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Key volatile aroma compounds of lactic acid fermented malt based beverages – impact of lactic acid bacteria strains

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β-Damascenone (PubChem CID: 5374527)
Furaneol (PubChem CID: 19309)
Phenylacetic acid (PubChem CID: 999)
2-Phenylethanol (PubChem CID: 6054)
Acetaldehyde (PubChem CID: 177)
Sotolon (PubChem CID: 62835)
Vanillin (PubChem CID: 1183)
Ethyl 2-methylbutanoate (PubChem CID: 24020)
4-Vinylguaiacol (PubChem CID: 332)
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ABSTRACT

This study aims to define the aroma composition and key aroma compounds of barley malt wort beverages produced from fermentation using six lactic acid bacteria (LAB) strains. Gas chromatography mass spectrometry–olfactometry and flame ionization detection was employed; key aroma compounds were determined by means of aroma extract dilution analysis. Fifty-six detected volatile compounds were similar among beverages. However, significant differences were observed in the concentration of individual compounds. Key aroma compounds (flavor dilution (FD) factors ≥16) were β-damascenone, furaneol, phenylacetic acid, 2-phenylethanol, 4-vinylguaiacol, sotolon, methional, vanillin, acetic acid, norfuraneol, guaiacol and ethyl 2-methylbutanoate. Furthermore, acetaldehyde had the greatest odor activity value of up to 4266. Sensory analyses revealed large differences in the flavor profile. Beverage from *L. plantarum* Lp. 758 showed the highest FD factors in key aroma compounds and was correlated to fruity flavors. Therefore, we suggest that suitable LAB strain selection may improve the flavor of malt based beverages.

1. Introduction

Due to increasing consumer awareness to the importance of healthy nutrition, the market for functional, natural and non-alcoholic beverages is steadily increasing all over the world (Fortitech., 2011). Cereal based beverages produced from lactic acid fermentation (LAFCB) are gaining a lot of attention because of their high nutritional values and functional properties that meet diverse and changing consumer nutrition demands such as lactose free, vegan, low cholesterol, non-alcoholic, gluten-free, natural and functional foods. However, their flavor was not accepted by consumers who requested flavor improvement as the most important aspect before nutritional properties (Yu & Bogue, 2013).

Cereals and cereal derived substrates are very rich in nutrients including proteins and bioactive compounds (Hassani, Zarnkow, & Becker, 2013). Barley is the cereal of choice with respect to its enzymatic activity, processability, nutrient content and sensory profile (Charalampopoulos, Pandiella, & Webb, 2003; Steiner, Gastl, & Becker, 2011). Malting and mashing, performed in breweries for malt wort production, and lactic acid fermentation further increase the nutrient availability and reduce anti-nutrient compounds such as phytates and oxalic acids occurring in cereals (Hassani, Procopio, & Becker, 2016; Kanauchi, Milet, & Bamforth, 2009; Singh, Rehal, Kaur, & Jyot, 2013). Hence, optimized malting and mashing conditions of diverse cereals were proposed for the production of lactic acid based beverages (Hassani et al., 2013; Zarnkow, Keßler, Back, Arendt, & Gastl, 2010). Furthermore, malt-

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ing and mashing improve the sensory quality and volatile composition of the resulted substrates which may significantly contribute to the final beverage flavor. Malt wort is therefore a complex nutrient rich substrate suited for LAFCB production than unmalted cereal substrates.

The volatile composition of LAFCB is affected by the type of cereal and processing treatment. Diverse types of cereal and substrates have been considered so far for LAFCB production. The volatile composition and sensory profile of LAFCBs produced from different cereal types were different even when same lactic acid bacteria (LAB) strain was used for fermentation. As such, a considerable amount of new volatiles was generated in barley malt than in non-malted cereal substrates during lactic acid fermentation (Coda, Rizzello, Trani, & Gobbetti, 2011; Salmerón, Fuciños, Charalampopoulos, & Pandiella, 2009; Salmerón, Thomas, & Pandiella, 2015).

However, LAB strains also influence the sensory and aroma profile of LAFCB. Significant differences in the acetaldehyde content and sensory acceptance were reported in malt based beverages formulated with *L. plantarum* and those with *L. acidophilus* and *L. reuteri*. Whether for acetaldehyde content or sensory acceptance, very large differences were observed in the values (Salmerón et al., 2015). Significant differences among LAB strains were described in the specific activity of some relevant enzymes involved in flavor formation during lactic acid fermentation (Smit, Smit, & Engels, 2005). Furthermore, beverage produced from *L. plantarum* NCIMB 8826 fermentation of barley malt had a better sensory acceptance than its counterparts produced with other LAB strains. It was tentatively attributed to differences in acetaldehyde content (Salmerón et al., 2015). Consequently, LAB strain selection was proposed as an approach for flavor improvement of LAFCB (Nsogning Dongmo, Procopio, Sacher, & Becker, 2016).

A prerequisite for flavor improvement is the thorough elucidation of the aroma composition and key aroma compounds in order to outline existing shortcomings. The aroma profile of LAFCB was not given an important focus while it is the main character which determines the acceptance. The most studied aroma compounds were acetoin, acetone, ethanol and acetaldehyde.

To the best of our knowledge, key aroma compounds that determine the flavor of LAFCB are not yet reported. The aim of this study is to define the aroma composition and key aroma compounds of barley malt wort beverages produced from lactic acid fermentation. Ultimately, the impact of six LAB strains on the aroma profile was investigated. This is an important basic knowledge for future attempts to the flavor improvement of cereal based beverages.

2. Materials and methods

2.1. Wort preparation and fermentation

Wort at 14% concentration was prepared from 72% standardized unhopped Bavarian pilsner barley malt extract from Weyermann® (Bamberg, Germany) using distilled water and autoclaved at 110 °C for 10 min. Hot break materials were separated after cooling. Six selected and previously identified strains of *L. plantarum* Lp. 758, Lp. 765 and Lp. 725, *L. brevis* Lb. 986 and *L. amylolyticus* La. TL3 and La. TL5 were obtained from the strain collection of the Institute of Brewing and Beverage Technology, Technische Universität München (München, Germany). These strains were preselected in preliminary screening experiments based on their current use in breweries (*L. amylolyticus, L. plantarum*) or their natural occurrence in malt wort (*L. brevis*). Cultures were stored in MRS broth containing 60% (v/v) glycerol at −80 °C. They were propagated twice in MRS broth (Sigma Aldrich, Germany) for 24–36 h and precultured in wort for 12 h at 28 °C (*L. brevis* and *L. plantarum)* and 48 °C (*L. amylolyticus)* before use for the experiments. Cells were washed thrice with sterile quarter strength Ringer's solution at 4000 rpm (rpm), 4 °C for 10 min. Fermentation was carried out in triplicate for each LAB strain at laboratory scale in 500 mL wort volume in static conditions for 72 h. The inoculation rate was $5.8 \pm 1.1 \times 10^6$ CFU (colony forming unit)/mL. Fermented beverages were immediately stored at 2 °C for sensory evaluation within 24 h and at −20 °C for aroma compounds analysis.

2.2. Volatile compounds extraction

2.2.1. Head-space-solid-phase-micro-extraction (HS-SPME)

An amount of 5 g fermented cold sample was weighed into a 20 mL glass headspace vial, tightly sealed with silicon septum and flanged cap and pre-equilibrated for 10 min at 36 °C. The septum was pierced with the SPME needle, and subsequently, the fiber was manually exposed to the sample headspace for an adsorption at 36 °C for 30 min for better estimation of aroma profile as perceived by the human nose (Sagratini et al., 2012). The SPME fiber material was 50/30 μm Divinylbenzol/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) (Supelco Inc., Bellefonte, USA). After adsorption, the fiber was directly transferred to the Gas-Chromatography–Mass Spectrometry–Olfactometry (GC–MS–O) injection port. Volatiles were then desorbed at 250 °C for 30 s.

2.2.2. Steam distillation

Volatile fraction was isolated using a Büchi distillation unit (Büchi K-314, Büchi Labortechnik, Germany) as previously described (Krahl, Zarnkow, Stürmer, & Becker, 2009). To 100 mL of cold samples, 9 mL ethanol (purity ≥99.5) and 1 mL of internal standards (methyl heptanoate and benzyl alcohol) were added. Samples were distilled at 100 °C into an ice cooled 100 mL volumetric flask and stored at 4 °C. Subsequently, 80 mL distillates, 22.2 g sodium chloride and 0.5 mL dichloromethane were given in a centrifuge glass tube, shaken for 30 min (Turbula-Shaker, Willy A. Bachhofen, Switzerland) and centrifuged at 0 °C with 2400 rpm for 15 min (Heraeus Megafuge 1.0 R, Thermo Fischer Scientific Inc., Massachusetts, USA). The supernatant was removed and the dichloromethane pearl in the bottom was pipetted into a GC–vial with integrated glass insert and filled with distilled water using a Pasteur pipette due to dichloromethane high volatility and to allow water separation from the dichloromethane pearl.

2.2.3. Solvent assisted flavor evaporation (SAFE)

The extraction was based on the procedure proposed by Schieberle (1996). A volume of 100 mL fermented sample was separated three times in a separatory funnel with diethyl ether (3×150 mL) after addition of 0.5 mL internal standard (methyl heptanoate and benzyl alcohol) The extracts were collected and washed twice with saturated NaCl solution (2×225 mL). The washed extract was dried by the addition of Na_2SO_4 and filtered through filter paper. The extract was subsequently concentrated to 30 mL using a Vigreux column (40 °C). The volatile fraction of the concentrated extract was isolated by means of SAFE (Engel, Bahr, & Schieberle, 1999). Isolated volatile fraction was further concentrated to 1 mL using the Vigreux column and stored at −20 °C prior to GC–MS–O analyses.

2.3. Analysis of volatile compounds by GC–MS–O and aroma extract dilution analysis

Identification of volatiles from SAFE distillate was performed on a Thermo Scientific GC Trace 1300 directly coupled to an ISQ-mass spectrometer (Thermo Fischer Scientific Inc, Darmstadt, Germany) as previously described (Roth, Schuster, Kollmannsberger, Jekle, & Becker, 2016). The column effluent was split by a 2-way μ-flow-