Continuous beer fermentation with yeast immobilized on alternative cheap carriers and sensorial evaluation of the final product

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Abstract. Continuous beer fermentation offers a wide range of advantages, mostly of economic nature over the traditional batch process. However, due to increased complexity of operation comparing to batch process, flavor problems, risk of contamination, yeast viability, carrier price and inconvenience of immobilization, the continuous beer fermentation has found few practical applications so far. The carrier cost represents a significant part of the investment costs and therefore the need for a cheap support material easy to regenerate is still relevant. This work deals with a complete continuous fermentation system for beer fermentation and maturation consisting of an airlift and a packed-bed reactor containing brewing yeast immobilized on spent grains and corncobs, respectively. The objective of this study was to verify the long-term performance of the system and the suitability of these new cellulose-based carrier materials made from brewing and agricultural by-products. Further the influence of feed rate and aeration rate on bioreactors fermentation performance, immobilized biomass load, ethanol production and flavor profile of both green and maturated beer was followed. The influence of process parameters on sensorial quality of beer has been studied by physicochemical methods as well as by sensorial analysis (acceptance and description tests) carried out by both consumers and experienced tasters. This work clearly demonstrated the technological feasibility of the continuous brewing based on yeast immobilization on cheap alternative carriers (spent grains, corncobs) for continuous production of a beer with a balanced flavor profile.

Keywords: Continuous, Beer fermentation and Sensorial evaluation.

1. Introduction

Today the brewing industry applies a broad spectrum of novel engineering, biochemical, microbiological and genetic inventions. Thanks to these contemporary achievements this traditional industry became similar to those referred to as “modern biotechnologies” (Pilkinkgton et al., 1998). Nevertheless, some of the new possibilities, e.g. continuous beer fermentation with immobilized brewing yeast, have still not been intensely commercialized. 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 (Mensour et al., 1997).

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 (Tata et al., 1999).

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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 (Linko et al., 1998). 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 favor 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 and temperature) in terms of productivity and sensorial quality of the final product.

2. Methodology

2.1. 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.

2.2. 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. For more detailed description see Brányik et al. 2001.

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.

2.3. 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 BT (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.

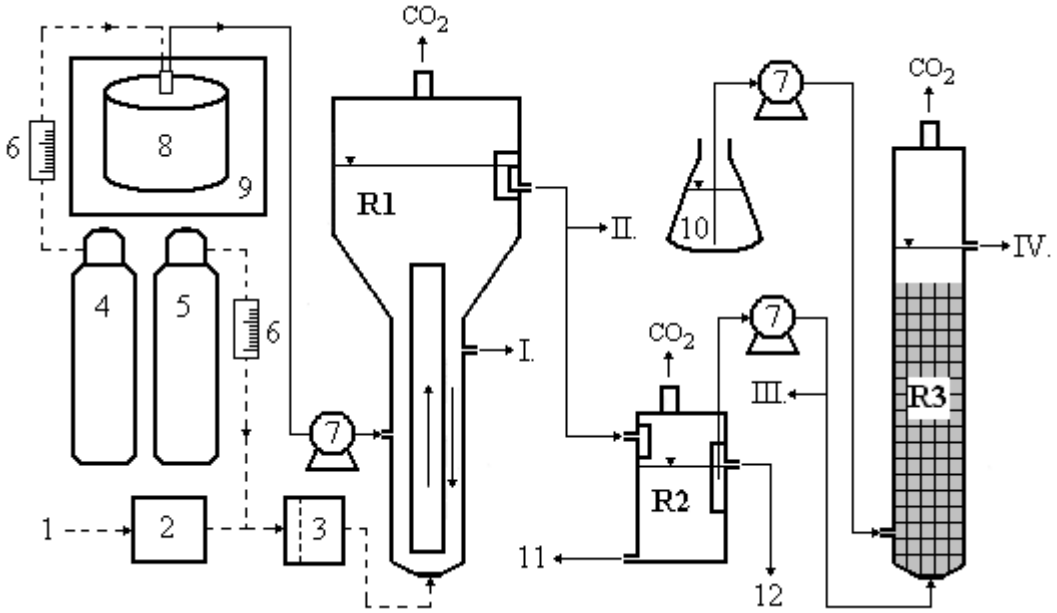

Fig. 1. Immobilized yeast reactor system for laboratory scale continuous beer fermentation: 1-air supply; 2-mass (gas) flow controller; 3-gas sterilization filter; 4-N_2 bottle; 5-CO_2 bottle; 6-rotameter; 7-peristaltic pump; 8-wort barrel; 9-refrigeration unit; 10-washing (physiological) solution; 11- 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 point; IV.-maturated beer sampling point.

2.4. 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 under N_2 atmosphere. 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}).

2.5. 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 (van Iersel et al. 1999). The flavour and aroma compounds (higher alcohols and esters) were measured according to the current European Brewery Convention recommended methods. The detailed procedure of the immobilized biomass (X_{im}) determination can be found in Brányik et al. 2004a. Cell viability was measured by counting dead cells stained with methylene blue (Analytica – Microbiologica – EBC 2001). 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 measurement.

2.6. 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 continuous beer for consumer tests were collected under N_2 atmosphere and both continuous beer and commercial beer brands were poured without foam into a dark colored glass (90 mL) and tasted at temperatures between 6 and 8°C. Results were analyzed by Analysis of Variance (ANOVA) and Tukey tests ($p \leq 0.05$), in order to quantify the variability in the average of the responses.

For descriptive evaluation tests, the samples of continuous beer were collected also 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. Panelists were asked to describe the flavor 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 flavor profile of each sample.

3. Results and Discussion

3.1. Primary fermentation

According to the results (Figure 2), it is in the primary reactor (R1) where the prevailing portion of the fermentation occurs. In average it corresponds to 83.5±8.5 % of the total apparent attenuation. Besides conversion of fermentable sugars into ethanol and CO_2 it is in this stage where the formation of by-products having considerable sensorial effect on beer takes place. From this point of view, the effect of operational conditions on continuous primary fermentation is crucial and has already been intensely studied (Brányik et al., 2004c; Norton and D'Amore, 1994). As it can be seen from 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}). The influence of aeration into R1 on the values of attenuation is less explicit, however, it will certainly affect the formation of flavor volatiles (higher alcohols and esters).

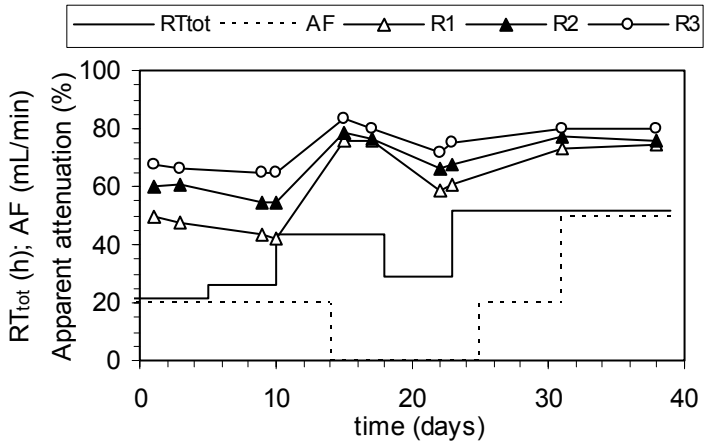

Fig. 2. The influence of different total residence times (RT_{tot}) and proportion of air flow (AF) in the constant total gas flow rate (air + CO_2 = 0.4 L/min) into R1 on the apparent attenuation of wort (12°P) in each stage of the immobilized cell reactor system (ICR): R1 – main fermentation reactor,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

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 significantly higher value (ca. 17 %) at the end of the experiment (Figure 4). Although the viability of immobilized brewing yeast in continuous beer fermentation has already been reported to decrease (Pilkington et al., 1999), 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/renewal.

3.2. Sedimentation tank

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 (RT_{R2}) was short (3 - 7 h) when compared to the total residence time (RT_{tot}). The percentage of dead cells both in the outflow from R2 did not exceed 8 % (Figure 4). 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 % (Figure 3). 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 in R2.

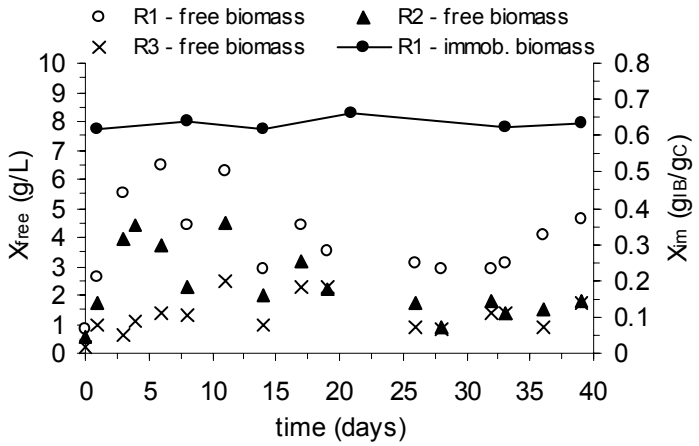

Fig. 3. Free biomass concentration in the outflow from each reactor of the continuous fermentation system (R1, R2 and R3) and immobilized biomass concentration inside the main fermentation reactor (R1).

3.3. Maturation

The apparent degree of attenuation in the outflow of the maturation column varied between 65 and 83 % depending on the total residence time of wort in the reactor system and on the intensity of aeration in the primary fermentation reactor (Figure 2). At RT_{tot} above 43 hours the apparent attenuation of the final beer was high, almost independent on the airflow into R1 and close to the attenuation limit of the wort.

The free biomass concentration in the inflow to the maturation column (R3) was relatively high, 2 to 4 g/L in average (Figure 3), due to the unsatisfactory sedimentation in R2. Therefore, 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.

The percentage of dead cells in the outflow from R3 was between 2 and 8 % slightly exceeding this range (12 %) at the beginning of the beer fermentation when synthetic medium was switched to wort (Figure 4). 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.

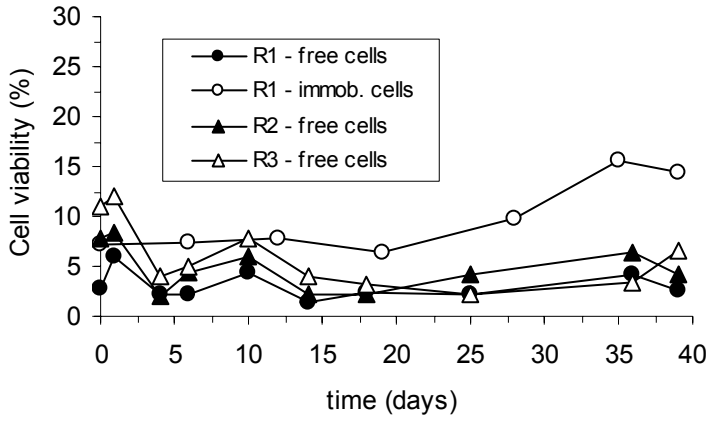

Fig. 4. Percentage of dead cells among free cells in the outflow from each reactor of the continuous fermentation system (R1, R2 and R3) and among immobilized cells inside the main fermentation reactor (R1).

3.4. 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 (lager 1 and 2). The consumer tests No. 1 and 2 revealed a preference in favor 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 test 1 and 2 (Table 1). The average classification of the other compared beer brands given by untrained tasters did not show any statistically significant difference.

Table 1. Average classification of continuous and commercial lager beers during consumer acceptance tests.

	Continuous beer 1[a]	Continuous beer 2[b]	Portuguese lager 1	Portuguese lager 2	Spanish lager	American lager	Czech lager
Comsumer test 1	6.1^{SE}		7.0^{SD}	6.8^{SD}			
Comsumer test 2	5.8^{SE}		6.8^{SD}		6.0^{SE}		
Comsumer test 3		5.9^{SE}				6.4^{SE}	6.0^{SE}

[a]Continuous Beer 1: Aeration - 20 mL/min, RT_{tot} (200 mL/h) – 26 h

[b]Continuous Beer 2: No aeration, RT_{tot} (180 mL/h) – 29 h

[SE]Statistically equal; [SD]Statistically different

When evaluating the quality criteria characterizing beer, one has to bear in mind that the expected beer flavor 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.

3.5. 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 flavor 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.

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.

Parameter	Conti. beer 2[a]	Conti. beer 3[b]	Conti. beer 4[c]	Control beer[d]
Original extract (w/w %)	12	12	12.4	12.1-12.5
Alcohol (v/v %)	5.06	5.30	5.51	5.6
Real attenuation (w/w %)	65.58	69.08	68.55	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
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

[a]Continuous Beer 2: No aeration, RT_{tot} (180 mL/h) – 29 h

[b]Continuous Beer 3: No aeration, RT_{tot} (120 mL/h) – 43 h

[c]Continuous Beer 4: Aeration - 20 mL/min, RT_{tot} (100 mL/h) – 52 h

[d]Control beer: unfiltered and non-pasteurized Portuguese lager 1

Apart from the total diacetyl concentration in final beer, the analytical parameters of continuously produced beers (Continuous beer 2, 2 and 3) were similar to those of the beer produced by traditional batch technology (Control beer). The concentration of total diacetyl in the final continuous beers was significantly higher than in

the control beer. Although it decreased with increasing total residence time (RT_{tot}) in the immobilized cell system (Table 2), at RT_{tot} = 52 h it still slightly exceeded the taste threshold (0.15 mg/l) in the final lager beers (Meilgaard 1975). 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}.

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).

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 (palatefulness, body) 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). Since the wort applied in this work was prepared for traditional fermentation, the more intense bitterness of continuous beers can be ascribed to lower adsorption of trubs (coagulates of proteins, hop resins, polyphenols etc.) to yeast biomass in the continuous system comparing to the traditional technology. Apart from the fine cold trub present in wort, further coagulates were probably formed during the additional wort sterilization carried out prior to utilization in the continuous fermentation experiments. However, the presence of these components influences not only the intensity of bitterness, but also its increased lingering character (Table 2). Moreover, the bitterness is apparently influenced by other taste features such as sweetness. For example, the sweeter was found the beer sample to be, the lower intensity of bitterness was attributed to it (Table 2). Therefore a preliminary conclusion can be drawn, namely that the preparation of wort for the immobilized cell reactor system requires fewer hops. Such hypothesis should be considered and studied carefully with respect to both the prevention of undesirable character of the final bitterness and its interplay with other taste features.

4. 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 flavor 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 can be ascribed to the continuous character and

insufficient optimization of the process (high bitterness, unbalanced flavor volatiles). 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). On the other hand, varying the process parameters provides an effective tool for adjusting the aroma (aeration) and taste (residence time) profile of the final beer.

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 flavor.

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