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Continuous Primary Beer Fermentation with Yeast Immobilized in Alginate–Chitosan Microcapsules with a Liquid Core

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Abstract: The application of continuous fermentation with immobilized cells in brewing is a challenge because of problems with carrier selection and reactor design, which have economic impacts on the beer produced. Moreover, immobilization alters yeast physiology, which significantly affects beer flavor and aroma. Therefore, the aim of this study was to investigate the feasibility of a continuous fermentation system, consisting of a packed bed column bioreactor, containing lager brewing yeast, immobilized in alginate–chitosan microcapsules with a liquid core, in the primary beer fermentation. The results showed that the system entered in a stationary mode on the 3rd day and worked stably in this mode for 6 days. The "green" beer was taken at every 24 h at the output of the reactor and used for secondary fermentation with the yeast cells leaked from the capsules during the primary fermentation. The extract consumption, ethanol production, and pH change during primary and secondary fermentation were investigated. Some of the secondary yeast metabolites such as vicinal diketones, higher alcohols, esters, and aldehydes in "green" and final beers were determined and it was found that the flavor profile of the final beer was comparable to two industrially produced Bulgarian beers.

Keywords: continuous fermentation; packed bed bioreactor; alginate–chitosan capsules; secondary metabolites

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1. Introduction

In recent decades, the need for innovation has encouraged the brewing industry to search for different strategies useful to achieve cost-effective products or products with a new taste and aroma. One of these approaches is immobilization, which aims to confine intact cells to a certain region of space whilst preserving their biological activity [1]. The immobilized system can be divided into four main categories based on the physical mechanism of cell localization and the nature of the support mechanisms: "attachment to a surface", "entrapment within a porous matrix", "containment behind a barrier", and "self-aggregation" [2]. Cell immobilization behind or within a porous barrier includes systems with a barrier formed around cells such as microcapsules, and systems with cells contained within a compartment separated by a preformed membrane such as hollow fiber and flat membrane modules [2,3]. The microencapsulation is achieved by the inclusion of cells within a porous material (mainly polymers such as alginate, etc.), thus limiting cell diffusion and simultaneously enabling the transfer of nutrients and metabolites through the matrix pores. Sometimes, in order to enhance the stability and to reduce the porosity of alginate microcapsules, they are covered with a coating agent such as chitosan [4]. Alginate and chitosan are used as carriers for cell encapsulation because of their good biocompatibility, low cost, easy availability, and ease of preparation [5]. Alginate–chitosan microcapsules have been used for the entrapment of bacterial and yeast cells in batch and continuous lactic acid fermentation [6], alcoholic fermentations [7], batch and continuous

beer fermentation [4,8,9], sparkling wine fermentation by the traditional method [10], and in the reduction in the volatile acidity of acidic wines [11] at a laboratory scale. However, the biggest problem for the industrial implementation of alginate–chitosan microcapsules is the change in yeast metabolism due to immobilization itself. It was found that the structure as well as the size of the produced capsules affects the metabolic behavior of encapsulated cells, and subsequently the volatile and sensory profile of the obtained beers [4].

Continuous beer fermentation is another strategy for breweries to produce cost-effective products because of great time savings. The main economic advantages of continuous immobilized cell fermentation are the possibility to use very short fermentation times and to minimize the downtimes (filling, cleaning, standby) [12]. Despite the great benefits of using continuous beer fermentation with immobilized cells, it has not yet been widely adopted in beer fermentation and maturation, mainly because of the complexity of the operations compared with batch processes, the lack of controllability of the changed metabolism and flavor, and the carrier price [13]. However, cheap carrier materials such as alginate and chitosan applied in suitable reactor configurations could inspire researchers and encourage brewing engineers to consider the industrial application of this process [14].

The aim of this study was to investigate the possibility for the application of a packed bed column bioreactor containing lager yeasts, immobilized in alginate–chitosan microcapsules with a liquid core, in a continuous primary beer fermentation process. The "green" beer produced was taken at every 24 h at the output of the reactor and used for batch secondary fermentation with yeast cells leaked from the microcapsules. Special attention was paid to the yeast metabolite synthesis during continuous primary fermentation and their change during secondary fermentation. Another aspect of the investigation was the comparison between the flavor profile of the laboratory-produced beer and two commercial beers.

2. Materials and Methods

2.1. Microorganisms

Fermentation was carried out with *Saccharomyces pastorianus* Saflager S-23 (Fermentis, Lille, France). The yeast was rehydrated before use according to the manufacturer's instruction.

2.2. Wort

Industrially produced wort (Kamenitza, Bulgaria) with an initial extract of 17.5 $\pm$ 0.5 °P was diluted with sterile distilled water to 9.7 $\pm$ 0.5 °P. The amount of water used was calculated on the basis of the initial wort extract, the desired wort extract, and the initial wort volume. The diluted wort with a volume of 20 L was autoclaved at 121 °C for 20 min and filtered with sterile cotton before using it for experiments.

2.3. Reagents

A commercial sodium alginate Algogel® 6021 (Cargill, Paris, France) and chitosan (Acros Organics, Brussels, Belgium) were used for yeast encapsulation. Vicinal diketones were determined by using o-phenilenediamine and 2,3-butanedione (Sigma-Aldrich, St. Louise, MO, USA). Ethyl acetate (purity $\geq$ 99.9%), acetaldehyde (purity $\geq$ 99.9%), 2-Methyl-1-butanol (purity $\geq$ 98%), 3-methyl-1-butanol (purity $\geq$ 98.5%), 2-methyl-1-propanol (purity $\geq$ 99.8%), 1-propanol (purity $\geq$ 99.9%), and 2-butanol (purity $\geq$ 99.8%) were purchased from Merck, Darmstadt, Germany (Buchs, Switzerland). All the other reagents were from different Bulgarian producers. All the chemical reagents were of analytical grade and were used without further purification.

2.4. Immobilization Procedure

The mixture of rehydrated yeast and 3% (*w/v*) sodium alginate solution was dropped into a 2% (*w*/*v*) $CaCl_2$ solution by means of a peristaltic pump. The cell concentration in

the beads was 10^7 cells/mL of gel. After a stay of 30 min in $CaCl_2$ solution with constant stirring, the beads were washed with sterile water and then were placed into a 0.38% (*w*/*v*) chitosan in 1% (*v*/*v*) acetic acid solution. The alginate beads stayed in the chitosan solution for 60 min. After that, the capsules produced were washed with sterile water in order to remove the excess of chitosan. The alginate–chitosan beads were left in 0.05 M sodium citrate solution for 30 min for dissolving the alginate and obtaining microcapsules with a liquid core [8,15].

2.5. Immobilized Cells Bioreactor

The immobilized cell bioreactor (Figure 1) used for the experiments was previously described in Vassilev et al. [9]. The alginate–chitosan microcapsules were packed into a water-jacketed glass column (6) with a diameter of 30 mm, a total height of 1000 mm, and a working volume of 565.2 mL. A grid with a thickness of 1 mm (10) was placed inside the column to allow the easier leaving of CO_2 formed during the fermentation. The immobilized cells were placed on a drainage bed (5) set at the bottom of the column. The column temperature was maintained by circulating water with a constant temperature via the outer water-jacket and the water bath (4). The wort from the storage tank (1) was continuously fed into the packed bed reactor from bottom to top using a peristaltic pump (2). The "green" beer from the outlet of the packed bed column bioreactor (9) was periodically taken out to monitor the fermentation parameters. The liquid level in the column was maintained by means of an overflow (7), and the pressure in the column could be controlled via a connection (8). The column was operated at a slight overpressure determined by the partial pressure of CO_2. When necessary, the pressure in the column was lowered using a small valve, thereby regulating the rate of CO_2 removal from the capsules. It could also be regulated through the clamping force of the plug in the top of the column.

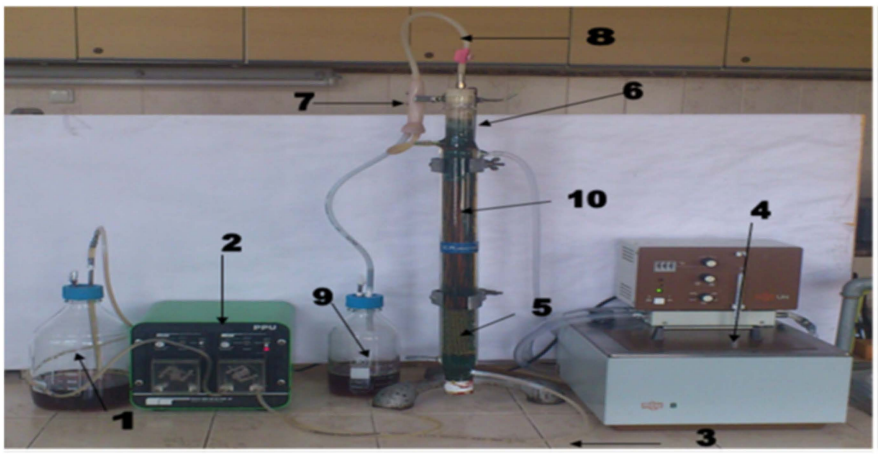

Figure 1. Immobilized cell system for continuous main fermentation: 1—wort tank; 2—peristaltic pump; 3—pipes; 4—water bath; 5—packed bed; 6—column; 7—overflow; 8—pressure control connection; 9—"green" beer tank; 10—grid [9].

2.6. The Start and Working of the Immobilized Cells Bioreactor

The system was "sterilized" in the cold with a 1% solution of neomycin (antibiotic) for 24 h, and then washed with sterile distilled water before use. The drainage bed (5) and the grid (10) were sterilized in a boiling water bath and they were put on the column bottom. Forty-eight grams of immobilized cells were pre-innoculated in 400 mL of sterile wort for 3–4 h to enhance the fermentation start and to reduce the transitional mode time. The mixture of fermenting wort and immobilized cells was transferred aseptically into the bioreactor to form a layer of approximately 20 cm, which corresponded to 1/3 of the column working height. The dilution rate of 0.163 h^{-1} was controlled by means of a peristaltic pump (2) in order to keep the constant volume of 400 mL of fermenting wort in the column. The continuous fermentation was carried out at a constant temperature of

16 ± 0.5 °C. The system worked continuously for 10 days. "Green" beer samples were taken every 24 h at the output of the system and part of them were used for analysis [9]. The other part was used for secondary fermentation at a temperature of 20 ± 0.5 °C. Secondary fermentation was carried out as a batch process with the available yeast in the "green" beer, which was leaked from the capsules. Secondary fermentation finished when the vicinal diketone content in the beer was below 0.2 mg/L.

2.7. Analytical Methods

The analyses of basic wort and beer parameters (extract, alcohol, pH, and vicinal diketones) were conducted according to the EBC methods of analysis [16]. Wort and beer extracts were measured by an Anton Paar DMA 35 density meter (Anton Paar, Graz, Austria). Alcohol content was also measured by the same density meter after simple distillation of the beer. The pH was determined by Sartorius PB–11 pH meter (Sartorius, Gottingen, Germany). Vicinal diketones were measured at 335 nm in a 1 cm quartz cuvette on a Shimadzu UV–VIS1800 spectrophotometer (Shimadzu, Kyoto, Japan) after steam distillation of the beers.

Esters, aldehydes, and higher alcohols were analyzed with a Perkin-Elmer Autosystem XL Gas Chromatograph (Perkin-Elmer, Waltham, MA, USA) equipped with a flame ionization detection (FID) system, a split–splitless injector, 2 columns of "HP-35" (30 m × 0.25 mm; ft 0.25 μm) and "DB-1701" (30 m × 0.25mm; ft 1.5 μm), and the Totalchrome program. The oven temperature was programmed from 50 °C to 130 °C at 7 °C/min, from 130 °C to 180 °C at 30 °C/min, and retained at 180 °C until the column was cleared. Injector and detector temperatures were maintained at 200 °C. Samples were injected in the split mode (1/70) using helium as a carrier gas (1 mL/min). The quantitative analysis was performed by the external calibration method with a series of standard solutions of esters, aldehydes, and higher alcohols.

2.8. Statistical Analysis

The presented data for the main parameters of the "green" beer at the exit of the column were the average of three consecutive fermentation processes under the conditions described in Section 2.6. The standard deviation (SD) was calculated from the pooled results of three parallel studies to assess the variability within the dataset.

3. Results and Discussion

In our previous research, the optimal fermentation conditions for batch beer fermentation with yeast immobilized in alginate–chitosan microcapsules with a liquid core were determined to be as follows: wort original extract—9.72 ± 0.5 °P; primary fermentation temperature—16 ± 0.5 °C; secondary fermentation temperature—20 ± 0.5 °C [17]. However, when the batch process was transferred to a continuous mode the optimal dilution rate and immobilized cells mass were also selected—0.163 h^{-1} and 48 g, respectively [9]. The dilution rate corresponded to a primary fermentation time of 6 h.

3.1. Basic Beer Parameter Changes during Fermentation

3.1.1. Changes in the Basic Beer Parameters during Primary Fermentation

The results for the extract consumption, the alcohol production, and the pH drop during continuous primary beer fermentation are presented on Figure 2.

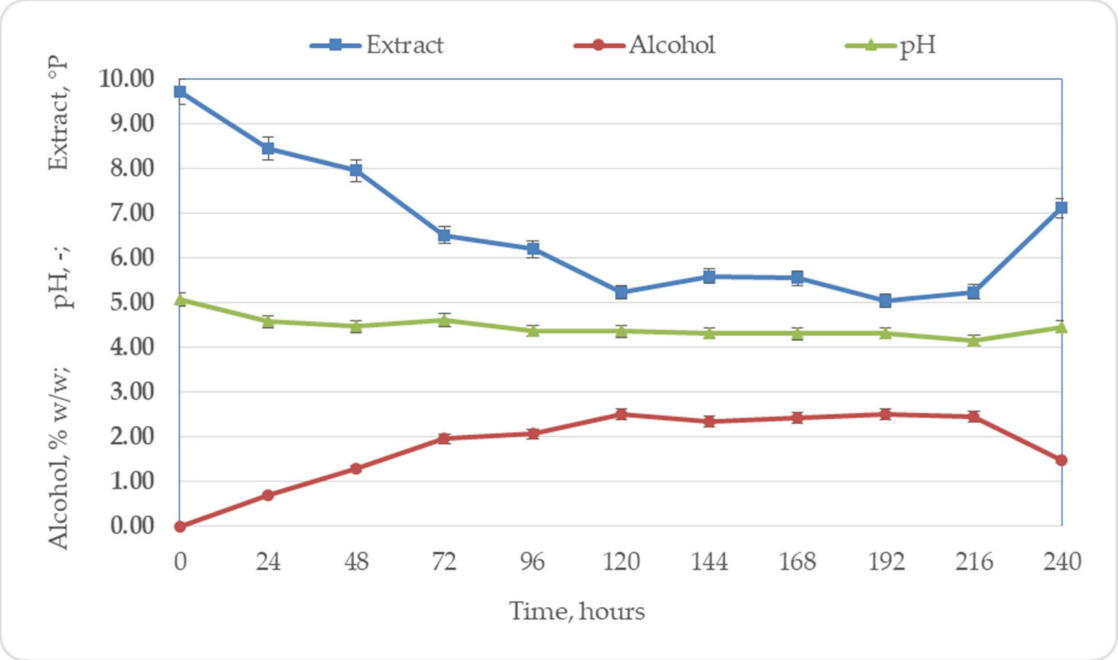

Figure 2. Changes in extract, alcohol, and pH during continuous primary fermentation.

The results for extract consumption and alcohol production show that the system entered the stationary phase after 72 h from the fermentation start. The results were in correspondence with the results presented by Vassilev et al. [9], who investigated continuous primary fermentation at three dilution rates—0.0825 h^{-1}, 0.163 h^{-1}, and 0.25 h^{-1}, and found that the transitional mode of the system continued between 60 and 72 h. The continuous fermentation system was stable for 9 days because the results for extract consumption and alcohol production at the 10th day showed that the extract consumption and alcohol production were 19% and 40%, respectively, lower than the previous day. In brewing terms, attenuation describes the level of wort extract that is converted into ethanol during fermentation and it is often desired to be a level of 70–80% in the final beer as the leftover sugars contribute a desirable sweetness and body composition to the final beer products [18]. The apparent attenuation degree of the "green" beer varied between 42% and 60% after the system entered the stationary mode. The average attenuation rate during the stationary period was approximately 50%, which allowed the remaining fermentable extract to be consumed by yeast during secondary fermentation. Alcohol concentration in "green" beer in a stationary mode varied between 1.95 and 2.51% (*w*/*w*). The pH drop during primary fermentation was approximately 1, which was mainly due to the excretion of organic acids, as well as the absorption of basic amino acids by the yeast [4].

3.1.2. Basic Beer Parameter Change during Secondary Fermentation

During the secondary beer fermentation, several objectives should be realized, such as the fermentation of the remaining extract; beer saturation with carbon dioxide; the removal of unwanted aroma compounds; and the excretion of flavor-active compounds from yeast to give body and depth to the beer [19]. The results for the major beer parameters at the end of the secondary fermentation are shown on Figure 3.

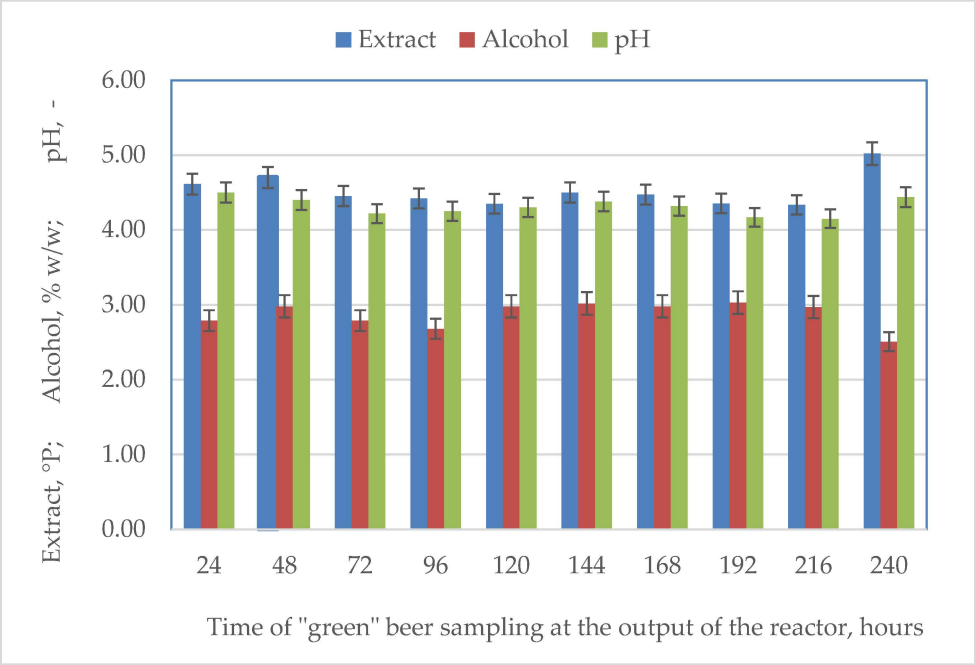

Figure 3. Basic beer parameters in the final beers, produced by continuous primary fermentation with immobilized cells and batch secondary fermentation with free cells.

In order to produce beer with similar quality, the secondary fermentation duration was between 144 h (samples were taken at the 192nd, the 216th, and the 240th hour) and 196 h (the sample was taken at the 24th hour). In all the final beers, the real extract was between 4.34 °P and 5.02 °P. However, the highest extract was measured in the sample taken at the 240th hour (10th day) of primary fermentation and we already mentioned that the system was stable for only 9 days. The average attenuation degree was approximately 69%, which showed that 19% of the fermentable extract was consumed during secondary fermentation. The attenuation degree was lower than the same degree given by the producer of the yeast used but it also depended on the wort composition [19]. Alcohol contents in all the final beers, except the last one, were between 2.7% (*w*/*w*) and 3.0% (*w*/*w*). The pH did not change significantly during secondary fermentation because pH decreased significantly during the initial and logarithmic growth phase of yeast, which were observed during the primary beer fermentation [20].

3.2. Secondary Yeast Metabolite Changes during Primary and Secondary Fermentation

A great number of yeast by-products of the alcoholic fermentation deeply contribute to the final taste/aroma of beer [21]. The combination between continuous beer fermentation and immobilization significantly affects the physiology of yeast cells and thus alters yeast metabolism [12]. Therefore, the knowledge of the synthesis and/or reduction of different groups of metabolites is essential for the selection of the fermentation regime. The results for the content of vicinal diketones, esters, aldehydes, and higher alcohols in the "green" and final beer are presented in Table 1.

The vicinal diketones—diacetyl and 2–3 pentanedione—are undesirable for beer quality. During fermentation, yeast cells excrete an intermediate of valine biosynthesis, α-acetolactate, that is spontaneously decarboxylated to diacetyl. Both diacetyl and 2,3-pentanedione have a strong aroma of toffee and butterscotch with very low flavor thresholds of 0.15 ppm and 0.9 ppm, respectively [22]. The vicinal diketone content was maximal during the first 24 h; afterwards its quantity decreased and when the system entered the stationary mode its amount in "green" beer was between 0.45 mg/L and 0.87 mg/L (Table 1). During the secondary fermentation, the vicinal diketones in the samples, taken during the transitional mode of the system, could not be reduced below

the flavor threshold. It could be explained by the relatively few yeast cells leaked from the capsules in the "green" beer to carry out the reduction of vicinal diketones. When the system entered the stationary mode, the capsules were already completely colonized and the vicinal diketones reduction started during the primary fermentation. It resulted in a decrease in the secondary fermentation duration from 196 to 144 h. Vicinal diketones reduction was also favored by high primary and secondary fermentation temperatures. The vicinal diketones concentration in all the finished beer produced after the primary fermentation in a stationary mode was below 0.14 mg/L. In the scientific literature there were data for the content of diacetyl and 2,3-pentanedione, which were determined by the means of gas chromatography. According to EBC standards [16], the spectrophotometric method for vicinal diketones is not directly comparable to the results obtained by gas chromatography because pentanedione is calculated as diacetyl and not all the amount of pentanedione was distilled during steam distillation. Therefore, it was not adequate to make a comparison between our results and the ones presented in the scientific literature.

Table 1. Secondary metabolite concentration in "green" and final beer, produced by continuous primary fermentation with immobilized cells and batch fermentation with free cells.

Secondary Metabolites, mg/L		Sample, Taken at the Reactor Output after Hours									
		24	48	72	96	120	144	168	192	216	240
Vicinal diketones	In "green" beer	2.6 ± 0.3	1.1 ± 0.2	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.8 ± 0.1	0.6 ± 0.1
	In final beer	0.5 ± 0.1	0.5 ± 0.1	0.17 ± 0.06	0.12 ± 0.04	0.14 ± 0.06	0.05 ± 0.02	0.10 ± 0.03	0.02 ± 0.01	0.04 ± 0.01	0.12 ± 0.06
Acetaldehyde	In "green" beer	11 ± 1	12 ± 1	13 ± 2	16 ± 2	15 ± 2	11 ± 1	10 ± 2	11 ± 1	8.1 ± 0.6	7.9 ± 0.6
	In final beer	6.8 ± 0.8	5.9 ± 0.8	4.1 ± 0.4	4.8 ± 0.4	2.3 ± 0.2	5.3 ± 0.6	4.9 ± 0.6	4.3 ± 0.6	2.1 ± 0.3	6.0 ± 0.4
Ethyl acetate	In "green" beer	11 ± 1	3.1 ± 0.8	4.2 ± 0.8	5.2 ± 0.6	9.6 ± 0.7	3.4 ± 0.4	3.6 ± 0.4	3.3 ± 0.4	6.2 ± 0.4	9.1 ± 0.8
	In final beer	10 ± 1	13 ± 2	8 ± 1	13 ± 2	26 ± 2	14 ± 2	13 ± 2	11 ± 2	14 ± 2	11 ± 2
1-propanol	In "green" beer	2.3 ± 0.6	2.2 ± 0.6	2.4 ± 0.4	2.8 ± 0.4	4.5 ± 0.5	n.d. *	1.1 ± 0.2	n.d.	n.d.	3.4 ± 0.3
	In final beer	14 ± 2	13 ± 1	4.8 ± 0.7	4.2 ± 0.8	3.8 ± 0.6	12 ± 1	13 ± 1	10 ± 1	9.1 ± 0.8	10 ± 2
Isobutanol	In "green" beer	4.6 ± 0.5	3.9 ± 0.4	5.0 ± 0.5	6.6 ± 0.7	6.3 ± 0.7	1.6 ± 0.7	5.5 ± 0.3	2.0 ± 0.2	n.d.	4.5 ± 0.3
	In final beer	16 ± 1	13.3 ± 0.9	5.7 ± 0.6	6.5 ± 0.4	n.d.	9.5 ± 0.8	6.6 ± 0.7	7.7 ± 0.8	10.4 ± 0.8	11 ± 1
Isoamyl alcohol	In "green" beer	25 ± 1	27 ± 1	41 ± 1	40 ± 2	65 ± 2	33 ± 1	37 ± 1	29 ± 2	32 ± 2	35 ± 1
	In final beer	73 ± 2	78 ± 3	46 ± 2	55 ± 2	65 ± 2	66 ± 2	65 ± 2	66 ± 2	78 ± 2	61 ± 2
Amyl alcohol	In "green" beer	3.3 ± 0.1	7 ± 2	13 ± 2	15 ± 1	38 ± 2	14 ± 1	9.4 ± 0.8	11 ± 1	12 ± 1	10.1 ± 0.6
	In final beer	17.2 ± 0.1	20.9 ± 0.6	13.9 ± 0.4	15.7 ± 0.4	17.1 ± 0.2	15.9 ± 0.3	16.2 ± 0.3	17.9 ± 0.2	18.9 ± 0.2	15.0 ± 0.2

* n.d.—not detected.

Acetaldehyde is produced by yeast via the decarboxylation of pyruvate during the active yeast growth and it is a precursor for ethanol formation. Depending on its concentration in beer, acetaldehyde's aroma can range from a pleasant fruity aroma to an unwanted green apple-like or grassy aroma [23,24]. Acetaldehyde concentrations in the "green" beers were higher than those in the final beers and varied between 7.9 mg/L and 16.1 mg/L. The results for the acetaldehyde concentration were comparable to the results reviewed by Branyik et al. [25]. In this paper, the acetaldehyde concentration in "green" beer produced by continuous main fermentation varied between 8.0 mg/L (kappa-carrageenan as carrier; air-lift bioreactor) and 17.2 mg/L (aspen chip as a carrier; fixed bed bioreactor). In all the final beers (Table 1), the acetaldehyde concentration was below its threshold of 10 mg/L.

Esters are formed during fermentation by the enzymatic condensation of alcohol and organic acid. Esters are formed during the active growth phase and the stationary phase. The most abundant ester in beer is ethyl acetate, which is formed after a condensation of ethanol and acetic acid [23,26]. Ethyl acetate has a flavor threshold of 25–30 mg/l and its aroma is described as solvent-like or fruity [27]. In all the studied variants, the ethyl acetate concentration in the final beers was higher than that in the "green" beer except for the sample taken at the 72nd hour. The results obtained for "green" beer were lower than the results cited by Djordjevic et al. [28], which varied between 11 mg/L (Ca-alginate beads; packed bed bioreactor) and 33.3 mg/L (DEAE-cellulose beads; fixed bed bioreactor). However, the ethyl acetate concentrations in the final beer were higher than those of the results reported by Ogawa et al. [13] for batch beer fermentation with alginate beads. The observed difference can be ascribed to the differences in wort composition, yeast strain used, and the fermentation regimes.

The major aliphatic higher alcohols found in beer are propanol, isobutanol (2-methyl-1-propanol), isoamyl alcohol (3-methyl-1-butanol), and amyl alcohol (2-methyl-1-butanol). Aliphatic higher alcohols contribute to the alcoholic or solvent-like aroma of beer and produce a warm feeling in the mouth. Higher alcohols are formed by yeast during fermentation via the catabolic (Ehrlich) and the anabolic (amino acid metabolism) pathways and can be used as precursors for ester synthesis [27]. "Green" beer contained a significantly lower amount of 1-propanol and isobutanol compared to the results presented in Willaert and Nedovic et al. [26]. In their review, the concentration of 1-propanol varied between 6.4 mg/L (calcium pectate beads; air-lift bioreactor) and 32.5 mg/L (kappa-carrageenan; gas-lift bioreactor) and the concentration of isobutanol was between 7.5 mg/L (alginate beads; packed bed bioreactor) and 32.3 mg/L (DEAE cellulose; packed bed bioreactor). The concentrations of amyl and isoamyl alcohols were comparable to the data cited by Djordjevic et al. [28], where the amyl alcohol concentration was in the range of 0 mg/L (ceramic hydroxylapatite; batch fermentation) to 34.5 mg/L (ceramic chamotte; batch fermentation) and isoamyl alcohol content varied between 31.0 mg/L (beech wood chips; packed bed bioreactor) and 60.4 mg/L (silicon carbide rods; monolith reactor). During the secondary fermentation, the concentration of all the higher alcohols increased.

3.3. A Comparison of the Volatile Profiles of the Beer Produced by Continuous Fermentation and Two Commercial Beers

It is important for consumer acceptance that the flavor profile of the final beer produced with immobilized cells is comparable to the conventional beer offered in the market. Therefore, such a comparison between the laboratory-scale-produced beer and two beers industrially produced by Kamenitza PLC (the company that supplied us with wort for our experiments) was made. The results of the comparison are presented in Table 2. The data for the volatile compounds in the laboratory-produced beer were average for all the samples, taken after the system entered the stationary mode. This was due to the fact that the vicinal diketones could not be reduced below their flavor threshold, when samples were taken when the continuous primary fermentation system was working in the transitional mode. The vicinal diketones concentration in beer with immobilized cells was below the flavor threshold and was comparable to the commercial beer content. The acetaldehyde content of the laboratory-produced beer was slightly higher than that of the commercial beer, but overall the results were comparable. Similar trends were reported by Branyik et al. [25]. The content of ethyl acetate in the laboratory beer was higher than that of the commercial network. The data described in the literature generally indicated a reduced amount of esters in beer with immobilized cells, but in this case the peculiarity of the yeast strain used, namely its ability to produce more esters, must be taken into account. Regarding the higher alcohols, an increase in the concentration of 1-propanol and a decrease in those of 1-butanol and 3-methylbutanol were observed, but the high threshold concentrations of the higher alcohols made these differences insignificant. Similar trends were found by Branyik et al. [25].

Table 2. Comparison between laboratory-produced beer with immobilized cells and two commercial beers.

Metabolites, mg/L	Laboratory Beer	Commercial Beer 1	Commercial Beer 2
Vicinal diketones	0.09 ± 0.01	0.09 ± 0.02	0.02 ± 0.01
Acetaldehyde	4.2 ± 0.1	3.7 ± 0.1	3.0 ± 0.1
Ethyl acetate	13.7 ± 0.1	5.0 ± 0.2	4.5 ± 0.2
1-propanol	8.4 ± 0.2	4.5 ± 0.1	6.8 ± 0.1
Isobutanol	8.2 ± 0.5	9.9 ± 0.5	13 ± 1
Isoamyl alcohol	63 ± 2	68 ± 2	83 ± 2
Amyl alcohol	16.3 ± 0.7	14.7 ± 0.7	17.8 ± 0.6

4. Conclusions

A technological regime for beer production by continuous primary fermentation with yeast immobilized in alginate–chitosan microcapsules with a liquid core and batch secondary fermentation with free cells was developed. The main fermentation lasted 6 h and the secondary fermentation duration was between 144 and 192 h. The data for some of the secondary metabolites produced by the yeast during the continuous primary fermentation differed significantly from the results reported in the scientific literature, but for other secondary metabolites they were comparable. The differences can be ascribed to the yeast strain, the carrier and the bioreactor used, the fermentation regime, and the wort composition. However, the beer produced at a laboratory scale had a flavor profile comparable to the flavor profile of two commercial beers produced by one of the big Bulgarian breweries.

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