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Red Wine Making by Immobilized Cells and Influence on Volatile Composition

ARGYRIS TSAKIRIS,[†] VASILLIOS SIPSAS,[‡] ARGYRO BEKATOROU,[‡] ATHANASIOS MALLOUCHOS,[§] AND ATHANASIOS A. KOUTINAS*,[‡]

Department of Oenology and Spirits Technology, Faculty of Food Technology and Nutrition, Technological Educational Institution of Athens, Ag. Spiridonos Str, Egaleo, GR-12210, Athens, Greece, Food Biotechnology Group, Department of Chemistry, University of Patras, GR-26500, Patras, Greece, and Laboratory of Food Chemistry, Department of Food Chemistry and Biotechnology, Agricultural University of Athens, Iera Odos 75, GR-118 55, Athens, Greece

Red wine making using yeast cells immobilized in two types of raisin berries, at various temperatures (6−30 °C), was studied. A modification of the batch bioreactor was used to separate the grape skins used for color extraction from the biocatalyst and the fermenting grape must. The evaluation of the immobilized biocatalysts was made on terms of productivity and organoleptic quality, including color intensity and formation of volatiles. The immobilized cells were found capable of low-temperature wine making, producing red wines containing more than 11% v/v alcohol in 8 days at 6 °C. The quality of wines was examined by gas chromatography (GC) and GC-MS analysis and sensory evaluation. Higher alcohol concentrations were decreased, and ethyl acetate concentrations increased by the drop of temperature. Many esters, alcohols, carbonyls, and miscellaneous compounds were identified in wines produced by immobilized cells, revealing no significant qualitative differences as compared to wines produced by free cells. The sensory evaluation showed that the best red wine was produced at 6 °C.

KEYWORDS: Red wine making; immobilization; bioreactor; raisins; volatiles; GC; GC-MS

INTRODUCTION

The use of immobilized cells in wine making is a rapidly expanding research area with potential advantages as compared to free cell systems. The purpose for using immobilized cells in wine making is to increase fermentation productivity (*1*−*3*), to improve quality through low-temperature fermentation (*3*, *4*), or to produce sparkling wines (*1*, *5*). Commercial preparations of selected yeasts immobilized on alginates (*6*, *7*) or confined by microfiltration membranes (*8*) are available for sparkling wine production for secondary fermentation in the bottle according to the traditional Champagne method. Furthermore, cell immobilization has also been applied for malolactic fermentation of wine (*9*, *10*) and distillates production (*11*, *12*). However, most research efforts concern white wine making. This may be attributed to the technical difficulties rising from the simultaneous extraction of colorings and the fermentation using immobilized cells. On the other hand, for successful industrial application of immobilized cells in wine making, the proposed support must be of food grade purity, abundant in nature, and of low cost. To satisfy these prerequisites, supports such as delignified cellulosic materials (*3*), gluten pellets (*13*), and fruit pieces (*4*, *14*) have been proposed for ambient and low-temperature wine making.

Raisins (dried seedless grapes) are an abundant and cheap product, usually available in most wineries, as their extracts are commonly used in wine making and potable alcohol production. Greece, like the United States, Turkey, and other countries, is a large raisin producer. Raisins include two distinct products, the black currants, which are produced exclusively in Greece, and the golden *Sultana* raisins produced also in other countries. The production of red wine using cells entrapped in raisin berries has not been reported, and it is a very attractive perspective, due to the full compatibility of this support with wine. Therefore, the aim of the present study was to investigate the suitability of immobilized cells entrapped in raisin berries for alcoholic fermentation and red wine making at various temperatures, as well as the influence of the immobilized biocatalyst on the volatile composition of the produced red wines.

MATERIALS AND METHODS

Yeast Strains and Media. Dried *Saccharomyces cerevisiae* strain Uvaferme 299, which is widely used in industrial wine making practice, and the alcohol resistant and cryotolerant *S. cerevisiae* AXAZ-1, isolated from Greek grapes (*15*), were used in the present study. Both strains

* To whom correspondence should be addressed. Tel: 0030-2610-997104. Fax: 0030-2610-997105. E-mail: a.a.koutinas@upatras.gr.
[†] Technological Educational Institution of Athens.
[‡] University of Patras.
[§] Agricultural University of Athens.

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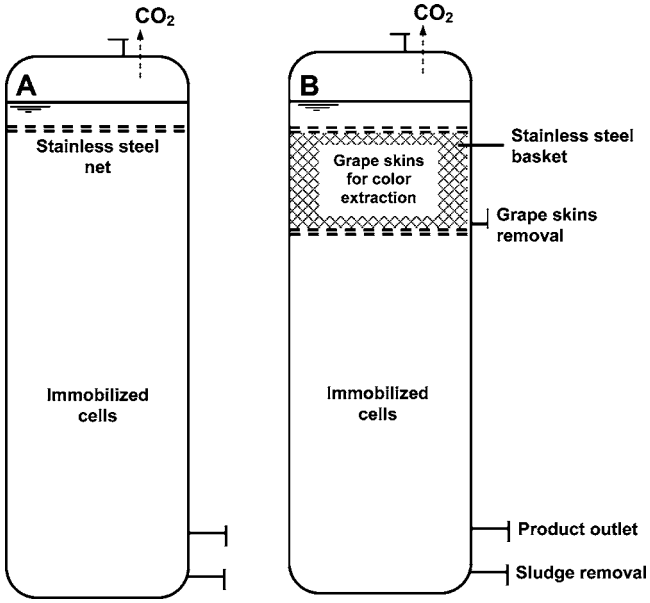

Figure 1. Bioreactor diagrams for white (**A**) and red (**B**) wine making.

were grown on culture medium consisting of 4 g yeast extract/L, 1 g $(NH_4)_2SO_4$/L, 1 g KH_2PO_4/L, 5 g $MgSO_4 \cdot 7H_2O$/L, and 40 g glucose monohydrate/L and harvested at 4000 rpm for 10 min. Glucose media of the above composition of nutrient salts were used for cell immobilization and fermentation experiments using free or immobilized cells. All media were sterilized at 130 °C and at 1−1.5 atm for 10 min. Must from red grapes of the Greek variety *Agiorgitiko* was used after adjustment of density at 12 °Be (approximately 200 g fermentable sugar/L).

Preparation of the Support and Immobilization of Cells. An amount of 100 g of golden *Sultana* raisins (dried white grapes, Achaia region, Greece) or *Corintiaki* black currants (dried black grapes, Corinth region, Greece) was placed in a 1 L glass cylinder and covered with 800 mL of 120 g glucose/L synthetic medium, in which 16 g of yeast had been suspended. The system was allowed to ferment for about 8 h, to a stable 0−1 °Be density. The raisin berries with entrapped cells were washed 2−3 times with 100 g glucose/L medium and used for batch fermentations of glucose and grape must.

Bioreactor. A 1.5 L glass tower bioreactor was used as shown in **Figure 1**. An opening at the top of the bioreactor allowed grape must and support filling and CO_2 escape, while two openings at the bottom were used for wine and sludge removal and for emptying the bioreactor. For the glucose fermentation experiments, a stainless steel net was adjusted near the top of the bioreactor to hold the raisin berries into the fermenting liquid (**Figure 1A**). In the case of red wine making, a stainless steel, basket-shaped net construction was adjusted in the bioreactor containing skins from red grapes for color extraction (**Figure 1B**), to avoid mixing the biocatalyst with the grape skins.

Fermentations. An amount of 100 g of the immobilized biocatalyst (*S. cerevisiae* AXAZ-1 cells entrapped in black currants or golden raisins) was introduced into 300 mL of 100 g glucose/L medium in the bioreactor (**Figure 1A**). The total bioreactor volume was 450 mL. Ten repeated batch fermentations were performed at 30 °C. After the end of each batch, the biocatalyst was washed with 400 mL of fresh 100 g glucose/L medium and was used for the next fermentation batch. During these fermentation batches, sugars contained in raisins were gradually extracted and fermented. After the 10th fermentation batch, all sugars from raisins were removed, which was confirmed by the fact that within 1 h time intervals from batch to batch no increase in the °Be density of the fermenting liquid was observed. Then, the immobilized biocatalyst was washed twice with 400 mL of grape must to remove free cells and residual glucose medium. The metal net construction was attached into the bioreactor (**Figure 1B**), holding skins from 300 g of red grapes (variety *Agiorgitiko*), obtained after crashing the grapes and quickly draining to remove the contained must and avoid loss of pigments. A 300 mL amount of fresh grape must (12 °Be initial density) was added, and the grape skins were totally covered with must. The total bioreactor volume before and after the addition of grape skins was 450 and 750 mL, respectively. Repeated fermentation batches followed reducing the temperature successively from 30 to 6 °C. Each time, the biocatalyst was washed with 400 mL of fresh grape must. Fresh grape skins for color extraction were used in each batch. When each fermentation batch was completed, the produced wine was collected and immediately analyzed for ethanol, residual sugar, color intensity (CI), free cell biomass, total acidity, volatile acidity, and volatiles. Red wine making was also performed at 25 °C using cells of the commercial strain *S. cerevisiae* Uvaferme 299, free and immobilized on golden raisins.

Determination of Ethanol, Residual Sugar, and Acidity. Fermentation kinetics were performed by measuring the °Be density at various time intervals. Residual sugar and ethanol were determined on a Shimadzu LC-9A HPLC system. A Shim-pack (SCR-101 N) column, a refractive index detector, three times distilled and filtered water as the mobile phase (0.8 mL/min), and 1-butanol (0.05% v/v) as internal standard were used. The column temperature was 60 °C. The sample dilution was 1% v/v, and the injection volume was 40 μL. Ethanol productivity was calculated as grams of ethanol per liter liquid volume produced per day. Wine productivity was calculated as grams of wine per liter total volume produced per day. CI of red wines was determined as the sum of absorbance at 420, 520, and 620 nm (*16*) on a UV−vis Milton Roy Com-Spectronic 20D spectrophotometer. Water was used as a blank. Total acidity of the wines (expressed as g tartaric acid/L) was determined by titration with 0.1 N NaOH. Volatile acidity (expressed as g acetic acid/L) was determined by titration with 0.1 N NaOH of the distillates obtained after steam distillation of the wine samples (*17*). The standard deviation for ethanol was $\leq \pm 0.3$; for residual sugar, $\leq \pm 0.2$; for color, $\leq \pm 0.1$; for total acidity, $\leq \pm 0.1$; and for volatile acidity, $\leq \pm 0.2$.

Determination of Volatiles. Volatiles were determined by means of gas chromatography (GC) 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% Bis(2-ethylhexyl) sebacate, with N_2 as the carrier gas (20 mL/min) and a FID detector. The injection port and detector temperatures were 210 °C, and the column temperature was 70 °C. The internal standard was 1-butanol (0.1% v/v). Samples of 4 μL of wine were injected directly in the column, and the concentrations of the above compounds were determined using both the standard curves and the internal standard method. Methanol and ethanol were determined on a similar Shimadzu GC-8A system consisting of 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 at 140−180 °C (10 °C/min). 1-Butanol (0.1% v/v) was used as the internal standard, and samples of 2 μL of wine were injected directly in the column. The standard deviations for volatiles were $\leq \pm 0.5$ for acetaldehyde, $\leq \pm 5$ for ethyl acetate, $\leq \pm 2$ for 1-propanol, $\leq \pm 2$ for isobutyl alcohol, $\leq \pm 12$ for amyl alcohols, and $\leq \pm 9$ for methanol.

GC-MS Analysis. The volatiles in a sample of fresh must and two samples of wines produced by entrapped cells on black currants and golden raisins, at 22 °C, were analyzed by means of GC-MS. Volatiles were isolated by the solid phase microextraction method (SPME). The fiber used for the absorption of volatiles was a PDMS-DVB (Supelco, U.S.A.). The conditions of headspace SPME sampling used were as follows: 5 mL of liquid sample and 1.5 g of NaCl were transferred into a 10 mL screw-capped glass vial with a rubber septum. The contents were magnetically stirred for 5 min at 40 °C, and the fiber was exposed to the headspace for 45 min. The length of the fiber in the headspace was kept constant. Desorption of volatiles took place in the injector of the gas chromatograph in the splitless mode, at 240 °C for 5 min. Before each analysis, the fiber was exposed to the injection port for 10 min to remove any volatile contaminants.

GC-MS analysis was performed on a Fisons 8000 series gas chromatograph (model 8060) coupled to a Fisons MD-800 quadrupole mass spectrometer. Helium was used as the carrier gas (2.0 mL/min). Separation of compounds was performed on a Chrompack WCOT fused silica column (CP-Wax 52CB, 60 m × 0.32 mm, DF = 0.25 μm). The

Table 1. Kinetic Parameters of the Repeated Batch Fermentations of 100 g Glucose/L Medium and Must (12 °Be) for Red Wine Making Using *S. cerevisiae* AXAZ-1 Immobilized on Black Currants and Golden Raisins at Various Temperatures

	temp (°C)	batch	initial density (°Be)	fermentation time (h)	free cells (g/L)	residual sugar (g/L)	ethanol (% v/v)	ethanol productivity (g/L/day)	wine productivity (g/L/day)
				black currants					
glucose	30	1–10	5.98 ± 0.1	35.6 ± 0.7					
grape must	30	11–15	12.0 ± 0.1	48.0 ± 0.0	0.5 ± 0.1	23.5 ± 6.6	11.5 ± 0.4	45.2 ± 1.6	200.0 ± 0.0
grape must	22	16–23	12.0 ± 0.1	90.0 ± 0.0	1.7 ± 0.3	26.9 ± 7.3	11.6 ± 0.9	24.4 ± 2.0	107.0 ± 0.0
grape must	6	24–26	12.0 ± 0.0	183.3 ± 5.8	0.5 ± 0.0	30.0 ± 1.3	11.6 ± 0.4	12.3 ± 0.6	52.3 ± 1.2
				golden raisins					
glucose	30	1–10	6.0 ± 0.1	38.8 ± 1.0					
grape must	30	11–15	12.0 ± 0.1	48.0 ± 0.0	0.4 ± 0.1	24.0 ± 4.1	11.1 ± 0.4	44.0 ± 2.0	200.0 ± 0.0
grape must	22	16–23	12.0 ± 0.1	91.5 ± 2.8	1.6 ± 0.3	29.4 ± 9.6	11.3 ± 0.7	22.5 ± 1.3	104.5 ± 2.8
grape must	6	24–26	12.0 ± 0.0	190.0 ± 0.0	0.6 ± 0.1	30.7 ± 1.6	11.7 ± 0.6	11.7 ± 0.6	50.0 ± 0.0

Table 2. Color, Acidity, and Volatiles of the Red Wines Produced by Repeated Batch Fermentations of Must with *S. cerevisiae* AXAZ-1 Immobilized on Black Currants and Golden Raisin Berries at Various Temperatures

temp (°C)	batch	color	volatile acidity (g acetic acid/L)	total acidity (g tartaric acid/L)	acetaldehyde (mg/L)	ethyl acetate (mg/L)	1-propanol (mg/L)	isobutyl alcohol (mg/L)	amyl alcohols (mg/L)	methanol (mg/L)	total volatiles (methanol excluded) (mg/L)
					black currants						
30	11–15	8.0 ± 0.1	0.3 ± 0.0	5.5 ± 0.4	24.8 ± 3.5	23.2 ± 5.3	8.2 ± 4.0	30.6 ± 7.3	260.0 ± 7.1	111.0 ± 13.2	346.8 ± 10.8
22	16–23	7.6 ± 0.0	0.3 ± 0.0	5.5 ± 0.3	34.9 ± 12.7	26.5 ± 6.5	21.0 ± 7.5	34.1 ± 10.8	206.4 ± 16.7	92.3 ± 37.0	322.9 ± 35.4
6	24–26	7.3 ± 0.0	0.3 ± 0.0	4.3 ± 0.1	37.0 ± 7.8	61.0 ± 11.1	13.0 ± 4.6	20.7 ± 1.5	136.7 ± 6.7	73.7 ± 7.0	268.3 ± 4.2
					golden raisins						
30	11–15	7.7 ± 0.0	0.3 ± 0.0	5.5 ± 0.4	36.6 ± 11.9	23.6 ± 4.5	12.4 ± 7.1	64.4 ± 15.5	239.8 ± 18.5	139.4 ± 24.8	376.8 ± 33.6
22	16–23	7.4 ± 0.0	0.3 ± 0.0	5.5 ± 0.3	33.1 ± 8.6	33.1 ± 19.6	25.4 ± 10.5	66.8 ± 15.2	226.0 ± 24.0	86.5 ± 38.0	384.4 ± 44.7
6	24–26	7.0 ± 0.0	0.3 ± 0.0	4.3 ± 0.1	35.3 ± 6.0	83.3 ± 5.5	24.0 ± 4.4	36.3 ± 4.2	142.3 ± 10.6	67.7 ± 4.2	321.3 ± 24.7

oven temperature was programmed at 35 °C for 5 min, and then, it was raised to 60, 200, and 250 °C with a rate of 2.0, 5.0, and 25.0 °C/min, respectively. It was held at 250 °C for 10 min. The injector, ion source, and interface temperatures were set at 240, 200, and 250 °C, respectively. Electron impact mass spectra were recorded at 70 eV of ionization energy in the 29−400 *m*/*z* mass range. Identification of compounds was done by comparing the retention times and MS data with those of standard compounds and by MS data obtained from Wiley and NIST libraries.

Electron Microscopy. Pieces of the immobilized biocatalysts (raisin berries with immobilized *S. cerevisiae* AXAZ-1 cells) were washed with deionized water and dried overnight at 30 °C. The samples were coated with gold in a Balzers SCD 004 Sputter Coater for 3 min and examined in a JEOL model JSM-6300 scanning electron microscope.

Sensory Evaluation. Samples of wines produced at 22 and 6 °C using *S. cerevisiae* AXAZ-1 cells immobilized in golden raisins and black currants were tested immediately after preparation by 10 nontrained testers (consumers), according to a preliminary taste test protocol (*11*), based on a preference, 0−10 scale (10, fine; 9, excellent; 8, very good; 7, good; 6, might be good; 5, medium; 4, acceptable; 3, might be bad; 2, bad; 1, very bad; 0, unacceptable). Two samples of the same wine were tested. Testers evaluated separately the aroma and taste of wines and compared them with samples of wines produced by free *S. cerevisiae* AXAZ-1 cells and a locally available commercial red wine (*Agiorgitiko*). The results are given in **Table 5** as average scores plus standard deviations.

RESULTS AND DISCUSSION

Alcoholic fermentation of glucose and red wine making using yeast cells immobilized in raisin berries, at various temperatures, was studied. A modification of the batch bioreactor was used in order to separate the grape skins used for color extraction from the biocatalyst and the fermenting grape must. The evaluation of the immobilized biocatalyst was made in terms of productivity and organoleptic quality, including CI and formation of volatiles. Two types of raisins were tested as supports for cell immobilization, the black currants and the golden (*Sultana*) raisins. To confirm cell immobilization on raisin berries and suitability of the immobilized biocatalyst for alcoholic fermentation, 10 repeated batch fermentations of glucose were carried out at 30 °C. Fermentation times were low and stable from batch to batch (35−37 h for cells immobilized in black currants and 38−40 h for cells immobilized in golden raisins) showing operational stability of the system and fast adaptation to the fermentation process (**Table 1**). Immobilization of yeast on the raisin berries was also confirmed by electron microscopy, showing yeast cells attached on the external raisin surface and entrapped in the interior, which was also obvious by the sludge of yeast cells collected inside the berry.

Repeated fermentation batches of grape must (initial density 12 °Be) were subsequently performed for red wine making (**Tables 1** and **2**). The use of two types of raisins was decided due to the fact that the two varieties differ in composition as well as in the mechanical properties of skins, which are related to the stability of the biocatalyst. In both cases, the immobilized biocatalysts retained their operational stability from batch to batch, even at low temperatures, and fermented grape must in times that ranged from 4 days at 22 °C to 8 days at 6 °C. Residual sugar (12−37 g/L) and ethanol concentrations (10.6−12.9% v/v) in all of the produced red wines were in the same levels showing the possibility for red wine making using both the tested biocatalysts in a wide range of temperatures (6−30 °C) (**Table 1**). Wine (53−200 g/L/day) and ethanol productivities (12−47 g/L/day) were high and were reduced as the temperature decreased. The final free cell concentrations in

Table 3. Kinetic Parameters of the Repeated Batch Fermentations of Must by Free and Immobilized Cells of *S. cerevisiae* Uvaferme 299 Immobilized on Golden Raisin Berries at 25 °C and Major Volatiles of the Produced Red Wines

batch	initial density (°Be)	color	ethanol (% v/v)	ethanol product (g/L/day)	wine product (g/L/day)	acetaldehyde (mg/L)	ethyl acetate (mg/L)	1-propanol (mg/L)	isobutyl alcohol (mg/L)	amyl alcohols (mg/L)
immobilized cells										
1–4	11.3 ± 0.0	7.8 ± 0.1	13.6 ± 0.1	27.0 ± 0.0	146.0 ± 0.0	24.5 ± 9.7	30.5 ± 16.4	28.3 ± 12.4	22.3 ± 6.6	147.8 ± 12.7
free cells										
1–4	12.8 ± 0.0	8.0 ± 0.1	13.6 ± 0.1	23.0 ± 0.0	195.0 ± 0.0	27.3 ± 12.1	39.5 ± 18.9	25.5 ± 10.7	22.0 ± 2.2	159.8 ± 16.0

wines produced with AXAZ-1 cells immobilized on raisins were low (0.3−2.1 g/L), and the wines had a fine clarity after the end of fermentation. Likewise, volatile acidity (0.26−0.34 g acetic acid/L) and total acidity (4.2−6.2 g tartaric acid/L) were within the accepted limits for commercial products (**Table 2**). Finally, the new wines had a CI (7.0−8.0) about similar to commercial wines produced by the same grape variety. For both supports, color extraction was reduced by approximately 0.7 units by the reduction of temperature from 30 to 6 °C, a decrease negligible to affect feasibility of red wine making at low temperatures.

Fermentations were performed in a similar way, using a commercial strain (*S. cerevisiae* Uvaferme 299) free and immobilized on golden raisin berries, to evaluate its suitability and the possibility of commercial applications (**Table 3**). Residual sugar was 0.0 g/L (100% conversion) at 25 °C, and volatile acidity (0.29−0.33 g acetic acid/L) and total acidity (4.4−6.0 g tartaric acid/L) were within desired limits. Generally, the *S. cerevisiae* Uvaferme 299, free or immobilized, did not show any significant differences regarding fermentation kinetics, volatiles, and CI as compared to the *S. cerevisiae* AXAZ-1 strain. However, according to the results of a previous study (*18*), *S. cerevisiae* Uvaferme 299 was not found suitable for low-temperature fermentation; therefore, low-temperature wine making (below 10 °C) is not possible using this strain.

Volatiles. Analysis by GC showed that the variety of raisins did not affect the concentration of the major volatiles (**Table 2**). However, the amounts of higher alcohols produced by both supports were reduced, while the ethyl acetate concentrations were increased as the temperature decreased. Furthermore, the percentages of ethyl acetate on total volatiles (methanol excluded) were increased while those of amyl alcohols were reduced as the temperature dropped. These results are in accordance with previous studies investigating low-temperature wine making using *S. cerevisiae* AXAZ-1 cells immobilized on gluten pellets, delignified cellulosic material, and apple pieces (*3*, *4*, *13*). The reduction of amyl alcohols and the increase of ethyl acetate concentrations with the drop of temperature have been related with improvement of organoleptic quality (*19*, *20*) and is one of the advantages of wine making at low temperatures. Methanol concentration was decreased by the drop of temperature (**Table 2**). This reduction may be attributed to the consequent decrease in pectinmethylesterase activity or to fast hydrolysis of pectin substances during the first fermentation batches as observed in previous studies (*21*).

GC-MS Analysis. The identification of volatiles by GC-MS was done to evaluate the differences between red wines produced at 22 °C by cells immobilized on the two types of raisins and free cells, as well as the contribution of grape must on the aromatic profile of the product. A SPME technique was employed, which gives more peaks than the common headspace technique. In both red wines produced by cells entrapped in black currants and golden raisins, a large number of compounds was identified, most of which with reliability (**Table 4**). Generally, more esters and alcohols were identified in the wine samples, and the opposite was observed for terpenoids and many carbonyl compounds, which were found only in grape must. Totally, 41 esters in 132 compounds were identified in the wine samples produced by immobilized cells. Most of them were acetic esters of higher alcohols and ethyl esters of fatty acids and, specifically, the ethyl esters of the C2−C16 fatty acids of the aliphatic series and the acetate esters of the C4−C8 higher alcohols of the aliphatic series. Some of them were not identified in grape must and were obviously produced by yeast metabolism. The main acids identified were the acetic, hexanoic, 3-hexenoic, 2-ethyl-hexanoic, octanoic, nonanoic, and decanoic acids. Alcohols identified in both wines produced by immobilized cells included the C2−C10 primary alcohols of the aliphatic series. Fusel alcohols (isobutanol and amyl alcohols) were in low levels in both wine samples (**Table 2**). Carbonyl compounds identified in all samples included mainly acetaldehyde and the C7−C13 primary aldehydes of the aliphatic series. A number of miscellaneous compounds, some groups of which are known to contribute to the complexity of wine aroma (*16*), such as acetals, terpenoids, norisoprenoids, phenolic acid derivatives, and furans, were also identified in the wine samples. Finally, traces of sulfur compounds such as 3-(methylthio)-1-propanol were also detected. The presence of such compounds demands special attention, although their effect on wine flavor is less intense than the effect of hydrogen sulfide or sulfur dioxide. It should be noted that various compounds that are reported in the literature to strongly affect wine flavor gave relatively big peaks, indicating high concentrations in the wines produced with immobilized cells. Specifically, distinctive was the big peak for 2-phenylethanol, which is a compound with a characteristic rose aroma, as well as the relatively high peak of β-damascenone present in both wine samples.

From the results shown in **Table 4** and data in the literature (*4*, *14*), it is obvious that the impact of fermentation on the formation of aroma compounds is big, although immobilization itself does not seem to alter the qualitative composition of wine aroma as compared to wines produced by free cells, as shown in previous works (*4*, *21*, *22*). The differences revealed by the sensory evaluation between wines produced by immobilized and free cells may be attributed to quantitative differences of the aromatic compounds, caused either by altered metabolism of the immobilized cells or by transfer of aromatic compounds from raisins to the product.

Sensory Evaluation. The sensory evaluation of wines at 22 and 6 °C showed that the testers (nontrained consumers) had a slightly bigger preference for wines produced by cells entrapped in black currants, as far as both aroma and taste are concerned (**Table 5**). Also, wines produced at lower temperatures (6 °C) were preferred than those produced at ambient temperatures. This can be attributed to the reduction of amyl alcohols, which are off-flavor compounds, at lower temperatures and therefore

Table 4. Volatile Compounds Identified in Grape Must (M) and in the Red Wines Produced by *S. cerevisiae* AXAZ-1 Immobilized on Black Currants (BC) and Golden Raisin Berries (GR) at 22 °C

R_t (min)	compound	reliability of identification	BC	GR	M	R_t (min)	compound	reliability of identification	BC	GR	M
					esters						
5.210	ethyl acetate	*a*	+	+	+	31.166	octyl acetate	*b*	+	+	–
7.394	ethyl propanoate	*a*	+	+	+	31.967	ethyl 4-octenoate	*b*	+	+	–
9.427	isobutyl acetate	*a*	+	+	–	33.017	ethyl nonanoate	*a*	+	+	–
10.494	ethyl butanoate	*a*	+	+	+	35.867	ethyl decanoate	*a*	+	+	+
12.228	3-methylbutyl formate	*b*	+	+	–	36.418	3-methylbutyl octanoate	*b*	+	+	–
15.095	3-methylbutyl acetate	*a*	+	+	+	36.818	diethyl butanedioate	*b*	+	+	+
15.745	ethyl pentanoate	*a*	+	+	–	37.251	ethyl 9-decenoate	*b*	+	+	–
19.047	methyl hexanoate	*a*	+	+	+	38.538	ethyl undecanoate	*b*	+	+	–
19.947	2-methyl-2-propenoic acid 1,2-ethanediyl ester	*b*	+	+	–	39.668	ethyl phenylacetate	*b*	+	+	+
21.731	ethyl hexanoate	*a*	+	+	+	39.935	ethyl 3-hydroxybutanoate	*b*	+	+	–
22.348	methyl 4-hexenoate or 3-hexenoate	*b*	+	+	–	40.402	2-phenylethyl acetate	*a*	+	+	+
23.631	hexyl acetate	*a*	+	+	–	40.769	isopropyl dodecanoate	*b*	+	+	+
24.631	ethyl 3-hexenoate	*b*	+	+	+	40.985	ethyl dodecanoate	*a*	+	+	+
26.199	ethyl heptanoate	*a*	+	+	+	41.452	3-methylbutyl decanoate	*b*	+	+	–
26.531	ethyl 2-hydroxypropanoate	*a*	+	+	+	41.736	2-methylpropanoic acid, 2-ethyl-1-propyl-1,3-propanediyl ester	*b*	+	+	+
27.716	heptyl acetate	*b*	+	+	–	45.320	isopropyl myristate	*b*	+	+	–
28.298	methyl octanoate	*a*	+	+	–	45.603	ethyl tetradecanoate	*a*	+	+	+
29.033	butyl 3-hexenoate	*b*	+	+	–	47.220	ethyl cinnamate	*b*	+	+	–
29.866	ethyl octanoate	*a*	+	+	+	48.704	1-methylethyl hexadecanoate	*b*	+	+	+
30.383	methyl 2,4-hexadienoate	*b*	+	+	+	48.971	ethyl hexadecanoate	*a*	+	+	+
30.682	3-methylbutyl hexanoate	*b*	+	+	–						
					organic acids						
30.516	acetic acid	*a*	+	+	+	43.369	2-ethyl hexanoic acid	*b*	+	+	+
33.933	2-methylpropanoic acid	*b*	+	+	–	45.803	octanoic acid	*a*	+	+	+
36.718	3-methylbutanoic acid	*b*	+	+	–	47.403	2,4-hexadienoic acid	*b*	+	+	+
41.100	hexanoic acid	*a*	+	+	+	47.670	nonanoic acid	*b*	+	+	+
42.652	3-hexenoic acid	*b*	+	+	+	49.254	decanoic acid	*b*	+	+	+
					alcohols						
5.793	ethanol	*a*	+	+	+	32.919	2,3-butanediol	*a*	+	+	–
10.578	1-propanol	*a*	+	+	+	33.250	linalool	*a*	+	+	+
14.062	isobutanol	*a*	+	+	+	33.516	1-octanol	*a*	+	+	+
16.729	1-butanol	*a*	+	+	+	34.000	1,3-butanediol	*b*	+	+	–
20.663	amyl alcohols	*a*	+	+	+	34.851	*p*-menth-1-en-4-ol	*b*	–	–	+
22.448	3-methyl-3-buten-1-ol or 3-pentenol	*b*	+	+	–	35.034	hotrienol	*b*	–	–	+
22.581	1-pentanol	*a*	+	+	+	35.234	2-(2-ethoxyethoxy)ethanol	*b*	+	+	+
25.365	4-methyl-1-pentanol	*a*	+	+	+	36.334	nonanol	*b*	+	+	+
25.632	2-heptanol	*b*	+	+	+	36.435	2-furanmethanol	*b*	–	–	+
25.881	3-methyl-1-pentanol	*a*	+	+	+	37.351	*a*-terpineol	*b*	+	+	+
26.882	1-hexanol	*a*	+	+	+	38.935	1-decanol	*b*	+	+	+
28.014	(*Z*)-3-hexen-1-ol	*b*	+	+	+	39.018	citronellol	*b*	+	+	+
29.265	2-octanol	*b*	+	+	–	39.868	nerol	*b*	+	+	+
30.266	7-octen-4-ol	*b*	+	+	+	42.586	2-phenylethanol	*a*	+	+	–
30.433	heptanol	*b*	+	+	+	43.919	2-ethyl-1-decanol	*b*	+	+	+
30.700	6-methyl-5-hepten-2-ol	*b*	+	+	+	44.720	phenol	*b*	+	+	+
31.533	2-ethyl-1-hexanol	*b*	+	+	+	49.404	farnesol	*b*	+	+	+
					carbonyl compounds						
3.193	acetaldehyde	*a*	+	+	+	29.516	2-octenal	*b*	+	+	+
10.995	hexanal	*b*	–	–	+	31.883	decanal	*b*	+	+	+
18.912	heptanal	*b*	+	+	+	32.617	benzaldehyde	*b*	+	+	+
23.698	cyclohexanone	*b*	–	–	+	32.934	2-nonenal	*b*	–	–	+
24.031	3-hydroxy-2-butanone (acetoin)	*a*	+	+	–	34.967	undecanal	*b*	+	+	+
24.281	octanal	*b*	+	+	+	36.017	2-decenal	*b*	+	+	+
26.298	6-methyl-5-hepten-2-one	*b*	+	+	+	36.085	benzeneacetaldehyde	*b*	+	+	+
27.115	4-hydroxy-4-methyl-2-pentanone	*b*	+	+	+	36.251	acetophenone	*b*	+	+	+
28.215	2-nonanone	*b*	+	+	+	37.768	dodecanal	*b*	+	+	+
28.399	nonanal	*b*	+	+	+	45.020	2-dodecanone	*b*	+	+	+
29.349	2-decanone	*b*	+	+	–	45.237	tridecanal	*b*	+	+	+

Table 4. Continued

R_t (min)	compound	reliability of identification	BC	GR	M	R_t (min)	compound	reliability of identification	BC	GR	M
					miscellaneous compounds						
5.277	1,1-diethoxy ethane	*b*	+	+	–	32.534	bornylene (2-bornene)	*b*	+	+	+
12.295	1,1-diethoxy-3-methyl butane (isovaleraldehyde diethyl acetal)	*b*	+	+	–	35.751	unknown peak		–	–	+
12.679	2-ethoxytetrahydro-2H-pyran	*b*	+	+	–	37.834	3-(methylthio)propanol	*b*	+	+	–
13.528	1-(1-ethoxyethoxy)pentane (acetaldehyde ethylamyl acetal)	*b*	+	+	–	38.318	citral	*b*	+	+	+
20.030	1-ethyl-4-methyl benzene	*b*	–	–	+	38.635	naphthalene	*b*	+	+	+
22.148	(1-methylethyl)benzene (cumene)	*b*	–	–	+	40.518	β-damascenone	*b*	+	+	+
28.682	2,6-dimethyl-2,6-octadiene	*b*	+	+	+	41.252	geranyl acetone	*b*	+	+	+
29.933	linalool oxide	*b*	–	–	+	41.650	cinnamaldehyde (3-phenyl-2-propenal)	*b*	+	+	+
30.816	2-furaldehyde	*b*	+	+	+	47.020	6,10,14-trimethyl-2-pentadecanone	*b*	+	+	–
30.883	linalool oxide	*b*	–	–	+	47.820	6-undecylamine	*b*	+	+	+
31.133	4,4-dimethyl-2-cyclo-penten-1-one	*b*	–	–	+	50.671	2-(phenylmethylene)octanal (β-hexyl cinnamaldehyde)	*a*	+	+	+
31.717	2,6-dimethyl-2,5-hepta-dien-4-one	*b*	–	–	+						

[a] Identification by comparison of gas chromatographic retention times and MS data with those of available pure compounds. [b] Tentatively identified.

Table 5. Sensory Evaluation of the Wines Produced by Repeated Batch Fermentations of Must at 22 and 6 °C, Using *S. cerevisiae* AXAZ-1 Immobilized on Black Currants, Golden Raisins, and Free Cells and a Locally Available Commercial Red Wine (*Agiorgitiko*)

	°C	black currants	golden raisins	free cells	commercial wine
aroma	22	7.7 ± 0.82	7.6 ± 1.07	6.8 ± 1.10	6.5 ± 0.95
aroma	6	8.4 ± 0.62	8.0 ± 1.02		
taste	22	7.5 ± 0.97	6.2 ± 0.92	6.0 ± 1.22	5.6 ± 1.01
taste	6	8.4 ± 0.71	8.3 ± 0.77		

an increase of the percentage of other aroma compounds on total volatiles. Sensory evaluation of wines produced by Uvaferme 299 cells showed a nonstatistically significant preference for wines produced by immobilized cells as compared to free cells, except in the case of samples that were tested after a storage period. This was attributed to a hydrogen sulfide smell in samples produced by free cells due to the prolonged contact of wine with the yeast cells, as it had been previously observed in the case of white wine making. No such odor was detected in the case of wines produced by immobilized cells. The wines produced by immobilized cells in both types of raisins had a fine clarity after the end of fermentation with low free cell concentrations and characteristic fruity−floral hints in their aroma.

Conclusions and Technological Consideration of Results. The immobilization of yeast cells in black currants and golden raisins was made by entrapment of cells in the berries and attachment on the external surface. The biocatalyst was found effective for red wine making, and the process was feasible using a modified bioreactor, designed to keep separately the grape skins from the immobilized biocatalyst. The good operational stability, proved by the performed repeated fermentation batches, will give the possibility to remove the biocatalyst once a year. This operation is a common practice in emptying extraction tanks during potable alcohol production, where raisins are used as raw material. Longer preservation of the biocatalyst may be achieved by cooling the bioreactor (*23*), and although this would increase the cost of the bioreactor as compared to the simple fermentation tanks, profits would be obtained by the improved quality of the wine produced at 6 °C and the improvement of productivity. The above study demonstrates that dried grape raisins meet the prerequisites for a cost effective industrial application using immobilized cells for red wine making. Raisins are a cheap and easily available raw material of food grade purity that needs no pretreatment and is fully compatible with wine. Their use in wine making imitates a natural process. This carrier, like gluten pellets, delignified cellulosic materials and fruit pieces, previously tested as immobilization supports in wine making, is a relatively resistant material that is not disrupted in the fermentation environment. Also, because of their shape and size, they could be applied in continuous processes and fluidized bed bioreactor systems. Additionally, the extracted residues of raisins from wineries and potable alcohol producing plants are undesirable solid wastes, which alternatively could be exploited as immobilization supports to produce commercial biocatalysts for wine making.

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