Technological Development of Brewing in Domestic Refrigerator Using Freeze-Dried Raw Materials

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Summary

Development of a novel directly marketable beer brewed at low temperature in a domestic refrigerator combined with yeast immobilization technology is presented in this study. Separately, freeze-dried wort and immobilized cells of the cryotolerant yeast strain *Saccharomyces cerevisiae* AXAZ-1 on tubular cellulose were used in low-temperature fermentation (2, 5 and 7 °C). The positive effect of tubular cellulose during low-temperature brewing was examined, revealing that freeze-dried immobilized yeast cells on tubular cellulose significantly reduced the fermentation rates in contrast to freeze-dried free cells, although they are recommended for home-made beer production. Immobilization also enhanced the yeast resistance at low-temperature fermentation, reducing the minimum brewing temperature value from 5 to 2 °C. In the case of high-quality beer production, the effect of temperature and initial sugar concentration on the fermentation kinetics were assessed. Sensory enrichment of the produced beer was confirmed by the analysis of the final products, revealing a low diacetyl concentration, together with improved polyphenol content, aroma profile and clarity. The proposed process for beer production in a domestic refrigerator can easily be commercialized and applied by dissolving the content of two separate packages in tap water; one package containing dried wort and the other dried immobilized cells on tubular cellulose suspended in tap water.

Key words: brewing, low-temperature fermentation, novel technology, immobilized cells, freeze-drying, tubular cellulose

Introduction

A combination of immobilized biocatalysts and low--temperature fermentation has led to products with acceptable sensory characteristics (*1,2*). For fermentations at extremely low temperatures (below 5 °C), the use of psychrotolerant yeast strains is necessary. Such microorganisms exhibit cold shock response phenotypes with the accumulation of cryoprotectants (*e.g.* trehalose, glycerol and heat shock proteins), modification of transcription activity, and membrane fluidity changes (*3,4*). Cold-adapted yeasts, such as the *S. cerevisiae* AXAZ-1 strain used in the present study, retain their viability and fermentation ability at low temperatures and have high potential for biotechnological applications. Immobilization of psychrophilic yeast strains has enabled the use of an extremely low brewing temperature (0–7 °C), leading to acceptable fermentation efficiency and product quality. Specifically, the combination of cell immobilization and low fermentation temperature has yielded products with low diacetyl concentration and improved ratios of the desired esters and unwanted volatile compounds (fusel alcohols) compared

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to free cell systems (*4*). Cell immobilization on tubular cellulose (*5*) resulted in high efficiency in fermentations at extremely low temperature, together with the high fermentation activity of freeze-dried cells (*6*). This opened a way for wine and beer making in a domestic refrigerator (*7*).

In previous studies, freeze-dried free and immobilized cells were used in low-temperature brewing with satisfactory fermentation kinetics and improved final beer quality (*8*). The aim of the present study is to develop a dry ready-to-use mixture of immobilized yeast biocatalyst on tubular cellulose and wort, and evaluate its suitability for brewing in a domestic refrigerator.

Materials and Methods

Microorganism and media

The alcohol-resistant, psychrotolerant lager yeast strain *Saccharomyces cerevisiae* AXAZ-1 (*9*) was grown in a synthetic medium containing (in g/L): NH_4SO_4 1, KH_2PO_4 1, $MgSO_4$ 5, glucose 40 and yeast extract 4 at 30 °C with aeration (500 cm^3/min, 700 Pa). The culture was harvested by centrifugation at 2000×*g* for 10 min. Synthetic media with the above composition of nutrients but containing 120 g/L of glucose were used for cell immobilization. Fermentation experiments were performed using hopped lager wort supplied by the Athenian Brewery S.A. (Patras, Greece). The pH value of wort was 5.0 and its relative density was 4.72 °Plato (specific gravity of 1.018). All media were autoclaved at 120 °C and 101 325 Pa for 15 min prior to use.

Preparation of the support and cell immobilization

The carrier used for cell immobilization in this study was a porous cellulosic material, tubular cellulose, produced after pine sawdust delignification with 1 % NaOH, as described by Koutinas *et al.* (*7*). Pine tree sawdust was obtained from a local lumber mill in Patras. After delignification, tubular cellulose was sterilized by autoclaving at 120 °C for 15 min and then dried at 37 °C for 48 h. Cell immobilization was carried out by suspending 7 g of the harvested wet yeast culture and 20 g of dry tubular cellulose in 500 mL of 120 g/L of glucose medium. The system was allowed to ferment for 24 h at 30 °C to a final density of 0.3 °Plato (specific gravity of 1.001). The liquid was then decanted and the immobilized biocatalyst washed with 200 mL of fresh glucose (120 g/L) medium.

Freeze-drying of raw materials

Amounts of the immobilized biocatalyst (65 g wet mass), yeast biomass (2 g wet mass) and wort were frozen separately to −45 °C at a cooling rate of 3 °C/min using methanol as the freezing liquid in a Bio-Cool freezer (FTS Systems, New York, NY, USA). No cryoprotecting media were added (*6*). The frozen materials were freeze-dried overnight at 500 Pa and −45 °C in a Freeze Dry System (Daihan Labtech Co., Ltd, Namyangju, South Korea).

Beer production using freeze-dried raw materials

The freeze-dried immobilized biocatalyst (18 g dry mass) was introduced into a 1-litre glass cylindrical fermenter. Sterilized deionized water was added to an appropriate amount of freeze-dried wort until the desirable sugar relative density (3.9, 4.7 and 6.9 °Plato; *i.e.* specific gravity of 1.015, 1.018 and 1.027) was achieved (final volume of 500 mL). Rehydrated wort and the biocatalyst were then mixed for fermentation and the effect of sugar concentration on fermentation kinetics was studied. Wort with optimum sugar concentration was used for fermentations in order to study the effects of temperature (2, 5 and 7 °C) on fermentation parameters. For comparison, freeze-dried free cells and wort were used for beer production under the same conditions as described above.

Assays

Fermentation kinetics was monitored by measuring relative density using the Plato hydrometer (°Plato) at various time intervals. Residual sugars were detected by high-performance liquid chromatography (HPLC) on a Shimadzu LC-9A HPLC (Shimadzu Co., Kyoto, Japan) system consisting of a Nucleogel ION 300 OA column, an LC-9A pump, an RID-6A refractive index detector, a CTO-10A column oven, and a DGU-2A degassing unit. The mobile phase was 0.004 M H_2SO_4 (0.5 mL/min; Merck Co., Darmstadt, Germany) and 1-propanol (1 % by volume; Merck Co.) was used as an internal standard. The column temperature was 30 °C, the sample dilution was 1 % (by volume), and the injection volume was 40 µL.

Ethanol and methanol were determined by gas chromatography (GC) on a Shimadzu GC-8A system (Shimadzu Co.), with a HayeSep Q column, a C-R6A Chromatopack integrator, with He as carrier gas (20 mL/min) and a flame ionization detector (FID). The column temperature was programmed at 80–180 °C (16 °C/min). The temperature of both the injector and detector was 200 °C. A volume of 2 µL of each sample was injected directly into the column and the concentration of ethanol/methanol was determined using standard curves. Ethanol productivity was calculated in grammes of ethanol per litre of liquid volume produced per day. Beer productivity was calculated in grammes of beer per litre of total working volume produced per day.

The polyphenol concentration, colour and bitterness of the beer samples were determined by the official methods of the European Brewery Convention (*10*).

Diacetyl was determined on an Agilent GC-7890-A GC system (Agilent Technologies Co., Los Angeles, CA, USA) with an Agilent HP-5 column, an Agilent 7697-A headspace sampler, and FID and electron capture detector (ECD) at 280 and 155 °C, respectively. Helium was used as carrier gas (3 mL/min). The column temperature was programmed to rise from 40 to 250 °C (equilibration time 0.5 min). Samples of 1 mL were injected into the column *via* the headspace sampler. Standard curves were constructed for quantitative analysis.

Headspace SPME GC/MS analysis

The identification and semi-quantitative analysis of the headspace volatiles was conducted using gas chromatography/mass spectrometry (GC/MS) and the solid phase microextraction method (SPME). Internal standard for semi-quantitative analysis was 2-pentanol (1.62 mg/L).

The conditions of headspace-SPME sampling and GC-MS analysis were performed according to Gialleli *et al.* (*11*).

Statistics

All analyses were carried out in triplicate and the results are presented as mean value±standard deviation. The significance of differences in the mean values among various groups was checked by one-way analysis of variance (ANOVA) at the 5 % level of significance.

Results and Discussion

Rationale

This research focuses on the development of an innovative, simple method that enables consumers to produce beer in their domestic refrigerators. The proposed technology is based on the use of freeze-dried tubular cellulose-supported biocatalyst in a dry mixture with freeze-dried wort, which can be dissolved in tap water and placed in a refrigerator for fermentation. The process is based on the feasibility of fermentation at extremely low temperature, positively affected by tubular cellulose, as reported in a previous study (*7*). Consequently, fermentation with wet tubular cellulose-supported biocatalyst and liquid wort has been studied due to the positive effect of tubular cellulose on volatiles during wort fermentation and beer characteristics (*7,12*). Therefore, the experiments were designed to examine the freeze-dried wort and the biocatalytic effect on fermentation kinetics at low temperatures on the quality of the beer.

Tubular cellulose – the key biocatalyst material

Pine tubular cellulose, a nano/microporous cellulosic material obtained after delignification of sawdust, is a suitable material for use in food bioprocessing as a cell immobilization carrier. It is an abundant, low-cost, food-grade material that can be easily prepared and handled (*13*). The immobilized cells are attached to tubular cellulose due to physical adsorption by electrostatic and Van der Waals forces as well as by entrapment into the carrier tubes. It has been reported that the tubular cellulose presence increases sugar uptake rate by the immobilized cells due to the attraction by hydrogen bonding onto the tubular cellulose surface and continuous pumping of the sugar towards the cells (*7*). Calculation of the activation energy of maltose fermentation at low temperatures (5, 10 and 15 °C) showed that it was reduced by an average 42 % when cells immobilized on tubular cellulose were used (*14*). This reduction of the activation energy was also observed during glucose alcoholic fermentations and supports the promotional activity of tubular cellulose and the extremely low temperature in wine making (*7*) and brewing (*12*).

Low-temperature fermentation with dry raw materials – fermentation kinetics

Fig. 1 shows the kinetics of freeze-dried wort fermentation by freeze-dried immobilized and free cells. In all cases, the freeze-dried immobilized cells promoted the fermentation of freeze-dried wort and required shorter adaptation periods than freeze-dried free cells, showing that these biocatalysts are ready to ferment after their drying. The protective activity of tubular cellulose on free yeast cells during freeze-drying and fermentation has also been illustrated in other studies (*6,12*). The fermentation rates of free cells at 7 °C were lower than those of freeze-dried immobilized cells with all wort densities (3.9, 4.7, 6.9 °Plato; *i.e.* specific gravity of 1.015, 1.018 and 1.027). Freeze-dried free cells did not exhibit the ability to ferment the wort at temperatures lower than 7 °C (data not shown). Furthermore, Fig. 2 indicates that the fermentation of freeze-dried wort by freeze-dried immobilized cells at 2 and 5 °C proceeds at similar fermentation rate and is completed after approx. 2 months. This result indicates the feasibility of fermenting freeze-dried wort with freeze-dried tubular cellulose-supported biocatalyst in a refrigerator.

In addition, the results in Table 1 show that ethanol and overall beer productivities during the fermentation using immobilized biocatalysts are higher than with free cells at 7 °C. The sugar relative density of 4.7 °Plato (specific gravity of 1.018) was used for further experiments at lower temperatures (5 and 2 °C) based on the higher fermentation rate than those of the other wort relative densities (3.9 and 6.9 °Plato; *i.e.* specific gravity of 1.015 and 1.027) at 7 °C. Carbohydrates in wort comprise up to 92 % of its total solids and include mainly the sugars maltose, maltotriose and glucose. Brewing strains utilize monosaccharides first and then the disaccharide maltose and finally the trisaccharide maltotriose (*15*). In the beer produced with freeze-dried immobilized cells and freeze-dried wort, the residual sugar was at low levels (0.8–3.2 g/L). However, higher residual sugar values (1.6–4.5 g/L) were obtained in fermentations with free cells at 7 °C at all sugar densities. Beer obtained at 7 °C from freeze-dried wort in aqueous solution with the same starting relative density and produced by freeze-dried immobilized cells had alcohol volume fraction about 0.5 % higher than those produced by freeze-dried free cells. This results in about a 10 % higher beer yield and significantly affects beer productivity.

Characteristics of beer produced in the refrigerator

Table 2 compares the characteristics of the beer produced with freeze-dried biocatalysts, free cells and freeze-dried wort. Beer produced with the freeze-dried immobilized cells had colour values in the range of 9.3–16.7 on European Brewery Convention (EBC) scale, whereas the values for commercial lager beer range within 6–15 EBC. The colour intensity was lower than that of the beer fermented by freeze-dried free cells (12.6–20.4 EBC). The pH value of all types of beer produced by freeze-dried raw material and immobilized biocatalyst ranged between 4.0 and 4.3.

Polyphenols in beer, derived from malt and hops, play a significant role in foam maintenance, physical and chemical stability as well as antioxidant activity (*16*). In commercial beer, the polyphenol content is within the range of 190–250 mg/L. Polyphenols in beer produced at 7 °C were reduced for about 20 % when using immobilized compared to free cells. The polyphenols and colour values were higher when using a denser wort. Likewise, bitterness values were reduced when immobilized cells
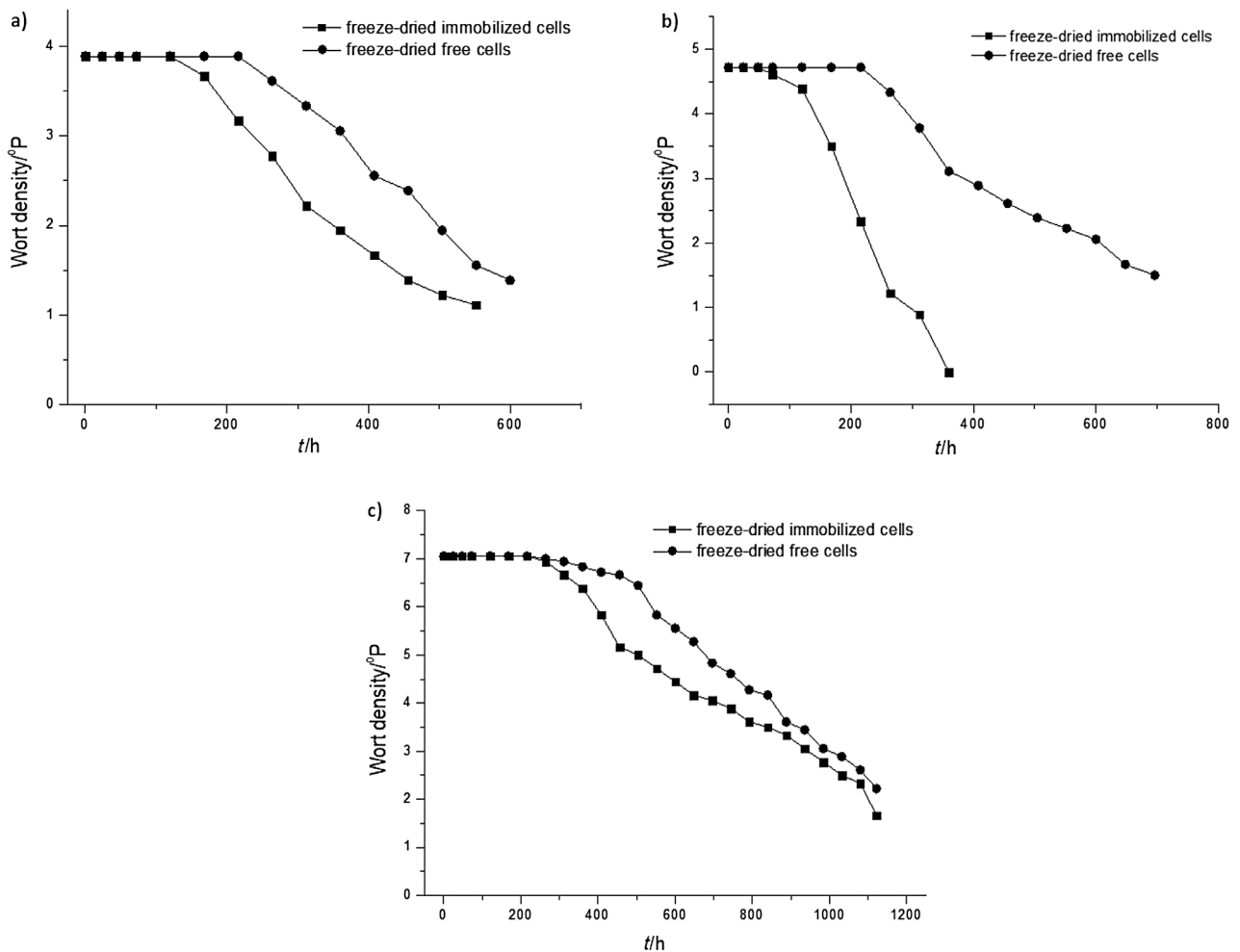
Fig. 1. Fermentation kinetics of freeze-dried wort at initial density of: a) 3.9 °Plato (specific gravity of 1.015), b) 4.7 °Plato (specific gravity of 1.018), and c) 6.9 °Plato (specific gravity of 1.027) using freeze-dried free and immobilized yeast at 7 °C
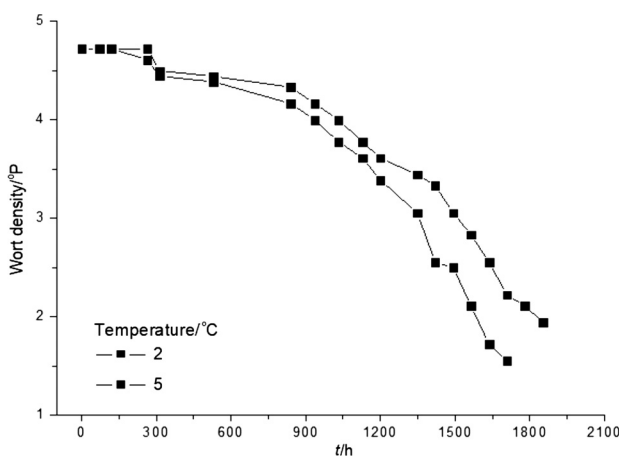

Fig. 2. Fermentation kinetics of freeze-dried wort (4.7 °Plato; *i.e.* specific gravity of 1.018) using freeze-dried immobilized yeast at 5 and 2 °C

10–40 European bitterness unit (EBU) range. In the case of fermentations at 2 and 5 °C using freeze-dried wort and immobilized cells, the bitterness and polyphenol values decreased along with the temperature reduction due to the longer contact of the fermenting wort with the tubular cellulose material. At all studied temperatures, the diacetyl concentration in beer was lower than its agreed taste threshold (0.15 mg/L), which is the optimum concentration for many types of beer (*15*). Diacetyl (2,3-butanedione), produced by yeast through the decarboxylation of α-acetolactate, adds a buttery (or stale milk) aroma to beer and is undesirable to most consumers in concentrations above this taste threshold. In fermented products resulting from this research, beer diacetyl concentrations ranged from 0.06 to 0.15 and from 0.06 to 0.07 mg/L when using free and immobilized cells, respectively. The diacetyl content achieved in the beer is satisfactorily low and the maturation stage traditionally used for diacetyl concentration reduction can be avoided.

Aroma profile

The semi-quantitative analysis of volatile compounds was performed by SPME GC/MS to evaluate the effect of freeze-dried wort density on the aroma concentration of

were used, due to adsorption of hop acids onto the tubular cellulose material, as reported in previous studies (*6,8*). In commercial beer, bitterness can be adjusted with the addition of hop extracts and usually varies within the

Table 1. Kinetic parameters of fermentations at various temperatures using freeze-dried wort with freeze-dried biocatalysts in aqueous solution

Wort density	Temperature	t	γ(residual sugar)	φ(ethanol)	Ethanol productivity	Beer productivity
°P	°C	h	g/L	%	g/(L·day)	g/(L·day)
Freeze-dried free cells						
3.9	7	600	1.6	5.4	1.7	33.6
4.7	7	696	2.1	5.8	1.6	29.0
6.9	7	1122	4.5	7.7	1.3	18.0
Freeze-dried immobilized cells						
3.9	7	552	1.1	6.0	2.1	36.5
4.7	7	360	0.8	6.2	3.3	56.0
4.7	5	1708	1.5	5.7	0.6	11.8
4.7	2	1852	2.2	5.4	0.6	10.9
6.9	7	1120	3.2	8.2	1.4	18.0

Table 2. Characteristics of beer produced at various temperatures by fermentation using mixtures of freeze-dried wort with freeze-dried biocatalysts

Wort density	Temperature	γ(polyphenols)	Colour	Bitterness	γ(diacetyl)
°P	°C	mg/L	EBC	EBU	mg/L
Freeze-dried free cells					
3.9	7	213±10	12.6±0.3	28.6±1.1	0.146±0.007
4.7	7	304±17	15.9±0.6	25.8±1.0	0.110±0.005
6.9	7	358±13	20.4±1.0	27.8±1.2	0.061±0.003
Freeze-dried immobilized cells					
3.9	7	180±7	9.3±0.4	14.2±0.5	0.065±0.003
4.7	7	256±14	14.3±0.6	16.5±0.7	0.057±0.001
4.7	5	211±10	9.8±0.5	8.0±0.5	0.062±0.001
4.7	2	179±8	8.8±0.3	6.7±0.3	0.079±0.001
6.9	7	302±16	16.7±0.7	16.1±0.7	0.058±0.001

EBC=European Brewery Convention, EBU=European bitterness unit

the beer produced by freeze-dried free and immobilized cells. In Table 3 (*17–20*), the majority of the identified compounds were esters that have low flavour thresholds and are considered to have a major impact on beer flavour. Various organic acids, carbonyl compounds, alcohols and terpenoids were also determined in all samples.

The semi-quantitative analysis, with 2-pentanol as internal standard, showed that in all cases the freeze-dried immobilized cells yielded products with higher total headspace volatile content. Specifically, the total amount of esters formed by freeze-dried immobilized cells was higher than that by free cells, at all wort sugar densities prepared using freeze-dried wort. The detected esters produced a flowery, fruity aroma in the products and are highly desirable (within certain limits) in beer. The concentrations of alcohols, carbonyl compounds, organic acids and terpenoids in the beer produced by freeze-dried free cells were not significantly different ($p>0.05$) from those produced by freeze-dried immobilized cells. The alcohols in the products are mainly aliphatic, amyl or aromatic fusel alcohols that are not highly favourable for beer aroma. However, phenyl ethyl alcohol provides a

sweet rose-like flavour that positively contributes to beer aroma and it was detected in higher concentration in the beer produced by freeze-dried immobilized cells. In addition, terpenes (Table 4), which are isoprene compounds derived from hops, add a flowery note to beer aroma (*15*) and their concentration was higher in the beer produced using freeze-dried immobilized cells on tubular cellulose.

Beer with the highest level of volatile compounds (6013.2 µg/L) compared to all other fermentation products was produced when fermenting freeze-dried wort of 4.7 °Plato (specific gravity of 1.018) by freeze-dried immobilized yeast cells. As shown in Table 4, the decrease in fermentation temperature leads to a reduction of the total concentration of volatile compounds (esters and alcohols), but the number of the total compounds detected was similar.

Practical applications of the novel brewing method

The proposed process is mainly based on the use of freeze-dried wort and brewer's phychrophilic yeast strain on freeze-dried tubular cellulose. Freeze-dried wort and a biocatalyst can be packaged separately or in the same

Table 3. Volatile compounds identified in the beer produced by fermentation using mixtures of freeze-dried wort with freeze-dried biocatalysts at 7 °C

KI	Compound	Wort density/°P					
		3.9	4.7	6.9	3.9	4.7	6.9
		γ(volatile compound)/(µg/L)					
		Freeze-dried free cells			Freeze-dried immobilized cells		
	Esters						
847	Ethyl acetate[a]	33.5	45.6	12.9	27.0	55.1	16.1
1011	Ethyl butanoate[b]	3.1	5.0	n.d.	10.9	36.3	20.0
1104	3-Methyl-butyl acetate[b]	10.8	12.3	20.1	72.3	367.3	76.7
1227	Ethyl hexanoate[a]	20.0	22.7	34.0	115.2	141.2	100.3
1259	Hexyl acetate[e]	n.d.	3.7	2.2	n.d.	n.d.	n.d.
1298	Ethyl heptanoate[c]	n.d.	n.d.	n.d.	31.5	3.0	8.4
1389	Ethyl octanoate[a]	47.8	52.4	81.2	837.1	1444.5	625.0
1425	Octyl acetate[e]	n.d.	6.1	n.d.	n.d.	n.d.	n.d.
1588	Ethyl decanoate[c]	n.d.	n.d.	29.3	123.3	482.4	221.8
1645	Ethyl-9-decenoate[e]	41.7	29.8	120.4	1005.8	1066.7	650.2
1782	2-Phenyl ethyl acetate[a]	n.d.	n.d.	n.d.	46.3	71.9	28.0
	N(esters)	6	8	7	9	9	9
	$\Sigma\gamma$(esters)	156.9	177.6	297.9	2269.4	3668.4	1746.5
	Organic acids						
2047	Octanoic acid[a]	148.7	43.2	104.0	447.1	400.0	n.d.
2252	Decanoic acid[b]	50.5	10.7	n.d.	290.7	512.1	426.5
2266	Undecanoic acid[d]	n.d.	n.d.	n.d.	n.d.	71.8	n.d.
2318	Undecylenic acid[b]	n.d.	n.d.	n.d.	306.5	244.1	277.0
	N(organic acids)	2	2	1	3	4	2
	$\Sigma\gamma$(organic acids)	199.2	53.9	104.0	1044.3	1228.0	703.5
	Alcohols						
1213	2-Methyl-1-butanol[d]	109.4	92.2	109.7	150.4	302.2	211.6
1322	1-Hexanol[b]	n.d.	2.5	n.d.	n.d.	n.d.	n.d.
1504	1-Octanol[e]	n.d.	9.4	n.d.	n.d.	2.7	n.d.
1611	2-Furanmethanol[c]	n.d.	n.d.	n.d.	34.2	n.d.	n.d.
1880	Phenyl ethyl alcohol[a]	55.5	52.8	56.4	111.4	764.8	309.0
	N(alcohols)	2	4	2	3	3	2
	$\Sigma\gamma$(alcohols)	164.9	156.9	166.1	296.0	1069.7	520.6
	Carbonyl compounds						
1353	Nonanal[b]	n.d.	n.d.	4.1	6.7	20.8	13.0
1448	Decanal[a]	3.4	2.2	n.d.	n.d.	7.5	n.d.
	N(carbonyl compounds)	1	1	1	1	2	1
	$\Sigma\gamma$(carbonyl compounds)	3.4	2.2	4.1	6.7	28.3	13.0
	Terpenoids						
1167	Limonene[e]	n.d.	n.d.	4.9	n.d.	18.8	n.d.
1652	α-terpineol[a]	5.4	n.d.	n.d.	n.d.	n.d.	2.8
1721	Citronellol[e]	3.1	n.d.	n.d.	12.0	n.d.	n.d.
2008	Nerolidol[b]	8.1	7.7	n.d.	13.2	n.d.	3.6
	N(terpenoids)	3	1	1	2	1	2
	$\Sigma\gamma$(terpenoids)	16.6	7.7	4.9	25.2	18.8	6.4
	N(all compounds)	14	16	12	18	19	16
	$\Sigma\gamma$(all compounds)	541.0	398.3	577.0	3641.6	6013.2	2990.0

KI=tentative identification by Kovats retention index; [a](*17*), [b](*18*), [c](*19*), [d]identification by mass spectra obtained from NIST107, NIST21 and SZTERP libraries, [e](*20*); n.d.=not detected

Table 4. Volatile compounds identified in the beer produced by fermentation using mixtures of freeze-dried wort (4.7 °Plato; *i.e.* specific gravity of 1.018) with freeze-dried biocatalysts

	Temperature/°C		
Compound	7	5	2
	γ(volatile compound)/(μg/L)		
N(esters)	9	8	6
Total concentration	3668.4	3115.7	2775.2
N(organic acids)	4	5	4
Total concentration	1228.0	1150.8	985.7
N(alcohols)	3	3	4
Total concentration	1069.7	985.4	910.9
N(carbonyl compounds)	2	2	3
Total concentration	28.3	30.9	24.1
N(terpenes)	1	1	1
Total concentration	18.8	14.4	13.5
N(detected compounds)	19	19	18
Total volatile compound concentration	6013.2	5297.2	4709.4

container. These dry raw materials can be used as commercial product. A consumer will be able to conveniently suspend the dry raw materials in water in a bottle or small barrel. The fermentation vessel is then stored in a domestic refrigerator and beer produced with 3–5 % alcohol by volume after 10–15 days, depending on the refrigerator temperature (2–7 °C). Carbon dioxide is contained in the beer after sealing the vessel when the relative density of fermented broth is below 0.8.

The home-scale application of the technology provides the opportunity for a consumer to produce good-quality, home-made beer easily and at low cost in the domestic refrigerator. In addition, the immobilization of cells on tubular cellulose, an abundant and cheap support, results in a clearer final product with improved aroma characteristics than beer produced with free yeast cells. This proposed technology is an interesting alternative to conventional home brewing methods and can provide potentially high-quality beer with possible commercialization potential.

Conclusions

The use of a freeze-dried biocatalyst of *Saccharomyces cerevisiae* cells immobilized on pine tubular cellulose has been proved effective for the low-temperature brewing of freeze-dried wort. Freeze-dried free cells cannot otherwise be used for brewing in a domestic refrigerator because they are not able to complete the fermentation at temperatures lower than 5 °C. The beer produced by freeze-dried immobilized cells generally had good clarity, satisfactory volatile compound concentrations together with lower diacetyl and polyphenol contents than commercial beer. The proposed technology of beer production in a domestic refrigerator shows high commercialization potential as brewing kit using single-use packages of freeze-dried wort and a freeze-dried biocatalyst on tubular cellulose.

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