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Extremely Low Temperature Fermentations of Grape Must by Potato-Supported Yeast, Strain AXAZ-1. A Contribution Is Performed for Catalysis of Alcoholic Fermentation

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This investigation announces the use of potato pieces as a suitable support for cell immobilization resulting in extremely low temperature wine making. The results showed an increase of the total esters by immobilized cells and reduction of higher alcohols. Likewise, percentages of total esters on total volatiles were increased by the drop in temperature, while percentages of higher alcohols were reduced in wines. Kinetics experiments at different temperatures allowed the calculation of activation energy (E_a) and showed reduction in the case of immobilized cells as compared with free cells. These results may lead to the conclusion that the increased productivities that are obtained by immobilized cells, can be attributed to the catalytic activity by the support to enzymes, which are involved in the process. Biocatalysts were prepared by immobilization of *Saccharomyces cerevisiae*, strain AXAZ-1, on whole potatoes and potato pieces, and their efficiency for alcoholic repeated batch fermentations of glucose and grape must in the range 2−30 °C was examined. To study the operational stability of biocatalyst, 35 repeated batch fermentations of grape must were performed without any significant reduction of the fermentation activity. Wines were analyzed for volatile byproducts determination by GC and GC-MS.

KEYWORDS: Immobilization; yeast; wine; volatiles; GC/MS; potato; activation energy

INTRODUCTION

In recent years there has been increasing interest in using cell immobilization for alcoholic fermentation (*1*), as a result of their numerous advantages compared to free cell systems. Therefore many immobilization supports have been proposed for use in alcoholic fermentations such as mineral kissiris (*2*), γ-alumina (*3*), alginates (*4*), DEAE-cellulose (*5*), Ca-pectate (*6*), and κ-carrageenan (*7*). However, especially the supports that are used for immobilization in food industry must be materials of food grade purity, of low cost, abundant in nature, and easy to handle. Furthermore, in the case of wine making the immobilization supports must also have some other characteristics such as nondegradable nature and suitability for low-temperature fermentations. Therefore, in the last years several supports, having these properties, have been proposed such as delignified cellulosic materials (*8*), gluten pellets (*9*), brewer's spent grains (*10*), dried fig (*11*), dried raisin berries (*12*), and fruits pieces (*13*). The use of alcohol-resistant and cryotolerant yeasts immobilized on these supports led to low-temperature fermentations producing wines and beers with excellent taste and aroma. In addition, productivities were high, even at extremely low temperatures (0−5 °C), which can be compared with those at high-temperatures fermentations. However, even though several immobilization supports have been proposed for alcoholic fermentation, the industrial application of this technology is limited mainly due to additional cost and problems are created in the industrial handling of the support. Potatoes are harder and cheaper than fruits, raisin beries, figs, and gluten pellets. Furthermore, they create less handling problems in fermentations than delignified cellulosic (DC) materials. Therefore, it is attractive to try cell immobilization on potatoes for possible low-temperature fermentation in wine making. Low-temperature fermentations with immobilized cells on suitable supports already have been reported in previous studies (*8, 14*) for wine making and brewing. In these studies the role of the support is promoting fermentation at lower temperatures as compared with free cells examined in theoretical base. A theoretical study of the Arrhenius equation in the case of DC materials-supported yeast cells (*8*), concluded that this support acts as a catalyst due to reduction of the activation energy E_a with the presence of this support. However, low-temperature alcoholic fermentations were also obtained by using as supports fruits (*13*) and gluten (*15*) in wine making. After the above presentation of literature it is attractive to study potatoes as immobilization support for low-temperature fermentation. Likewise, we undertake experiments to calculate the speed reaction coefficients and activation energy E_a of the alcoholic fermenta-

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tion in order to prove that the increase in the rate of fermentation in the case of immobilized cells can be attributed to catalysis by the support to enzymes involved in the process.

MATERIALS AND METHODS

Yeast Strains and Media. The alcohol resistant and cryotolerant *S. cerevisiae* AXAZ-1, isolated from Greek vineyard plantation (*16*), was used in the present study. It was grown on culture medium consisting of 4 g of yeast extract/L, 1 g of $(NH_4)_2SO_4$/L, 1 g of KH_2PO_4/L, 5 g of $MgSO_4 \cdot 7H_2O$/L, and 40 g of glucose monohydrate/L at 30 °C and harvested at 5000 rpm for 10 min. This glucose media was also used for cell immobilization and fermentation experiments, using free or immobilized cells. All media were sterilized at 130 °C and 1.5 atm for 15 min. Must of the roditis grape variety was provided from the local wine industry "Achaia Clauss", with 11.3−12.1 initial °Be density (197.5−224.5g/L), total acidity of 6−7 g tartaric acid/L, and sulfur dioxide content of ∼60 mg/L.

Preparation of Support and Cell Immobilization. Whole potatoes were treated with 1% w/v NaOH solution for 15 min at 80−85 °C for lignin removal and then washed several times with hot water (70 °C). After cooling, the half of them were used directly for cell immobilization and the other half were first cut into pieces of about 10 g and used for cell immobilization. Cell immobilization was carried out at 30 °C by mixing 300 g of the support and 16 g wet weight AXAZ-1 cells in a 1-L glass cylinder containing 800 mL of 12% (w/v) glucose culture medium. The system was allowed to ferment until the density of the fermented liquid reached 0−0.5 °Be. The fermented liquid was decanted and the support was washed two times with 12% (w/v) glucose culture medium and used for batch glucose fermentations.

Enumeration of Immobilized Cells. Representative 10-g portions of duplicate potato samples taken after immobilization and at the end of fermentations were blended with 90 mL of sterilized ringer solution (1/4 strength) and subjected to serial dilutions. The enumeration of immobilized yeast cells was made on Malt agar and on an agar with the following (w/v) content: glucose 4%, yeast extract 0.4%, $(NH_4)_2SO_4$ 0.1%, KH_2PO_4 0.1% and $MgSO_4 \cdot 7H_2O$ 0.5% after incubation at 30 °C for at least 72 h. The number of immobilized cells on potato pieces was approximately constant during fermentations (7.1×10^8 cells g^{-1} wet potato pieces corresponding to 4.69×10^{-3} g dry weight cells g^{-1} wet potato pieces) with a small reduction with the drop of temperature.

Fermentations. The immobilized biocatalyst of 300 g was introduced in 500 mL of 12% (w/v) glucose medium. Repeated batch fermentations, without any agitation, were carried out initially at 30 °C and then the temperature was successively decreased to 25, 20, 15, 10, 5, and 2 °C. When the fermentation was completed the liquid was collected for analyses and the support was washed twice with 200 mL of 12% (w/v) glucose culture medium and then used for the next fermentation batch. After the completion of glucose fermentations the potato pieces were used for repeated batch fermentations of 400 mL of must at the same temperatures. For comparison must fermentations with free cells were also carried out in duplicate.

Analyses. Fermentation kinetics were performed by measuring the °Be density at various time intervals. Residual sugar was determined by high performance liquid chromatography, using a Shimadzu chromatograph (Kyoto, Japan) with a SCR-101N stainless steel column, a LC-9A pump, a 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 1-butanol (Sigma-Aldrich, Poole, UK) was used as an internal standard. Samples of 0.5 and 2.5 mL of a 1% (v/v) solution of 1-butanol were diluted to 50 mL and 40 μL was injected directly into the column. Residual sugar concentrations were calculated using standard curves prepared by at least 7 standard solutions (0, 5, 10, 15, 20, 25, 30 g/L) with the corresponding ratio of residual sugar peak areas/1-butanol peak areas to residual sugar concentrations.

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*). Wet weight free cell concentrations in the fermentation liquid were determined according to a previous study (*18*). Wine productivity was calculated as grams of wine per liter total volume produced per day. Ethanol productivity was expressed as g of ethanol produced per day per L liquid volume of the bioreactor. Conversion was calculated by the following equation:

$$(\text{initial sugar concentration} - \text{residual sugar concentration})/\text{initial sugar concentration} \times 100$$

Determination of Ethanol and Volatile Byproducts. Ethanol was determined by gas chromatography by using a Shimadzu GC-8A Gas−Liquid Chromatograph (Kyoto, Japan) with a Porapac S column. Nitrogen was used as carrier gas at 40 mL/min. The column temperature was settled at 120−170 °C at a rate of 10 deg/min. The temperatures of the injector and FID detector were 210 and 220 °C, respectively. For the ethanol determination, a total volume of 2 μL for each sample was injected directly into the column. 1-Butanol was used as internal standard at a concentration of 0.5% (v/v). Ethanol concentrations were calculated by using standard curves prepared by at least 7 standard solutions (0, 2, 4, 6, 8, 10, 12% v/v) with the corresponding ratio of ethanol peak areas/1-butanol peak areas to ethanol concentrations.

Volatiles such as acetaldehyde, ethyl acetate, 1-propanol, isobutanol and amyl alcohols were determined by gas chromatography by using a Shimadzu GC-8A Gas−Liquid Chromatograph (Kyoto, Japan), with a stainless steel column packed with Escarto-5905 consisting of 5% squalene, 90% Cabowax-300, and 5% bis(2-ethylhexyl) sebacate. Nitrogen was used as carrier gas at 20 mL/min. The injection port and FID detector temperatures were 210 °C, and the column temperature was 70 °C. The internal standard was 1-butanol (0.5% v/v). Samples of 4 μL were injected directly in the column, and the concentrations of the above compounds were calculated by using standard curves prepared with at least 7 standard solutions (0, 25, 50, 75, 100, 125, and 150 mg/L for acetaldehyde, ethyl acetate, amyl alcohols and 0, 5, 10, 15, 20, 25, and 30 mg/L for 1-propanol and isobutanol) with the corresponding ratio of each compound peak areas/1-butanol peak areas to each compound concentration.

Headspace SPME-GC/MS. The volatile constituents of the produced wines were determined by means of gas chromatography−mass spectroscopy. The volatiles were isolated by the headspace solid-phase microextraction (SPME) method. The fiber used for the absorption of volatiles was a 2-cm fiber coated with 50/30 mm Divinylbenzene/Carboxen on poly(dimethylsiloxane) bonded to a flexible fused silica core (Supelco, Bellefonte, PA, USA). The conditions of headspace SPME sampling were as follows: 10 mL of liquid sample, 3 g of NaCl, and internal standard (4-methyl-2-pentanol) were transferred into a 20-mL headspace vial fitted with a Teflon-lined septum sealed with an aluminum crimp seal. The contents were magnetically stirred for 5 min at 60 °C, and then 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 3 min. Before each analysis, the fiber was exposed to the injection port for 5 min to remove any volatile contaminants. GC/MS analysis was performed on a Shimadzu GC-17A gas chromatograph coupled to a Shimadzu MS QP5050 mass spectrometer. Helium was used as the carrier gas (1.8 mL/min). Separation of compounds was performed on a capillary column (Supelco CO Wax-10 60 m, 0.32 mm i.d., 0.25 μm film thickness). Oven temperature was programmed at 35 °C for 6 min and then it was raised to 60, 200, and 250 °C with a rate of 2, 5, and 25 deg/min, respectively. It was then held at 250 °C for 6 min. The injector and interface temperatures were set at 240 and 240 °C, respectively. The mass spectrometer was operated in scan range of 45−400 *m*/*z*. Identification of the compounds was effected by comparing (i) the linear retention indices based on the even *n*-alkanes (C10−C24) with those of standard compounds and by the literature retention indices and (ii) MS data with those of standard compounds and by MS data obtained from Wiley and NIST libraries. Semiquantitative analysis was performed by dividing the peak area of a compound with the peak area of the internal standard and multiplying the result with the concentration of the internal standard (1.62 mg/L).

Calculation of E_a. Fermentations of 500 mL of 12% (w/v) glucose medium were carried out with immobilized and free cells at various

temperatures (300 g wet potato pieces corresponding to 1.41 g dry weight cells). The activation energies of the fermentation systems were calculated by using an equation based on the Arrhenius equation according to a previous study (*19*) by a curve obtained by plotting $\ln(dP/dt)$ versus $1/T$.

Electron Microscopy. Pieces of the immobilized biocatalysts prepared with potatoes having 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.

Experimental Design and Statistical Analysis. All analyses were carried out in triplicate and the mean values are presented (standard deviation for all values was about ±5% in most cases). In the experiments conducted, the effect of temperature and immobilization on fermentation parameters and formation of volatiles (ethanol, acetaldehyde, ethyl acetate, isobutanol, 1-propanol, and amyl alcohols) during glucose synthetic medium and must fermentations were studied. In addition the effect of temperature and immobilization on activation energy E_a, rate coefficient k, and viable immobilized cells populations were also studied. The experiments were designed and analyzed statistically by ANOVA. Duncan's multiple range test was used to determine significant differences among results [coefficients, ANOVA tables and significance ($p < 0.05$) were computed by using Statistica v.5.0 (StatSoft, Inc., Tulsa, USA)].

RESULTS AND DISCUSSION

Alcoholic fermentation of glucose and wine making using yeast cells immobilized on whole potatoes and potato pieces, at various temperatures, has not yet been reported and it was studied in the present investigation. Furthermore, fermentations were performed with immobilized and free cells at several temperatures and were used to calculate alcoholic fermentation speed coefficients and activation energy E_a. These results were used to prove that the increased fermentation efficiency of immobilized cells can be attributed to catalysis by the support to the enzymes that are involved in the process.

Cell Immobilization. The potatoes were first washed with water and then treated with hot sodium hydroxide solution for lignin removal. Parts **A** and **B** of **Figure 1** show the skin of potatoes before and after this treatment, respectively, without any significant differences. When potatoes were cut and the interior was studied the differences were more obvious. More specifically, prior to sodium hydroxide treatment, the electron micrograph of the interior of potatoes (**Figure 1D**) showed clearly the presence of characteristic large (diameter usually >40 μm) oval potato starch granules (*20*). After treatment the electron micrograph of the interior of potatoes was completely different, without any starch granule but a rough surface instead (**Figure 1E**). This is attributed to a process known as retrogradation in which the starch granules when heated above their gelatinization temperature undergo irreversible swelling and as they cool crystallization is favored and an elastic gel is formed (*21, 22*). In addition, this process was facilitated and accelerated by using sodium hydroxide solution (*23*). The treatment of potatoes with hot sodium hydroxide solution prior to immobilization made more stable and not easily disrupted supports than those produced with raw potatoes where the release of starch in the fermentation liquid was high (data not shown). After that treatment, immobilization took place by mixing each of the supports of whole potatoes and potato pieces with a liquid culture of yeast cells and the whole was left to ferment for ~8 h. The immobilization in both supports was confirmed by electron microscopy (**Figures 1**, parts **C** and **F**), showing yeast cells attached to the skin of potatoes, while in the case of potato pieces, yeast cells were also mixed and entrapped inside the gel that was formed after retrogradation of starch granules. To strengthen further the view that cell immobilization occurred on potatoes, repeated batch fermentations were performed using potatoes supported biocatalyst.

Repeated Batch Glucose Fermentations. Repeated batch fermentations of glucose were carried out at various temperatures ranging from 2 to 30 °C (**Table 1**). Enumeration of immobilized viable cells after immobilization resulted in a yeast cell population of 7.37 logcfu/g and 6.13 logcfu/g for potato pieces and whole potatoes, respectively. Fermentation temperature and immobilization significantly affected ($p < 0.01$) the immobilized viable cells. In both systems the yeast population was increased during the repeated batch fermentations and the highest value was counted to 8.80 logcfu/g after the 20th batch for potato pieces and 7.00 logcfu/g after the 16th batch for whole potatoes. As the batch fermentations were continued and the temperature was decreased, a reduction of the immobilized viable yeast cell population was observed, which was more rapid in the case of whole potatoes. At the end of the last fermentation batch the viable immobilized yeast cell population was 8.12 and 5.90 logcfu/g for potato pieces (2−3 °C, 42nd batch) and whole potatoes (5 °C, 37th batch), respectively. The higher numbers of viable cells in immobilization on potato pieces may be attributed to the protective role of gelatinized starch that appears only in the interior of potatoes, as is proved by scanning electron microscopy. Both systems operated for a period longer than 4 months and the ethanol concentration in the final product ranged from 5.5% to 6.8% (v/v), while the conversion remained at a high level. However, the potato pieces presented lower fermentation times and therefore better ethanol productivities than the whole potatoes, especially at low temperatures. More specifically, the ethanol productivities were 2-fold and even higher in the case of potato pieces at temperatures lower than 20 °C. The biocatalyst prepared with whole potatoes showed a decrease in fermentation activity with the drop of temperature, compared with that of potato pieces. This may be attributed to the increase of potato surface available for immobilization, and to better immobilization of cells on the interior of potatoes as was mentioned above. In addition, this can explain the ability of the biocatalyst prepared with potato pieces to ferment even at 2 °C with satisfactory fermentation times. Fermentation kinetics of immobilized cells compared to free cells are presented to **Figures 2** and **3**, showing that the immobilization on potato pieces proved to increase drastically the fermentation rates, especially at low temperatures.

Regarding the effect of the proposed biocatalysts on the aroma, the formation of major volatile byproducts was examined and the results are summarized in **Table 2**. Temperature affected significantly all the major volatile byproducts ($p < 0.05$ for acetaldehyde with immobilized cells on whole potatoes and $p < 0.01$ for the rest of the parameters). A strong interaction between temperature and immobilization affecting ethyl acetate ($p < 0.01$), 1-propanol ($p < 0.05$), and amyl alcohols ($p < 0.01$) was also observed. More specifically the concentration of amyl alcohols was higher in potato pieces compared to whole potatoes especially at higher temperatures. The amounts of higher alcohols in both systems were reduced as the temperature decreased. At 5 °C the concentrations of amyl alcohols were lower by 50% than those at 30 °C in both systems. Likewise, at this low temperature, extremely low concentrations were found for 1-propanol and isobutyl alcohol. These concentrations of higher alcohols were lower than those observed in previous studies (*11, 24*). Ethyl acetate was determined in expected amounts (*11, 24*) and an increase was observed at low

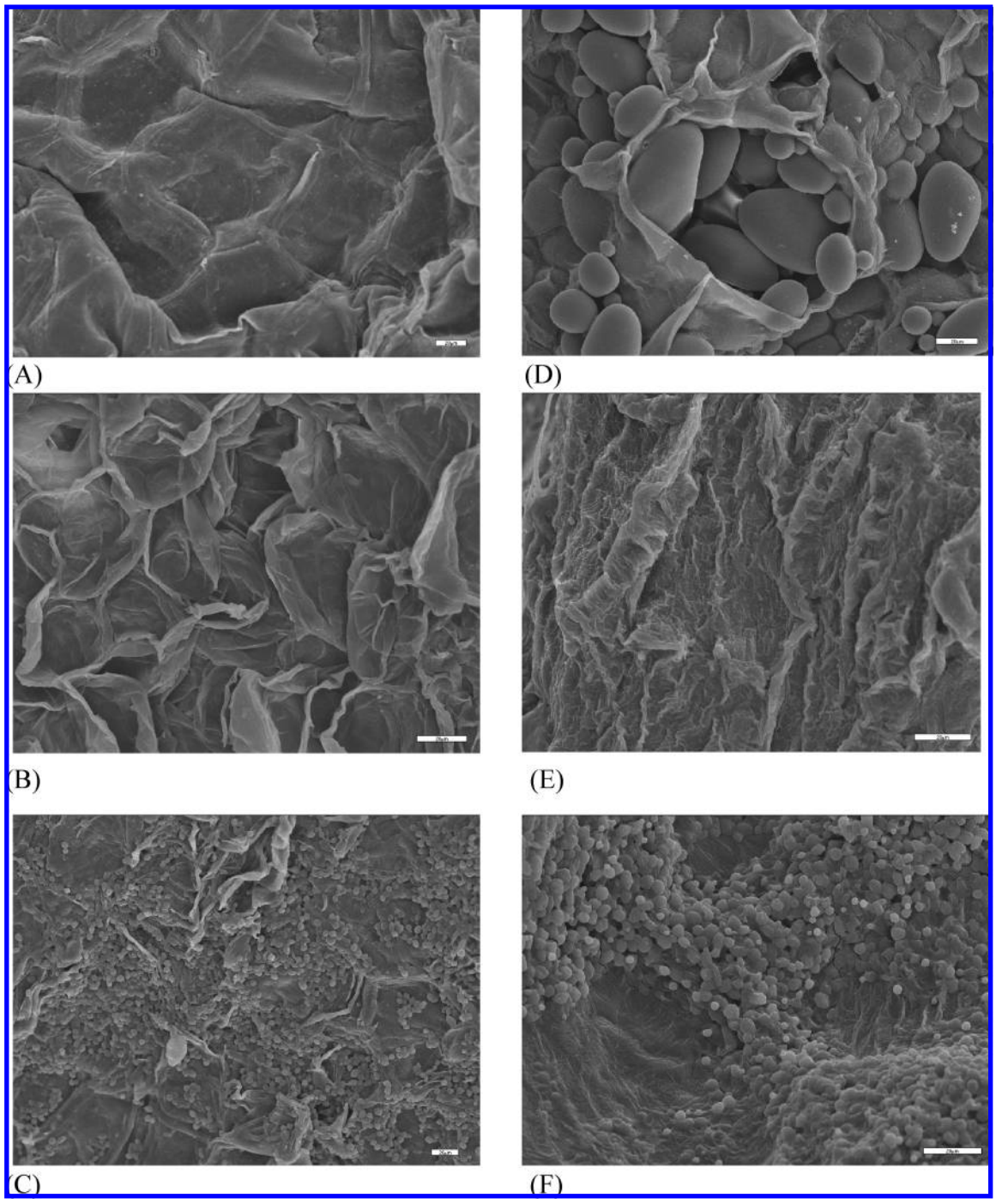

Figure 1. Electron micrographs of the skin surface of whole potatoes (**A**) before any treatment, (**B**) after delignification, and (**C**) after yeast cell immobilization and of potato pieces (**D**) before any treatment, (**E**) after delignification, and (**F**) after yeast cell immobilization.

temperatures. The reduction of higher alcohols and the increase of ethyl acetate with the drop of temperature are positively considered (*24*). The acetaldehyde content ranged from traces up to 62 mg/L in both systems and was increased as the temperature was decreased.

Wine Making by Potato Pieces-Supported Biocatalyst. After the completion of repeated batch fermentations of glucose the biocatalyst prepared with potato pieces was used directly for the fermentation of must and the production of white wine at the same range of temperatures starting from 30 °C. All fermentations were carried out by using grape must with an initial sugar concentration ranging between 197 and 217 g/L. Fermentation temperature affected significantly fermentation time ($p < 0.01$), final free cell concentration ($p < 0.01$), ethanol and wine productivity ($p < 0.01$), and residual sugar ($p < 0.05$), while it did not affect ethanol concentration ($p > 0.05$). The immobilized biocatalyst retained its operational stability for the long period of 9 months, even at low temperatures, and produced wines in 1.5 days at 25−30 °C and 30 days at 5 °C (**Table 3**). The wines produced were of fine clarity and contained alcohol at concentrations similar to those of dry table wines. The final free cell concentrations were low, especially at low temperatures, meaning that the fermentation was carried out by immobilized cells. Ethanol and wine productivities were relatively high while total acidity and volatile acidity were in the level of commercial dry wines and were affected ($p < 0.01$) by the temperature of fermentation (**Table 4**). The high concentration of residual sugar

Table 1. Kinetic Parameters Observed at Repeated Batch Fermentations of 12% w/v Glucose Medium, Using Immobilized *Saccharomyces cerevisiae* AXAZ-1 Yeast Cells on Whole Potatoes and Potato Pieces

temp (°C)	batch	initial sugar (g/L)	fermentation time (h)	ethanol (% v/v)	ethanol productivity (g/L)/day	residual sugar (g/L)	conversion (%)
			potato pieces				
30	1−5	120.5±1.5	25.8± 4.5	5.8±0.3	44.1±6.4	3.4±1.3	97.2±1.0
30	6−10	121.8±3.3	21.3± 0.3	6.1±0.3	54.1±3.0	2.1±0.4	98.3±0.4
30	11−15	121.5±2.2	19.6± 1.3	6.1±0.2	59.8±6.1	1.5±0.9	98.7±0.7
25	16−20	122.1±3.3	17.5± 0.4	6.1±0.1	65.8±1.9	1.6±0.5	98.7±0.4
20	21−25	121.4±2.7	24.6± 0.5	6.3±0.2	48.6±2.2	1.2±0.8	99.0±0.7
15	26−30	119.5±1.6	42.4± 0.4	6.0±0.3	26.9±1.4	3.0±1.3	97.8±1.4
10	31−35	121.2±2.0	68.8± 7.8	6.5±0.3	18.1±2.5	1.1±0.7	98.8±0.8
5	36−40	121.0±1.6	147.8± 8.1	6.2±0.3	8.0±0.7	2.0±1.2	98.3±1.0
2	41	122.3	357	6.2	3.3	1.5	98.8
2	42	119.4	408	5.6	2.6	4.7	95.3
			whole potatoes				
30	1−5	121.1±2.3	26.4± 0.3	6.3±0.5	45.1±2.3	1.5±1.1	98.8±1.0
30	6−10	120.7±2.8	24.6± 1.2	6.2±0.3	48.1±1.8	1.9±0.9	98.5±0.7
30	11−15	119.4±1.9	24.3± 0.6	6.0±0.4	46.8±2.5	2.7±1.1	97.7±0.9
25	16−20	121.2±2.3	31.2± 0.6	6.1±0.4	37.1±3.0	1.8±1.0	98.6±0.8
20	21−25	120.0±2.4	48.5± 1.0	6.3±0.3	24.8±1.1	1.8±0.9	98.5±0.7
15	26−30	122.5±3.4	102.8± 5.4	6.4±0.2	11.9±0.4	0.9±0.3	99.3±0.3
10	31−35	120.1±1.3	274.8±52.2	6.1±0.3	4.3±0.8	2.0±1.1	98.3±0.9
5	36	118.5	671	5.7	1.6	4.5	95.2
5	37	120.8	1107	3.3	0.6	58.9	52.2

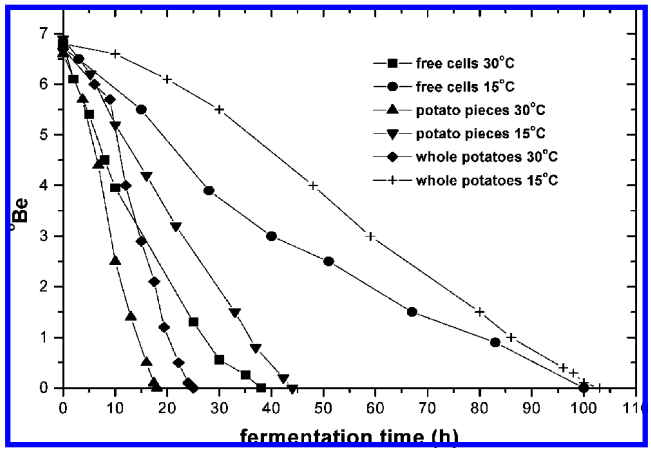

Figure 2. Fermentation kinetics observed with use of 12% w/v glucose at 30 and 15 °C by free and immobilized yeast cells on whole potatoes and potato pieces.

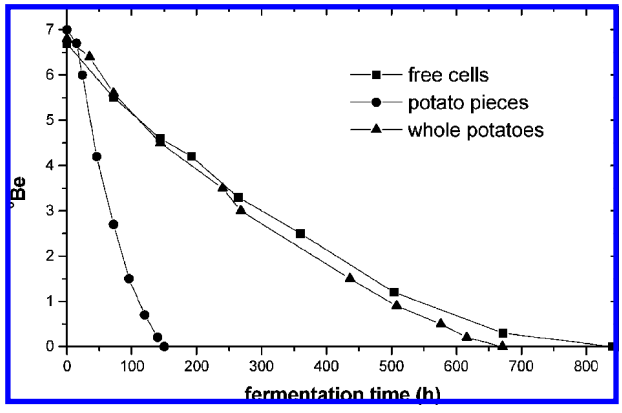

Figure 3. Fermentation kinetics observed with use of 12% w/v glucose at 5 °C by free and immobilized yeast cells on whole potatoes and potato pieces.

was observed because the fermentations were stopped deliberately when an approximate 0.5 °Be density was reached to retain cell viability (*25, 26*).

Volatile Byproducts. Most of the compounds that contribute to the aroma of wines are produced during must fermentation. Major volatile byproducts are acetaldehyde, ethyl acetate, 1-propanol, isobutanol, and amyl alcohols, accounting for more than half of them (*27*). Therefore, the produced wines were analyzed separately for major volatile byproducts and the results are summarized in **Table 4**. Fermentation temperature affected significantly ($p < 0.01$) all the major volatile byproducts. Acetaldehyde ranged in relatively high concentrations from 70 to 115 mg/L, which is still inside the usual levels produced by *Saccharomyces cerevisiae* strains according to refs *28* and *29*. The presence of SO_2 in the grape must used and its low pH value may explain the relatively high amounts of acetaldehyde in wines produced (*30*−*32*). Ethyl acetate is the most important and abundant ester in wines (*33*−*35*). In the present study its concentration ranged from 20 mg/L at 30 °C to 100 mg/L at 2−5 °C. It is considered that such low concentrations of ethyl acetate (50−80 mg/L) contribute to wine olfactory complexity having a positive impact on wine quality and only at concentrations >120 mg/L may spoil the bouquet with an unpleasant, pungent tang (*34*). In addition many studies support that any factor that decreases the speed of fermentation like temperature, pH, and low oxygen conditions simultaneously increases the amount of ethyl ester and acetaldehyde (*32, 36*). Higher alcohols are considered as the largest group of flavor compounds in wines and among them amyl alcohols and isobutanol have high importance (*31*). The concentrations of amyl alcohols and isobutanol in the wines produced ranged from low levels up to 140 and 22 mg/L, respectively. A decrease was observed with the drop of temperature, which complies with the results using other supports in wine making (*36, 37*). However, the concentration of higher alcohols in all cases ranged in levels that contribute to the pleasant flavor of the wines produced (*35, 34*).

For the evaluation of the aromatic profile, wine samples, produced by free and immobilized cells, were analyzed by using a SPME GC/MS technique and the results are presented in **Table 5**. In total, 95 compounds were detected: 42 in must, 47 in wines produced by free cells at 15 °C, and 66 in wines produced at the same temperature by immobilized cells on potato pieces.

Table 2. Effect of Temperature on the Production of Volatiles in the Fermentation of 12% w/v Glucose Medium, Using Immobilized *Saccharomyces cerevisiae* AXAZ-1 Yeast Cells on Whole Potatoes and Potato Pieces[a]

temp (°C)	batch	acetaldehyde (mg/L)	ethyl acetate (mg/L)	1-propanol (mg/L)	isobutyl alcohol (mg/L)	amyl alcohols (mg/L)
			potato pieces			
30	1−5	19.2 ± 15.4	32.8 ± 3.8	21.1 ± 1.4	15.8 ± 4.2	77.8 ± 2.2
30	6−10	29.4 ± 12.7	30.4 ± 2.9	18.4 ± 1.7	22.4 ± 2.9	80.8 ± 4.6
30	11−15	17.4 ± 13.9	31.8 ± 3.4	20.6 ± 1.9	17.4 ± 2.7	84.8 ± 4.6
25	16−20	16.2 ± 13.0	35.0 ± 5.2	12.8 ± 5.1	15.6 ± 1.5	83.4 ± 5.3
20	21−25	25.4 ± 10.3	49.8 ± 1.8	11.6 ± 1.8	14.4 ± 1.9	73.4 ± 12.7
15	26−30	22.6 ± 9.0	51.0 ± 2.0	6.8 ± 0.6	10.0 ± 0.8	69.8 ± 3.0
10	31−35	31.6 ± 12.6	54.4 ± 2.7	2.2 ± 1.8	7.2 ± 0.6	57.0 ± 5.6
5	36−40	53.7 ± 6.8	58.4 ± 5.3	Tr	5.6 ± 2.9	42.4 ± 1.9
2	41	48	62	Tr	Tr	36
2	42	50	65	Tr	Tr	45
			whole potatoes			
30	1−5	19.6 ± 15.7	29.6 ± 1.5	14.0 ± 1.2	22.8 ± 1.0	55.8 ± 2.6
30	6−10	29.6 ± 12.5	29.8 ± 1.4	13.4 ± 1.7	19.2 ± 0.6	58.4 ± 2.1
30	11−15	25.8 ± 10.6	35.6 ± 1.7	11.4 ± 1.7	17.6 ± 1.3	58.4 ± 3.3
25	16−20	28.6 ± 12.9	40.2 ± 1.4	8.6 ± 1.9	13.0 ± 1.6	52.2 ± 1.8
20	21−25	30.8 ± 13.4	42.0 ± 3.6	4.0 ± 3.2	9.8 ± 0.6	48.4 ± 1.9
15	26−30	30.8 ± 13.8	44.2 ± 1.0	2.6 ± 2.1	8.6 ± 0.9	46.4 ± 2.3
10	31−35	55.4 ± 6.1	47.6 ± 2.1	Tr	3.6 ± 3.6	41.4 ± 2.3
5	36	58	50	Tr	4	33
5	37	50	40	Tr	Tr	30

[a] Tr = trace.

Table 3. Kinetic Parameters of the Repeated Batch Fermentations of Grape Must with Immobilized *Saccharomyces cerevisiae* AXAZ-1 Yeast Cells on Potato Pieces

temp (°C)	batch	initial density (°Be)	initial sugar (g/L)	fermentation time (h)	Free cells (g/L)	residual sugar (g/L)	ethanol (% v/v)	ethanol productivity ((g/L)/day)	wine productivity ((g/L)/day)
30	1−5	11.7 ± 0.1	205.9 ± 1.6	27.4 ± 2.3	2.0 ± 0.1	12.4 ± 4.3	11.1 ± 0.6	77.0 ± 8.1	504.6 ± 44.3
30	6−10	11.7 ± 0.0	205.6 ± 1.2	30.8 ± 0.8	1.5 ± 0.2	14.2 ± 5.4	11.1 ± 0.3	67.7 ± 1.8	445.5 ± 9.3
25	11−15	11.5 ± 0.1	204.2 ± 1.8	33.7 ± 0.9	1.2 ± 0.1	9.8 ± 5.3	11.0 ± 0.5	61.6 ± 2.7	407.4 ± 11.8
20	16−20	12.0 ± 0.1	217.6 ± 6.9	60.0 ± 1.2	1.3 ± 0.1	14.0 ± 7.7	11.6 ± 0.5	36.3 ± 1.2	228.7 ± 4.6
15	21−25	11.7 ± 0.2	210.5 ± 7.8	98.4 ± 2.1	1.0 ± 0.1	16.9 ± 3.6	11.5 ± 0.1	21.9 ± 0.6	139.4 ± 2.9
10	26−30	11.6 ± 0.3	207.6 ± 10.1	334.8 ± 39.4	0.7 ± 0.1	15.6 ± 4.7	11.3 ± 0.1	6.5 ± 0.8	41.7 ± 4.7
5	31−33	11.6 ± 0.1	203.8 ± 1.1	840 ± 48	0.3 ± 0.1	9.4 ± 2.2	11.4 ± 0.1	2.6 ± 0.2	16.3 ± 0.9
2	34	11.5	204.4	1402	0.1	8.5	11.4	1.5	9.8
2	35	11.5	203.9	1450	0.1	10.1	11.3	1.5	9.4

Table 4. Volatiles and Acidity of Wines Produced by Repeated Batch Fermentations of Grape Must, Using Immobilized *Saccharomyces cerevisiae* AXAZ-1 Yeast Cells on Potato Pieces

temp (°C)	batch	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)	total volatiles (mg/L)
30	1−5	0.24 ± 0.07	5.4 ± 0.6	81.8 ± 10.2	33.5 ± 13.6	17.7 ± 2.4	20.0 ± 2.0	127.3 ± 12.3	265.3 ± 12.9
30	6−10	0.34 ± 0.08	5.1 ± 0.3	94.8 ± 13.1	55.6 ± 11.8	13.7 ± 2.5	17.5 ± 1.2	110.8 ± 12.4	272.4 ± 28.9
25	11−15	0.41 ± 0.05	5.8 ± 0.3	104.3 ± 8.7	72.0 ± 5.8	11.7 ± 1.8	16.4 ± 2.4	111.3 ± 11.2	292.8 ± 24.9
20	16−20	0.34 ± 0.05	5.9 ± 0.3	102.1 ± 7.3	84.8 ± 3.4	7.9 ± 1.0	15.5 ± 1.3	116.6 ± 4.2	309.9 ± 12.1
15	21−25	0.40 ± 0.07	5.1 ± 0.3	95.0 ± 5.1	87.3 ± 5.6	7.1 ± 0.4	14.0 ± 1.8	106.0 ± 3.8	296.4 ± 11.0
10	26−30	0.32 ± 0.07	5.7 ± 0.4	86.8 ± 5.7	95.8 ± 6.4	4.5 ± 1.8	15.0 ± 1.4	88.8 ± 8.2	285.9 ± 12.6
5	31−33	0.35 ± 0.04	5.6 ± 0.3	78.6 ± 2.8	93.3 ± 5.7	3.6 ± 1.5	13.5 ± 1.0	81.6 ± 5.3	255.6 ± 2.0
2	34	0.35	5.5	80.2	90.5	3.4	13.5	80.5	268.1
2	35	0.31	5.3	75.9	94.3	3.0	13	82.1	268.3

Further reduction of temperature in fermentations by immobilized cells did not affect significantly the qualitative aroma profile. As regards the quantitative aroma profile, immobilized cells produced significantly higher concentrations of "fruity" esters and other compounds provide improved characteristic flavor. The reduction of fermentation temperature led to lower concentrations of all compounds (*38, 26*). However, an increase in the percentage of total esters and a decrease in the percentage of higher alcohols were also observed. Similar results were recently published (*26*). A total of 36 esters were detected in the present study in wines produced by immobilized cells while only 18 were detected by free cells. Very few esters are present in grapes and the majority of them were produced during fermentation as a product of yeast metabolism (*39*). The main ester in our samples and wines in general was ethyl acetate. Other esters present in all wine samples were those of fusel

Table 5. Volatile Compounds (mg/L) Identified in Grape Must and in Wines Produced by Free (15 °C) and Immobilized *Saccharomyces cerevisiae* AXAZ-1 Yeast Cells on Potato Pieces (15, 10, and 5 °C), Using the SPME GC/MS Technique

			free cells	immobilized cells on potato pieces		
RI	compd	grape must	15 °C	15 °C	10 °C	5 °C
		esters				
925	ethyl formate[b]			0.008	0.003	0.044
1040	ethyl butanoate[a]	0.012		0.551	0.244	0.332
1258	ethyl hexanoate[a]	0.139	0.606	1.499	1.372	1.417
1307	hexyl acetate[c]			0.013	0.011	
1386	ethyl 2-hydroxypropanoate[c]		1.691	0.357	0.338	0.500
1396	heptyl acetate[c]				0.005	
1401	methyl octanoate[a]			0.046		0.025
1451	ethyl octanoate[b]	0.181	2.395	2.931	2.206	2.025
1477	3-methylbutyl hexanoate[b]			0.010		
1497	octyl acetate[b]			0.015		
1506	ethyl 7-octenoate[b]		0.022	0.013	0.049	0.092
1532	Ethyl 2,4-hexadienoate[c]					0.007
1553	ethyl 3-hydroxybutanoate[c]			0.221	0.061	0.248
1563	3-furanmethanol acetate[c]			0.036	0.019	
1564	2-furanmethanol acetate[c]				0.006	
1567	butyl octanoate[b]			0.004		
1652	ethyl decanoate[b]		1.297	3.198	2.980	2.678
1676	3-methylbutyl octanoate[b]			0.076		
1679	3,7-dimethyl 6-octen-1-ol acetate[c]			0.188	0.071	0.182
1700	diethyl butanedioate[b]	0.206	2.382	0.334	0.144	0.574
1709	ethyl 9-decenoate[b]	0.128	2.166	2.390	2.033	1.703
1776	3,7-dimethyl 2,6-octadien-1-ol acetate[c]			0.123	0.056	0.155
1808	methyl salicylate[c]	0.009	0.018	0.065	0.030	0.119
1809	ethyl benzeneacetate[b]	0.003	0.038			0.043
1847	2-phenylethyl acetate[b]	1.425	0.585	5.874	4.239	3.995
1850	ethyl dodecanoate[b]	0.049	0.184	1.451	1.108	1.036
1889	3-methylbutyl pentadecanoate[c]			0.164	0.089	
2001	phenylethyl butanoate[c]					0.910
2094	ethyl tetradecanoate[c]	0.040	0.154	0.108	0.064	0.020
2185	dibutyl phthalate[c]		0.269			
2260	isopropyl palmitate[c]	0.087	0.370	0.315	0.374	0.216
2271	ethyl hexadecanoate[c]		0.590	0.339	0.406	0.319
2279	3,7,11-trimethyl 2,6,10-dodecatrien-1-ol acetate[c]			0.277	0.280	0.200
2292	ethyl 9-hexadecenoate[b]		0.443	1.625	1.108	0.493
2365	diethyl phthalate[c]	0.055	0.121	0.224	0.407	0.214
2416	ethyl octadecanoate[c]		0.100	0.129	0.293	0.137
	sum of esters	***2.334***	***13.431***	***22.584***	***17.996***	***17.684***
	total esters	**12**	**18**	**30**	**27**	**26**
		organic acids				
1534	acetic acid[c]		6.689			0.157
1633	butanoic acid[b]					0.001
1962	hexanoic acid[c]		0.085	1.671	0.880	
2156	octanoic acid[c]	1.397	1.145	19.105	7.719	5.913
2213	nonanoic acid[c]		0.790	0.181	0.216	0.098
2336	decanoic acid[c]	0.508		3.622	2.744	2.396
2390	undecylenic acid[c]			0.661	0.834	1.130
	sum of acids	***1.905***	***8.709***	***25.240***	***12.393***	***9.695***
	total acids	**2**	**4**	**5**	**5**	**6**
		alcohols				
1179	1-butanol[b]		0.114	0.348	0.116	0.056
1312	4-penten-1-ol[b]		0.323	0.405	0.240	0.156
1329	4-methyl-1pentanol[b]			0.013	0.006	
1350	3-methyl-1-pentanol[b]	0.002	0.014	0.056	0.040	0.019
1370	1-hexanol[a]	0.094		0.152	0.039	0.160
1419	3-ethoxy-1-propanol[c]			0.042		
1470	1-heptanol[b]	0.009	0.079	0.024	0.038	0.045
1520	2-ethyl-1-hexanol[c]	0.015	0.013		0.018	0.022
1538	6-methyl-5-hepten-2-ol[c]	0.028		0.040		
1556	β-linalool[b]	0.053		0.031	0.022	0.022
1559	2.3-butanediol[b]	0.339	2.883	7.456	6.709	6.036
1570	1-octanol[a]	0.137				
1590	1.3-butanediol[b]	0.540	1.343	2.152	2.405	2.422
1675	isopinocampheol[c]				0.035	
1680	2-furanmethanol[a]	0.212		0.588	0.344	0.448
1715	terpineol[b]	0.287		0.177	0.139	0.237
1730	3-(methylthio) 1-propanol[b]	0.077	0.244	0.676	0.446	0.631
1783	decanol[c]		0.150	0.098	0.125	0.135
1790	3.7-dimethyl-6-octe-1-ol[c]		0.101	0.125	0.144	0.261

Table 5. Continued

RI	compd	grape must	free cells 15 °C	immobilized cells on potato pieces 15 °C	10 °C	5 °C
1823	2-(2-butoxyethoxy) ethanol[c]				0.011	
1843	anthenol[c]		1.105			
1916	benzyl alcohol[a]	0.032		0.059	0.027	0.028
1928	isophytol[c]	0.020	0.025		0.026	
1933	2-phenylethanol[a]	3.292	3.086	6.537	4.994	4.656
1998	dodecanol[a]	0.030	0.105			0.134
2090	nerolidol[c]		0.058	0.124	0.109	0.076
2312	2.4-bis-(1.1-dimethylethyl) phenol[c]	0.270	0.537	0.201	0.181	0.103
2343	3.7.11-trimethyl 2.6.10-dodecatrien-1-ol[c]		0.181		0.066	
2359	hexadecanol[c]		0.395			
	sum of alcohols	***5.437***	***10.756***	***19.304***	***16.28***	***15.647***
	total alcohols	**17**	**18**	**20**	**23**	**19**
	carbonyl compounds					
958	3-methyl-butanal[b]	0.034				
1195	5-methyl-3-heptanone[c]	0.004				
1358	6-methyl 5-hepten-2-one[c]	0.003				
1390	nonanal[b]			0.012		
1403	4-nonanone[c]			0.017		
1445	5-methyl 2-furanone[c]	0.001				
1486	furfural[a]	0.930	0.135			0.108
1533	benzaldehyde[b]	0.022		0.049	0.042	0.039
1578	5-methyl-furfural[c]	0.180		0.020		0.013
1741	geranial[c]		0.056			
1834	β-damascenone[b]	0.038	0 024	0 040		
	sum of carbonyl compounds	***1.212***	***0.191***	***0.098***	***0.042***	***0.160***
	total carbonyl compounds	**8**	**3**	**5**	**1**	**3**
	miscellaneous compounds					
690	chloromethane[c]	0.008		0.001	0.015	0.010
700	heptane[a]			0.110	0.079	0.024
850	tetrahydro furan[c]	0.005				
930	1.1-diethoxy ethane[c]		0.429			
1063	3-fluoro-1-propene[c]		0.388	1.033	0.970	0.316
1189	limonene[a]	0.038	0.081			
1236	1-dodecene[c]			0.083		
1454	*cis*-linalool oxide[b]		0.022	0.011	0.011	
1738	α-fernesene[c]				0.154	0.103
1909	2-(methoxymethyl)-tetrahydro furan[c]				0.019	
2072	ethoxy-benzene[c]				0.076	
2382	2.3-dihydro benzofuran[c]			0.177	0.176	0.209
	sum of miscellaneous compounds	***0.051***	***0.920***	***1.415***	***1.500***	***0.662***
	total miscellaneous compounds	**3**	**4**	**6**	**8**	**5**
	sum of compounds detected	***10.939***	***34.007***	***68.641***	***48.211***	***43.848***
	total compounds detected	**42**	**47**	**66**	**64**	**59**

[a] MS data and retention index in agreement with those of authentic compound. [b] MS data and retention index in agreement with those in the literature. [c] MS data in agreement with those in NIST and Wiley libraries.

alcohols and short chain fatty acids, the so-called "fruit esters". In the case of immobilized cells their content was higher compared to that of free cells. As the carbon number of acids increased the concentration was reduced. 2-Phenylethyl acetate, detected in all wine samples, contributes to the aromatic complexity of wines giving a banana-apple aroma (*34*). Its content was 10-fold higher in the case of immobilized cells at 15 °C compared to that of free cells. Acetates other than ethyl acetate were detected in our samples which are responsible for a pleasant fruit-like aroma (*35*). Their number and concentration were relatively high in the immobilized cells compared to those of free cells. Another ester with a higher concentration in immobilized cells was ethyl-9-decenoate, which was described as exhibiting a very pleasant odor (*36*). Fatty acids due to their low odor threshold values and rather high concentrations in wines are considered to have flavor impact in wines (*36*). As shown in **Table 5**, the number and

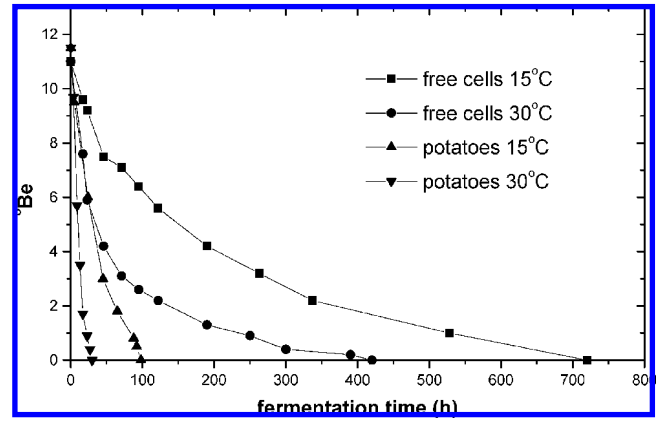

Figure 4. Fermentation kinetics observed with use of grape must at 30 and 15 °C by free and immobilized yeast cells on potato pieces.

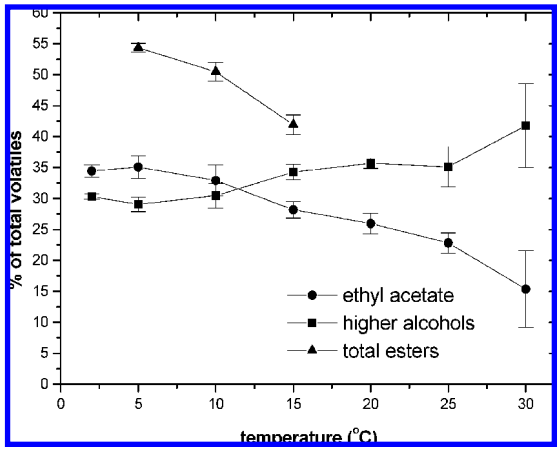

Figure 5. Effect of temperature on percentages of total esters, ethyl acetate, and higher alcohols on total volatiles were determined of wines produced by immobilized cells.

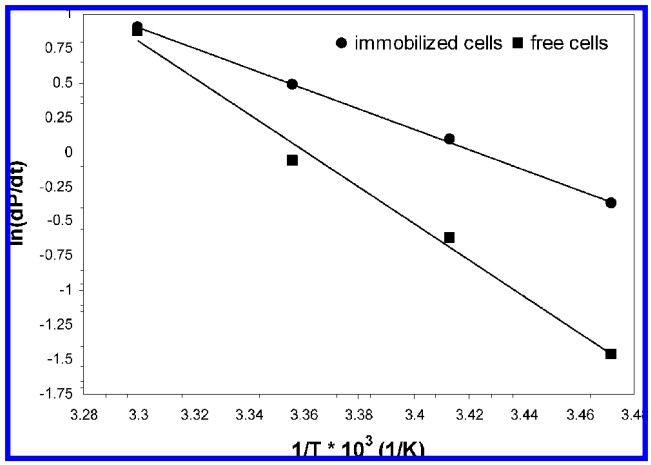

Figure 6. Arrhenius plot for the graphic evaluation of the activation energy and the pre-exponential factor of alcoholic fermentation performed with free and immobilized cells.

concentration of the volatile fatty acids is relatively higher in the case of immobilized cells compared to those of free cells. Similar findings have been reported in other studies with immobilized cells (*26*). The higher content of volatile acidity (0.70 g of acetic acid/L) in wines produced by free cells compared to that of wines produced by immobilized cells (up to 0.41 g of acetic acid/L) may explain the detection of acetic acid by GC/MS in the case of free cells. 2-Phenylethanol is one of the few fusel alcohols described with the pleasant odor of old rose (*12, 36*). It was detected in all wines but immobilized cells resulted to relatively higher contents than free cells. A reduction in its content with the decrease of temperature was observed, which is in accordance with other studies (*40*). Benzaldehyde detected in must and wines with immobilized cells has a bitter almond odor. β-Damascenone detected in must and wines with free and immobilized cells at 15 °C has a complex smell of flowers, tropical fruit, and stewed apple (*34, 41*). Furfural and 5-methyl furfural detected in wine samples are characterized by a toasted almond odor; however, in our samples these compounds were detected in very low levels probably because they are formed mainly during aging of wine in the barrel (*35*).

Effect of Immobilization on E_a. Figures 2, **3** and **4** show the fermentation kinetics observed with 12% w/v glucose and grape must at several temperatures by free and immobilized cells. The results from these figures are in agreement with several previous studies (*8, 15*), which used immobilized cells for wine making, and suggested the possible catalytic effect of the immobilization support on alcoholic fermentation. More specifically these studies after a theoretical consideration had concluded that the support may behave as a catalyst or as a promoter of the catalytic activity of the enzymes involved in the fermentation process, causing a reduction in the activation energy E_a. For these reasons experiments were undertaken to calculate the activation energy in the case of free and immobilized cells used for alcoholic fermentation. The activation energy of the immobilized cells on potato pieces was 44% smaller than that of free cells (61.1 and 109.6 kJ/mol, respectively), confirming the catalytic activity of the immobilization support. The activation energies were calculated by using the following equation based on the Arrhenius equation according to a previous study (*19*)

$$\ln(dP/dt) = \ln(AX) - E_a/RT \quad (1)$$

This equation describes the curve obtained by plotting $\ln(dP/dt)$ versus $1/T$ (**Figure 6**). From the slope and intercept of this straight line were calculated the activation energy E_a and the Arrhenius pre-exponential factor A.

Scientific and Technological Consideration of Results. It is the first time calculations of the activation energy E_a in the case of immobilized yeast and separately for free cells led to calculations of the fermentation rate constant k for free cells and immobilized cells on a solid support. Using the Arrhenius equation and substituting E_a and A, k_{30} and k_{15} for free and immobilized cells at 30 and 15 °C were calculated. Immobilization affected significantly ($p < 0.01$) the rate coefficient even at 30 °C ($p < 0.01$). Temperature also affected significantly ($p < 0.01$) the rate coefficient and a strong interaction between immobilization and temperature ($p < 0.01$) was also observed. More specifically the reaction rate constant at 30 °C, k_{30}, was higher for immobilized compared to free cells (26.2×10^{-2} and 23.2×10^{-2} h^{-1}, respectively), while at 15 °C the reaction rate constant, k_{15}, of immobilized cells was three times higher

Table 6. Comparison of Potatoes with Fruits and Other Food Grade Purity Supports in Grape Must Fermentation at 15 °C with Yeast Strain AXAZ-1[a]

immobilization support	wine productivity ((g/L)/day)	amyl alcohols (mg/L)	total volatiles (mg/L)	esters (mg/L)	ref
pear	91	167	408		*25*
quince	188	195	346		*42*
apple	200	246	428		*43*
gluten pellets	400	146	332		*15, 44*
DCM	857	124	315		*8, 44*
DRB	117	52	160		*12*
BSG[b]	132	137	267	84	*26*
potatoes	139	106	296	34	present study

[a] DCM: delignified cellulosic material. DRB: dried raisin berries. BSG: Brewer's spent grains. [b] Visanto-1 yeast strain used.

compared to that of free cells (7.4×10^{-2} and 2.4×10^{-2} h^{-1}, respectively). These calculations validate the theoretical approach previously published for delignified cellulosic materials (*8*). Therefore, the reduction of the activation energy supports the idea that catalysis of the alcoholic fermentation is performed by the solid support that holds the immobilized cells, in comparison with that of free cells. This reduction of the activation energy also explains the higher activity of immobilized cells as compared with that of free cells. Furthermore, it explains why extremely low temperature fermentations by immobilized cells on some supports are performed.

The quantitative analysis of esters and various alcohols made by GC-MS provided the possibility of determining the effect of temperature on percentages of total esters and total alcohols on total volatiles. **Figure 5** shows that the reduction of temperature results to an increase of total esters percentage and reduction of higher alcohols percentage. This increase of total ester percentages is an original result and in combination with the reduction of higher alcohol percentages it is an incident that explains the improvement of wine quality at low temperatures (*26*). Also, the reduction of activation energy that has been calculated for the first time for immobilized cells shows why immobilized cells are much more active at low temperatures than free cells. Furthermore, **Table 6** shows that immobilized cells on potatoes results in comparable wine productivities with those of other fruity supports that have been used in wine making. It also forms normal amounts of amyl alcohols and total volatiles as compared with other supports of food grade purity.

All of the above results of this study lead the potato pieces being a suitable support for yeast cell immobilization that can be used in white wine making as a biocatalyst of food grade purity. The formation of esters is increased by using immobilized cells on potato as compared with free cells. The percentages of total esters on total volatiles are increased as the temperature drops while the percentages of total higher alcohols are reduced. Potato pieces are a convenient support for low-temperature white wine making, form similar volatiles, and lead to similar productivities as fruity supports. After experimental kinetic consideration, potatoes reduce the activation energy E_a and seem to behave as a catalyst or as a promoter of the enzymes involved in the process. This experimental result validates previous theoretical investigation (*8*). This result may lead also to the conclusion that the increased productivities of immobilized cells as compared with those of free cells can be mainly attributed to catalysis by some supports. This results in the possibility of extremely low temperature fermentation, using immobilized cells on some supports. However, further research is necessary for various supports to ensure this conclusion.

ABBREVIATIONS USED

GC/MS, gas chromatography/mass spectrometry; SPME, solid phase microextraction; E_a, activation energy (kJ/mol); P, alcohol concentration (g/L); T, temperature (K); k, reaction rate constant (h^{-1}); t, fermentation time (h); X, cell mass concentration (g/L); R, ideal gas constant (kJ/(mol K)); A, Arrhenius pre-exponential factor of fermentation (h^{-1}); RI, retention index.

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