ol (**15**), 3-methylbutan-1-ol (**23**), hexan-1-ol (**32**) and the aromatic alcohol phenylethyl alcohol (**57**). With exception of phenylethyl alcohol, which has a rose-like odour, remain alcohols are responsible for a malty and burnt flavour (Christoph & Bauer-Christoph, 2007) and so their presence should be well balanced to deliver a fruity character, otherwise a strong pungent and 'fusel-like' smell and taste can occur (Dragone, Mussatto, Oliveira, & Teixeira, 2009). After ethyl alcohol (**7**), 3-methylbutan-1-ol (**23**) was found the most abundant alcohol in beer flavour and this has an important contribution to the consumer acceptance as it affects beer drinkability. Accordingly, sensory analysis reported in the literature described beer flavour as heavier as 3-methylbutan-1-ol content increases (Humia et al., 2019).

Aliphatic esters are responsible for the fruity-flowery aroma notes that are much appreciated in fresh beer and several of them were identified in this work. This included two of the most abundant VOMs identified in this work, the ethyl hexanoate (**26**) and ethyl octanoate (**36**), both having and apple-like flavour and belonging to the ethyl or fatty acid esters family, that result from fatty acids and ethanol (Brányik, Vicente, Dostálek, & Teixeira, 2008). Several other acetate esters, as ethyl acetate (**4**; fruity, solvent-like), isoamyl acetate (**16**; banana) and phenylethyl acetate (**54**; roses, honey, apple), that result from acetyl-CoA and alcohol (Hrivňák et al., 2010; Humia et al., 2019; Pires, Teixeira, Brányik, & Vicente, 2014), were also identified in this work. Overall, these esters are the most significant for the beer aroma and have been identified in similar works analysing the volatile profile of different beers (Gonzalez Viejo et al., 2019; Martins et al., 2018; Ocvirk et al., 2018). It should be emphasized in this context that most of these VOMs have very low odour thresholds and the synergistic effects elicit by the presence of several esters can easily interfere with the beer

Table 2
Relevant VOMs identified in this work and respective aroma.

VOMs	Structure	Chemical Family	Characteristic aroma	Ref.
Most abundant VOMS				
Phenylethyl acetate		Aliphatic ester	Rose, floral, fruity, sweet, honey	[1, 2]
Ethyl octanoate			Fruity, sour apple, fatty, floral, green, anise, sweet, fresh	[1–3]
Ethyl hexanoate			Fruity, sour apple strawberry, anise, wine gum, sweet	[1–3]
Isoamyl acetate			Fresh, banana, sweet, fruity, apple	[1–3]
Phenylethyl alcohol		Alcohol	Honey, sweet, yeast, floral, spicy, herbal, rose	[1–3]
3-Methylbutan-1-ol			Whiskey, malt, burnt, alcohol	[2, 3]
Ethyl alcohol			Pungent, sweet	[1, 3]
Low[a] abundant VOMS				
Furfuryl alcohol		Furan compound	Burnt sugar, fermented, Creamy, Caramel	[1, 3]
Acetic acid		Fatty acid	Sour, vinegar, pungent	[1, 3]
Hexanoic acid			Sweat, Pungent, Cheesy, Goat-like, Rancid	[1, 3]
Octanoic acid			Sweat, cheese, fatty, fresh, moss	[1, 3]
Acetaldehyde		Aldehyde	Pungent, ether, green leaves, fruity	[2, 3]
3-Methyl butanal			Fruity, almond, toasted, malty, green, herbal	[1]
Nonanal			Fatty, citrus, green, gravy, fruity, floral, waxy, sweet, lavender	[1, 3]

[a] Low abundant VOMs previously described as being very important for the aroma composition of different matrices (including beverages as beer)- References: 1. The Pherobase: Database of pheromones and semiochemicals [cited 2020 March 21]. Available from: https://www.pherobase.com/; 2. Olaniran AO, Hiralal L, Mokoena MP, Pillay B. Flavour-active volatile compounds in beer: production, regulation and control. 2017; 123: 13–23; 3. Acree T, Arn H. Flavornet and human odor space. [cited 2020 March 21]. Available from: http://www.flavornet.org/flavornet.html.

flavour perception (reviewed in (Holt et al., 2019; Humia et al., 2019)). Terpenoids have an important role in the definition of beer flavour, although many of them, particularly the hydrophobic terpenoids, are not retained in the finished beer (Takoi et al., 2010). Generically, these compounds add spicy and citrus notes to the beer flavour and β-myrcene (**19**), D-limonene (**22**) and (Z)-β-ocimene (**43**) and the sesquiterpene α-caryophyllene (**48**) have been detected in this work. In contrast to the previous VOMs, there are many compounds whose presence can be beneficial to the beer flavour, but above a certain concentration, an opposite effect can arise. Fatty acids, for instance, can contribute with fruity, cheesy and fatty odours to beer's sensory properties, but also to bitterness, astringency and rancidity when their presence become excessive (Rodrigues et al., 2008). The straight-chain acids hexanoic (**56**) and octanoic (**58**), for instance, were identified in this work as two of the most abundant fatty acids, although in very low levels that decrease with the brewing progression. Hexanoic acid has a pungent, sweaty, cheesy aroma, while octanoic acid has an unpleasant, fatty, oily, rancid aroma (Oliver, 2012). Nevertheless, their presence should be below the odour threshold because such unpleasant flavours are not perceived in the lager beer here analysed. Similarly, the presence of acetic acid (**37**) in beer is obviously not desired due to its typical vinegar-like off-flavour, but this compound was detected in vestigial levels in the final product.

There are several other metabolites, as carbonyl compounds, that are also often associated to unpleasant flavours and aromas and present low flavour thresholds (Saison, De Schutter, Uyttenhove, Delvaux, & Delvaux, 2009). This means that even a vestigial presence of such compounds can have a dramatic effect in the beer flavour (Nedović et al., 2015). This is the case of 3-methylbutanal (**5**) that is described in the literature as potent flavour compound, being responsible for the "malty" character (Smit, Engels, & Smit, 2009). Also, linear aldehydes from hexanal to decanal provide grassy, green, citrus and fatty odour characteristics (Eßlinger, 2009). Included in this group, nonanal (**35**) was detected during the brewing process, including in the final product. In contrast, acetaldehyde (**1**), which has an unpleasant 'grassy' flavour and aroma, was detected during the fermentation and maturation stages, but not thereafter, in the filtrated and final product. This is certainly related with its role as immediate precursor of ethyl alcohol (Supplementary Fig. 2). Ketones are generically associated with pleasant fruity aromas and nine of them were identified in this work. However, some ketones add undesirable flavours to beer. This is the case of vicinal diketones (VDKs), reported in the literature as the most important ketones in beer (Preedy, 2009). VDKs are normally formed during fermentation (Olaniran et al., 2017) and their level can decrease bellow their odour threshold by yeast-mediated reduction at low temperature (often 7 °C). The immediate products of such reduction are 3-hydroxybutan-2-one (**28**) and butane-2,3-diol, from which the first was detect in this work. Another ketone, β-damascenone (**53**), has been associated to off-flavours which arise during beer ageing (Bezman et al., 2005). Accordingly, this ketone was only detected during wort preparation.

Beyond the aspect of adding unpleasant or off-flavours to beer, the

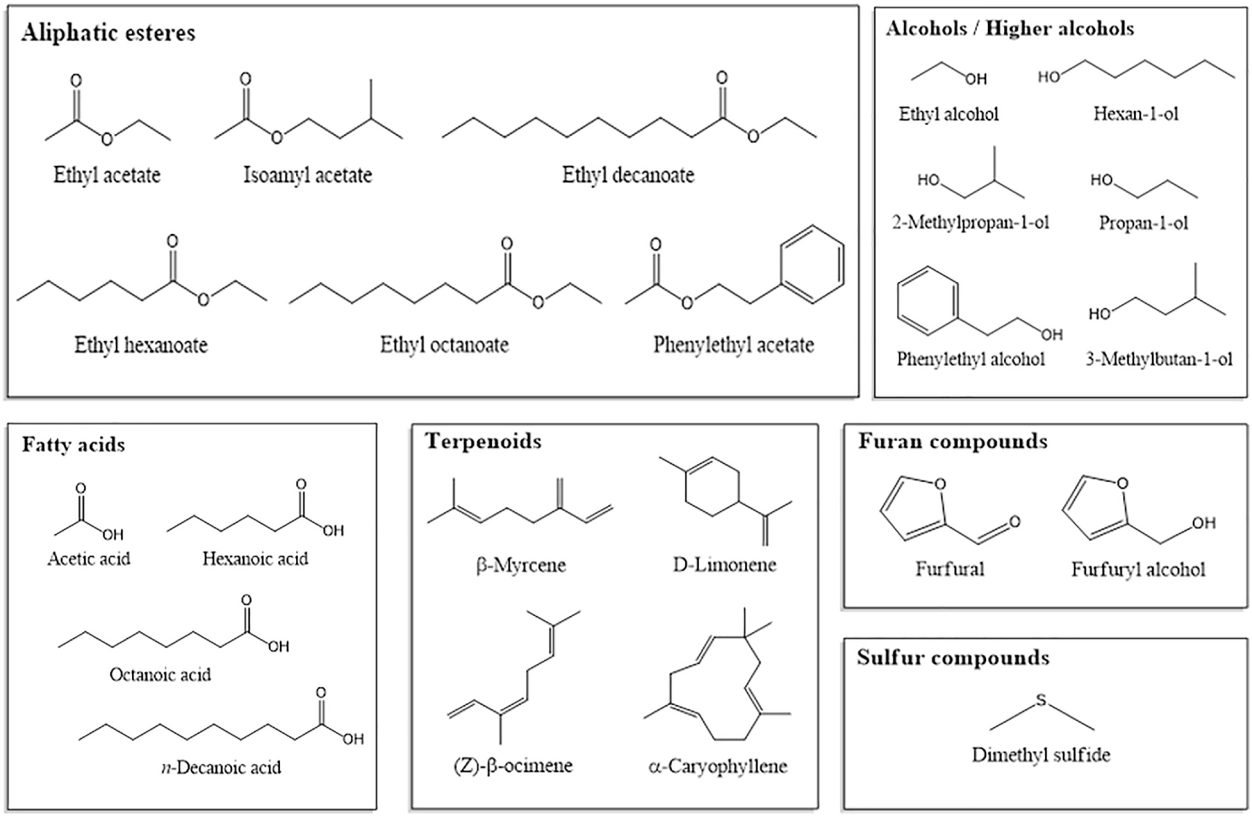


Fig. 4. Structures of the major VOMs identified during the selected brewing steps studied in this work.

presence of some VOMs can be used to diagnose the evolution and quality of the brewing step. Acetic acid, for instance, can be produced during and after fermentation by oxidation of ethanol, but also by bacteria *Acetobacter* (Christoph & Bauer-Christoph, 2007), being in this case associated to bacterial spoilage. In the same context, dimethyl sulphide (**2**, DMS) is an essential contributor to lager beer character and one of the most important markers for brewers while boiling (De Schutter et al., 2007). DMS results from the thermal decomposition of S-methylmethionine (SMM), a molecule present in the barley embryo. However, wort spoilage bacteria, as *Obesumbacterium proteus*, are capable of converting dimethyl sulfoxide (DMSO) to DMS (Oliver, 2012) originating high concentrations of this compound and a consequent unpleasant flavour and aroma usually described as 'cooked sweet-corn' or 'cooked vegetable' (Guadayol, Cortina, Guadayol, & Caixach, 2016). The wort quick cooling is therefore essential to slow DMS production and prevents the growth of bacterial wort spoilers (Oliver, 2012).

Overall and unlike most of the reports in the literature, this work provides a complete volatile fingerprint of the major brewing points, from the wort to the final beer. Such volatile fingerprints are certainly important to get insights about each beer profile, helping the producers to obtain beers with added aroma value. Also, beer volatomic fingerprints can be very valuable tool for beer differentiation and certification of authenticity.

4. Conclusion

In this paper, the beer volatile fingerprinting throughout brewing was profiled using a HS-SMPE/GC–MS methodology. This approach allowed to establish the volatile pattern of a lager beer from wort preparation, fermentation at 8 °C, 12 °C, 14 °C and 7 °C, maturation, filtration to final product. The results show that although the wort composition and its preparation are of utmost importance for the quality of the final product, fermentation is the step of the brewing process that determines the final beer flavour. During fermentation, a burst in the number and abundance of VOMs previously reported to influence the taste, aroma, and other properties that characterize each beer style and flavour was observed The volatile fingerprint constitutes a valuable approach providing useful and comprehensive insights to evaluate the impact of each brewing step on the beer volatile composition and understand the formation of VOMs, helping producers to make high quality beers with added organoleptic properties. In addition, it might act as a powerful tool on quality control, beer differentiation and certification of authenticity.

CRediT authorship contribution statement

Vera Alves: Methodology, Writing - original draft. **João Gonçalves:** Methodology, Writing - original draft. **José A. Figueira:** Methodology, Writing - original draft. **Laura P. Ornelas:** Methodology. **Ricardo N. Branco:** Methodology. **José S. Câmara:** Conceptualization, Supervision. **Jorge A.M. Pereira:** Data curation, Writing - original draft, Writing - review & editing.

Declaration of Competing 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.

Acknowledgements

This work was supported by FCT – Fundação para a Ciência e a Tecnologia (CQM Project PEst-OE/QUI/UI0674/2019, CQM, Portuguese Government funds), and the Madeira 14-20 Program, project PROEQUIPRAM – Reforço do Investimento em Equipamentos e Infraestruturas Científicas na RAM (M1420-01-0145-FEDER-000008) and ARDITI - Agência Regional para o Desenvolvimento da Investigação

Tecnologia e Inovação, through the project M1420-01-0145-FEDER-000005 – Centro de Química da Madeira – CQM+ (Madeira 14-20). JAMP and JAF were supported, respectively, by Post-Doctoral and Doctoral fellowships given by ARDITI (Project M1420 – 09-5369-FSE-000001), JG and VA were supported by FCT (Doctoral fellowships SFRH/BD/116895/2016 and SFRH/BD/117426/2016, respectively). The authors also acknowledge the Empresa de Cervejas da Madeira (ECM) for the supply of the raw material.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.126856.

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