Sensorial evaluation of continuously fermented beer and the role of process parameters in adjusting its flavour profile

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SUMMARY

Although traditional batch overwhelmingly prevails over continuous fermentation, a breakthrough in quality and savings could be achieved by a simple, flexible and cheap fermentation system. This work deals with a complete continuous beer fermentation consisting of an airlift and a packed-bed reactor containing yeast immobilized on spent grains and corncobs, respectively. The goal was to study the influence of process parameters on bioreactor performance and flavour profile of beer. Consumers considered the continuously fermented beer to be of a regular quality. The possibility of flavour adjustments by changing the process parameters was proved by a panel of experienced tasters.

INTRODUCTION

Traditional beer fermentation and maturation processes using open fermentation and lager tanks had previously been considered indispensable. However, during the last decades a broad spectrum of novel inventions revolutionized the conventional fermentation equipment. This led many breweries to the introduction of large production units (cylindroconical tanks), which have proved to be successful both providing operating advantages and ensuring the quality of the final beer. Another promising contemporary technology, namely continuous beer fermentation using immobilized brewing yeast, by contrast, has found only a limited number of industrial applications. The reason for this lies in the often legitimate objections of the industry towards technical difficulties accompanying the process as well as in the desire of the brewers to preserve the traditional image approved by the consumer (10).
Fermentation and maturation are the most time consuming steps in the production of beer. In such a competitive market, the potential time savings offered by continuous

fermentation present a challenging dilemma to be addressed. The continuous fermentation process based on immobilized yeast cell technology would allow brewing companies to produce an acceptable end product with great time savings. Immobilized yeast cell technology allows the production of beer to be accomplished in as little as 2-3 days (4, 15).

In spite of the economic advantages that continuous beer fermentation offers, the technical difficulties such as demanding process control, flavour problems, risk of contamination, yeast viability, fear of yeast mutation, carrier price and the inconvenience of immobilization retard the implementation of the process at industrial scale (8, 12). For example, the total investment costs depend significantly on the carrier costs and on the technology applied. Thus the use of cheap carrier materials in a suitably designed bioreactor could favour the economics of the immobilized process, inspire researchers and encourage brewing engineers.

The goal of this paper is to describe the use of spent grain particles and corncobs, new cellulose-based carrier materials made from brewing and agricultural by-products, as a carrier for brewing yeast immobilization and its application in continuous beer fermentation system consisting of a gas-lift and a packed-bed bioreactor, respectively. Attention will be also paid to the optimization of operational conditions (aeration, flow rate and temperature) in terms of productivity and sensorial quality of the final product.

METHODOLOGY

Yeast strain and culture conditions

The brewing yeast *Saccharomyces uvarum (carsbergensis)* was supplied by the brewing company UNICER, SA. The yeast for inoculation of the continuous airlift reactor were cultivated in 500 mL of synthetic medium under aerobic conditions on a rotary shaker (120 rpm) at 30 ºC for 30 h. The composition of the synthetic medium was as follows (g/L): KH_2PO_4, 5.0; $(NH_4)_2SO_4$, 2.0; $MgSO_4.7H_2O$, 0.4; yeast extract, 1.0; glucose, 10.0. Medium with the same composition was used in continuous experiments during biomass attachment. The all malt wort used in this work had an original gravity of 12 ºP and was supplied by UNICER, SA.

Carrier preparation

Dry spent grains were mixed in 3 vol % HCl to hydrolyse the residual starchy endosperm and embryo of the barley kernel present in the spent grains. Then the mixture was washed with water and dried. The remaining solids mainly the husks of the barley grain were partially delignified by shaking in 2 % (wt/vol) NaOH. After being washed several times with water (until neutral pH) and dried, the carrier was ready to be used (3).

Cylindrical corncobs were cut into slices with a diameter of ca. 2 – 3 cm (width) and a height of approximately 1 cm and these small cylinders were further cut in two pieces along the width. The total volume of the corncob carrier (140 g in dry weight) was 3 times sterilized in distilled water. Between sterilizations the carrier was washed in running water (20 L) in order to remove all the flavour and aroma active compounds that could interfere with the quality of the final beer.

The immobilized cell reactor system (ICR)

The ICR used in this work (Figure 1) consists of a concentric draught tube type gas-lift reactor (GLR) for primary beer fermentation with a total working volume of 2.9 L (R1), a sedimentation tank (ST) for excess biomass removal by sedimentation from green beer with 700 mL volume (R2), and a packed-bed reactor (PBR) for beer maturation with total working volume of 1.6 L (R3). The immobilization matrix applied in R1 were spent grain particles, R3 was filled with corncob cylinders while R2 did not contain carrier material. The dimensions of the concentric draught tube type GLR (R1) with an enlarged top section for degassing are: down comer length - 44 cm, inside diameter - 7 cm; draught tube length - 41 cm, diameter – 3.2 cm, thickness - 4 mm; cylindrical part length – 8 cm, diameter – 14 cm. The angle between the conical sector and the main body was 51°. Gas injection was made through a perforated plate with 5 holes, each of 0.5 mm diameter, placed 2.5 cm below the annulus of the riser. The outflow of the reactor was placed behind a sedimentation barrier thus minimizing carrier losses. The temperature inside the R1 (16 °C) was maintained by means of a cooling coil connected to a refrigeration bath. Air flow rate was adjusted using a mass flow controller (Hastings 202D, Hastings Instruments, USA) while CO_2 flow rate was regulated by a rotameter. Both ST (R2) and PBR (R3) were cylindrical reactors with an inside diameter of 8.5 and 7 cm and total working height of 12.5 and 42 cm, respectively. The internal temperatures in R2 (8 – 10 °C) and R3 (2 – 3 °C) were maintained by means of a cooling coil connected to a refrigeration bath. R3 was operated in upward flow.

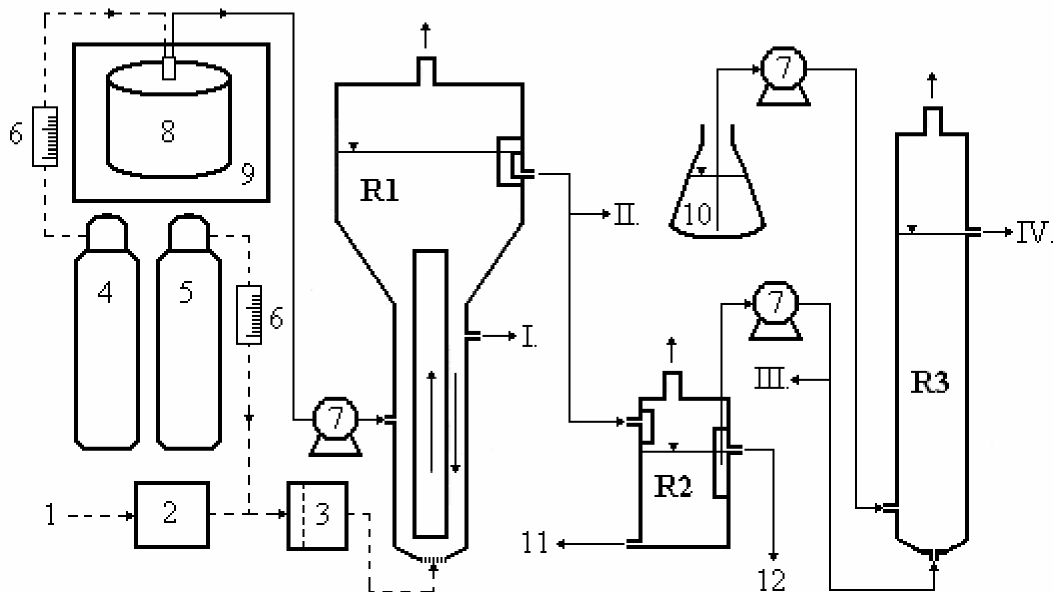

Figure 1: Immobilized yeast reactor system for laboratory scale continuous beer fermentation: 1-air supply; 2-mass (gas) flow controller; 3-gas sterilization filter; 4-N_2 bottle; 5-CO_2 bottle; 6-rotameter; 7-peristaltic pump; 8-wort barrel; 9-refrigeration unit; 10-washing (physiological) solution; 11- excess (flocculated) biomass outlet; 12-excess green beer outlet; R1-main fermentation reactor (GLR); R2-sedimentation tank (ST); R3-maturation reactor (PBR); I.-spent grains (carrier) sampling point; II. and III.-green beer sampling points; IV.-maturated beer sampling point.

Starting and operating of ICR

The whole Plexiglas ICR system was sterilized using sodium hypochlorite solution (2 % active chlorine) at least 4 days prior to fermentation. After draining the reactors the sterile gas supply into R1 was started at a total flow rate (mixture of air and CO_2) of 0.4 L/min and the reactor was filled with a sterilized slurry consisting of spent grains (40 g dry state) in distilled water (1.5 L). Similarly, the R3 was filled with 140 g (in dry weight) of corncob cylinders through the top of the reactor. Likewise R1 and R3, the sedimentation tank (R2) was also drained. Prior to inoculation, the whole ICR containing fresh carrier was washed with 100 L of sterile water. Subsequently, the R1 was charged with concentrated medium to obtain the desired concentration of the synthetic medium and then inoculated with 2×500 mL of yeast cell suspension grown on a rotary shaker. At the end of 24 h batch growth, synthetic medium started being fed into R1 and continued through the whole ICR system at a total dilution rate (D_{tot}) of 0.06 h^{-1}, which was after 168 h of operation increased to 0.16 h^{-1}. At 225 h, the synthetic medium was changed to sterilized wort (50 L, sterilized 40 min at 120 ºC), which was used throughout the whole fermentation experiment at a desired dilution rate (D). In order to prevent contamination and oxidation, wort was kept during the whole experiment in a refrigeration unit at 6 – 8 °C while N_2 was sparged into the wort barrel. During wort fermentation the total gas flow rate in the reactor (mixture of air and CO_2) was kept at 0.4 L/min, with different proportions of air in the mixture. The continuous system was considered to be in steady state conditions after a period of 5 total residence times (RT_{tot}).

Analytical methods

Characterization of wort, green beer and beer (specific gravity, original extract, degree of attenuation, alcohol, pH, and colour) was performed by SCABA 5600 (Automatic Beer Analyser, Tecator AB, Sweden). Total diacetyl was determined by gas chromatographic analysis of the static headspace (16). The flavour and aroma compounds (higher alcohols and esters) were measured according to the current European Brewery Convention recommended methods (1). The detailed procedure of the immobilized biomass (X_{im}) determination can be found in (5). Cell viability was measured by counting dead cells stained with methylene blue (2). To make possible the analysis of the cells immobilized on spent grain particles in R1, the biocatalyst (carrier+ immobilized cells) was washed with distilled water (4 × 100 mL), then agitated with a magnetic stirrer (2 cm bar, 200 rpm) for 20 min in 50 mL of synthetic medium without glucose and yeast extract. The biomass released from the carrier was used for vital staining.

Sensorial analysis

The consumer acceptance tests were carried out by at least 30 untrained consumers of Portuguese nationality. The consumer panel participating on acceptance tests had the following average profile: 52±6% within age from 26 to 35 years, 73±8% with completed higher education and 80±8% with a consumption frequency of one or more beers per week. For each group of samples the tasting was performed on the same day in a controlled room (temperature, noise, individuality of the taster) so that unbiased results were obtained. Samples of both continuous beer and commercial beer brands were poured without foam into a dark coloured glass (90 mL) and tasted at temperatures between 6 and 8 °C. Results were analyzed by Analysis of Variance (ANOVA) and Tukey tests ($p \leq 0.05$), to quantify the variability in the average of the responses. The samples of continuous beer for both consumer acceptance and

descriptive evaluation tests were collected under N_2 atmosphere and stored in PET bottles at 4 °C for ca. 24 hours before tasting.
Seven tasters of the internal sensory panel of UNICER (the main Portuguese Brewery Group) with at least one year of sensory experience were recruited based on their good sensory ability. The descriptive tests took place once per week in the morning hours in an adequately isolated taste room. Panellists were asked to describe the flavour profile according to a special form (description test). The beer samples were tasted at 12 °C and evaluated with control beer using a 9 point scale (0 = absent, 1 - 3 = low, 4 - 6 = moderate, 7 - 9 = strong). An average of the experimental values to each sensorial attribute was calculated in order to evaluate the flavour profile of each sample.

RESULTS AND DISCUSSION

Extract consumption

According to the results (Figure 2), it is in the gas-lift reactor (R1) where the prevailing portion of the fermentation occurs during the primary fermentation. In average 83.5±8.5 % of the total apparent attenuation occurs in R1. The contribution of the sedimentation tank (R2) to the fermentation was in average 9±6 % of the total apparent attenuation. This contribution is not excessive, both because a significant portion the biomass in R2 was flocculated on the bottom of the reactor and the residence time in R2 was short (RT_{R2} = 3 - 7 h) when compared to the total residence time (RT_{tot}). At RT_{tot} above 43 hours the apparent attenuation of the final beer from R3 was almost independent on RT_{tot} and close to the attenuation limit of the wort. As it can be seen (Figure 2), the degree of fermentation in the immobilized cell reactor system (ICR) can be controlled mainly by the total residence time of wort (RT_{tot}).

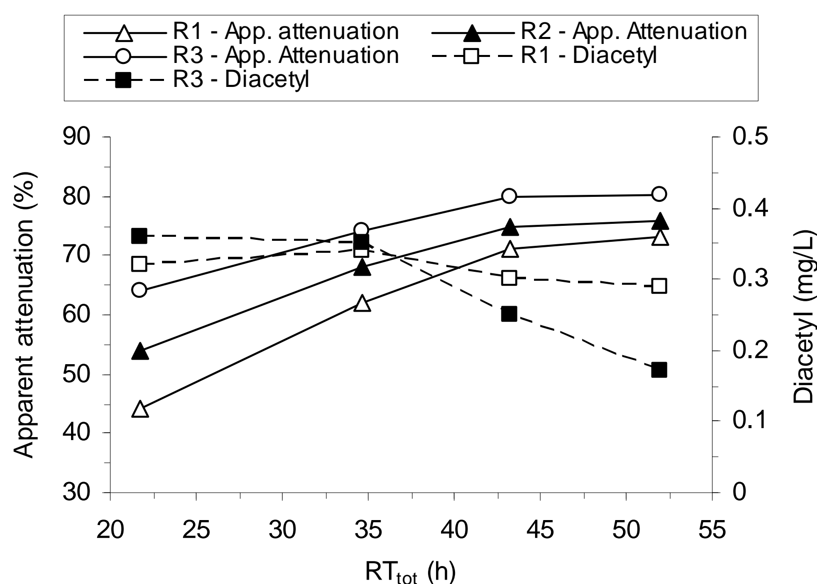

Figure 2: The influence of different total residence times (RT_{tot}) on the apparent attenuation of wort (12°P) and diacetyl formation in each stage of the immobilized cell reactor system (ICR): R1 – main fermentation reactor, R2 – sedimentation tank, R3 – maturation column. Both the air flow (AF = 20 mL/min) and the total gas flow rate (air + CO_2 = 0.4 L/min) into R1 were constant.

The influence of aeration rate into R1 on the values of attenuation was less explicit (data not shown) due to the oxygen ingress into the system through silicone feed tubing (2.5 m long, 3 mm diameter). Thus, in spite of the nitrogen sparging into the wort barrel, the dissolved oxygen level in the feed wort was in the range from 5 to 7 mg/L corresponding to the range of applied flow rates from 260 to 100 mL/h, respectively. These levels of dissolved oxygen in wort suppressed the effect of the direct aeration into R1 (0 – 50 mL/min) on attenuation.

Biomass growth and cell viability

In the course of the continuous fermentation experiment the percentage of dead immobilized cells from R1 increased from a value which is comparable with the one of free cells in ICR (7.5 %) at the beginning of the beer fermentation to a higher value (ca. 17 %) at the end of the experiment (Figure 3). Although the viability of immobilized brewing yeast in continuous beer fermentation has already been reported to decrease (6, 13), there is little known on the senescence and aging process of immobilized yeast in continuous beer fermentation systems and on their impact in product quality. The aging of the immobilized yeast biomass and all its consequences (altered fermentation rate, metabolism and sensorial profile) have also a great practical importance, namely the need for a regular biocatalyst replacement and/or renewal.

The percentage of dead cells both in the outflow from R2 did not exceed 8 % (Figure 3). Due to the regular removal of excess biomass from R2 the average viability of flocculated cells was equal to that of suspended cells (ca. 92 %). The main reason for the inclusion of R2 into ICR was the removal of free yeast and gaseous CO_2 from the green beer entering the maturation column (R3). While the removal of CO_2 in R2 was complete, the effectiveness of the free biomass removal was only 45±10 %. This insufficient sedimentation and flocculation of free cells could be in the future improved by increasing the height to diameter ratio of R2 (H/D>1.5), applying a sedimentation tank with a conical shaped bottom and decreasing the temperature.

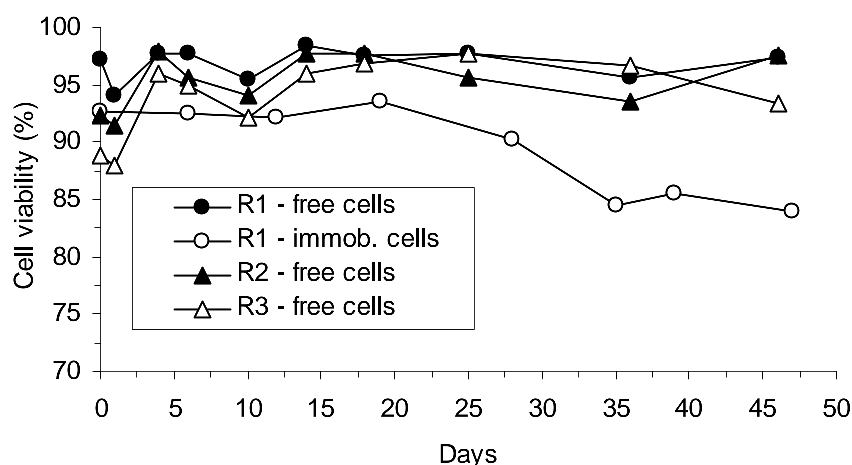

Figure 3: Percentage of viable cells among free cells in the outflow from each reactor of the continuous fermentation system (R1, R2 and R3) and among immobilized cells inside the main fermentation reactor (R1).

As a consequence of the incomplete free biomass removal in R2, the concentration of cells in the inflow to from the maturation column (R3) was 3±1 g/L. Since the average cell content in the outflow was 1.2±0.5 g/L, it was necessary to remove the excess

biomass gradually accumulating in R3. Such removal was carried out by a periodical (every 10 days) upward flushing of the R3 with 4 L of sterile physiological solution (9 g/L NaCl).
The viability of cells in the outflow from R3 was between 92 and 98 % slightly exceeding this range (88 %) at the beginning of the beer fermentation when synthetic medium was switched to wort (Figure 3). Difficulties with sterile carrier sampling from the maturation column disabled the measurement of immobilized cell biomass and its viability in this reactor. However at the end of the fermentation experiment, after 50 days of reactor operation (10 days of immobilization + 40 days of beer fermentation), the immobilized biomass distribution on corncob particles at the top, middle and bottom of the R3 was 0.354, 0.56 and 0.61 g_{IB}/g_C , respectively. The corresponding immobilized cell viabilities were 93, 86.3 and 75 %, thus the average cell viability in maturation column (ca. 85 %) can be considered satisfactory after 50 days of operation.

Flavour formation
The formation of diacetyl is linked through amino acid metabolism with biomass growth and thus wort fermentation. Since the majority of the wort attenuation occurs in the primary fermentation reactor (R1), the total diacetyl level in the green beer is relatively stable (0.35 to 0.29 mg/L) and independent on RT_{tot} (Figure 2). At short residence times (RT_{tot} = 22 – 35 h), the continuing wort attenuation in R2 and R3 resulted in increased diacetyl concentration in the outflow from the ICR (Figure 2). Consequently at short RT_{tot} the R3 does not complete its role of a maturation reactor. Conversely, at low wort flow rate through ICR the green beer entering into R3 is almost fully attenuated allowing thus the biomass immobilized on corncob particles to re-assimilate the diacetyl formed mainly in R1 (Figure 2).
The concentration of total diacetyl in the final continuous beers was higher than in the control beer (Table 2). Although the total diacetyl in the outflow from R3 decreased with increasing total residence time (RT_{tot}) in the immobilized cell system (Figure 2), at RT_{tot} = 52 h it still slightly exceeded the taste threshold (0.15 mg/L) for lager beers (9). We assume that in order to decrease the diacetyl concentration below its taste threshold, an increased residence time in the maturation column (RT_{R3}) would be recommended rather than a further increase of RT_{tot}.
Besides diacetyl there are other by-products of fermentation, namely higher alcohols and esters, having considerable sensorial effect on beer. The effect of operational conditions on the formation of these compounds during continuous beer fermentation is crucial and has already been intensely studied (7, 11, 14). Especially the oxygen supply in primary fermentation is critical for adequate beer flavour formation. The excess oxygen entering into the wort stream through silicon tubing led to low ester production, in the range from 5 to 11 mg/L of total esters, resulting in a higher than optimum fusel alcohol to esters (A/E) ratio (Table 2).

Consumer acceptance tests
In order to find out how the general public will receive beer from continuous immobilized fermentation a group of at least 30 untrained tasters participated on consumer tests. Untrained tasters carried out three acceptance tests, classifying in each of them three different lager beers on a 9 point scale (1 – the worst possible result conceivable, 9 – the best result imaginable). The results of the comparison of the unfiltered and non-pasteurized continuous beer from the immobilized fermentation system with various bottled commercial lager beer brands are shown in Table 1. The

highest scores among the compared lager beers were given to both Portuguese brands (PL 1 and PL 2). The consumer acceptance tests 1 and 2 revealed a preference in flavour of Portuguese lager beer. In the case of Portuguese beers there was a statistically significant difference when comparing to continuous beer 1 and a Spanish lager beer brand during CAT 1 and CAT 2 (Table 1). The average classification of the beer brands compared in CAT 3 given by untrained tasters did not show any statistically significant difference.

	CB1	CB2	PL1	PL2	SL	AL	CL
CAT 1	6.1^{SD}		7.0^{SE}	6.8^{SE}			
CAT 2	5.8^{SE}		6.8^{SD}		6.0^{SE}		
CAT 3		5.9^{SE}				6.4^{SE}	6.0^{SE}

Table 1: Average classification of continuous and commercial lager beers during consumer acceptance tests (CAT). CB1: Continuous Beer 1, aeration = 20 mL/min, RT_{tot} (200 mL/h) = 26 h; CB2: Continuous Beer 2, no aeration, RT_{tot} (180 mL/h) = 29 h; PL1: Portuguese lager 1; PL2: Portuguese lager 2; SL: Spanish lager; AL: American lager; CL: Czech lager; ^{SE} Statistically equal; ^{SD} Statistically different

When evaluating the quality criteria characterizing beer, one has to bear in mind that the expected beer flavour is influenced by the type of beer and circumstances depending on country and fashion. Moreover, beer is a drink the taste of which easily becomes a familiar one and this information stays registered in the memory. In other words, the beer which someone drinks regularly tastes best to him/her. This can explain the highest average classification of the local beer brands (Portuguese lager 1 and 2) during consumer tests. Taking this into account, the absence of statistically significant difference between continuous beers and the compared foreign beer brands (Table 1) means, that the local consumers considered the taste of the continuous beers fully acceptable, although slightly distinctive from the Portuguese-made products.

Analytical and descriptive sensorial tests

Some substances in beer can be measured specifically; however, there are taste and aroma attributes in beer which can not be determined analytically. Many of these (e.g. cleanness, full body, bitterness, tingle etc.) make the beer attractive to the consumers. The taste and aroma profile of unfiltered and non-pasteurized continuous beer produced in the immobilized fermentation system at different conditions (residence time, aeration) was compared with an unfiltered and non-pasteurized commercially produced Portuguese lager used as a control beer. Experienced tasters carried out three descriptive sensorial tests, classifying in each of them one continuous beer and the control beer. The intensities of several flavour features of the examined beer were estimated on a 9 point scale (0 = absent, 1 - 3 = low, 4 - 6 = moderate, 7 - 9 = strong). The results of the descriptive sensorial analysis complemented with results of physicochemical analysis can be seen in Table 2.

A higher fruity (estery) fragrance was observed in all tasted continuous beers comparing to control beer (Table 2). In the case of continuous beer 2 this parameter was even classified, in the opinion of the trained tasters' team, in the "strong" category. The intensity of alcoholic/solvent and sulphury aroma also surpassed in

some samples of continuous beers the commercial product but in all cases the samples were classified in the "moderate" category (Table 2).

Parameter	CB2	CB3	CB4	Control
Original extract (°P)	12	12	12.4	12.1 - 12.5
Alcohol (v/v %)	5.06	5.30	5.51	5.6
Real attenuation (%)	65.5	69	68.5	67 - 73
pH	4.45	4.23	4.33	4.0 - 4.4
Bitterness (EBU)	21	25	21	20 - 24
Total diacetyl (mg/L)	0.29	0.25	0.17	0.03
A/E	7	18	27	4 - 5
Average intensities of examined aroma/taste features:				
Fruity	7	6	6	5
Alcoholic/Solvent	6	5	6	5
Hoppy	3	3	3	3
Malty	3	2	2	3
Sulphury	4	5	5	4
Sweet	4	2	5	4
Bitter	7	8	6	5
Linger	7	6	7	4

Table 2: Analytical and descriptive sensorial tests of beer produced in the continuous immobilized cell reactor system compared with a control beer produced by traditional industrial batch fermentation. CB2: Continuous Beer 2, no aeration, RT_{tot} (180 mL/h) = 29 h; CB3: Continuous Beer 3, no aeration, RT_{tot} (120 mL/h) = 43 h; CB4: Continuous Beer 4, aeration = 20 mL/min, RT_{tot} (100 mL/h) = 52 h; Control: unfiltered and non-pasteurized Portuguese lager 1; A/E: higher alcohols to esters ratio.

As for the "sweet" taste intensity, the continuous beers were in average awarded points in the range from 2 to 5, while the control beer received an average note of 4. When assessing the sweetness one has to take into account the original extract and the degree of attenuation of the sample. Generally, deeply fermented beers lose their sweet character. In the case of continuous fermentation systems, this feature of the final product can be controlled through residence time and temperature in each fermentation stage.

Contrary to sweetness, the bitterness was considered by the taster panel significantly stronger in the continuous beers (Table 2). It can be hypothesised that the more intense bitterness of continuous beers is caused by lower adsorption of trubs to yeast biomass in the continuous system comparing to the traditional technology. However, it was not only the intensity of bitterness that was found different in continuous beers but also its increased lingering character (Table 2).

The final character of the beer results from an interplay of different taste features. For example, the bitterness is apparently influenced by other taste features such as sweetness. Thus, the sweeter was found the beer sample to be, the lower intensity of bitterness was attributed to it (Table 2). Therefore the preparation of wort for

continuous beer fermentation in immobilized cell reactor system has to be also adjusted with respect to the prevention of undesirable character of the final product.

CONCLUSIONS

In order to convince the brewing engineers and economists that continuous brewing can produce both quality and savings, the researchers should not lose sight of the applicability, simplicity and economic attractiveness of the suggested fermentation systems. Thus, the investment costs (e.g. carrier price) of the continuous beer fermentation should be kept as low as possible. The results above show that the continuous reactor system (ICR) containing brewer's yeast immobilized on cheap alternative carriers (spent grains, corncobs) was able to operate steadily for almost 2 months. During that period, the viability of both immobilized and free cells in the system remained high.

The finished product from the ICR was found by consumers to be quite acceptable from a flavour perspective, although a little distinct from the local lager beer brands produced by conventional batch processes. The descriptive sensorial analysis carried out by experienced tasters also found differences between the products of continuous and batch fermentation. Some of these differences though can be eliminated by an optimization of the process. For instance (i) the diacetyl content can be decreased by a prolonged maturation in an enlarged maturation column; (ii) the prevention of over-aeration ensures an optimum formation of volatile compounds; (iii) the palatefulness (body) of the final beer can be controlled through the residence time in the system determining the degree of wort attenuation; (iv) an ideal bitterness would require an adjusted hop addition. Generally, varying the process parameters provide an effective tool for adjusting the aroma and taste profile of the final beer. However, other "faults" of the continuous beer found by trained tasters arise from the necessity (in this particular work) of additional wort sterilization (leading to oxidized or burnt aftertaste).

The presented continuous fermentation system stands out by its significantly shorter fermentation times, cheap carrier materials reducing the investment costs and beer with generally acceptable and sufficiently balanced flavour.

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