Aroma enhancement and enzymolysis regulation of grape wine using β-glycosidase

Feng-Mei Zhu¹, Bin Du² & Jun Li¹

¹College of Food Science and Technology, Hebei Normal University of Science and Technology, Qinhuangdao 066600, China
²Analysis and Testing Center, Hebei Normal University of Science and Technology, Qinhuangdao 066600, China

Keywords
Aroma enhancement, enzymolysis regulation, gas chromatography-mass spectrometry, grape wine, Kramer sensory evaluation

Abstract
Adding β-glycosidase into grape wine for enhancing aroma was investigated using gas chromatography-mass spectrometry (GC-MS) and Kramer sensory evaluation. Compared with the extract from control wines, the extract from enzyme-treated wines increased more aromatic compounds using steam distillation extraction (SDE) and GC-MS analyses. These aromatic compounds were as follows: 3-methyl-1-butanol formate, 3-pentanol, furfural, 3-methyl-butanoic acid, 2-methyl-butanoic acid, 3-hydroxy-butanoic acid ethyl ester, hexanoic acid, hexanoic acid ethyl ester, benzyl alcohol, octanoic acid, octanoic acid ethyl ester, dodecanoic acid, and ethyl ester. The enzymolysis regulation conditions, including enzymolysis temperature, enzymolysis time, and enzyme amount, were optimized through L₉(3⁴) orthogonal test. Kramer sensory evaluation was performed by an 11-man panel of judges. The optimum enzymolysis regulation conditions were found to be temperature of 45°C, enzymolysis time of 90 min, and enzyme amount of 58.32 U/mL grape wine, respectively. The Kramer sensory evaluation supported that the enzyme-treated wines produced a stronger fragrance.

Introduction
Grape is a non-climacteric fruit of the genus Vitis that grows on the perennial and deciduous woody vines. There are about 60 species of Vitis, which are mainly found in the temperate zones of the Northern Hemisphere and almost equally distributed between America and Asia (Mullins et al. 1992). Grape wine is an alcoholic beverage, typically made of fermented grape juice. Its composition and properties are related to the wine’s origin and age. The constituents of wine are water, ethanol, saccharides, amino acids, phenolic compounds, and other pigments, and trace metal (Monaci et al. 2003; Roig and Thomas 2003; Katalinic et al. 2004; Nilsson et al. 2004).
Grape wine aromas are made up of several hundreds of volatile compounds. The aroma potential of grape wine derives from aromatic free volatiles and from non-volatile, odorless precursor, which may be hydrolyzed during the winemaking process (Villena et al. 2006). Grape-derived aroma and flavor compounds are present as free volatiles and, in part, as sugar-bound precursor including glycosides (McMahon et al. 1999). Glycosides which contain aroma and flavor aglycones may affect wine quality after hydrolysis. The liberation of free aglycones from glycosides is performed by acid-catalyzed reactions or by the action of endogenous enzmes from glycosides (McMahon et al. 1999). Glycosides (Villena et al. 2006). The aroma of grape wine is enhanced and the wine can be liberated by hydrolysis of the insufficient activity of endogenous activity in the grapes. The potential aromatic compounds in grape wine are exogenous glycosidase, can thereby accelerate the formation of odor-active volatiles during conservation due to the reactivity of liberated aglycones in wine pH. Winemaking is a biotechnological process in which the use of exogenous enzyme preparations helps to solve the problem of the insufficient activity of endogenous activity in the grapes. The potential aromatic compounds in grape wine can be liberated by hydrolysis of β-glycosidase. Therefore, the aroma of grape wine is enhanced and the quality of grape wine is improved. It would be very available to study the aroma enhancement using β-glycosidase for grape wine. Due to the limited effect of glycosidase from grape and Saccharomyces cerevisiae in winemaking, a large part of glycosides is still present in young wines (Cabaroglu et al. 2003). Therefore, increasing interest has been devoted in the past years to the study of using exogenous glycosidase from yeast and from filamentous fungi to enhance wine aroma.

Some biotechniques have been of fundamental importance in oenology, among these technological innovations, enzymatic treatments by commercial preparation in free or immobilized form, selected yeasts, improvement of microbial starters, and enzyme immobilization (Palmer and Spagna 2007). But the use of the enzmes in the wine industry remains limited for several reasons that can be summarized as follows: traditionalism of winemakers, influence on enzymatic activities related to physicochemical characteristics of musts and wines (pH, temperature, ethanol, sugars, polyphenols, etc.) on enzymatic activities (Colagrande et al. 1994). The aromatic component of a wine is, moreover, closely related to its sensory quality, which is determined by the consumer’s acceptability (Varrela and Gambara 2006; Vilanova 2006). Recently, sensory analysis has defined its role in the oenological industry identifying the causes of variation in perceived quality, the corrective actions, thereby becoming instrument of quality control (Verzera et al. 2008). The main aim of this work was to investigate the release of glycosidically bound aroma compounds by adding exogenous β-glycosidase into wines and using gas chromatography-mass spectrometry (GC-MS) analyses and Kramer sensory evaluation.

Materials and Methods

Sampling

Two samples of Cabernet Sauvignon dry red wine, one was obtained from the Experimental Winery at the College of Food Science and Technology, Hebei Normal University of Science and Technology, the other was obtained from the Langgesi (Qinhuangdao) Ltd. Company (Changli County, Hebei Province, China).

The crude β-glycosidase obtained from our laboratory (Zhu et al. 2010) was added into two wines. The wine without adding enzymes was used as control.

Microorganism

Aspergillus oryzae 3.481 and Aspergillus niger 3.316 strain were obtained from China General Microbiological Culture Collection Center. The fused protoplasts of A. oryzae 3.481 and A. niger 3.316 have been regenerated on regeneration medium containing (in g L⁻¹): sucrose 0.5, glucose 2.0, peptone 2.0, yeast extract 1.0, and agar 20.0. The fusion strain was selected for further studies.

Inoculum

The protoplasts of A. oryzae and A. niger high-producing β-glycosidase were prepared, formed, regenerated, and fused for screening strain, which were attained by our laboratory (Zhu et al. 2010).

Enzyme assay

β-Glycosidase activities were determined in duplicate, using 0.5% (w/v) salicin as substrate. Assays were performed in acetate buffer pH4.8 and incubated at 65°C for 20 min. The amount of released glucose was determined by 3,5-dinitrosalicylic acid (DNS) reagent method. β-Glycosidase activity is expressed in units (U/mL), defined as micromoles of salicin hydrolyzed by β-glycosidase per minute, under the conditions of assay.

Enzyme treatment

The wine samples added with β-glycosidase were kept in water bath oscillator for heating oscillation. The Lₐ(3³) orthogonal design was arranged (Tables 1, 2) with the
three independent variables of enzymolysis temperature, enzymolysis time, and enzyme amount.

**Aromatic components extraction**

The minor volatile components in grape wine were fractionated using the simultaneous distillation extraction (SDE) technique (Cai et al. 2001). Sample concentration was carried out by using a SDE apparatus. A quantity of 100 mL of wine was heated in a 500 mL round-bottomed flask. The liquid phase extractions were achieved and continuous reflux of water was maintained during the extraction time (1.5 h). The distillate was extracted three times with 200 mL anhydrous ether and combined. Extracts were desiccated over anhydrous sodium sulfate, filtered, and concentrated using a rotary evaporator. Finally, the concentrates were kept in vials at 4°C until GC-MS analysis.

**Aromatic components analysis: GC-MS**

The GC-MS system comprised a gas chromatograph (model HP6890) and a Saturn mass spectrometer (Hewlett Packard, Palo Alto, CA). Separation was performed through a cross-linked polymethylsiloxane capillary column (HP-5, 30 m x 0.25 mm x 0.25 μm). Carrier gas was helium with a flow rate of 1 mL/min. The injector (the split flow ratio was 40:1) was set to 230°C. The GC temperature program was 60°C, 5°C/min to 200°C, held for 10 min. The mass spectrometer was operated in the electron impact (EI) mode at ionization energy of 70 eV and the mass range scanned was 20–500 amu in the full scan acquisition mode. The temperature of EI mode was 230°C. The ion trap was set to 150°C. Mass spectrometer with an electron bombardment source was used for the sample analysis.

Major volatile compounds were analyzed by direct injection of 0.5 μL. Volatile compounds were identified by the NIST and WILEY Mass Spectral Search Program. The identification of the volatile compounds was confirmed by comparing the retention indices with standard values of authentic samples. The relative content was calculated from the area ratio. Following equations were used to determine their percentage concentration of the identified compounds (w/w):

\[ C_i = \frac{A_i}{\sum A_i} \times 100\% \]

where \( C \) is concentration of one compound, \( A \) is peak area counts, \( \Sigma A \) is summation of all peak area counts, and subscript \( i \) represents one component.

**Sensory analysis**

Wines were assessed by 11 judges from College of Food Science and Technology, Hebei Normal University of Science and Technology, Qinhuangdao, China, who had previous experience in wine sensory analysis. Assessment took place in a standard sensory-analysis chamber (ISO 8589, 2007) equipped with separate booths. All evaluations were conducted from 10.00 to 12.00 AM in individual booths illuminated with white light. Water was provided for rinsing between wines. The order of presentation was randomized among judges and sessions. Triangle tests were performed to determine if the control samples and nine enzyme-treated wines from L9 (3^4) were significantly different and the significance of the test was established from the statistical tables.

**Statistical analysis**

The statistical significance of the effect of enzyme treatment on free and bound volatiles analyzed in triplicate was determined by analysis of variance (ANOVA) using the following software packages: SPSS 11.5 (SPSS Inc., Chicago, IL) for Windows statistical package.

**Results and Discussion**

**GC-MS results**

The aromatic components from control wine were analyzed by GC-MS, the total ionic chromatography is shown in Figure 1 and the relative content is shown in Table 3. The volatiles of wines were dominated by esters and alcohols, representing almost 90% of the volatiles (Table 3).
The aromatic components from bound compounds with enzymolysis were analyzed by GC-MS, the total ionic chromatography is shown in Figure 2 and the relative content is shown in Table 4.

The use of β-glycosidase increased considerably the total level of volatiles in wines compared to the controls. Figure 2 and Table 4 report that more aromatic components were released from the enzymolysis wine compared with the control wine. The aromatic components include 1-butanol, 3-methyl-, formate (0.75%), 3-pentanol (0.25%), furfural (0.12%), 3-methyl-butanoic acid (1.29%), 2-methyl-butanoic acid (1.07%), butanoic acid, 3-hydroxy-, ethyl ester (0.25%), hexanoic acid (1.93%), hexanoic acid, ethyl ester (1.16%), benzyl alcohol (0.41%), octanoic acid

Table 3. GC-MS identification and relative content of free aromatic components from control wine.

| Number | Retention time (min) | Chemical constituent | Formula | Molecular weight | Relative content (%) |
|--------|----------------------|----------------------|---------|------------------|----------------------|
| 1      | 3.55                 | Propanoic acid, 2-hydroxy-, ethyl ester | C₅H₁₀O₃ | 118.13           | 64.80               |
| 2      | 4.57                 | 1-Hexanol            | C₆H₁₄O  | 102.18           | 0.70                |
| 3      | 4.71                 | 1-Butanol, 3-methyl-, acetate | C₇H₁₄O₂ | 130.18           | 2.23                |
| 4      | 4.76                 | 1-Butanol, 2-methyl-, acetate | C₇H₁₄O₂ | 130.18           | 0.23                |
| 5      | 5.53                 | Butyrolactone        | C₄H₆O₂  | 86.09            | 0.15                |
| 6      | 11.25                | Phenylethyl alcohol  | C₈H₁₀O  | 122.16           | 17.80               |
| 7      | 12.78                | Butanedioic acid, diethyl ester | C₈H₁₄O₄ | 174.20           | 3.29                |
| 8      | 14.92                | Acetic acid, 2-phenylethyl ester | C₁₀H₁₄O₂ | 164.21           | 0.58                |
| 9      | 18.52                | Decanoic acid, ethyl ester | C₁₀H₂₀O₂ | 200.32           | 0.18                |
| 10     | 25.43                | Hexanedioic acid, bis(2-methylpropyl)ester | C₁₄H₂₆O₄ | 258.35           | 0.40                |
| 11     | 29.39                | Dibutyl phthalate    | C₁₀H₁₄O₄ | 278.35           | 1.78                |
Figure 2. Total ionic chromatography of aromatic components from bound compounds with enzymolysis.

Table 4. GC-MS identification and relative content of aromatic components from bound compounds with enzymolysis.

| Number | Retention time (min) | Chemical constituent | Formula | Molecular weight | Relative content (%) |
|--------|----------------------|----------------------|---------|------------------|---------------------|
| 1      | 3.18                 | 1-Butanol, 3-methyl-, formate | C₆H₁₂O₂  | 116.16           | 0.75                |
| 2      | 3.43                 | 3-Pentanol           | C₅H₁₀O   | 88.15            | 0.25                |
| 3      | 3.57                 | Propanoic acid, 2-hydroxy-, ethyl ester | C₅H₁₀O₃  | 118.13           | 38.7                |
| 4      | 3.97                 | Furfural            | C₄H₅O₂   | 96.09            | 0.12                |
| 5      | 4.28                 | 3-Methyl-butanolic acid | C₅H₁₀O₂  | 102.13           | 1.29                |
| 6      | 4.51                 | 2-Methyl-butanolic acid | C₅H₁₀O₂  | 102.13           | 1.07                |
| 7      | 4.62                 | 1-Hexanol           | C₆H₁₂O   | 102.15           | 0.86                |
| 8      | 4.74                 | 1-Butanol, 3-methyl-, acetate | C₅H₁₀O₂  | 130.18           | 3.21                |
| 9      | 4.79                 | 1-Butanol, 2-methyl-, acetate | C₅H₁₀O₂  | 130.18           | 0.51                |
| 10     | 5.58                 | Butyrolactone       | C₄H₆O₂   | 86.09            | 0.16                |
| 11     | 6.06                 | Butanoic acid, 3-hydroxy-, ethyl ester | C₆H₁₂O₃  | 132.15           | 0.25                |
| 12     | 7.47                 | Hexanoic acid       | C₇H₁₄O   | 116.16           | 1.93                |
| 13     | 7.67                 | Hexanoic acid, ethyl ester | C₇H₁₄O₂  | 76.10            | 1.16                |
| 14     | 8.73                 | Benzyl alcohol      | C₇H₁₄O   | 108.13           | 0.41                |
| 15     | 11.03                | Phenylethyl alcohol | C₈H₁₀O    | 122.16           | 31.06               |
| 16     | 12.72                | Butenedioic acid, diethyl ester | C₈H₁₄O₄  | 174.20           | 4.06                |
| 17     | 12.80                | Octanoic acid       | C₉H₁₈O₂  | 144.21           | 0.78                |
| 18     | 13.16                | Octanoic acid, ethyl ester | C₁₀H₂₀O₂ | 172.26           | 1.22                |
| 19     | 13.22                | Dodecane           | C₁₂H₂₆   | 170.34           | 0.35                |
| 20     | 14.87                | Acetic acid, 2-phenylethyl ester | C₁₀H₁₄O₂ | 164.21           | 0.33                |
| 21     | 18.48                | Decanoic acid, ethyl ester | C₁₂H₂₄O₂ | 200.32           | 0.27                |
| 22     | 23.33                | Dodecanoic acid, ethyl ester | C₁₄H₂₈O₂ | 228.37           | 0.29                |
| 23     | 25.39                | Hexanedioic acid, bis(2-methylpropyl)ester | C₁₄H₂₆O₄ | 258.35           | 0.38                |
| 24     | 29.33                | Dibutyl phthalate    | C₁₆H₁₄O₄ | 278.35           | 0.72                |
| 25     | 31.72                | Hexadecanoic acid, methyl ester | C₁₇H₃₂O₂ | 270.00           | 0.26                |
| 26     | 33.63                | 9,12-Octadecadienoic acid, methyl ester | C₁₉H₃₄O₂ | 294.47           | 0.48                |
Kramer sensory evaluation

Kramer sensory evaluation is one of the sensory-analysis methods using ranking. In this method, samples are ranked according to the degree of a specific attribute or liking. Kramer test is used to analyze the data of ranking and to identify the significant differences between samples. Three wines were presented at each session, in coded standard wine tasting glasses according to standard (ISO 3591, 1997) and covered with a watch glass to minimize the escape of volatile components. Testing temperature was 10°C. The panelists arranged the order of wines by aromatic intensity level. The strongest level was “aroma strongly perceptible” and the weakest level was “aroma not perceptible.” When there are no differences between different samples, the same bit-level was indicated in the evaluation table.

The Kramer sensory evaluation results are shown in Table 5. The 11 panelists had a very good sort for control wine samples and enzyme-treated wines. The rank and rank sum of 10 wine samples are shown in Table 6 according to Kramer sensory evaluation method. According to the table (Sun et al. 1999), the upper range was 32–89 and the lower range was 39–121 (P < 0.01). The 10 samples were significantly different from each other (P < 0.01) due to Rimax < RF = 105 and Rimin > RA = 25. The C, E, and B were as a group owing to 39 < RC < RC < RC < RA < 82. The F, G, and N were as a group owing to RF > RC > RC > RN > 82. The A, P, D, and O were as a group owing to RA = RF < RP = RO = 39. Enzyme-treated wines were found to be more intense in lime, honey, and smoky attributes than control wines. Similar descriptors were found on hydrolysis of glycosidase from Emir wine (Cabaroglu et al. 2003).

The optimization of enzymolysis regulation conditions of wine

The analysis results are shown in Table 7 according to the rank sum of Kramer sensory evaluation. The effecting order of enzymolysis regulation conditions was enzymolysis temperature > enzymolysis time > enzyme amount. The results showed that the optimal conditions were A2B3C1, namely temperature at 45°C, enzymolysis time for 90 min, and enzyme addition amount of 58.32 U/mL grape wine.

Table 5. Kramer sensory evaluation result (from weak to strong).

| Evaluator | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|-----------|---|---|---|---|---|---|---|---|---|----|
| 1         | P | C | A | D | O | E | B | N | G | F  |
| 2         | P | O | D | A | C | N | E | B | F | G  |
| 3         | A | P | C | O | D | B | E | N | G | F  |
| 4         | O | A | D | C | E | B | G | A | F | N  |
| 5         | D | A | P | O | C | B | N | E | G | F  |
| 6         | O | P | A | C | D | B | E | G | N | F  |
| 7         | P | A | O | D | C | N | B | E | F | G  |
| 8         | D | A | P | O | C | B | N | E | G | F  |
| 9         | A | C | P | D | O | E | N | B | G | F  |
| 10        | P | O | D | A | C | N | B | G | E | F  |
| 11        | A | O | D | C | P | E | G | F | B | N  |

Table 6. Rank and rank sum of 10 samples of grape wine.

| Evaluator | P | C | A | D | O | E | B | N | G | F |
|-----------|---|---|---|---|---|---|---|---|---|----|
| 1         | 1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9  |
| 2         | 1 | 1 | 5 | 4 | 2 | 3 | 7 | 8 | 6 | 10 |
| 3         | 2 | 3 | 1 | 5 | 4 | 7 | 6 | 8 | 9 | 10 |
| 4         | 3 | 4 | 2 | 3 | 1 | 5 | 6 | 10| 7 | 9  |
| 5         | 3 | 5 | 2 | 1 | 4 | 8 | 6 | 7 | 9 | 10 |
| 6         | 2 | 4 | 3 | 5 | 1 | 7 | 6 | 9 | 8 | 10 |
| 7         | 1 | 5 | 2 | 4 | 3 | 8 | 7 | 6 | 10| 9  |
| 8         | 3 | 4 | 2 | 1 | 5 | 7 | 8 | 6 | 9 | 10 |
| 9         | 3 | 2 | 1 | 4 | 5 | 6 | 8 | 7 | 9 | 10 |
| 10        | 1 | 5 | 4 | 3 | 2 | 9 | 7 | 6 | 8 | 10 |
|           | 11 | 5 | 4 | 1 | 3 | 2 | 6 | 9 | 10| 7  |

| Rank sum | 25 | 43 | 25 | 35 | 35 | 76 | 78 | 83 | 95 | 105 |

Table 7. Experiment result of L9(34) orthogonal design and range analysis.

| Number | Temperature (°C) | Time (min) | Enzyme volume (mL) | Sample code | Rank sum |
|--------|------------------|------------|--------------------|-------------|----------|
| 1      | 1 (40)           | 1 (30)     | 1 (58.32)          | B           | 78       |
| 2      | 1 (40)           | 2 (60)     | 2 (72.9)           | C           | 43       |
| 3      | 1 (40)           | 3 (90)     | 3 (87.48)          | D           | 35       |
| 4      | 2 (45)           | 1 (30)     | 2 (72.9)           | E           | 76       |
| 5      | 2 (45)           | 2 (60)     | 3 (87.48)          | F           | 105      |
| 6      | 2 (45)           | 3 (90)     | 1 (58.32)          | G           | 95       |
| 7      | 3 (50)           | 1 (30)     | 3 (87.48)          | P           | 25       |
| 8      | 3 (50)           | 2 (60)     | 1 (58.32)          | O           | 35       |
| 9      | 3 (50)           | 3 (90)     | 2 (72.9)           | N           | 83       |

| K1     | 156              | 179        | 208                |
| K2     | 276              | 183        | 202                |
| K3     | 143              | 213        | 165                |
| R      | 44.3             | 11.3       | 14.3               |

The Aroma Enhancement of Grape Wine

F.-M. Zhu et al.

© 2014 The Authors. Food Science & Nutrition published by Wiley Periodicals, Inc.
ple, the enzyme-treatment wines, which were extracted by SDE, were analyzed by GC-MS, and the yield of aromatic compounds was more: 3-methyl-1-butanol formate, 3-pentanol, furfural, 3-methyl-butanoic acid, 2-methyl-butanoic acid, 3-hydroxy-butanoic acid ethyl ester, hexanoic acid, hexanoic acid ethyl ester, benzyl alcohol, octanoic acid, octanoic acid ethyl ester, dodecanoic acid, ethyl ester, and so on. The results obtained provide a reliable indication of the aroma potential of the wines. This assay will thus be valuable when taking decisions on the use of enzyme treatments (dose, duration) applied to enhance aroma release.

Acknowledgments

This study was financially supported by Institution of Higher Education Science and Technology Research Foundation of Hebei Province of China (approved no. 2010247), Science and Technology Research and Development Project Foundation of Hebei Province of China (approved no. 09221009), and Startup Foundation for Doctor of Hebei Normal University of Science & Technology (approved no. 2009YB005).

Conflict of Interest

None declared.

References

Bothlho, G., C. Paulino, A. Mendes-Fala, and M. C. Climaco. 2007. A method to analyse bound aroma compounds in non-aromatic red grape juices. Cienc. Tec. Vitiv. 22:21–26.

Cabaroglu, T., S. Selli, A. Canbas, J. P. Lepoutre, and Z. Gunata. 2003. Wine flavor enhancement through the use of exogenous fungal glycosidase. Enzyme Microb. Technol. 33:581–587.

Cai, J. B., B. Z. Liu, and Q. D. Su. 2001. Comparison of simultaneous distillation extraction and solid-phase microextraction for the determination of volatile flavor components. J. Chromatogr. A 930:1–7.

Colagrande, O., A. Silva, and M. D. Fumi. 1994. Recent application of biotechnology in wine production-review. Biotechnol. Prog. 10:2–18.

Katalinic, V., M. Milos, D. Modum, I. Music, and M. Boban. 2004. Antioxidant effectiveness of selected wines in comparison with (+)-catechin. Food Chem. 86:593–600.

McMahon, H., B. W. Zoecklein, K. Fugelsang, and Y. Jasinaki. 1999. Quantification of glycosidase activities in selected yeasts and lactic acid bacteria. J. Ind. Microbiol. Biotechnol. 23:198–203.

Monaci, F., R. Bargagli, and S. Focarid. 2003. Element concentrations in Chianti Classico appellation wines. J. Trace Elem. Med. Biol. 17:45–50.

Mullins, M. G., A. Bouquet, and L. Williams. 1992. Biology of the grapevines. Cambridge University Press, New York, NY.

Nilsson, M., I. F. Duarte, C. Almeida, I. Delgadillo, B. Goodfellow, A. M. Gil, et al. 2004. High-resolution NMR and diffusion-ordered spectroscopy of port wine. J. Agric. Food Chem. 52:3736–3743.

Palmeri, R., and G. Spagna. 2007. β-Glucosidase in cellular and acellular form for winemaking application. Enzyme Microb. Tech. 40:382–389.

Roig, B., and O. Thomas. 2003. UV monitoring of sugars during wine making. Carbohydr. Res. 338:79–83.

Sun, S. H., J. S. Du, and Y. Xue. 1999. Food sensory evaluation. South China University of Technology Press, Guangzhou, China.

Varela, P., and P. Gambara. 2006. Sensory descriptive analysis of Uruguayan Tannat wine: correlation to quality assessment. J. Sens. Stud. 21:203–217.

Verrera, A., M. Zion, A. Scacco, C. M. Lanza, A. Mazzaglia, V. Rome, et al. 2008. Volatile compound and sensory analysis for the characterization of an Italian white wine from “inzolia” grapes. Food Anal. Methods 1:144–151.

Vilanova, M. 2006. Sensory descriptive analysis and consumer acceptability of Godello wines from Valdeorras appellation origen controlee (North West Spain). J. Sens. Stud. 21:362–372.

Villena, M. A., J. D. Perz, J. F. Ubeda, E. Navascuse, and A. I. Briones. 2006. A rapid method for quantifying aroma precursors: application to grape extract, musts and wines made from several varieties. Food Chem. 99:183–190.

Zhu, F. M., B. Du, H. S. Gao, C. J. Liu, and J. Li. 2010. Purification and characterization of an intracellular β-glucosidase from the protoplast fusant of Aspergillus oryzae and Aspergillus niger. Appl. Biochem. Microbiol. 46:626–632.