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Control of selected fermentation indices by statistically designed experiments in industrial scale beer fermentation

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Abstract: Fermentation indices of a bottom-fermented lager brew from high gravity wort (15.5 °P) were analysed using the response surface methodology (RSM, Box-Behnken design). Fermentation parameters like pitching rates (6–10 mln cells mL^{-1}), wort aeration (8–12 mg O_2 mL^{-1}), different times (4.5–13.5 h) of filling CCTs (cylindroconical fermentation tanks; 3 850 hL) and fermentation temperatures (8.5–11.5 °C) were modulated to assess their impact on the fermentation indices. Within the studied ranges of fermentation parameters the experimental factors had a significant influence (R^2 for the model 73%) on alcohol content, pH (83%), extract drop (86%), FAN consumption (70%), bitterness loss (73%) and sensory analysis (71%). Based on the multiple response optimisation analysis, the values of independent factors that optimised alcohol content at the level of 6.94% (v/v), extract drop at 1.77 °P per day with maximization of FAN consumption (ca. 128 mg L^{-1}) and pH drop to the level of 4.69 with minimized bitter substances losses (6.2 BU) were as follows: pitching rate 6 mln cells mL^{-1}; fermentation temperature 11.2 °C; aeration level 10.5 mg L^{-1}; and CCTs filling time 13.5 h.

Keywords: bottom fermentation; fermentation indices; production scale; response surface methodology

In the brewing process, beer fermentation and maturation are the two most time-consuming phases. In order to increase the plant capacity, fermentation of wort can be optimised by several approaches like by an increase in wort gravity or by acceleration of the extract to the alcohol conversion rate (Lima et al. 2011).

Generally, beer containing 5% (v/v) of ethanol is produced from 12 °P worts, while higher contents of ethanol are obtained from high gravity brews (HGB). The mashing of HGB became a very useful approach that created a possibility of bottling different beers from the same highly concentrated batch.

In beer fermentation, the factor that limits high levels of ethanol biosynthesis is often recognised as the availability of nutrients. The concentration of assimilable nitrogen (free amino nitrogen – FAN) seems to be a key factor in fermentation of high-gravity worts. Nitrogen substances determine both the enhancement of the yeast performance and their tolerance to ethanol (Dragone et al. 2004).

The production of HGB worts may guarantee increased volumetric productivity, reduced energy, labour and material costs. Using faster fermentations with concentrated worts results in measurable financial benefits (Jones et al. 2007). There have been a few studies comprising a series of single-factor experiments where the effects of yeast pitching rate (Erten et al. 2007; Verbelen et al. 2009a), aeration level (Verbelen et al. 2009b; Kucharczyk & Tuszyński 2017), wort filling time (Jones et al. 2007; Kucharczyk & Tuszyński 2015) and temperature (Kobayashi et al. 2006; Ramirez & Maciejewski 2007) on fermentation and maturation parameters like ethanol synthesis, pH of beer, and rate of fermentation expressed as the FAN and bitterness

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consumption, as well as the sensory properties of beer were investigated. In other studies by Andres-Toro et al. (1998), Dragone et al. (2004), Lima et al. (2011), and Cui et al. (2015), experimental modelling for optimisation of brewing processes was applied.

The purpose of the present study was to develop empirical models by the response surface methodology (RSM) for optimising alcohol content, pH of beer, FAN consumption and losses of bitter components during fermentation and maturation of a lager beer produced on an industrial scale by variations in yeast pitching rates, fermentation temperature, wort aeration, and time of CCTs filling.

MATERIAL AND METHODS

Process description. The process of beer fermentation and maturation was investigated in industrial cylindroconical tanks (CCTs). Each fermentation tank was filled with three brews. The final wort volume in every three CCT was 3 090 hL, whereas the gross capacity was 3 850 hL (HGB worts, high gravity 15.5 °P). The process of infusion mashing took place at a standard scale temperature of 60–76 °C. Afterwards, the mash was transferred to a lauter tun. After boiling, the wort was cooled to 8.5°C and then aerated. Worts were aerated with compressed sterile air during wort transfer to each of the CCT and with various intensity so as to have 8–12 mg O_2 L^{-1} of the wort. The concentrations of dissolved oxygen were measured in the pitching wort and after filling CCTs using an optical oxygen meter (Mettler Toledo, Columbus, USA). The pitching rates of 6, 8 and 10 mln yeast cells per mL were used. For each fermentation, the yeast pitching temperature was the same –8.5 °C. Fermentation was conducted in isothermal fermentation tanks. A new technology was used in the processes of fermentation – higher temperatures without a slow decrease at the end of fermentation. Primary fermentation was performed at 8.5, 10 and 11.5 °C whereas the temperature of the final phase of fermentation was 13 °C. The third generation (yeasts used twice before) of *Saccharomyces pastorianus* (strain WS34/70) brewers' yeast was used for pitching. The yeast was added to the first of the three brews to each CCT. Yeasts were pitched using ABER Instruments Ltd (Aberystwyth, UK) for the rate control, which determined the total viable cell count.

Analytical procedures. Alcohol and pH marking were performed using an automatic wort and beer analyser (DMA 4500; Anton Paar; Graz, Austria). Tabarié's formula was the basis for 'Alcolyzer' beer calculations (Miedaner 2002). Ethanol content was measured in fully fermented beer during a lagering process. In order to achieve the same conditions, the malt was used from the same malt houses in the same proportions. The mashing and boiling processes were conducted at identical conditions.

Free amino nitrogen in wort and beer was determined by a ninhydrin-based method, according to the standard method as defined by the European Brewery Convention (EBC 1998). In the experiment, FAN uptake (mg L^{-1}) means a difference between the initial FAN concentration in wort minus the final FAN concentration in matured beer on the 18th day of the production process.

The bitter compounds were extracted with isooctane from the acidified beers and the absorbance was measured at 275 nm in a quartz cuvette (EBC 1998).

In this study, bitterness uptake (in EBC units) means a difference between the initial bitterness concentration in wort minus the final bitterness concentration in matured beer on the 18th day of the production process.

The fermentation rate was determined as a daily drop of apparent extract after 5 days from the start of fermentation. The extract difference was then divided by 5 to show the average rate of fermentation over the 5 days of the process.

Statistical analysis. Processing factors that influenced alcohol content, pH, extract drop (rate of fermentation), FAN and bitterness uptake and sensory properties of beer were tested using the experimental design module of the Statgraphics Centurion XVII 17.1.12 (Statpoint Technologies Inc., Warrenton, Virginia). The design employed was a fully randomised Box-Behnken design with four factors at three levels each and two blocks, including three centre points per block, which provided 38 error degrees of freedom in 54 runs. Independent variables, their codes and actual values are presented in Table 1. Results were subjected to analysis of variance (ANOVA) and Pareto chart analyses, and non-significant ($P > 0.05$) components were removed from the model. To evaluate the statistical significance of the secondary-order polynomial model, the coefficient of determination (R^2) and the probability of the lack-of-fit values were calculated.

Sensory analysis. Fresh experimental beers from each of the 54 runs were subjected to sensory evaluation. The beer samples were coded and distributed in dark coloured bottles. Sensory evaluation of bottled beer consisted of a comparison test where a tested

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Table 1. Independent variables, their codes and actual values of the optimisation parameters

Independent variables	Unit	Symbol	Coded levels		
			−1	0	+1
Pitching rate	mln cells mL^{-1}	x_1	6	8	10
Fermentation temperature	°C	x_2	8.5	10	11.5
Aeration level	mg L^{-1}	x_3	8	10	12
Total time of CCT filling	h	x_4	4.5	9	13.5

CCT – cylindroconical fermentation tank

sample was compared with a reference beer. The comparison tests involved the evaluation of aroma esters, hops, bitterness, sulphur compounds, sweetness, acidity, fullness, balance and flavour. The beer was evaluated by a trained panel of nine brewers according to a scale from 50 to 75 points (very good: 70–75; good: 65–69; neither good nor poor: 60–64; poor: 55–59; very poor: 50–54). Sensory evaluation of bottled beer used a comparison test, with the test sample compared with the reference beer profile as described earlier (Kucharczyk et al. 2020).

RESULTS AND DISCUSSION

Model fitting. Within the studied ranges of yeast pitching rate, fermentation temperature, wort aeration level, and filling time of CCTs, a significant influence ($R^2 \geq 70\%$) on ethanol content, pH, extract drop, FAN consumption, and bitterness losses were exerted by the experimental factors (Table 2).

Polynomial equations. In the text below whose length is limited, a detailed analysis of only three parameters will be presented: ethanol content, extract drop and FAN consumption.

Ethanol content. Seventy-three percent of the observed variability in ethanol concentrations in beer can be attributed to the effects of changes in fermentation parameters evaluated in this study. Table 3 shows the ANOVA for ethanol content in matured beer after removing insignificant components from the model.

The relationship between the independent factors and the predicted responses of ethanol concentrations was calculated to be:

$$y_1 = 7.161 - 0.062x_1 + 0.023x_2 - 0.109x_3 + 0.057x_4 + 0.014x_1x_3 - 0.007x_1x_4 \quad (1)$$

where: y_1 – ethanol concentration (%; v/v); x_1 – pitching rate (mln cells mL^{-1}); x_2 – fermentation temperature (°C); x_3 – aeration level (mgO_2 L^{-1}); x_4 – the total time of CCT filling (h).

The subsequent analysis by means of the response optimisation module revealed that over the studied range of independent factors x_1 = 7.8, x_2 = 9.3, x_3 = 10.4, and x_4 = 10.1 were optimal to keep a predicted ethanol concentration at the fixed value of 6.9% (v/v). Convincing evidences were provided that by optimising the yeast pitching rate, fermentation temperature, and level of wort oxygenation, high gravity wort (25 °P) may be completely attenuated. Furthermore, different tendency of pitching rate on the efficiency of alcohol synthesis was shown by Verbelen et al. (2009a). A fourfold increase in the yeast dose (from 10 to 40 mln cells mL^{-1}) lowered alcohol production from 6.77 to 6.61% (v/v).

Dragone et al. (2004) applied a 2^3 full factorial design of experimental factors, namely wort gravity (x_1), fermentation temperature (x_2), and nutrient supplementation (x_3), to report a simple model that allowed the prediction of ethanol production rates:

$$y_2 = 0.421 + 0.155x_2 + 0.0575x_2x_3 \quad (2)$$

where: y_2 – ethanol production rate (g hL^{-1}); x_2 – fermentation temperature (°C); x_3 – nutrient supplementation (g L^{-1}).

Similar conclusions can be drawn from our study, where the amounts of ethanol synthesised during the fermentation of 15.5 °P worts were modulated mostly by fermentation temperature. This finding is also in line with data reported by Jones et al. (2007) and Lima et al. (2011).

Extract drop (speed of fermentation process). After removing insignificant components from the model, the ANOVA for the extract drop is given in Table 4. As already stated, the model explained eighty-six percent of the observed variations in the extract drop.

The relationship between technological parameters and predicted responses of the extract drop values was calculated by the following equation:

$$y_3 = -7.490 + 0.079x_1 + 1.394x_2 + 0.022x_4 - 0.054x_2^2 \quad (3)$$

where: y_3 – extract drop; x_1 – pitching rate (mln cells mL^{-1}); x_2 – fermentation temperature (°C); x_4 – the total time of CCT filling (h).

The subsequent data analysis by means of the response optimisation formula revealed that over the ex-

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Table 2. Analysis of variance (ANOVA) of selected brewery fermentation indices: significance of model components and assessment of the adequacy of the model*

Dependent parameter	Analysis of variance							
	R^2 (%)	Lack-of-fit	x_1	x_2	x_3	x_4	Significant components of the model	
		probability						
Alcohol content (v/v)	73	ns	0.054	0.028	ns	ns	0.034	x_1x_3
							0.022	x_1x_4
pH	83	ns	0.001	0.001	0.002	0.001	0.012	x_1^2
							0.005	x_1x_4
							0.002	x_2x_4
							0.003	x_3^2
							0.017	x_3x_4
							0.001	x_4^2
Extract drop	86	ns	0.009	0.001	ns	0.041	0.046	x_2^2
FAN consumption	70	ND	ns	ns	0.001	ns	0.001	x_1x_2
							0.001	x_2x_3
							0.026	x_4^2
Bitterness loss	73	ND	0.028	0.001	ns	0.025	0.001	x_1^2
							0.006	x_1x_2
							0.001	x_1x_3
							0.001	x_2^2
							0.001	x_2x_4
							0.010	x_3^2
							0.001	x_3^2
Sensory analysis	71	0.0631	0.015	0.001	0.005	0.032	0.001	x_1^2
							0.029	x_1x_2
							0.038	x_1x_4
							0.004	x_2^2
							0.002	x_2x_3
							0.006	x_3^2
							0.016	x_4^2

*published already in Kucharczyk et al. (2020); FAN – fee amino nitrogen; ns – not significant; ND – not detected; x_1 – pitching rate (mln cells mL^{-1}); x_2 – fermentation temperature (°C); x_3 – the aeration level (mg L^{-1}); x_4 – the total time of CCT (cylindroconical fermentation tank) filling (h)

perimental range of independent factors, x_1 = 8.1, x_2 = 9.2, x_3 = 10.5, and x_4 = 9.9 optimised the extract drop to the target value of 1.6 °P per day during the first five days of fermentation. Verbelen et al. (2009a), who reviewed data on the pitching rate, clearly demonstrated that the initial cell concentration had a significant impact on the extract drop (°P day^{-1}). This parameter was improved from 1.6 to 2.0 when the pitching rate was increased from 10 to 20 mln cells 1 mL^{-1}. Similar results were reported by Erten et al. (2007), who confirmed at a laboratory scale that an increase in the rate of fermentation by about 25% resulted from a 10-fold increase in the pitching rate (from 10 to 100 mln yeast cells per mL of wort).

There seems to be a common opinion in the literature that an increase in fermentation temperature improves the dynamics of fermentation. Dragone et al. (2004) recommended the fermentation temperature of 14 °C to activate yeast metabolism at high-gravity brewing. Ramirez & Maciejewski (2007) confirmed that a similar temperature (13 °C) had the most effective influence on the process of bottom fermentation.

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Table 3. ANOVA table for ethanol concentration

Source	Sum of squares	df	Mean square	*F*-ratio	*P*-value
x_1	0.0182	1	0.0182	7.26	0.0544
x_2	0.0287	1	0.0287	11.48	0.0276
x_3	0.0011	1	0.0011	0.43	0.5493
x_4	0.0007	1	0.0007	0.28	0.6237
x_1x_3	0.0253	1	0.0253	10.12	0.0335
x_1x_4	0.0338	1	0.0338	13.52	0.0213
Blocks	0.0031	1	0.0031	1.25	0.3270
Lack-of-fit	0.0983	42	0.0023	0.94	0.6157
Pure error	0.0100	4	0.0025		
Total (corr.)	0.2192	53			

For x_1–x_4 see Table 2

Table 4. ANOVA table for extract drop

Source	Sum of squares	*df*	Mean square	*F*-ratio	*P*-value
x_1	0.6016	1	0.6016	22.07	0.0093
x_2	5.1894	1	5.1894	190.32	0.0002
x_4	0.2400	1	0.2400	8.80	0.0413
x_2^2	0.2243	1	0.2243	8.22	0.0456
Blocks	0.0091	1	0.0091	0.36	0.5949
Lack-of-fit	0.9833	34	0.0289	0.33	0.2489
Pure error	0.1091	16	0.0273		
Total (corr.)	7.7595	53			

For x_1, x_2 and x_4 see Table 2

Table 5. ANOVA table for fee amino nitrogen (FAN)

Source	Sum of squares	*df*	Mean square	*F*-ratio	*P*-value
x_3	1 027.04	1	1 027.04	11.33	0.002
x_1x_2	2 346.13	1	2 346.13	25.88	0.000
x_2x_3	1 512.50	1	1 512.50	16.68	0.001
x_4^2	382.823	1	382.823	4.220	0.046
Blocks	75.8519	1	75.8519	0.840	0.365
Total error	4 079.35	45	90.6522		
Total (corr.)	9 800.81	53			

For x_1–x_4 see Table 2

FAN consumption. Table 5 shows the ANOVA for the FAN values in matured beer after removing insignificant components from the model.

Aeration rate appeared to be the most important linear parameter affecting the FAN uptake of the lager beer. Additionally, a significant interaction of the pitching rate with fermentation temperature and aeration level as well as a positive effect of the quadratic component of the CCT filling time was predicted by the following equation:

$$y_4 = 29.064 + 49.104x_3 + 5.708x_1x_2 - 4.583x_2x_3 - 0.264x_4^2 \quad (4)$$

where: y – FAN uptake of beer (mg L^{-1}); x_4 – pitching rate (mln cells mL^{-1}), x_1 – fermentation temperature in °C, x_2 – the aeration level (mg L^{-1}), x_3 – the total time of CCT filling (h).

The equation of the fitted model explained seventy percent of the variability in the FAN uptake. Using the above formula in the response optimisation

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Table 6. Values of independent factors that optimised alcohol content pH, extract drop, FAN consumption, bitterness loss in the tested beer, its sensory quality, and corresponding predicted values after optimisation

Technological parameters	Levels alcohol		Optimum/Target							
			pH	extract drop	FAN	bitterness		production indices	all aspects	sensory
	−1	+1	opt.	min.	opt.	max.	min.	opt.	opt.	max.
Pitching rate (mln cells mL^{-1})	6.0	10.0	7.81	10.0	8.11	10.0	10.0	6.00	6.0	6.0
Temperature of fermentation (°C)	8.5	11.5	9.47	11.5	9.04	11.49	8.53	9.29	11.24	11.24
Wort aeration level (mg L^{-1})	8.0	12.0	10.32	10.5	10.16	8.0	8.01	10.10	10.5	10.1
Total filling time of CCT (h)	4.5	13.5	9.87	6.60	10.04	8.19	13.50	13.5	13.5	13.5
Predicted values										
Alcohol content (% v/v)			6.90					6.94		
pH				4.6				4.69		
Extract drop (°P day^{-1})					1.60			1.77		
FAN consumption (mg L^{-1})						146		128		
Bitterness loss (EBC)							0.14	6.2		
Sensory (pts)								66.5		67

FAN – fee amino nitrogen; CCT – cylindroconical fermentation tanks

module allowed to predict the highest FAN uptake 144 mg L^{-1} at x_1 = 10, x_2 = 11.5, x_3 = 8, x_4 = 9.4 (see Table 6). The highest FAN uptake was characteristic of beer brew with pitching rate and fermentation temperature values set at the high levels.

Verbelen et al. (2009a) reported that the FAN consumption depended on the pitching rate used. The authors underlined that FAN uptake was enhanced by 40, 61 and 66% when two-, four- and six-fold higher than normal pitching rates were used. The results presented by Nguyen & Viet Man (2009) confirmed such a relationship. The researchers observed that the enhancement (by about 20%) in FAN absorption was attributed to yeast cells with the increased pitching rate from 15 to 75 mln yeast cells per 1 mL of wort. In another study Verbelen et al. (2009b) researched the effect of the wort aeration level on the FAN uptake. Experiments showed that the highest FAN consumption resulted from applying pure oxygen (51.8 ppm) and in relation to the normal aeration with air the authors reported a twice higher reduction of FAN.

Multiple response optimisation procedures. Multiple response optimisation as a part of the experimental design module allowed to find areas where the levels of independent factors maximized FAN consumption, minimized pH, extract drop and bitterness losses while keeping the ethanol content at the desired value of 6.9% (v/v). Furthermore, the last step of optimisation ("optimise all") involved simultaneous optimisation of all fermentation indices as well as maximization of the beer sensory quality (details of this assessment were given in our previous work Kucharczyk et al. 2020). The comparison of results from the single response optimisation, which were described earlier, with those originated from the multiple response optimisation procedures as well as predicted values of alcohol, pH, extract drop, FAN consumption and bitterness loss, and the sensory quality of beer are presented in Table 6.

CONCLUSION

The concentrations of alcohol, pH value, rate of fermentation (expressed by extract drop), FAN uptake and bitterness loss were significantly improved by changing the values of technological parameters. The multiple response optimisation method allowed for simultaneous optimisation of the above process parameters and sensory quality of beer. Levels of independent factors that led to the enhanced sensory quality of beer, pitching rate of 6 mln cells mL^{-1}, fermentation temperature of 11.2 °C, aeration level of 10.5 mg L^{-1}, and time of CCTs filling in 13.5 h were a compromise between individual process parameters.

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