K. Schwarz and F.-J. Methner

Styrene Concentrations during Wheat Beer Production

The screening of styrene concentrations during different stages of wheat beer production shows that in opposite to thermal processes like mashing and wort boiling the primary and secondary fermentation contribute most to the styrene content in final beers. Therefore, the enzymatic decarboxylation of cinnamic acid by brewers' yeast is the predominant kind of reaction. Styrene concentrations in bottled beer were up to 25 ppb. In addition, the results of the screening were compared with the precursor concentration of cinnamic acid in the corresponding pitching wort. A linear correlation could not be found. Moreover, the influence of various manufacturing considerations like the kind of fermentation or the ratio of wheat grist load is shown. For example, the cinnamic acid content in wort decreases with an increasing amount of wheat malt.

Descriptors: styrene, wheat beer, aroma, cinnamic acid, 4-vinylguaiacol

1 Introduction

Styrene is a sweet-smelling fluid and serves as a starting product during polystyrene production. It can be used as packing material for dairy products or insulating materials. Styrene can be found in low concentration as a natural component in numerous food products, such as coffee, meat, cereals, and nuts. Higher levels (0.17–39.2 mg/kg) are partly found in cinnamon, where it is formed from cinnamon aldehyde under unfavorable conditions during storage and transport [1, 2].

From the toxicological point of view, styrene is a harmful component with a low carcinogenic potential. The possible carcinogenic effect of styrene in human tissues which is based on the proper toxicological relevant metabolite styrene-7.8-oxide, was stated for the first time in a press release from 2006 by the German Federal Institute on Risk Assessment (BfR) [3]. Additionally, styrene is classified as possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC). For those classified substances the principle of minimizing strategy has to be applied due to the fact that a toxicological limit can not be stated [4]. Consequently, the WHO derived two important reference values. The tolerable daily intake (TDI) of styrene in drinking water with 7.7 µg/kg body weight (BW) and the preliminary acceptable daily intake (ADI) with 4 µg/kg BW [5].

The formation of styrene, as derived from cinnamic acid (CA) occurs analog to the decarboxylation of ferulic- and p-cumaric acid to 4-vinylguaiacol and 4-vinylphenol (Fig. 1). These reactions can proceed both as an enzymatic [6, 7, 8] and as a thermal decarboxylation [9, 10]. The enzymatic decomposition of cinnamic acid to styrene is encoded by the same phenyl acrylic acid decarboxylase (PAD1) and ferulic acid decarboxylase (FDC1) gene, as the decomposition of phenol carboxylic acids to the corresponding phenols [11]. Only phenolic off-flavor positive (POF+) yeast strains, e.g. top-fermenting *Saccharomyces cerevisiae*, which are used for wheat beer production possess this enzyme equipment [12, 13].

Purpose of this study was to screen the brewing process to identify the production stage which makes the greatest contribution to the styrene content and the ranges in which the concentrations of styrene in bottled wheat beer can be found. Furthermore, the predominant kind of reaction can be defined.

2 Materials and methods

2.1 Samples

Up to 24 samples of full kettle wort, pitching wort, green beer, maturated beer and bottled beer were provided from 19 German wheat beer breweries.

2.2 Chemicals and Reagents

All analytes and reagents were of analytical or HPLC grade, respectively. Cinnamic acid, styrene, methanol and acetic acid were obtained from Sigma-Aldrich (Steinheim, Germany). Toluene and sodium hydroxide (solid) were obtained from Merck (Darmstadt, Germany).

2.3 Styrene

For the determination of styrene, the samples were decarbonized with 12 N NaOH (0.2 mL for 10 mL) and tempered at 20 °C. 10 mL were transferred into a headspace (HS) vial and 100 µL toluene containing 2 µg/mL was added as an internal standard.

Prior to injection, the samples were tempered at 80 °C for 45 min. The injection volume of the gas was 1 mL. The HS-syringe was tempered at 100 °C. A HS-GC-FID (Hewlett-Packard 6890)

Authors

Dipl.-LMChem. Katrin Schwarz, Prof. Dr.-Ing. Frank-Jürgen Methner, Technische Universität Berlin, Institut für Biotechnologie, Fachgebiet Brauwesen, Berlin, Germany; corresponding author: katrin.schwarz@tu-berlin.de

Figures see Appendix

equipped with a 60 m x 0.32 mm I.D. x 0.5 µm DB-WAX capillary column (J&W Scientific) and a Headspace autosampler (Gerstel MPS 2) were used. Hydrogen was used as carrier gas at a flow rate of 5.4 mL/min. The injector temperature was 200 °C. The temperature of the FID was 200 °C. The GC program started at 40 °C for 10 min, then increased to 130 °C at 10 °C/min, followed by an increase to 220 °C at 22.5 °C/min, and a final holding time of 2 min. The quantification of styrene was performed by an external calibration between 0.5 and 100 ppb.

2.4 Cinnamic acid

The quantification of CA in full kettle and pitching wort was performed by an Agilent 1100 HPLC-DAD. Prior to injection, the samples were centrifuged with 10.000 rpm for 10 min at 15 °C. The supernatant fluid was transferred into auto sampler vials and frozen at –18 °C until analyzed. Peak areas were analyzed with Agilent ChemStation. The mobile phase consisted of two solvents: Solvent (A) water/acetic acid (98:2; v/v) Solvent (B) methanol/acetic acid (98:2; v/v). Cinnamic acid was eluted under following conditions: 20 µL automatic sample injection, 1 mL/min flow rate, temperature was set at 40 °C. For separation a Purospher® STAR RP18e LiChroCHART 250 mm x 4 mm x 6 µm (Merck, Darmstadt) was used. The identification of cinnamic acid was obtained by retention time and ultra-violet-visible spectra. Quantification was performed by external calibration with standard at 280nm. The following elution program was used at 40 °C: 0–7 min, 95–90 % A; 7–28 min, 90–65 % A; 28–45.5 min, 65–40 % A; 45.5–49 min, 40–5 % A; 49–50.5 min 5 % A; 50.5–52.5 min, 5–95 % A; 52.5–56 min, 95 % A. The flow rate was set at 1.0 mL/min.

3 Results and discussion

Styrene concentrations of full kettle wort (Fig. 2) ranged from 0 to 2 ppb. One third of those were between 0.5 and 1 ppb. The values of the styrene content in pitching wort (Fig. 3) were sim-ilar (0–1.5 ppb). Over 40 % of the samples had a concentration between 0.5 and 1 ppb. In green beer (Fig. 4), the styrene concentrations increased up to 25 ppb and 74 % of these samples had a concentration up to 10 ppb. During the next production stage, the maturated beer (Fig. 5), styrene concentrations decreased slightly. About 60 % of all samples had a styrene concentra-tion up to 10 ppb. The analyzed samples of bottled beer (Fig. 6) contained concentrations between 1 and 25 ppb. The majority of these samples had a styrene content of 10 ppb (90 %). Up to 35 % contained concentrations between 20 and 25 ppb. Therefore, there is an increase from maturated to bottled beer.

In addition to the styrene concentration, the content of the pre-cursor cinnamic acid was quantified in full kettle and pitching wort. In figure 7, the cinnamic acid concentrations of full kettle wort samples are plotted against the styrene content of the corresponding bottled beers. A linear correlation was not found. The range of cinnamic acid in the samples ranged from 75 ppb to 350 ppb as calculated on an extract of 12 °P. The majority (71 %) had a concentration between 175 ppb and 275 ppb.

In contrast, a linear correlation between the cinnamic acid content in wort and the used ratio of wheat could be observed (Fig. 8). The concentration of CA decreases with a rising amount of wheat malt. The high standard variance can be explained by the use of different types of barley, especially colored malt. In this context, own investigations pointed out, that darker malt has a higher CA content. This correlates with the screening of different types of beer and special manufacturing processes (Fig. 9). Darker beers had a higher average in styrene concentration than pale beers. Furthermore, bottle fermented beers had also a higher average concentration than beers without bottle fermentation.

4 Conclusions

The formation of styrene in wheat beer is determined by the enzymatic decarboxylation of cinnamic acid. This is confirmed by the large increase during the fermentation processes. In contrast to thermal processes, like mashing, wort boiling and flash pasteurization, which have no high contribution to the styrene content of wheat beer. However, thermally formed styrene can be found in condensates of the wort boiling process. The non-existent correlation between cinnamic acid concentration in wort and the concentration of styrene in beer does not suggest a disconnection between these two substances but the importance of the individual process control. Especially, the used yeast strain, the fermentation temperature and time and the kind of fermentation (e.g. open, closed or bottle fermentation) appear to have significant influences of the styrene content in the final beer (Fig. 9). It also has been shown that the cinnamic acid content in wort is dependent on the ratio of wheat malt. This leads to a decreasing concentration of CA with a rising amount of wheat. Due to the fact that wheat has also a lower level of ferulic acid (FA) and both phenolic acids were metabolites of the phenylpropanoid pathway [14, 15]. Moreover, analyzed dark wheat beers had a higher average concentration of styrene than pale beers.

Acknowledgments

This research project (AiF 16301 N) was supported by the German Ministry of Economics and Technology (via AiF) and the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn. We also thank the Wissenschaftsförderung der Deutschen Brauwirtschaft (Wifö) for the financial support and the Bayerischer Brauerbund e.V. and the involved breweries for the samples.

5 References

1. Steele, D.H.; Thornburg M.J.; Stanley, J.S.; Miller, R.R.; Brooke, R.; Cushman, J.R. and Cruzan, G.: Determination of Styrene in Selected Foods, Journal of Agricultural and Food Chemistry, **42** (1994), pp. 1661-1665.
2. Fragnièr, C.; Aebischer, J.-N. and Dudler, V.: A short study on the formation of styrene in cinnamon, Mitteilungen aus Lebensmitteluntersuchung und Hygiene A, **94** (2003), pp. 609-620.
3. Bundesinstitut für Risikobewertung: Hinweise auf tumorauslösende Wirkung von Styrol beim Menschen, Pressinformation, http://www.

bfr.bund.de/de/ presseinformation/2006/25/hinweise_auf_tumorausloesende_wirkung_von_styrol_beim_menschen-8281.html **25** (2006).
4. International Agency for Research on Cancer (IARC): The Evaluation of Carcinogenic Risks to Humans: Some traditional herbal medicines, some mycotoxins, naphthalene and styrene, WHO, **82** (2002).
5. Word Health Organisation (WHO): Guidelines for drinking-water quality. 2nd ed., vol. 2. Health criteria and other supporting information. World Health Organization, Genf, 1996.
6. Wackerbauer, K.; Krämer, P. and Siepert, J.: Phenolische Aromastoffe im Bier, BRAUWELT, **122** (1982), pp. 618-626.
7. Kieniger, H.; Narziß, L.; Miedaner, H. and Hecht, S.: Über die Veränderung wertbestimmender Stoffgruppen bei der Herstellung von bayrischen Weizenbieren, Monatsschrift für Brauwissenschaft, **55** (1984), pp. 9-18.
8. Wackerbauer, K.; Kossa, T. and Tressl, R.: Bildung von Phenolen durch Hefen, Monatsschrift für Brauwissenschaft, **31** (1978), pp. 52-55.
9. Dorfner, R.; Ferge, T.; Kettrup, A.; Zimmermann, R. and Yeretzian, C.: Real-time monitoring of 4-vinylguajacol, guajacol and phenol during coffee roasting by resonant laser ionization time-of-flight mass spectrometry, Journal of Agricultural and Food Chemistry, **51** (2003), pp. 5768-5773.
10. Tressl, R.; Kossa, T.; Renner, R. and Köppler, H.: Gaschromatographisch-massenspektrometrische Untersuchungen über die Bildung von Phenolen und aromatischen Kohlenwasserstoffen in Lebensmitteln, Zeitschrift für Lebensmittel-Untersuchung und -Forschung, **162** (1976), pp. 123-130
11. Mukai, N.; Masaki, K.; Fujii, T.; Kawamukai, M. and Iefuji, H.: PAD1 and FDC1 are essential for the decarboxylation of phenylacrylic acids in *Saccharomyces cerevisiae,* Journal of Bioscience and Bioengineering, **109** (2010), pp. 564-569.
12. Thurston, P.A. and Tupp, R.S.: Screening yeast strains for their ability to produce phenolic off-flavours – a simple method for determining phenols in wort and beer, Journal of the Institute of Brewing, **87** (1981), pp. 177-179.
13. Goddey, A.R.; and Tubb, R.S.: Genetic and Biochemical Analysis of the Ability of *Saccharomyces cerevisiae* to Decarboxylate Cinnamic Acids, Journal of General Microbiology, **128** (1982), pp. 261 5-2620.
14. Coghe, S.; Benoot, K.; Delvaux, F.; Vanderhaegen, B. and Delvaux, F.R.: Ferulic Acid Release and 4-Vinylguaiacol Formation during Brewing and Fermentation: Indications for Feruloyl Esterase Activity in *Saccharomyces cerevisiae*, Journal of Agricultural and Food Chemistry, **52** (2004), pp. 602-608.
15. Bourne, L.C. and Rice-Evans, C.: Bioavailability of Ferulic Acid, Biochemical and Biophysical Research Communications, **253** (1998), pp. 222-227.

Received 24 October 2011, accepted 20 November, 2011

Appendix

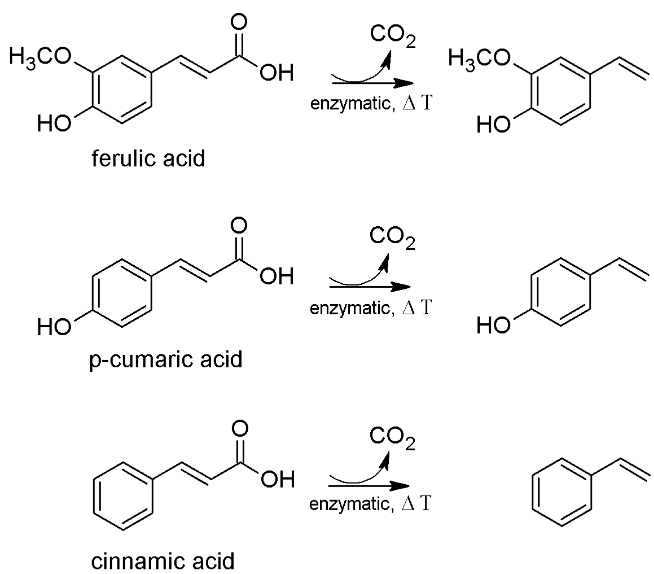

Fig. 1 Formation of 4-vinyl aromates

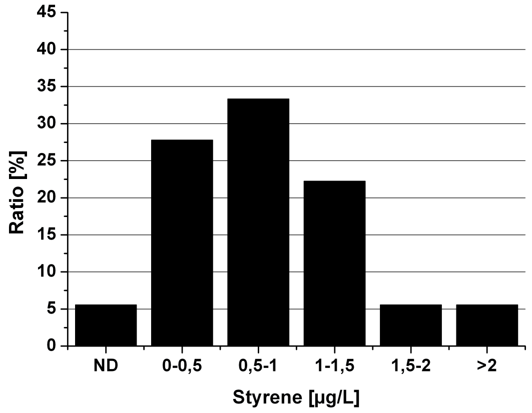

Fig. 2 **Styrene concentration in full kettle wort, n = 18**

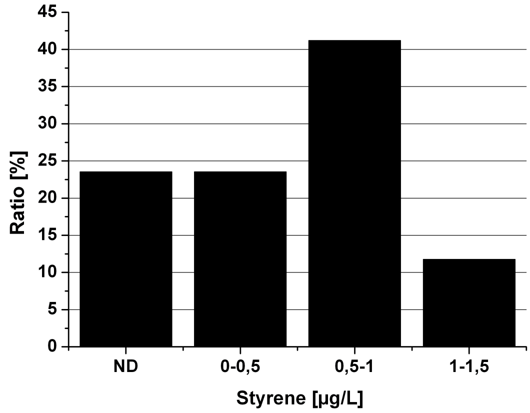

Fig. 3 **Styrene concentration in pitching wort, n = 17**

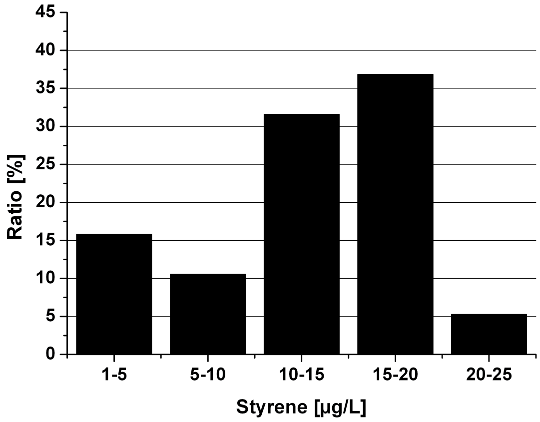

Fig. 4 **Styrene concentration in green beer, n = 19**

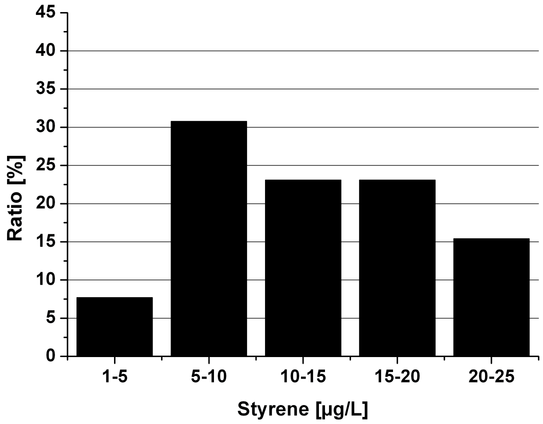

Fig. 5 **Styrene concentration in maturated beer, n = 13**

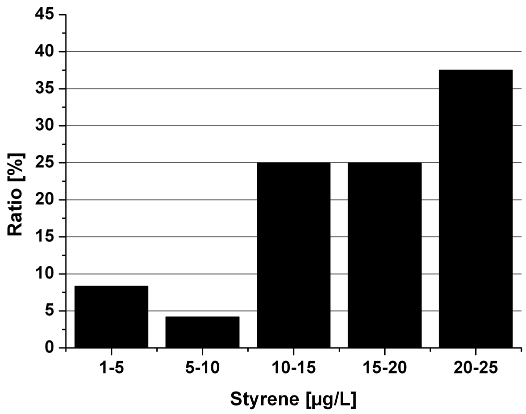

Fig. 6 **Styrene concentration in bottled beer, n = 24**

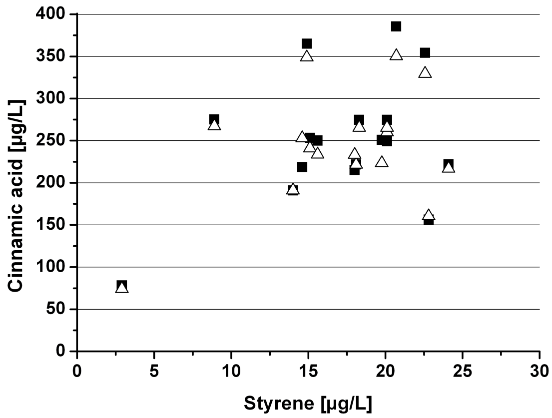

Fig. 7 **Correlation between cinnamic acid concentration in full kettle wort and styrene concentration in wheat beer:**

■ absolute concentrations

Δ normalized at 12 °P

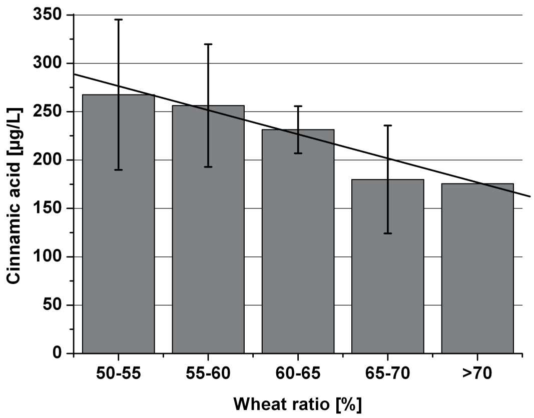

Fig. 8 **Normalized cinnamic acid concentrations (12 °P) in full kettle wort dependent on the wheat ratio, n = 15**

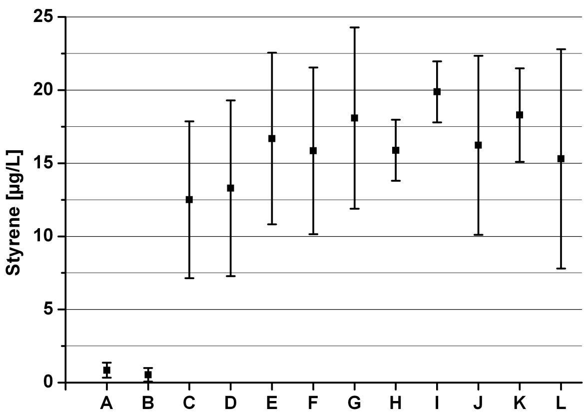

Fig. 9 **Average concentrations of styrene in different production stages and wheat beer types. A: Full kettle wort (n = 18) B: Pitching wort (n = 17) C: Green beer (n = 19) D: Maturated Beer (n = 13) E: Bottled Beer (n = 24) F: Without flash pasteurization (n = 9) G: With flash pasteurization (n = 8) H: Crystal Beer (n = 3) I: Dark beer (n = 3) J: Pale beer (n = 21) K: Bottle fermented (n = 6) L: Without bottle fermentation (n = 7)**