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Analytical Methods

A powerful methodological approach combining headspace solid phase microextraction, mass spectrometry and multivariate analysis for profiling the volatile metabolomic pattern of beer starting raw materials

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ABSTRACT

The volatile metabolomic patterns from different raw materials commonly used in beer production, namely barley, corn and hop-derived products – such as hop pellets, hop essential oil from *Saaz* variety and tetra-hydro isomerized hop extract (tetra hop), were established using a suitable analytical procedure based on dynamic headspace solid-phase microextraction (HS-SPME) followed by thermal desorption gas chromatography–quadrupole mass spectrometry detection (GC–qMS). Some SPME extraction parameters were optimized. The best results, in terms of maximum signal recorded and number of isolated metabolites, were obtained with a 50/30 μm DVB/CAR/PDMS coating fiber at 40 °C for 30 min. A set of 152 volatile metabolites comprising ketones (27), sesquiterpenes (26), monoterpenes (19), aliphatic esters (19), higher alcohols (15), aldehydes (11), furan compounds (11), aliphatic fatty acids (9), aliphatic hydrocarbons (8), sulphur compounds (5) and nitrogen compounds (2) were positively identified. Each raw material showed a specific volatile metabolomic profile. Monoterpenes in hop essential oil and corn, sesquiterpenes in hop pellets, ketones in tetra hop and aldehydes and sulphur compounds in barley were the predominant chemical families in the targeted beer raw materials. β-Myrcene was the most dominant volatile metabolite in hop essential oil, hop pellets and corn samples while, in barley, the predominant volatile metabolites were dimethyl sulphide and 3-methylbutanal and, in tetra hop, 6-methyl-2-pentanone and 4-methyl-2-pentanone. Principal component analysis (PCA) showed natural sample grouping among beer raw materials.

1. Introduction

Beer is one of the most popular beverages worldwide. It is a very complex matrix containing volatile and semi-volatile metabolites, many of them contributing to its flavour, originating from raw materials, namely malted barley and hops or hop-derived products such as hop pellets, CO_2 hop extract and tetra hop extract, as well as from the brewing process (Bamforth, 2003; Hughes, 2003; Preedy, 2009; Silva, Augusto, & Poppi, 2008). Often, other raw materials are used, like unmalted barley, wheat or corn (Preedy, 2009).

In traditional brewing, barley (*Hordeum vulgare*) is the grain of choice. It is readily available, fairly inexpensive, and presents numerous health benefits. In addition, it is a source of starch, proteins, and cytolytic, proteolytic and amylolytic enzymes, which are necessary for the efficient production of wort (Goode, Wijngaard, & Arendt, 2005). Typically, barley is subject to hydration, partial germination and then kilning, which arrests germination but ensures that specific enzyme activities survive. This process starts to release simple sugars, amino acids and other low molecular weight compounds that, under the influence of heat, yield a complex portfolio of Maillard reaction products (Preedy, 2009). Malted barley can have an impact on beer stability due to the presence of some metabolites with antioxidant properties, namely phenolic compounds, ascorbic acid, melanoidins, and several enzymes. Although barley malt is the most important cereal, corn is also used as starch-containing adjuncts or extenders and sources for fermentable sugars (Erbe & Brückner, 2000).

Hops (*Humulus lupulus* L.) and hop-derived products including hop pellets and tetra hop also impart attractive aromas as well as the typical bitter taste. Hops are an economically important crop

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for the brewing industry where they are used to impart flavour and aromas such as floral, spicy, herbal, woody and fruity characteristics. There are a large number of hop varieties commercially available with distinct odour characteristics. Geographical location, climate and agronomical factors are the main factors that affect volatile composition. Generally, hops are added during or after the wort-boiling process to provide the bitter taste, characteristic aromas, and allow the chemical isomerization of α-acids to the more bitter iso-α-acids. To minimize evaporation of essential oil and retain aroma compounds, premium aroma hops are added at the end of boiling (late hopping) (Fernandes, Passos, Medeiros, & da Cunha, 2007; Preedy, 2009).

Understanding the differences in volatile metabolomic patterns from natural products requires reliable and sensible analytical methods. Several extraction and concentration methods, including liquid–liquid extraction (LLE) (Wei, Mura, & Shibamoto, 2001), stir bar sorptive extraction (SBSE) (Horák et al., 2009; Perestrelo, Nogueira, & Câmara, 2009) and solid-phase extraction (SPE) (Mendes, Gonçalves, & Câmara, 2012) have been reported for the analysis of a great number of volatile compounds belonging to heterogeneous chemical classes, such as esters, higher alcohols, fatty acids, aldehydes, ketones, hydrocarbons, ethers, sulphur-, alicyclic-, aromatic-and heterocyclic compounds, among others. These techniques, however, present some drawbacks such as the use of organic solvents and expensive devices with a limited lifetime as well as carryover or cross-contamination problems. Consequently, in order to overcome these drawbacks, solid phase microextraction (SPME) and more recently microextraction by packed sorbent (MEPS) (Jönsson, Hagberg, & van Bavel, 2008) have emerged as efficient extraction/pre-concentration methods and reliable alternatives to traditional sample preparation techniques because of their simplicity, low cost, selectivity, and sensitivity when combined with appropriate detection modes (Caldeira, Rodrigues, Perestrelo, Marques, & Câmara, 2007; Câmara et al., 2007; Dietz, Sanz, & Cámara, 2006). SPME, developed by Pawliszyn and co-workers (Arthur, Killam, Buchholz, Pawliszyn, & Berg, 1992), is selective, rapid, simple and solvent-free allowing the pre-concentration of volatile compounds. Moreover, SPME can integrate sampling, extraction, concentration and injection into a single uninterrupted process, resulting in high sample throughput. Since its introduction, publications of using this promising technique have described the analysis of volatiles in alcoholic beverages (Caldeira et al., 2007; Perestrelo, Caldeira, Rodrigues, & Camara, 2008; Rodrigues, Caldeira, & Camara, 2008) and in different food samples, such as fruit (Pereira, Pereira, & Câmara, 2011), meats (Théron et al., 2010), vinegars (Cirlini, Caligiani, Palla, & Palla, 2011) and fish (Iglesias, Gallardo, & Medina, 2010).

The volatile metabolomic profile of raw materials contains valuable information for brewers. However, no literature can be found describing this. Thus, in the present study, a simple and solvent-free technique, based on volatile extraction using SPME in headspace mode (HS-SPME) combined with gas chromatography–mass spectrometry (GC–qMS) for identification, was used to establish the metabolomic profile of volatile compounds in different raw materials used in Coral beer production (Empresa de Cervejas da Madeira, Portugal), mainly barley, corn, hop pellets, hop essential oil from *Saaz* variety and tetra hop. The procedure was optimized by selection of the appropriate fibre, and extraction temperature and time. Potential differences in the sample composition were investigated. The data obtained were subjected to correlation analysis to determine the relationships amoung the different beer raw materials samples analyzed.

To our knowledge, the metabolomic patterns of volatile compounds in the raw materials for beer have not been investigated previously. The results could provide useful information regarding the distinctive metabolomic profile among the raw materials, some of which contribute directly to flavour while others are important in building up the background flavour of the final product.

2. Materials and methods

2.1. Reagents and materials

The SPME fibre coated with divinylbenzene/carboxen on polydimethylsiloxane (DBV/CAR/PDMS; StableFlex, 50/30 μm), SPME holder for manual sampling, temperature controlled six-vial agitator tray and clear glass screw cap vials for SPME with PTFE/silica (film thickness 1.3 mm) septa were purchased from Supelco (Bellefonte, PA, USA).

2.2. Samples

The samples of milled barley and corn, hop pellets and hop essential oil, obtained by supercritical CO_2 extraction, and tetrahydro isomerized hop extract (tetra hop) were kindly provided by Empresa de Cervejas da Madeira (ECM), Madeira Island (Portugal). Samples were transported under refrigeration (ca. 2–5 °C) to the laboratory and stored at −20 °C until analysis.

2.3. HS-SPME extraction procedure

The HS-SPME experimental parameters were established and optimized previously by Gonçalves, Figueira, Rodrigues, and Câmara (2012). The SPME holder for manual sampling and fibre were purchased from Supelco (Aldrich, Bellefonte, PA, USA). The SPME device included a fused silica fibre coating partially cross-linked with 50/30 μm DVB–CAR–PDMS. The advantage of use a triphasic fibre, such as the DVB/CAR/PDMS chosen for this study, is the recovery of volatile metabolites with both high and low polarity (Cirlini et al., 2011; Théron et al., 2010). The CAR-phase is highly adsorptive (Iglesias et al., 2010) and, thus, increases the retention capacity of the fibre (Silva et al., 2008). Prior to use, the SPME fibre was conditioned at 270 °C for 60 min in the GC injector, according to the manufacturer's instructions. Blank runs were completed, before sampling, each day to ensure no carry-over of analytes from previous extractions.

For the HS-SPME assay, aliquots of 0.5 ± 0.001 g of milled barley, milled corn, hop pellets or hop essential oil were placed into a 4 mL glass vial. The vial was closed and placed in a thermostatic controlled water bath adjusted to 40 ± 0.1 °C. The SPME fibre was manually inserted into the sample vial headspace during 30 min. After extraction, the fibre was retracted prior to removal from the sample vial and immediately inserted into the GC injection port for desorption at 250 °C for six minutes in splitless mode.

For headspace extraction of volatile compounds from tetra hop extracts, aliquots of 0.58 mL (≈0.5 g) were placed into a 4 mL glass vial and subjected to the same procedure to determine the metabolomics profile.

2.4. GC–qMS conditions

GC-qMS analysis of the SPME-collected volatile metabolites were performed on an Agilent Technologies 6890N Network gas chromatograph equipped with a BP-20 fused silica capillary column (30 m length × 0.25 mm i.d.; film thickness 0.25 μm, SGE) 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 of 12 psi). The injections were performed in the splitless mode (5 min). The GC temperature program was from 40 °C (held for 1.0 min) up to 200 °C at a rate of 1.7 °C min^{-1} (held for 1.0 min)