ISSN 1330-9862
(FTB-2760)

scientific note

Low-Temperature Winemaking by Thermally Dried Immobilized Yeast on Delignified Brewer's Spent Grains

*Konstantina Tsaousi[1,2], Asimina Velli[1], Filippos Akarepis[3], Loulouda Bosnea[1], Chryssoula Drouza[2], Athanasios A. Koutinas[1] and Argyro Bekatorou[1]**

[1]Food Biotechnology Group, Department of Chemistry, University of Patras, GR-26500 Patras, Greece

[2]Department of Agricultural Production and Food Science and Technology, Cyprus University of Technology, CY-3603 Limassol, Cyprus

[3]Technical Enological Institute, Ag. Spyridonos Str., GR-12243 Egaleo, Athens, Greece

Received: November 20, 2010
Accepted: February 3, 2011

Summary

Preservation, risk of contamination, transportation and storage costs (*e.g.* refrigeration) are the main problems associated with wet, active starter cultures such as yeast. To deal with these aspects, drying of commercial cultures is required. A thermally dried biocatalyst has been prepared by immobilization of the psychrotolerant yeast strain *Saccharomyces cerevisiae* AXAZ-1 on delignified brewer's spent grains, followed by simple thermal drying or by air stream at 30 and 35 °C. Repeated batch fermentations of grape must (11.5 °Be, corresponding to 196 g/L of fermentable sugar) using the dried biocatalysts were performed at successively reduced temperatures (15, 10 and 5 °C). The fermented samples were analyzed for residual sugar, alcohol and free cell concentrations. The alcohol yield, alcohol productivity and sugar conversion were calculated in order to estimate the optimum conditions of drying. The volatile constituents in the produced wines were analysed by GC analysis. The results showed that drying of immobilized cells by the proposed techniques did not affect their viability and fermentative activity. High alcohol productivity, increased esters and lower concentrations of higher alcohols obtained by low-temperature fermentation using the dried biocatalysts indicate a potential improvement of product quality.

Key words: brewer's spent grains, immobilization, thermal drying, winemaking

Introduction

A wide variety of pure yeast cultures, mainly *Saccharomyces*, are produced industrially for the use in induced wine fermentations, according to the industrial demands for fermentation efficiency and productivity (*1*). In the last decades many researchers have focused, among other applications, on immobilization of *S. cerevisiae* on various natural solid materials such as cellulose, gluten pellets, pieces of fruit, orange peel, grape skins, *etc.*, for alcoholic beverages and fermented food production (*2–5*). The results of these efforts were outstanding regarding the improvement of fermentative activity, quality of the products and potential for industrial application. Nevertheless, for full commercialization, development of suitable drying methods is required for the production of dried biocatalysts easy to store, preserve, and transport. Various drying methods such as freeze-drying and spray-drying have been evaluated (*5*) and are mainly used in the food industry. The main disadvantage of these methods is the higher equipment cost and energy demand compared to simpler methods like convective, thermal drying, which in turn may have some disadvantages

*Corresponding author; Phone: ++30 2610 997 123; Fax: ++30 2610 997 105; E-mail: ampe@chemistry.upatras.gr

such as reduced cell viability and undesired rheological properties of the dried cultures.

A yeast cell suspension (*S. cerevisiae*) spread in thin layers was dried successfully by a simple thermal drying method at low temperatures and the dried cells showed higher glucose fermentation activity compared to the freeze-dried ones (*6*). Storage of the dried culture for over a month did not lead to loss of fermentative ability and the thermally dried cells could effectively ferment glucose at low temperatures. The chemical composition of volatiles produced during glucose fermentation was similar to that obtained using freeze-dried and fresh yeast cultures. In the present study, the effect of low-temperature thermal drying of yeast immobilized on brewer's spent grains (BSG) and the use of the dried biocatalyst for winemaking are investigated. Thermal drying was performed at low temperatures (30 and 35 °C) in an oven with or without air stream supply. The fermentative activity of the biocatalyst, the alcohol productivity, alcohol yield, and major volatile compounds produced during winemaking were evaluated.

Materials and Methods

Yeast strain, immobilization support and media

The yeast used was the alcohol-resistant and psychrotolerant strain *S. cerevisiae* AXAZ-1 isolated from an agricultural area in Greece. It was grown in a medium containing (in g/L): $(NH_4)_2SO_4$ (Merck, Darmstadt, Germany) 1, KH_2PO_4 (Fluka, Buchs, Switzerland) 1, $MgSO_4{\cdot}7H_2O$ (Merck) 5, yeast extract (Fluka) 4, and glucose monohydrate (Fluka) 40, and harvested by centrifugation at 5000 rpm for 10 min. Brewer's spent grains (BSG) were obtained from the Athenian Brewery S.A. (Patras, Greece). They contained 26.2 g of dry matter per 100 g of sample and crude protein 24.0, ash 4.7, and fat 6.8 (in g/100 g of dry matter, respectively), and had pH=5.0. Grape must was provided by the Achaia Clauss winery (Patras, Greece), and was diluted to an initial density of 11.5 °Be, corresponding to 196 g/L of fermentable sugar, without the addition of extra nutrients. All media were sterilized by autoclaving at 130 °C and at 150–200 Pa for 15–20 min.

Immobilization of yeast

BSG were pretreated with a solution of 10 g/L of NaOH for the removal of lignin (*7*) and then the delignified brewer's spent grains (DBSG) were sterilized by autoclaving at 120 °C for 10 min. A mass of 170 g of DBSG was mixed with 16 g of *S. cerevisiae* AXAZ-1 in 800-mL glucose synthetic medium (120 g/L) and left to ferment for 6–8 h for the immobilization of the yeast. The supernatant liquid was decanted and the biocatalyst was washed twice with 200 mL of fresh glucose medium.

Drying of the biocatalyst in thin layers

Drying was performed by thermal drying (*i*) in an oven (WTCbinder, Tuttlingen, Germany), and (*ii*) in an oven equipped with air circulation (J.P. Selecta, Barcelona, Spain), at 30 and 35 °C. A mass of 170 g of the biocatalyst was spread in thin layer (~5 mm) on glass plates. The drying process was carried out up to constant mass.

Winemaking

Repeated batch fermentations of 400 mL of grape must were performed at 15, 10 and 5 °C using the dried immobilized biocatalyst. Fermentation kinetics was monitored by measuring the density at various time intervals. At the end of each fermentation batch, the liquid was collected and analysed for the determination of produced ethanol, residual sugars and volatile by-products. The data were analysed using the one-way analysis of variance (ANOVA). The biocatalyst was washed twice with fresh glucose medium and used for a subsequent batch fermentation.

Determination of ethanol and residual sugar

Ethanol was determined by gas chromatography (GC) on a Shimadzu GC-8A gas liquid chromatograph system (Kyoto, Japan), with Porapak S column (130 °C, 10 °C/min), flame ionization detector (FID) (220 °C), nitrogen as carrier gas (40 mL/min), and injector temperature 210 °C. A volume of 2 μL of each sample was injected directly into the column and the concentration of ethanol was determined using standard curves. Internal standard was 1-butanol solution of 0.5 % (by volume). Residual sugars were determined by HPLC on a Shimadzu LC-9A HPLC system, with Shim-pack SCR-101N column (60 °C), RID-6A refractive index detector, triple distilled water as mobile phase (0.8 mL/min), and 1-butanol as internal standard. Volumes of 0.5 mL of the sample and 2.5 mL of 1 % (by volume) solution of 1-butanol were diluted to 50 mL, and then 40 μL of the final solution were injected into the column after filtration through 0.2-μm microfilters.

Determination of volatiles

Major volatiles were determined by GC on a Shimadzu GC-8A gas-liquid chromatograph with a stainless steel column packed with Escarto-5905 (5 % squalene, 90 % Carbowax 300, and 5 % bis(2-ethylhexyl) sebacate), nitrogen as carrier gas (20 mL/min), injection port and FID detector temperature 210 °C, and column temperature 70 °C. The internal standard was 1-butanol (0.5 % by volume). Samples of 4 μL were injected directly into the column, and the concentrations of the above compounds were calculated using standard curves.

Scanning electron microscopy

Examination of the dried immobilized biocatalysts was carried out by electron scanning microscopy using a Jeol model JSM-6300 SEM (JEOL Ltd., Tokyo, Japan), and coating with gold in a Balzers SCD 004 sputter coater (OC Oerlikon Balzers AG, Balzers, Liechtenstein) for 3 min to increase electron conductivity.

Results and Discussion

A biocatalyst was prepared by immobilization of the psychrotolerant yeast strain *S. cerevisiae* AXAZ-1 on delignified brewer's spent grains, and dried by simple thermal drying techniques at 30–35 °C. BSG have high nutritive value, mainly protein and fibre, and they are suitable as carriers for yeast immobilization to promote its fer-

mentation activity (*8*,*9*). Furthermore, thermal drying methods at low temperatures are simple, low-cost and mild drying techniques that do not affect viability and fermentation activity of the yeast (*6*). In order to study the effect of thermal drying on the fermentation activity of the dried immobilized biocatalyst and on wine quality, repeated fermentations of grape must were carried out at low temperatures of 15, 10 and 5 °C. Kinetics of the first fermentation batch at 15 °C is presented in Fig. 1. It can be observed that a few more hours were needed for the start-up of fermentation when the biocatalyst was dried at 35 °C using both drying techniques, but there were no differences in the average fermentation time ($p<0.05$) among the tested biocatalysts. Statistically significant differences between fermentation times ($p<0.05$) seem to appear between the biocatalysts dried in an oven or by air stream at 35 °C during fermentations at 15 or 10 °C. Immobilization of yeast cells was observed by electron microscope (Fig. 2). The immobilized cells seemed to remain adherent and intact on the supporting material even after drying, while no differences were observed between *S. cerevisiae* cells dried at 30 or 35 °C. The cells sustained their shape and the integrity of the cell walls, indicating potential protective effect of the carrier (DBSG).

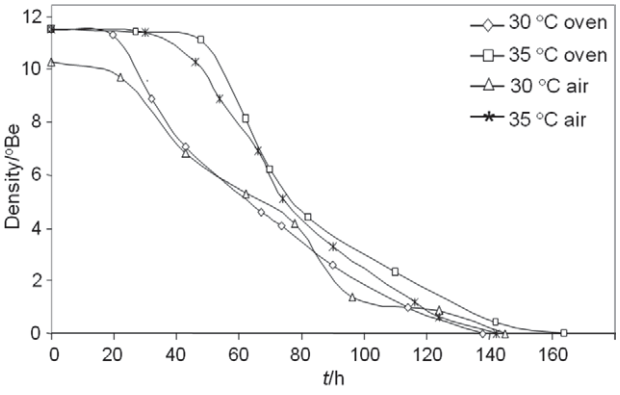

Fig. 1. Kinetics of the first fermentation batch of grape must at 15 °C by *S. cerevisiae* immobilized on DBSG and thermally dried in an oven at 30 or 35 °C, or by air stream at 30 or 35 °C

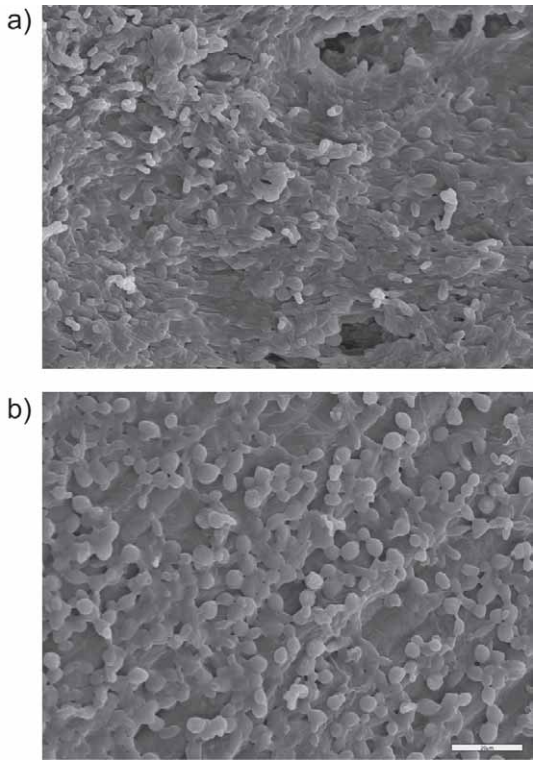

Fig. 2. Scanning electron microscopy of *S. cerevisiae* immobilized on DBSG, thermally dried: a) in an oven at 35 °C, and b) by air stream at 30 °C

Winemaking using the thermally dried biocatalysts

To estimate the viability and maintenance of fermentation ability of *S. cerevisiae* cells after both thermal drying processes (oven drying or drying by air stream at 30 and 35 °C), the dried biocatalysts were used for repeated fermentation batches of grape must at low temperatures (15, 10 and 5 °C). Fermentation kinetic parameters of the biocatalysts dried in an oven at 30 or 35 °C are presented in Tables 1 and 2, respectively. The biocatalyst dried at 35 °C required more time to complete the fer-

Table 1. Kinetic parameters of the repeated fermentations of grape must at 15, 10 and 5 °C by the oven-dried biocatalyst at 30 °C

Temperature	Batch	Fermentation time	γ(residual sugars)	φ(ethanol)	Ethanol yield	Ethanol productivity	Conversion
°C		h	g/L	%	g/g	g/(L·day)	%
15	1	138	4.12	11.41	0.46	15.67	97.94
15	2	95	6.65	11.32	0.46	22.59	96.67
15	3	75	10.44	10.82	0.45	27.34	94.78
15	4	71	7.90	11.34	0.47	30.28	96.05
15	5	68	12.40	10.82	0.46	30.17	93.80
10	6	131	12.92	10.96	0.46	15.86	93.54
10	7	152	14.50	10.84	0.46	13.52	92.75
10	8	143	17.73	10.03	0.44	13.30	91.13
10	9	139	11.93	10.79	0.45	14.71	94.03
10	10	141	14.70	10.29	0.44	13.84	92.65
5	11	462	11.65	10.25	0.43	4.21	94.17
5	12	576	17.64	10.37	0.45	3.41	91.18
5	13	720	16.24	10.20	0.44	2.69	91.88

Table 2. Kinetic parameters of repeated fermentations of grape must at 15, 10 and 5 °C by the oven-dried biocatalyst at 35 °C

Temperature	Batch	Fermentation time	γ(residual sugars)	φ(ethanol)	Ethanol yield	Ethanol productivity	Conversion
°C		h	g/L	%	g/g	g/(L·day)	%
15	1	164	6.80	11.44	0.47	13.22	96.60
15	2	127	12.29	10.34	0.44	15.44	93.85
15	3	122	8.06	11.48	0.47	17.84	95.97
15	4	119	12.69	11.00	0.46	17.52	93.65
15	5	117	13.90	10.54	0.45	17.08	93.05
10	6	192	14.76	10.98	0.47	10.85	92.62
10	7	196	16.14	10.99	0.47	10.63	91.93
10	8	282	14.91	11.51	0.49	7.74	92.54
10	9	332	14.48	11.30	0.48	6.45	92.76
10	10	381	11.34	11.11	0.47	5.53	94.33
5	11	720	12.72	10.89	0.46	2.87	93.64
5	12	749	13.65	10.90	0.46	2.76	93.18
5	13	801	14.32	11.10	0.47	2.63	92.84

mentation. Furthermore, a decrease of fermentation temperature led to an increase of the difference in fermentation time of the biocatalysts dried at 30 or 35 °C, although both were able to ferment the must efficiently. Fermentations at 15 °C were completed in 3–6 days. Even at 5 °C, which is extremely low temperature for winemaking, fermentations were completed in 25–30 days. Differences between the tested biocatalysts were also observed in alcohol productivity (due to variations of yield and fermentation time). Therefore, taking into account the lower energy demand, lower fermentation time and higher alcohol productivity, drying at 30 °C seems to be more advantageous.

Kinetics of fermentation carried out with biocatalysts dried by air stream at 30 or 35 °C is shown in Tables 3 and 4, respectively. In this case, the different drying temperatures did not seem to affect the fermentation time and alcohol productivity ($p<0.05$). This is probably due to the fact that by using air stream, the drying time is shorter, hence the exposure of yeast cells under these conditions is shorter and cell viability is not affected. Therefore, comparing the drying methods, drying by air stream is more advantageous because the drying time is shorter and the fermentation efficiency of the biocatalyst is not affected. These results are in agreement with previous observations regarding an extensive study of drying times, moisture levels and effect of these drying methods on yeast fermentation activity (*9*).

Formation of volatiles during winemaking using the thermally dried biocatalysts

Analysis of the major volatile compounds in wines made using the thermally dried biocatalysts was performed. Fig. 3 presents the concentrations of the major volatiles in wines produced by biocatalysts dried in an oven or by air stream at 30 °C. Significant variation of the total amount of volatiles in wine was not observed, although a small reduction was recorded at lower tem-

Table 3. Kinetic parameters of repeated fermentations of grape must at 15, 10 and 5 °C by the air stream-dried biocatalyst at 30 °C

Temperature	Batch	Fermentation time	γ(residual sugars)	φ(ethanol)	Ethanol yield	Ethanol productivity	Conversion
°C		h	g/L	%	g/g	g/(L·day)	%
15	1	145	4.08	11.23	0.45	14.68	97.96
15	2	76	2.16	11.46	0.46	28.59	98.92
15	3	71	9.80	11.23	0.47	29.99	95.10
15	4	71	13.30	10.84	0.46	28.95	93.35
15	5	74	10.46	11.02	0.46	28.24	94.77
10	6	117	12.95	10.80	0.46	17.50	93.52
10	7	142	12.41	10.83	0.46	14.46	93.79
10	8	168	12.50	10.90	0.46	12.31	93.75
10	9	187	9.62	11.40	0.47	11.56	95.19
10	10	197	17.89	10.39	0.45	10.00	91.06
5	11	681	17.04	10.58	0.46	2.95	91.48
5	12	699	11.36	10.98	0.46	2.98	94.32
5	13	716	16.98	10.43	0.45	2.76	91.51

Table 4. Kinetic parameters of repeated fermentations of grape must at 15, 10 and 5 °C by the air stream-dried biocatalyst at 35 °C

Temperature	Batch	Fermentation time	γ(residual sugars)	φ(ethanol)	Ethanol yield	Ethanol productivity	Conversion
°C		h	g/L	%	g/g	g/(L·day)	%
15	1	142	4.97	11.47	0.47	15.32	97.51
15	2	72	4.63	11.47	0.46	30.21	97.69
15	3	75	0.86	10.78	0.43	27.26	99.57
15	4	70	1.91	10.97	0.44	29.72	99.05
15	5	66	0.80	11.03	0.44	31.69	99.60
10	6	116	10.08	9.69	0.40	15.83	94.96
10	7	141	14.95	10.23	0.44	13.75	92.53
10	8	181	16.75	10.03	0.43	10.51	91.62
10	9	184	17.36	10.21	0.44	10.52	91.32
10	10	182	15.82	10.35	0.44	10.78	92.09
5	11	480	15.84	11.23	0.48	4.44	92.08
5	12	648	15.90	10.70	0.46	3.13	92.05
5	13	696	12.57	11.27	0.48	3.07	93.71

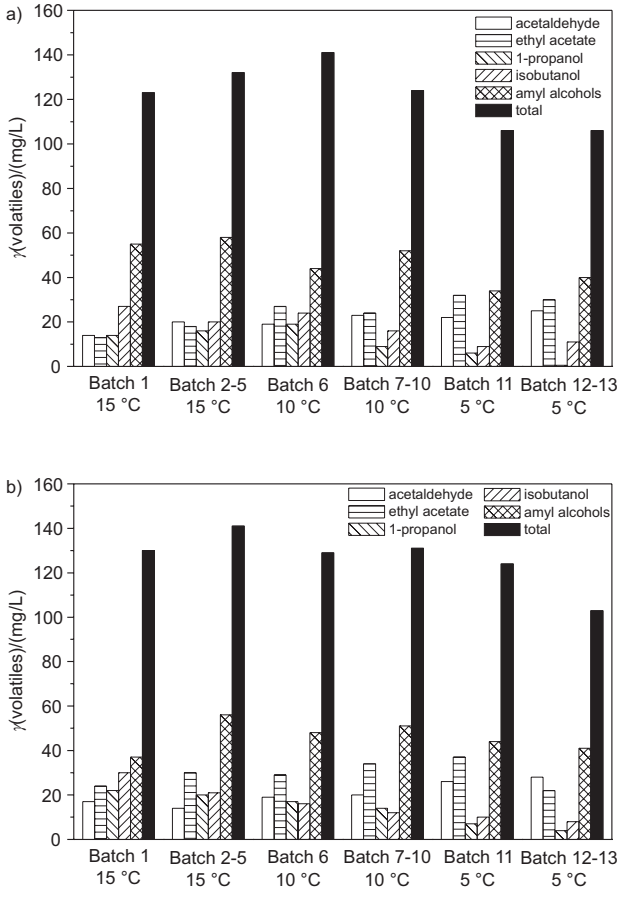

Fig. 3. Concentrations of major volatiles in wine produced by biocatalysts dried: a) in an oven, and b) by air stream, at 30 °C

peratures. Also, as shown in previous studies regarding low fermentation temperatures using immobilized cells (*5*), the concentrations of higher alcohols were reduced and ethyl acetate concentrations were slightly increased. Furthermore, the concentrations of acetaldehyde were low, which is known to contribute to fruity aromas in wines. Ethyl acetate also contributes to pleasant aroma at concentrations below or up to threshold values. On the other hand, 1-propanol and amyl alcohols cause unpleasant aromas and bitter flavours of ripe fruit and alcohol (*10*). Therefore, the use of the immobilized biocatalysts dried with the described methods can potentially affect and improve the quality of wine.

Conclusions

The development of low-cost, simple and mild drying techniques is essential for commercialization of biocatalysts such as the ones proposed in the present study. DBSG is a suitable material for yeast immobilization and might protect yeast cells during drying and promote its fermentation activity. It has been shown that biocatalysts produced by immobilization of *S. cerevisiae* AXAZ-1 on BSG and DBSG can improve the quality of the product, especially at low fermentation temperatures (*11–13*). Drying ensures increased shelf-life, easier handling and transportation, lower mass, and protection from spoilage, so that the dried biocatalysts can be produced commercially and be available for various applications. In this study it was demonstrated that thermal drying of immobilized cells in an oven or by air stream did not affect their viability and fermentative activity. High alcohol productivity, increased esters and lower concentrations of higher alcohols obtained by low-temperature fermentation using the dried biocatalysts were the main results obtained, indicating a potential improvement of wine quality. The dried biocatalysts were efficient regarding fermentation ability and the drying methods used are simple and require low cost for equipment and energy demand.

Acknowledgements

This paper is part of research project no. 03ED922, implemented within the framework of the 'Reinforcement Programme of Human Research Manpower' (PENED) and co-financed by National and Community Funds (20 % by the Greek Ministry of Development, General Secretariat of Research and Technology and 80 % by the E.U. European Social Fund).

References

1. A. Bekatorou, C. Psarianos, A.A. Koutinas, Production of food grade yeasts, *Food Technol. Biotechnol. 44* (2006) 407–415.
2. M. Iconomopoulou, M. Kanellaki, K. Psarianos, A.A. Koutinas, Delignified cellulosic material supported biocatalyst as freeze-dried product in alcoholic fermentation, *J. Agric. Food Chem. 48* (2000) 958–961.
3. S. Plessas, A. Bekatorou, A.A. Koutinas, M. Soupioni, I.M. Banat, R. Marchant, Use of *Saccharomyces cerevisiae* cells immobilized on orange peel as biocatalyst for alcoholic fermentation, *Bioresour. Technol. 98* (2007) 860–865.
4. A. Mallouchos, P. Skandamis, P. Loukatos, M. Komaitis, A.A. Koutinas, M. Kanellaki, Volatile compounds of wines produced by cells immobilized on grape skins, *J. Agric. Food Chem. 51* (2003) 3060–3066.
5. Y. Kourkoutas, A. Bekatorou, I.M. Banat, R. Marchant, A.A. Koutinas, Immobilization technologies and support materials suitable in alcohol beverages production: A review, *Food Microbiol. 21* (2004) 377–397.
6. K. Tsaousi, D. Dimitrellou, A.A. Koutinas, Low temperature thermal drying of *Saccharomyces cerevisiae* starter culture for food production, *Food Chem. 110* (2008) 547–553.
7. E.P. Bardi, A.A. Koutinas, Immobilization of yeast on delignified cellulosic material for room temperature and low-temperature wine making, *J. Agric. Food Chem. 42* (1994) 221–226.
8. S.I. Mussatto, G. Dragone, I.C. Roberto, Brewer's spent grain: Generation, characteristics and potential applications, *J. Cereal Sci. 43* (2006) 1–14.
9. K. Tsaousi, A.A. Koutinas, A. Bekatorou, P. Loukatos, Fermentation efficiency of cells immobilized on delignified brewer's spent grains after low- and high-temperature thin layer thermal drying, *Appl. Biochem. Biotechnol. 162* (2010) 594–606.
10. M. Gil, M.J. Cabellos, T. Arroyo, M. Provanov, Characterization of the volatile fraction of young wines from the Denomination of Origin 'Vinos de Madrid' (Spain), *Anal. Chim. Acta, 563* (2006) 145–153.
11. N. Kopsahelis, M. Kanellaki, A. Bekatorou, Low temperature brewing using cells immobilized on brewer's spent grains, *Food Chem. 104* (2007) 480–488.
12. A. Mallouchos, M. Komaitis, A.A. Koutinas, M. Kanellaki, Wine fermentations by immobilized and free cells at different temperatures. Effect of immobilization and temperature on volatile by-products, *Food Chem. 80* (2003) 109–113.
13. A. Mallouchos, P. Loukatos, A. Bekatorou, A.A. Koutinas, M. Komaitis, Ambient and low temperature winemaking by immobilized cells on brewer's spent grains: Effect on volatile composition, *Food Chem. 104* (2007) 918–927.