A Very Efficient β-Glucosidase Catalyst for the Hydrolysis of Flavor Precursors of Wines and Fruit Juices

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Candida molischiana 35M5N β-glucosidase was immobilized to Duolite A-568 resin. Higher immobilization efficiency (86%) was achieved with citrate−phosphate buffer (0.1 M) at pH 4. The study of the immobilized β-glucosidase demonstrated that the physicochemical properties were similar to those of the free enzyme. Free and immobilized β-glucosidase were used to treat muscat wine and apricot fruit juice. GC−MS analysis indicated a significant increase in the flavor compounds nerol, geraniol, linalool, 2-phenylethanol, and benzyl alcohol in the muscat wine and linalool, α- and γ-terpinene, α-terpineol, 2-phenylethanol, and α-pinene in the apricot fruit juice. The immobilized β-glucosidase was found to be very stable under fruit juice or wine conditions and could be used repeatedly for several hydrolyses of bound aroma. The efficiency of this experimental catalyst was successfully tested with several fruit juices and wines containing various amounts of precursors.

Keywords: *Wines; fruit juices; aroma precursors; β-glucosidase; immobilization*

INTRODUCTION

The study of the aromatic potential of fruit juices and wines (Engel and Tressl, 1983; Schwab and Schreier, 1988; Williams et al., 1982a,b; Gunata et al., 1985; Vasserot et al., 1993) has revealed that besides a free fraction of volatile terpenols there exist naturally nonodorous and nonvolatile precursors and they represent an important source of fragant compounds (Cordonnier et al., 1986). In general, bound glycoside forms are more abundant than the free ones (Dimitriadis et al., 1984; Gunata et al., 1985).

This aromatic potential is naturally revealed during fruit maturation by endogeneous enzymes identified as β-glucosidases (Gunata, 1984). Since these enzymes show low activities and cannot liberate the whole aromatic potential, hydrolytic experiments were performed with exogeneous β-glucosidases (Cordonnier et al., 1986, 1989; Grossmann et al., 1987; Dubourdieu et al., 1988; Shoseyov et al., 1988; Gunata et al., 1990a,b; Vasserot et al., 1993). These enzymes appeared to be more efficient than acid hydrolysis in the liberation of bound terpenes without producing modification of the aromatic character (Gunata, 1984).

Recently, Janbon et al. (1994) have isolated a glucose-derepressed mutant of *Candida molischiana* 35M5N that is able to produce large quantities of a β-glucosidase with unique properties. The enzyme possesses a wide spectrum of activity including α-L-arabinofuranosidase and α-L-rhamnosidase activities. These are interesting because studies (Gunata et al., 1988) on the enzymatic hydrolyses of grape monoterpenyl diglycosides resulted in the proposal of a sequential mechanism involving three enzymes (α-L-arabinofuranosidase, α-L-rhamnosidase, and β-D-glucosidase).

The β-glucosidase could function at low pH value (optimum pH 3.5); it is also very stable (78% activity recovered after 145 h at pH 3.5 and 30 °C) and had enhanced activity in the presence of ethanol (Janbon et al., 1995).

This paper presents a method for the immobilization of *C. molischiana* 35M5N β-glucosidase (EC 3.2.1.21) to Duolite A-568 resin, evaluates its efficiency, and demonstrates the potential use in industry of the immobilized enzyme for flavor enrichment of wine and fruit juice. The possibility of rapidly developing the aroma of terpenes in a continuous process may therefore be of great interest in enology and in the fruit juice industry.

EXPERIMENTAL PROCEDURES

Culture Conditions. The strain used was *C. molischiana* 35M5N (Janbon et al., 1994). The medium for basal culture was G medium (Galzy, 1964), to which carbon (cellobiose, glucose) was added to a final concentration of 0.5% (w/v). The cultures were incubated at 28 °C in Erlenmeyer flasks filled to 1/10 of their volume. Cultures were incubated at 28 °C and shaken (80 oscillations per min, 8 cm amplitude).

Enzyme Assay. β-Glucosidase activity against *p*-nitrophenyl β-D-glucopyranoside (pNPG) was determined by adding 0.1 mL of enzyme solution to 4.9 mL of citrate−phosphate buffer (0.1 M) containing pNPG (5 mM final) (Blondin et al., 1983). The reaction mixture was incubated at 30 °C. Samples (0.5 mL) were taken at regular intervals and added to 1.0 mL of carbonate buffer (0.2 M; pH 10.2). Liberated *p*-nitrophenol (pNP) in this mixture was assayed by spectrophotometry at 400 nm. The molar extinction coefficient used was 18 300 mol^{-1} cm^{-1}.

One β-glucosidase activity unit (U) was defined as the quantity of enzyme required for hydrolysis of 1 μmol of substrate (pNPG)/min (U mL^{-1}) under the above experimental conditions.

Enzyme Immobilization. The β-glucosidase of *C. molischiana* 35M5N was immobilized on Duolite A-568 resin (Rohm and Haas France S.A., Paris). Duolite A-568 is a highly porous granular weak base anion exchange resin based on cross-linked phenol−formaldehyde polycondensate (principal functional group: tertiary amine). The resin was washed with distilled water and then with 0.1 M citrate−phosphate buffer at pH 4. It was then dried under vacuum overnight. A culture supernatant (200 mL) containing 800 U of β-glucosidase activity was agitated with the dry resin for 1 h at room

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S0021-8561(95)00360-8 CCC: $12.00

Table 1. Immobilization of the β-Glucosidase of *C. molischiana* 35M5N with Different Resins[a]

	Duolite A-568	Duolite A-7	Amberlite XAD-4
pH 4	1.65	73.9	95
pH 5	4.9	93.6	100
pH 6	4.3	100	100
pH 7	5.1	100	100

[a] Percent of β-glucosidase activity not retained on the resin at different pH values (citrate−phosphate buffer 0.1 M is used).

temperature. The resin was washed sequentially with 100 mL of water (two times) and 100 mL (two times) of 0.1 M citrate−phosphate buffer, pH 4. The immobilized enzyme was kept at 4 °C.

The immobilization yields were expressed as

$$R = \frac{\text{no. of units put into contact with the carrier}}{\text{no. of units retained by the carrier}} \times 100$$

To select a resin, the percent of activity not immobilized on the resin was determined.

Enzymatic Treatment and Flavor Extraction. Fifty units of immobilized β-glucosidase was added to 50 mL of wine or fruit juice obtained from the local market. The experiments were done with fresh fruits and young wine (1 year old) because during wine aging, and depending on the differents aroma compounds, slow hydrolysis of glycoside precursors occurs naturally; however, this natural hydrolysis is very low (Gunata et al., 1986). The enzymatic reactions were run in stoppered glass bottles at 30 °C, with shaking. After addition of 4-nonanol (190 μg) as standard, wine and apricot juice were passed through a solvent-washed Amberlite XAD-2 column (1 cm i.d. × 35 cm) at a flow rate of 2.0 mL/min (Gunata et al., 1985). The column was then rinsed with 100 mL of distilled water to eliminate sugars, acids, and other water-soluble compounds. The fractions containing free aroma were eluted by 50 mL of pentane/dichloromethane (2/1v/v). The eluate was dried over anhydrous calcium sulfate, concentrated to 50 μL under reduced pressure (rotavapor), and then subjected to GC analyses.

Gas Chromatography. GC analyses were performed using a Varian 3300 chromatograph (Varian Associates, Inc., Sunnyvale, CA) equipped with a flame ionization detector and a fused silica capillary column (15 m × 0.22 mm i.d., 0.25 μm film thickness) coated with DB1 (J&W Scientific, Folsom, CA) and an inlet system using the split (1:18) injection technique. Injector and detector temperatures were 200 and 250 °C, respectively. The column temperature was kept at 70 °C for 1 min and then raised to 200 °C at a rate of 1 °C/min. The carrier gas was helium at a flow rate of 2.7 mL/min.

The volatile compounds were primarily identified by comparing the retention times of the gas chromatographic peaks with those of commercial standards (Sigma). The identity of peaks was verified by gas chromatography−mass spectrometry using a Hewlett-Packard 5870 Series II apparatus (Hewlett-Packard France, Les Ulis, France).

For each wine and fruit juice, the results represent the average of two experiments.

RESULTS AND DISCUSSION

Immobilization of *C. molischiana* 35M5N β-Glucosidase on Duolite A-568 Resin. The immobilization of a β-glucosidase provides a biocatalyst of potential interest to produce release of volatiles in fruit juice and wine. The advantage of using immobilized enzymes over traditional batchwise treatment for industrial process is primarily due to better control of the enzymatic process. Other advantages include the possibility of the repeated use of the biocatalyst and the feasibility of a continuous process.

Three resins (Amberlite XAD-4, Duolite A-568, and Duolite A-7) were tested, at different pH values. The results are summarized in Table 1. The immobilization

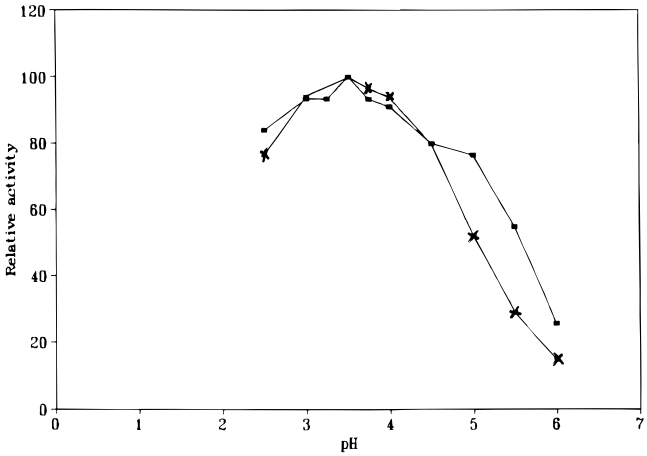

Figure 1. Effect of pH on the β-glucosidase activity of both free (×) and immobilized (■) β-glucosidase.

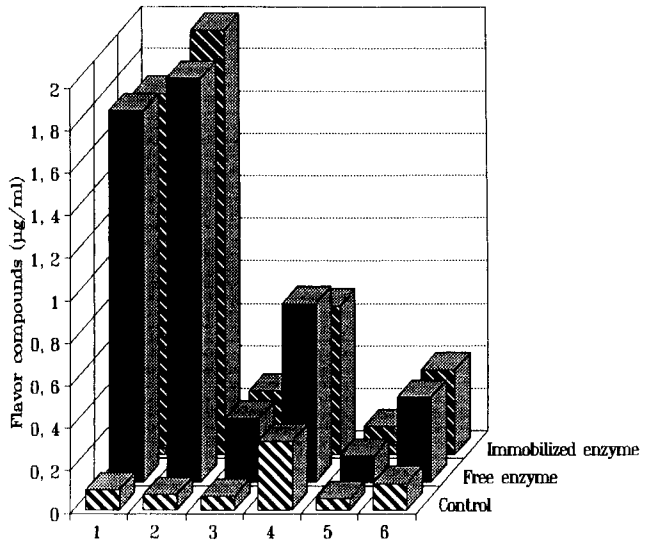

Figure 2. Effect of the use of the immobilized and free β-glucosidase of *C. molischiana* 35M5N on the level of flavor compounds in apricot fruit juice. Fifty milliliters of juice is treated with 50 of β-glucosidase for 12 h at 30 °C. (1) α-Pinene, (2) α-terpinene, (3) γ-terpinene, (4) α-terpineol, (5) linalool, (6) 2-phenylethanol. The results represent the average of two experiments.

Table 2. Comparison of the Physicochemical Properties of the Immobilized and the Free β-Glucosidase of *C. molischiana* 35M5N

property	free β-glucosidase	immobilized β-glucosidase
activity immobilization, %		82
optimal pH	3.5	3.5
optimal temp, °C	55	55
activation energy (E_a), kJ/mol^{-1}	45.4	38.3
K_m (pNPG), mM	0.2	0.6
K_i (glucose), mM	7.3	5.3

using Amberlite XAD-4 was found to be very inefficient. The results shown in Table 1 indicate that pH 4 should be the pH of choice to immobilize the β-glucosidase on Duolite A-568. The immobilization yields were calculated with this resin (Table 2), and the results show that the *C. molischiana* 35M5N β-glucosidase was immobilized very efficiently on Duolite A-568 resin. The immobilization was rapid (1 h), and the activity retained after immobilization is 86% of that of the original soluble enzyme. The immobilization yield is similar to that obtained by Fu-mian et al. (1994) (75%) with the β-glucosidase of *Aspergillus niger* immobilized on γ-alu-

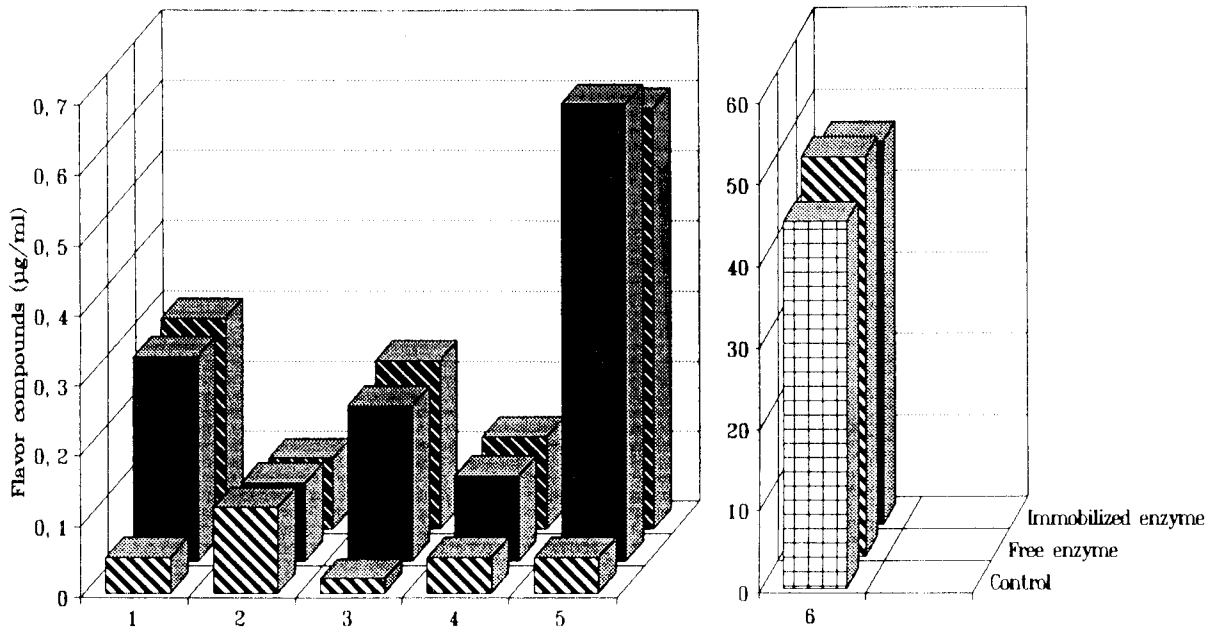

Figure 3. Effect of the use of the immobilized and free β-glucosidase of *C. molischiana* 35M5N on the level of flavor compounds in muscat wine. Fifty milliliters of juice is treated with 50 of β-glucosidase for 12 h at 30 °C. (1) Nerol, (2) citronellol, (3) geraniol, (4) 2-phenylethanol, (5) benzyl alcohol, (6) linalool. The results represent the average of two experiments.

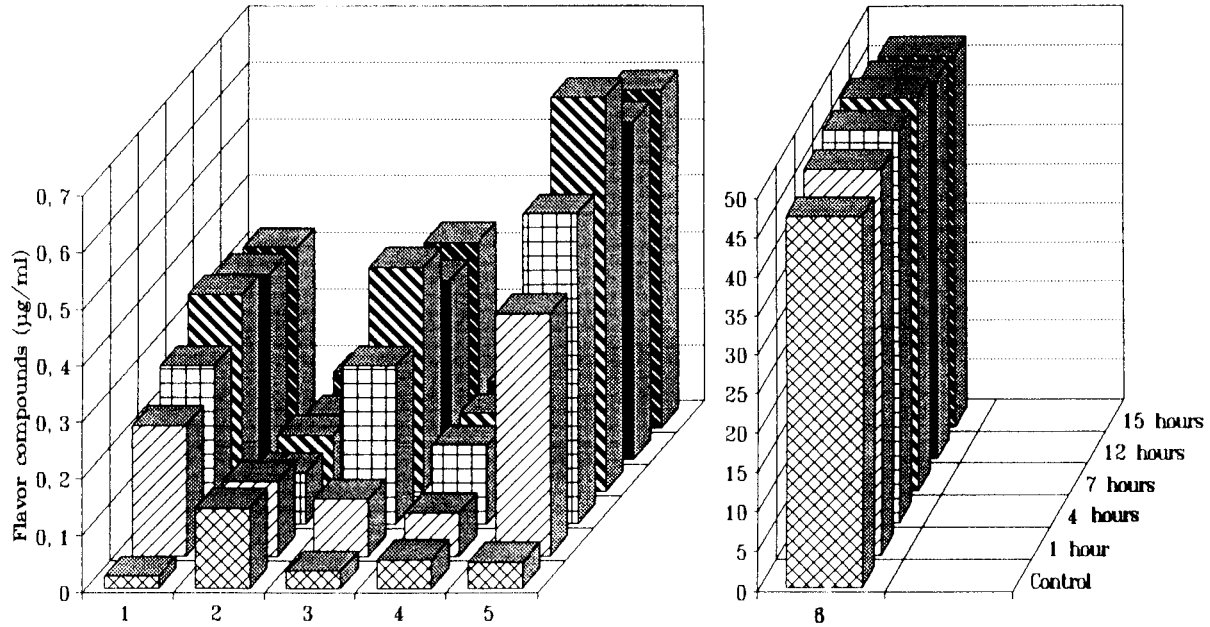

Figure 4. Study of reaction time on the enzymatic hydrolysis of flavor precursors of muscat wine catalyzed by the immobilized β-glucosidase of *C. molischiana* 35M5N. (1) Nerol, (2) citronellol, (3) geraniol, (4) 2-phenylethanol, (5) benzyl alcohol, (6) linalool. The results represent the average of two experiments.

mina, but it is higher than that obtained by Shoseyov et al. (1990) with the β-glucosidase of another strain of *A. niger* immobilized to corn stover cellulose (30%).

The optimum pH of both free and immobilized enzyme on Duolite A-568 resin is 3.5 (Figure 1). Our findings show that the activity of the immobilized enzyme as a function of temperature is similar to that of the free enzyme (Table 2). This demonstrates that the immobilized β-glucosidase retains its original structure. The K_m value for pNPG for the immobilized enzyme is notably higher (0.6 M) than for the free enzyme (0.2 M). Fu-Mian et al. (1994) obtained the same results with the β-glucosidase of *A. niger*. This increase may be due to diffusional factors that originate from (1) the repulsion between the charges of the substrates and the support or (2) steric hindrance, which affects the interactions between the substrate and the catalytic site arranged on the support, as well as the loss of product from the matrix. For β-glucosidase inhibition, two factors must be taken into account, i.e. ethanol and glucose. When pNPG was used as substrate, ethanol was an activator up to 1 M concentration and 35% higher activity was obtained as compared to reference for both free and immobilized β-glucosidase. No inhibition was observed by 2 M ethanol in the medium (average value in wine). Glucose has been demonstrated to be a competitive inhibitor for the free (K_i = 5.3 mM) and the immobilized (K_i = 7.3 mM) β-glucosidase.

Enzymatic Treatment of Apricot Fruit Juice and Muscat Wine. Apricot fruit juice (pH 3.6) was enzymatically treated. The GC−MS analysis of the

treated juice compared to the control indicates a severalfold increase of many volatiles (Figure 2) including linalool, α-terpineol, 2-phenylethanol, α- and γ-terpinene, and α-pinene. There is no difference between the treatment with the free enzyme and that with the immobilized one; 100% of the immobilized β-glucosidase activity is retained at the end of the treatment.

Muscat wine (pH 3.8; 15% alcohol) was treated with the free and the immobilized enzyme. The GC−MS analysis of the treated wine compared to the control (Figure 3) indicates that the enzyme treatment increased the concentrations of free monoterpene alcohols such as geraniol, nerol, linalool, and cyclic alcohols such as benzyl alcohol and 2-phenylethanol. One hundred percent of the immobilized β-glucosidase activity is retained at the end of the treatment. The results of the treatment show that there is a weak hydrolysis of linalool. This phenomenon has already been noted (Gunata et al., 1985). According to its origin, the β-glucosidase is more or less strongly influenced by the nature of the aglycon. Some fungal enzyme and plant β-glucosidases act on primary alcohol β-glucosides such as nerol and geraniol glucosides, while β-glucosides of tertiary alcohol such as linalool or α-terpineol are not substrates (Gunata et al., 1985). *C. molischiana* 35M5N β-glucosidase has poor activity toward these latter glycosides, but it is possible, too, that there is a small quantity of linalool precusors in this wine.

Sensory evaluations of the treated wine and fruit juice compared to the control were conducted: 9 of 10 judges identified correctly the treated Muscat wine and found a significant increase in the flavor and a richer Muscat flavor; 10 of 10 judges correctly identified the treated apricot juice. In many cases, treated juice was preferred, in particular because of an intense fruitiness flavor.

In an attempt to achieve the liberation of the volatile terpenols in a short time, an experiment was carried out at various reaction times using Muscat wine. The results show that 7 h is necessary to obtain the best hydrolysis of the bound aroma precursors (Figure 4). The results of Gunata et al. (1985) showed that hydrolysis of grape glycosidic extract was complete after 12 h of incubation at pH 5 and 40 °C. Our results are interesting because the hydrolysis is more rapid and the experiments are done directly in wine conditions.

To establish operational stability over longer periods of operation, enzyme activity in the treated juice and wine was analyzed. It was found that there is no enzyme desorption up to a monitoring period of five repeated batches and that 100% of activity is retained.

Enzymatic Treatment of Fruit Juices and Wines. Because of the previous interesting results, the efficiency of the immobilized β-glucosidase was also tested for several fruit juices and wines. Determinations of the free volatile compounds (terpenes, cyclic alcohols) indicated that concentrations, in enzyme-treated fruit juices and wines, greatly increased (Table 3). For example, the free volatile compounds increased by 1250%, 705%, 141%, and 12% in mango, strawberry, and apple juice and sauvignon wine, respectively. The results obtained are better than or identic to those obtained by the different researches on the hydrolysis of aroma precursors (Gunata et al., 1990c; Shoseyov et al., 1990; Vasserot et al., 1993).

In this way, the use of β-glucosidases during winemaking or fruit juice processing can contribute to

Table 3. Effects of the Immobilized β-Glucosidase of *C. molischiana* 35M5N on Some Flavor Compounds of Different Fruit Juices and Wines[a]

	α-pinene		β-pinene		α-terpinene		γ-terpinene		α-terpineol		linalool		geraniol		nerol		benzyl alcohol		2-phenylethanol	
	control	treated	control	treated	control	treated	control	treated	control	treated	control	treated	control	treated	control	treated	control	treated	control	treated
fruits																				
peach	0.06	7.1			0.2	0.67	0.06	0.1	0.13	0.26	0.3	0.42	0.05	0.84			0.15	1.12	0.11	0.23
red grape	0.02	0.1			0.03	0.5	0.02	0.1			0.75	0.9							0.04	0.2
white grape									0.16	0.21	0.72	0.94					0.08	0.45	0.1	0.3
apple											0.17	0.25	0.01	0.25					0.11	0.2
cherry											0.48	1.18					2.6	3.7		
papaya											1.8	2.2								
orange											0.11	0.87						0.2		
strawberry											0.1	0.77						0.18	0.13	0.9
mango											0.1	1.3						0.05		
kiwi fruit			0.1				0.11	0.11	0.1	0.3	1.5	1.5								0.1
passion fruit			0.15				0.1	0.3	3.7	3.7	1.8	2					2.5	5		
wines																				
sauvignon											20	20	0.14	0.18	0.04	1.6	0.46	1.2		
chardonnay		0.1		0.15			0.23	0.51			23.1	25					0.36	0.97		

[a] The level of volatile compounds (μg/mL of juice) is measured in control and treated fruit juices and wines. Fifty milliliters of juices and wines was treated with 50 U of immobilized β-glucosidase of *C. molischiana* 35M5N, 10 h at 30 °C. The results represent the average of two experiments.

increase greatly the amount of flavor compounds in wine and fruit juices and also improve their flavor quality.

Enzyme Stability. After 6 months of storage at 4 °C, the activity of the immobilized β-glucosidase was 100% of the original activity. In addition, the stability of the immobilized β-glucosidase was investigated by measuring the residual activity after each enzymatic treatment in the fruit juices or the wines: 100% of activity was recovered in all cases.

Conclusion. The immobilized β-glucosidase system using Duolite A-568 resin was found to be quite stable for the hydrolysis of bound aroma precursors under fruit juice or wine conditions. This enzyme can be used repeatedly for different treatments and suggests that it may be possible to enhance the fruit juice or wine flavor by enzymatic hydrolysis of glycoside precursors. We hope to extend the work with the study of a reactor permitting the continuous hydrolysis of flavor precursors of beverages.

LITERATURE CITED

Blondin, B.; Ratomahenina, R.; Arnaud, A.; Galzy, P. Purification and properties of the β-glucosidase of a yeast capable of fermenting cellobiose to ethanol: *Dekkera intermedia*. *Eur. J. Appl. Microbiol. Biotechnol.* **1983**, *17*, 1−6.

Cordonnier, R.; Bayonove, C.; Baumes, R. Données récentes sur les précurseurs d'arôme du raisin, perspectives de leur exploitation en vinification (Update results on grape wine aroma precursors and their possible applications in wine making). *Rev. Fr. Oenol.* **1986**, *102*, 29−41.

Cordonnier, R.; Gunata, Y.; Baumes, R.; Bayonove, C. Recherche d'un matériel enzymatique adapté à l'hydrolyse de précurseurs d'arôme de nature glycosidique du raisin (Research on an enzymic material suitable to the hydrolysis of precursors of glycosidic aroma of grape wine). *Connais. Vigne Vin.* **1989**, *23*, 7−23.

Dimitriadis, E.; Williams, P. J. The development and use of a rapid analytical technique for estimation of free and potentially volatile monoterpene flavorants of grapes. *Am. J. Enol. Vitic.* **1987**, *35*, 66−71.

Dubourdieu, D.; Darriet, P. H.; Ollivier, D.; Boidron, J. N.; Ribereau-Gayon, P. Rôle de la levure *Saccharomyces cerevisiae* dans l'hydrolyse enzymatique des hétérosides terpéniques du jus de raisin (Role of *Saccharomyces cerevisiae* in enzymic hydrolysis of terpenic heterosides found in grape juice). *C. R. Acad. Sci.* **1988**, *306*, 489−493.

Engel, K. H.; Tressl, R. Formation of aroma components from non volatile precursors in passion fruit. *J. Agric. Food Chem.* **1983**, *31*, 998−1002.

Fu-Mian, C.; Pifferi, P. G.; Setti, L.; Spagna, G.; Martino, A. Immobilization of an anthocyanase (β-glucosidase) from *Aspergillus niger*. *Ital. J. Food Sci.* **1994**, *1*, 31−42.

Galzy, P. Etude génétique et physiologique du métabolisme de l'acide lactique chez *Saccharomyces cerevisiae* Hansen (Genetic and physiological study of lactic acid metabolism in *Saccharomyces cerevisiae* Hansen). *Ann. Technol. Agric.* **1964**, *13*, 109−259.

Gunata, Y. Z. Recherches sur la fraction liée de nature glycosidique de l'arôme du raisin: importance des terpénylglycosides, action des glycosidases (Research on the bound glycosidic fraction of grape aroma: importance of terpenylglycosides, action of glycosidases). Ph.D. Dissertation, The University of Montpellier, 1984.

Gunata, Y. Z.; Bayonove, C.; Baumes, R.; Cordonnier, R. E. The aroma of grapes. Extraction and determination of free and glycosidically bound fractions of some grape aroma composants. *J. Chromatogr.* **1985**, *331*, 83−90.

Gunata, Y. Z.; Bayonove, C.; Baumes, R.; Cordonnier, R. E. Stability of free and bound fractions of some aroma components of grapes cv. Muscat during the wine processing: preliminary results. *Am. J. Enol. Vitic.* **1986**, *37*, 112−114.

Gunata, Y. Z.; Bitteur, S.; Brillouet, J. M.; Bayonove, C.; Cordonnier, R. E. Sequentiel enzymatic hydrolysis of potential aromatic glycosides from grape. *Carbohydr. Res.* **1988**, *184*, 139−149.

Gunata, Y. Z.; Bayonove, C.; Cordonnier, R. E.; Arnaud, A.; Galzy, P. Hydrolysis of grape monoterpenyl-β-D-glucosides by *Candida molischiana* and *Candida wickerhamii* β-glucosidases. *J. Sci. Food Agric.* **1990a**, *50*, 499−506.

Gunata, Z.; Bayonove, C.; Tapiro, C.; Cordonnier, R. Hydrolysis of grape monoterpenyl β-D-glucosides by various β-D-glucosidases. *J. Agric. Food Chem.* **1990b**, *38*, 1232−1236.

Gunata, Y. Z.; Dugelay, I.; Sapis, J. C.; Baumes, R.; Bayonove, C. Action des glycosidases exogènes au cours de la vinification: libération de l'arôme à partir de précurseurs glycosidiques (Action of added glycosidases during wine making: aroma release from glycosidic precursors). *J. Int. Sci. Vigne Vin.* **1993**, *13*, 59−72.

Janbon, G.; Arnaud, A.; Galzy, P. Selection and study of a *Candida molischiana* mutant derepressed for β-glucosidase production. *FEMS Microbiol. Lett.* **1994**, *118*, 207−212.

Janbon, G.; Derancourt, J.; Chemardin, P.; Arnaud, A.; Galzy, P. A very stable β-glucosidase from a *Candida molischiana* mutant strain: enzymatic properties, sequencing and homology with other yeast β-glucosidases. *Biosci., Biotechnol., Biochem.* **1995**, *59*, 1320−1322.

Schwab, W.; Schreier, P. Simultaneous enzyme catalysis extraction: a versatile technique for the study of flavor precursor. *J. Agric. Food Chem.* **1988**, *36*, 1238−1242.

Shoseyov, O.; Bravdo, B. A.; Siegel, D.; Goldman, A.; Cohen, S. Shoseyov, L.; Ikan, R.; Immobilized endo-β-glucosidase enriches flavor of wine and passion fruit juice. *J. Agric. Food Chem.* **1990**, *38*, 1973−1976.

Vasserot, Y.; Arnaud, A.; Galzy, P. Evidence for marc monoterpenol glycosides hydrolysis by free or immobilized yeast β-glucosidase. *Bioresource Technol.* **1993**, *43*, 269−271.

Voirin, S.; Baumes, R.; Sapis, J. C.; Bitteur, S.; Gunata, Z.; Bayonove, C. Novel monoterpene disaccharide glycosides of *Vitis vifera* grapes. *J. Agric. Food Chem.* **1990**, *38*, 1373−1378.

Williams, P. J.; Strauss, C. R.; Wilson, B.; Massy-Westropp, R. Novel monoterpene disaccharide glycosides of *Vitis vinifera* grapes and wines. *Phytochemistry* **1982a**, *18*, 785−786.

Williams, P. J.; Strauss, C. R.; Wilson, B.; Massy-Westropp, R. Studies on the hydrolysis of *Vitis vinifera* monoterpene precursor compounds and model monoterpene β-D-glucosides rationalizing the monoterpene composition of grapes. *J. Agric. Food Chem.* **1982b**, *30*, 1219−1223.

Received for review June 15, 1995. Accepted April 29, 1996.[⊗]

JF950360J

[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1996.