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Continuous Wine Making by γ-Alumina-Supported Biocatalyst

Quality of the Wine and Distillates

P. Loukatos,[1] M. Kiaris,[2] I. Ligas,[2] G. Bourgos,[2] M. Kanellaki,[2] M. Komaitis,[1] and A. A. Koutinas*,[2]

[1]Food Chemistry Laboratory, Department of Food Science, Agricultural University of Athens, Votanicos, Athens; and [2]Food Biotechnology Group, Department of Chemistry, University of Patras, Patras, Greece

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Abstract

The main objective of the present work was the removal of aluminum from wines produced by γ-alumina-supported yeast cells. Reagents such as Na_2CO_3, NH_4OH, albumin, and $Ca(OH)_2$ were used. Calcium in the presence of albumin was effective, whereas other reagents were not so effective. Because of the improved aroma and taste of distillates produced by γ-alumina-supported biocatalyst, volatile byproducts of distillates were analyzed. They were also assessed by sensory tests. Methanol, acetaldehyde, ethyl acetate, propanol-1, isobutyl alcohol, and amyl alcohols were determined in distillates. It was noted that the amounts of higher alcohols and amyl alcohols decreased as the temperature of fermentation dropped, leading to a product of improved quality and reduced toxicity.

Index Entries: Wine; distillates; aluminum; volatiles; quality.

Introduction

Cell immobilization of yeasts on various supports and preparation of suitable biocatalysts for continuous wine making have been objects of research interest for many years *(1–9)*. However, to utilize immobilized cells in wine making and further in the production of distillates, researchers must satisfy some prerequisites: the low cost of the support material, its availability in nature, and the nondestructible nature of the material in industrial processing. Furthermore, for wine making a hygienic support is

*Author to whom all correspondence and reprint requests should be addressed.

necessary, a prerequisite not conditional for the production of distillates. In the framework of the aforementioned requirements, γ-alumina pellets have been proposed as support for immobilization of yeast cells *(10)* and of *Zymomonas mobilis* for batch *(11)* and continuous processes *(12)*. Recently, a study on low-temperature wine making using γ-alumina-supported biocatalyst *(13,14)* and its effect on the formation of volatiles *(15)* was reported. It was observed that the wines produced had an improved aroma. These reports indicate that γ-alumina-supported biocatalyst has to be investigated (1) to remove the aluminum that is liberated in the wines produced by the use of γ-alumina-supported biocatalyst and (2) to examine the quality of distillates on the basis of volatile byproducts during low-temperature wine making. The present study focuses on these two points.

Materials and Methods

Grapes of Roditis cultivar were used. Must, of a total acidity in the range of 4.7–6.2, was employed in all experiments. It was sterilized before fermentation. The yeast strain Visanto-1, psychophilic and alcohol resistant, was used. It was isolated from grapes of a vineyard on the Aegean Island Santorini *(16)* and was grown on complete medium. Pressed wet weight cells (15–20 g) were prepared as in ref. *16* and were employed directly in fermentations.

The porous, cylindrical γ-alumina pellets Houndry Ho415 (length, 5 mm; diameter, 2.5 mm; pore volume, 0.4 cm^3/g; surface area, 1.40 m^2/g) were used as the support for immobilization. The immobilization of cells was performed as described previously *(10)*.

Batch and Continuous Wine Making

Repeated batch fermentations were performed at 30°C in a 500-mL multistage fixed batch tower bioreactor. It consisted of a glass cylinder containing five packed sections of 7 cm depth. Every packed section was supported via a vertical center beam. The netting, which holds γ-alumina, was also connected with the vertical center beam. γ-Alumina (40 g) was charged to each of the five levels and then used as immobilization support.

The series of fermentations were performed using the same γ-alumina-supported biocatalysts from batch to batch. The preparation of Visanto-1 inoculum, its immobilization on γ-alumina pellets and biomass attachment, and the continuous fermentation of must at various low and ambient temperatures were conducted according to the method described by Bakoyianis et al. *(14)*.

Removal of Aluminum from Wine

Samples of wine produced by batch fermentations using γ-alumina-supported biocatalyst were treated after each experiment separately with Na_2CO_3, ammonia solution, $Ca(OH)_2$, albumin, and a mixture of albumin and $Ca(OH)_2$. NH_4OH (Merck) was used as supplied. For Na_2CO_3, a 10% sodium solution was used, while concentrated $Ca(OH)_2$ solutions were used. Egg-white albumin was used in a suspension form prepared by adding the white of one egg to 0.5 L of commercial wine. This solution was added dropwise to the wine sample. Subsequently, all samples were filtered and the aluminum in the filtrate was determined.

Determination of Aluminum

The aluminum content in wines was determined by atomic absorption spectrometry using an atomic absorption frame emission spectrophotometer (Shimadzu model AA-6501) equipped with an acetylene-N_2O flame and aluminum hollow cathode lamp. An acetylene flow rate of 7 L/min was used. Wine samples were used directly for the analysis after being centrifuged. Aluminum concentrations were determined using a standard curve plotted in the range of 0–200 mg/L and diluting standard solutions of 1000 mg/L of $Al(NO_3)_3$.

Production of Distillates

When the steady state of the continuous fermentation of must was obtained at each temperature, five 1-L wine samples were collected during a 10-d period. The 1-L samples were distilled in a single distillation apparatus. The distillation lasted 2 h, and 150 mL of distillate was collected in an Erlenmeyer flask cooled by ice, each time.

A second series of wine samples was collected after passing the wine (just after the outlet of the bioreactors) through a cooler (cooling mixture of 2°C). The samples were collected in an Erlenmeyer flask cooled by ice (Fig. 1). This process resembles the process of Greek distillates Tsipouro and Tsicoudia.

Determination of Ethanol and Byproducts

Ethanol was determined as alcoholic degrees (milliliters of ethanol/100 mL of distillate) together with methanol by gas chromatography. The quantitative determinations of the constituents and volatiles were made with a Shimadzu Gas Chromatograph GS-8A connected with the integrator C-R 6A Chromatopac. For ethanol and methanol, Porapak S column material and nitrogen as the carrier gas (40 mL/min) were employed. The injector port and detector temperatures were 180°C. The column temperature was programmed between 140 and 180°C. 3-Pentanol was used as internal standard, and 1-μL samples of the wine were injected directly into the column.

Ethanol, ethyl acetate, propanol-1, isobutyl alcohol, and amyl alcohols were determined using a stainless-steel column, packed with Escarto 5905 (consisting of 5% squalene, 90% Carbowax 300, and 5% [v/v] bis (2-ethylexyl) sebacate), with N_2 as the carrier gas (20 mL/min). The injection port and detector temperatures were 210°C, and the column temperature was 58°C.

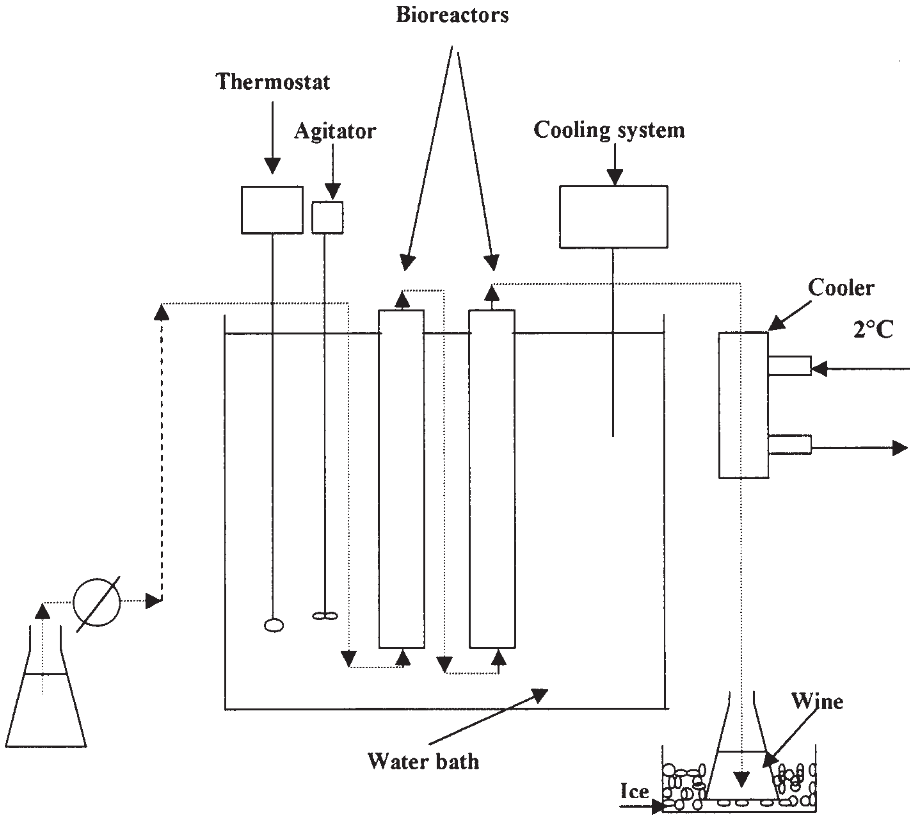

Fig. 1. Continuous wine making with cooling of the wine at the outlet.

The internal standard was 3-pentanol, at a concentration of 0.5% (v/v). Samples of 2 μL of the distillate were injected directly in the column.

Sensory Evaluation

Distillates produced by use of free cells and γ-alumina-supported biocatalysts were evaluated by 14 taste and aroma panelists. The evaluation was performed on a 0–10 scale. Tables 1 and 2 present the product characterization and the results.

Results

To use γ-alumina-supported biocatalyst in wine making, the aluminum content of the wines produced from batch to batch and ways to remove it had to be examined. Figures 2 and 3 present the results of this investigation.

Figure 2 shows clearly that the aluminum content of the wines in repeated batch fermentations was on the order of 200 ppm. This was observed after the third batch and remained constant for at least 15 repeated batches. Attempts to remove aluminum from the wine by precipitation

Table 1
Sensory Test results of Distillates Produced
by γ-Alumina-Supported Biocatalyst at 30 (Δ), 20 (○), and 16°C (+) Compared with Free Cells at 16°C (□)

Sample		Scale (0–10)	Tester 1	2	3	4	5	6	7	8	9	10	11	12	13	14
γ-Alumina-supported biocatalyst	Fine	10														
	Excellent	9						+						+		
	Very good	8		+	+		+	○		+	+					
	Good	7	+	Δ		+	○		○	○		+	Δ+	Δ○	+	
	Might be good	6	○	○	Δ○		Δ	Δ	+	Δ	○	Δ○	○		○	
	Medium	5	Δ			Δ○			Δ						Δ	+
	Acceptable	4									Δ					Δ○
	Might be bad	3														
	Bad	2														
	Very bad	1														
	Unsuitable	0														
Free cells	Fine	10														
	Excellent	9														
	Very good	8														
	Good	7														
	Might be good	6				□										
	Medium	5														
	Acceptable	4							□	□						□
	Might be bad	3						□			□	□	□	□	□	
	Bad	2	□	□			□									
	Very bad	1														
	Unsuitable	0			□											

Table 2
Aroma Test Results of Distillates Produced
by γ-Alumina-Supported Biocatalyst at 30 (Δ), 20 (○), and 16°C (+) Compared with Free Cells at 16°C (□)

Sample		Scale (0–10)	Tester 1	2	3	4	5	6	7	8	9	10	11	12	13	14
γ-Alumina supported biocatalyst	Fine	10														
	Excellent	9						+		+				+		
	Very good	8		+	+				○		+	+		Δ	+	
	Good	7	Δ○		○	+	+	Δ○	+					○		+
	Might be good	6	+	Δ○		Δ○	○			○	Δ○	○	+		Δ○	
	Medium	5			Δ		Δ		Δ	Δ		Δ	○			○
	Acceptable	4											Δ			Δ
	Might be bad	3														
	Bad	2														
	Very bad	1														
	Unsuitable	0														
Free cells	Fine	10														
	Excellent	9														
	Very good	8														
	Good	7														
	Might be good	6														
	Medium	5														
	Acceptable	4		□		□								□		
	Might be bad	3					□	□	□	□	□		□			
	Bad	2	□									□			□	□
	Very bad	1														
	Unsuitable	0			□											

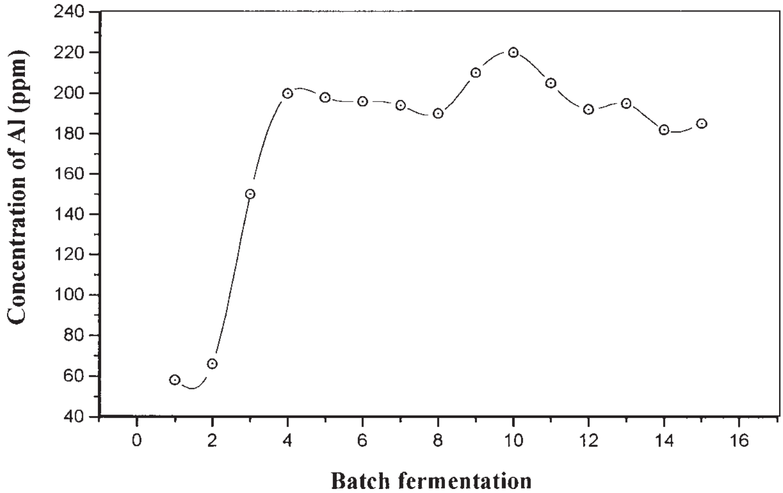

Fig. 2. Aluminum content in wines produced by γ-alumina-supported biocatalyst in relation to a series of repeated fermentation batches.

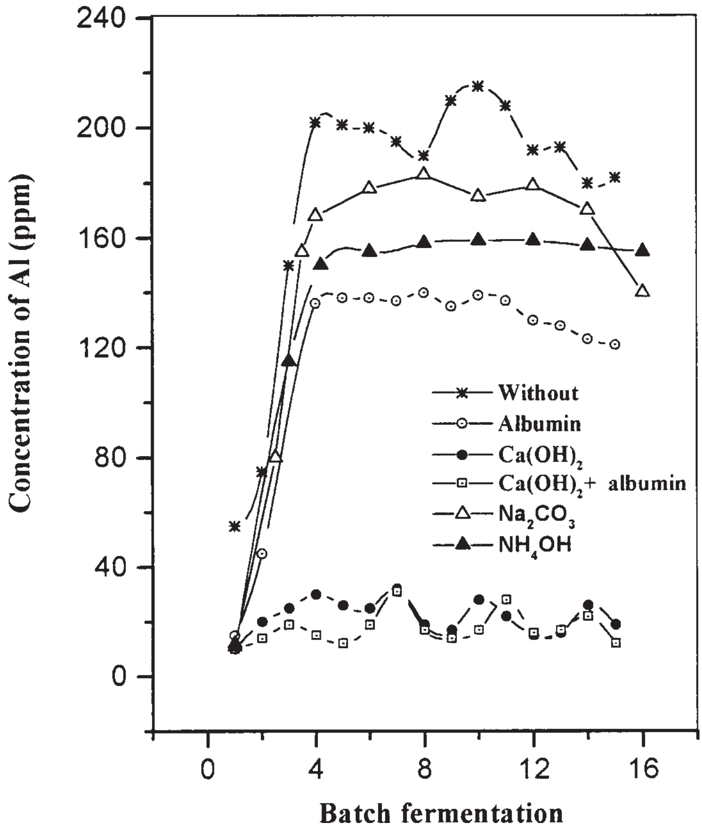

Fig. 3. Aluminum content in wines produced by repeated batch fermentations using γ-alumina-supported biocatalyst after treatment with Na_2CO_3, NH_4OH, albumin, $Ca(OH)_2$, $Ca(OH)_2$ in a mixture with albumin, and without treatment.

using sodium carbonate failed. Only 10% of the original amount was removed (Fig. 3). The use of NH_4OH improved the percentage of aluminum removal, but it was not sufficient enough to be safely used in wine production (Fig. 3). Egg albumin further increased the aluminum removal, but still the aluminum concentration of the wine remained high. However, by employing $Ca(OH)_2$, the aluminum concentration dropped to a very low concentration of 25 ppm, whereas the use of a mixture of egg albumin and $Ca(OH)_2$ further reduced the average aluminum content to a level of 17 ppm (Fig. 3).

However, wines produced by γ-alumina-supported biocatalyst even in the presence of high concentrations of aluminum are considered as suitable raw material for the production of distillates. Therefore, a study of volatiles in distillates in relation to the sensory test had to be performed. Tables 3 and 4 summarize the results.

Table 3 shows the effect of temperature on volatiles present in distillates. Ethyl acetate seems to remain constant as the temperature decreased whereas the propanol-1 content was reduced. The concentration of propanol-1 at 16°C was about half of that at 30°C. Likewise, the higher alcohols, isobutyl alcohol and amyl alcohol, decreased as the temperature was lowered. Specifically, isobutyl alcohol at 16°C was about 40% of that at 30°C. The decrease in the content of amyl alcohols was less as the temperature decreased. Finally, total volatiles also decreased by lowering the temperature from 30 to 16°C. The observed decrease was equal to 16%.

Furthermore, to keep most of the volatiles in the wines produced by continuous fermentation using γ-alumina-supported biocatalyst, cooling of the wine to 2°C in the outlet of the bioreactor and during collection of the wine was done. Table 4 presents the results obtained for volatile byproducts for distillates. The results are similar to those of distillates produced by wines with or without cooling. The concentrations of total volatiles in distillates from wines with cooling are about the same as those without cooling: 786 and 793 ppm, respectively.

To verify the improvements in the volatile content made by the use of immobilized cells on γ-alumina pellets, taste and aroma tests were done. Distillates by γ-alumina-supported biocatalyst were compared with those of free cells. Note that traditional production of distillates is affected with fermentations using free cells. Table 1 presents the sensory test results. All panelists preferred the distillates produced by use of γ-alumina-supported biocatalyst. It is also obvious that panelists preferred the distillates by fermentation at low temperatures. Half of the panelists characterized the distillate by γ-alumina-supported biocatalysts and by fermentation at 16°C as very good and excellent and 50% better than acceptable. By contrast, 78% of the panelists characterized the distillates produced by free cells as might be bad, and unsuitable. Regarding the aroma of the distillates, similar results were obtained (Table 2).

Table 3
Effect of Temperature on Volatiles Content
in Distillates Produced by Continuous Wine Making Using Immobilized Cells on γ-Alumina

Temperature (°C)	Ethanol concentration (% [v/v])	Methanol (mg/L)	Acetaldehyde (mg/L)	Ethyl acetate (mg/L)	Propanol-1 (mg/L)	Isobutyl alcohol (mg/L)	Amyl alcohols (mg/L)	Total volatiles (mg/L)
30	52	352	73	171	123	85	370	822
30	50	344	75	182	115	80	290	722
30	56	374	83	167	131	80	374	845
30	54	474	78	185	127	79	320	789
30	51	397	74	174	119	86	297	750
20	54	375	88	165	102	55	287	697
20	55	387	94	170	99	57	322	742
20	51	380	91	162	93	51	299	696
20	51	295	89	172	98	60	283	702
20	53	370	98	167	106	54	286	711
16	50	396	103	173	60	35	274	645
16	52	394	116	166	58	37	286	663
16	49	368	98	159	55	31	275	618
16	51	425	124	170	61	34	268	657
16	48	386	109	160	63	25	280	637

Table 4
Effect of Temperature on Volatiles Content in Distillates
from Continuous Fermentation by Immobilized Cells on γ-Alumina and After Cooling of Emitted Gases from Bioreactor

Temperature (°C)	Ethanol concentration (% [v/v])	Methanol (mg/L)	Acetaldehyde (mg/L)	Ethyl acetate (mg/L)	Propanol-1 (mg/L)	Isobutyl alcohol (mg/L)	Amyl alcohols (mg/L)	Total volatiles (mg/L)
30	55	383	83	187	120	92	410	892
30	60	422	79	182	108	83	380	832
30	59	450	69	189	97	80	302	737
30	56	390	63	172	113	86	315	749
30	54	484	82	164	99	78	330	753
26	53	407	72	159	110	73	303	717
26	57	387	67	164	115	79	328	753
26	52	364	74	175	96	82	290	717
26	55	374	69	168	101	75	298	717
26	54	415	78	163	95	80	307	723
23	58	398	80	182	94	72	295	723
23	56	387	76	177	102	70	289	714
23	55	388	82	174	90	68	305	719
23	59	280	78	169	92	75	278	692
23	53	410	84	181	89	69	280	703

Discussion

The improvement in the aroma of wines produced *(13)* and the possible use of immobilized cells in wine making and production of distillates was the reason for this investigation. It was obvious that γ-alumina will liberate in the wine a known toxic heavy metal. Therefore, a detailed study of the aluminum content and its removal was necessary. Repeated batch fermentations were used for the investigation of the presence of aluminum in wine. The results clearly showed that the aluminum concentrations were at a relatively high level. They remained constant from batch to batch. To remove the aluminum, various reagents such as Na_2CO_3, NH_4OH, egg albumin, and $Ca(OH)_2$ were employed. $Ca(OH)_2$ as well as a mixture of egg albumin and $Ca(OH)_2$ were the most effective. Moreover, these materials are cheap and abundant in nature. The aluminum remaining after the treatment (17 ppm) is considered to be near the higher limit permitted by state legislation. The better results obtained by the use of $Ca(OH)_2$ are owing to the fact that calcium ions cause better coagulation of colloid aluminum hydroxide than Na^+ and NH_4^+ *(17)*. Likewise, that the presence of albumin with $Ca(OH)_2$ further increased the precipitation observed can be attributed to its known reaction with tannins and the formation of bulky precipitates *(18)*, which remove additional amounts of aluminum hydroxide.

The decrease in volatile compounds such as higher and amyl alcohols in the distillates as the temperature of fermentation decreased resulted in reduced toxicity of the distillates. Furthermore, the reduction in the concentration of these volatiles resulted in the production of distillates of improved flavor. This is clearly shown by the sensory tests (Tables 1 and 2). The latter may be attributed to the diminution of volatiles such as propanol-1, isobutyl alcohol, and amyl alcohol, and the relatively high ethyl acetate concentration, which was also a result of the temperature decrease. The increase in the percentage of ethyl acetate and the reduction in the percentage of isobutyl alcohol and propanol-1, calculated on total volatiles determined plus methanol, significantly contributed to an improvement in the taste and aroma of the distillates (Fig. 4). The latter was observed in wines produced by continuous fermentation using kissiris-supported biocatalyst *(19)*. Improvement in the composition of distillates produced by γ-alumina-supported biocatalyst seems to acquire a processing stability as indicated by the consistency of volatiles determined from batch to batch (Fig. 5). Finally, cooling of the wine outlet stream and the gases emitted at low temperature (2°C) marginally increased the amount of the total volatiles; this is considered undesirable.

One can conclude that γ-alumina is a good support for cell immobilization when the wines are employed for the production of distillates. γ-Alumina-supported biocatalyst leads to the production of distillates of better quality compared with those produced by the use of free cells. Distillates produced at low temperatures also have better quality characteristics. In the case of wine making, the removal of aluminum from the final product is needed. The most effective reagent is $Ca(OH)_2$, especially in the presence of albumin.

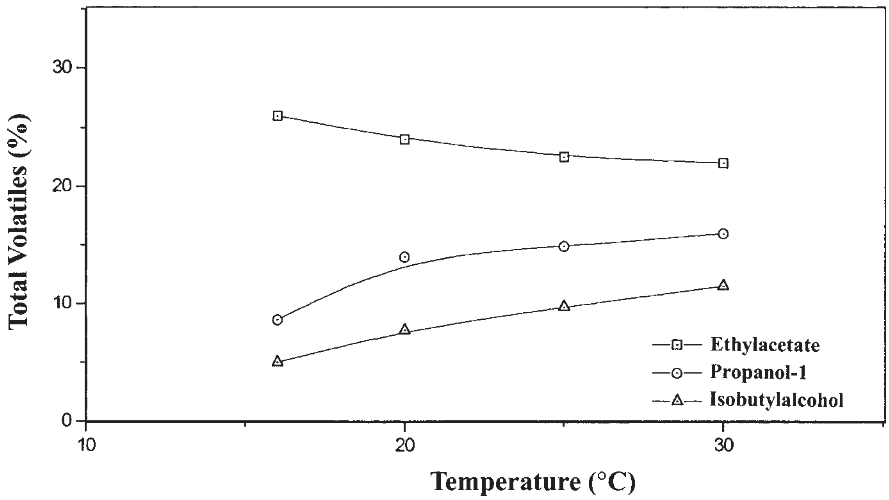

Fig. 4. Contents of each volatile on total volatiles determined in distillates from continuous fermentation by immobilized cells on γ-alumina.

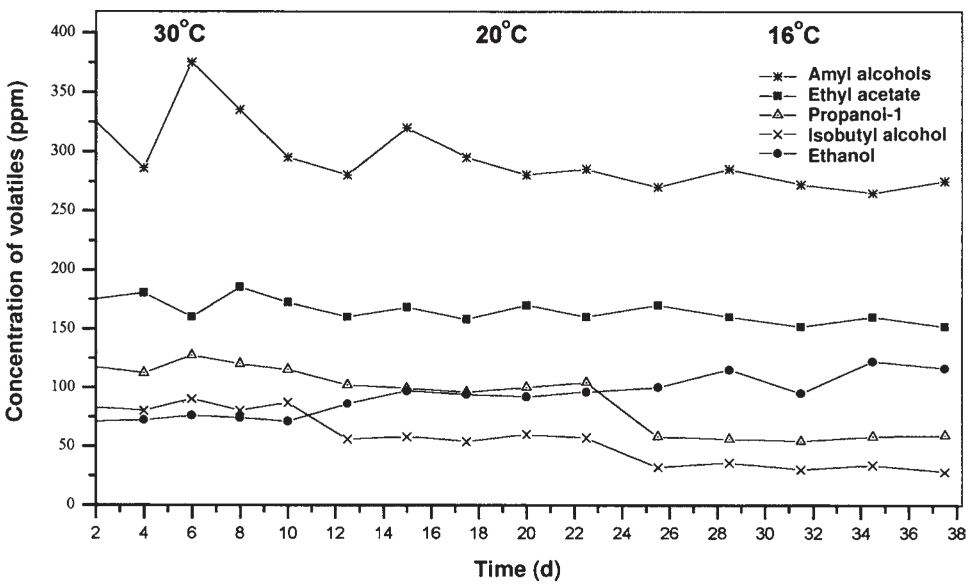

Fig. 5. Operational stability regarding the volatile contents in distillates produced by continuous fermentation by immobilized cells on γ-alumina.

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