Purification and Characterization of a Novel α-L-Arabinofuranosidase from Pichia capsulata X91

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An intracellular α-L-arabinofuranosidase from Pichia capsulata X91 was purified and characterized. The enzyme was purified to homogeneity from a cell-free extract by ammonium sulfate treatment, Concanavalin A-Sepharose, ion-exchange chromatography with DEAE Bio-Gel A agarose, arabinoase-Sepharose 6B affinity chromatography, and hydroxyapatite column chromatography. The apparent molecular mass of the enzyme was estimated to be 250 kDa by native-PAGE. The enzyme was suggested to be a tetramer with a subunit molecular mass of 72 kDa by SDS-PAGE. The enzyme had an isoelectric point at 5.1, and was most active at pH 6.0 and at around 50°C. The α-L-arabinofuranosidase was active at ethanol concentrations of wine. The enzyme was inhibited by Cu^{2+}, Hg^{2+}, and p-chloromercuribenzoate. The enzyme hydrolyzed beet abrinan and arabinogalactan, and efficiently released monoterpenols from an aroma precursor extracted from Muscat grape juice. A considerable amount of monoterpenols was produced in the Muscat wine coupled with the enzyme addition.

Key words: α-L-arabinofuranosidase; Pichia capsulata; aroma enhancement; monoterpenol

α-L-Arabinofuranyl residues are widely distributed in hemicellulose such as arabinans, arabinogalactans, and arabinoxylans, and in glycoconjugates. Arabinans consist of α-L-1,5-linked L-arabinofuranosyl residues, some of which are substituted with α-L-1,2- and α-L-1,3-linked side chains of L-arabinose in the furanose conformation. In arabinogalactans, the β-L-1,3,5-galactopyranose backbone is mainly branched by β-L-galactopyranose at O-6 and α-L-arabinofuranose residues at O-3 with an average of two residues per side-chain. α-L-Arabinofuranosidases (EC 3.2.1.55) are exo-type enzymes that hydrolyze terminal α-L-arabinofuranosyl groups from L-arabinose-containing polysaccharides. α-L-Arabinofuranosidases are also involved in the hydrolysis of grape monoterpenyl glycosides.

It is now well established that certain monoterpenols of grapes, which are linked to diglycosides, such as 6-O-α-L-arabinofuranosyl-, 6-O-α-L-rhamnopyranosyl-, and 6-O-β-apiofuranosyl-β-D-glucosides, significantly contribute to the flavor of wine. These glycosidically bound volatile compounds can be released by enzymatic hydrolysis. In the first step, the glycosidic linkage is cleaved by either an α-L-arabinofuranosidase, an α-L-rhamnosidase, or a β-D-apiosidase, and then a β-D-glucosidase liberates the monoterpenols. As the major terminal sugar in these glycosides is α-L-arabinofuranose, α-L-arabinofuranosidases have been attracting attention for enhancing aromas in wine by the hydrolyzing ability of grape monoterpenyl glycosides. α-L-Arabinofuranosidases have been purified and characterized from fungi, actinomycetes, bacteria, and plants. However, the information on the enzyme in yeast is quite limited. Only one species of yeast, Rhodotorula flavia, was found to be a good source of this enzyme.

We have found that Pichia capsulata X91 produced an intracellular α-L-arabinofuranosidase that was able to hydrolyze arabinan and grape glycosides. Here we report results on the purification and properties of the α-L-arabinofuranosidase produced by P. capsulata X91. Furthermore, we describe the liberation of monoterpenols from the grape glycosides, and its application in winemaking using the enzyme.

Materials and Methods

Chemicals. p-Nitrophenyl α-L-arabinofuranoside, p-nitrophenyl α-L-rhamnopyranoside, p-nitrophenyl β-D-glucopyranoside, p-nitrophenyl β-D-xylpyranoside, p-nitrophenyl β-D-celllobioside and larchwood arabinogalactan were obtained from Sigma (St. Louis, MO.). Sugar-beet arabinan, arabinobiose, arabinotriose, arabinotetraose, arabinopentaose and arabinohexaose were supplied by Megazyme Pty., Ltd. (Sydney, Australia). Concanavalin A-Sepharose was purchased from Amersham Pharmacia Biotech. DEAE-Bio-Gel A agarose and hydroxyapatite were

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obtained from Bio-Rad Laboratories (Hercules, Calif.). All other chemicals used were of reagent grade.

**Organism, culture conditions, and enzyme production.** Yeast strains from stock cultures maintained in our laboratory were examined for their ability to produce α-l-arabinofuranosidase. *Pichia capsulata* X91 was selected as the best strain, and was used for further studies. The basal medium (YM) consisted of 0.5% peptone, 0.3% yeast extract, and 0.3% malt extract. To induce the α-l-arabinofuranosidase activity, *P. capsulata* was cultured with 1.0% arabinose in 100 ml of YM medium (pH 4.0) in a 500-ml flask at 28°C with 220-rpm agitation for 2 days. The cells were harvested by washing twice with 20 mM Bis/Tris, pH 6.5, and the 40 g of wet cells were resuspended in 400 ml of the same buffer. The cells were ground with glass beads, and the cell debris was eliminated by centrifugation. The resulting supernatant fluid was ultracentrifuged at 100,000 × g for 90 min, and the supernatant fluid (crude extracts) contained the soluble intracellular α-l-arabinofuranosidase.

**Enzyme assays.** α-l-Arabinofuranosidase activity was measured by the spectrophotometric method with p-nitrophenyl α-l-arabinofuranoside as the substrate. The assay mixture contained 200 μl of the substrate solution (2 mM p-nitrophenyl α-l-arabinofuranoside in 50 mM sodium phosphate buffer, pH 6.5), and 200 μl of appropriately diluted enzyme solution. After incubation at 30°C for 10 min, the reaction was stopped by the addition of 1.6 ml of 1 M Na2CO3. The liberated p-nitrophenol in the mixture was measured by spectrophotometry at 405 nm. One unit of α-l-arabinofuranosidase activity was defined as the amount of enzyme releasing 1 μmole of p-nitrophenol per min in the reaction mixture under these assay conditions. α-l-Rhamnopyranosidase, β-α-glucopyranosidase, β-α-dxylopyranosidase and β-α-d-celllobiohydrolase were measured under the same conditions with the corresponding p-nitrophenyl glycosides.

**Purification of α-l-arabinofuranosidase.** All purification steps were done at 4°C, unless otherwise stated.

(i) **Ammonium sulfate treatment.** The crude extract was fractionated with ammonium sulfate. The precipitate obtained with 30 to 80% saturation was collected, and dialyzed against 100 mM sodium acetate buffer (pH 6.0) containing 1 M NaCl, 1 mM MnCl2, 1 mM CaCl2, and 0.003 mM NaN3.

(ii) **Concanavalin A-Sepharose column chromatography.** The dialyzed enzyme solution was put onto a Concanavalin A Sepharose column (2.6 × 6.5 cm) equilibrated with the same buffer. The active proteins were unabsorbed the column. The enzyme fractions were pooled, concentrated by ultrafiltration with a PM 10 membrane, and dialyzed against 20 mM MOPS, pH 7.0.

(iii) **DEAE Bio-Gel A agarose column chromatography.** The dialyzed solution was put on a DEAE Bio-Gel A agarose column (2.6 × 6.0 cm) equilibrated with 20 mM MOPS, pH 7.0. The enzyme was eluted with a linear gradient of 0 to 300 mM NaCl in the same buffer. The active fractions were combined and dialyzed against 50 mM sodium acetate buffer, pH 5.0.

(iv) **Arabinose-Sepharose 6B affinity chromatography.** An Arabinose-Sepharose 6B affinity matrix was prepared by coupling arabinose to epoxy-activated Sepharose 6B according to the manufacturer’s recommendations. The enzyme obtained after DEAE Bio-Gel A agarose was put on an Arabinose-Sepharose 6B column (1.5 × 7.2 cm) equilibrated with 50 mM sodium acetate buffer, pH 5.0. The column was washed with the same buffer, and the adsorbed proteins were eluted with 1 mM NaCl in the same buffer. The active enzyme fractions were pooled and dialyzed against 10 mM sodium phosphate buffer (pH 6.8).

(v) **Hydroxyapatite chromatography.** The dialyzed enzyme solution was put on a hydroxyapatite column (1.5 × 2.8 cm) equilibrated with 10 mM sodium phosphate buffer (pH 6.8). The column was extensively washed with the same buffer, and the enzyme was eluted with a linear gradient of 10 to 200 mM sodium phosphate buffer (pH 6.8).

**Protein assay.** Protein content was measured by the Bradford method with the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Hercules, Calif.), using bovine serum albumin as the standard.

**Polyacrylamide gel electrophoresis (PAGE).** Polyacrylamide gel electrophoresis (PAGE) was done with a PhastSystem apparatus (Amersham Pharmacis Biotech). Sodium dodecyl sulfate (SDS)-PAGE and native PAGE were done with 12.5% polyacrylamide gel and 8 to 25% polyacrylamide gel, respectively. Isoelectric focusing (IEF) PAGE was done with PhastGel IEF 3-9 precast gel. The low- and high-molecular mass calibration kits and the broad pI calibration kit were purchased from Amersham Pharmacia Biotech. The proteins in the polyacrylamide gels were stained with Coomassie brilliant blue R-250.

**N-terminal amino acid sequencing.** The amino acid sequence of the N-terminal region of α-l-arabinofuranosidase was analyzed on a PVDF membrane blotted with the enzyme using a Hewlett Packard G 1005 A protein sequencer.
Enzymatic hydrolysis of arabino-oligosaccharides. We used arabinobose, arabinotriose, arabino-tetraose, arabinopentaose, and arabinoxhexaose as substrates. The hydrolytic reactions were done at 30°C for 18 h in a reaction mixture consisting of 1 mg of substrate, 0.02 U of purified α-L-arabinofuranosidase and 50 mM sodium acetate buffer, pH 6.5 (final volume 200 μl). The reaction temperature (30°C) was selected by the stability of the enzyme protein. The products were then analyzed by HPLC on Shodex RS pak DC-613 column (Showa Denko K.K. Tokyo, Japan) equilibrated and eluted with CH₃CN:H₂O (70:30) at 1.0 ml/min. Peaks were detected with a RI detector and identified by comparing the elution times with those of appropriate standards.

Thin-layer chromatography (TLC). Sugar-beet arabinian or arabino-galactan digestion products were analyzed by TLC (silica gel 60 F₂₅₄, Merck, NJ, USA). The reaction mixture contained 0.5 ml of α-L-arabinofuranosidase solution (0.2 U), 0.4 ml of 50 mM sodium phosphate buffer (pH 6.5), and 0.1 ml of 10% sugar-beet arabinian or arabino-galactan. After incubation at 30°C, the reaction mixture was heated in boiling water for 10 min to stop the reaction. Arabiniane was used as the standard, and the running solvent consisted of 1-butanol-pyridine-water (6:4:3, v/v/v) or 1-propanol-water (8:5:1.5, v/v). Sugars on the plate were detected by heating at 140°C for a few minutes after spraying with a 1% p-anisidine methanol solution.

Enzymatic hydrolysis of the glycosides. Grape glycosides were isolated from the juice of Muscat of Alexandria grapes. The hydrolysis was done at 30°C for 2 days in a reaction mixture consisting of 10 mg of the glycoside sample, 0.5 U of α-L-arabinofuranosidase, and 50 mM sodium phosphate buffer, pH 6.5 (final volume 2 ml). The liberated aglycones were extracted twice with 5 ml of pentane/ether (1:1). The extract was dried over anhydrous sodium sulfate, concentrated in vacuo, and analyzed by the GC method described later. In the same manner, a blank test without the addition of the enzyme was done.

Application of the enzyme in winemaking. Fermentation experiments were done using 500 ml of Muscat grape juice. The vinification process was as described previously. At the end of fermentation, α-L-arabinofuranosidase (2 U) was added to the young Muscat wine and incubated at 20°C for 24 h.

Capillary gas chromatography (GC). A Hewlett Packard 5980 gas chromatograph equipped with a fused silica DB-WAX capillary column [30 m × 0.25 mm (i.d.), J&W Scientific] and a flame ionization de-
tector was used to analyze the volatile monoter-
penols. The He carrier flow rate was 2.0 ml/min. The temperature program was increased from 75 to 220°C at 4°C/min and held at 220°C for 15 min. The injector port was kept at 230°C and the detector port was at 220°C. Injection was done by a split mode (1:15) and the peak identification was based on the retention time using 2-ethyl-1-hexanol as the internal standard. The sample injection volume was 2 μl.

The volatile compounds were identified by com-
paring the retention times of the peaks with those of commercial standards. The identification of peaks verified by gas chromatography-mass spectrometry (GC-MS) using a HP 6890 series apparatus (Hewlett-Packard).

Results

Production of α-L-arabinofuranosidase and the location of enzyme activity

P. capsulata X91 produced the α-L-arabinofuranosidase in media containing l-arabinose, arabino-galactan, or arabitol as the substrate. The enzyme is not produced when glucose is used as the car-
bond source. The highest enzyme activity of α-L-arabinofuranosidase was obtained by cultivating with l-arabinose. The location of the enzyme activity was studied after cell growth on YM medium contain-
ing l-arabinose. No activity was detected in the culture medium. Almost all activity was found in the disrupted cells, and in the supernatant fluid after an ultracentrifugation at 100,000 × g for 90 min. The en-
zyme was found to be a soluble intracellular enzyme.

Purification of α-L-arabinofuranosidase

An intracellular α-L-arabinofuranosidase was puri-
ified to homogeneity from crude extracts of P. caps-
ulata grown on l-arabinose. A summary of the purifi-
cation procedures is presented in Table 1. The final purification resulted in a yield of 6.5% of the ac-
tivity and a 56.7-fold increase in specific activity. The specific activity of the purified α-L-arabinofuranosidase was 36.3 U/mg of protein. SDS-PAGE analysis of the purified enzyme indicated the presence of a single band when stained with Coomassie brilliant blue (Fig. 1-1).

Properties of α-L-arabinofuranosidase

The molecular mass of native α-L-arabinofuranosi-
dase was estimated to be around 250 kDa by native PAGE (Fig. 1-2) and was 72 kDa by SDS-PAGE (Fig. 1-1). The purified enzyme was composed of four subunits of equal molecular weight. The isoelectric point of the protein was 5.1 (Fig. 2). The influence of pH and temperature on the enzymatic ac-
tivities is summarized in Figs. 3 and 4, respectively. The enzyme showed optimum activity at pH 6.0 and at around 50°C. It was stable from pH 6.0 to 8.0 and
Table 1. Summary of Purification of α-l-Arabinofuranosidase from Pichia epasulata X91

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total activity (units)</th>
<th>Total protein (mg)</th>
<th>Specific activity (units/mg)</th>
<th>Purification (fold)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>336</td>
<td>526</td>
<td>0.6</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>Ammonium sulfate</td>
<td>212</td>
<td>265</td>
<td>0.8</td>
<td>1.3</td>
<td>63</td>
</tr>
<tr>
<td>Concanavalin A-Sepharose</td>
<td>153</td>
<td>77</td>
<td>2</td>
<td>3.1</td>
<td>45</td>
</tr>
<tr>
<td>DEAE-BioGel A</td>
<td>127</td>
<td>10</td>
<td>13.4</td>
<td>20.9</td>
<td>38</td>
</tr>
<tr>
<td>Arabinose-Sepharose</td>
<td>50.6</td>
<td>2.1</td>
<td>24.6</td>
<td>38.4</td>
<td>15</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>21.7</td>
<td>0.6</td>
<td>36.3</td>
<td>56.7</td>
<td>6.5</td>
</tr>
</tbody>
</table>

Fig. 1-1. SDS-PAGE Gel of α-l-Arabinofuranosidase.
Lane M, MW standards; lane A, purified α-l-arabinofuranosidase. The standards were phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14 kDa).

Fig. 1-2. Native PAGE Gel of α-l-Arabinofuranosidase.
Lane M, MW standards; lane A, purified α-l-arabinofuranosidase. The standards were thyroglobulin (669 kDa), ferritin (440 kDa), catalase (232 kDa), lactate dehydrogenase (140 kDa), and bovine serum albumin (67 kDa).

below 30°C.

The influence of various cations at 10^{-2} M on the enzyme activity was studied (Table 2). The enzyme did not show any requirement for divalent cations for the activity. 2-Mercaptoethanol, Ca^{2+}, Mn^{2+}, Fe^{2+}, Mg^{2+}, Co^{2+}, and Al^{3+} had little effect on the activity, but Cu^{2+}, Hg^{2+}, and p-chloromercuribenzoate strongly depressed the activity.

Fig. 2. IEF PAGE Gel of α-l-Arabinofuranosidase.
Lane M, pI standards; lane A, purified α-l-arabinofuranosidase. The standards were human carbonic anhydrase B (pI 6.55), bovine carbonic anhydrase B (pI 5.85), β-lactoglobulin A (pI 5.20), soybean trypsin inhibitor (pI 4.55), and amylloglucosidase (pI 3.50).

Fig. 3. Effects of pH on Stability and Activity of α-l-Arabinofuranosidase.
For stability, the enzyme solutions in 50 mM buffer at various pHs were incubated for 30 min at 30°C. The residual activity was assayed by the standard method. The enzyme activity was assayed by the standard assay method by changing the buffer to obtain the desired pH. The buffer used were citrate-phosphate (pH 3.0 to 5.0), sodium phosphate (pH 6.0 to 8.0), and glycine-NaOH (pH 9.0 to 10.0).
Fig. 4. Effects of Temperature on Stability and Activity of α-l-Arabinofuranosidase.
For stability, the enzyme solution in sodium phosphate buffer (50 mM, pH 6.5) was incubated for 30 min at various temperatures, and then the residual enzyme activities were assayed. For activity, the enzyme activity was assayed at various temperatures by the standard assay method.

Table 2. Effects of Various Metal Ions and Organic Compounds on Activities of α-l-Arabinofuranosidase from P. capsulata X91

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No addition</td>
<td>100</td>
</tr>
<tr>
<td>CuCl₂</td>
<td>6</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>103</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>79</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>105</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>96</td>
</tr>
<tr>
<td>CoCl₂</td>
<td>93</td>
</tr>
<tr>
<td>MnCl₂</td>
<td>100</td>
</tr>
<tr>
<td>AlCl₃</td>
<td>96</td>
</tr>
<tr>
<td>HgCl₂</td>
<td>2</td>
</tr>
<tr>
<td>EDTA*</td>
<td>87</td>
</tr>
<tr>
<td>2-Mercaptoethanol</td>
<td>102</td>
</tr>
<tr>
<td>p-CMB**</td>
<td>6</td>
</tr>
</tbody>
</table>

* EDTA, ethylenediaminetetraacetic acid. ** p-CMB, p-chloromercuribenzoate.

Table 3. Substrate Specificity of α-l-Arabinofuranosidase

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-nitrophenyl α-l-arabinofuranoside</td>
<td>100</td>
</tr>
<tr>
<td>p-nitrophenyl α-l-rhamnopyranoside</td>
<td>0</td>
</tr>
<tr>
<td>p-nitrophenyl β-D-glucopyranoside</td>
<td>0</td>
</tr>
<tr>
<td>p-nitrophenyl β-D-xylopyranoside</td>
<td>0.1</td>
</tr>
<tr>
<td>p-nitrophenyl β-D-cellubioside</td>
<td>0</td>
</tr>
</tbody>
</table>

At the normal concentrations found in wine, ethanol had a stimulating effect on the activity. The enzymatic activity increased 16% in the present of 15% (v/v) ethanol (Fig. 5-1). A glucose inhibition test was done with p-nitrophenyl α-l-arabinofuranoside as the substrate. The enzyme had 63% of its maximal activity even under the presence of 500 mM glucose (Fig. 5-2).

Relative rates of hydrolysis of various p-nitrophenyl glycosides by the enzyme were examined (Table 3). The enzyme was active towards p-nitrophenyl α-l-arabinofuranoside, but showed very little (<0.1%) or no activity towards p-nitrophenyl α-l-rhamnopyranoside, p-nitrophenyl β-D-glucopyranoside, p-nitrophenyl β-D-xylopyranoside, or p-nitrophenyl β-D-cellubioside.

We further examined the hydrolytic activity of α-l-arabinofuranosidase towards α-1,5-linked arabinogalactan oligosaccharides with chain lengths from 2 to 6. We found that the enzyme reacted with all the substrates tested. The HPLC profiles of the enzyme reaction on arabinobiose, arabinopentaose, and arabinohexaose are shown in Fig. 6. After 18 h of incubation, the degradation degree of arabinobiose, arabinopentaose, and arabinohexaose was 81.4, 35.7, and 40.2%, respectively. The enzyme hydrolyzed arabinobiose, forming arabinose as the single product. When arabinopentaose was used as the substrate, the major product formed were
arabinotetraose, and the main hydrolysis product from arabinohexaose was arabinopentaose.

Figure 7 shows the courses of hydrolysis of beet arabinan by the enzyme. After 3 h of incubation, arabinose was detected and the amount of arabinose released increased as the reaction proceeded. No other sugar was detected during 48 h of incubation. The enzyme released arabinose from larchwood arabinogalactan, but did not liberate galactose (Fig. 8).

**N-terminal amino acid sequences**

The first 21 amino acids of N-terminus of the enzyme were X-Ile-Gln-X-Leu-Asp-X-Phe-Ser-Gly-X-Glu-X-Ile-X-Ile-Asp-Lys-Glu-Phe-Val-Thr- (X represents an ambiguous residue). A search through the Genbank and EMBL Nucleotide Sequence Databases using FASTA software showed no significant similarity with other α-L-arabinofuranosidase genes already sequenced.

**The effects of α-L-arabinofuranosidase on the glycosides extracted from Muscat juice and in Muscat wine**

An enzymatic preparation of *P. capsulata* was added to a sodium phosphate buffer (pH 6.5) containing the glycosides extracted from Muscat juice, and incubated at 30°C for 2 days. The results on the liberation of monoterpenols by the α-L-arabinofuranosidase are shown in Table 4. Among the glycosidically linked monoterpenols in grape juice, the arabinofuranosyglucosides of geraniol, nerol, and linalool are the most abundant. The enzyme efficiently liberated monoterpenols, especially linalool, citronellol, and geraniol from the glycoside extract.

Muscat wine (pH 3.8, 12% ethanol) was treated with the enzyme at 20°C for 24 h. The GC-MS analysis of the treated wine comparing to the control (Table 5) indicated that the enzyme increased the concentrations of some monoterpenols such as linalool, citronellol, and geraniol.

**Discussion**

*P. capsulata* X91 produced an intracellular α-L-arabinofuranosidase. Although induction of α-L-arabinofuranosidase by beet arabinan was reported for a yeast, *R. flava*, the α-L-arabinofuranosidase activity was extracellular.

The α-L-arabinofuranosidase from *P. capsulata* is a homotetramer with an apparent native molecular
Table 4. Monoterpenols Released by P. capsulata X91 α-L-Arabinoferanosidase from the Muscat Glycoside Extract (1 mg)

<table>
<thead>
<tr>
<th>Monoterpenol</th>
<th>P. capsulata X91 blank</th>
<th>α-L-Arabinoferanosidase enzyme treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linalool</td>
<td>0</td>
<td>362</td>
</tr>
<tr>
<td>α-Terpineol</td>
<td>0</td>
<td>70</td>
</tr>
<tr>
<td>Citronellol</td>
<td>0</td>
<td>233</td>
</tr>
<tr>
<td>Nerol</td>
<td>0</td>
<td>89</td>
</tr>
<tr>
<td>Geraniol</td>
<td>0</td>
<td>252</td>
</tr>
</tbody>
</table>

Table 5. Effects of α-L-Arabinoferanosidase from P. capsulata X91 on the Concentration of Monoterpenols in Muscat Wine

<table>
<thead>
<tr>
<th>Monoterpenol</th>
<th>Without enzyme (µg/l)</th>
<th>With enzyme (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linalool</td>
<td>234</td>
<td>469</td>
</tr>
<tr>
<td>α-Terpineol</td>
<td>67</td>
<td>82</td>
</tr>
<tr>
<td>Citronellol</td>
<td>82</td>
<td>162</td>
</tr>
<tr>
<td>Nerol</td>
<td>96</td>
<td>94</td>
</tr>
<tr>
<td>Geraniol</td>
<td>166</td>
<td>261</td>
</tr>
<tr>
<td>Total terpenols</td>
<td>675</td>
<td>1069</td>
</tr>
</tbody>
</table>

mass of 250 kDa (Fig. 1-2) and a subunit molecular mass of 72 kDa (Fig. 1-1). Molecular masses of α-L-arabinofuranosidases range from 31 kDa to 495 kDa, several of these enzymes consist of subunits. The molecular mass of α-L-arabinofuranosidase of Bacillus stearothermophilus T-6 was 284 kDa and the enzyme consisted of four subunits of molecular weight 64 kDa. The optimal activity of the enzyme from P. capsulata was observed at pH 6.0 and 50 °C (Figs. 2 and 3). Both pH and temperature optimum are similar to those reported for a rumen bacterium, Butyribrio fibrisolvens GS113 α-L-arabinofuranosidase. In the pH range (3.0–3.8) usually found in winemaking P. capsulata α-L-arabinofuranosidase activity was 20% of its maximum value. With respect to the temperature (20–30 °C) in winemaking, the enzyme showed around 40% of its initial activity. Another yeast α-L-arabinofuranosidase from R. flavus is highly acid stable, retaining 82% of its activity after being maintained for 24 h at pH 1.5, and has an optimum activity at pH 2.0. However, there is no information about R. flavus α-L-arabinofuranosidase activity in winemaking.

The enzyme activity was significantly inhibited by a sulfhydryl reagent (p-chloromercuribenzoate) and sulfhydryl oxidant metal (Hg²⁺), suggesting some importance of a sulfhydryl group for the expression of the enzyme activity.

It was interesting to observe 16% stimulation of enzyme activity in the presence of 15% (v/v) ethanol. A similar effect was observed for Aspergillus terreus CECT 2663 α-L-arabinofuranosidases. The activity increase by ethanol has been reported for β-glucosidases from yeast, Dekkera intermedia, Candida molischiana, and Debaryomyces Hansenii, and the fungus Botrytis cinerea and the actinomycete Streptomyces sp. (ATCC 11238). This activation may be due to the glycosyltransferase activity of the enzyme.

The α-L-arabinofuranosidase was highly tolerant to glucose, and it maintained more than 60% of its maximal activity in 500 mM glucose. As grape juice has about 300 mM glucose concentration, the enzyme will be active in grape juice.

The enzyme cleaved p-nitrophenyl α-L-arabinofuranoside, but had very little or no activity toward a variety of other p-nitrophenyl glycosides. It was highly specific to the α-L-arabinofuranoside configuration. The purified α-L-arabinofuranosidase released only arabinose from arabinan and larchwood arabinogalactan. The main products of α-L-arabinino-oligosaccharides hydrolysis were an oligosaccharide, which was shorter by one arabinose unit when compared to the substrate. This suggests that the enzyme was an exo-type α-L-arabinofuranosidase. Furthermore, the α-L-arabinofuranosidase liberated monoterpenols from monoterprenyl arabinofuranosylglycosides from grape juice. Therefore, the enzyme may be able to cleave the α-1,6-linkage between the terminal arabinofuranosyl unit and the intermediate glucose, and then hydrolyze the released monoterprenyl β-D-glucoside. However, it has almost no activity on p-nitrophenyl β-D-glucopyranoside. This apparent difference of the enzyme’s action on β-D-glucosides might relate to the structure of substrates. Additional studies are required to characterize the specificity of this α-L-arabinofuranosidase.

Brillouet et al. showed that a major polysaccharide of grape origin from a red wine was an arabinino-3,6-galactan of low intrinsic viscosity (molecular mass 178 kDa) and the α-L-arabinofuranosidase from Dichomitus squalens was able to hydrolyze the wine arabinogalactan. As the α-L-arabinofuranosidase from P. capsulata is capable of releasing of arabinose from arabinogalactan, the enzyme might be useful for degradation of wine arabinogalactan, strongly retained by microfiltration membranes.

The enzyme increased the amount of flavor compounds in wine examined by enzymatic hydrolysis of glycoside flavor precursors, and also improved its flavor. The positive results of this test suggest that it might be applicable in other wines having the same type of aroma precursors.

With regard to technological applications during juice processing and winemaking, the P. capsulata α-L-arabinofuranosidase may be useful due to the high tolerance to glucose and activity stimulation by ethanol and their specificity for the aglycone moieties of grape glycosides.
References


