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Optimisation of Lab-Scale Continuous Alcohol-Free Beer Production

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Abstract

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In order to study the formation and conversion of the most important flavour compounds, the real wort used in alcohol-free beer fermentation was mimicked by a complex model medium containing glucose, yeast extract, and selected aldehydes. The fermentation experiments were carried out in a continuously operating gas-lift reactor with brewing yeast immobilised on spent grains (brewing by-product). During the continuous experiment, parameters such as oxygen supply, residence time (Rt), and temperature (T) were varied to find the optimal conditions for the alcohol-free beer production. The formation of ethanol, higher alcohols (HA), esters (ES), as well as the reduction of aldehydes and consumption of glucose were observed. The results suggest that the process parameters represent a powerful tool in controlling the degree of fermentation and flavour formation brought about by immobilised biocatalyst. Subsequently, the optimised process parameters were used to produce real alcohol-free beer during continuous fermentation. The final product was compared with batch fermented alcohol-free beers using the methods of instrumental and sensorial analysis.

Keywords: alcohol-free beer; continuous reactor; immobilised yeast; beer flavour

In EU countries, the beer with ethanol content up to 0.5% by volume is termed alcohol-free. Although it is still a minor product of the brewing industry, the increasing production of alcohol-free beer worldwide reflects the global trend for healthier lifestyle. There are two main strategies for its production (*i*) removal of alcohol from regular beer (dialysis, reverse osmosis, vacuum distillation or evaporation) and (*ii*) controlled (suppressed) alcohol formation. While the former approach requires special equipment for alcohol removal, the latter is carried out in conventional brewery facilities. The continuous alcohol-free beer fermentation is also based on controlled alcohol formation, however, it requires modified reactor systems. It can outperform the traditional technologies in various aspects (productivity, investment, and operating costs). However, it can be successful only on condition that the continuous system produces a competitive final product (Brányik *et al.* 2006). This can be achieved by high quality yeast, optimised process parameters, and sensitive process control.

Alcohol-free beers incline to have worty off-flavour (aldehydes) and to lack the pleasant estery aroma found in regular beers (Perpete & Collin

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1999). Such taste defects result from either a shortened fermentation procedure (insufficient wort aldehyde reduction, lack of fusel alcohol and ester production) or loss of volatiles (higher alcohols, esters) accompanying ethanol removal.

It is known that wort carbonyls contribute to the unpleasant worty taste of alcohol-free beers (Perpete & Collin 1999) and that yeast metabolism reduces these substances to less flavour active ones (Peppard & Halsey 1981). Several alcohols, other than ethanol, are formed in beer during fermentation (*n*-propanol, isobutanol, and isoamyl alcohols contribute most significantly to the beer flavour). The control of higher alcohol formation in continuous systems can be achieved by the choice of an appropriate yeast strain (Linko *et al.* 1997), wort composition, fermentation conditions, immobilisation method, and reactor design (Norton & D'Amore 1994; Yamauchi *et al.* 1995). The synthesis of aroma-active esters by yeast is also of a great importance because they represent the largest group of flavour active compounds in beer. The control of ester formation during continuous fermentation is rather difficult due to many factors involved in the regulation of the enzyme activity or substrate availability (Verstrepen *et al.* 2003).

The fermentation experiments were carried out in a continuously operating gas-lift reactor with brewing yeast immobilised on spent grains, a brewing by-product (Brányik *et al.* 2001). During the optimisation study, parameters such as aeration, residence time (Rt), and temperature (T) were varied to elucidate their influence on the continuous production of alcohol-free beer with balanced flavour. Subsequently, the optimised process parameters were used to produce real alcohol-free beer by continuous fermentation and the product was compared with traditional batch fermented alcohol-free beers both analytically and sensorially.

MATERIAL AND METHODS

Microorganisms and medium. Different bottom fermenting brewing yeast strains of *Saccharomyces pastorianus* (*carlsbergensis*) were used throughout the experiments. For the optimisation of the process parameters, an industrial strain (PT) was used (UNICER. Bebidas de Portugal, S.A., S. Mamede de Infesta, Portugal). The continuous production of real alcohol-free beer was tested with the aforementioned strain PT and strains 96 and 55 (collection of the Research Institute of Brewing and Malting Prague, Czech Republic). The brewing yeast strains and their origins are indicated in connection with the results. The yeasts were cultivated in a complex model medium (CMM) mimicking the wort composition used for the alcohol-free beer production. The composition of CMM was (in g/l): 5, KH_2PO_4; 2, $(NH_4)_2SO_4$; 0.4, $MgSO_4 \cdot 7H_2O$; 2, yeast extract (Merck, Darmstadt, Germany); 20, glucose p.a. (Penta, Chrudim, Czech Republic). During the experiments focused on the aldehyde removal capacity of the immobilised cell reactor system, the aldehydes were added into CMM in the following concentrations (in µg/l): 100, 2-methyl propanal; 200, 3-methyl butanal; 100, hexanal; 100, furfural (Fluka Chemie GmbH, Steinheim, Switzerland). In the experiments aimed at quantifying the stripping of beer volatiles, the following amounts of alcohols and esters were added into CMM (in mg/l): 3945, ethanol; 3, isobutanol; 10, isoamyl alcohol; 10, amyl alcohol; 2, ethyl acetate; 2, amyl acetate; 2, isoamyl acetate; 1, ethyl caproate; 1, ethyl caprylate (Fluka Chemie GmbH, Steinheim, Switzerland). Barrels with 20 l of CMM were sterilised at 121°C, 100 kPa, for 30 minutes. The real all-malt wort (original extract 5% wt) used in this work was prepared from powdered wort concentrate (Research Institute of Brewing and Malting, Plc., Prague, Czech Republic) by dissolving the appropriate amount of the concentrate in sterile water (121°C, 60 min) and heating the solution to 100°C during 20 minutes. The industrially produced commercial alcohol-free beers analysed in this work were made of worts with an original extract from 4 to 6% wt.

The immobilised cell reactor (ICR). The gas-lift reactor (GLR) used in this work was of the concentric draught tube type with an enlarged top section for degassing and a total working volume of 2.9 l. The dimensions of the reactor were: total height – 76 cm; downcomer lengh – 44 cm and inside diameter – 7 cm; draft tube (riser) length – 41 cm, diameter – 3.2 cm and thickness – 0.4 cm; cylindrical part length – 8 cm and diameter – 14 cm. The angle between the conical sector and the main body was 51°. Gas was injected through a sparger (perforated plate, diameter – 1 cm) with seven holes of 0.5 mm each and placed 2.5 cm below the annulus of the riser. The outflow of the reactor was placed behind the sedimentation

barrier, thus minimising the carrier losses. The whole ICR was placed into a thermostated cold room (Figure 1). The desired gas flow (pure air or air + CO_2 mixture) was adjusted with a mass flow controller (Aalborg GFC17, Aalborg Instruments, Orangeburg, New York, USA). Dry spent grains were cleaned by acidic hydrolysis (3% v/v HCl, 2.5 h, 60°C) followed by delignification in 2% w/v NaOH (30°C 24 h). Prior to the use, the carrier was washed with water until pH 7 was reached and was then dried (12 h, 60°C) (Brányik *et al*. 2001).

Starting and operating of ICR. The Plexiglas GLR was sterilised using sodium hypochlorite solution (2% active chlorine) at least 4 days prior to fermentation. After draining the reactor, the sterile air supply into GLR was started at total flow rate of 0.25 l/min and the GLR was washed with 30 l of sterile water. Prior to inoculation, the reactor was filled with sterilised slurry consisting of 40 g of spent grains in dry state in 1.5 l of distilled water. Subsequently, the GLR was charged with CMM and then inoculated with 500 ml of yeast

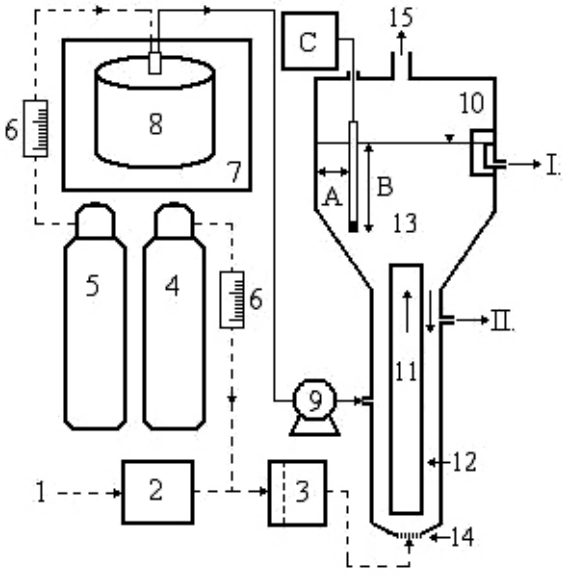

Figure 1. Schematic diagram of the continuous immobilised cell reactor system (ICR) kept in a thermostated cold room: 1 – air supply, 2 – mass flow controller, 3 – gas sterilisation filter, 4 – CO_2 cylinder, 5 – N_2 cylinder, 6 – rotameters, 7 – refrigerator, 8 – barrel with complex model medium, 9 – peristaltic pump, 10 – gas-lift reactor (GLR), 11 – riser, 12 – downcomer, 13 – enlarged separator zone, 14 – sparger (gas inlet), 15 – gas outlet; I – medium outflow, II – sampling port, A – distance of oxygen probe from reactor wall, B – depth of oxygen probe below liquid surface, C – data acquisition system

cell suspension (ca 30 × 10^6 cells/ml) grown on a rotary shaker at 20°C for 48 hours. After 24 h of the batch growth, the start-up period of the ICR was initiated. The CMM was fed at a total residence time of 6.5 h and the temperature inside GLR was maintained at 8°C. Within 10 days, a fully developed yeast biofilm was formed around the spent grain particles and afterwards the intensity of aeration was decreased by switching to air + CO_2 mixture, while the total gas flow rate was kept constant (0.25 l/min). In order to prevent contamination and oxidation, CMM was kept in a refrigeration unit at 4°C during the whole experiment. The continuous system was considered to be in the steady state after a period of 5 residence times (Rt).

Batch fermentation of alcohol-free beer. Prior to fermentation, 5 l of wort (5% wt.) was prepared from the powdered wort concentrate. The wort was then cooled to 8°C and aerated for 15 min (airflow 4 l/h). The aerated wort was inoculated with the yeast strain studied to a final concentration of 3.25 × 10^6 cell/ml. Yeasts were previously grown on wort (8°C, 10% wt) and were collected by centrifuging (6000 rpm, 5°C, 10 min). The main fermentation was carried out in a 10 l container at 8°C until the ethanol concentration was reached of approximately 0.4% by volume. Subsequently, the young beer obtained was matured at 2°C for 48 hours.

Analytical methods. Ethanol and glucose were analysed by means of HPLC (Pump LCP 4000, Column oven LCO 101, ECOM Ltd., Prague, Czech Republic) using a Polymer IEX Ca form column (250 × 8 mm, Watrex International Inc., San Francisco, USA), and a RIDK 102 refraction index detector (Laboratorní přístroje Praha, Prague, Czech Republic). The elution was performed with Nanopure-filtered water at 85°C, the flow rate being 0.7 ml/minute. The flavour and aroma compounds (higher alcohols, esters, and vicinal diketones) contents were measured according to the current European Brewery Convention recommended methods (ANALYTICA-EBC, 2000). The aldehydes were determined by solid-phase microextraction using on-fiber derivatisation with *O*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBOA). The carbonyl compounds selectively reacted with PFBOA, and the oximes formed were desorbed into a gas chromatograph injection port (6890N, Agilent Technologies, Santa Clara, CA) and quantified by mass spectrometry (5975, Agilent Technologies, Santa Clara, CA) (Vesely *et al*.

2003). The cell viability was measured by counting the dead cells stained with methylene blue. Immobilised biomass was determined according to Brányik *et al.* (2004).

The samples of free and immobilised cells were collected at the sampling port of ICR (Figure 1). The immobilised cells were liberated from the biocatalyst (carrier + immobilised cells) by agitation. The biocatalyst taken from ICR was washed in running water (4 × 100 ml), and then agitated (2 cm magnetic bar, 200 rpm) in 50 ml of physiological solution (9 g/l NaCl) for 20 minutes. The biomass released from the carrier was used for viability determination.

Organic acids were determined by means of a capillary zone electrophoretic analyser (EA 101, Labeco-Villa, Ltd., Slovak Republic), with the separation capillaries (PTFE, lenght 90 mm, diameter 0.8 mm, 150 μA) connected to an analytical capillary (length 90 mm, diameter 0.3 mm, 20 μA) and a detector (conductive and UV detection at 254 nm). The analyses were carried out in a leading electrolyte (5mM HCl + 10mM glycylglycine + 0.05% hydroxyethylcellulose) and a finishing electrolyte (10mM caproic acid).

The volumetric gas-liquid mass transfer coefficient $k_L a$ in the three-phase (gas-liquid-solid) GLR used in this work was determined by the dynamic gassing-out method. The $k_L a$ was calculated from the slope of the increasing dissolved oxygen concentration (Chisti & Jauregui-Haza 2002).

Sensorial analysis of alcohol-free beers. The sensorial analyses of all alcohol-free beer samples were registered in a modified form based on the pattern sensory beer analysis of EBC (European Brewery Convention). The intensity of the selected flavour and odour parameters was described as absent, very weak, weak, normal, strong, and very strong. The overall impression was evaluated using a 9 point scale, where 1 was considered as extremely good and 9 as extremely bad. The arithmetical average of the overall impression was calculated to evaluate the quality of samples. Seven tasters of the internal sensory panel of the Institute of Chemical Technology in Prague, Czech Republic with at least one year of sensory experience each were recruited based on their good sensory ability. The sensorial panel consisted of members aged between 22–43 years. The beer tasting took place in a noiseless room at a temperature around 21°C. All samples were previously degassed and served at a temperature of 8°C in degustation glasses.

RESULTS AND DISCUSSION

During the beer production, a well-balanced aroma and flavour of the final product are equally or even more important than the efficient fermentation and high ethanol yield. Therefore, prior to the trials with real alcohol-free beer production in a lab-scale continuous bioreactor with immobilised yeast, a set of experiments aimed at the optimisation of the process parameters was carried out. These preliminary experiments with continuous alcohol-free beer production were studied in a complex model medium (CMM) containing inorganic salts, nutrients and aldehydes (hexanal, 2-methyl propanal, 3-methyl butanal, furfural) and mimicked real brewery wort with an advantage of constant composition.

Optimisation of process parameters in model medium: Aeration

Although the immobilised and free cell population in a continuous fermenter lacks the distinct growth phases of a batch culture, the optimum oxygen supply is indispensable for both the yeast physiology and by-product formation. In order to avoid the often observed under- or over-aeration in continuous beer fermentation systems, it was necessary to determine the volumetric oxygen mass transfer coefficient ($k_L a$) under real fermentation conditions. The oxygen transfer rate (OTR) during real fermentation experiments was calculated using the $k_L a$ ($0.98 \pm 0.5\ h^{-1}$) determined for the solid loading of 14 g of dry spent grains per unit reactor volume (Lehnert *et al.* 2008). The influence of the OTR on the flavour active compounds was studied in a range from 1 to 12 mg O_2/lh at a constant temperature (8°C) and residence time (6.5 h). The results showed that the most advantageous composition of the fermented CMM was achieved at OTR = 1 mg O_2/lh. At this oxygen supply, the total higher alcohol and ester concentration both in the fermented CMM from ICR and commercial alcohol-free beers produced by traditional batch fermentation was in the same range (Table 1). Besides the concentrations of the individual flavour active compounds, it is the ratio between the total higher alcohols to total esters (HA/ES) which is particularly important in terms of the beer flavour. In regular beers, the HA/ES ratio is considered favourable around 3:1. The best HA/ES

Table 1. Major groups of flavour active compounds in complex model medium (CMM) from the continuous immobilised cell reactor system (ICR) and in three commercial alcohol-free beers produced by traditional industrial batch fermentation

Parameter	CONT[a]	COM1[b]	COM2[b]	COM3[b]
Ethanol (v/v %)	0.50	≤ 0.50	≤ 0.50	≤ 0.50
Total higher alcohols[c] (mg/l)	11.80	11.45	9.30	9.18
Total esters[d] (mg/l)	0.85	0.86	0.17	0.77
HA/ES[e]	13.9	13.70	54.70	11.92
Vicinal diketones[f] (mg/l)	0.06	0.05	0.08	0.03

[a]fermentation conditions in ICR (CMM, strain PT, OTR = 1 mg O_2/lh, temperature 8°C, residence time was adjusted to result in ethanol content 0.5–1.0 v/v %). The data presented were obtained after dilution with distilled water to ethanol concentration of 0.5 v/v %

[b]commercial alcohol-free beers brewed in the Czech Republic

[c]sum of propanol, isobutanol, amyl alcohol, and isoamyl alcohol concentrations

[d]sum of ethyl acetate, amyl acetate, isoamyl acetate, ethyl caproate, and ethyl caprylate concentrations

[e]total higher alcohols to total esters ratio

[f]2,3-butanedione and 2,3-pentanedione

ratio achieved in the continuous system (13.9:1) was that at the lowest oxygen supply studied, i.e. 1 mg O_2/lh (Table 1). The concentrations of the undesirable vicinal diketones (VDKs) found in all analysed samples did not exceed the taste threshold limit of VDKs (0.15 mg/l) (Meilgaard 1975).

Optimisation of process parameters in model medium: Residence time and temperature

The influence of temperature (5°C, 10°C and 15°C) and residence time (Rt) in ICR on the glucose consumption, formation of ethanol, flavour active volatiles (HA-higher alcohols, ES-esters), and reduction of aldehydes was studied. During these experiments the oxygen transfer rate (OTR) to free and immobilised yeasts in ICR was kept constant at a previously optimised level (1 mg O_2/lh). As expected, the increasing residence time (Rt) of the CMM resulted in a gradual increase of the glucose consumption and ethanol formation (Figure 2). Simultaneously with the progress in the degree of fermentation, the amounts of sensorially active metabolic by-products (HA and ES) increased approximately to the point at which glucose depletion in the effluent occurred. A further increase in the residence time led to losses of volatiles (Figure 2B), most probably by stripping with the gas mixture injected into ICR.

Besides microbial formation of volatiles, the stripping of the same compounds by driving gas responsible for mixing in ICR was also quantified. It was found that under the operating conditions of 10°C, 0.25 l/min gas flow, and residence time from 3 to 10 h, the losses of total aldehydes added to CMM by stripping were from 18% to 87% of their original concentration. Under the same conditions the losses of esters by stripping were in the range from 21% to 40%, while higher alcohol and ethanol losses were negligible.

By changing the fermentation temperature and Rt, the HA/ES ratio varied in the range from 14 to 22 (Figure 2B). Not surprisingly, since the final concentration of higher alcohols is determined also by the extent of carbohydrate metabolism (Sablayrolles & Ball 1995). In other words, the initial increase of HA/ES ratio can be ascribed mainly to a more intensive higher alcohol formation as a result of the progress in fermentation and biomass growth. At a high Rt, the HA/ES ratio further increases since the esters are generally more volatile than higher alcohols. Hence, the ratio of the volatile fermentation by-products (HA/ES) is more favourable at a lower degree of fermentation leading to 0.5% vol. ethanol and at higher temperatures (Figure 2). When evaluating the fermentation condition, the economic aspect is not negligible. Therefore, achieving the same product composition at a lower temperature will result in savings on cooling expenses.

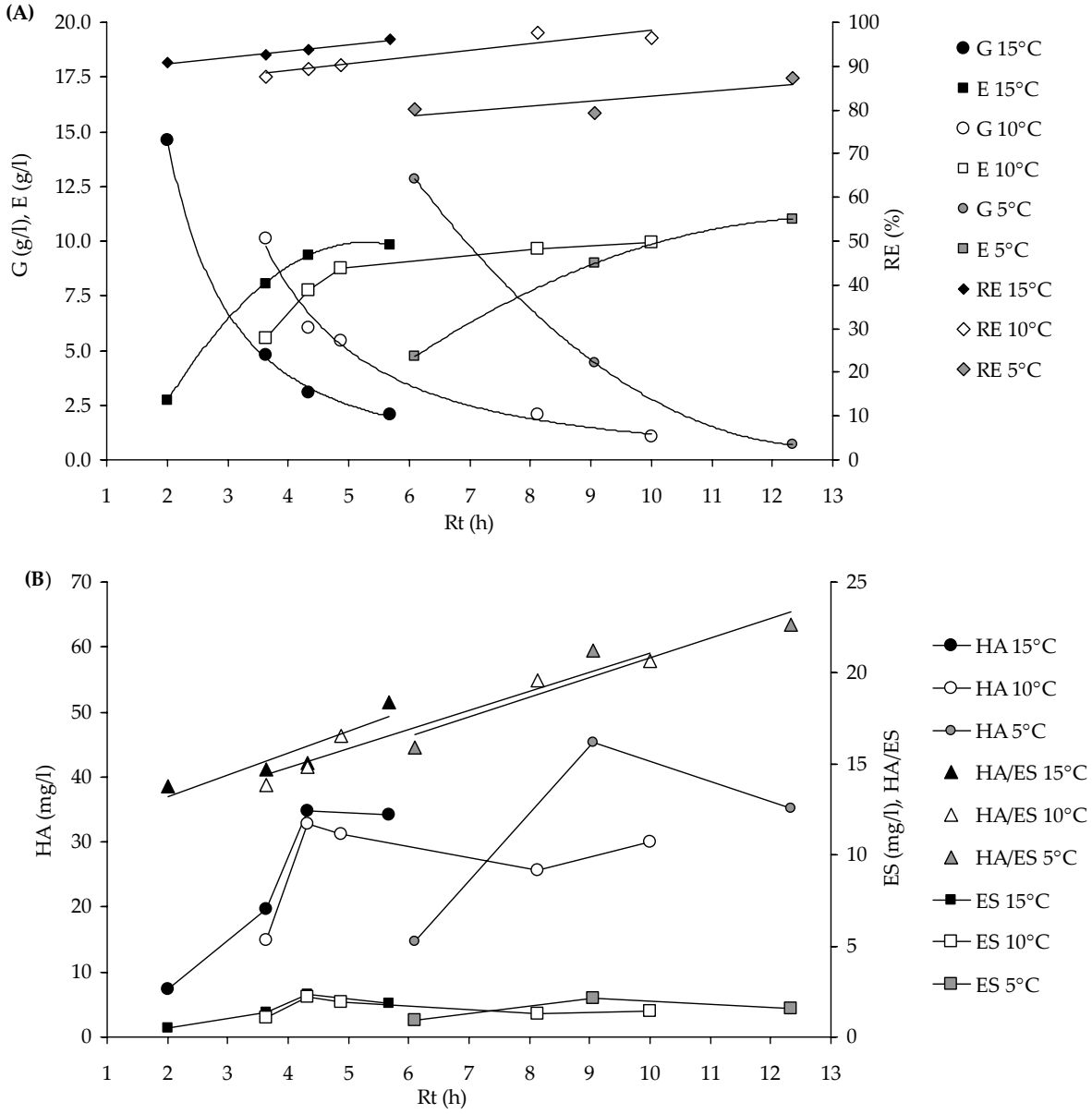

Figure 2. The composition of the complex model medium (CMM) in the effluent from ICR (E – ethanol, G – glucose, HA – total higher alcohols, ES – total esters, HA/ES – total higher alcohols to total esters ratio, RE – total aldehyde reduction efficiency) versus residence time (Rt) in ICR at different temperatures and constant oxygen transfer rate (1 mg O_2/lh)

The total aldehyde reduction efficiency (RE) in the fermented CMM containing 0.5% vol. ethanol (3.945 g/l) was approximately 91, 85, and 75% at 15, 10, and 5°C, respectively (Figure 2A). This suggests that the increasing process temperature is favourable for aldehyde reduction. Although the RE increases with the prolonging contact (residence time) between cells and CMM, the RE values at 0.5% vol. ethanol in the fermented CMM can be considered satisfactory taking into account that aldehydes were added into CMM in excess.

Real alcohol-free beer fermentation

The results obtained during the optimisation experiments carried out with complex model medium (CMM) allowed us to choose a range of suitable process parameters applicable in real wort fermentation. It was found out that a low oxygen supply (OTR) results in the lowest HA/ES ratio desirable for balanced flavour. Therefore, during real alcohol-free beer fermentation, the OTR was kept at the lowest level achievable on

our laboratory scale ICR (1 mg O_2/lh). Similarly, the highest temperature tested (15°C) led to the lowest HA/ES ratio and highest aldehyde reduction efficiency. However, at high fermentation temperatures the short residence time (approximately 4 h) in ICR resulted in an exaggeratedly high wort consumption (> 20 l/day). In order to decrease the expenditures on raw material (powdered wort concentrate) and labour demands, the real wort fermentation experiments were carried out at a lower temperature (8°C). The goal of the experiments with real alcohol-free beer fermentation was to obtain the data on the extent to which the medium composition, brewing yeast strain, and applied technology influence the formation of flavour substances.

The volatile (HA+ES) production in CMM and wort under identical process conditions and by the same strain (PT) can be seen in Tables 1 and 2. The results show that the strain PT formed somewhat less total volatiles in wort and simultaneously a slightly lower HA/ES ratio, namely 12.2:1 in wort and 13.9:1 in CMM (Tables 1 and 2).

The comparison of different production strains revealed a significant heterogeneity in sensorially important by-product formation. The best performance (high total volatiles content, low HA/ES ratio) in ICR was achieved with strain 96 while the worst flavour balance was observed with strain 55 (Table 2). The alcohol-free beer continuously produced by strain 96 was fully comparable in its basic features with the commercial products of batch fermentation (Tables 1 and 2). This shows how important the choice is of the production strain for each reactor arrangement (batch or continuous) or immobilisation technique. The selection of the brewing strains especially suited to continuous brewing has been underestimated. Yeast strains performing well in traditional batch fermentations were often automatically applied in continuous reactors, regardless of a possible mismatch between the requirements of the continuous process arrangement, immobilisation, aging, flavour production etc. on one side and genetic potential of the yeast cell on the other. The production strain for a continuous beer fermentation system should be carefully selected from a pool of strains in view of the particular conditions of a certain plant design and taste features of the intended final product (Brányik *et al.* 2008).

The influence of the applied technology can be evaluated by comparison of the selected parameters of alcohol-free beers produced by the same strain (96) and from the same wort by either continuous (ICR) or batch fermentation. The comparison revealed that the influence of the fermentation arrangement on the product composition is not negligible and in the case of strain 96 surprisingly more favorable (higher total volatiles content, lower HA/ES ratio) for continuously fermented alcohol-free beer (Table 2). The viability of both the immobilised and free cells during the experiments was below 4% of dead cells as determined by methylene blue staining.

The continuous fermentation process based on the immobilised yeast cell technology allows producing an acceptable end product with great time savings (Brányik *et al.* 2008). The main economic advantage of the continuous, immobilised

Table 2. Selected parameters of real alcohol-free beer from the continuous immobilised cell reactor system (ICR) produced by different yeast strains and of two alcohol-free beers produced by traditional batch fermentation

Parameters	CONT1[a,b]	CONT2[a,b]	CONT3[a,b]	BATCH[a]	COM4[c]
Ethanol (v/v %)	0.50	0.50	0.50	≤ 0.50	≤ 0.50
Total higher alcohols (mg/l)	8.46	12.42	9.10	8.84	16.9
Total esters (mg/l)	0.69	1.6	0.35	0.77	1.82
HA/ES	12.22	7.76	26.0	11.48	9.3
pH	4.62	4.64	4.95	4.91	5.05

[a]CONT1 – Strain PT; CONT2 – Strain 96; CONT3 – Strain 55; BATCH – Strain 96 (produced at the ICT Prague by batch fermentation from wort with original extract 5% wt.)

[b]fermentation conditions in ICR (5% wt. wort, OTR = 1 mg O_2/lh, temperature 8°C, residence time was adjusted to result in ethanol content 0.5–1.0 v/v %). The data presented were obtained after dilution with distilled water to ethanol concentration of 0.5 v/v %

[c]commercial alcohol-free beer brewed in the Czech Republic

Table 3. Sensorial evaluation of the samples of alcohol-free beers from continuous immobilised cell reactor system (ICR) produced by different yeast strains and of alcohol-free beers produced by traditional batch fermentation

Parameters	CONT1[a]	CONT2[a]	CONT3[a]	BATCH[a]	COM1[b]	COM2[b]
Aroma	weak	normal	normal	normal	normal	normal
Foreign odour	worty	esters	medicinal	yeasty	worty	worty
Fullness	weak	normal	weak	weak	normal	normal
Bitterness	normal	normal	weak	weak	strong	weak
Aftertaste	medicinal	oxidised	medicinal	autolysed	worty	sweet
Overall impression	5.86	6.57	7.67	7.33	4.33	6.88

[a]CONT1 – Strain PT; CONT2 – Strain 96; CONT3 – Strain 55; BATCH – Strain 96 (produced at the ICT Prague by batch fermentation)

[b]commercial alcohol-free beers brewed in the Czech Republic

cell fermentation is the possibility to minimise the investment costs (small reactor size) since the productivity of the process is very high. The continuous fermentation presented using immobilised yeast cell technology allows the production of alcohol-free beer to be accomplished in as little as 3–4 h compared to 2–4 days in the traditional limited batch fermentation.

Sensorial evaluation of real alcohol-free beers

The final arbiter of beer quality is the palate of the consumer which can show wide variations between individuals, geographical areas, and even fashion tendencies. In this work, the sensorial assesment was carried out with the purpose to compare the alcohol-free beers produced by different yeast strains both in continuous and batch fermentation systems. The results show that the overall evaluation of two continuously fermented alcohol-free beers (CONT1 and CONT2) was in the range of commercial products (COM1 and COM2), while the remaining two samples produced under laboratory conditions (CONT3 and BATCH) were considered the worst (Table 3). As regards foreign odours and aftertastes, only those are depicted found by the sensory panel as the most typical for each sample, although the organoleptic impression is always complex. The most common foreign odour and aftertaste found in all evaluated samples was worty and medicinal, respectively. However, the variability of the detected off-flavours/aromas was considerable (Table 3).

Considering that the main analytical parameters of continuously fermented and commercial (batch) alcohol-free beers were similar (Table 2), it was somewhat surprising that the sensory evaluation of the continuously fermented samples was rather poor (Table 3). According to the panellists, there was a characteristic and complex off-flavour present in all samples produced in laboratory, but not in commercial products. Since this was reported repeatedly, we assume that it was the powdered wort concentrate (produced by spray drying) which was responsible for the worse overall evaluation of the laboratory fermented products. Of course, in the industrial scale production the risk of flavour deterioration could be eliminated by using non-spray dryed wort directly from the brewhouse.

CONCLUSIONS

In order to demonstrate that continuous brewing can produce both quality and savings, a set of experiments with continuous alcohol-free beer production was carried out. The objective of the work was to show that continuous fermentation systems are not only highly productive but that fine tuning of their process parameters can also result in alcohol-free beer comparable with commercial products.

As regards the product quality, it can be concluded that the process parameters (oxygen transfer rate, residence time, temperature) are instruments which allow us to control to some extent the flavour formation and process economy in a continuous immobilised cell reactor system (ICR). Simultaneously, the experiments with real alcohol-free beer production indicate the importance of the selection of a proper yeast strain in connection with the continuous fer-

mentation systems to achieve the desired product composition. The continuous system presented stands out by its short fermentation time, cheap carrier material reducing the investment costs, and alcohol-free beer with a composition and flavour close to commercial products.

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