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Gluten pellets to immobilise yeast for brewery fermentations

Beers produced in two-stage continuous immobilised systems were compared to classical control beer. Primary wort fermentation was conducted in a gas-lift reactor using calcium-alginate (residence time 12 h), secondary fermentation in two parallel packed-bed systems using calcium alginate (residence time 57 h) or gluten (residence time 61 h) for yeast immobilisation. Diacetyl, acetaldehyde, total higher alcohols and total esters concentrations were lower when using immobilised systems. Concentrations of total nitrogen, free amino nitrogen and polyphenols were lower in beer produced by gluten-immobilised yeast compared to alginate.

BC 2 Malting and brewing/ 23 fermentation

(Descriptors: beer, yeast, immobilisation, continuous fermentation, gluten, calcium alginate.

Descriptoren: Bier, Hefe, Immobilisierung, kontinuierliche Gärung, Gluten, Calciumalginat).

1 Introduction

Beer production process is an energy consuming one, mainly when it comes down to the primary and secondary fermentation processes, because of the beer cool-down necessity. Therefore, it is important to shorten the fermentation time. New technological ways are looked for. However, it is a difficult task to achieve, thus the final product must have the same analytical and sensorical properties as classical beer. The classical technology was replaced by a semi-continuous process, or the one-phase fermentation in the cylindro-conical tanks. The current efforts are to speed-up the wort fermentation process as much as possible through its full continualisation. The conditions and needs of beer production theory and technology were the driving force behind the initialisation of the continuous-wort-fermentation-process research at the Department of Biochemical Technology of the Slovak University of Technology in Bratislava. The immobilised cell technology in beer production has exhaustively been reviewed recently (1). Entrapment of yeast in hydrogel matrices of a polysaccharide nature, such as calcium alginate (2) or sodium κ-carrageenan (3) is preferred by many researchers because of extremely mild immobilisation conditions and high cell density. *Šmogrovičová* and co-workers (4) paid attention to primary fermentation process. Two types of reactors were compared – a packed-bed column and a gas-lift bioreactor. Effect of immobilised yeast concentration on beer quality in continuous fermentation was published (5). Purified gluten seems to be a good carrier for yeast immobilisation due to its really low solubility in wort and young beer, natural origin and large surface area. To test gluten pellets as a carrier for continual beer production we verified results of *Bardi* and co-workers (6). Starch processing of wheat has a high amount of gluten and a low commercial value. Its market is strictly controlled, prices restricted and new applications are welcome, especially on a mass scale (7).

The main focus of this work was to produce beer using yeasts immobilised in gluten – a carrier not very frequently used for beer fermentation.

2 Materials and methods

2.1 Yeast strain and medium

Saccharomyces cerevisiae W 96 (bottom-fermented yeast), was obtained from a local brewery (the number of the strain refers to Collection of the Research Institute of Brewing and Malting, Prague, Czech Republic) and maintained on the malt-extract agar at 5 °C. The cultivation medium was wort with original extract 12.4 % (w/w), the content of saccharides at 112 to 114 g/l and the concentration of unfermentable saccharides in the range 18 – 20 g/l (Brewery Codecon, Sväty Jur, Slovakia).

2.2 Carriers and cell immobilisation

Gluten pellets were prepared according to *Bardi* et al. (6) with some modifications. Wheat flour was mixed with tap water, kneaded to viscous dough to establish stronger gluten binding. Then the dough was washed with cold water to remove residual starch until negative observable reaction on starch presence occured. Gluten of 1.5 – 2 cm in diameter were prepared manually and then dried in two phases. First at 80 °C for 2 hours to desiccate the surface and then at 105 °C for approximately 3 hours to reach complete dryness. Using the recommended 105 °C from the beginning causes an enormous increase in volume of pellets with weaker stability to compression in the packed bed bioreactor. Dried gluten pellets were cultivated in wort with a yeast suspension of 10^9 cells/ml for 48 hours on a rotary shaker at 28 °C. The cell concentration in beads reached 54 g cell dry mass per litre of gluten.

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Alginic acid sodium salt (Protanal LF 20/60) was provided by Pronova Biopolymers (Drammen, Norway). The yeast suspension was added to a solution of sodium alginate (25 g/litre) by means of a bead generator (8) and subsequently dropped into 0.1 mol/litre $CaCl_2$ solution (alginate) forming beads of 2 mm in diameter. Cells were immobilised at an ambient temperature. The cell concentration in beads was 40 g/litre (g of cell dry mass per litre of gel) for primary fermentation and 54 g/litre for secondary fermentation.

2.3 Continuous fermentation

Primary fermentation was carried out in an up-flow gas lift bioreactor using calcium-alginate immobilised yeast with the carrier-to-medium ratio (v/v) 0,27:1 (5). The volume of the reactor was 2860 ml, an internal diameter 8.8 cm. The carrier gas was the recovered carbon dioxide formed during wort fermentation and recycled at a flow rate of 0.230 litre/min. The wort contained 6 mg/litre of oxygen. Secondary fermentation of young beer was carried out in two parallel column reactors consisting of eight packed-beds filled with yeast immobilised in two different carries – calcium alginate or gluten. Volume of the reactors was 468 ml, an internal diameter 4.6 cm. The immobilised carriers filled up 60% of the total volume of the reactors. The operating temperatures were kept at 15 °C in both, primary and secondary fermentations.

2.4 Analytical methods

Ethanol concentration was determined by gas chromatography using a flame ionisation detector (FID) on a column filled with PORAPAK Q. Volatile compounds were determined after distillation and extraction in a Likens-Nickerson apparatus (9) by gas chromatography using a FID detector on a column filled with 8% free fatty acid phase (FFAP). Amino acids were determined by gas chromatography using a flame ionisation detector (FID) on a column filled with SE 30. Total nitrogen, free amino nitrogen, total polyphenols, bitterness and colour were all measured according to the current European Brewery Convention Recommended Methods (10). Diacetyl concentration was expressed as the total vicinal diketones which were measured after thermal conversion of precursors to vicinal diketones (VDK) as described by *Acker* (11). Multistage parameter characterisation of beer was determined by a Servo Chem Automatic Beer Analyser (SCABA 5600, Tecator AB, Sweden). The yeast dry mass in beads was determined gravimetrically as follows: the calcium alginate and calcium pectate gel beads were dissolved in a sodium citrate solution (50 g/l) and the sodium carrageenan beads underwent thermoreversion. The solution was then filtered through a membrane filter (0.45 µ; Sartorius – Membranfilter GmbH, FRG) and washed with physiological saline and subsequently determined gravimetrically. The yeast vitality was determined by vitality test (12).

3 Results and Discussion

Based on our previous results with immobilised continuous systems (4) we proposed and constructed a two-stage immobilised continuous system for wort fermentation (Fig. 1) using bottom-fermented yeast strain *Saccharomyces cerevisiae*.

Before primary fermentation of wort, the initial concentration of yeast in calcium alginate was 40 g/litre. During the first four days the oxygen concentration in the gas-lift reactor was maintained at 8 mg/litre and the residence time at 8 hours, to reach maximum yeast concentration in gel. After 96 hours, when the yeast concentration in gel was 71 g/litre, the oxygen regulation was stopped,

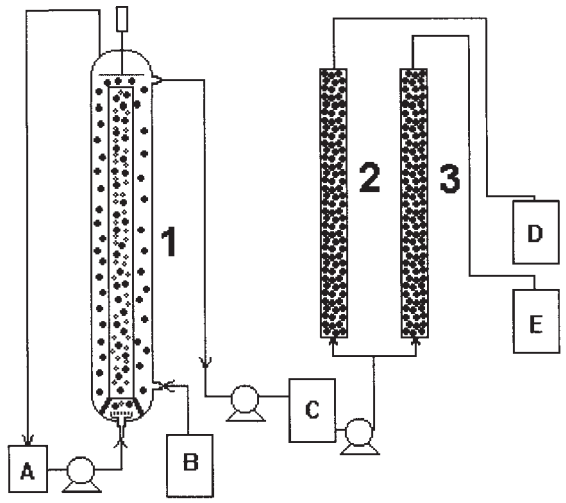

Fig. 1 Flow diagram of beer fermentation using a two-stage continuous reactor system with immobilised yeast

1 – primary fermentation in calcium-alginate immobilised gas-lift reactor. Secondary fermentation in packed-bed reactors using calcium alginate (2) or gluten (3) for immobilisation A – CO_2 reservoir, B – wort, C – young beer, D, E – final beer.

only wort entering the reactor was aerated at oxygen concentration 6 mg/litre. The flow rate of young beer into the secondary fermentation bioreactors was adjusted so that the outlet saccharide concentration reached 20 g/litre. The residence time was then 12 hours. Young beer was separated into two output streams for consequent secondary fermentation.

The first variant of secondary fermentation used immobilised packed-bed systems with calcium alginate. The initial yeast cell

Table 1 Characteristics of beers produced in two-stage continuous system. Young beer – after primary fermentation in calcium-alginate immobilised gas-lift bioreactor. Secondary fermentation in packed-bed reactors using calcium alginate or gluten for immobilisation. Control – beer fermented in a cylindro-conical vessel. Temperature 15 °C

Parameter	Young beer	Alginate	Gluten	Control
Original extract, w/w %	12.4	12.4	12.4	12.1
Apparent extract, w/w %	3.61	2.65	2.61	4.21
Real extract, w/w %	5.1	3.38	3.38	4.81
Alcohol, w/w %	3.2	4.67	4.7	4.08
Real attenuation, w/w %	58.8	72.7	72.5	65.2
Colour, °EBC	14.1	14.2	14.9	14
pH	4.42	4.12	4.11	4.1
Bitterness, BU	19.6	17.9	18.6	18
Total nitrogen, mg/100 ml	107.8	82.9	71.6	82.9
FAN,* mg/100ml	41.2	30.1	21.4	31.4
Total polyphenol, mg/l	28.7	20.1	16.7	21.4

**free amino nitrogen*

concentration in gel was 54 g/litre, final concentration 56 g/litre. The second system used the gluten-pellets-immobilised yeast cells with the same initial cell concentration (54 g/litre), but the final concentration during secondary fermentation reached 60 g/l. The residence time of beer in the bioreactors was different for particular immobilisation carriers. It was 57 hours for calcium alginate and 61 hours for gluten. The main parameters of beers are summarised in Table 1. Immobilised yeast in both systems for secondary fermentation produced beer with higher attenuation and higher content of alcohol compared to control beer. Higher levels of free amino nitrogen in beers indicate a lower rate of amino acid uptake by yeast. This fact has been frequently reported in alginate-immobilised systems (13, 14, 15). We obtained the same results after primary fermentation, but after secondary fermentation the content of free amino nitrogen in experimental beers was lower than in the reference beer. The concentrations of total nitrogen, free amino nitrogen and polyphenols were significantly lower in beer produced by gluten-immobilised yeast compared to control beer and beer produced using alginate-immobilised yeast. The content of individual amino acids in wort and their utilisation in experimental beers are showed in Table 2. In primary fermentation, the utilisation of alanine, phenylalanine and histidine is only 70%, opposite, the utilisation of arginine is complete and utilisation of serine, threonine, asparagine and lysine higher than 95%. After secondary fermentation levels of isoleucine and leucine in gluten-immobilised system are higher compare to alginate-immobilised system or control. The utilisation rate of different amino acids is comparable to the one measured by *Van de Winkel* et al. (16). Experimental beers show differences in concentration of flavour active compounds and volatile concentration profile (Table 3). Diacetyl, acetaldehyde, total higher alcohols and total esters concentrations are lower using immobilised systems. On the other hand, 2- and 3-methylbutanol concentrations are higher. The flavour of a beer depends not only on the contents of its compounds, but also on their ratio. The optimum higher alcohols-to-esters ratio for lagers is 4.1 to 4.7:7 according to *Poledníková* et al. (14). The values for our experimental beers are within this range.

The results show, that gluten is a suitable immobilisation carrier for beer fermentation and beer parameters and quality are comparable to control beer or beer produced using calcium-alginate immobilised yeast.

Table 2 Amino acids utilisation in beers produced in two-stage continuous system. Young beer – after primary fermentation in calcium-alginate immobilised gas-lift bioreactor. Secondary fermentation in packed-bed reactors using calcium alginate or gluten for immobilisation. Control – classically fermented beer in two stages. Temperature 15 °C

Amino acid		Amino acid utilisation (%)			
Name	Wort concentration mg/l	Young beer	Alginate	Gluten	Control
Serine	34.7	97	98	97	96
Threonine	512.2	96	98	97	98
Asparagine	187.4	95	100	100	95
Arginine	115.7	100	100	100	100
Isoleucine	152.9	87	95	91	97
Leucine	292.6	92	95	91	93
Lysine	87.4	95	98	96	98
Methionine	73.7	76	84	84	85
Valine	223.7	75	88	87	86
Glycine	101.5	85	96	91	89
Alanine	110.4	64	82	83	71
Phenylalanine	281.1	63	77	74	68
Tyrosine	211.4	72	85	84	75
Tryptophane	20.1	71	96	91	74
Histidine	137.2	48	77	76	69

Table 3 Low volatile compounds of beers produced by two-stage continuous system. Young beer – after primary fermentation in calcium-alginate immobilised gas-lift bioreactor. Secondary fermentation in packed-bed reactors using calcium alginate or gluten for immobilisation. Control – classically fermented beer in two stages. Temperature 15 °C

Low volatile compounds	Young beer	Alginate	Gluten	Control
Diacetyl	0.123	0.09	0.09	0.118
Acetaldehyde	3.08	2.61	2.61	2.84
n-Propanol	7.4	6.4	6.1	6.8
2-Methylpropanol	41.1	36.9	37.3	35.9
i-Butanol	14.3	13.4	12.4	16.9
2- and 3-Methylbutanol	62.1	56.8	57.2	51.7
2-Phenylethanol	11.2	10.1	10.1	16.3
Total alcohols	136.1	123.1	123.1	127.6
Ethyl formate	1.61	1.49	1.14	1.59
Ethyl acetate	11.7	11.6	12.4	12.1
Propyl acetate	1.18	1.19	1.11	1.24
2-Methylpropyl acetate	0.16	0.25	0.26	0.17
3-Methylbutyl acetate	3.44	3.19	3.14	3.14
Ethyl hexanoate	2.1	1.89	1.74	2.19
Hexyl acetate	4.9	4.49	4.51	4.47
Ethyl lactate	1.1	1.09	1.12	1.01
2-Methylpropyl hexanoate	0	0.11	0.11	0.19
Ethyl octanoate	0.26	0.21	0.24	0.24
3-Methylbutyl hexanoate	0.36	0.14	0.16	0.19
Ethyl decanoate	2.3	1.9	2.2	3.4
Ethyl phenylacetate	0	0.15	0.09	0
Ethyl dodecanoate	0.12	0.17	0.11	0.24
Ethyl tetradecanoate	0.18	0.12	0.14	0.17
Total esters	29.25	27.99	28.47	30.34
Alcohols : Esters	**4.66:1**	**4.39:1**	**4.32:1**	**4.21:1**

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4 Zusammenfassung

Šmogrovičová, D., Dömény, Z., Slugeň, D., Pátková, J., und Bafrncová, P.: Gluten-Pellets zur Immobilisierung von Bierhefe für die Brauereigärung— Monatsschrift für Brauwissenschaft 52, Nr. 7/8, 119 – 122, 1999

BC 2 Malz- und Bierbereitung/ BC 23 Gärung

In zweistufigen, kontinuierlichen immobilisierten Systemen produzierte Biere wurden mit nach dem klassischen Verfahren hergestellten Bier verglichen. Die primäre Gärung der Bierwürze wurde in einem Gasliftreaktor unter Verwendung von Kalziumalginat (Verweilzeit 12 Std.) durchgeführt, die sekundäre Fermentation in zwei parallelen Festbettsystemen unter Verwendung von Kalziumalginat (Verweilzeit 57 Std.) oder Gluten (Verweilzeit 61 Std.) zur Immobilisation der Bierhefe durchgeführt. Die Konzentration von Diacetyl, Acetaldehyd, der gesamten höheren Alkohole und der Gesamtester waren bei der Verwendung immobilisierter Systeme niedriger. Die Konzentrationen an Gesamtstickstoff, freiem Aminostickstoff und Polyphenolen waren bei Bier, das mit Hilfe von Gluten-immobilisierter Bierhefe produziert wurde, im Vergleich zu Alginat niedriger.

Šmogrovičová, D., Dömény, Z., Slugeň, D., Pátková, J., et Bafrncová, P.: Des pellets de gluten pour l'immobilisation de levures de brasserie pour la fermentation brassicole — Monatsschrift für Brauwissenschaft 52, Nr. 7/8, 119 – 122, 1999

BC 2 Fabrication du malt et de la bière/ 23 Fermentation

Des bières produites dans un système immobilisé continu à deux étages ont été comparées à des bières fabriquées suivant le procédé classique. La fermentation primaire du moût de brasserie a été effectuée dans un réacteur en boucle utilisant de l'alginate de calcium (temps de séjour 12 heures), la fermentation secondaire a été réalisée dans deux systèmes à lit fixe utilisant de l'alginate de calcium (temps de séjour 57 heures) ou du gluten (temps de séjour 61 heures) pour l'immobilisation des levures brassicoles. La concentration en diacétyle, acétaldéhyde, alcools supérieurs totaux et ester totaux pour l'utilisation des systèmes immobilisés était inférieure à celle des systèmes classiques. La concentration en azote total, azote aminé libre et polyphénols dans la bière produite à l'aide de levures immobilisées sur gluten est inférieure à celle de la bière sur alginate.

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(Manuskripteingang: 4. 3. 1999)

Erratum

In der letzten Ausgabe der Monatsschrift für Brauwissenschaft, Heft 5/6, 1999 wurden im Artikel von **L. Narziß, H. Miedaner und P. Eichhorn, Untersuchungen zur Geschmacksstabilität des Bieres (Teil 2),** auf Seite 84 die Förderer des Projektes ungenau zitiert. Richtig muß es heißen:

Das Vorhaben wurde aus Haushaltsmitteln des Bundesministeriums für Wirtschaft und Technologie (MBWi) über die Arbeitsgemeinschaft industrieller Forschungsvereinigungen „Otto von Guericke" eV (AiF) und der Wissenschaftsförderung der Deutschen Brauwirtschaft e.V. gefördert.