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Low-Temperature Brewing Using Yeast Immobilized on Dried Figs

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Dried figs, following exhaustive extraction of their residual sugars with water, were used for immobilization of *Saccharomyces cerevisiae* AXAZ-1. The immobilized biocatalyst was used in repeated batch fermentations of glucose at 30 °C, where significant reduction of the fermentation time was observed, falling from 65 h in the first batch to 7 h after the sixth batch. Repeated fermentations of wort at room and low temperatures resulted in fermentation times that fell from 26 to 20 h and from 27 to 24 days at 18 and 3 °C, respectively. Ethanol and beer productivities were high, showing suitability of the biocatalyst for low-temperature brewing. Diacetyl concentrations were low (0.3−0.5 mg/L), and polyphenols were lower than in commercial products and decreased as the fermentation temperature was decreased (126−50 mg/L). Ethyl acetate concentrations increased from 53 to 88 mg/L as the temperature was decreased, while the concentration of amyl alcohols at 3 °C (58 mg/L) was lower than half of that at 18 °C (125 mg/L). The beers produced at the end of the main fermentation had a fine clarity and a special fruity figlike aroma and taste, distinct from commercial products and more intense than beers produced by cells immobilized on other food-grade supports (gluten pellets or delignified cellulosic materials). GC−MS analysis did not show significant differences in the qualitative composition of the aroma compounds of the beers produced by immobilized and free cells.

KEYWORDS: Fermentation; brewing; immobilized cells; dried figs; volatile byproducts; organoleptic quality

INTRODUCTION

The advantages of immobilized cell systems over free cell systems are well known (*1*). For the past 30 years, brewing research has focused on cell immobilization for batch and continuous processes, mainly to shorten the maturation time (secondary fermentation) and consequently reduce the overall production cost. Immobilized cell systems have also been applied in the brewing industry for the production of alcohol-free or low-alcohol beer, but they have not yet been successfully applied for primary beer fermentation (*2*−*4*). At present, the only carriers reported to have been used in full scale are porous glass and DEAE-cellulose with additions of titanium dioxide and polystyrene. These novel biocatalysts have not yet been commercialized due to the desire to maintain the traditional character of the product and because of the uncertainty of consumers toward them. Additionally, the carrier cost, the operational stability of the immobilized system, and the regeneration of the carrier are important parameters as far as economic feasibility is concerned (*2*, *3*, *5*).

Low-temperature brewing using cryotolerant and ethanol-resistant yeast cells immobilized on food-grade supports, such as gluten and delignified cellulosic material, resulted in the production of beer with improved taste and aroma, while ethanol and beer productivities were high even at extremely low temperatures (0−5 °C) (*6*−*8*). The combination of immobilization and freeze-drying was applied successfully for the above biocatalysts, solving the problem of supplying breweries or wineries with preserved and marketable ready-to-use immobilized cells (*9*−*11*). However, for industrial application of the above supports, major consideration must be given to suitable bioreactor design and consumer acceptance. Pieces of fruits such as apple and quince can be easily accepted by the consumers and have been used as immobilization supports of yeast cells for wine-making at room and low temperatures, leading to higher productivities, compared with traditional processes, and better sensory characteristics of the special type of wines produced (*12*). The use of dried figs as support for

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Table 1. Kinetic Parameters Obtained in the Repeated Batch Fermentations of Glucose and Wort with AXAZ-1 Cells Immobilized on Dried Figs at Various Temperatures (3–30 °C)

fermentation temp (°C)	fermentation batch	initial density (°P)	fermentation time (h)	residual sugar (g/L)	final biomass (g/L)	ethanol (% v/v)	ethanol productivity (g/L/day)	beer productivity (g/L/day)
				Glucose				
30	1	13.1	65	2.3	2.0	5.1	15	
30	2	13.0	45	1.4	2.0	5.7	24	
30	3	13.4	26	0.3	1.7	6.0	44	
30	4	13.3	11	0.3	1.9	6.1	105	
30	5	13.1	8	1.4	1.8	5.8	138	
30	6	13.2	8	0.2	1.7	6.4	152	
30	7	13.5	7	0.3	1.6	6.0	163	
30	8	13.4	7	0.2	1.2	6.4	173	
30	9	13.3	7	0.2	2.3	6.3	171	
30	10	13.1	7	0.2	2.1	6.2	168	
30	11	13.5	7	1.1	2.0	5.9	160	
				Wort				
30	12	14.2	20	0.0	2.6	6.0	57	500
30	13	14.2	18	0.2	2.7	6.1	64	556
30	14	14.1	16	0.0	1.8	6.2	74	625
30	15	14.2	13	0.0	1.6	6.2	90	769
18	16	14.3	26	0.9	1.1	6.3	46	385
18	17	14.1	22	0.8	1.6	6.0	52	455
18	18	14.0	20	0.9	3.2	5.9	56	500
18	19	14.2	20	0.3	1.6	6.1	58	500
7	20	13.7	90	0.8	1.0	5.8	12	111
7	21	13.6	80	1.7	0.8	5.2	12	125
7	22	13.6	85	1.1	1.2	5.7	13	118
7	23	13.6	85	1.0	0.8	5.9	13	118
3	24	13.7	650	0.8	0.7	5.7	2	15
3	25	13.9	580	1.0	0.9	5.8	2	17
3	26	13.8	580	0.8	0.9	5.7	2	17

yeast immobilization for wine-making or brewing has not been reported. Dried figs (*Ficus carica*) are rich in fiber, trace minerals, antioxidant polyphenols, proteins, sugars, and volatile compounds that provide a pleasant characteristic aroma. They are abundant in the Mediterranean area, and they are safe and inexpensive, comprising an interesting new support for cell immobilization in brewing.

The aim of this investigation was to study the efficiency of an alternative natural support (dried figs) for cell immobilization and the use of the immobilized biocatalyst in brewing, especially at low temperatures.

MATERIALS AND METHODS

Yeast Strain and Wort. *Saccharomyces cerevisiae* AXAZ-1, which was alcohol resistant and cryotolerant, was isolated from Greek grapes (*13*). The isolate was grown on complete culture medium consisting of 4 g/L yeast extract, 1 g/L $(NH_4)_2SO_4$, 1 g/L KH_2PO_4, 5 g/L $MgSO_4 \cdot 7H_2O$, and 40 g/L glucose monohydrate, harvested at 4000 rpm for 10 min and used in the present study. Wort was obtained from the Athenian Brewery S.A., hopped, filtered, and sterilized. The pH of the wort was 5.0, and the °Be density was fixed at 7.5−7.6.

Preparation of Support and Immobilization of Cells. Dried figs were used after the exhaustive extraction of their residual sugars with deionized water. An amount of dried figs was placed in a conical flask and covered with water. The flask was shaken at 25−30 °C, measuring the °Be density of the extract until all sugars were extracted. The carrier was autoclaved for 15 min at 125 °C and 1.5−2 atm, and cell immobilization followed by mixing 170 g of the carrier and 16 g of AXAZ-1 cells in a 1-L glass cylinder containing 800 mL of 12% (w/v) glucose culture medium (pH 4.8). The system was allowed to ferment for 6−8 h until the density of the fermented liquid reached a final value of 0−0.5 °Be. The biocatalyst was washed two or three times with 12% w/v glucose medium and used for batch fermentations of glucose and wort.

Glucose Fermentation. An amount of 600 g of the immobilized biocatalyst was introduced into 450 mL of 12% (w/v) glucose culture medium in a 1-L glass cylinder. The total bioreactor volume was 1 L. Eleven repeated batch fermentations were performed at 30 °C. After the end of each fermentation batch, the support was washed with 400 mL of 12% (w/v) glucose medium, and the biocatalyst was used for the next fermentation batch. The fermented liquids were immediately analyzed for ethanol, residual sugar, and volatile byproducts (**Tables 1** and **2**).

Room- and Low-Temperature Brewing. After the glucose fermentations, 350 g of the above biocatalyst was introduced in 250 mL of pasteurized wort, with an initial density of 14 °Plato (**Figure 1**). The total bioreactor volume was 600 mL. Repeated fermentation batches were carried out initially at 30 °C for the adaptation of cells in wort, and fermentations followed upon reducing the temperature successively to 18, 7, and 3 °C. When each fermentation batch was completed, the produced beer was collected and immediately analyzed for ethanol, residual sugar, free cell biomass, diacetyl and polyphenol concentrations, bitterness, color, and volatile byproducts (**Tables 1−3**). The support was washed with 400 mL of fresh wort, and the biocatalyst was used for the next fermentation batch.

Assays. The ethanol concentration in the final products was determined by means of both gas chromatography and high-pressure liquid chromatography. Ethanol productivity was calculated as the grams of ethanol per liter of liquid volume produced per day. Beer productivity was calculated as grams of beer per liter of total volume produced per day, considering that beer density is equal to 1 g/mL. Residual sugar and ethanol were determined using HPLC on a Shimadzu LC-9A liquid chromatograph. A Shim-pack (SCR-101 N) column, a refractive index detector, three times distilled and filtered water as mobile phase, and butanol-1 as internal standard were used. The column temperature was 60 °C, with a flow rate of 0.8 mL/min. The standard deviation for ethanol was $\leq \pm 0.4$, and that for residual sugar was $\leq \pm 0.1$.

Beer characteristics such as wort and beer gravities (% sugar per weight), diacetyl (mg/L), polyphenols (mg/L), bitterness (EBU), color (EBC), and refractive index (at 18 °C) were determined in decarbonated and paper-filtered beer samples. All the above values were determined as in the industrial routine, according to the EBC methods of analysis (*14*). The initial wort gravity and final beer gravity (apparent extract)

Table 2. Volatile Byproducts Obtained by the Repeated Batch Fermentations of Glucose and Wort with AXAZ-1 Cells Immobilized on Dried Figs at Various Temperatures (3–30 °C)

fermentation temp (°C)	fermentation batch	acetaldehyde (mg/L)	ethyl acetate (mg/L)	propanol-1 (mg/L)	isobutyl alcohol (mg/L)	amyl alcohols (mg/L)	methanol (mg/L)	total volatiles (methanol excluded) (mg/L)
				Glucose				
30	1	3	85	74	19	91	589	272
30	2	11	90	65	27	132	238	325
30	3	3	89	50	25	132	240	299
30	4	3	97	32	23	120	177	275
30	5	14	94	92	18	85	168	303
30	6	4	84	34	34	119	156	275
30	7	3	55	43	27	137	145	265
30	8	3	92	44	31	144	89	314
30	9	3	76	36	34	156	92	305
30	10	3	64	37	32	145	81	281
30	11	13	46	42	31	137	89	269
				Wort				
30	12–15							
18	16	13	53	42	33	125	73	266
18	17	10	54	31	33	111	84	239
18	18	11	68	33	33	107	72	252
18	19	10	67	20	26	106	72	229
7	20	13	76	28	18	90	71	225
7	21	13	82	21	17	62	66	195
7	22	15	73	19	16	62	64	185
7	23	16	75	18	16	61	65	186
3	24	19	86	16	13	66	45	200
3	25	20	84	17	12	60	32	193
3	26	19	88	15	12	58	35	192

Figure 1. Yeast immobilized on dried figs in the fermentation of wort.

were measured in °P units using a thermohydrometer. The original and real extracts (% w/w) (corrected values of initial gravity and apparent extract, respectively) were determined using a nomogram furnished by the Athenian Brewery S.A. Free cell concentrations (g/L) were determined by measurement of the optical density at 700 nm using a standard curve (*15*, *16*). The standard deviations were $\leq \pm 0.2$ for final biomass, $\leq \pm 9$ for polyphenols, $\leq \pm 0.03$ for diacetyl, and $\leq \pm 0.9$ for bitterness.

Volatile byproducts were determined by means of gas chromatography using a Shimadzu GC-8A gas−liquid chromatograph, with a stainless steel column packed with Escarto-5905 consisting of 5% squalene, 90% Cabowax-300, and 5% di-2-ethylhexyl sebacate, with N_2 as the carrier gas (20 mL/min) and an FID detector (*17*). The injection port and detector temperatures were 210 °C, and the column temperature was 70 °C. The internal standard was butanol-1 at a concentration of 0.1% (v/v). Samples of 4 μL of beer were injected directly in the column, and the concentrations of the above compounds were determined using standard curves. Methanol and ethanol were determined by a Shimadzu GC-8A gas−liquid chromatograph, with a column packed with Porapac-S, N_2 as the carrier gas (20 mL/min), and an FID detector. The injection port and detector temperatures were 210 °C, and the column temperature was programmed between 140 and 180 °C. Butanol-1 used as the internal standard, and samples of 2 μL of beer were injected directly in the column. The standard deviations for volatile byproducts were $\leq \pm 0.6$ for acetaldehyde, $\leq \pm 3$ for ethyl acetate, $\leq \pm 2$ for propanol-1, $\leq \pm 1$ for isobutyl alcohol, $\leq \pm 9$ for amyl alcohols, and $\leq \pm 11$ for methanol.

Gas Chromatography−Mass Spectroscopy (GC−MS). The volatiles in a sample of fig extract, a sample of beer produced by free cells, and a sample of beer produced by immobilized cells at 18 °C were analyzed by means of gas chromatography−mass spectroscopy, and the results are presented in **Tables 4−6**. The volatiles in the above samples were isolated by an extraction technique (*18*, *19*). An amount of 200 mL of sample, 5 mL of CH_2Cl_2, and 30 g of NaCl were placed into a spherical-bottom flask of a specific extraction device. The flask was cooled in melting ice, and the sample−CH_2Cl_2 mixture was stirred at 500 rpm for 2 h. The formed emulsion was broken down by passing it, with a gastight syringe, through a specific filter (C_THP Acrodisc syringe filters GP 0.45 μm). The organic extract was dried over anhydrous sodium sulfate in a small glass vial and stored at −5 °C until further analysis. All compounds in the extracts were identified with a GC−MS from Fisons Instruments (GC 8000 series, MS: MD 800). A Chrompack WCOT fused silica column was used (CP-Sil 8,

Table 3. Characteristics of Beers Produced by the Fermentation of Wort with AXAZ-1 Cells Immobilized on Dried Figs at Various Temperatures (3–18 °C)

fermentation temp (°C)	fermentation batch	initial density (°P)	original gravity (% w/w)	apparent extract (E_a) (% w/w)	real extract (E_r) (% w/w)	refractive index (R_o)	color (EBC)	polyphenols (mg/L)	diacetyl (mg/L)	bitterness (EBU)
30	12–15									
18	16	14.3	10.0	2.0	3.5	1.3415	10.0	118	0.3	6.2
18	17	14.1	9.6	2.4	3.7	1.3415	10.0	126	0.3	9.6
18	18	14.0	8.9	2.7	3.9	1.3415	11.0	111	0.5	11.7
18	19	14.2	9.8	2.4	3.7	1.3420	12.2	73	0.3	12.3
7	20	13.7	7.8	3.2	4.1	1.3420	12.2	86	0.5	12.9
7	21	13.6	7.5	3.5	4.4	1.3430	13.2	67	0.5	14.9
7	22	13.6	7.7	3.3	4.2	1.3430	14.2	57	0.3	15.2
7	23	13.6	7.6	3.3	4.2	1.3432	14.1	55	0.3	14.8
3	24	13.7	8.5	3.2	4.1	1.3420	13.9	50	0.3	14.1
3	25	13.9	8.8	3.4	4.3	1.3430	13.5	53	0.3	13.9
3	26	13.8	8.7	3.3	4.2	1.3430	13.3	51	0.3	14.0

Table 4. Volatile Compounds Identified in Beer Produced by AXAZ-1 Cells, Free and Immobilized on Dried Figs, at 18 °C

t_R (min)	compound	reliability of identification[a]	t_R (min)	compound	reliability of identification[a]
2.992	acetaldehyde	a	35.278	2-furanmethanol	b
5.987	ethyl acetate	a	35.595	3-methylbutanoic acid	b
9.393	1-propanol	a	36.611	5-ethyldihydro-2(3*H*)-furanone	c
9.676	ethyl butanoate	a	36.995	3-(methylthio)-1-propanol	b
11.860	2-methyl-1-propanol (isobutanol)	a	37.645	1,3-propanediol, diacetate	a
12.576	2,7-dimethyl-2,6-octadiene	b	37.711	pentanoic acid	a
13.693	3-methyl-1-butyl acetate	a	38.328	1-decanol	b
16.327	cyclopentanone	b	39.628	ethyl 3-hydroxybutanoate	a
17.860	3- and 2-methyl-1-butanol	a	40.028	2-phenylethyl acetate	c
19.043	ethyl hexanoate	a	40.895	hexanoic acid	c
21.177	3-hydroxy-2-butanone	a	41.328	*N*-(3-methylbutyl)acetamide	c
21.994	4-penten-1-ol	a	42.895	2-phenylethanol	a
22.277	4-methyl-1-pentanol	b	43.878	heptanoic acid or hex-3-enoic acid	b
23.427	ethyl heptanoate	b	44.478	1-(1*H*-pyrrol-2-yl)ethanone	b
23.644	3-hydroxy-2-pentanone	c	46.045	dihydro-5-pentyl-2(3*H*)-furanone	c
23.777	ethyl 2-hydroxypropanoate	b	46.729	octanoic acid	c
24.160	1-hexanol	a	49.062	dihydro-5-hexyl-2(3*H*)-furanone	a
24.427	4-hydroxy-4-methyl-2-pentanone	c	49.445	nonanoic acid	c
25.011	3-ethoxy-1-propanol	b	50.329	4-hydroxy-2-methylacetophenone	c
26.177	heptane	b	51.596	ethyl dodecanoate	c
27.444	ethyl octanoate	a	52.046	decanoic acid	c
28.244	acetic acid	a	52.246	ethyl 9-hexadecenoate	c
30.028	1-(2-furanyl)ethanone	c	53.562	ethyl pentadecanoate	b
30.794	dihydro-2-methyl-3(2*H*)-thiophenone	b	54.496	undecylenic acid	c
31.178	2-furanmethanol, acetate	c	54.612	dihydro-5-octyl-2(3*H*)-furanone	c
31.328	propanoic acid	a	55.062	3-(2-hydroxyphenyl)-2-propenoic acid (cinnamic acid-*O*-hydroxy)	c
31.778	1-octanol	b	55.913	benzoic acid	b
32.294	isobutyric acid	a	56.913	*N*,*N*-bis(2-hydroxyethyl)dodecanamide	c
34.244	dihydro-2(3*H*)-furanone	b	58.496	tetradecanoic acid	c
34.511	ethyl decanoate	a	58.963	benzeneacetic acid	c

[a] a, identification by comparison of gas chromatographic retention times and mass spectroscopic data with those of the available pure compounds; b, mass spectrum in agreement with spectra in the literature; c, tentatively identified.

30 m, 0.32 mm i.d., 0.25 μm film thickness). Helium was used as carrier gas (linear velocity of 1 mL/min). The oven temperature was programmed to start at 35 °C for 2 min and then rise to 50 °C at a rate of 4 °C/min. After a period of 5 min at 50 °C, the temperature was raised to 230 °C at a rate of 4 °C/min. The injector temperature was 230 °C. The mass spectrometer was set at 70 eV. The identification was performed by comparison with standard compounds and data obtained from NIST and Wiley libraries.

Electron Microscopy. A piece of the immobilized biocatalyst (AXAZ-1 cells immobilized on dried figs) was washed with deionized water and dried overnight at 30 °C. The sample was coated with gold in a Balzers SCD 004 Sputter Coater for 3 min and examined in a JEOL model JSM-6300 (Japan) scanning electron microscope (**Figure 2**).

Preliminary Taste Test. Samples of the beers produced by yeast cells immobilized on dried figs were kept at 4−5 °C. A chill haze, which can settle as sediment, was not developed during storage. The beers were not carbonated and were tested immediately after preparation by five nontrained testers (consumers), according to a preliminary taste test protocol based on a preference scale (**Table 7**), and pointing out the special characteristics of the produced beers compared to the commercial products (*10*, *20*).

RESULTS AND DISCUSSION

We proceeded to a step-by-step investigation of the new immobilized biocatalyst (yeast cells immobilized on dried figs) to evaluate its efficiency for glucose and wort fermentation, even at low temperatures, in terms of productivity and organoleptic quality, which are factors directly related to commercial application and consumer acceptance.

Table 5. Differences between the Volatiles Identified in Beer Produced by AXAZ-1 Cells, Free and Immobilized on Dried Figs, at 18 °C

t_R (min)	compound	immobilized cells	free cells	reliability of identification[a]
7.410	ethyl propanoate	−	+	a
7.992	propyl acetate	−	+	a
8.893	isobutyl acetate	−	+	a
20.794	hexyl acetate	+	−	a
23.077	3-methyl-1-pentanol	+	−	a
26.794	2-heptanol	+	−	c
27.561	linalool oxide	+	−	b
27.794	3,4-dimethyl-2-hexanol	+	−	c
27.977	1-octen-3-ol	+	−	c
28.144	1-heptanol	+	−	b
29.411	2-ethyl-1-hexanol	+	−	c
32.961	5-methyl-2-furancarboxaldehyde	+	−	c
36.178	ethyl dec-9-enoate	+	−	b

[a] a, identification by comparison of gas chromatographic retention times and mass spectroscopic data with those of the available pure compounds; b, mass spectrum in agreement with spectra in the literature; c, tentatively identified.

Table 6. Volatile Compounds Identified in Dried Fig Extract

t_R (min)	compound	not found in[a]	reliability of identification[b]	t_R (min)	compound	not found in[a]	reliability of identification[b]
3.002	acetaldehyde		a	35.295	2-furanmethanol		b
5.998	ethyl acetate		a	35.612	3-methylbutanoic acid		b
11.660	hexanal	I, F	a	35.661	5-ethenyldihydro-3-methyl-2(3*H*)-furanone	I, F	c
13.610	3-penten-2-one	I, F	c	36.195	5-methyl-3-hexen-2-one	I, F	c
15.510	4-hexen-2-one	I, F	c	36.645	5-ethyldihydro-2(3*H*)-furanone		c
17.244	2,3-dihydro-4-methylfuran	I, F	c	37.562	3,4-dimethyl-2,5-furandione	I, F	c
17.677	3- and 2-methyl-1-butanol		a	37.762	pentanoic acid		c
21.211	3-hydroxy-2-butanone		a	38.412	5-ethyl-2(5*H*)-furanone	I, F	c
22.327	4-methyl-1-pentanol		b	40.929	hexanoic acid		a
22.944	1-octene	I, F	c	40.195	α-damascenone	I, F	c
24.194	1-hexanol		a	42.812	2-phenylethanol		a
25.861	nonanal	I, F	c	43.929	heptanoic acid or 3-hexenoic acid		c
27.561	linalool oxide	F	c	46.079	dihydro-5-pentyl-2(3*H*)-furanone		c
27.794	2-ethyl-6-methyl-1,5-heptadiene	I, F	c	46.779	octanoic acid		a
28.011	1-octen-3-ol	F	a	49.496	nonanoic acid		b
28.328	acetic acid		b	50.013	2-octenoic acid	I, F	c
28.611	2-furancarboxaldehyde	I, F	c	50.346	4-hydroxy-2-methyl-acetophenone		c
29.978	2,7-dimethyloctane	I, F	c	51.646	ethyl dodecanoate		c
30.061	1-(2-furanyl)ethanone		b	52.279	ethyl 9-hexadecenoate		c
30.661	benzaldehyde	I, F	c	57.597	5-(hydroxymethyl)-2-furancarboxaldehyde	I, F	c
32.945	5-methyl-2-furancarboxaldehyde	F	b	58.030	13-tetradecenal	I, F	c
34.311	dihydro-2(3*H*)-furanone						

[a] I, beer produced by immobilized cells; F, beer produced by free cells. [b] a, identification by comparison of gas chromatographic retention times and mass spectroscopic data with those of the available pure compounds; b, mass spectrum in agreement with spectra in the literature; c, tentatively identified.

Glucose Fermentation. To confirm that the immobilization of AXAZ-1 cells on dried figs is possible and the immobilized biocatalyst can be used successfully for alcoholic fermentation, 11 repeated batch fermentations of 12% (w/v) glucose solutions were carried out at 30 °C. An impressive reduction of the fermentation time was observed, from 65 h in the first batch to 45 h in the second, and to a stable 7 h after the sixth fermentation batch (**Table 1**). This reduction can possibly be attributed to a continuous growth of cells in the carrier and their adaptation to the fermentation process up to a steady state. The carrier was not disrupted and remained intact throughout the fermentation experiments, while its shape and size allowed easy handling (decanting, washing out, refilling of fresh wort, etc). Foaming, which was not vigorous since carbon dioxide was not recycled as in industrial practice, did not cause floating of the biocatalyst, as observed previously in the case of lighter supports such as delignified cellulosic materials. Consequently, the attachment of a special gauze construction (**Figure 1**) to keep the biocatalyst deep in the fermenting liquid proved to be unnecessary.

The successful immobilization of yeast on dried figs was confirmed by the electron micrograph showing the morphology of the dried figs' surface after the immobilization of yeast cells (**Figure 2**). The cells were not only enclosed in natural cavities, they were also attached on smooth edges of the carrier surface.

Ethanol concentrations in the final product ranged from 5.1 to 6.4% (v/v) for initial densities 7.0−7.4 °Be, while ethanol productivities were very high (**Table 1**). The residual sugar was very low (0.2−2.3 g/L), indicating that the biocatalyst was very active and suitable for alcoholic fermentation. From the analysis of volatile byproducts (**Table 2**), a remarkable fluctuation of methanol concentration from batch to batch was observed. From a high of 589 mg/L in the first batch, it was reduced to half in the second, while in the eleventh batch it was 6 times lower than its initial value. This reduction can be obviously attributed to the hydrolysis of pectin substances contained in the carrier, which was limited during the next fermentation batches. Similar fluctuation was not observed for the concentration of the other

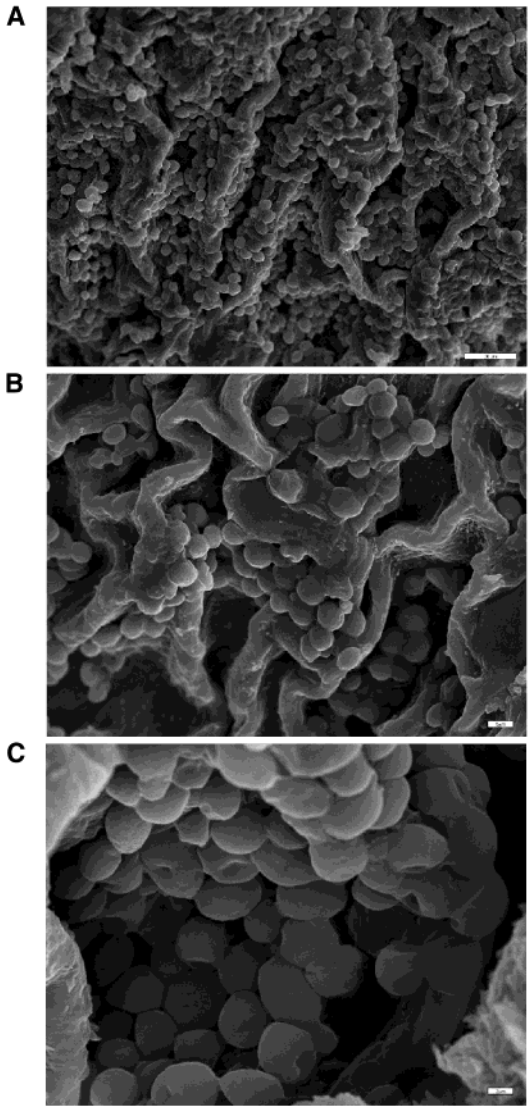

Figure 2. Electron micrograph showing the morphology of the dried fig surface after the immobilization of AXAZ-1 cells. (A) ×600; (B) ×1200; (C) ×3000.

Table 7. Preliminary Taste Test Results of Beers Obtained by the Batch Fermentations of Wort, with AXAZ-1 Cells Immobilized on Dried Figs, at Various Temperatures [18 (○), 7 (□), and 3 (▽) °C]

	tester				
scale	1	2	3	4	5
fine					
excellent	▽□	▽	▽□	▽□	▽□
very good	○	□○	○	○	○
good					
acceptable					
bad					
very bad					
unacceptable					
sweet	▽□○	▽□○	▽□○	▽□○	▽□○
fine clarity	▽□○	▽□○	▽□○	▽□○	▽□○
body	▽□○	▽□○	▽□○	▽□○	▽□○
after taste	▽□○	▽□○	▽□○	▽□○	▽□○
fruity aroma (figlike)	▽□○	▽□○	▽□○	▽□○	▽□○

volatiles that were determined (acetaldehyde, amyl alcohols, propanol-1, isobutyl alcohol, and ethyl acetate).

Brewing at Room and Low Temperatures. Repeated fermentation batches of wort at low temperatures (18, 7, and 3 °C) followed, and the fermentation kinetics are given in **Table 1**. It is obvious that the immobilized cells were recovered after each fermentation batch at low temperatures, and fermented wort during fermentation times that ranged from 20−26 h at 18 °C to 24−27 days at 3 °C. Fermentation times were reduced from batch to batch at each temperature and were lower than those obtained by free AXAZ-1 cells (*10*) at all studied temperatures (**Figure 3**). Residual sugar concentrations in the green beers were very low (0−1.7 g/L), and ethanol was present in the same high levels (5.2−6.3% v/v) as in those beers produced with cells immobilized on gluten pellets and delignified cellulosic material (*6*, *10*), for the same initial density even at low temperatures. Beer and ethanol productivities were very high and were reduced as the temperature decreased. The final free cell concentrations in beers produced with AXAZ-1 cells immobilized on dried figs were low (0.2−3.2 g/L), especially at temperatures lower than 7 °C, and the green beers had a fine clarity after the end of main fermentation (**Table 1**). At all the studied temperatures, the diacetyl concentrations in the green beers were low (0.3−0.5 mg/L), while polyphenols were lower than 126 mg/L and decreased as the fermentation temperature decreased (**Table 3**), as in the case with cells immobilized on gluten pellets and delignified cellulosic material (*6*, *10*). The original gravities, real and apparent extracts in the green beers were in the range of most commercial products. Bitterness values in beers produced with AXAZ-1 cells immobilized on dried figs ranged from 9.6 to 15.2 EBU and were slightly increased as the temperature was decreased (**Table 3**). In commercial beers,

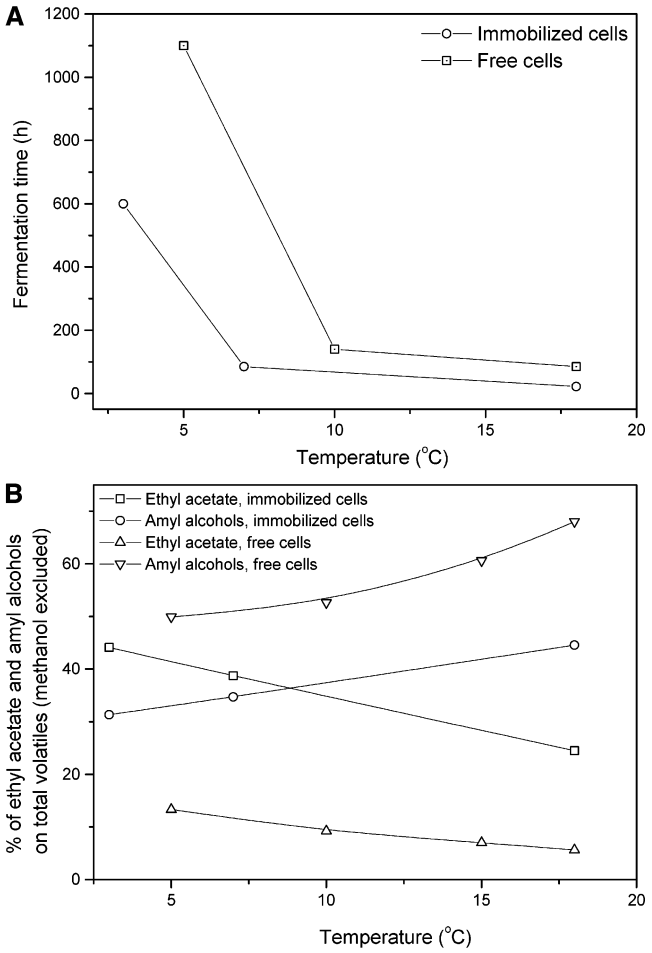

Figure 3. Fermentation times (A) and volatiles (B) obtained in the repeated batch fermentations of wort with AXAZ-1 cells, free and immobilized on dried figs, at various temperatures (3–18 °C).

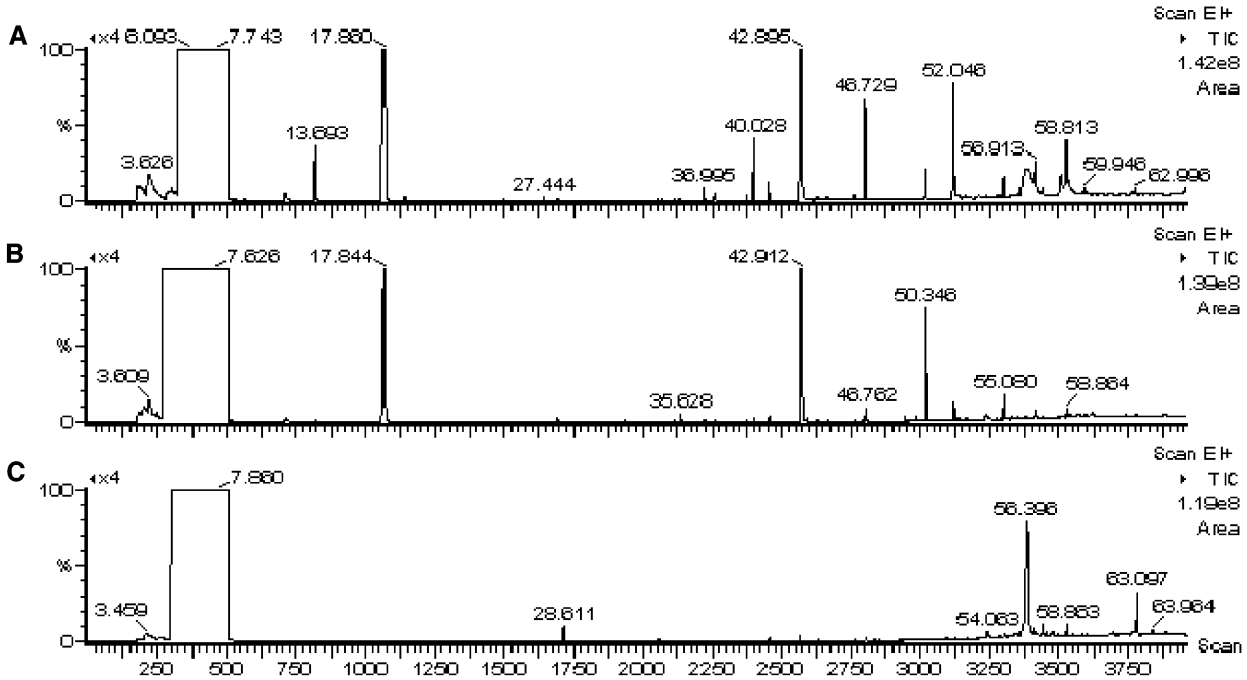

Figure 4. Chromatographs of aroma compounds identified by GC–MS after isolation with the CH_2Cl_2 extraction technique. (A) Beer produced by AXAZ-1 immobilized on dried figs at 18 °C; (B) beer produced by free AXAZ-1 cells at 18 °C; (C) fig extract in water.

bitterness varies in the range 10−40 EBU, depending on the type of the product, and can be further increased by the addition of hops extracts. Finally, the green beers had a fine clarity after the end of the main fermentation and were slightly darker compared with beers produced with cells immobilized on gluten pellets and delignified cellulosic material. Their colors were in the range 10.0−14.2 EBC (**Table 3**).

Volatile Byproducts. Table 2 shows that the amounts of higher alcohols in beers produced with AXAZ-1 cells immobilized on dried figs were reduced as the temperature decreased. Specifically, amyl alcohols, propanol-1, and isobutyl alcohol concentrations at 3 °C were lower than 50% of those at 18 °C. Furthermore, the concentrations of amyl alcohols were lower than those in beers produced by free AXAZ-1 cells (**Figure 3**) (*10*). On the other hand, the ethyl acetate concentrations (53−88 mg/L) were higher in beers produced by immobilized cells than in beers produced by free cells (**Figure 3**) and were increased as the temperature was decreased. These results are in accordance with previous studies investigating brewing with fresh or freeze-dried cells immobilized on gluten pellets and delignified cellulosic material (*7*, *10*). The reduction of amyl alcohols and the increase of ethyl acetate concentrations with the drop of temperature, as previously stated, lead to an improvement of the organoleptic quality of the product (*21*, *22*). Acetaldehyde concentrations in the green beers (**Table 2**) were slightly increased as the temperature was decreased, while methanol concentrations were relatively low and were reduced as the temperature was decreased from 84 mg/L at 18 °C to 32 mg/L at 3 °C.

GC−MS Analysis. The identification of volatiles by GC−MS was necessary in order to evaluate the differences in aroma between beers produced by free and immobilized cells and the contribution of the support (dried figs) on the volatile composition. For the evaluation of the aromatic profile of the beer produced at 18 °C, an extraction technique was employed which gives more peaks than the headspace technique. In both beers produced by free and immobilized cells, a large number of compounds were identified, 60 of which were identified with reliability (**Figure 4**; **Tables 4** and **5**). All of these compounds are important for beer flavor, as reported in the literature (*22*). Most of the identified compounds were acetic or ethyl esters of higher alcohols and fatty acids, which are responsible for the fruity effect on beer flavor. The major esters identified were ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate, 2-phenyl acetate, and 3-methyl-1-butyl acetate. These compounds, that are usually found in most beers, have very low threshold values, and their important contribution to beer flavor is well known (*22*).

The main acids identified were acetic, propanoic, pentanoic, hexanoic, heptanoic, octanoic, nonanoic, and decanoic. They are mainly byproducts of yeast metabolism, usually responsible for the soapy and caprylic off-flavors, and have a positive effect on beer flavor only as precursors of esters after their reaction with various alcohols (*22*). The most flavor-active are the C2 and C6 acids. Benzoic, tetradecanoic, benzenacetic, undecylenic, 3-methylbutanoic, and isobutyric acids were also traced, but their contribution to beer flavor is small since they do not exceed their threshold values.

Alcohols identified included 1-propanol, isobutanol, amyl alcohols, hexanol-1, 3-ethoxy-1-propanol, 1-octanol, 2-furanmethanol, decanol-1, 2-phenylethanol, and 4-penten-1-ol. Alcohols, except ethyl alcohol and amyl alcohols, have high threshold values, and their individual effect on flavor is usually negligible compared with that of esters and carbonyl compounds. Yet, in total they are considered as the principal group of flavor compounds in beer (*22*).

The major carbonyl compounds in beer are acetaldehyde and diacetyl, and their effect on beer flavor is very important as they usually exceed their threshold values (*22*). Aldehydes that are responsible for green-leaf off-flavors, like butanal, pentanal, hexanal, and octanal, were not detected. A number of other carbonyl compounds were also traced, including cyclopentanone, 3-hydroxy-2-butanone, 3-hydroxy-2-pentanone, and 4-hydroxy-

4-methyl-2-pentanone. These compounds have a small effect on beer flavor.

Other important compounds, such as 1-(2-furanyl)ethanone, dihydro-2(3*H*)-furanone, 5-ethyldihydro-2(3*H*)-furanone, 1-(1*H*-pyrrol-2-yl)ethanone, dihydro-5-hexyl-2(3*H*)-furanone, 4-hydroxy-2-methylacetophenone, and dihydro-5-octyl-2(3H)-furanone, were also identified. These compounds have relatively low threshold values and are responsible for desirable fruity, floral, or caramel flavors. Additionally, they are reported to provide antioxidant activity in beer (*22*).

Traces of sulfur compounds such as dihydro-2-methyl-3(2*H*)-thiophenone and 3-(methylthio)-1-propanol were detected. The presence of these compounds demands special attention, although their negative effect on beer flavor is less intense than the effect of hydrogen sulfide or sulfur dioxide.

GC−MS analysis of volatiles was also carried out in a sample of fig extract (**Figure 4**). The compounds that were identified in the extract and were not found in beer produced by free or immobilized cells are presented in **Table 6**. These compounds were obviously removed after the extraction of dried figs with water. From data that are shown in **Tables 4** and **5**, it is obvious that cell immobilization does not significantly alter the qualitative composition of the aroma compounds, as reported by other researchers (*12*). Wort is already a liquid rich in volatile compounds deriving from barley and hops. The characteristic fruity, figlike aroma of the new beers must be attributed to nondetected traces of miscellaneous flavor compounds such as furanones, acetals, pyrones, phenols, ethers, terpenes, sesquiterpenes, and lactones deriving from figs, or probably to the quantitative change of the most flavor-active volatiles such as esters, which are abundant in figs (*22*, *23*).

Organoleptic Quality of Beer. In previous studies (*6*−*8*, *10*) the production of beer with wet or freeze-dried yeast cells immobilized on gluten pellets and delignified cellulosic material was investigated. The concentrations of volatile byproducts in those systems were in the same levels as in beers produced by cells immobilized on dried figs, and the green beers had a characteristic aroma and taste, distinct from commercial products. Similarly, in the preliminary taste test, the green beers produced with cells immobilized on dried figs were characterized as specifically sweet, smooth, with a fruity, figlike aroma, with body and aftertaste clearly distinct from commercial products (**Table 7**). The fruity character of the flavor was more intense and clear than in beers produced with yeast immobilized on gluten pellets and delignified cellulosic material. Finally, the flavor was more intense and was characterized as better in beers produced at low temperatures, due to the higher ethyl acetate and lower amyl alcohol and diacetyl concentrations as the temperature was reduced. The green beers had a fine clarity, and a chill haze during storage at 4 °C did not develop due to the lower concentrations of free cells and polyphenols, which are usually responsible for haze development after reaction with the proteins of wort. This, in combination with the low diacetyl concentrations, gives the possibility to reduce or even avoid the maturation stage of the green beer.

Conclusions. From the above results, it can be concluded that dried figs are an interesting carrier for cell immobilization to be used for alcoholic fermentation in brewing. Fermentation kinetics were satisfactory and can be accepted by the industry even at low temperatures. This carrier, as gluten pellets and delignified cellulosic material, is a relatively resistant material that is not disrupted in the fermentation environment, especially compared with pieces of other fruits (e.g., apples) due to their high content of fiber. Also, due to their shape and size, they do not demand the design of complex bioreactors (e.g., multibed towers) to hold the support and allow easy flow of the influent, which are important parameters, especially in continuous processes. In addition, dried figs are inexpensive and abundant in the Mediterranean area, they are edible, and in combination with the special organoleptic character of the produced beers and consumer acceptance, they fulfill the basic prerequisites for future commercialization in brewing. The distinct character of the new product, with low polyphenol and diacetyl concentrations, and the fine clarity after the end of primary fermentation, give the possibility to reduce maturation time. This is of great economic importance, due to the reduction of the demand for energy and maturation tank capacity. The overall process creates a new beer distinct from commercial products that can be easily accepted by consumers. However, for application in the brewing industry, as in the case of all novel biocatalysts, further research is needed with pilot plant schemes, design of novel bioreactors according to the support properties, and application of continuous processes.

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