EFFECT OF SOME TECHNOLOGICAL FACTORS ON THE CONTENT OF ESTERS IN BEER

Gunka N. Jonkova, Nelly V. Georgieva*

University of Chemical Technology and Metallurgy, Department of Biotechnology, 8 Kliment Ohridski Blvd., 1756 - Sofia, Bulgaria

*Corresponding author: nelly.georgieva@yahoo.com

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Abstract: The purpose of this work was to examine the influence of the fermentation conditions such as temperature, the pitching rate of yeast and the "Free Amino Nitrogen" (FAN) in wort on the content of esters in beer. It has been found out that the higher temperature of fermentation stimulates the formation of esters and quantity in the beer is higher. Beer, obtained by increased pitching rate (26.10^6 and 35.10^6 cells/mL), contains the same or less amount of esters compared to that in the control beer. Lower content of FAN in wort must result in the replacement of 5 to 10% of the malt with rice, sugar or a combination thereof, leads to a high content of esters in beer.

Keywords: *brewing yeast, beer, esters, free amino nitrogen, wort*

INTRODUCTION

More than 400 aromatic substances [1], determining specific flavor profile of the beer are known today.Their total quantity is only 0.5‰, as the amount of the individual components ranged from 10^{-2} to 10^{-9} g/L [2 - 4]. The flavor of beer is driven by

substances formed as a result of biochemical processes in the fermentation of wort with the active participation of the enzyme complex of brewing yeast.
According to the most of authors, esters are amongst the most important flavor compounds in beer [5 - 7]. Arkima and Jounela-Erikson [8] established that 15% of the total aroma of beer is due to the esters, of which 5% of ethyl acetate, 9% of isoamyl acetate and 1% of ethyl caproate.
The esters are the most numerous group of aromatic-active compounds formed during the fermentation of the wort [9, 10]. About 90 esters have been identified in beer [7]. They are two main categories of flavor-active esters in beer: acetate esters and ethyl esters of medium chain fatty acids [11]. Biosynthesis of esters represents the condensation process between an alcohol (ethanol and higher alcohols) and fatty acids, which are activated by the inclusion of CoASH [12]. Recent studies on the formation of acetate esters show that the biosynthesis is catalysed by enzymes of the yeast alcohol acetyltransferase (AAT) [13], which inhibits the formation of unsaturated fatty acids [6, 14]. It has been found out that the composition of wort, yeast strain and fermentation conditions are the main critical factors for both - the course of the fermenting process and quality of the beer in terms of its flavor [12, 15, 16].
The purpose of this work was to examine the influence of the fermentations conditions such as temperature, the pitching rate of yeast and the "Free Amino Nitrogen" (FAN) in wort on the content of esters in beer.

MATERIALS AND METHODS

In two of the series of experiments, the wort used, has the following characteristics: extract content – 11.78%, pH – 5.6, FAN – 176 mg/L, dissolved oxygen – 7.5 mg/L. In the third series of experiments in order to achieve a different content of FAN, different raw materials have been used – malt, sugar and rice in the following variants: K_1 (control) – 100% malt, B_1 – 90% malt and 10% sugar, B_2 – 90% malt and 10% rice, B_3 – 90% malt, 5% sugar and 5% rice. The scheme of experimental variations in different temperatures of fermentation is: starting temperature 6.5 °C, final temperature – 5.0 °C and maximum temperature: K_1 – 8.5 °C, B_1 – 6.5 °C, B_2 – 12.0 °C, B_3 – 16 °C. Pitching rate of yeast is 18.10^6 cells/mL. The studies on the effect of pitching rate of yeast was held at maximum temperature of 8.5 °C in the following variants: control – 18.10^6 cells/mL, B_1 – 10.10^6 cells/mL, B_2 – 26.10^6 cells/mL, B_3 – 35.10^6 cells/mL. The experiments were conducted in a 2.5 L fermenter using yeast strain *Saccharomyces carlsbergensis*. After completion of primary fermentation green beer is transferred for maturation in watertight bottles at 2 – 4 °C for 20 days. The used tests are accepted in Bulgaria and in Europe, the EBC (European brewery convention) methods.
The amount of esters was determined according to the method described by Peynand [17]. Analysis of total esterified compounds is based on the color reaction between hydroxylamine hydrochloride and esters in alkali medium. The obtained color complex after addition of ferric chloride was determined spectrophotometrically (λ = 530 nm).

RESULTS AND DISCUSSION

The test results concerning the temperature effect are shown in Fig. 1. The results showed that the lowest value of the content of the ester-active components in the green beer has been observed in B_1, where the fermentation temperature is the lowest. It remains the lowest in the product. In B_2, where the selected temperature corresponds to the commonly used in the modern accelerated methods (12 °C), the quantity of esters during fermentation was increased about 50% and in B_3 – about 80% compared to the control. This shows that the temperature as a technological factor leads to significant changes in the metabolic activity of yeast. The reason for this is probably the increased activity of cellular enzymes in high temperature fermentation conditions.

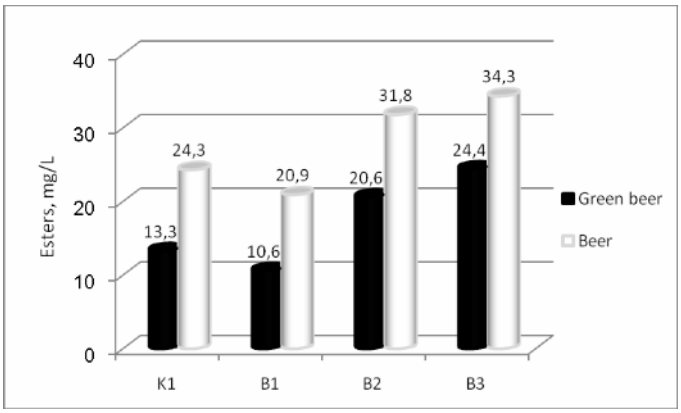

Figure 1. *Effect of fermentation temperature on esters content*

That trend is maintained in the beer, further enzymes formation being observed during the maturation. According to Narziss [18] their content in beer can reach 35 – 60 mg/L. In treatment 1, 2 and control the total content of the esters is lower than the sweet taste threshold of ethyl acetate, esters with the highest concentration (33 mg/L). This shows that when working with other yeast strains, which have a strong ester synthesis ability, their quantity at high temperatures may exceed the flavor taste threshold, leading to the emergence of a fruity aroma in beer.

Tasting evaluation of experimental beer shows the following:

- Control – clean, fine, harmonious taste and pleasant aroma;
- Treatment 1 – clean taste without taste defects, less intense flavor;
- Treatment 2 – slightly fruity aroma, no taste defects;
- Treatment 3 – harsh tasting, fruity yeast flavor.

The results regarding the effect of pitching rate of yeast on the esters content in beer are shown in Fig. 2.

It is known that pitching rate of yeast affects the multiplication of yeast and the duration of fermentation. The lower the initial concentration of yeast cells is, the more intense is the multiplication and the amount of esters increased (B_1). For B_2 the amount of esters in the green beer is equal to that of the control, indicating that in the range $(18 – 26).10^6$ cells/mL there are no significant differences. Further increase of yeast (35.10^6 cells/mL) reduced the content of the esters. That trend is maintained also in the final product.

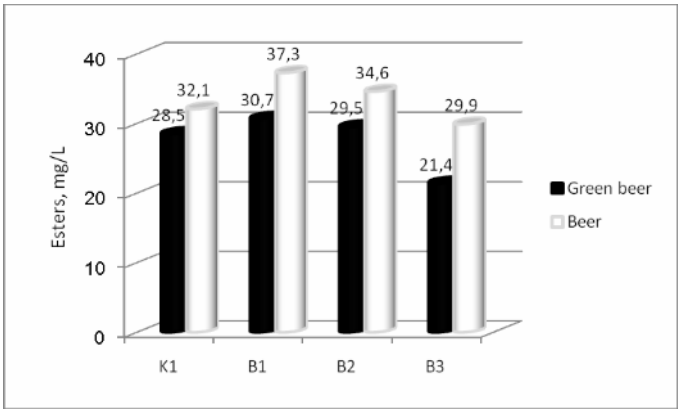

Figure 2. *Influence of pitching rate of yeast on the content of esters in beer*

Tasting evaluation shows that beers are close in their flavor and taste properties. Best neated in terms of aroma and taste is the control beer, which is close to that of B_2. In beer B_1 is established an intensive aroma, but not hop flavor one.

A third important medium parameter is the nitrogen content of wort, which can be "consumed" or "assimilated" by the yeast; it is called "free amino nitrogen" (FAN). The replacement of a part of the malt with additives such as starch containing substitute materials or sugar leads to lower nitrogen content in wort. Research in the amount of the percentage contribution substitute malt quantity of FAN is in the range 125-155 mg/L. The lowest value for the esters was found in the control malt beer, where the FAN was the highest (155 mg/L), and the highest is in B_1, where we have the lowest FAN (125 mg/L) – Fig. 3.

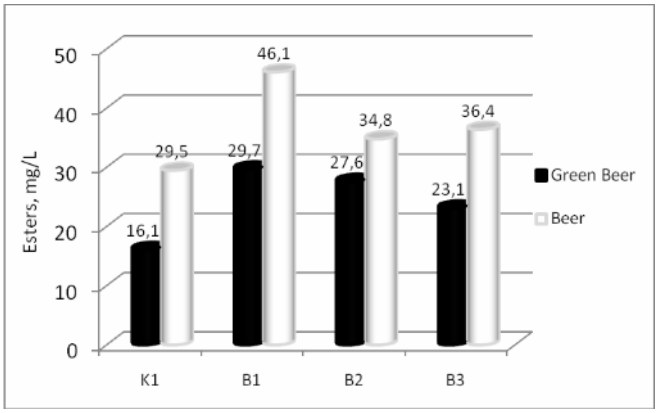

Figure 3. *Content of esters in beer obtained from different FAN in wort*

The amount of FAN in wort is a key technologycal parameter which has a decisive importance for the normal course of fermentation. The yeast cells require a certain amount of assimilative nitrogen for their development. The nitrogen is needed for synthesis of the components ensuring cell's growth and reproduction. Nitrogen metabolism of the cell is linked to the formation of many of the substances involved in the formation of flavor complex beer, in the case of higher alcohols, which leads to a change in the content of the esters.

Beer tasting evaluation has shown the following results:

- Control – clean, harmonious taste and pleasant aroma;
- Treatment 1 – clean taste, pleasant aroma;
- Treatment 2 – light, secondary taste, a lack of taste;
- Treatment 3 – neat, without taste defects.

The beer tasting evaluation shows some differences between samples in terms of flavor and taste, but they are not distinctly. Perhaps this is due to the different behavior of the individual representatives of this group, namely the acetate esters and ethyl esters of medium chain-fatty acids. The neatest beer is made from malt. Beer of B_1 and B_3 did not differ significantly from the control beer while in beer from B_2 is established light, secondary taste.

CONCLUSIONS

It was found that the highest temperature stimulates the formation of fermentation esters. The temperature ranging from 8.5 °C to 12 °C could be evaluated as the most suitable for generation of normal amount of esters. The beer produced by increased pitching rate of yeast (26.10^6 and 35.10^6 cells/mL), contains the same or less amount of esters compared to that in the control beer. The replacement of 5 to 10% of malt with rice, sugar or a combination thereof, which leads to lower amount of FAN in produced wort, causes an increased content in higher alcohols, and hence to higher values of esters in beer.

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