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Evaluation of non-volatile metabolites in beer stored at high temperature and utility as an accelerated method to predict flavour stability

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ABSTRACT

Flavour stability is vital to the brewing industry as beer is often stored for an extended time under variable conditions. Developing an accelerated model to evaluate brewing techniques that affect flavour stability is an important area of research. Here, we performed metabolomics on non-volatile compounds in beer stored at 37 °C between 1 and 14 days for two beer types: an amber ale and an India pale ale. The experiment determined high temperature to influence non-volatile metabolites, including the purine 5-methylthioadenosine (5-MTA). In a second experiment, three brewing techniques were evaluated for improved flavour stability: use of antioxidant crowns, chelation of pro-oxidants, and varying plant content in hops. Sensory analysis determined the hop method was associated with improved flavour stability, and this was consistent with reduced 5-MTA at both regular and high temperature storage. Future studies are warranted to understand the influence of 5-MTA on flavour and aging within different beer types.

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1. Introduction

Flavour stability is vital to success in the brewing industry, as beer is stored, often for long periods of time and under variable conditions. Beer quality is known to be affected by storage conditions, as factors such as temperature and light can influence chemical processes related to taste, aroma, mouthfeel and appearance (Stewart, 2004; Vanderhaegen, Neven, Verachtert, & Derdelinckx, 2006). Volatile compounds, such as aldehydes and sulphur-based compounds, are major contributors to an aged flavour profile (stale flavours, reviewed in (Vanderhaegen et al., 2006)). Additionally, non-volatile compounds, such as polyphenols (contribute to haze and astringent mouthfeel) and hop-derived iso-α-acids (bitter taste), can also vary during storage (Cooman, Aerts, Overmeire, & Keukeleire, 2000; De Clippeleer, De Rouck, De Cooman, & Aerts, 2010; Mikyška, Hrabak, Hašková, & Šrogl, 2002).

Standard packaging and storage procedures (e.g., refrigeration, coloured glass bottles, and providing external carbonation) have been widely adopted in the beer industry to inhibit age-related flavour changes. Nevertheless, flavour stability is variable among beer types, and therefore manufacturers continue to refine brewing methods to inhibit flavour changes during storage. Examples of modifications to the brewing process that may improve beer flavour stability include the addition of antioxidants (e.g., use of glutathione (Gijs et al., 2004)) or oxygen-scavenging crown liners (Gohil & Wysock, 2014; Miltz & Perry, 2005)), chelation of pro-oxidant metals (Wietstock & Shellhammer, 2011; Zufall & Tyrell, 2008), and modified hopping schemes to affect polyphenolic and bitter acid content (Aron & Shellhammer, 2010; Karabín et al., 2014; Kunz, Frenzel, Wietstock, & Methner, 2014).

In addition to modifying brewing techniques, there is a need to develop standard analytical procedures that can quickly and accurately measure small molecules and their association to flavour stability. For example, a method was described that estimated oxidative deterioration based on the *trans/cis* ratio of iso-α-acids

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(Araki, Takashio, & Shinotsuka, 2002), or volatile profiling combined with multivariate analysis (Rodrigues et al., 2011). These assays are direct measures of components that contribute to flavour; however, such methods require storing and testing the beer over several months for the analytical data to correlate with sensory changes in the beer. The need for analytical methods that can rapidly predict sensory shelf life are critical for brewers when developing new beers or making process changes to improve stability, evaluate new materials, or make process efficiency improvements. There are rapid predictive methods that measure individual components or classes of compounds (e.g., free radical formation measured by electron spin resonance spectroscopy). However, flavour is derived as an integration of many non-volatile and volatile compounds (e.g. aldehydes, bitter acids), and flavour stability is related to many types of chemical processes (e.g., oxidation, Maillard reactions). Therefore, measuring a single class of flavour compounds may fail to estimate the overall effect of brewing modifications to flavour.

Previously, we conducted a study that utilised a non-targeted metabolomics workflow encompassing ultra-performance liquid chromatography coupled to mass spectrometry (UPLC–MS) to profile the non-volatile metabolite content of beer stored cold or at room temperature (Heuberger et al., 2012). The putative non-volatile markers of aging have not been previously described to affect flavour, specifically the purines guanosine, guanine, deoxyadenosine, and 5-methylthioadenosine (5-MTA). Together, 5-MTA and other purines may act as indirect markers of beer quality. While these markers have potential utility for assessing overall flavour stability, the observed trends were evaluated over a traditional aging timeline of 16 weeks, which limits their application and advantage over sensory panel evaluation.

Here, we describe the development and validation of an accelerated aging model based on a purine marker. The method reduces the time required to evaluate flavour stability from 16 weeks to 3–5 days, thus enabling high throughput screening to evaluate the impact of experimental brewing techniques on flavour stability. To test the utility of the method, three brewing techniques that were hypothesised to improve the stability of two types of brews (an amber ale and an India pale ale) were evaluated. The amber ale was the same brew as previously reported (Heuberger et al., 2012) and an India pale ale was chosen to determine if similar aging trends are observed in a beer with very different flavour characteristics. Beer was aged under both traditional and accelerated time scales. Both sensory and molecular analysis was performed on beer stored in regular conditions (16 weeks), and non-volatile metabolite variation was measured in the beer aged under accelerated conditions. The data presented herein support that an accelerated aging model based on non-volatile molecular markers may expedite the evaluation of different brewing modifications to improve beer stability.

2. Materials and methods

2.1. Brewing parameters and storage

Beer was brewed at New Belgium Brewing Company (Fort Collins, CO) to produce an amber ale (AA) or India pale ale (IPA) style beer. Modified brewing methods included (1) the use of antioxidant crowns (Pelliconi, Bologna, Italy) (2) late-hopping to reduce α-acid isomerisation and chelate iron (Wietstock & Shellhammer, 2011) and (3) adding hops with low (control), medium ($MBhop_1$), or high plant content (MB_{hop2}), determined by small, medium, or large particle size. Long-term cold storage (“regular storage”) consisted of storing bottles for 30 days at ambient temperature (~21 °C) and then at 4 °C for an additional 12 weeks (“regular storage”). For metabolite analysis, beer was collected at 0 and 16 week time points and stored at −80 °C until further analysis.

2.2. Qualitative sensory analysis

Beer was sampled for sensory evaluation every 4 weeks and served blind to a trained expert sensory panel at four intervals (0, 4, 8 and 12 weeks). Sample temperature, volume, time, location and method of analysis were standardised to minimise evaluation bias. For each interval, ten or more expert panellists were instructed to generate robust, qualitative sensory descriptions for each sample and determine a perceived level of age by integrating data for several traits, including components of taste, aroma, mouthfeel and appearance. The final data points consisted of whether or not the AA or IPA still tasted true to brand after 12 weeks of storage.

2.3. Accelerated aging

Beer was incubated at 37 °C (or 4 °C as a control) for 0–14 days. Each sample consisted of an individual bottle brewed from the same AA or IPA batch. Bottle caps were removed and bottles equilibrated at room temperature until bubbles were not present when pipetting (~1 min of equilibration). A 1-mL aliquot of beer was transferred to a 2-mL microcentrifuge tube and stored at −80 °C until further analysis.

2.4. Non-volatile metabolite detection and processing

One microlitre of beer was injected into an Acquity UPLC system (Waters Corporation, Milford, MA). Separation was conducted with an Acquity UPLC T3 column (1.8 μm, 1.0 × 100 mm; Waters Corporation), using a gradient from solvent A (water, 0.1% formic acid) to solvent B (acetonitrile, 0.1% formic acid). Injections were made in 100% A and held for 1 min. A 12 min linear gradient to 95% B was then applied and held at 95% A for 3 min. Solvent composition was returned to starting conditions over 0.05 min and allowed to re-equilibrate for 3.95 min. Flow rate was constant at 200 μL/min for the entire run. The column was held at 50 °C and samples were held at 5 °C. Column eluent was coupled to a Xevo G2 Q-Tof MS (Waters Corporation) fitted with an electrospray source. Data was collected in positive ion mode, scanning from m/z 50–1200 at a rate of 5 scans s^{-1}, alternating between MS and MS^E mode. Collision energy was set to 6 V for MS mode, and ramped from 15 to 30 V for MS^E mode. Calibration was performed prior to sample analysis *via* infusion of sodium formate solution, with mass accuracy within 1 ppm. The capillary voltage was held at 2200 V, the source temperature at 150 °C, and the desolvation temperature at 350 °C at a nitrogen desolvation gas flow rate of 800 L/h.

For each sample, a matrix of molecular features (defined by retention time and mass (m/z)) was generated using XCMS software (Smith, Want, O'Maille, Abagyan, & Siuzdak, 2006). Samples were normalised to the total ion current and the relative quantity of each molecular feature was determined by the mean area of the chromatographic peak among replicate injections ($n = 2$). Mass spectra were generated using an algorithm that clusters masses into spectra (‘spectral clusters’) based on co-variation and co-elution in the data set (Broeckling, Afsar Minhas, Neumann, Ben-Hur, & Prenni, 2014). 5-Methylthioadenosine was annotated based on retention time and spectral matching of an authentic standard within an in–house library (a Level 1 identification as described in Sumner et al. (2007)).

2.5. Statistical analysis

Principal component analysis was performed using the pcaMethods package in the R statistical environment after mean-centring and Pareto scaling (R Core Team, 2012). For regular

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