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Wine production using yeast immobilized on quince biocatalyst at temperatures between 30 and 0 °C

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Abstract

A biocatalyst was prepared by immobilization of *Saccharomyces cerevisiae*, strain AXAZ-1, yeast cells on quince pieces and its suitability for fermentation of glucose and grape must was investigated. The immobilized yeast showed operational stability and no decrease in activity, even at low temperatures (0–10 °C). Total and volatile acidities in the wines produced were similar to dry wines. The concentrations of higher alcohols (propan-1nol and isobutyl alcohol) were low. The production of amyl alcohols proved to be temperature-dependent and decreased with lower temperatures. Ethyl acetate concentrations were relatively high, up to 113 mg/l. This probably contributes to the fruity aroma and high quality taste of the wines. GC-MS analysis of wines produced using the immobilized biocatalyst and free cell fermentations showed no significant differences in the qualitative composition of aroma-related constituents.
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1. Introduction

Cell immobilization in alcoholic fermentation is a rapidly expanding research area because of its attractive technical and economic advantages compared to the conventional free cell system (Margaritis & Merchant, 1984; Stewart & Russell, 1986). However, for industrial wine production, it is important to identify a suitable support for cell immobilization that is of food grade, readily available and cost-effective and which may contribute to an overall improvement in the sensory characteristics of the final wine product. Another criterion that is sought after in such an immobilized system is suitability for use in low temperature fermentations, since low temperature fermentation leads to a higher quality product. A host of support materials for cell immobilization, suitable for winemaking, have been proposed (Ageeva, Merzhanian, & Sobolev, 1985; Bakoyianis, Kanellaki, Kaliafas, & Koutinas, 1992). Many such support systems have involved the use of inorganic materials or alginates, which were later considered inappropriate for winemaking (Fumi, Trioli & Colagrande, 1987; Mori, 1987; Nakanishi & Yokotsuka, 1987; Shimobayashi & Tominaga, 1986) and they were eventually abandoned. The use of delignified cellulosic material as an immobilization support was then employed and proved to be a more effective alternative for low-temperature alcoholic fermentation (Bardi & Koutinas, 1994).

We have sought a biocatalyst which is suitable for industrial use, is cheap and abundant and could be used a number of times. The use of fruits in developing such a biocatalyst was an obvious alternative that has been tried in several food-related industries. Kourkoutas, Komaitis, Koutinas, & Kanellaki (2001) have already reported the use of apple pieces as a support material for cell immobilization. They suggested that this biocatalyst led to higher productivity and a product with better aroma and taste. Quince was also used for biocatalyst production using *Kluyveromyces marxianus*

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thermotolerant yeast and proved to be suitable for high temperature ethanolic fermentation (unpublished data).

In this paper we report the use of quince as a suitable support material for immobilization of yeast cells and its use in wine making at both low-temperature and room temperature.

2. Materials and methods

2.1. Yeast strain

Saccharomyces cerevisiae AXAZ-1 is an alcohol-tolerant and psychrophilic strain isolated from the Greek agricultural area by Argiriou, Kalliafas, Psarianos, Kana, Kanellaki, and Koutinas (1992). It was grown on a medium consisting of glucose 4%, yeast extract 0.4%, $(NH_4)_2S0_4$ 0.1%, KH_2P0_4 0.1% and $MgSO_4$ 0.5%. Pressed wet weight cells (15–20 g) were prepared accordingly to Argiriou et al. (1992) and employed directly in the fermentation process.

2.2. Preparation of must

Concentrated grape must was diluted with distilled water to a final °Be density range 10.3–12.6 °Be (≈18–22% w/v initial sugar concentration). The must was used without any nutrient addition or adjustments after sterilization at 130 °C for 15 min.

2.3. Support and immobilization of cells

Yeast cell immobilization was carried out on pieces of quince (400 g) in a 1-liter glass cylinder containing 500ml of synthetic medium. The culture medium contained (w/v): 12% glucose, 0.4% yeast extract, 0.1% $(NH_4)_2S0_4$, 0.1% KH_2P0_4 and 0.5% $MgS0_4$ in distilled water with no pH adjustment. Ten grammes (wet weight) of yeast cells biomass were also added to the cylinder and allowed to ferment until the must density was approximate 0.5°Be. The fermented liquid was decanted and the support was washed twice with 400 mL of the medium that was used for wine production. The first two batches were carried out with a 500 ml volume and the subsequent batches with 350 ml, due to the decrease of the volume of quince in the bioreactor (see Results and Discussion). A total of 40 repeated batch fermentation runs were carried out using the same immobilized biocatalyst. The volume of the quince remaining in the bioreactor was monitored during the repeated fermentations.

2.4. Repeated wine making batches at different temperatures

The quince-supported biocatalyst, was introduced to 350 ml of medium containing (w/v) glucose 20%, yeast extract 0.4%, $(NH_4)_2S0_4$ 0.1%, KH_2P0_4 0.1% and $MgSO_4$ 0.5% or must, in a 1-liter glass cylinder. Repeated batch fermentations were carried out without agitation at various temperatures that gradually decreased as follows: 30, 15, 10, 5 and 0 °C. Towards the end of each batch the fermentation liquid was decanted and the biocatalyst support was washed twice (2×350 ml) with synthetic medium containing glucose or must and reused in the next fermentation. A total of 40 batch fermentation runs were carried out, collecting samples at the end of most for analysis of ethanol, residual sugar, free cells, total and volatile acidity and volatile by-products (acetaldehyde, ethyl acetate, propan1-ol, isobutanol, amyl alcohols and methanol).

2.5. Analytical assays

Alcohol was distilled and measured using a Gay-Lussac alcoholmeter and ethanol productivity calculated as the grammes of ethanol per litre of liquid volume produced per day. Wine productivity was calculated as grammes of wine per litre of total volume produced per day.

Residual sugar was determined by high performance liquid chromatography, using a Shimadzu chromatograph with a SCR-101N stainless steel column, a LC-9A pump, an CTO-10A oven at 60 °C and a RID-6A refractive index detector. Three times distilled water was used as mobile phase with a flow rate of 0.8 ml/min and butan 1-ol was used as an internal standard. Samples of 0.5mL wine and 2.5 mL of a 1% (v/v) solution of butanol-1 was diluted to 50 and 40 μl were injected directly on to the column. The residual sugar concentration was calculated using a standard curve and expressed as grammes of residual sugar per litre.

Total acidity was estimated by titration of samples with 0.1 M NaOH solution and volatile acidity by titration with 0.1 M NaOH of distillates obtained by steam distillation of wine samples (Zoeklein, Fugelsang, Gump, & Nury, 1990). Free cell concentrations (wet weight/l) in the fermentation liquid were determined according to Klein and Kressdorf (1983).

2.6. Volatile by-products determination

Acetaldehyde, ethyl acetate, propan1-ol, isobutanol and amyl alcohol were determined by gas chromatography using a stainless steel column, packed with Escarto-5905, consisting of Squalene, 5%, Carbowax-300, 90% and di-ethyl-hexyl sebacate, 5% (v/v) (Cabezudo, Gorostiza, Herraiz, Fernadez-Biarange, Garcla-Dominguez, & Molera, 1978). Nitrogen was used as carrier gas at 20 ml/min. Injection port and FID detector temperatures were 210 °C and 220 °C, respectively. The column temperature was 70 °C. In all cases, the

Table 1
Fermentation parameters obtained in repeated batch fermentations with immobilised *Saccharomyces cerevisiae* AXAZ-1 yeast cells on quince pieces

Temp. (°C)	Sugar source	Repeated batch fermentation	Initial °Be density	Fermentation time (h)	Daily wine productivity (g/l)	Ethanol concen.(% v/v)	Daily ethanol productivity (g/l)	Residual sugar (g/l)	Free cells concen. (g/l)	Total acidity (g of tartaric acid/l)	Volatile acidity (g of acetic acid/l)
30	Glucose	1	11.4	72	–	10.1	27	1.1	5.0	–	–
30	Glucose	3	11.2	25	–	10.6	80	0.01	6.4	–	–
30	Glucose	5	10.3	28	–	10.1	68	1.7	4.2	–	–
30	Glucose	6	11.3	26	–	11.0	80	0.03	3.5	–	–
30	Glucose	8	11.3	27	–	10.7	75	0.2	4.2	–	–
30	Glucose	9	11.4	31	–	10.7	65	0.9	10.8	–	–
30	Glucose	10	11.4	28	–	10.3	70	0.2	4.7	–	–
30	Glucose	11	11.3	28	–	10.4	70	1.7	3.9	–	–
30	Glucose	12	11.3	30	–	11.1	70	0.1	4.1	–	–
30	Glucose	14	11.5	32	–	10.3	61	1.1	8.9	–	–
30	Glucose	15	11.4	32	–	10.3	61	1.8	6.2	–	–
30	Must	17	10.9	28	357	10.7	73	0.1	17.4	5.4	0.08
30	Must	18	10.9	14	678	10.7	150	0.3	17.2	5.9	0.08
30	Must	19	11.7	13	743	11.4	166	0.6	10.7	5.9	0.08
30	Must	20	11.5	13	730	12.0	175	0.2	9.2	6.1	0.10
30	Must	21	12.0	15	636	11.9	150	0.7	7.7	6.5	0.11
30	Must	23	11.4	13	726	11.4	166	1.7	8.3	5.5	0.12
30	Must	24	11.6	12	787	11.5	182	0.4	7.5	5.4	0.10
30	Must	25	12.0	14	678	11.1	150	0.4	8.0	5.9	0.12
30	Must	26	11.4	14	670	11.3	153	0.4	7.6	5.5	0.14
15	Must	27	11.6	57	169	11.5	38	0.5	5.0	4.6	0.08
15	Must	28	11.8	50	196	11.7	44	0.4	7.5	5.0	0.11
15	Must	29	11.6	51	193	11.0	41	1.2	5.0	5.9	0.12
15	Must	30	11.7	51	193	11.6	43	0.9	6.0	4.3	0.14
15	Must	31	11.6	50	190	11.2	43	1.1	5.7	5.5	0.16
10	Must	32	11.3	70	140	11.1	30	0.1	3.8	5.6	0.16
10	Must	33	11.8	94	100	11.9	24	0.2	4.5	4.5	0.17
10	Must	34	12.3	101	94	12.7	24	0.1	4.4	5.3	0.22
10	Must	35	12.3	100	95	12.0	23	1.3	4.3	4.7	0.22
5	Must	36	12.6	474	20	12.6	5	1.5	3.4	4.5	0.19
5	Must	37	11.7	461	21	12.7	5	0.9	3.6	4.5	0.18
5	Must	38	12.2	575	17	13.1	4	1.1	2.9	3.9	0.20
0	Must	39	11.8	1153	9	12.5	2	2.1	3.1	4.0	0.24
0	Must	40	11.7	1245	8	12.3	2	2.6	3.4	4.1	0.26

internal standard was butan1-ol at a concentration of 0.5% (v/v). Samples of 4 μl of wine were directly injected into the column and the concentrations of the above volatile compounds were determined using standard curves. Methanol was also determined by gas chromatography using a Porapac S column. Nitrogen was used as carrier gas at 40ml/min. The column temperature was programmed at 120–170 °C at a rate 10 °C/min. The temperatures of the injector and FID detector were 210 and 220 °C, respectively. For the methanol determination, 2 μl samples were injected directly into the column and the concentration of methanol was determined using standard curves. Butan 1-ol was used as internal standard at a concentration of 0.5% (v/v).

2.7. GC/MS headspace analysis

Ten millilitres of the sample containing 2.2 g of Na_2SO_4 was incubated in closed container at 60 °C for 45 min prior to analysis of head-space. 1ml of sample was injected into the GC/MS (GC 8000 series, MS: MD 800) operated in split injector mode with a chromapack WCOT fused silica column (CP-WAX CB52 60 m, 0.32 mm i.d., 0.25 μm film thickness). Helium was used as carrier gas at 1ml/min. Oven temperature was programmed from 35 °C for 2 min and then raised to 50 °C with a rate of 4 °C/min. After a period of 5 min at 50 °C the temperature was raised to 230 °C with a rate of 4 °C/min. The injector temperature was 230 °C. The mass spectrometer was operated in the electron impact mode with the electron energy set at 70 eV. The scan range was 29–400 *m/z*. Identification was effected by comparison of retention times with those of standard compounds and data obtained from NIST and Wiley libraries.

2.8. Electron microscope investigation

A piece of quince with immobilized biocatalyst was coated with gold in a Balzers SCD 004 sputter coater for 2 min to increase the electron conductivity. The prepared sample was studied in an electron microscope (Japan Electron Optics Limited).

2.9. Preliminary taste evaluation

Samples of the wine were kept at 4–5 °C for one month and then tested for their aroma and taste characteristics compared to a commercial dry wine produced locally using similar must types. Ten tasters, familiar with wine tastes, were asked to give scores on a 0–10 scale using locally approved protocols in our laboratories for taste and aroma. The sensory evaluation was a blind test in a coloured glass under no light.

3. Results & discussion

3.1. Immobilization and fermentation

For immobilization of yeast cells pieces of quince were mixed with a liquid culture of yeast biomass and left to ferment for 8 h. The biocatalyst produced was used in 40 repeat batch fermentation runs, 15 using glucose and 25 using grape must at room and at low temperatures. The results are summarised in Table 1.

The volume of quince that remained in the cylinder after each batch was measured. A decrease in the volume of the quince biocatalyst was observed. The main decrease of the quince volume was observed after the first two fermentation batches. This was probably due to the conversion of the quince sugar to ethanol and/or cells. The quince volume remained stable until the end of the experiments (duration 8 months) which is mainly due to the residual cellulose content of quince which is an unfermentable constituent (Fig. 1).

Fig. 2 shows the clarity of the fermented liquid produced during the fermentation process with immobilized cells. A closer look, using the electron microscope, shows the predominance and proliferation of the yeast cells within the biocatalyst tissue structure (Fig. 3).

Table 1 shows the results of repeated batch fermentations of glucose and grape must. The whole process was carried out by quince-supported biocatalyst. The first 15 repeated batch fermentations using glucose were carried out without any significant change in the fermentation time. Similar initial °Be densities were employed. Initial °Be densities of grape must were in the range 10.9–12.6, and wines produced contained alcohol at concentrations similar to those of dry table wines. The fermentations continued for ≈8 months without any significant loss of the biocatalyst activity. Fermentation at very low temperatures (< 10 °C) continued and wine production was achieved within 4 days at 10 °C using the biocatalyst. This is less time than required for traditional fermenta-

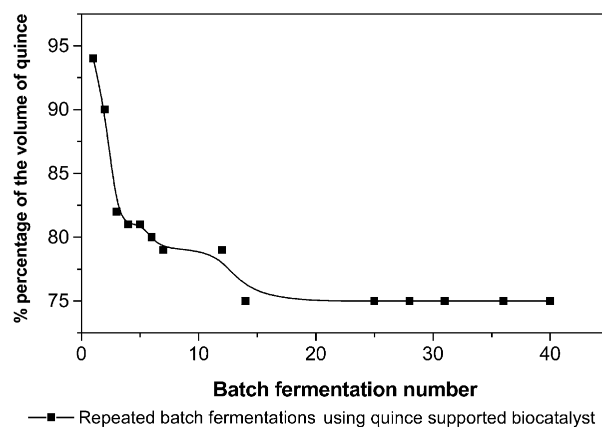


Fig. 1. Percentage volume of quince remaining within the bioreactor during 40 repeated batch fermentations.

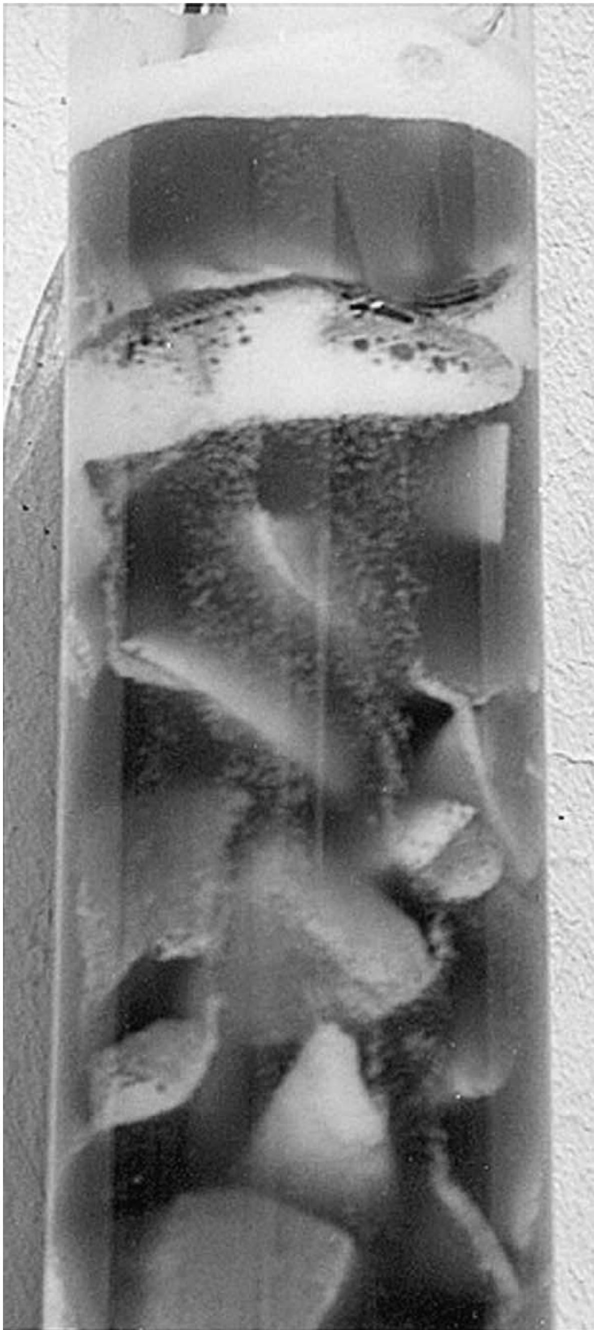
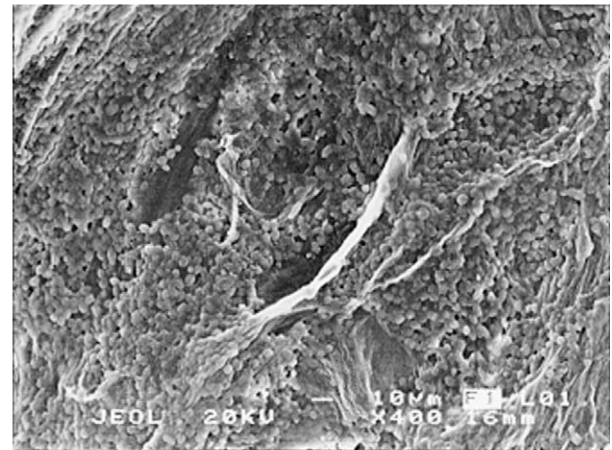
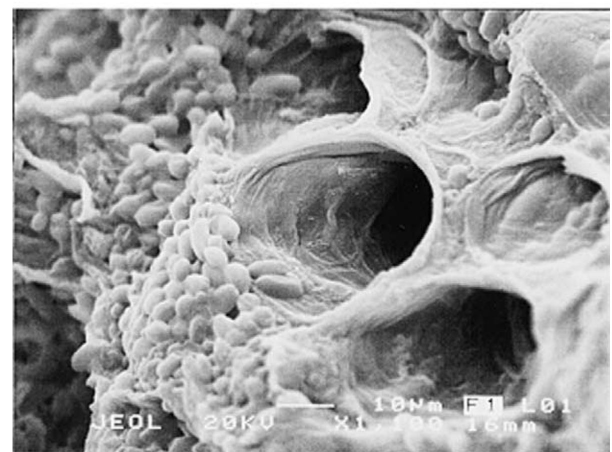


Fig. 2. Bioreactor with active fermentation using quince-immobilized yeast biocatalyst.

tion of grape must at this temperature. Wine making at 0 °C was achieved after a period of 50 days. This is very useful for the wine industry as it may be used in the scaling-up of the winemaking process. Residual sugar concentrations show that this process might be suitable for dry wine production. Likewise, wine and ethanol productivities were at least 4- or 5-fold higher than in traditional fermentations. Free cell biomass concentrations for the duration of the experiment at all temperatures ranged from 2.9 to 17.4 g/l while total and volatile acidities were similar to values in dry wines.



(a)



(b)

Fig. 3. Electron micrographs showing yeast cells proliferation within the quince biocatalyst support. A. surface cover B. impregnation of structural cavities.

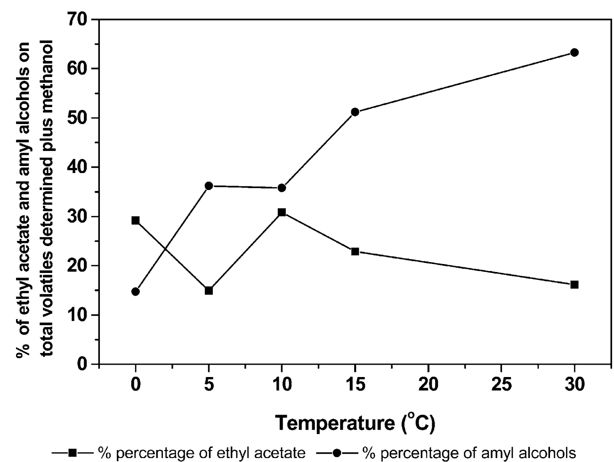


Fig. 4. Percentages of ethyl acetate and amyl alcohols in the wine product at different temperatures.

Table 2
Effect of temperature on the production of volatiles in the fermentation of grape must using immobilised yeast on quince biocatalyst

Temp (°C)	Batch	Acetaledyde (ppm)	Ethyl acetate (ppm)	Propan 1-ol (ppm)	Isobutyl alcohol (ppm)	Amyl alcohol (ppm)	Methanol (ppm)
30	17	Tr	42	19	26	233	72
30	18	27	58	20	27	265	27
30	19	9	55	13	17	155	10
30	20	27	56	20	26	263	5
30	21	17	83	19	18	294	17
30	23	16	42	15	9	159	16
30	24	1	52	16	12	197	17
30	25	6	85	20	15	238	18
30	26	Tr	30	17	11	167	36
15	27	14	105	25	20	215	13
15	28	Tr	97	30	25	225	35
15	29	22	87	29	21	189	19
15	30	28	79	26	21	194	67
15	31	20	69	18	17	154	45
10	32	Tr	113	36	27	93	19
10	33	6	95	42	27	92	20
10	34	22	91	48	26	87	6
10	35	59	62	29	24	147	Tr
5	36	11	19	16	11	94	21
5	37	9	25	5	6	60	72
5	38	28	34	13	10	35	53
0	39	31	41	8	8	23	68
0	40	23	50	5	7	23	25

Tr, traces.

3.2. Volatile by-products

3.2.1. Higher alcohols

The concentrations of higher alcohols (propanl-ol and isobutyl alcohol) were extremely low at 0–5 °C and the maximum concentration detected was 48 ppm (Table 2). The concentration of amyl alcohols decreased with the temperature decrease. At 0 °C the amount of amyl alcohols was approximately a tenth of that detectable at 30 °C. Low temperature generally led to a reduction of higher alcohol concentrations, which is considered a positive factor in product quality. Such a reduction in amyl alcohols in wines produced at low temperatures has been reported in the literature (Bardi, Koutinas, Psarianos, & Kanellaki, 1997; Etievant, 1991; Jackson, 1994; Kourkoutas, Komaitis, Koutinas, & Kanellaki, 2001).

3.2.2. Ethyl acetate

Ethyl acetate was analyzed due to its effect on the organoleptic characteristics of the wine. Concentrations up to 113 mg/l were detected but, in most cases, it was much lower (Table 2). Although some of the values of ethyl acetate concentrations were relatively high, there was no vinegar odour in the final product. On the contrary, the product had a fruity aroma and a fine taste.

3.2.3. Other volatiles

The acetaldehyde content in wines usually ranges from 13 to 40 mg/l (Longo, Velazquez, Siero, Ansado, Calo, & Villa, 1992) but may reach 75 mg/l (Koutinas & Pefanis, 1992). Low concentrations were generally detected in our product with a maximum of 59 mg/l (Table 2). Methanol, which is derived from methylated pectic substances (pectins) by the action of pectin esterase, was negligible in the wine from batch fermentation of must using the yeast immobilized on quince biocatalyst.

3.3. GC-MS analysis

As the immobilized cells show differences in their specific metabolic activities in comparison with free cells, the use of GC/MS was necessary. This analysis allowed a comparison of the aroma-related compounds present in wine produced by the immobilized biocatalyst and wines that are produced using the traditional free cell fermentation (Table 3).

The most important compounds identified by this technique were: ethanol, acetaldehyde, acetic acid ethyl ester, 2-propanone, 2-methyl-1-propanol, 3-methyl butyl acetate, 2-methyl butyl acetate, 2-methyl-1-butanol, 3-methyl-1-butanol, hexanoic acid ethyl ester, octanoic acid ethyl ester, acetic acid phenethyl ester, acetic acid, hexanoic acid, 2-ethyl-hexanoic acid, nonanoic acid, decanoic acid, undecanoic acid and 2-ethyl-1-decanol. Most of these compounds are esters known to make a positive contribution to the aroma of wines. Most of the volatile compounds detected in the product

Table 3
GC/MS headspace analysis of volatile components of wine produced using yeast immobilized on quince biocatalyst (IQB) and free cells fermentation both carried out at 15 °C

Time	Compound	Certainty of identification	
		IQB	Free cells
3.21	Acetaldehyde	a	a
3.58	2-propanone	a	a
3.99	Acetic acid ethyl ester	a	a
4.94	Ethanol	a	a
6.09	Propionic acid ethyl ester	ND	a
6.48	Acetic acid isobutyl ester	ND	a
6.63	Butyric acid ethyl ester	a	a
7.26	1-propanol	a	a
9.64	*n*-pentane	b	ND
9.73	2-methyl-1-propanol	a	a
10.53	1-butanol, 2-methyl-, acetate	b	b
10.64	1-butanol, 3-methyl-, acetate	b	b
13.98	1-butanol	ND	a
16.51	2-methyl-1-butanol	a	a
17.31	3-methyl-1-butanol	a	a
17.60	Hexanoic acid, ethyl ester	a	a
20.00	2-hexyl-1-octanol	ND	c
20.45	Acetic acid hexylester	ND	b
23.23	2-butanone,4-hydroxy acetate	ND	c
24.88	Acetic acid	a	ND
27.06	Octanoic acid ethyl ester	a	a
33.50	Decanoic acid ethyl ester	ND	b
40.02	Acetic acid, phenethyl ester	b	ND
40.85	Hexanoic acid	b	ND
43.80	2-ethyl-hexanoic acid	b	c
43.83	2-ethyl-1-decanol	c	ND
44.15	Dodecyl alchol	b	ND
44.51	*n*-pentadecanol	ND	b
45.84	myristaldehyde	c	ND
46.86	Hexadecanol	b	ND
49.54	Nonanoic acid	c	ND
52.07	Decanoic acid	c	ND
53.89	Undecanoic acid	c	ND
55.97	Benzenecarboxylic acid	c	ND
56.99	Unknown	b	ND

a = Positive identification from MS and retention times. b = Positive identification from MS data only. c = Tentative identification from mass spectra data. ND = None detected

of the immobilized cells on quince were present in traditionally produced wines. No significant changes in the aroma compounds were therefore noticeable.

With regard to the most abundant components of the volatiles in wine, a reduction of the percentage of higher alcohols and a relative increase in the percentage of ethyl acetate in the total volatiles were observed in our product particularly at low-temperatures (Fig. 4). High concentrations of ethyl acetate (>150 mg/l) contribute to the sour vinegar off-flavour in wines. However, in the present experiment, ethyl acetate concentrations were consistently lower than 150 mg/l. The recorded volatile acidity was also low (<0.26 g/l).

3.4. Sensory investigation

Preliminary sensory tests, carried out in the laboratory, indicated some improvement in aroma and taste of the wines produced by using cells immobilized on quince pieces, particularly at low temperatures, when compared to other commercially available wines (Table 4). The commercial wine had both lower scores and much more variable preference results. The sensory results, although preliminary and in need of proper statistical confirmation, have indicated that the new wines are characterised by a pleasant, soft aroma and fruity taste.

4. Conclusions

Quince-immobilized yeast biocatalyst offers a good and effective support material for alcoholic fermentation, particularly at low temperatures. Its use led to higher productivity and to a product with an improved aromatic quality, especially at the lower temperatures. The immobilization and fermentation process is simple technology that is readily available for adoption in industrial-scale winemaking.

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Table 4
Results of aroma and taste for wines produced fermenting with *Saccharomyces cerevisiae* AXAZ-1 yeast immobilized on quince biocatalyst and as free cell in comparison to a commercial, locally-available wine expressed as an average score plus standard deviation

Test	*S. cerevisiae*		Commercial wine
	Quince	Free cells	
Aroma	7.6±1.07	6.9±1.20	6.8±1.40
Taste	6.2±0.92	6.3±2.06	4.3±1.57

0: Unacceptable, 10: Excellent.

References

Ageeva, N. M., Merzhanian, A. A., & Sobolev, E. M. (1985). Effect of yeast adsorption on the functional activity of the yeast cells and composition of wine. *Mikrobiologiya*, *54*, 830–834.

Argiriou, T., Kalliafas, A., Psarianos, K., Kana, K., Kanellaki, M., & Koutinas, A. A. (1992). New alcohol resistant strains of S. cerevisiae species for potable alcohol production using molasses. *Applied Biochemistry & Biotechnology*, *36*, 153–161.

Bakoyianis, V., Kanellaki, M., Kalliafas, A., & Koutinas, A. A. (1992). Low temperature wine making by immobilized cells on mineral Kissiris. *Journal of Agricultural & Food Chemistry*, *40*(7), 1293–1296.

Bardi, E. P., Koutinas, A. A., Psarianos, C., & Kanellaki, M. (1997). Volatile by-products formed in low-temperature wine-making using immobilized yeast cells. *Process Biochemistry*, *32*(7), 579–584.

Bardi, E. P., & Koutinas, A. A. (1994). Immobilization of yeast on delignified cellulosic material for room temperature and low-temperature wine-making. *Journal of Agricultural & Food Chemistry*, *42*(1), 221–226.

Cabezudo, M. D., Gorostiza, E. F., Herraiz, M., Fernadez-Biarange, J., Garcia-Dominguez, J. A., & Molera, M. J. (1978). Mixed columns made to order in gas chromatography. IV. Isothermal selective separation of alcoholic and acetic fermentation products. *Journal of Chromatographic Science*, *16*, 61–67.

Etievant, X.P. (1991). Wine. In *Volatile compounds in foods and beverages*, pp. 495. Henk Maarse (Ed).

Jackson, S.R. (1994). In *Wine science*. *Principles and applications*, pp.185. Academic Press.

Fumi, M., Trioli, G., & Colagrande, O. (1987). Preliminary assessment on the use of immobilized yeast cells in sodium alginate for sparkling wine processes. *Biotechnology Letters*, *9*(5), 339–342.

Klein, J., & Kressdorf, B. (1983). Improvement of productivity and efficiency in ethanol production with Ca-alginate immobilized Z. mobilis. *Biotechnology Letters*, *5*(8), 497–502.

Kourkoutas, Y., Komaitis, M., Koutinas, A. A., & Kanellaki, M. (2001). Wine production using yeast immobilized on apple pieces at low and room temperatures. *Journal of Agricultural & Food Chemistry*, *49*, 1417–1425.

Koutinas, A.A., & Pefanis. S. (1992). *Biotechnology of foods and drinks,* pp.74. University of Patras (Ed), Patras, Greece.

Longo, E., Velazquez, J. B., Siero, C., Ansado, C. J., Calo, P., & Villa, T. G. (1992). Production of higher alcohols, ethyl acetate, acetaldehyde and other compounds by *Saccharomyces cerevisiae* wine strains isolated from the same region (Salnes NW Spain). *World Journal of Microbiology & Biotechnology*, *8*, 539–541.

Margaritis, A., & Merchant, F. J. A. (1984). Advances in ethanol production using immobilized cell systems. *CRC Crit*. *Reviews in Biotechnology*, *1*(4), 339–393.

Mori, S. (1987). Fruit wine or sake manufacture by bioreactor. *Japanese Kokai Tokyo Koho JP 62*, *61*, 577 (March 18).

Nakanishi, K., & Yokotsuka, K. (1987). Fermentation of white wine from Koshu grape using immobilized yeast. *Nippon Shokuhin Kogyo Gakkaishi*, *34*(6), 362–369.

Shimobayashi, Y., & Tominaga, K. (1986). Application of biotechnology in the food industry. I. Brewing of white wine by a bioreactor. *Hokaidoritsu Kogyo Shikenjo Hokoku*, *285*, 199–204.

Stewart, G. G., & Russell, I. (1986). One hundred years of yeast research and development in the brewing industry. *Journal of the Institute of Brewing*, *92*, 537–558.

Zoeklein, B., Fugelsang, K., Gump, B., & Nury, F. (1990). Volatile Acidity. In *Production Wine Analysis*, pp. 105–110. Van Norstrand Reinhold: New York.