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Food Chemistry 87 (2004) 509–521
Food Chemistry
www.elsevier.com/locate/foodchem

Plant and microbial glycoside hydrolases: Volatile release from glycosidic aroma precursors

Jean-Emmanuel Sarry [a], Ziya Günata [b,*]

[a] Department of Biology, Plant Science Institute, University of Pennsylvania, 3800 Hamilton Walk, Philadelphia, PA 19104-6018, USA
[b] UMR UMII/ENSAM/INRA Ingénierie des Réactions Biologiques, Bioproductions, Université de Montpellier II, Place Eugène Bataillon, F-34095 Montpellier Cedex 5, France

Received 15 October 2003; accepted 6 January 2004

Abstract

Following a brief look at the structure and occurrence of glycosidic flavour precursors in plants and fruits, attention is given to mechanisms of enzymatic hydrolysis, the properties of relevant glycosidases, as well as endogenous and exogenous glycosidases affecting flavour release in plants, in fruit juice processing and in winemaking. The constraints for technological applications and future prospects are discussed.

Keywords: Glycosides; Aroma; Glycosidases; Fruits; Plants; Aroma enhancement

1. Introduction

Phytochemists have identified some 70,000 plant chemicals, many thousands of which are glycosylated (Williams & Harborne, 1994). From a chemical point of view, the glycosylation invariably results in enhanced water solubility and lower chemical reactivity. Glycosylated compounds are, therefore, often considered as transportable storage compounds or detoxification products assumed to lack physiological activity. The widespread occurrence of glycosylated secondary metabolites, including flavonols, anthocyanins, monoterpenes, norisoprenoidic compounds and plant hormones shows that both glycoside hydrolases and glycoside transferases responsible for their metabolism play a central role in a large number of major biological processes. The *O*-glycoside hydrolases (EC 3.2.1.x) are a widespread group of enzymes of significant biological, biomedical and industrial importance. They catalyse the hydrolysis of the glycosidic bond between two or more carbohydrates or between a carbohydrate and a non-carbohydrate moiety. A classification of the glycoside hydrolases into 82 families, based on the amino acid sequence similarities, has been proposed and is now widely used (Henrissat, 1991; Henrissat & Davies, 1997).

Among the glycoside hydrolases, β-glucosidases have been the subject of much work because of their importance: (i) in numerous biological processes: growth and development via release of phytohormones (auxins, gibberellins, cytokinins) from their inactive glucoconjugated forms (Duroux, Delmotte, Lancelin, Keravis, & Jay-Allemand, 1998), host-parasite interactions (Osbourn, 1996; Sue, Ishihara, & Iwamura, 2000), lignification (Dharmawhardana, Ellis, & Carlson, 1999; Hösel, Surholt, & Borgmann, 1978), cell wall degradation in the endosperm during germination (Leah, Kigel, Svendsen, & Mundy, 1995), circadian rhythm of leaf movements (Ueda & Yamamura, 2000) and (ii) in biotechnological applications: food detoxification (Birk, Bravdo, & Shoseyov, 1996), biomass conversion (Pemberton, Brown, & Emert, 1980; Woodward & Wiseman, 1982) and, over the past decade, flavour enhancement in beverages (Günata, Dugelay, Sapis, Baumes, & Bayonove, 1993). Indeed the intensive research carried out over the past two decades has demonstrated that, in a great number of fruit and other plant tissues, important flavour

* Corresponding author. Tel.: +33-467149321; fax: +33-04-67-41-42-92/+33-467144292.
E-mail address: gunata@arpb.univ-montp2.fr (Z. Günata).

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doi:10.1016/j.foodchem.2004.01.003

compounds accumulate as non-volatile and flavourless glycoconjugates, which make up a reserve of aroma to be exploited (Stahl-Biskup, Intert, Holthuijzen, Stengele, & Schulz, 1993; Winterhalter & Skouroumounis, 1997).

This review brings up to date research that has been undertaken on the involvement of endogenous and exogenous glycosidases for the release of volatile compounds in plants, fruits and beverages and future prospects in this field. A brief presentation will be given of the structure of glycosidically bound volatile compounds.

2. Chemical composition of glycosidic aroma precursors and their occurrence

Glycosides of volatile compounds identified in plants and fruits are mainly *O*-β-D-glucosides or *O*-diglycosides. In few cases, trisaccharide glycoconjugates were observed (Winterhalter & Skouroumounis, 1997). The aglycone moiety is always linked to β-D-glucopyranose. In the case of diglycosides, the glucose moiety was further substituted with one of the following monosaccharides: α-L-arabinofuranose, α-L-arabinopyranose, α-L-rhamnopyranose, β-D-glucopyranose, β-D-apiofuranose and β-D-xylopyranose (Fig. 1). Trivial names are attributed to some disaccharidic substrates according to the plant species from which they have been isolated: 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranosides (vicianosides), 6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosides (rutinosides), 6-*O*-β-D-glucopyranosyl-β-D-glucopyranosides (gentiobiosides), 6-*O*-β-D-xylopyranosyl-β-D-glucopyranosides (primeverosides) and 6-*O*-β-D-apiofuranosyl-β-D-glucopyranosides (Guo et al., 1993; Stahl-Biskup et al., 1993; Williams, Strauss, Wilson, & Massy-

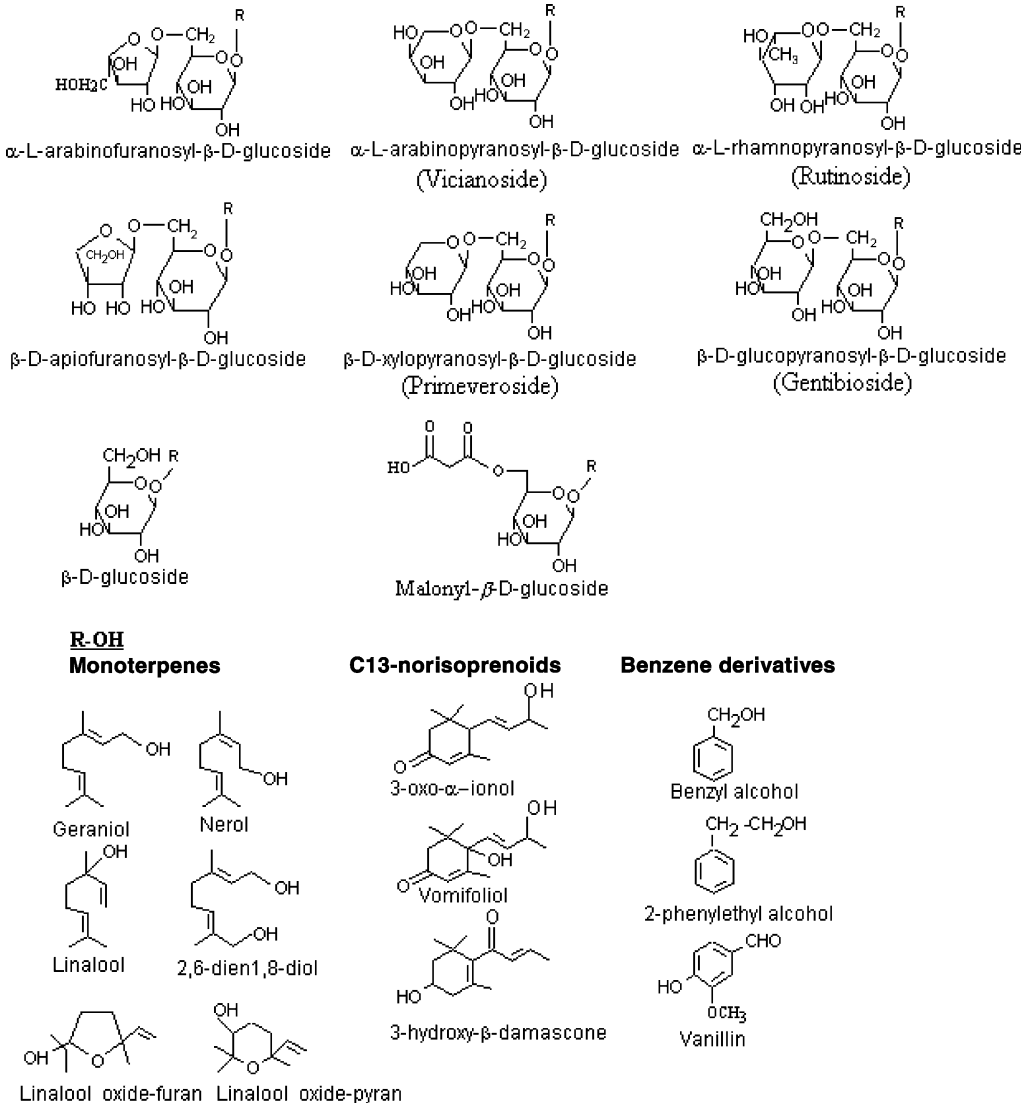

Fig. 1. Structure of glycosidic aroma precursors from plants.

Westropp, 1982a; Winterhalter & Skouroumounis, 1997).

Malonylated-β-D-glucopyranosides of some volatiles from fruit and plant tissues have also been reported (Withopf, Richling, Roscher, Schwab, & Schreier, 1997). Most of the quantitative studies on glycosidic flavour compounds in fruits and plants were performed by the analysis of hydrolytically liberated aglycones because the reference glycosides are not commercially available. Analyses carried out by direct analysis of glycosides, i.e., before hydrolysis, have shown that most glycosides are diglycosides in grape berries (Voirin, Baumes, Sapis, & Bayonove, 1992) and in tea leaves (Mizutani et al., 2002). The aglycone moiety of glycosides is often dominated by monoterpenes, C_{13}-norisoprenoids and benzene derivatives (Fig. 1). Some linear alcohols have also been detected. About 200 aglycones have been identified hitherto in 150 plant species (Stahl-Biskup et al., 1993; Vasserot, Arnaud, & Galzy, 1995; Winterhalter & Skouroumounis, 1997). The structures of some abundant aglycones are given in Fig. 1.

Some liberated aglycones may already be odorous, such as linalool, geraniol and nerol, or some may give rise to potent flavour compounds, such as β-damascenone, vitispirane and theaspirane by further enzymatic and/or chemical transformations during fruit juice processing or leaf products processing (Winterhalter, 1992).

The techniques mainly used for the isolation of glycosides of volatiles from plants and fruits involve the selective retention of glycosides from aqueous extracts on hydrophobic adsorbents: C_{18}-reversed phase (Williams, Strauss, Wilson, & Massy-Westropp, 1982b) and Amberlite XAD-2 (Günata, Bayonove, Baumes, & Cordonnier, 1985a). Recovered glycosides were analyzed by different chromatography techniques: TLC, HPLC and GC, either by direct analysis of glycosides or by analysis of aglycones following enzymatic or acid hydrolysis of glycosides. For identification purposes, chromatographic and spectral techniques (GC-MS, GC-FTIR, HPLC-MS/MS, and FAB-MS/MS) were performed. A recent review (Winterhalter & Skouroumounis, 1997) gives general approaches to the analysis of glycosides with abundant references.

Volatiles occurring as glycosides have been detected in several fruits, usually at concentrations from 2–8 fold greater than the free counterparts: grape berries (Günata et al., 1985a, 1985b), banana (Perez, Cert, Rios, & Olias, 1997), apricot (Krammer, Winterhalter, Schwab, & Schreier, 1991; Salles, Jallageas, Fournier, Tabet, & Crouzet, 1991), peach (Krammer et al., 1991), nectarine (Aubert, Günata, Ambid, & Baumes, 2003), yellow plum (Krammer et al., 1991), quince (Lutz & Winterhalter, 1992), sour cherry (Schwab, Scheller, & Schreier, 1990), passion fruit (Chassagne, Crouzet, Bayonove, Brillouet, & Baumes, 1996), kiwi (Young & Paterson, 1995), papaya (Schwab, Mahr, & Schreier, 1989), pineapple (Wu, Kuo, Hartman, Rosen, & Ho, 1991), mango (Sakho, Chassagne, & Crouzet, 1997), lulo (Suarez, Duque, Wintoch, & Schreier, 1991), raspberry (Pabst, Barron, Etiévant, & Schreier, 1991), strawberry (Roscher, Herderich, Steffen, Schreier, & Schwab, 1997) and tomato (Marlatt, Ho, & Chien, 1992). Furthermore, glycosidic flavour precursors were detected in green parts, roots, rhizomes and seeds in the plant kingdom (Winterhalter & Skouroumounis, 1997). No data are available in the literature as regards the tissue localisation of glycosidic flavour precursors. Their accumulation in vacuoles seems plausible due to their higher solubility than aglycones.

3. Enzymatic release of volatiles from glycosides: Mode of action of glycosidases

Acid or enzyme hydrolysis of glycosides leads to the liberation of volatiles (Günata et al., 1985a, 1985b; Williams, 1983). The rate of acid hydrolysis is closely dependent on the pH and temperature of the medium and on the structure of the aglycone moiety. Glycosides of tertiary alcohols such as linalool, linalool oxides and α-terpineol are more readily hydrolyzed than those of primary alcohols such as geraniol and nerol as it was observed in wine (Günata, Bayonove, Baumes, & Cordonnier, 1985b; Park & Noble, 1993). After 2 years of storage at 10 °C, more than half of the glycosides of geraniol were still present in a Muscat wine, while glycosides of linalool were totally hydrolyzed (Park & Noble, 1993).

The enzymatic release of volatiles, monoterpenes (Bourquelot & Bridel, 1913) and vanillin (Goris, 1924), from corresponding β-D-glucosides through the action of β-glucosidase (E.C. 3.2.1.21), was already suggested at the beginning of the 1900s. The enzymatic hydrolysis of glucovanillin, β-D-glucoside of vanillin, stimulated much interest since a major portion of vanillin in vanilla beans is glycosylated (Leong, Archavlis, & Derbesy, 1989). With regard to enzymatic hydrolysis of diglycosidic flavour precursors, the first reports appeared much later, in 1988 (Günata, Bitteur, Brillouet, Bayonove, & Cordonnier, 1988) and in 1997 (Ogawa et al., 1997) and 1998 (Günata et al., 1998) because of the discovery of the relevant substrates in 1982 (Williams et al., 1982a, 1982b). A two-step (sequential hydrolysis) or one step (diglycosidase) enzyme-catalyzed reaction is involved in the release of aglycones from diglycosides (Fig. 2).

In sequential mode, first one of the following exo-glycosidases: α-arabinofuranosidase, α-rhamnopyranosidase, β-xylopyranosidase, α-arabinopyranosidase or β-apiofuranosidase, make the cleavage of the inter-sugar linkage liberating corresponding sugars and

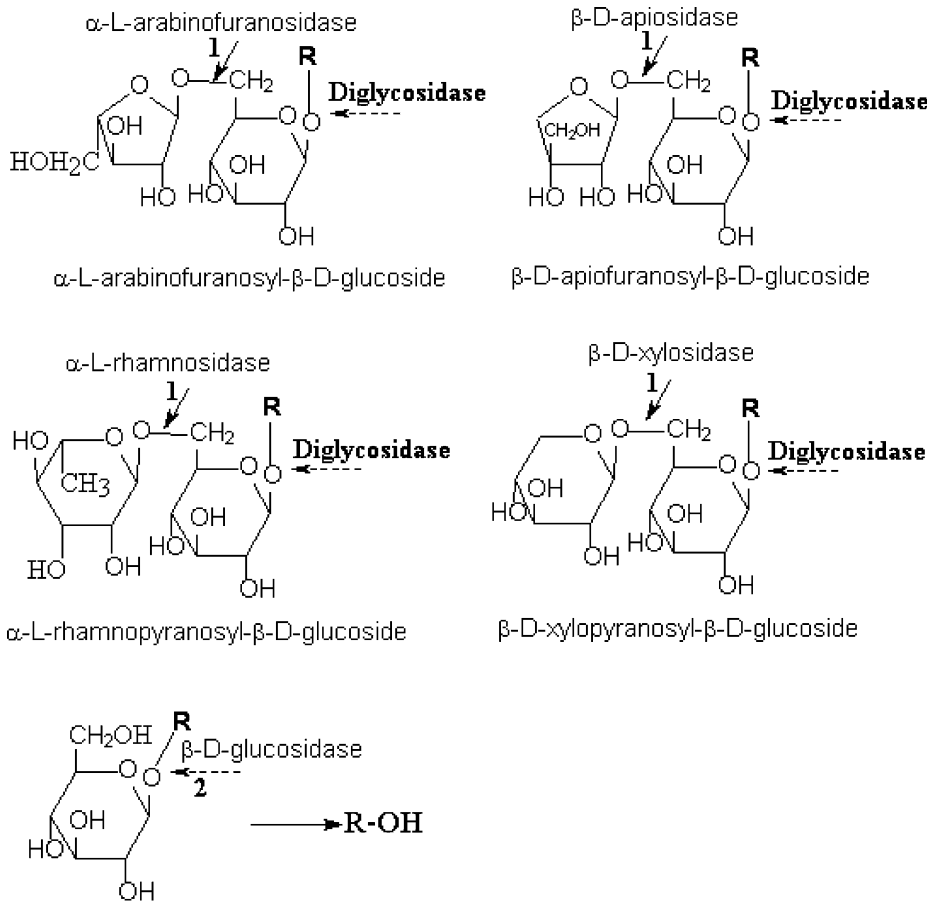

Fig. 2. Two-step and one-step (diglycosidase) enzymatic hydrolysis of glycosidic aroma precursors.

β-D-glucosides. In the second step, β-glucosidase catalyzes the hydrolysis of β-D-glucoside and liberates the corresponding aglycone and glucose (Günata et al., 1988).

In one-step mode, a diglycosidase catalyzes the cleavage of the aglyconic linkage of diglycosides, which results in the release of corresponding aglycone and disaccharide (Günata et al., 1998; Ogawa et al., 1997; Sarry et al., 2003).

The mechanism of enzymatic hydrolysis of glycosides is presumed to proceed in a way similar to the acid-catalyzed cleavage of glycosidic bonds, implicating a carbocation intermediate (Sinnott, 1990). His, glu, and asp are the amino acids thought to be involved in the enzymatic hydrolysis.

4. Plant and microbial glycosidases

4.1. Types of enzymes

The plant and microbial enzymes presented here are mainly those studied in relation to the hydrolysis of glycosidic flavour precursors.

4.2. Plant glycosidases

4.2.1. Exoglycosidases and β-glucosidase

Exoglycosidases mentioned here, and particularly β-glucosidases, are widely distributed among plants (Günata et al., 1993; Hösel, 1981) and micro-organisms (Leclerc, Arnaud, Ratomahenina, & Galzy, 1987; Woodward & Wiseman, 1982). Among fruits, clear evidence of glycosidic flavour precursors was first reported in grape berry, where they constitute an important flavour potential (Günata et al., 1985a, 1985b). Consequently, glycosidases from this fruit have been the subject of several studies; β-glucosidase (Aryan, Wilson, Strauss, & Williams, 1987; Lecas, Günata, Sapis, & Bayonove, 1991; Sarry et al., 2003); α-arabinofuranosidase (Aryan et al., 1987; Günata, Biron, Sapis, & Bayonove, 1989) and α-rhamnopyranosidase (Aryan et al., 1987; Günata et al., 1989) activities were detected in various grape varieties, but not β-apiosidase. Most of the studies concerned β-glucosidase activity as it is a key enzyme in flavour release. Furthermore, it is the most abundant glycosidase activity in grape berry as well as in vine leaves (Sarry et al., 2003). The enzyme occurs in

multiple forms in grape berry. Among different parts of the grape berry, the skin was the richest in activity (Sarry et al., 2003). A histochemical study has shown that enzyme is localized in the hypodermic walls of grape berry skins (Sarry et al., 2003). This assumption was further supported by the possibility of the solubilization of the enzyme in the presence of polyethylene glycol and at high ionic strength. β-glucosidase activity from melon fruit was solubilized under similar conditions (Fils-Lycaon & Buret, 1991). In contrast, enzymes from papaya (Schreier & Schreier, 1986), orange (Cameron, Manthey, Baker, & Grohmann, 2001) and sweet cherry (Gerardi, Blando, Santino, & Zacheo, 2001) fruits and from tea leaves (Ogawa et al., 1997) were readily soluble in the extraction medium. Sweet cherry enzyme was mainly localized in cytosol and in the apoplast. β-glucosidase and exoglycosidase activities were found to increase during ripening of grape berries (Aryan et al., 1987; Sarry et al., 2003) and melon fruit (Fils-Lycaon & Buret, 1991).

4.2.2. Diglycosidase

Diglycosidase activity has recently been reported in grape berry (Günata et al., 1998; Sarry et al., 2003), vine leaves (Sarry et al., 2003) and tea leaves (Ogawa et al., 1997). The enzyme, isolated from grape berry skins, was partially characterized (Sarry et al., 2003). Although needing confirmation, grape disaccharidase seems to possess β-glucosidase activity, contrary to the enzyme from tea (Guo et al., 1996; Mizutani et al., 2002).

The enzyme from tea leaves was named primeverosidase, after primeverosides, which are the most abundant glycosidic aroma precursors in this plant. It was quite recently sequenced and has a strong homology with common β-glucosidases from various plants, belonging to the glycoside hydrolase family GH1 (Mizutani et al., 2002). As for β-glucosidase activity, the recovery of diglycosidase activity from grape berries and vine leaves required the use of PEG (Sarry et al., 2003). In contrast, primeverosidase from tea leaves was readily soluble in the extraction medium (Guo et al., 1996). Among different grapevine tissues, berry skins were the richest in diglycosidase activity (Sarry et al., 2003). Activity increased during grape berry maturation, showing a similar tendency to changes with β-glucosidase activity (Sarry et al., 2003). Primeverosidase activity was found to be more abundant in young tea leaves than in old leaves (Ogawa et al., 1995). Importantly, high quality oolong and black tea is traditionally obtained from young tea leaves (Mizutani et al., 2002).

4.3. Microbial glycosidases

4.3.1. Exoglycosidases and β-glucosidase

4.3.1.1. Yeasts. Saccharomyces cerevisiae is the main yeast involved in alcoholic fermentation of grape juice. Under fermentation conditions, this yeast displays low levels of α-arabinofuranosidase, α-rhamnosidase and β-glucosidase activities (Delcroix, Günata, Sapis, Salmon, & Bayonove, 1994; Dubourdieu, Darriet, Ollivier, Boidron, & Ribéreau-Gayon, 1988), the latter being the most abundant. Consequently, non-*Saccharomyces* yeast species from grape berry flora or from other sources were studied: (i) for producing glycosidases, mainly β-glucosidase during grape juice fermentation in mixed culture with *S. cerevisiae* (Garcia et al., 2002); (ii) for constructing recombinant *S. cerevisiae* strains expressing the relevant activity (Raynal & Guerineau, 1984; Sanchez-Torres, Gonzalez-Candelas, & Ramon, 1998); and (iii) for obtaining glycosidase preparations for flavour enhancement in fruit juice processing and winemaking (Rosi, Vinella, & Domizio, 1994; Vasserot, Chemardin, Arnaud, & Galzy, 1989; Yani & Sato, 1999).

The yeast species from the genera *Dekkera* (Blondin, Ratomahenina, Arnaud, & Galzy, 1983), *Debaryomyces* (Belancic, Günata, Vallier, & Agosin, 2003; Rosi et al., 1994), *Kloeckera* (Vasserot, Chemardin, Arnaud, & Galzy, 1990), *Hansenula* (Vasserot et al., 1989) and *Candida* (Gondé, Ratomahenina, Arnaud, & Galzy, 1985) are able to synthesize β-glucosidase when cultured on a suitable culture medium. Membrane and cytoplasm localisation of the enzyme in yeasts were reported (Kaplan, 1965; Leclerc et al., 1987). Except for *Candida* and *Debaryomyces* species, β-glucosidases from yeasts are often intracellular (Leclerc et al., 1987).

4.3.1.2. Filamentous fungi. Mold-contaminated grape berries usually contain the fungus *Botrytis cinerea.* The development of this fungus enriches grape juice in β-glucosidase, α-arabinofuranosidase and α-rhamnosidase activities (Günata et al., 1989). However, mold-contaminated juices contain glucono-δ-lactone, which is a strong inhibitor of β-glucosidase activity (Heyworth & Walker, 1962).

Pectic and hemicellulase enzyme preparations, widely used in fruit juice processing and winemaking, especially to improve juice clarification and juice yield, are mainly obtained from filamentous fungi, particularly *Aspergillus spp.* (Pilnik & Voragen, 1991; Villettaz & Dubourdieu, 1991). They contain ''side activities'', among them glycosidases, as shown in Table 1 where only glycosidases involved in the hydrolysis of grape glycosidic flavour precursors were reported (Günata et al., 1993). β-glucosidase and α-arabinofuranosidase are usually the most abundant white α-rhamnosidase, and particularly β-apiosidase activities, are either very low or absent. β-apiosidase production by *A. niger* was found to be inducible (Dupin et al., 1992). Among enzyme preparations, a noticeable difference in terms of glycosidase activities was observed. Indeed, they are formulated for their pectinase or cellulase activities.

Table 1
Glycosidase activities (nkat/mg) involved in volatile release from some enzyme preparations

	β-glucosidase	α-arabinofuranosidase	α-rhamnosidase	β-apiosidase
Cellulase A	6.1	0.6	0.007	nd
Hemicellulase	7.1	7.0	0.9	nd
Pectinol VR	0.2	0.1	nd	nd
Rohament CW	3.3	0.7	0.4	nd
Pectinol D5S	0.5	0.7	nd	nd
Pektolase 3PA	1.5	3.8	0.04	0.3
Ultrazym 100	0.5	0.1	nd	0.03
Pectinase 263	7.2	1.4	0.3	0.2
AR 2000	5.6	9.2	0.32	1.08

Source: CellulaseA, Hemicellulase, Pectinase 263, AR 2000:Gist-Brocades
Pectinol VR, Rohament CW, Pectinol D5S: Röhm
Pektolase 3PA: Grinsted
Ultrazym 100: Ciba-Geigy;
nd: not detected.

4.3.1.3. Bacteria. β-Glucosidase from *Bacillus polymyxa*, which had been expressed in *S. cerevisiae* (Adam, Rubio-Texeira, & Polaina, 1995), was able to hydrolyse monoterpenic β-glucosides (Günata et al., 1996). Recent reports have shown that strains from *Oenococcus oeni* involved in the malolactic fermentation of wine possess exoglycosidase and β-glucosidase activities (Bodio, Lloret, Medina, Carrau, & Dellacassa, 2002; Grimaldi, McLean, & Jiranek, 2000). Hydrolysis of glycosides during this biological process has been reported. However, the levels of free aglycones did not increase (Bodio et al., 2002). More studies are needed to understand the implication of malolactic fermentation bacteria on the hydrolysis of glycosidic flavour precursors.

4.3.2. Diglycosidase

To date, there is only one paper reporting a diglycosidase from *A. niger* hydrolysing geranyl-β-rutinoside. The enzyme was induced when the fungus was cultured on rutin as the sole carbon source (Shoseyov, Bravdo, Ikan, & Chet, 1988).

5. Properties of glycosidases

5.1. General

Properties of glycosidases mainly in relation to their role in flavour release are now considered.

5.2. Effects of pH and temperature

The optimum pH activities of plant (Aryan et al., 1987; Lecas et al., 1991; Schreier & Schreier, 1986) and microbial (Leclerc et al., 1987; Woodward & Wiseman, 1982) β-glucosidases are in general from 4.0 to 6.0. In the pH of fruit juices (2.8–3.8), only 5–15% of the maximum activity of most β-glucosidases is observed.

The optimum pH activity of fungal α-arabinofuranosidase (Günata, Brillouet, Voirin, Baumes, & Cordonnier, 1990a; Kaji, 1984) and α-rhamnosidase (Cordonnier, Günata, Baumes, & Bayonove, 1989; Roitner, Schalkhammer, & Pittner, 1984) is close to that of fungal β-glucosidase (Günata, Dugelay, Vallier, Sapis, & Bayonove, 1997). Both α-arabinofuranosidase and α-rhamnosidase show more than 50% of their maximum activities in the pH range of fruit juices.

A good stability of glycoside hydrolases at the acidic pH of fruit juices is important for technological applications. The exoglycosidases and β-glucosidase from *A. niger* (Günata et al., 1997) and a β-glucosidase from a mutant strain of *C. molishiana* (Janbon, Derancourt, Chemardin, Arnaud, & Galzy, 1995; Vasserot, Chemardin, Arnaud, & Galzy, 1991) have shown very good stability in fruit juices, contrary to the enzymes from grape berry (Günata et al., 1993) and *S. cerevisiae* (Delcroix et al., 1994).

The optimum temperature activities of plant (Lecas et al., 1991; Schreier & Schreier, 1986) and microbial (Leclerc et al., 1987; Woodward & Wiseman, 1982) glycosidases are generally 40–50 °C. Thermal denaturation is accelerated at temperatures above 50 °C (Dekker, 1986; Leclerc et al., 1987). In general, glycosidases from filamentous fungi are more heat-resistant than those from plants and yeasts.

5.3. Specificity

5.3.1. Exoglycosidases and β-glucosidase

The activity of exoglycosidases: α-arabinofuranosidase (Günata et al., 1988; Günata et al., 1990a), α-arabinopyranosidase (Chassagne, Bayonove, Crouzet, & Baumes, 1995), α-rhamnosidase (Günata et al., 1988) and β-apiofuranosidase (Dupin et al., 1992) was not affected by the aglycone moiety of the diglycosides. This can be explained by the fact that the orientation of the terminal sugar of diglycosides remains the same, what-

ever the aglycone moiety (Voirin, Baumes, Bayonove, M'Bairaroua, & Tapiero, 1990). However, the aglycone structure of β-D-glucosides has a great effect on the activity of β-glucosidases. β-D-Glucosides of primary and secondary alcohols are good substrates for plant (Aryan et al., 1987; Günata, Bayonove, Tapiero, & Cordonnier, 1990b) and microbial enzymes (Günata et al., 1990a; Günata, Bayonove, Cordonnier, Arnaud, & Galzy, 1990c), while tertiary alcohol β-D-glucosides are effectively hydrolyzed by β-D-glucosidases from fungi, mainly those from *A. niger* (Günata et al., 1990b), *Candida* (Günata et al., 1990c) and *Debaryomyces spp* (Belancic et al., 2003). The pattern of liberated aglycones, and therefore the sensory properties of the products, can be influenced by the source of enzyme.

5.3.2. Diglycosidase

Diglycosidase from grape berry skin was found to have much broader substrate specificity (Sarry et al., 2003) than that (primeverosidase) from tea leaves (Mizutani et al., 2002). Grape enzyme was able to catalyse hydrolysis of β-rutinosides, β-primeverosides, β-vicianosides and arabinofuranosyl-l,6-β-D-glucosides, while tea enzyme showed a pronounced specificity for β-primeverosides. Fungal diglycosidase from *A. niger* was able to hydrolyze β-rutinosides (Shoseyov et al., 1988). The activity of this enzyme against other diglycosidic flavour precursors was not studied.

5.4. Inhibitors

There is no clear evidence of a cofactor requirement for *O*-glycosidases. β-Glucosidase activity is inhibited by glucose, the extent of inhibition depending on the source of enzyme (Günata & Vallier, 1999; Leclerc, Arnaud, Ratomahenina, Galzy, & Nicolas, 1984; Sarry et al., 2003). Therefore, the use of glucose-tolerant β-glucosidase is important for flavour release in glucose-rich products, such as fruit juices. Other sugars, mono or disaccharides, at the levels encountered in fruit juices, have no significant influence on β-glucosidase activity (Shoseyov et al., 1988).

The inhibition by glucose is often of a competitive type. β-Glucosidases from almond (Heyworth & Walker, 1962) and grape (Lecas et al., 1991) are resistant to glucose inhibiton with Ki values of 170 and 210 mM, respectively. Enzymes from yeasts *S. cerevisiae* (Ki = 6.7 mM) (Inamdar & Kaplan, 1966), *C. molischiana* (Ki = 7 mM) (Gondé et al., 1985) and *Dekkera intermedia* (Ki = 3 mM) (Blondin et al., 1983) are more sensitive to glucose inhibition than those from yeasts *C. wickerhamii* (Ki = 230 mM) (Leclerc et al., 1984), *C. peltata* (Ki = 1400 mM) (Saha & Bothast, 1996) and *D. vanriji* (439 mM) (Belancic et al., 2003). Bacterial and filamentous fungal-originated β-glucosidases are generally strongly inhibited by glucose with a Ki of 0.6–10 mM (Dekker, 1986; Hoh, Yeoh, & Tan, 1992). A highly glucose-resistant β-glucosidase from *A. oryzae* (Ki = 953 mM) (Günata & Vallier, 1999) and *A. niger* (Ki = 543 mM) (Yan & Lin, 1997) was recently reported.

Fungal exoglycosidase activities, i.e., α-arabinofuranosidase (Le Clinche, Pinaga, Ramon, & Vallès, 1997), β-apiofuranosidase (Dupin et al., 1992) and α-rhamnosidase (Romero, Manjon, Bastida, & Iborra, 1985), are not significantly inhibited by glucose in the concentrations at which this sugar occurs in fruit juices. Grape diglycosidase was less inhibited by glucose than grape β-glucosidase (Sarry et al., 2003). *A. niger* diglycosidase was more strongly inhibited by glucose (Ki = 40 mM) (Shoseyov et al., 1988) than grape enzyme.

Glucono-δ-lactone, a transition state glucose analogue, is one of the most powerful inhibitors of β-glucosidases from plants (Heyworth & Walker, 1962; Lecas et al., 1991) and micro-organisms (Dekker, 1986; Leclerc et al., 1987) with Ki values often lower than 1 mM. The concentration of this compound can rise up to 10 mM in grape juices and wines obtained from mold-contaminated grapes (McCloskey, 1974).

In general, glycoside hydrolase activities are not significantly influenced by ethanol (10%, v/v) (Cordonnier et al., 1989; Leclerc et al., 1984) and sulfur dioxide (50–200 ppm) (Sanchez-Torres, Gonzalez-Candelas, & Ramon, 1996; Shoseyov et al., 1988) at the usual levels found in fruit juice processing and in wines. Metal ions, such as Ag^{+}, Hg^{2+}, Cu^{2+}, Mg^{2+}, Ca^{2+}, Fe^{2+}, and Fe^{3+}, are potent inhibitors of plant and fungal glycosidases (Leclerc et al., 1987; Sarry et al., 2003). The strongest inhibition often occurs with Ag^{+}, Hg^{2+} and Cu^{2+}. The inhibition can be reversed by reducing agents. The target of these cations on the enzyme may be the catalytic nucleophile glutamate.

6. Flavour release by plant and microbial glycosidases

6.1. By endogenous enzymes

Although glycosidase activities increase in fruit during the ripening process (Fils-Lycaon & Buret, 1991; Sarry et al., 2003), no clear evidence is yet available of a relationship with the hydrolysis of glycosidic flavour precursors. This phenomenon may not occur, since there is compartmentation of substrates and enzymes in plant cells (Hösel, 1981). On the contrary, the implication of glycosidases in cell-wall degradation has been suggested (Nunan, Davies, Robinson, & Fincher, 2001). The floral aroma formation during the tea manufacturing process is attributed to the endogenous primeverosidase. It has been suggested that β-primeverosides and β-primeverosidase are localized separately in tea leaves and contact takes place during the tea manufacturing process when leaf tissues are stressed and

wounded (Mizutani et al., 2002). The formation of flower fragrance compounds in *Gardenia jasmonoides* flowers was attributed to a glycosidase activity, reaching a maximum level at the flower opening stage (Nakajima et al., 1993).

Due to the ionically bound cell-wall localization of glycosidases from grape berry (Sarry et al., 2003), their solubilization during winemaking may be of little importance. This may explain, among other things, the quite low hydrolysis of glycosides during grape juice processing (Delcroix et al., 1994; Günata et al., 1985b).

Glycosidases from yeast *S. cerevisiae*, originating from grape berry flora, have little effect on the hydrolysis of glycosidic flavour compounds during grape juice fermentation because of the quite low levels of activities, together with poor stability in the pH of grape juice (Delcroix et al., 1994).

When a Muscat grape juice was co-fermented in a mixed culture with *S. cerevisiae* and *D. vanriji*, able to synthesize high levels of β-glucosidase in a synthetic culture medium (Belancic et al., 2003), the amounts of volatiles increased in wines compared to the control. This could be attributed to the β-glucosidase activity of *D. vanriji*. However, β-glucosidase synthesis by *D. vanriji* was quite low under grape juice fermentation conditions, which could explain why most of the glycosides were still present in wines (Garcia et al., 2002).

6.2. Exogenous enzymes

Most applications of exogenous enzymes have concerned wines because of the abundance of glycosidic flavour precursors and the flavour is a wine's most important distinguishing characteristic. Moreover, wines originating from complete metabolism of fermentable sugars do not contain glucose, or only minute amounts. Alternatively, the enzymes were used in fruit juice processing to enhance flavour.

6.3. Wines

Commercial enzyme preparations (pectinases and hemicellulases) containing glycosidase activities (Bertrand & Beloqui, 1996; Cabaroglu, Selli, Canbas, Lepoutre, & Günata, 2003; Günata et al., 1993; Rogerson, Grande, & Silva, 1995) and β-glucosidase preparations from yeasts (Gueguen, Chemardin, Pien, Arnaud, & Galzy, 1997; Yani & Sato, 1999), or filamentous fungi in solution or immobilized forms, have been used to enhance wine flavour (Spagna et al., 1998).

Numerous studies have been published. Table 2 gives some examples of the effect of the use of exogenous enzymes on volatiles in winemaking, from several grape varieties. Since the reported quantitative data were obtained by different enzyme preparations, grape varieties and analytical techniques, a direct comparison is not feasible. Nevertheless, the general tendency is a significant increase of volatiles, by several-fold in some cases, corresponding to three classes of chemical compounds: monoterpenes, norisoprenoids and benzene derivatives. Generally, the highest increase among monoterpenes was often observed for geraniol, nerol, linalool, linalool oxides and some monoterpene polyols, among norisoprenoids for 3-hydroxy-β-damascone, 3-oxo-α-ionol and vomifoliol, and among benzene derivatives for benzyl alcohol, 2-phenylethanol, tyrosol and zingerone. For the benzene derivatives, the increases of 4-vinylphenol and 4-vinylguaiacol could be the consequence of cinnamate esterase activity from enzyme preparations (Chatonnet, Barbe, Canal-Llauberes, Dubourdieu, & Boidron, 1992; Dugelay, Günata, Sapis, Baumes, & Bayonove, 1993). Glycosidase-treated wines, from monoterpene-rich grape varieties, Muscat, Riesling and Gewurztraminer, are often perceived to have more intense floral attributes, due to the enrichment of the wines in monoterpenes largely exceeding their perception threshold values (Bertrand & Beloqui, 1996; Günata

Table 2
Effect of the use of glycosidase-rich pectinase preparations[1] **or** β-glucosidase preparations from yeasts[2] on the release of volatiles (μg/l) from glycosides in winemaking

	Monoterpenes		Norisoprenoids		Benzene derivatives[a]		References
	Control	Treated	Control	Treated	Control	Treated	
Muscat of Frontignan[1]	4384	7718	9	542	101	392	Günata et al. (1993)
Riesling[1]	2418	3119	nd	407	107	693	Günata et al. (1993)
Gewurztraminer[1]	129	248	nr	nr	nr	nr	Bertrand and Beloqui (1996)
Emir[1]	14	25	48	74	809	1105	Cabaroglu et al. (2003)
Trajadura[1]	100	233	nr	nr	nr	nr	Rogerson et al. (1995)
Muscat[2]	805	1407	nr	nr	nr	nr	Yani and Sato (1999)
Muscat of Lunel[2]	3916	4646	nr	nr	nr	nr	Gueguen et al. (1997)
Sauvignon[2]	2018	2178	nr	nr	46	120	Gueguen et al. (1997)
Chardonnay[2],[b]	2310	2500	nr	nr	36	97	Gueguen et al. (1997)

nd: not detected; nr: not reported.
[a] 4-vinylphenol and 4-vinylguaiacol, although detected were not included since their formation is often due to the other enzymatic activities.
[b] Increases observed for pinene and terpinene in treated sample were not included since they cannot derive from the hydrolysis of glycosides.

Table 3
Effect of the use of yeast β-glucosidase preparations on the release of volatiles (μg/l) from glycosides in fruit juices

	Monoterpenes		Benzene derivatives		References
	Control	Treated	Control	Treated	
Peach[a]	350	1260	260	1350	Gueguen et al. (1996)
Cherry	480	1180	2600	3700	Gueguen et al. (1996)
Strawberry	100	770	130	1080	Gueguen et al. (1996)
Passion fruit[a]	180	200	250	500	Gueguen et al. (1996)
Orange	110	870	nd	200	Gueguen et al. (1996)
Papaya	1800	2200	nd	nd	Gueguen et al. (1996)
Apple	180	500	110	200	Gueguen et al. (1996)
Grape (Muscat of Rosada)	1492	2685	nr	nr	Belancic et al. (2003)

nd: not detected; nr: not reported.
[a] Increases observed for pinene and terpinene in treated samples were not included since they cannot derive from the hydrolysis of glycosides.

et al., 1993). For other varieties, although an increase can be observed in monoterpene levels, a direct contribution of these compounds to flavour of wines seems limited because enrichment does not often exceed the perception threshold of the monoterpenes (Cabaroglu et al., 2003; Günata et al., 1993; Rogerson et al., 1995). Glycosidase-treated wines can contain unusually high levels of citronellol (citrus notes), as the consequence of yeast reductase activity on geraniol and nerol (Di Stefano, Magiorotto, & Gianotti, 1992; Dugelay, Günata, Sapis, Baumes, & Bayonove, 1992).

The norisoprenoid levels liberated by exogenous enzymes in wines are generally quite similar among grape varieties (Günata et al., 1993). In some papers, these compounds are not analyzed because the reference compounds are not commercially available and the relevant GC/MS identification library is usually not complete. The liberated norisoprenoids are odourless, but some are able to generate odour-active compounds, such as β-damascenone, vitispirane and theaspirane by further acid-catalyzed reactions occurring during wine conservation (Winterhalter, 1992). Hence, the rapid liberation of aglycones by enzymatic hydrolysis could accelerate the formation of odorous compounds in wines. Indeed, the aglycones are not chemically reactive when glycosylated and the acid hydrolysis of glycosides takes place slowly under traditional wine storage conditions (Günata et al., 1985b; Park & Noble, 1993). Benzene derivatives, liberated by enzyme treatment, may not contribute to the flavour of wine on the basis of their perception threshold value (Cabaroglu et al., 2003; Günata et al., 1993).

Two parameters should be taken into consideration in the choice of glycosidase-rich pectinase/hemicellulase preparations for flavour increase in winemaking. First, these preparations should not contain significant levels of cinnamate esterase activity, which leads, in connection with a decarboxylase activity from *S. cerevisiae*, to the formation of high levels of volatile phenols, imparting a phenolic off-flavour to wine (Chatonnet et al., 1992; Dugelay et al., 1992). Second, β-glucosidase activity from enzyme preparations should possess quite low activities towards glucosylated anthocyanidins to avoid colour loss in red wines (Le Traon-Masson & Pellerin, 1998).

β-Glucosidase preparations from yeasts *C. molischiana* and *D. hanseni* allowed increases of volatiles in treated wines (Table 2) (Gueguen et al., 1997). In this case, the hydrolysis of β-glycosides should mainly concern β-D-glucosides because of the possible lack of exoglycosidase and diglycosidase activities.

6.4. Fruit juices

Due to the fact that most fungal β-glucosidases are inhibited by glucose, little work has focused the use of exogenous enzymes for aroma enhancement in fruit juices.

β-Glucosidase from *C. molischiana* significantly increased the levels of linalool, benzyl alcohol and 2-phenylethanol in the juices of several fruits, such as peach, cherry, strawberry, passion fruit, orange, apple and papaya (Table 3) (Gueguen et al., 1996). *D. vanriji* β-glucosidase addition to a Muscat grape juice resulted in increase of the amounts of, mainly, linalool, geraniol and α-terpineol (Belancic et al., 2003). Diglycosidase from *A. niger* increased the levels of linalool, benzyl alcohol, and benzaldehyde in a passion fruit juice (Shoseyov, Bravdo, Ikan, & Chet, 1990).

7. Conclusion and perspectives

It is now well established that glycoconjugated volatile compounds represent an important potential for enhancing the flavour of fruit juices and derived beverages. Moreover, they constitute an interesting source of potent odour compounds in plant tissue products. Flavour enhancement in fruit juices, and particularly in wine processing, has nowadays become possible through

the use of exogenous glycosidases. For a better control of flavour recovery from glycosides in plants, fruits and derived products, new challenges are opened through genetic engineering by constructing fungal strains, recombinant yeast strains or transformed plants overexpressing target enzymes: diglycosidases from grape berry and tea, β-glucosidases from fungi tolerant to glucose inhibition and β-apiosidase from fungi.

References

Adam, A. C., Rubio-Texeira, M., & Polaina, J. (1995). Induced expression of bacterial β-glucosidase activity in *Saccharomyces*. *Yeast, 11*, 395–406.

Aryan, A. P., Wilson, B., Strauss, C. R., & Williams, P. J. (1987). The properties of glycosidases of *Vitis vinifera* and comparison of their β-glucosidase activity with that of exogenous enzymes. An assessment of possible applications in enology. *American Journal of Enology and Viticulture, 38*, 182–188.

Aubert, C., Günata, Z., Ambid, C., & Baumes, R. (2003). Changes in physicochemical characteristics and volatile constituents of yellow and white-fleshed nectarines during maturation and artificial ripening. *Journal of Agricultural and Food Chemistry, 51*, 3083–3091.

Belancic, A., Günata, Z., Vallier, M. J., & Agosin, E. (2003). β-Glucosidase from the grape native yeast *Debaryomyces vanriji*: Purification, characterization and its effect on monoterpene concentration of a Muscat grape juice. *Journal of Agricultural and Food Chemistry, 51*, 1453–1459.

Bertrand, A., & Beloqui, A. A. (1996). Essais d'utilisation d'enzymes pectolytiques à activités complémentaires de type glucosidase dans les vins. Influence sur l'arôme. *Revue Française d'Oenologie, 157*, 1–6.

Birk, R., Bravdo, B., & Shoseyov, O. (1996). Detoxification of cassava by *Aspergillus niger* B-1. *Applied Microbiology and Biotechnology, 45*(3), 411–414.

Blondin, B., Ratomahenina, R., Arnaud, A., & Galzy, P. (1983). Purification and properties of the β-glucosidase of a yeast capable of fermenting cellobiose to ethanol: *Dekkera intermedia* Van der Walt. *European Journal of Applied Microbiology and Biotechnology, 17*, 1–6.

Bodio, E., Lloret, A., Medina, K., Carrau, F., & Dellacassa, E. (2002). Effect of β-glucosidase activity of *Oenococcus oeni* on the glycosylated flavor precursors of Tannat wine during malolactic fermentation. *Journal of Agricultural and Food Chemistry, 50*(2), 2344–2349.

Bourquelot, E., & Bridel, M. (1913). Synthèse du géranyl glucoside á l'aide de l'émulsine: sa présence dans les végétaux. *Compte Rendu de l'Académie des Sciences, Paris, 157*, 72–74.

Cabaroglu, T., Selli, S., Canbas, A., Lepoutre, J. P., & Günata, Z. (2003). Wine flavor enhancement through the use of exogenous fungal glycosidases. *Enzyme and Microbial Technology, 33*, 581–587.

Cameron, R. G., Manthey, J. A., Baker, R. A., & Grohmann, K. (2001). Purification and characterization of a β-glucosidase from *Citrus sinensis* var. Valencia fruit tissue. *Journal of Agricultural and Food Chemistry, 49*, 4457–4462.

Chassagne, D., Bayonove, C., Crouzet, J., & Baumes, R. (1995). Formation of aroma by enzymic hydrolysis of glycosidically bound components of passion fruit. In P. Etiévant & P. Schreier (Eds.), *Bioflavour* (Vol. 95, pp. 14–17). Paris: INRA.

Chassagne, D., Crouzet, J. C., Bayonove, C. L., Brillouet, J. M., & Baumes, R. L. (1996). 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranosides as aroma precursors from passion fruit. *Phytochemistry, 41*, 1497–1500.

Chatonnet, P., Barbe, C., Canal-Llauberes, R. M., Dubourdieu, D., & Boidron, J. N. (1992). Incidences de certaines préparations pectolytiques sur la teneur en phénols volatils des vins blancs. *Journal International des Sciences de la Vigne et du Vin, 26*, 253–269.

Cordonnier, R. E., Günata, Y. Z., Baumes, R. L., & Bayonove, C. L. (1989). Recherche d'un matériel enzymatique adapté à l'hydrolyse des précurseurs d'arômes de nature glucosidique du raisin. *Connaissance de la Vigne et du Vin*, 1–17.

Dekker, R. F. H. (1986). Kinetic, inhibition and stability properties of a commercial β-D-glucosidase (cellobiase) preparation from *Aspergillus niger* and its suitability in the hydrolysis of lignocellulose. *Biotechnology and Bioengineering, 28*, 1438–1442.

Delcroix, A., Günata, Z., Sapis, J. C., Salmon, J. M., & Bayonove, C. (1994). Glycosidase activities of three enological yeast strains during wine making. Effect on the terpenol content of Muscat wine. *American Journal of Enology and Viticulture, 45*, 291–296.

Dharmawhardana, D. P., Ellis, B. E., & Carlson, I. E. (1999). cDNA cloning and heterologous expression of coniferin β-glucosidase. *Plant Molecular Biology, 40*, 365–372.

Di Stefano, R., Magiorotto, G., & Gianotti, S. (1992). Transformazioni di nerolo e geraniolo indotte dai lieviti. *Rivista di Viticultura e di Enologia*, 43–49.

Dubourdieu, D., Darriet, P., Ollivier, C., Boidron, J. N., & Ribéreau-Gayon, P. (1988). Role de la levure Saccharomyces cerevisiae dans l'hydrolyse enzymatique des hétérosides terpéniques du jus de raisin. *Compte Rendu de l'Académic des Sciences, Paris*, 489–493.

Dugelay, I., Günata, Z., Sapis, J. C., Baumes, R., & Bayonove, C. (1992). Etude de l'origine du citronellol dans les vins. *Journal International des Sciences de la Vigne et du Vin, 26*, 177–184.

Dugelay, I., Günata, Z., Sapis, J. C., Baumes, R., & Bayonove, C. (1993). Role of cinnamoylesterase activities from enzyme preparations on the formation of volatile phenols during winemaking. *Journal of Agricultural and Food Chemistry, 41*, 2092–2096.

Dupin, I., Günata, Z., Sapis, J. C., Bayonove, C., M'Bairaroua, O., & Tapiero, C. (1992). Production of a β-apiosidase by *Aspergillus niger*. Partial purification, properties and effect on terpenyl apiosylglucosides from grape. *Journal of Agricultural and Food Chemistry, 40*, 1886–1891.

Duroux, L., Delmotte, P. M., Lancelin, J. M., Keravis, G., & Jay-Allemand, C. (1998). Insight into naphthoquinone metabolism: β-Glucosidase-catalysed hydrolysis of hydrojuglone β-D-glucopyranoside. *Biochemical Journal, 333*, 275–283.

Fils-Lycaon, B., & Buret, M. (1991). Changes in glycosidase activities during development and ripening of melon. *Postharvest Biology and Technology, 1*, 143–151.

Garcia, A., Carcel, C., Dulau, L., Samson, A., Aguera, E., Agosin, E., & Günata, Z. (2002). Influence of a mixed culture with *Debaryomyces vanrijii* and *Saccharomyces cerevisiae* on the volatiles of a Muscat wine. *Journal of Food Science, 67*(3), 1138–1143.

Gerardi, C., Blando, F., Santino, A., & Zacheo, G. (2001). Purification and characterization of a β-glucosidase abundantly expressed in ripe sweet cherry (*Prunus avium L.*) fruit. *Plant Science, 160*, 795–805.

Gondé, P., Ratomahenina, R., Arnaud, A., & Galzy, P. (1985). Purification and properties of the exocellular β-glucosidase of *Candida molischiana* (Zikes) Meyer and Yarror capable of hydrolyzing soluble cellodextrines. *Canadian Journal of Biochemistry and Cell Biology, 63*, 1160–1166.

Goris, M. A. (1924). Sur la composition chimique des fruits verts de vanille et le mode de formation du parfum de la vanille. *Compte Rendu de l'Académie des Sciences, Paris, 179*, 70–72.

Grimaldi, A., McLean, H., & Jiranek, V. (2000). Identification and partial characterization of glycosidic activities of commercial

strains of the lactic acid bacterium, *Oenococcus oeni*. *American Journal of Enology and Viticulture, 51*(4), 362–369.

Gueguen, Y., Chemardin, P., Janbon, G., Arnaud, A., & Galzy, P. (1996). A very efficient β-glucosidase catalyst for the hydrolysis of flavor precursors of wines and fruit juices. *Journal of Agricultural and Food Chemistry, 44*, 2336–2340.

Gueguen, Y., Chemardin, P., Pien, S., Arnaud, A., & Galzy, P. (1997). Enhancement of aromatic quality of Muscat wine by the use of immobilized β-glucosidase. *Journal of Biotechnology, 55*, 151–156.

Günata, Y. Z., Bayonove, C. L., Baumes, R. L., & Cordonnier, R. E. (1985a). The aroma of grapes: I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. *Journal of Chromatography, 331*, 83–90.

Günata, Y. Z., Bayonove, C., Baumes, R., & Cordonnier, R. (1985b). Stability of free and bound fractions of some aroma components of grape *cv* Muscat during the wine processing. *American Journal of Enology and Viticulture, 37*, 112–114.

Günata, Z., Bitteur, S., Brillouet, J. M., Bayonove, C., & Cordonnier, R. (1988). Sequential enzymic hydrolysis of potentially aromatic glycosides from grape. *Carbohydrate Research, 184*, 139–149.

Günata, Y. Z., Biron, C., Sapis, J. C., & Bayonove, C. (1989). Glycosidase activities in sound and rotten grapes in relation to hydrolysis of grape monoterpenyl glycosides. *Vitis, 28*, 191–197.

Günata, Z., Brillouet, J. M., Voirin, S., Baumes, B., & Cordonnier, R. (1990a). Purification and some properties of an α-L-arabinofuranosidase from *Aspergillus niger*. Action on grape monoterpenyl arabinofuranosyl glucosides. *Journal of Agricultural and Food Chemistry, 38*, 772–776.

Günata, Z., Bayonove, C., Tapiero, C., & Cordonnier, R. (1990b). Hydrolysis of grapemonoterpenyl β-D-glucosides by various β-glucosidases. *Journal of Agricultural and Food Chemistry, 38*, 1232–1236.

Günata, Z., Bayonove, C., Cordonnier, R., Arnaud, A., & Galzy, P. (1990c). Hydrolysis of grape monoterpenyl glycosides by *Candida molischiana* and *Candida wickerhamii* β-glucosidases. *Journal of the Science of Food and Agriculture, 50*, 499–506.

Günata, Z., Dugelay, I., Sapis, J. C., Baumes, R., & Bayonove, C. (1993). Role of the enzymes in the use of the flavour potential from grape glycosides in winemaking. In P. Schreier & P. Winterhalter (Eds.), *Progress in flavour precursor studies* (pp. 219–234). Carol Stream IL: Allured Publishers.

Günata, Z., Vallier, M. J., Sapis, J. C., Bayonove, C., Arnaudon, V., Madarro, A., & Polaina, J. (1996). Hydrolysis of monoterpenyl-β-D-glucosides by cloned β-glucosidases from *Bacillus polymyxa*. *Enzyme and Microbial Technology, 18*, 286–290.

Günata, Z., Dugelay, I., Vallier, M. J., Sapis, J. C., & Bayonove, C. (1997). Multiple forms of glycosidases in an enzyme preparation from *Aspergillus niger*: Partial characterization of an apiosidase. *Enzyme and Microbial Technology, 21*, 39–44.

Günata, Z., Blondeel, C., Vallier, M. J., Lepoutre, J. P., Sapis, J. C., & Watanabe, N. (1998). An endoglycosidase from grape berry skin of *cv*. M. Alexandrie, hydrolyzing potentially aromatic disaccharide glycosides. *Journal of Agricultural and Food Chemistry, 46*, 2748–2753.

Günata, Z., & Vallier, M. J. (1999). Production of highly glucose-tolerant extracellular β-glucosidase by three *Aspergillus* strains. *Biotechnology Letters, 21*, 219–223.

Guo, W., Sakata, K., Watanabe, N., Nakajima, R., Yagi, A., Ina, J., & Luo, S. (1993). Geranyl 6-*O*-β-D-xylopyranosyl-β-D-glucopyranoside isolated as an aroma precursor from tea leaves for oolong tea. *Phytochemistry, 6*, 1373–1375.

Guo, W., Ogawa, K., Yamauchi, K., Watanabe, N., Usui, T., Luo, S., & Sakata, K. (1996). Isolation and characterization of a β-primeverosidase concerned with alcoholic aroma formation in tea leaves. *Bioscience, Biotechnology and Biochemistry, 60*, 1810–1814.

Henrissat, B. (1991). A classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochemical Journal, 280*, 309–316.

Henrissat, B., & Davies, G. (1997). Structural and sequence-based classification of glycosyl hydrolases. *Current Opinion Structure in Biology, 7*, 637–644.

Heyworth, R., & Walker, P. O. (1962). Almond-emulsin β-D-glucosidase and β-D-galactosidase. *Biochemical Journal, 83*, 331–335.

Hoh, Y. K., Yeoh, H. H., & Tan, T. K. (1992). Properties of β-glucosidase purified from *Aspergillus niger* mutants USDB 0827 and USDB 0828. *Applied Microbiology and Biotechnology, 37*, 590–593.

Hösel, W., Surholt, E., & Borgmann, E. (1978). Characterization of β-glucosidase isoenzymes possibly involved in lignification from chick pea (*Cicer arietinum* L.) cell suspension cultures. *European Journal of Biochemistry, 84*, 487–492.

Hösel, W. (1981). Glycosylation and glycosidases. In P. K. Stumpf & E. E. Conn (Eds.), *The biochemistry of plants* (pp. 14–17). New York: Academic Press.

Inamdar, A. N., & Kaplan, J. G. (1966). Purification and properties of an inducible β-glucosidase of bakers' yeast. *Canadian Journal of Biochemistry, 44*, 1099–1108.

Janbon, G., Derancourt, J., Chemardin, P., Arnaud, A., & Galzy, P. (1995). A very stable β-glucosidase from a *Candida molischiana* mutant strain. Enzymatic properties, sequencing and homology with other yeast strains. *Bioscience, Biotechnology and Biochemistry, 59*, 1320–1322.

Kaji, A. (1984). L-arabinosidases. *Advances in Carbohydrate Chemistry and Biochemistry, 42*, 383–394.

Kaplan, J. G. (1965). An inducible system for the hydrolysis and transport of β-glucosidase in yeast. *The Journal of General Physiology, 48*, 873–885.

Krammer, G., Winterhalter, P., Schwab, M., & Schreier, P. (1991). Glycosidically bound aroma compounds in the fruits of *Prunus species*: Apricot (*P. armeniaca*, L.), peach (P. persica, L.), yellow plum (*P. domestica*, L. ssp. Syriaca). *Journal of Agricultural and Food Chemistry, 39*, 778–781.

Le Clinche, F., Pinaga, F., Ramon, D., & Vallès, S. (1997). α-L-Arabinofuranosidases from *Aspergillus terreus* with potential application in enology: Induction, purification and characterization. *Journal of Agricultural and Food Chemistry, 45*, 2379–2383.

Le Traon-Masson, M. P., & Pellerin, P. (1998). Purification and characterization of two β-D-glucosidases from an *Aspergillus niger* enzyme preparation: Affinity and specificity toward glycosylated compounds characteristic of the processing of fruits. *Enzyme and Microbial Technology, 22*, 374–382.

Leah, R., Kigel, J., Svendsen, I., & Mundy, J. (1995). Biochemical and molecular characterization of a barley seed β-glucosidase. *Journal of Biological Chemistry, 26*, 15789–15797.

Lecas, M., Günata, Y. Z., Sapis, J. C., & Bayonove, C. (1991). Purification and partial characterization of β-glucosidase from grape. *Phytochemistry, 30*, 451–454.

Leclerc, M., Arnaud, A., Ratomahenina, R., Galzy, P., & Nicolas, M. (1984). The enzyme system in a strain of *Candida wickerhamii* Meyer and Yarrow participating in the hydrolysis of cellodextrins. *Journal of General Applied Microbiology, 30*, 509–521.

Leclerc, M., Arnaud, A., Ratomahenina, R., & Galzy, P. (1987). Yeast β-glucosidases. *Biotechnology and Genetic Engineering Reviews, 5*, 269–295.

Leong, G., Archavlis, A., & Derbesy, M. (1989). Research on the glucoside fraction of the vanilla. *Journal of Essential Oil Research, 1*, 33–41.

Lutz, A., & Winterhalter, P. (1992). Isolation of additional carotenoid metabolites from quince fruit (*Cydonia oblonga* Mill.). *Journal of Agricultural and Food Chemistry, 40*, 1116–1120.

Marlatt, C., Ho, C., & Chien, M. J. (1992). Tomato: Studies of aroma constituents bound as glycosides in tomato. *Journal of Agricultural and Food Chemistry, 40*(2), 249–252.

McCloskey, L. P. (1974). Gluconic acid in California wines. *American Journal of Enology and Viticulture, 25*, 198–201.

Mizutani, M., Nakanishi, H. N., Ema, J. I., Ma, S. J., Noguchi, E., Inohara-Ochiai, M., Fukuchi-Mizutani, M., Nakao, M., & Sakata, K. (2002). Cloning of β-primeverosidase from tea leaves, key enzyme in tea aroma formation. *Plant Physiology, 130*, 2164–2176.

Nakajima, R., Moon, J. H., Shimokihara, K., Watanabe, N., Watanabe, S., Inagaki, J., Etoh, H., Asai, T., Sakata, K., & Ina, K. (1993). Formation of flower fragrance compounds from their precursors by enzymatic action during flower opening. *Bioscience, Biotechnology and Biochemistry, 57*(7), 1101–11106.

Nunan, K. J., Davies, C., Robinson, S. P., & Fincher, G. B. (2001). Expression patterns of cell-wall modifying enzymes during grape berry development. *Planta, 214*, 257–264.

Ogawa, K., Moon, J. H., Guo, W., Yagi, A., Watanabe, N., & Sakata, K. (1995). A study on tea aroma formation mechanism: Alcoholic aroma precursor amounts and glycosidase activity in parts of tea plant. *Zeitschrift fur Naturforschung, 50*, 493–498.

Ogawa, K., Yasuyuki, I., Guo, W., Watanabe, N., Usui, T., Dong, S., Tong, Q., & Sakata, K. (1997). Purification of a β-primeverosidase concerned with alcoholic aroma formation in tea leaves (Cv. *Shuixian*) to be processed to Oolong Tea. *Journal of Agricultural and Food Chemistry, 45*, 877–882.

Osbourn, A. E. (1996). Preformed antimicrobial compounds and plant defense against fungal attack. *The Plant Cell, 8*, 1821–1831.

Pabst, A., Barron, A., Etiévant, P., & Schreier, P. (1991). Studies on the enzymatic hydrolysis of bound aroma constituents from raspberry fruit pulp. *Journal of Agricultural and Food Chemistry, 39*, 173–175.

Park, S. K., & Noble, A. C. (1993). Monoterpenes and monoterpene glycosides in wine aromas. In *ACS symposium series. Beer and wine production* (pp. 99–109). Washington DC: ACS.

Pemberton, M. S., Brown, R. D., & Emert, G. H. (1980). The role of β-glucosidase in the by conversion of cellulose to ethanol. *Canadian Journal of Chemical Engineering, 58*, 723–729.

Perez, A. G., Cert, A., Rios, J., & Olias, J. M. (1997). Free and glycosidically bound volatile compounds from two banana cultivars, Valery and Pequena Enana. *Journal of Agricultural and Food Chemistry, 45*(11), 4393–4397.

Pilnik, W., & Voragen, A. G. J. (1991). The significance of endogenous and exogenous pectic enzymes in fruit and vegetable processing. In P. F. Fox (Ed.), *Food enzymology* (Vol. 1, pp. 303–336). London: Elsevier Applied Science.

Raynal, A., & Guerineau, M. (1984). Cloning and expression of the structural gene for β-glucosidase of *Kluyveromyces fragilis* in *Escherichia coli* and *Saccharomyces cerevisiae*. *Molecular and General Genetics, 195*, 108–115.

Rogerson, F. S. S., Grande, H. J., & Silva, M. C. M. (1995). Enzymatic enhancement of the free monoterpenol content of a Portuguese wine from a single, native grape variety "Trajadura". *Biotechnological Letters, 17*, 35–40.

Roitner, M., Schalkhammer, T., & Pittner, F. (1984). Preparation of prunin with the help of immobilised naringinase pretreated with alkaline buffer. *Applied Biochemistry and Biotechnology, 9*, 483–488.

Romero, C., Manjon, A., Bastida, J., & Iborra, J. L. (1985). A method for assaying the rhamnosidase activity of naringinase. *Analytical Biochemistry, 149*, 566–571.

Roscher, R., Herderich, M., Steffen, J. P., Schreier, P., & Schwab, W. (1997). 2,5-dimethyl-4-hydroxy-3(2H)-furanone 6′-*O*-malonyl-β-D-glucopyranoside in strawberry fruits. *Phytochemistry, 43*, 155–159.

Rosi, I., Vinella, M., & Domizio, P. (1994). Characterization of β-glucosidase activity in yeasts of enological origin. *Journal of Applied Bacteriology, 77*, 519–527.

Saha, B. C., & Bothast, R. J. (1996). Production, purification and characterization of a highly glucose-tolerant novel β-glucosidase from *Candida peltata*. *Applied Environmental Microbiology, 62*, 3165–3170.

Sakho, M., Chassagne, D., & Crouzet, J. (1997). African mango glycosidically bound volatile compounds. *Journal of Agricultural and Food Chemistry, 45*, 883–888.

Salles, C., Jallageas, J. C., Fournier, F., Tabet, J. C., & Crouzet, J. C. (1991). Apricot glycosidically bound volatile components. *Journal of Agricultural and Food Chemistry, 39*, 1979–1983.

Sanchez-Torres, P., Gonzalez-Candelas, L., & Ramon, D. (1996). Expression in a wine yeast strain of the *Aspergillus niger* abfB gene. *FEMS Microbiology Letters, 145*, 189–194.

Sanchez-Torres, P., Gonzalez-Candelas, L., & Ramon, D. (1998). Heterologous expression of a *Candida molischiana* anthocyanin-β-glucosidase in a wine yeast strain. *Journal of Agricultural and Food Chemistry, 46*, 354–360.

Sarry, J. E., Grimplet, J., Vallier, M. J., Mondolot-Cosson, L., Andary, C., Günata, Z., & Romieu, C. (2003). Purification and characterization of cell-wall glycoside hydrolase from grapewine (*Vitis vinifera* L.) berry skins. *Phytochemistry* (submitted).

Schreier, J. H., & Schreier, P. (1986). Purification and partial characterization of β-glucosidase from papaya fruit. *Phytochemistry, 10*, 2271–2274.

Schwab, W., Mahr, C., & Schreier, P. (1989). Studies on the enzymatic hydrolysis of bound aroma components from carica papaya fruit. *Journal of Agricultural and Food Chemistry, 37*, 1009–1012.

Schwab, W., Scheller, G., & Schreier, P. (1990). Glycosidically bound aroma components from sour cherry. *Phytochemistry, 29*, 607–612.

Shoseyov, O., Bravdo, B. A., Ikan, R., & Chet, I. (1988). Endo-β-glucosidase from *Aspergillus niger* grown on a monoterpene glycoside-containing medium. *Phytochemistry, 27*, 1973–1976.

Shoseyov, O., Bravdo, B. A., Ikan, R., & Chet, I. (1990). Immobilized endo-β-glucosidase enriches flavor of wine and passion fruit juice. *Journal of Agricultural and Food Chemistry, 27*, 1973–1976.

Sinnott, M. L. (1990). Catalytic mechanisms of enzymic glycosyl transfer. *Chemical Reviews, 90*, 1171–1202.

Spagna, G., Adreani, F., Salatelli, F., Romagnoli, D., Casarini, D., & Pifferi, P. G. (1998). Immobilization of the glycosidases: α-L-arabinofuranosidase and β-D-glucopyranosidase from *Aspergillus niger* on a chitosan derivative to increase the aroma of wine. Part II. *Enzyme and Microbial Technology, 23*, 413–421.

Stahl-Biskup, E., Intert, F., Holthuijzen, J., Stengele, M., & Schulz, G. (1993). Glycosidically bound volatiles – A review 1986–1991. *Flavor and Fragrance Journal, 8*, 61–80.

Suarez, M., Duque, C., Wintoch, H., & Schreier, P. (1991). Glycosidically bound aroma compounds from the pulp and the peelings of Lulo fruit (*Solanum vestissimum* D.). *Journal of Agricultural and Food Chemistry, 39*, 1643–1645.

Sue, M., Ishihara, A., & Iwamura, H. (2000). Purification and characterization of a hydroxamic acid glucoside β-glucosidase from wheat (*Triticum aestivum* L.) seedlings. *Planta, 210*, 432–438.

Ueda, M., & Yamamura, S. (2000). Chemistry and biology of plant leaf movements. *Angewandte Chemie International, 39*, 1400–1414.

Vasserot, V., Arnaud, A., & Galzy, P. (1995). Monoterpenol glycosides in plants and their biotechnological transformation. *Acta Biotechnology, 15*, 77–95.

Vasserot, V., Chemardin, P., Arnaud, A., & Galzy, P. (1991). Purification and properties of the β-glucosidase of a new strain of *Candida molischiana* able to work at low pH values: Possible use in the liberation of bound terpenols. *Journal of Basic Microbiology, 31*, 301–312.

Vasserot, V., Chemardin, P., Arnaud, A., & Galzy, P. (1989). Purification and properties of a β-glucosidase of *Hanseniaspora vineae* Van der Walt and Tscheuschner with the view to its utilization in fruit aroma liberation. *Journal of Applied Bacteriology, 66*, 271–279.

Vasserot, V., Chemardin, P., Arnaud, A., & Galzy, P. (1990). Evidence for the 3-glucosidase activity and cellobiose fermentation by various Kloeckera strains. *Acta Biotechnologica, 10*, 439–445.

Villettaz, J. C., & Dubourdieu, D. (1991). Enzymes in winemaking. In P. F. Fox (Ed.), *Food Enzymology* (pp. 427–453). London: Elsevier Applied Science.

Voirin, S., Baumes, R., Bayonove, C., M'Bairaroua, O., & Tapiero, C. (1990). Synthesis and NMR spectral properties of grape monoterpenyl glycosides. *Carbohydrate Research, 207*, 39–56.

Voirin, S. G., Baumes, R. L., Sapis, J. C., & Bayonove, C. L. (1992). Analytical method for monoterpene glycosides in grape and wine. Part 2: qualitative and quantitative determination of glycosides in grape. *Journal of Chromatography, 595*, 269–281.

Williams, C. A., & Harborne, J. B. (1994). Flavone and flavonol glucosides. In J. B. Harborne (Ed.), *The Flavonoids advances in research since 1986* (pp. 337–385). London: Chapmand and Hall.

Williams, P. J., Strauss, C., Wilson, B., & Massy-Westropp, R. A. (1982a). Novel monoterpene disaccharide glycosides of Vitis vinifera grapes and wines. *Phytochemistry, 21*, 2013–2020.

Williams, P. J., Strauss, C. R., Wilson, B., & Massy-Westropp, R. A. (1982b). Use of C_{18} reversed-phase liquid chromatography for the isolation of monoterpene glycosides and non-isoprenoid precursors from grape juice and wines. *Journal of Chromatography, 235*, 471–480.

Williams, P. J. (1983). Hydrolytic flavour release in fruits and wines through hydrolysis of nonvolatile precursors. In T. E. Acree & R. Teranishi (Eds.), *Flavor science. Sensible principles and techniques* (pp. 287–308). Washington DC: American Chemical Society.

Winterhalter, P. (1992). Oxygenated C_{13}-norisoprenoids. Important flavor precursors. In R. Teranishi, G. R. Takeoka, & M. Gúntert (Eds.), *Flavor precursors. Thermal and enzymatic conversions* (pp. 98–115). Washington DC: American Chemical Society.

Winterhalter, P., & Skouroumounis, G. K. (1997). Glycoconjugated aroma compounds: occurrence, role and biotechnological transformation. In T. Scheper (Ed.), *Advances in Biochemical Engineering/Biotechnology* (pp. 74–105). Heidelberg-Berlin: Spring-Verlag.

Withopf, B., Richling, E., Roscher, R., Schwab, W., & Schreier, P. (1997). Sensitive and selective screening for 6′-*O*-malonylated glucoconjugates in plants. *Journal of Agricultural and Food Chemistry, 45*, 907–911.

Woodward, J., & Wiseman, A. (1982). Fungal and other β-glucosidases. Their properties and applications. *Enzyme and Microbial Technology*, 4,73–79.

Wu, P., Kuo, M. C., Hartman, T. G., Rosen, R. T., & Ho, C. T. (1991). Free and glycosidically bound aroma compounds in pineapple (*Ananas comosus* L. Merr). *Journal of Agricultural and Food Chemistry, 39*, 170–172.

Yan, T. R., & Lin, C. L. (1997). Purification and characterization of a glucose-tolerant β-glucosidase from *Aspergillus niger* CCRC 31494. *Bioscience, Biotechnology and Biochemistry, 61*, 965–970.

Yani, T., & Sato, M. (1999). Isolation and properties of β-glucosidase produced by *Debaryomyces hansenii* and its application in winemaking. *American Journal of Enology and Viticulture, 50*(33), 231–235.

Young, H., & Paterson, V. J. (1995). Characterization of bound flavour components in kiwi fruit. *Journal of the Science of Food and Agriculture, 68*, 257–260.