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Ruslana Kosiv, Tetiana Kharandiuk, Lyubov Polyuzhyn, Liubov Palianytsia and Natalia Berezovska

OPTIMIZATION OF MAIN FERMENTATION OF HIGH-GRAVITY WORT

Lviv Polytechnic National University,
12 S. Bandera St., 79013 Lviv, Ukraine; r.kosiv@online.ua

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Abstract. The mathematical model has been developed to calculate the content of higher alcohols and esters depending on wort initial concentration and fermentation temperature. The process was optimized in accordance with the developed model. To obtain high-quality beer *via* high-gravity brewing technology the wort concentration was found to be 14-15 % relative to dry matter and main fermentation temperature of 284–285 K.

Keywords: high-gravity brewing, fermentation by-products, optimization.

1. Introduction

The traditional technology of wort fermentation and organoleptic evaluation of beer quality does not satisfy both producers and customers. Critical quality parameters of beer as a food product are the by-products of fermentation. Their content depends on raw material quality, yeast genetic properties and metabolism. Even slight deviation from the regulated technological regimes significantly alters the organoleptic properties of beer. This problem is of particular importance due to the implementation of high-concentration wort fermentation technology – high-gravity brewing (HGB).

During fermentation, yeast eliminates a number of metabolic products that are the subject to quantitative and qualitative changes due to their partial interaction. Apart from the main fermentation products (ethanol and carbon dioxide) by-products are produced as well: glycerol, higher alcohols, vicinal diketones (VDK), volatile and fatty acids, esters, aldehydes and sulfur-containing compounds. By-products are a small part of beer dry matter (DM) (except glycerol), but they play an important role to form the taste and aroma of the drink. The increase in these microcomponents concentration causes strange tastes and flavors.

HGB technology has been proposed to increase the production efficiency and reduce energy costs. Its essence is the preparation and fermentation of high-gravity wort with high extract content and dilution of beer to the desired condition before filtration. As a matter of fact, the wort fermentation time should not be greater than that in traditional technology. However, there are problems associated with a decrease in the yeast activity and their early flocculation which are consequences of "osmotic" and "ethanol" stresses. As a result, the fermentation rate decreases and fermentation time increases.

The increase in temperature may intensify the wort fermentation. Unexpectedly, the temperature control suddenly turned into the problem of regulation of beer taste and aroma because of different temperature optima of yeast metabolism and formation of volatiles. High temperatures have a positive stimulatory effect on the metabolism and growth of yeast, but fermentation by-products are formed more actively in that case. Moreover, the increase in wort initial concentration also affects the process of metabolites accumulation.

So, a lot of researches on improving HGB technology is carrying out to reduce the concentration of fermentation by-products and produce beer of high quality. It was found that organoleptic properties of conditioned beer are reduced with increasing concentration of wort. M. Tonyuk *et al*. [1] suggest single-parameter dependence of microcomponent concentrations (VDK, acetaldehyde, esters, higher alcohols) on the DM content in original wort within 13–19 %, but they do not include the temperature effect on the formation of fermentation by-products. G. Stewart [2] found that for beer with wort concentration of more than 16 % DM disproportionately higher content of esters, especially ethyl acetate and isoamyl acetate, is typical.

E. Faradjeva *et al.* [3] determined mathematical dependencies of higher alcohols and diacetyl content in beer obtained under the participation of H yeast on wort initial concentration (9–15 % DM), fermentation temperature (280–288 K) and yeast amount (10–30 mln/cm^3). The authors pointed to the greatest effect of wort concentration and temperature on the amount of by-products. However, for HGB technology other parameters are important as well. K. Sigler *et al.* [4] expected greater effect of yeast strain, temperature and other factors on the content and ratio of fermentation by-products compared with the effect of wort initial concentration.

Thus, there is no consensus on the effect of wort concentration and temperature on the synthesis and transformation of by-products that are essential for the formation of beer taste and flavor.

The aim of the present work is to optimize the process of the main fermentation of high-gravity wort relative the content of alcohol and ester microcomponents.

2. Experimental

We investigated Saflager W-34/70 brewer's yeast, hopped wort with the concentration of 14, 16 and 18 % DM and distillates of young beer. The yeast was cultivated in sterile non-hopped wort with 12% DM concentration at 298 K in three stages of 24 h each. Yeast biomass was separated for 10 min using centrifuge with 4000 rev/min. Then it was washed with sterile water and used for the fermentation of hopped wort (200 cm^3) with the concentration of 14, 16 and 18 % DM at 282, 287 and 292 K for 7 days.

The content of fermentation by-products was determined in young beer distillates. The content of vicinal diketons was investigated using spectrophotometry after their interaction with ortho-phenylenediamine [5].

Crystal-4000m-LUX chromatograph was used for chromatographic studies. To calibrate the chromatograph we used calibrated water-ethanol solutions: Gweru-6.1 (ester) and 6.2-Gweru (alcohol) according to the standards. Chromatography conditions: HP-FFAP capillary chromatographic column; stationary liquid phase – monoester of nitro-terephthalic acid and polyethylene glycol (molar ratio 2:1) with a film thickness of 0.5 μm; column length 50 m; internal diameter 0.32 mm; column temperature program: isotherm 313 K for 5 min, heating from 313 to 433 K with a rate of 4 K/min, 433 K isotherm (10 min); injector temperature 523 K; temperature detector 473 K; carrier gas – argon with the flow rate of 1 cm^3/min; flame ionization detector; air flow 250 cm^3/min; hydrogen flow rate 30 cm^3/min. System dead time used to calculate the retention index was 2.66 min. ML-1 microsyringe was used for sampling (the samples volume was 1 μm^3). Software – NetChrom for Windows version 2.1.

3. Results and Discussion

Higher alcohols, acids, esters, diketones, aldehydes and their derivatives play a significant role for beer aroma and taste. High concentrations of diketones and aldehydes negatively affect the beer quality.

3.1. Determination of Vicinal Diketones

The increase in wort concentration increases VDK content in the young beer (Table 1), the increase in fermentation temperature leads to its decrease to the level typical of matured beer (0.1 mg/dm^3). The maximum VDK concentration is observed in the young beer obtained at 282 K. This value of VDK concentration corresponds to the value typical of beer transferring for secondary fermentation (0.3–0.6 mg/dm^3).

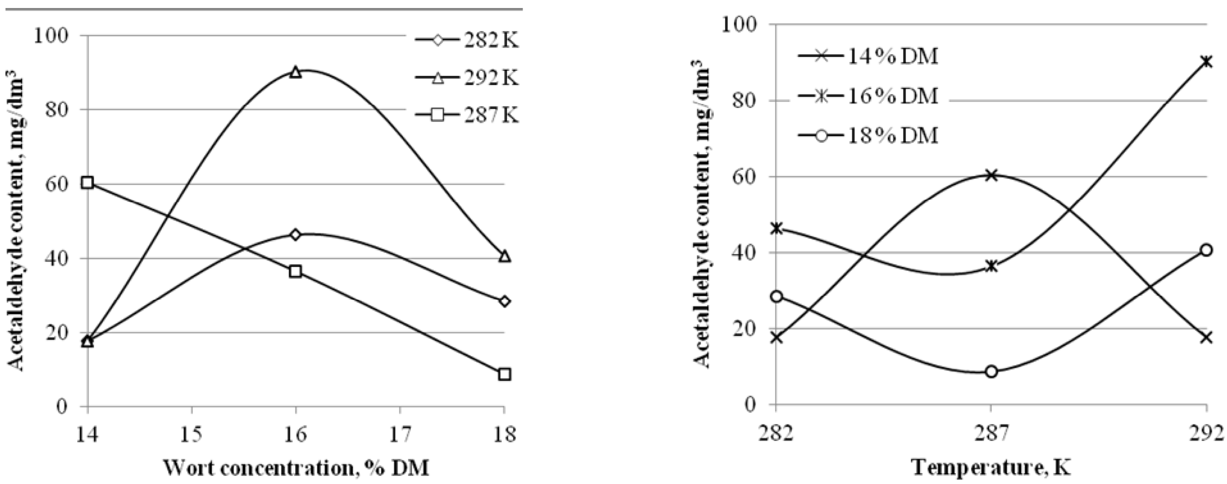
3.2. Determination of Acetaldehyde

The increase in main fermentation temperature from 287 to 292 K and wort concentration from 14 to 16 % provides the formation of acetaldehyde (Fig. 1). The further increase in wort concentration from 16 to 18 % DM retards the aldehyde formation. Usual content of acetaldehyde is 20–40 mg/dm^3 typical of the young beer obtained by fermentation of 14 and 18 % wort at 282–287 K. In most samples acetaldehyde concentration exceeds the threshold concentration of 10–25 mg/dm^3 but during secondary fermentation and maturation of beer the aldehyde concentration is reduced by biochemical method.

Table 1

VDK content (mg/dm^3) in the young beer

Wort concentration, % DM	Main fermentation temperature, K		
	282	287	292
14	0.49	0.10	0.10
16	0.89	0.13	0.14
18	0.35	0.12	0.12



a) b)

Fig. 1. Acetaldehyde content *vs*. wort concentration (a) and temperature (b)

3.3. Determination of Alcohol and Ester Microcomponents

To describe the process by means of the design of experiments [6] with two factors the regression equation (1) is used:

$$Y_i = b_0 + b_1 X_1 + b_2 X_2 + b_{12} X_1 X_2 \quad (1)$$

where Y – response function (parameter of the process efficiency) depends on process conditions; X – independent coded variables (process conditions).

On the basis of previous investigations [7] the temperature within 282–292 K and wort concentrations within 14–18 % DM are chosen as process conditions.

The estimation criteria of the mentioned parameters effect are the amount of higher alcohols and esters because these by-products have an essential influence on beer taste and flavor but cannot be eliminated from the beverage during manufacturing, ***e.g.*** by beer maturation. Taking into account that pentanols concentration in the samples of young beer is 2–5 times higher than that of other alcohols and nature of change of pentanol and other alcohols the amount depending on process conditions is the same, the process should be optimized relative to pentanols content Y_1, mg/dm^3. Ethyl acetate is the main among esters, therefore it was necessary to investigate the effect of temperature and wort concentration on ester content taking ethyl acetate as an example – Y_2, mg/dm^3.

The design of experiments for two parameters requires four experiments at the corners of the plan square. According to Eq. (1), four regression coefficients b_i are defined that causes uniqueness of the surface response after four experimental points and thus eliminates the statistic nature of the mathematical model. So, for the statistic significance of the regression equation in the form of Eq.(1) we applied a least squares method for solving this system of equations: the number of defined parameters (b_i) – four and the number of experiments is equal to the number of equations – nine. Additional experiments were conducted in the center of the plan and in the middle of the plan square sides. Redefined system of equations was solved in the matrix form (2), using MathCAD.

$$b = (X^T \cdot X)^{-1} \cdot (X^T \cdot Y) \quad (2)$$

Table 2 shows the experimental values of concentration of pentanol total amount and ethyl acetate depending on experimental conditions.

For pentanol total amount and ethyl acetate we obtained Eqs. (3) and (4) for physical variables Z_1 in Celsius degree and Z_2 in wt %:

$$Y_1 = 675.1 - 54.6 \cdot Z_1 - 50.9 \cdot Z_2 + 4.7 \cdot Z_1 \cdot Z_2 \quad (3)$$

$$Y_1 = 284.6 - 29.4 \cdot Z_1 - 20.7 \cdot Z_2 + 2.2 \cdot Z_1 \cdot Z_2 \quad (4)$$

The content of microcomponents was calculated according to Eqs. (2) and (3) with the step of temperature change 1 K and concentration change of 0.4 %. The estimation and search of optimum temperature and concentration ranges may be carried out according to graphic dependencies in the form of surface areas (Fig. 2a) or plots (Fig. 2b).

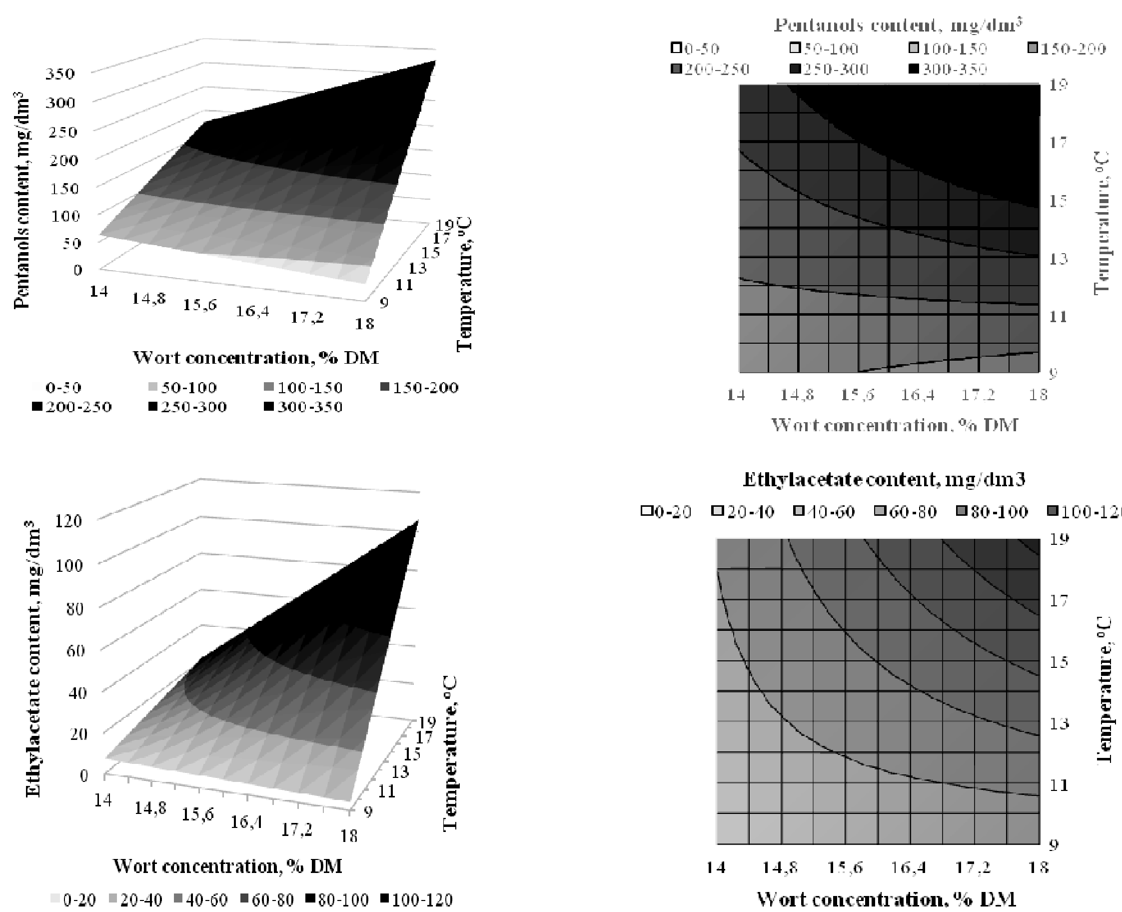
It is still not clear which range of esters concentration is the optimum one. It should be noted that at ethyl acetate concentration > 30 mg/dm^3 the taste of beer becomes unacceptable. The investigated range of esters concentration varied from 1.1 to 108 mg/dm^3. By previous experiments it was established that a rate of beer dilution (according to HGB technology) should not exceed 15–20 % to achieve standards relative to the height of beer head and its retention. Thus the limiting value of ethyl acetate content in young beer is 35–37.5 mg/dm^3. One can see from Fig. 2b that within the temperature range of 282–285 K the ethyl acetate content does not exceed the standard value. Main fermentation of high-gravity wort can be conducted at elevated temperatures (286–292 K), but wort concentration should be lower and be within 14–14.4 % DM.

The content of higher alcohols above 100 mg/dm^3 impairs taste and beneficial properties of beer. Taking into account the rate of beer dilution during its conditioning and the presence of other higher alcohols in beer the limiting value of pentanol content was found to be 100 mg/dm^3. For the studied range the pentanols content varies from 21 to 324 mg/dm^3. It is obvious from Fig. 2b that within the temperature interval of 282–284.5 K their content does not exceed the standard value for the whole tested range of DM content in wort. However, at 284–285 K, which provide a satisfactory process rate to ensure its efficiency, the wort concentration is within a narrower range of 14–15 % DM.

Table 2

Content of pentanols (Y_1) and ethyl acetate (Y_2) in mg/dm^3 in young beer

Temperature, K	Wort concentration, % DM					
	14		16		18	
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2
282	58.0	1.1	51.0	8.0	21.0	1.3
287	124.0	19.0	155.0	38.0	190.0	53.0
292	173.0	19.0	254.0	65.0	324.0	108.0



a) b)

Fig. 2. Model graphic dependencies in the form of surface areas (a) and plots (b)

If we combine optimal areas of ethyl acetate and pentanols content in beer the optimal conditions of the main fermentation are: temperature of 284–285 K and DM concentration in wort 14–15 %.

4. Conclusions

The mathematical model of fermentation by-products accumulation under high-gravity brewing conditions was developed that allows to calculate the content of higher alcohols and esters depending on the wort initial concentration and fermentation temperature. The optimal modes of the process were determined to provide high quality of finished beverage.

References

[1] Tonyuk M. and Varankina O.: Vestnik Kharkov.Politechn.Inst., 2012, **9**, 105.
[2] Stewart G.: Cerevisia, 2007, **32**, 37.
[3] Faradjeva E. and Chusova A.: Izv. VUZov. Pishchevaya Technol., 2001, **5-6,** 62.
[4] Sigler K., Matoulková D., Dienstbier M. and Gabriel Sigler P.: Appl. Microbiol. Biotechnol., 2009, **82**, 1027.
[5] Pfenninger H.: Brautechnische Analysenmethoden, Band III. Selbstverlag der MEBAK 1996.
[6] Meledina T. and Danina M.: Metody Planirovaniya i Obrabotki Rezultatov Nauchnykh Issledovaniy. IXiBT, Sankt-Peterburg 2015.
[7] Kharandiuk T., Kosiv R., Palianytsia L. and Berezovska N.: 81 Miznarodna Nauk. Conf. Molodykh Vchenykh, Aspirantiv i Studentiv. Ukraine, Kyiv 2015, 226.

ОПТИМІЗАЦІЯ ГОЛОВНОГО БРОДІННЯ ВИСОКОГУСТИННОГО ПИВНОГО СУСЛА

Анотація. *Створено математичну модель, яка дає можливість розрахувати вміст вищих спиртів та естерів залежно від початкової концентрації сусла та температури бродіння. За рівняннями математичної моделі здійснено оптимізацію процесу. Встановлено, що для одержання пива високої якості за технологією високогустинного пивоваріння концентрація сусла повинна становити 14–15 % сухих речовин, а температура головного бродіння 284–285 К.*

Ключові слова: *високогустинне пивоваріння, побічні продукти бродіння, оптимізація.*