Monitoring Volatile Substances in Beer in Relation to Beer Production Technology

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Abstract—**The content of volatile substances was analysed in relation to beer production technology using a gas chromatograph along with a mass spectrometric detector (GC-MS). Wort boiling increases the percentage of β-myrcene compared with other components of the essential oil of hops while reducing the level of malt volatiles except for furanmethanol. Maltol loss through evaporation exceeds the production during the Maillard reaction. The concentration of fermentation products was detected to be the highest on day 4 of fermenting. Secondary fermentation results in a noticeable decrease in the content of esters over the initial weeks of the process. The content of volatiles in the beer wort is not proportional to their production during wort boiling and fermentation. The evaporation of volatile substances can be reduced by using high-pressure wort pans. During fermentation the volatile substances are expelled by carbon dioxide; the process can be limited using closed fermentation tanks.**

Index Terms—**beer, gas chromatography, volatile compounds, solid phase microextraction, hops**

I. Introduction

Beer production leads to significant changes in the chemical composition of the product, which primarily occurs as part of wort boiling and fermentation. First, Maillard reaction products develop during malt kilning and roasting operations. These compounds are formed by the amino group of nitrogen compounds binding to reducing sugars, the ratio determined by the kilning and roasting temperature applied [1, 2]. Melanoidins, the final product of the reactions, are mostly formed at 160 °C. These high-molecular weight substances however are not critical for the aroma to develop. Instead, intermediate products termed chromophores are more sensorially active according to olfactometry analysis. This non-enzymatic browning continues during brewing, with however a larger proportion of melanoidins and chromophores volatilising out of the raw material [3, 4, 5].

Another group of beer volatiles consists of hop oils. Hops are added to the wort in several batches; the varieties with high resin content are used for the initial stage [6]. The stage 2 and stage 3 make use of aromatic varieties, characterized by the high content of volatile substances. Aromatic varieties of hop vines are split into groups as per the level of β-farnesene - a sesquiterpene of which up to 20 % are present in the finest-aroma variety Saaz [7, 8].

The fermentation process is the most important stage in terms of formation of volatile substances and one that develops a number of by-products with a low boiling point. The ratio and amount of these differs mainly between the bottom- and top-fermented beers, when fermentation at higher temperatures results in more aromatic substances [9, 10, 11].

II. Material and Methods

A. Gas Cromatography

All chemicals were purchased from Supelco (USA) with the highest purity available. Hop samples were kindly donated by Hop Research Institute (Czech Republic). The analysis involved using 15 ml of the sample placed in a 20 ml vial and mixed with 5 g of sodium chloride. The samples were heated for 10 minutes to 50 °C on a hotplate which was followed by 30 minutes of headspace micro extraction using a SPME 100 μm polydimethylsiloxane fibre (Supelco, the USA). The samples were analysed using a gas chromatograph, HP-6890/5973 (GC-MS). The separation of substances used the HP-5MS column under the conditions as follows: He flow rate: 1 ml per min; splitless injector; temperature: 250 °C; ramp: T_1 = 40 °C, t_1 = 5 min, 20 °C per min on T_2 = 250 °C, t_2 = 5 min. Statistically significant differences between the measured values were determined by two-sample T-test.

B. Brewing technology

The beer production used 18 kg of malt of Czech origin. The malt was mashed with 140 l of water at 52 °C. Mashing was underway using the infusion method as follows: T_1 = 52 °C, t_1 = 30 min, 15 min to T_2 = 62 °C, t_2 = 30 °C, 5 min to T_3 = 65 °C, t_3 = 25 min, 10 min to

$T_4 = 70$ °C, $t_4 = 20$ min, 5 min to $T_5 = 72$ °C, $t_5 = 5$ min, 20 min to $T_6 = 78$ °C. After lautering and pumping, the wort was heated to the boiling point of 100 °C. Of the 250 g of hops (Premiant variety), the first third was added to the wort kettle at the beginning of wort boiling, with the other parts following in 30 min periods. The overall wort boiling time was 90 min. This was followed by separating the sludge (40 min), transferring the wort into the fermentation tank with a temperature of 10 °C and inoculating the wort by bottom-fermentation brewing yeasts. The temperature of the main fermentation stage was set to be 10 °C for seven days. Subsequently, the temperature inside the tank was reduced to 4 °C and the beer left five weeks for conditioning.

III. Results and Discussion

A. The concent of hop oil components in the intermediate products as part of beer production and in the beer

Of the hop oils components, the highest concentration of β-myrcene was present in hop cones. Although β-farnesene is typical of the Saaz semi-early red bine hops variety, the amount contained in the analysed samples averaged a mere 0.18 g per kg of this hop oil component, which is only 8.73% of total hop oils while a standard level is 10% to 20% of β-farnesene present in the variety [12, 13].

The level of hop oils in the wort was not proportional to the quantity supplied. Compared with other components, the percentage of β-myrcene entering the wort as part of extraction is rather high. Caused by the high boiling point of the substance (167 °C), this is not desirable due to its grassy aroma but can be reduced by choosing a more preferable variety with a lower content of this hop oil component [6, 7, 14].

The relatively high content of hop oils in the wort gradually decreased during the fermentation and was found to be below the threshold values of sensory perception. The reason was the removal of the hop oils by resulting carbon dioxide. The results outlined above are shown on Fig. 1.

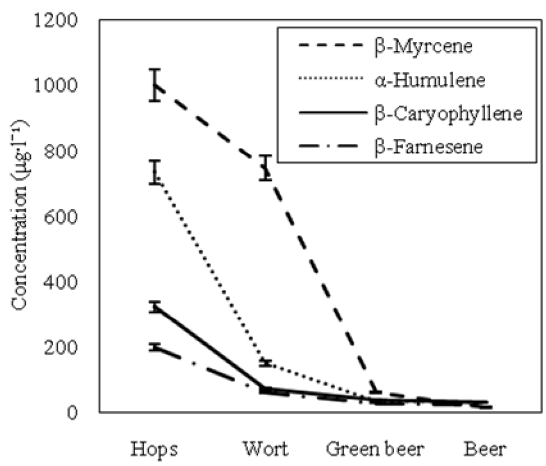

Figure 1. The decreased content of hop oil components in beer production

B. Determining pentanol and Maillard reaction products in the malt extract, wort and beer

In wort boiling, the content of pentanol and maltol decreases, while the concentration of furanmethanol increases. The formation of Maillard reaction products is induced by the high temperature during the boiling process [15, 16, 17]. As the loss of maltol through evaporation exceeds the production, the content of the substance decreases compared with furanmethanol. The difference in furanmethanol concentrations between the fresh mash, beer wort and beer was statistically highly significant (Fig. 2).

The increased content of furan derivatives in the wort and the beer reveals a deeper thermal action, i.e. the incidence of agents causing the "old beer" taste (carbonyl compounds): their occurrence is unwanted, although it completes the overall quality of the beverage [3, 5, 17, 18]. The toxicity of maltol, furfural and furanmethanol is another negative aspect. Since the beer concentrations of these are much lower than the lethal dose, they do not pose any health risk.

C. The formation of esters and alcohols as part of the main stage of fermentation and conditioning of beer

Over the main fermentation stage, the volatile substances are mostly produced in the initial four days of fermentation. As this production stage comes to its end, the loss of volatiles exceeds the production. Of the volatile substances analysed, the concentration of phenyl ethyl alcohol was the highest in the beer. The difference in the concentration of phenyl ethyl alcohol and that of other volatiles was statistically highly significant at the end of the main fermentation stage.

The sensory perception threshold is 3.9 mg per litre for ethyl acetate, while for phenyl ethyl alcohol and 3-methyl-butanol it equals 1 mg per litre [19, 20, 21]. In this regard, the differences between the analysed volatiles are more significant than shown on Fig. 3 with the phenyl ethyl alcohol content exceeding the threshold of sensory perception nearly ten times and the concentration of 3-methyl-butanol in the beer being five times higher.

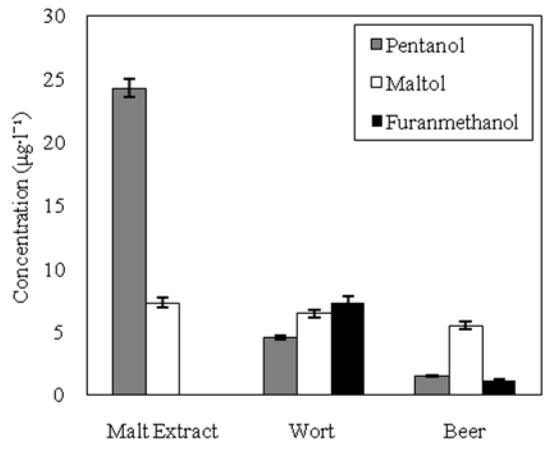

Figure 2. The effect of wort boiling and fermentation on the level of pentanol, furanmethanol and maltol in beer

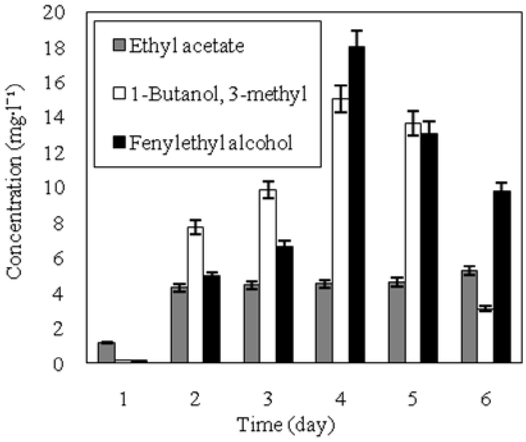

Figure 3. The content of volatile substances in the beer in the main beer fermentation stage

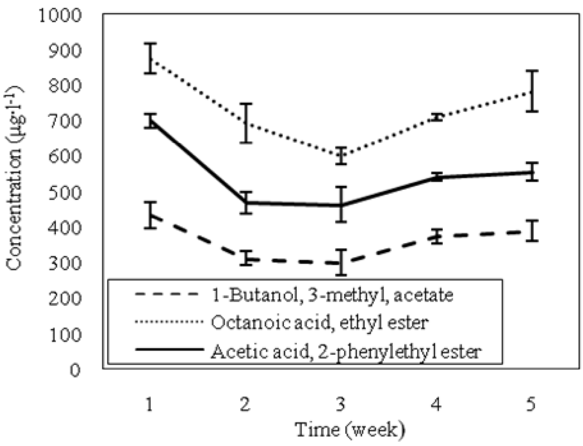

Figure 4. The content of 3-methyl-1-butyl acetate, ethyl octanoate and 2-phenyl ethyl acetate during beer conditioning

The ethyl acetate content exceeds the sensory sensation threshold to only a moderate extent. Since any higher concentration of ethyl acetate is undesirable in beer, the results are in agreement with quality requirements for the beverage [22, 23, 24].

Initially, the beer conditioning stage brings about a decrease in the content of esters, while three weeks after the concentration increases. The decrease in the content of volatiles in the beer as part of fermentation is not caused by reduced formation. Rather, displacing by carbon dioxide is the reason for the reducing level [20, 22]. With the beer becoming saturated after three weeks, the extent of the loss is not so considerable and the level of volatiles gradually increases (see Fig. 4).

IV. Conclusion

With their low boiling point, volatile substances form highly unstable intermediate products in beer production and in the beer. The highest temperatures are reached during wort boiling. Subsequent critical technological sages involve the separation of coarse sediments and beer fermentation. The concentration of native volatile substances of barley and hop oil components gradually decreases as part of beer production down to the threshold of sensory perception. The content of developing volatiles is not proportional to their production. The evaporation can be reduced by high-pressure boiling the drawbacks of which involve a thermal load put on the beer wort and accumulating undesirable, low-boiling volatiles such as dimethyl sulphide and carbonyl compounds. Eliminating the compounds makes use of additional vacuum evaporation after the separation of coarse sediments in the whirlpool.

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