Wine and Tea: Applications for β-glycosidase

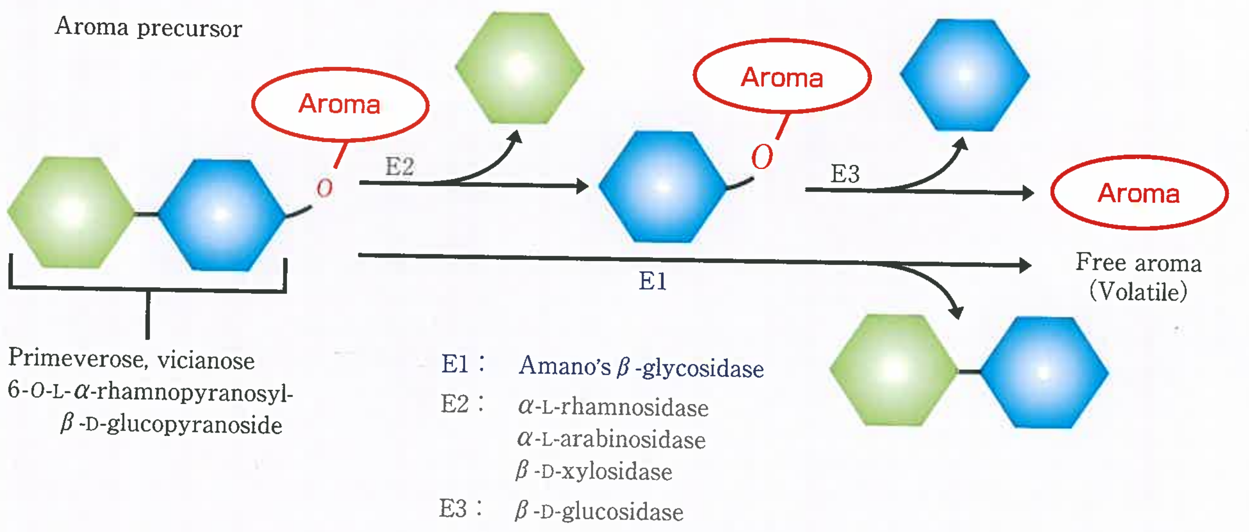
Wine Aroma
The aroma of wine, especially Muscatel, Riesling and Gewüztraminer, is defined by the presence of specific aromatic compounds, including monoterpene alcohol (e.g., α-terpineol, geraniol and citronellol), green alcohol ((Z)-3-hexenol) and phenolic alcohol (benzyl alcohol and 2-phenyl ethanol) (1). These aromatic compounds impart the fresh, floral and flowery sweet aromas characteristic of the wine; likewise, the characteristic aroma of Chardonnay is imparted by the presence of C13 norisoplenoids (e.g., vitispirane and damascenone).

The aromatic compounds in grape, grape juice and wine are present as diglycosidic precursors. The precursor compounds (6- O-α-L-rhamnopyranosyl β-D-glucopyranoside or 6- O-α-L-arabinofuranosyl β-D-glucopyranoside) are hydrolyzed during the process of wine making releasing the aromatic compounds that characterize the wine (2). The hydrolysis of the precursor compounds is carried out by a combination of glycosidases, including β-D-glucosidase, α-L-rhammosidase and α-L-arabinofuranosidase, originating from the yeast utilized in wine making (3) (figure 1). However, the hydrolysis of the precursors during wine making is not optimal; the activity of α-L-rhammosidase and α-L-arabinofuranosidase is relatively low and the crucial enzyme β-D-glucosidase is severely inhibited by free glucose.

Tea Aroma
The aroma of tea is one of the most important characteristics used to rank the quality of tea. The presence of aromatic compounds including (Z)-3-hexenol, 1-hexanol, geraniol, linalool, benzyl alcohol, 2-phenyl ethanol and methyl salicylate imparts the fresh and flowery sweet aroma found in green tea, oolong tea and black tea (4). These aromatic compounds also exist as diglycoside precursors (6- O-D-xylopyranosyl β-D-glucopyranoside (β-primeveroside)) that are hydrolyzed during tea preparation by endogenous β-primeverosidase which is found in tea leaves. This enzyme is apparently responsible for the hydrolysis of all aromatic precursor compounds found in tea (5). It has been reported that a significant amount of aromatic precursors remains in tea leaf extract after tea preparation and thus it should be possible to increase the aroma of tea by adding exogenous enzyme (6).

Amano's β-glycosidase
In order to develop enzymes to enhance the aroma of wine and tea, Amano Enzyme Inc. initiated a search for an activity capable of hydrolyzing *p*-nitrophenyl β-D-primeveroside to *p*-nitrophenol and primeverose. Several β-primeverosidase - like enzymes were found from microbial sources; these enzymes are referred to

Figure 1: One step hydrolysis of aroma precursor by Amano's β-glycosidase

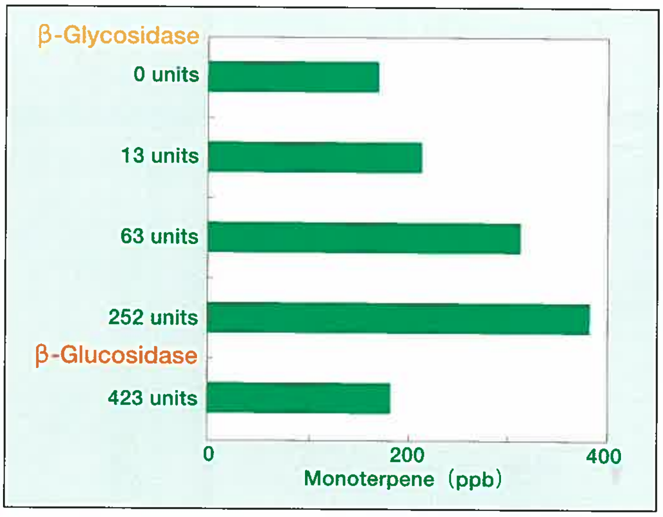


as β-glycosidase until they are more extensively characterized.

The addition of β-glycosidase during fermentation of Muscatel wine results in an increase in the total amount of five monoterpenes (linalool, α-terpineol, geraniol, nerol and citronellol) (figure 2) as well as a clear increase in floral and sweet aroma as monitored by an olfactory panel test. As a control, α-D-glucosidase from *Aspergillus niger* was added to a separate batch of Muscatel wine. The effect of β-glycosidase was superior to that of β-D-glucosidase (figure 2) as expected since β-D-glucosidase is greatly inhibited by free glucose and the effectiveness of β-D-glucosidase is dependent on the presence of other enzymes, especially α-L-rhamnosidase and α-L-arabinosidase, in a two step reaction to liberate aromatic compounds. In contrast, β-glycosidase is not inhibited by free glucose and liberates aromatic compounds directly from disaccharide precursors in a one step reaction and would therefore be expected to be more effective in aroma production.

The aroma of tea was also significantly improved by the addition of Amano's β-glycosidase as measured by the concentration of aromatic compounds in tea after enzyme treatment (table 1) and also by an olfactory panel test that found an increase in freshness in green tea (and corresponding decrease in the typical aroma characteristic of old or low grade green tea) and an increase in the floral and sweet aroma of black and oolong tea (characteristic of high grade tea). The results obtained with Amano's β-glycosidase were significantly better than results with β-D-glucosidase (data not shown).

Figure 2



Total amount of 5 monoterpenes (linalool, geraniol, nerol, citronellol, α-terpineol) in Muscatel wine. Amano's β-glycosidase was added in the process of fermentation. Commercial β-glucosidase was also tested for comparison.

Table 1: Effect of Amano's β-glycosidase on tea extract

Compound	Ratio		
	GT	OT	BT
Z-3-Hexenol	1.8	9.1	6.6
Z-Linalool oxide	1.6	7.4	
E-Linalool oxide		∞	1.7
Benzaldehyde	1.8	2.9	32.6
Linalool	1.2	6.1	4.4
Geranial	1.5	∞	∞
Methyl salicylate	2.9	∞	∞
Geraniol	6.6	7.0	2.4
Benzyl alcohol		∞	∞

GT; green tea, OT; oolong tea, BT; black tea.
Analyzed by GC/MS. Values on the table show the ratio of peak area to control (no enzyme addition).

The application work described above suggests that Amano's β-glycosidase can not only reinforce the characteristic aroma of wine and tea, but can also improve the aroma by releasing new aromatic compounds not typically found in wine or tea. It is also clear that the application of Amano's β-glycosidase may be extended to other materials as well as wine and tea.

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