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Purification and Characterization of an α-D-Galactosidase from Grape Berry

Han-Chul Kang* and Tae-Su Kim

Grape Experiment Station, Chungbuk Institute of Agricultural Technology, Okcheon 373-880, Korea

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Glycosidase activities were tested from the grape berries, *Vitis labruscana* B. Takasumi. Among various glycosidases, α-D-galactosidase was found to be the most active in the flesh and other glycosidases were considerably active in the order of the following: α-D-mannosidase>α-D-glucosidase>β-D-glucosidase >β-D-galactosidase. In the seeds, α-D-glucosidase activity was the highest and other glycosidases such as α-D-galactosidase, β-D-glucosidase, and β-D-galactosidase were still significantly active. The α-D-galactosidase in the grape flesh was purified over 83-folds through salting-out with $(NH_4)_2SO_4$ and a series of chromatographies employing Sephadex G-50, Octyl-Sepharose, Q-Sepha- rose, and Biogel P-100. The enzyme was a monomer of 45 kDa as determined through SDS-PAGE and Sephacryl S-200 chromatography. The purified enzyme showed a preference of α-D-galactose to β-D-galactose as a substrate about 5.4 times. Sulfhydryl specific reagents such as N-ethylmaleimide and iodoacetamide significantly inhibited the enzyme activity to the extents of 48 and 52% of its initial activity, respectively. The optimum pH range of α-D-galactosidase was around 6.5-7.0. The enzyme activity increased by 46% in the presence of 1 mM Fe^{2+}.

Key words: *α-D-galactosidase, grape.*

α-Galactosidases are widely distributed in intracellular or extracellular forms in microorganisms, plants, and animals. The enzyme in plants, especially, has been much investigated from seeds in relation with germination.[1-5] Considerable evidence for the occurrences and properties of the enzyme in the extracts of germinating seed has been reported.[6] In many plant species, the α-galactosidases appear to be occurred in their seeds where the enzymes are synthesized during maturation of tissues and rapidly disappear during the initial stage of germination.[7] However, the enzymes have been also found in leaves and other parts.[8-10] Different molecular forms of the enzymes occur in several plants, showing the diverse properties.[1-6,9,11,12]

α-D-Galactosyl derivatives of sucrose including raffinose and stachyose have been known to occur in many seeds, roots, and tubers.[12] α-Galactosidase is involved in the metabolic utilization of oligosaccharides such as raffinose, stachyose, melibiose, and galactomannan in these storage organs. The enzyme activity was known to increase during seed germination concomitant with the depletion of galactomannans. α-D-galactosidases have been implicated also with the metabolism of galactolipids.[14,15] It was suggested that α-galactosidases may play a role in the regulation of carbohydrate metabolism during the leaf development. In many plants, stachyose and raffinose are the principal sugars synthesized and transported to the growing region.[16,17] α-Galactosidases have many potentials in biotechnology processes such as hydrolysis of raffinose from beet sugar syrups[18] and raffinose and stachyose from soybean milk.[19] Enzymatic hydrolysis of galactomannans is important in the course of pulp industry and the product also useful for the gelling properties of the polysaccharides.[20]

Despite such wide distributions and diversities, α-D-galactosidase seems to be less focussed on the fruits compared with other plant organelles. As a preliminary stage in the study of oligosaccharide metabolism, ripening process, and other physiological aspects, it is necessary to look in more detail the α-D-galactosidase from grape berry. In this paper, we purified an α-D-galactosidase associated with the grape flesh and presented some of its biochemical characteristics.

Materials and Methods

Plant materials. *Vitis labruscana* B. Takasumi was cultivated in a green house under 14 h of day light and 10 h of darkness at temperatures between 21 and 30°C. Fully ripe grapes after 3 months from the berry setting were harvested, and healthy and unblemished grapes were selected. After surface sterilization with 60% ethanol, fruits were rinsed with sterile water and immediately stored at -70°C until use.

Purification of α-D-galactosidase. The grapes were separated into exocarp, flesh (mesocarp plus placental tissue), and seeds. The grape flesh (1.2 kg) was homogenized in the presence of 0.1% insoluble polyvinyl-

*Corresponding author
Phone: 82-43-220-5410; Fax: 82-43-220-5419
E-mail: oksipo@chollian. net

Abbreviation: PNP-α-D-Gal, *p*-nitrophenyl galactopyranoside.

polypyrrodine and 5% glycerol. The homogenate was filtered through two layers of nylon cloth and the resulting filtrate was centrifuged at 8,000 g for 20 min. After discarding the pellet, the supernatant was dissolved at 30% ammonium sulfate and centrifuged at 10,000 *g* for 20 min. The supernatant was saturated at 55% amonium sulfate, then the precipitate recovered by centrifugation. The resultant pellet was dissolved in 100 mL of 40 mM potassium phosphate buffer (pH 7.0) containing 2% glycerol (buffer A). The sample was clarified by dialysis with the same buffer and centrifuged at 10,000 g for 20 min.

The supernatant was applied to a Sephadex G-50 (Sigma) column equilibrated with buffer A. Proteins were eluted with the same buffer at a flow rate of 12 mL/h. All of the purification procedures including this step were performed at 4°C. The α-D-galactosidase activity was monitored using PNP-α-D-Gal as a substrate throughout the purification procedures.

The fractions containing α-D-galactosidase activity from the Sephadex G-50 chromatography were pooled, and then clarified by centrifugation at 8,000 g for 20 min. The fractions were loaded on a Octyl-Sepharose column (2 × 10 cm) equilibrated with buffer A. After washing the column, proteins were eluted with 200 mL of buffer A containing a linear gradient of KCl (0-500 mM) at a flow rate of 15 mL/h. The fractions with α-D-galactosidase activity were pooled and dialyzed with buffer A. The active fractions were loaded onto a Q-Sepharose column equilibrated with buffer A and proteins were eluted by the same way as that of Octyl-Sepharose chromatography. The active fractions containing α-D-galactosidase were further purified through Biogel P-100 chromatography. After loading the fractions, elution of proteins was performed with 50 mM potassium phosphate buffer (pH 7.0) and 2% glycerol at a flow rate of 12 mL/h. The purification was monitored by measuring the absorbance at 280 nm and assaying the enzyme activity. Unless otherwise mentioned, all of the experiments on α-D-galactosidase were performed with the samples purified from this chromatography.

Assay of glycosidase activities. For the measurement of glycosidases in the grape, whole grapes were separated into exocarp, flesh, and seeds. The exocarp was homogenized for 2 min with 40 mM K-phosphate (pH 7.0) and 0.5% glycerol. The paste was filtered through two layers of nylon cloth. The resulting filtrate was centrifuged at 8,000 g for 20 min and pellets discarded. The supernatant obtained was used for measurement of glycosidases in the exocarp. Crude extracts from the flesh and seeds were prepared by the same way, except that the flesh was directly homogenized without any additive buffer.

The standard assay of glycosidases was performed using *p*-nitrophenyl glycosides (PNP-glycosides) as substrates. Test samples were added to K-phosphate (50 mM, pH 7.0 and 1 mL of final volume) in the presence of 5 mg/mL of BSA and 2 mM of substrate. After 30 min of incubation at 37°C, the release of *p*-nitrophenol was measured spectrophotometrically by taking the increase in absorbance at 410 nm. One unit of the enzyme activity was defined as the hydrolysis of one mol of free *p*-nitrophenol/min produced.

The pH effect of α-D-galactosidase was tested using the following buffer with similar ionic strength: a three-buffer mixture (25 mM acetic acid, 25 mM Mes, and 50 mM Tris)[17] titrated to the different pH values with KOH or HCl.

Chemical modification of α-D-galactosidase. For elucidation of active site, the purified enzyme was preincubated with various chemical modification reagents in 0.5 mL of 20 mM potassium phosphate (pH 7.0) containing 0.4% glycerol and 5 mg/mL BSA. After the preincubation for 30 min, the reaction mixture was adjusted to 1 mL of a final volume with a 0.5 ml standard assay mixture. The reaction was, then, continued for another 30 min and the residual activity measured spectrophotometrically as described.

Determination of the molecular mass of a native form. The apparant molecular mass of purified α-D-galactosidase was estimated by a gel filtration chromatography through Sephacryl S-200. The column (2 × 90 cm) was equilibrated and eluted with 50 mM potassium phosphate buffer (pH 7.0) containing 5% glycerol. The following proteins were used for a calibration: aldolase (Mr; 158,000), phosphorylase b (97,400), BSA (66,200), ovalbumin (43,000), and cytochrome c (12,000). The fractions eluted were assayed for the enzyme activity using PNP-α-D-Gal as a substrate.

Other analytical methods. SDS-PAGE were performed according to the method of Laemmli.[22] A slab gel consisted of 12.5% acrylamide resolving gel and 5% acrylamide stacking gel was used. The gel was stained with Coomassie brilliant blue R-250 and silver nitrate.[23] Protein concentrations were determined through the dyebinding assay of Bradford using BSA as a standard protein reference for Bradford assay.[24]

Results

Distribution of glycosidases in the grape berry. Various glycosidase activities were tested for each soluble protein fraction, that were prepared from exocarp, flesh, and seeds in the grape berry (Table 1). Distribution of glycosidases was significantly different among the grape tissues.

Among the different substrates tested, glycosidase activity was the highest for PNP-α-D-Gal in the grape flesh. The activities for PNP-α-D-Man, PNP-β-D-Gal, PNP-α-D-Glc, and PNP-β-D-Glc were still considerable. Various glycosidases were also found in the exocarp fraction but the overall activities per g · tissue were lower than those of the other fractions from flesh and seeds. In the seeds, α-D-glucosidase and α-D-galactosidase activities were considerable and overall glycosidases activities per gtissue were much higher than those of other tissues in the grape berry.

Table 1. Distribution of various glycosidases in the grape berry. The activity was tested for the grape (Vitis labruscana B. Takasumi) harvested 3 months after the berry setting. The grapes were separated into seeds, exocarp, and flesh, and homogenized. The resulting supernatants were used for glycosidase test. The enzyme assay was run using various p-nitrophenyl glycosides (2 mM) in K-phosphate (pH 7.0, 50 mM) and 5 mg/ml of BSA. Glycosidase activities were represented as units per g·tissue.

Substrate	Glycosidase activity (unit/g · tissue)		
	Flesh	Exocarp	Seed
α-D-Gal	716.2	22.7	825.7
β-D-Gal	124.1	31.	124.8
α-D-Glc	290.4	31.5	838.1
β-D-Glc	205.6	20.9	320.5
α-L-Fuc	27.5	12.7	72.6
β-D-Fuc	24.3	4.4	69.8
α-D-Man	296.9	16.4	123.6
α-L-Ara	37.8	22.5	74.2
α-L-Rha	17.0	10.2	1
β-D-GlcUA	5.4	1	1
α-D-GlcNAc	1	1	12.4
β-D-GlcNAc	1	1	1.6
β-D-GalNAc	12.5	1	1

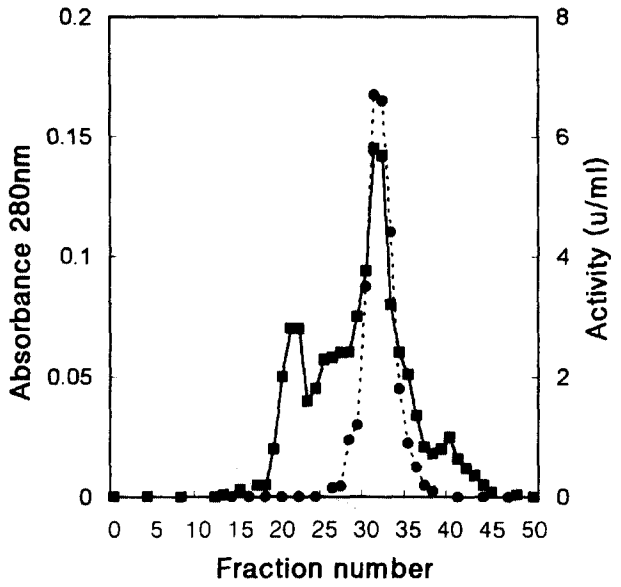

Fig. 1. Elution of the α-D-galactosidase from Biogel P-100 chromatography. The active fractions from Q-Sepharose chromatography were loaded onto a Biogel P-100 column equilibrated with potassium phosphate buffer (pH 7.0, 50 mM) plus 2% glycerol. Proteins were eluted with the same buffer along with measurement of absorbance at 280 nm (-■-). The activity of α-D-galactosidase (-●-) was monitored using PNP-α-D-Gal as a substrate.

Purification of α-D-galactosidase. The α-D-galactosidase was purified about 83 fold through salting out with $(NH_4)_2SO_4$, Sephadex G-50, Octyl-Sepharose, Q-Sepharose, and Biogel P-100 chromatographies (Table 2). α-D-galactosidase from the grape flesh appears to be stable during the initial steps of purification procedures. Dilution of the enzyme slightly accelerated the decrease in activity (data not shown). The activity loss was considerably protected by addition of glycerol in the equilibration/elution buffers during purification. The chromatography of Sephadex G-50 removed pigments that interfered with chromatography in subsequent purification steps and protein solubility. The fractions obtained from the Q-Sepharose chromatography were further purified using Sephacryl S-200 column (Fig. 1). SDS-PAGE analysis of the main fractions with peak activity from this final step of purification showed one major band with a monomeric molecular mass of 45 kDa (Fig. 2). This band was considered as an α-D-galactosidase since the intensities of bands were roughly proportional to the activity peak. The enzyme was stored in 20% glycerol at -20°C, and over 85% of the initial activity was retained up to 3 months after storage.

Substrate specificity of α-D-galactosidase. The substrate specificity of purified enzyme was assayed for its activity using different PNP-glycosides (Table 3). Among the PNP-glycosides tested, PNP-α-D-Gal was the most preferred substrate. The enzyme showed relatively low activity against PNP-β-D-Gal, showing 18.4% activity of PNP-α-D-Gal. PNP-β-D-Lac was slowly hydrolyzed by the purified α-D-galactosidase and its activity was lower than that for PNP-β-D-Gal. Another galactose related compound, PNP-β-D- GalNAc was not essentially hydrolyzed.

Chemical modification of α-D-galactosidase. In an attempt to elucidate an active site of the α-D-galactosidase in the grape flesh, the enzyme activity was assayed in the presence of various chemical modification reagents (Table 4). The activity was decreased to 48 and 52% of control by 2

Table 2. Purification of α-D-Galactosidase from the grape flesh (*Vitis labruscana* B. Takasumi).

Purification Step	Total Protein (mg)	Total activity (unit)	Specific activity (unit/mgprotein)	Purification fold
Crude extract	420.0	244.2	0.58	1.00
Ammonium sulfate	132.2	142.3	1.08	1.86
Sephadex G-50	32.1	101.9	3.17	5.46
Octyl-Sepharose	12.4	92.4	7.45	12.84
Q-Sepharose	3.4	69.9	20.56	35.45
Biogel P-100	1.1	53.1	48.27	83.22

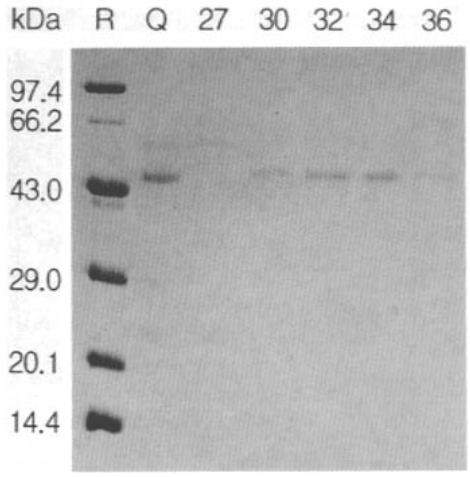

Fig. 2. SDS-polyacrylamide gel electrophoresis of purified α-D-galactosidase from grape flesh. R: reference proteins consisted of phosphorylase b (Mr; 97,400), BSA(66,200), ovalbumin (43,000), carbonic anhydrase (29,000), trypsin inhibitor (20,100), and lysozyme (14,400). Other lanes: Q, the active fraction from Q-Sepharose chromatography; 27-36, the fraction numbers from Biogel P-100 chromatography. The gel was stained with Coomassie brilliant blue R-250 and then with silver nitrate.

Table 3. Substrate specificities of α-D-galactosidase purified from the grape flesh. The enzyme was assayed for its activity using different p-nitrophenyl glycosides (2 mM) in K-phosphate (50 mM, pH 7.0) comprising 5 mg/mL of BSA. The activities were compared with that of α-D-gal (100%), which was equivalent to 28 units/mg·protein. All the values were the means of two determinations.

Substrate	Glycosidase activity (%)
α-D-Gal	100.0
β-D-Gal	18.4
β-D-Lac	12.1
α-D-Glc	11.6
β-D-Glc	9.4
α-L-Fuc	7.9
β-D-Fuc	2.4
α-D-Man	3.2
α-L-Ara	1
α-L-Rha	2.7
β-D-GlcUA	1
α-D-GlcNAc	1
β-D-GlcNAc	1
β-D-GalNAc	1

mM N-ethylmaleimide and iodoacetamide, respectively. This result suggests that a sulfhydryl group could participate in the enzyme catalysis and exists at or near the active site. Similarly, α-D-galactosidase from coconut was reported to be inhibited by a sulfhydryl specific reagent, iodoacetic acid[11] and the enzyme from *Poterioochromonas malhamensis* was also inhibited by the other SH reagent, *p*-chloromercuribenzoate.[25]

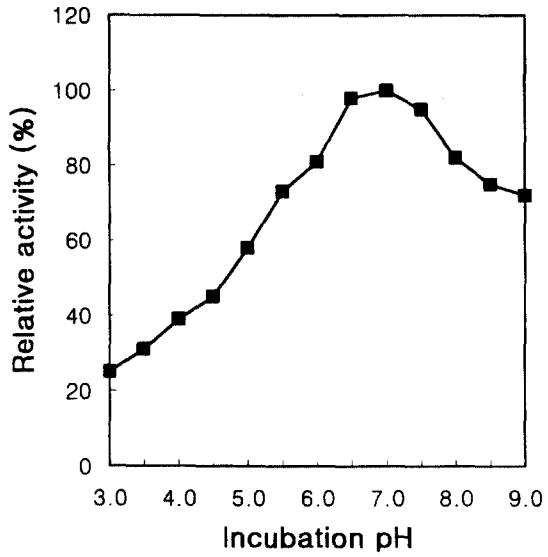

Fig. 3. Effect of pH on α-D-galactosidase activity. The purified α-D-galactosidase was assayed using PNP-α-D-Gal as a substrate in a range of different pH. The activities calculated by duplicate tests were compared with the maximum activity (100%) that was equivalent to 26 units/mg·protein.

Other characteristics. The molecular weight of the purified α-D-galactosidase was determined by gel filtration using a Sephacryl S-200 column. The active fractions from the Biogel P-100 chromatography were loaded onto the Sephacryl S-200 column along with size marker. The activity peaks were monitored and the molecular mass of the purified enzyme was calculated to be about 42 kDa.

The hydrolysis of PNP-α-D-Gal by the purified enzyme was tested in the presence of free α-D-Gal to see if the product inhibits the enzmatic reaction. The activity was linearly decreased with increasing galactose concentrations. At 5 mM galactose, the activity was 56% of the initial activity and glucose did not essentially inhibit the enzyme activity (detailed data not shown). A similar inhibition by galactose has been reported for the α-galactosidase purified from the leaves of *Cucurbita pepo*.[10]

The purified α-D-galactosidase activity was tested in buffers of varying pH using 2 mM PNP-α-D-Gal as a substrate (Fig. 3). The optimum pH for the activity was found to be around 7.0. The enzyme activity was rapidly increased from pH 4.0 to pH 7.0 and slowly decreased at alkaline pH.

The eventual requirement of cofactor for the α-D-galactosidase was examined by determining the activity in the presence of various divalent cations with chloride as counter part ions (Table 5). Additions of most divalent ions (1 mM) to the assay mixture did not significantly increase or decrease the activity. But Fe^{+2} ion increased the activity by 46% of the initial activity, and Cd^{+2} decreased about 49% of the initial activity.

Heat stability was tested using the enzyme fractions purified from the final step of chromatography. The enzyme

Table 4. Effect of activators or inhibitors on the α-D-galactosidase. The purified α-D-galactosidase was assayed for its activity in the presence of various 2 mM chemical modification reagents using PNP-α-D-Gal as a substrate. The activities determined by the standard assay method were represented as percent activity of control (100%), which was equivalent to 31 units/mg·protein.

Chemical reagents	Relative activity (%)
None (control)	100
N-ethylmaleimide	48
Iodoacetamide	52
PMSF	89
p-Hydroxyphenylglyoxal	101
Sulfo-NHS-acetate	112
2,3-butanedione	94
2-iminothiolane	76
BNPS-skatole	75
Acetic anhydride	64
Citraconic anhydride	67

Table 5. Effect of divalent cations on the α-D-galactosidase. The activity of α-D-galactosidase was measured in the presence of various 1mM divalent cations using PNP-α-D-Gal as a substrate. The activities determined by the standard assay method were represented as percent activity of control (100%), which was equivalent to 24 units/mg·protein.

Cations	Relative activity (%)
Control (None)	100
$CaCl_2$	99
$MgCl_2$	96
$CoCl_2$	107
$ZnCl_2$	104
$FeCl_2$	146
$CuCl_2$	107
$MnCl_2$	108
$CdCl_2$	51

was preincubated at 30°C, 45°C, and 60°C for 10 min and assayed using PNP-α-D-Gal. The activity was decreased to 62% of initial activity when preincubated at 60°C (detailed data not shown).

Discussion

Distribution of various glycosidases was tested with the grape berry, *Vitis labruscana* B. Takasumi. Glycosidase activity in the flesh was the highest against PNP-α-D-Gal and other glycosidases activities were considerable by the order of activity as follows: α-D-mannosidase>α-D-glucosidase>β-D-glucosidase>β-D-galactosidase. The highest activity of α-D-galactosidase might implicate that the enzyme may play an important role in the grape flesh.

α-D-galactosidase in the grape flesh was purified to homogeneity and the active fractions from the final Biogel P-100 chromatography gave one major band corresponding to 45 kDa on the SDS-PAGE. This 45 kDa band was considered to be an α-D-galactosidase since 42 kDa of molecular weight determined from Sephacryl S-200 chromatography approached to this value and the intensities of the 45 kDa bands on the SDS-PAGE were roughly proportional to the activity peak. The purified enzyme hydrolyzed the most rapidly PNP-α-D-Gal and showed low activity against PNP-β-D-Gal, implying anomer specific galactosidase. The enzyme hydrolyzed very slowly β-D-Lac and had no essential activity against β-D-GalNAc. Such low or scarce activities against these galactose related compounds implicate that the α-D-galactosidase in the grape flesh has a somewhat narrow specificity.

The α-D-galactosidase assay with PNP-α-D-Gal showed a optimum pH of 6.5-7.0. This pH optimum is similar to the α-D-galactosidase from *Poterioochromonas malhamensis*,[25] whereas many higher plant α-galactosidases display pH optima in the acidic pH.[6] The increase of α-D-galactosidase activity in the grape flesh from pH 4.0 to 7.0 might have a correlation with the environment of flesh since pH in flesh increases for weaker acidity as ripening proceeds.

References

1. Barham, D., Dey, P. M., Griffiths, D. and Pridam, J. B. (1971) Studies on the distribution of α-galactosidases in seeds. *Phytochemistry* **10**, 1759-1763.
2. Corchette, M. and Guerra, H. (1987) α- and β-galactosidase activities in protein bodies and cell walls of lentil seed cotyledons. *Phytochemistry* **26**, 927-932.
3. Dey, P. M. and Pridam, J. B. (1969) Purification and properties of α-galactosidases from *Vicia faba* seeds. *Biochem. J.* **113**, 49-55.
4. Dey P. M., Del Campillo, E. M. and Lezica, R. P. (1983) Characterization of a glycoprotein α-galactosidase from lentil seeds (*Lens culinaris*). *J. Biol. Chem.* **258**, 923-929.
5. McCleary, B. V. and Matheson, N. K. (1974) α-D-galactosidase activity and galactomannan and galactosylsucrose oligosaccharide depletion in germinating legume seeds. *Phytochemistry* **13**, 1747-1757.
6. Dey, P. M. and Pridam, J. B. (1972) Biochemistry of α-D-galactosidase. In *Advance in Enzymology*, Meister, A. (ed.) vol **36**, pp. 91-130, Academic Press, New York.
7. Amuti, K. S. and Pollard, C. J. (1977) Soluble carbohydrates of dry and developing seeds. *Phytochemistry* **16**, 529-532.
8. Gatts, S. and Baker, E. A. (1970) Purification and separation of α- and β-galactosidases from spinach leaves. *Biochim. Biophys. Acta.* **206**, 125-135.
9. Smart, E. L. and Pharr, D. M. (1980) Characterization of α-galactosidase from cucumber leaves. *Plant Physiol.* **66**, 731-734.
10. Thomas, B. and Webb, J. A. (1978) Distribution of α-galactosidase in *Cucurbita pepo*. *Plant Physiol.* **62**, 713-717.
11. Mujer, C. V., Ramirez, D. A. and Mendoza, E. M. T.

(1984) Coconut α-D-galactosidase isoenzymes: Isolation, purification and characterization. *Phytochemistry* **23**, 1251-1254.

12. Haibath, F., Hata, J., Mitra, M., Dhar, M., Harmata, M., Sun, P. and Smith, D. (1991) Purification and characterization of a *Coffea canephora* α-D-galactosidase isozyme. *Biochem. Biophys. Res. Comm.* **181**, 1564- 1571.
13. Kandler, O. and Hopf, H (1980) Occurrence, metabolism and function of oligosaccharides. In *A Comprehensive Treatise: The Biochemistry of Plants,* Stumpf, P. K. and Conn, E. E. (eds.) vol. **3**, pp. 221-270, Acadmic press, New York.
14. Sastry, P. S. and Kates, M. (1964) Hydrolysis of monogalactosyl and digalactosyl diglycerides by specific enzymes in runner-bean leaves. *Biochemistry* **3**, 1280-1287.
15. Pridam, J. B. and Dey, P. M. (1974) The nature and function of higher plant α-galactosidases. In *Plant Carbohydrate Biochemistry*, Pridam, J. B. (ed.) pp. 83-96, Academic press, New York.
16. Webb, J. A. and Gorham, P. R. (1964) Translocation of ^{14}C-assimilates in the straight necked squash. *Plant Physiol.* **39**, 663-672.
17. Zimmerman, M. H. (1957) Translocation of organic substances in Trees. I. The nature of the sugars in the sieve tube exudate of trees. *Plant Physiol.* **32**, 288-291.
18. Kobayashi, H. and Suzuki, H. (1972) Studies on the decomposition of raffinose by α-galactosidase of mold. *J. Ferment. Technol.* **50** 625-632
19. Thananunkul, D., Tanaka, M., Chicheester, C. O. and Li, T. (1976) Degradation of raffinose and stachyose in soybean milk by α-galactosidase from *Mortierella vinacea.* Entrapment of α-galactosidase within polyacrylamide gel. *J. Food Sci.* **41**, 173-175.
20. Bulpin, P. V., Gidley, M. J., Jeffcoat, R. and Underwood, D. J. (1990) Development of a biotechnological process for the modification of galactomannan polymers with plant α-galactosidase. *Carbohyd. Polym.* **12**, 155-168.
21. Ellis, K. J. and Morrisson, J. F. (1982) Buffers of constant ionic strength for studying pH-dependent processes. *Methods Enzymol.* **87**, 405-426.
22. Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage. *Nature* **227**, 680-685.
23. Wray, W., Boulikas, T., Wray, V. P. and Hancock, B. (1981) Silver staining of proteins in polyacrylamide gels. *Anal. Biochem.* **118**, 197-203.
24. Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254.
25. Dey, P. M. and Kauss, H. (1981) α-Galactosidase of *Poterioochromonas malhamensis. Phytochemistry* **20**, 45-48.