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ScienceDirect
Food Chemistry 102 (2007) 691–698
Food Chemistry
www.elsevier.com/locate/foodchem

Mango ripening – Role of carbohydrases in tissue softening

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Received 16 December 2005; received in revised form 30 May 2006; accepted 1 June 2006

Abstract

A range of cell wall degrading enzymes, both glycanases and glycosidases, was identified in ripe mango (*Mangifera indica* cv. Alphonso) fruits. Reduction in the mannose content of the hydrolyzed polymeric fractions of ripe mango revealed the possible involvement of an endomannanase and α-mannosidase, the two major enzymes, in mango fruit softening and ripening phenomena. α-Mannosidase was resolved into isoforms I and II by chromatographic methods and their kinetic and inhibition properties were studied.

Keywords: Mango; Ripening; Mannosidase; Isoforms; Mannose; Polysaccharides

1. Introduction

Textural softening during ripening and storage, a crucial phenomenon directly affecting fruit shelflife and quality, is essentially due to depolymerization of the cell wall polysaccharides by a variety of hydrolytic enzymes. A host of cell wall hydrolases is shown to increase in activity during ripening, especially at the climacteric stage, thus resulting in disassembly, depolymerization and dissolution of pectic and other hemicellulosic polysaccharides (Fry, 1995). Pectin degrading enzymes such as polygalacturonase, pectate lyase and pectin methylesterase are implicated in ripening and textural softening of fruits such as tomato, banana and guava (Aina & Oladunjoye, 1993; El Zoghbi, 1994; Selvaraj & Kumar, 1989). Galactosidase and galactanase enzymes are involved in ripening associated changes in fruits like kiwi (Ross, Redgwell, & MacRae, 1993), apple (Ross, Wegrzyn, MacRae, & Redgwell, 1994) and tomato (Carey et al., 1995). An increased activity of cellulase was reported in the ripening of pears (Yamaki & Kakiuchi, 1979) and avocado (Pesis, Fuchs, & Zauberman, 1978), whereas excessive pithiness of some varieties of ripened pear fruit was also attributed to the action of hemicellulases (Yamaki & Kakiuchi, 1979). Expansins, a group of extracellular proteins with characteristic cell wall loosening property, regulate cell wall extension during plant cell growth by disrupting hydrogen bonds between cellulose microfibrils and xyloglucans (Cosgrove et al., 2002; Li, Jones, & McQueen-Mason, 2003; Rose, Catala, Gonzalez-Carranza, & Roberts, 2003). They are shown to play an important role, together with other cell wall degrading enzymes (Rose & Bennet, 1999), in fruit softening phenomenon (Rose et al., 2003), and they are shown to be expressed at the point of radicle emergence in generating tomato seeds, suggesting that they may play a crucial role in promoting cell wall dissolution (Chen & Bradford, 2000). β-Endo-mannanase and α-mannosidase were recently reported to be involved in textural softening of banana, capsicum and papaya (Prabha & Bhagyalakshmi, 1998; Priya Sethu, Prabha, & Tharanathan, 1996; Suvarnalatha & Prabha, 1999). Nevertheless, the exact physiological role of some of the glycosidases involved in tissue softening during ripening is not well established. Scanty reports are available on the molecular basis of tissue softening of ripening mango of a few varieties other than Alphonso mango (Lazan, Ali, Wah, Voon, & Chaplin, 1986; Roe & Bruemmer, 1981). The main objective of this study was to look for the various enzyme activities involved

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doi:10.1016/j.foodchem.2006.06.001

in the ripening of mango (*Mangifera indica*, cv. Alphonso), especially of those involved in mannan hydrolysis in vivo.

2. Materials and methods

2.1. Materials

Freshly harvested mature mango fruits (*Mangifera indica*, cv. Alphonso) were obtained from the local mango dealers. They were allowed to ripen under ambient conditions (27 °C). Mango fruit pulp at various stages of ripening was used for enzyme extraction. Freshly harvested fruits were taken immediately to denote unripe stage, while the subsequent stages of ripening were picked up from the fruits kept for normal ripening. The four stages of mango ripening were as follows (determined subjectively by firmness measurements):

Stage I: mature, unripe – stony hard, dark green.
Stage II: pre-climacteric – intermediate, light green.
Stage III: post-climacteric – intermediate, yellowish green.
Stage IV: ripe – soft, yellow.

At each stage, five fruits were used and extracts were prepared in triplicates. All the synthetic and other substrates used for various enzyme assays were procured from Sigma Chemical Co., MO, USA.

2.2. Isolation of non-starch polysaccharides

The unripe (stage I) and ripe (stage IV) mango fruits were selected to make alcohol insoluble residues (AIR). A known weight of fruit pulp was separately soaked in three volumes of 95% ethanol. Slicing the tissue and plunging them into alcohol were done instantaneously in order to avoid endogenous metabolic activity due to cutting. They were homogenized in a Sorvall omni mixer and the resulting slurry was kept at 60 °C for 40 min to arrest endogenous enzyme activities and to facilitate protein coagulation (Carrington, Greve, & Labavitch, 1993). The slurry was cooled to room temperature and filtered through two layers of cotton cloth. The residue was repeatedly extracted in 80% ethanol (four to five times) to remove all the free soluble sugars, washed with acetone and diethyl ether, and air dried.

Based on differential solubility, the AIR was sequentially extracted with (1) cold water for CWS, (2) hot water for HWS, (3) 0.5% EDTA for pectins, (4) 1.8 N KOH (under N_2 atmosphere for hemicellulosic fractions) the alkali extract was adjusted to pH 4.5 to precipitate out hemicellulose A, whereas the supernatant on precipitation with ethanol (3 vol.) gave hemicellulose B (Yashoda, Prabha, & Tharanathan, 2006). The final alkali-insoluble residue was designated as cellulosic fraction (Salimath & Tharanathan, 1992). Each of the above fractions from unripe and ripe mango were subjected to DEAE-cellulose ion exchange chromatography and fractions were eluted stepwise from the column by water and with increasing molarities of $(NH_4)_2CO_3$ and NaOH. The major fractions eluted were then subjected to gel permeation chromatography (GPC) on Sepharose CL-4B. The GPC purified fractions were designated as endogenous substrates for α-mannosidase isoforms I and II activities (Yashoda et al., 2006).

2.3. Enzyme isolation and purification

Acetone dried powders were used for enzyme extraction (Priya Sethu et al., 1996). Unripe and ripe mango fruits, peeled and sliced (1 g), were extracted with sodium phosphate buffer (10 ml, pH 7.0, 0.2 M) containing polyvinyl pyrrolidone (0.1%) and homogenized in a blender and immediately poured into chilled (−18 °C) acetone (3 vol.). The precipitate (AIP, acetone insoluble powder) was filtered through muslin cloth, and dried. AIP (10 g) was extracted (×3) with sodium phosphate buffer (0.05 M, pH 6.6 at 4 °C for 10 h) containing 0.25 M NaCl, centrifuged (7000 rpm, 15 min) and the clear filtrate was dialyzed against sodium acetate buffer (10 mM, pH 4.4 at 4 °C) and used for enzyme assay.

2.4. β-Endomannanase

The reaction mixture consisting of enzyme extract (0.13 ml) and konjac glucomannan (Sigma Chemical Co., USA, 0.1% in 100 mM sodium acetate buffer pH 5.0, 1 ml) was incubated at 37 °C for 1 h. The liberated reducing sugar was determined by taking 1 ml sample solution, into which was added 1 ml of dinitrosalicylic acid (DNS) reagent and the tubes were kept in a boiling water bath for 10 min, cooled to room temperature and the colour developed was read at 550 nm against a reagent blank. A standard curve was prepared using glucose solution (1–100 μg ml^{-1}). One unit of enzyme activity is defined (Miller, 1959) as the amount of enzyme that catalyzed the formation of 1 μM reducing group min^{-1}.

2.5. Cellulase

To 0.1% solution of carboxymethyl cellulose (0.5 ml) in 100 mM sodium acetate buffer (pH 5.0) was added 0.25 ml of enzyme extract and incubated at 37 °C for 1 h. The liberated reducing sugar was determined. One unit of enzyme activity is defined (Abu-Sarra & Abu-Goukh, 1992) as the amount of enzyme that catalyzed the formation of 1 μM reducing group min^{-1}.

2.6. Other glycanases

Similarly, the activities of hemicellulase (using cereal arabinoxylan as the substrate), xylanase (xylan), glucanase (glucan), arabinanase (arabinogalactan), polygalacturonase (pectic acid) and amylase (corn starch, pregelatinized)

were determined by measuring the liberated reducing sugars (Miller, 1959). The pectin methyl esterase (PME) activity was measured by the rate of demethylation of citrus pectin with decrease in pH at room temperature, as determined by a decrease in the absorbance at 620 nm (Hagerman & Austin, 1986). One unit of PME activity is defined as 1 μM of uronic acid released min^{-1}.

2.7. Glycosidases

The activities of α-mannosidase, α/β-galactosidase, α/β-glucosidase, β-glucosaminidase, β-galactosaminidase, β-xylosidase and α-fucosidase were assayed in 0.05 M sodium acetate buffer using the respective *p*-nitrophenyl (*p*NP)-sugar glycosides at 37 °C for 15 min. The reaction was terminated by the addition of Na_2CO_3 solution (0.25 M, 1 ml) and the released *p*-nitrophenol was measured at 420 nm against a suitable reagent blank. One unit of enzyme activity is defined (Priya Sethu et al., 1996) as the amount of 1 μM pNP released min^{-1}.

2.8. Extraction and purification of α-mannosidase

The crude enzyme extract in a dialysis bag was kept immersed in saturated sucrose solution overnight and later dialyzed against 10 mM sodium phosphate buffer (pH 6.0 at 4 °C). The concentrated enzyme extract was subjected to chromatography on DEAE-Sephadex A-50 (3.2 × 12.5 cm) equilibrated with 0.05 M NaCl and eluted with 20 mM sodium phosphate buffer (pH 6.0, 200 ml) followed by increasing gradients of NaCl (0–1 M, 400 ml). Fractions (2.1 ml) were monitored by measurement of OD at 280 nm. The enzyme rich fractions (I and II) were individually concentrated by sucrose gradient and subjected to GPC on Sephadex G-200 (1.6 × 140 cm), by eluting with 0.05 M sodium phosphate buffer containing 0.05 M NaCl. The column was calibrated with standard protein markers, viz. BSA (66,000 Da), ovalbumin (45,000 Da) and cytochrome *C* (12,300 Da). The protein content and enzyme activity of each (1.6 ml) of the purified fractions were determined. The enzyme active fractions (isoforms I and II) were concentrated and characterized.

2.9. Kinetic parameters

The effect of pH in the range 3–8 was determined by dissolving *p*NP-glycosides in different buffers, adding isoforms I and II and incubating the reaction mixture at ambient temperature for 15 min followed by the absorbance measurement at 420 nm, whereas the effect of temperature was studied between 24 and 60 °C at optimal pH. The stability was measured by pre-incubating the enzyme with 0.1 M sodium phosphate buffer at different temperatures for 15 min. After incubation, the solution was immediately cooled and assayed for the remaining activity at optimal temperature. The temperature at which 50% of the activity is retained (T_m) was calculated. The activity of the untreated enzyme was used as the control (100%). The Michaelis–Menton constants were determined by incubating the enzyme at optimum temperature/pH with different concentrations of substrate. The kinetic parameters K_m and V_{max} were calculated by the double reciprocal Line Weaver–Burk plot.

2.10. Inhibition study

The effect of metal ions (Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Hg^{2+}) and EDTA on enzyme activity was determined by pre-incubating the enzyme in sodium phosphate buffer (pH 4.8) containing 1.0 mM concentration of each metal ion for 30 min prior to addition of the substrate, and the remaining enzyme activity was later assayed.

2.11. Effect of product analogues on enzyme activity

The effect of product analogues on enzyme activity was determined by pre-incubating the enzyme in sodium phosphate buffer (pH 4.8) containing 2.0 mM (upto 50 mM) galactose, glucose or mannose at 4 °C for 20 min. Later the remaining enzyme activity was assayed.

2.12. Substrate specificity

The substrate (synthetic) specificity of the enzyme was determined by incubating with various *p*NP glycosides (13 mM) at 37 °C for 15 min and determining the *p*NP released.

2.13. Activity on natural substrates

The activity on natural substrates like polygalacturonic acid (0.06%), pectin (0.06%), galactomannan (0.45%) and carboxymethyl cellulose (0.45%) was measured by incubating the substrates with the enzyme at 37 °C for 7 h.

2.14. Activity on endogenous substrates

The activity on endogenous substrates (0.5–1.0 mg) was determined by incubating the enzyme with the respective substrates at 37 °C for 7 h and later arresting the enzyme activity by adding potassium ferricyanide reagent (1.0 ml), kept in a boiling water bath for 15 min and estimating the reducing sugar released (Imogo & Yagishita, 1971).

2.15. Polyacrylamide gel electrophoresis

Native and SDS–PAGE was carried out using discontinuous buffer system as described by Laemmli (1970). The polyacrylamide gel (10%T) (containing 0.1% SDS for SDS–PAGE) was casted in 1.5 mm slab gel apparatus. The electrophoresis was carried out at 100 V in 0.025 M Tris–0.3 M glycine buffer, pH 8.3 (containing 0.1% SDS as electrode buffer for SDS–PAGE). The protein was

mixed with the sample buffer, pH 6.8 containing 10% (V/V) glycerol, (2% (W/V) SDS for SDS–PAGE), and 0.1% bromophenol blue. Samples were heated in a boiling water bath for 15 min and subjected to electrophoresis. The molecular weight markers were also treated similarly and electrophoresed. The protein bands in the gel were visualized by staining with Coomassie brilliant blue G-250.

3. Results and discussion

3.1. Carbohydrases

The climacteric period of mango fruit was seen around 8 days after harvest and ready-to-eat ripe stage by 12th day (Chen & Bradford, 2000), as also reported before (Krishnamurthy, Patwardhan, & Subramanyam, 1971). The activity of hydrolases gradually increased while that of an esterase (PME) decreased during ripening (Fig. 1 and Table 1), most of them showing maximum activity around climacteric stage. The rise in enzyme activity naturally resulted in increased charge density by diminishing the degree of esterification and/ or decreasing the polymer molecular weight. It may also be due to increase in pH from 3.4 to 6.0 as ripening progresses. Among glycanases, mannanase, arabinanase and galactanase showed high activities, the latter two are known to degrade arabinan- and galactan-type polysaccharides, respectively, which are common constituents of pectic complex, and which correlated with a significant loss of the corresponding sugar residues (arabinose and galactose) from the polymeric fractions derived from ripe mango fruits (Yashoda, Prabha, & Tharanathan, 2005). Enhanced mannanase activity has been reported in tomato (Pressey, 1989) and pear fruit (Yamaki & Kakiuchi, 1979).

The enhanced activity of PME in the initial stages of ripening is perhaps required for preparing pectic acid (by demethylation of pectins) for subsequent action by polygalacturonase (PG). Decreased activity of PME during ripening was shown in banana, guava and date (El Zoghbi, 1994; Hultin & Levine, 1965). PG activity was barely detected in unripe fruits, but an increase in its activity was observed in banana, tomato, papaya and other fruits (Brownleader et al., 1999; Selvaraj & Kumar, 1989). In climacteric fruits the rapid synthesis of PG activity coincides with considerable textural alteration (loss of firmness) during ripening, and conversion of pectic polysaccharides into water-soluble galacturonides (Lazan et al., 1986).

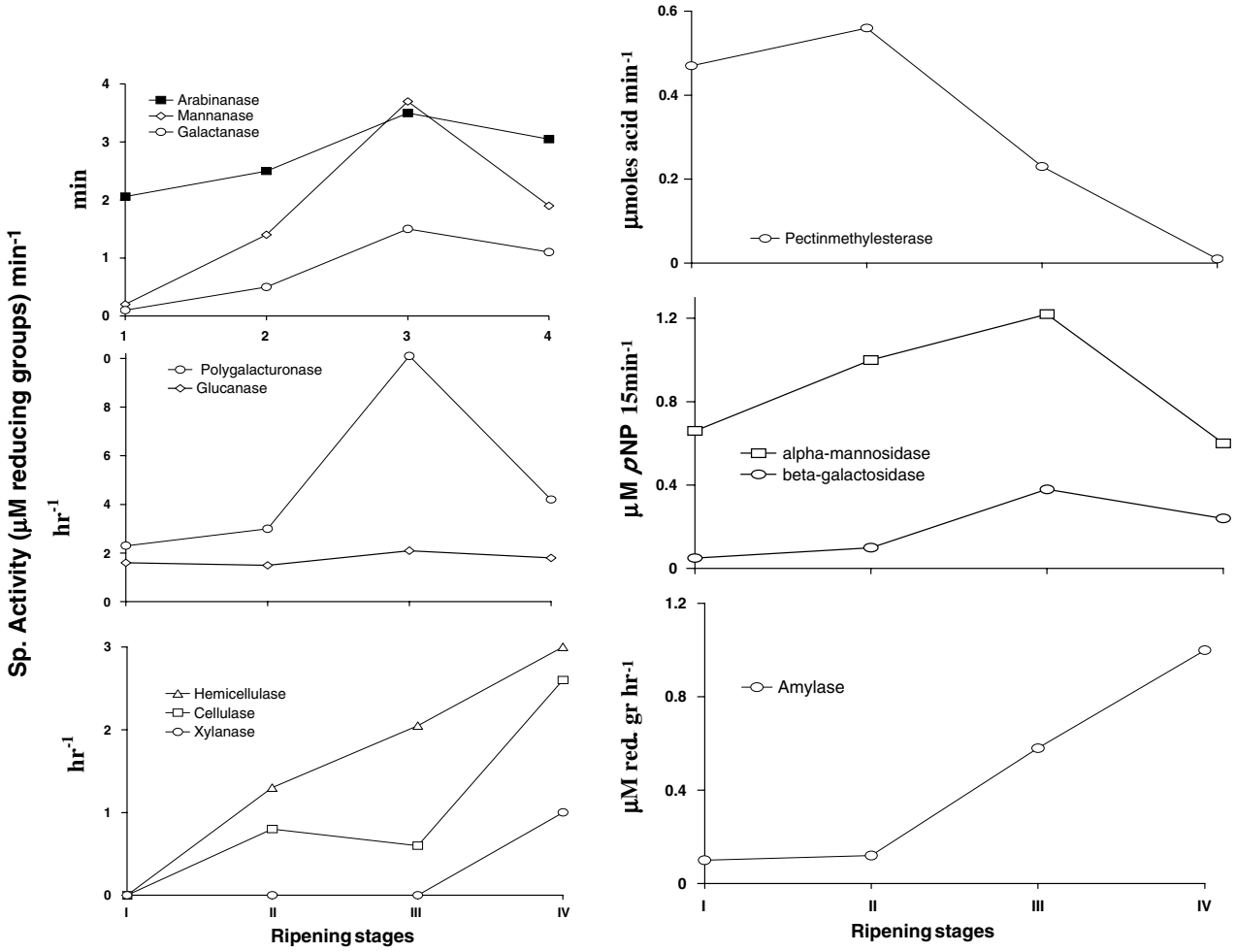

Fig. 1. Activity profiles of carbohydrate hydrolases in ripening mango.

Table 1
Activities of glycanases and glycosidases of ripe mango

Glycanases	Sp. activity (1 μM reducing group min^{-1} mg^{-1} protein)	Glycosidases	Activity (1 μM *p*NP released min^{-1} g^{-1} AIP)
β-Endomannanase	228	α-Mannosidase	130
Arabinanase	216	α-Galactosidase	37
Galactanase	96	β-Galactosidase	28
Glucanase	3	β-Glucosaminidase	26
Hemicellulase	2.6	β-Glucosidase	16
Amylase	1	β-Galactosaminidase	10
Polygalacturonase[a]	2.2	α-Glucosidase	9
Pectin methylesterase	0.55	α-Fucosidase	5
Xylanase	0.25	β-Xylosidase	4

AIP, acetone insoluble powder.
[a] 1 μM Galacturonic acid equi. min^{-1} mg^{-1} protein. The values are mean of three replicates, SD ± 0.5%.

Among glycosidases, α-mannosidase activity was highest followed by α-/β-galactosidases, β-glucosaminidase and β-glucosidase activities. Such an inference has also been reported in several other fruits. In avocado, despite its low PG activity, the high in vivo pectin degradation was attributed to strong β-galactosidase activity (De Veau, 1993). In capsicum and tomato the major glycosidase was β-hexosaminidase followed by α-galactosidase and α-mannosidase (Priya Sethu et al., 1996). All these data clearly show fruit tissue specificity in some of these enzymes.

To study the endogenous mannan solubilization, the composition of some of the major carbohydrate fractions, viz., cold water soluble (1), hot water soluble (2), pectic substances (3) and hemicelluloses (4) was studied and it was found that the mannose content was relatively less in the polymeric fractions of ripe mango (Fig. 2) (Yashoda et al., 2005). Both mannanase and α-mannosidase implicated in mannan solubilization, exhibited a peak in the activity around climacteric stage of ripening (Fig. 3). Mannosidase has a major role in hydrolyzing small molecular weight oligomers or mannose containing side chains linked to other core polysaccharides or glycoproteins, whereas mannanase acts internally on high molecular weight mannan-type polysaccharides present either as homo- or heteropolymers. The reduction in mannose content in ripe mango polymeric fractions could be due to combined action of both these enzymes, which are also shown to be involved in fruit softening during ripening of tomato (Watkins, Haki, & Frenkel, 1988).

3.2. α-Mannosidase

α-Mannosidase being the major glycosidase in ripe mango, some attempts were made to purify this enzyme and study its kinetic properties. The enzyme from ripe mango has not been purified and characterized so far. The activity of α-mannosidase was reported to increase

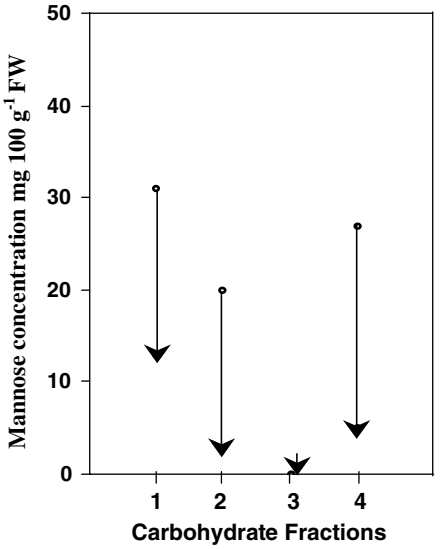

Fig. 2. In vivo mannan hydrolysis in ripening mango. Arrows indicate change in mannose levels from unripe to ripe stage, in different carbohydrate polymeric fractions after hydrolysis. 1, cold water soluble (CWS); 2, hot water soluble (HWS); 3, EDTA soluble (pectic); 4, alkali soluble (hemicellulose).

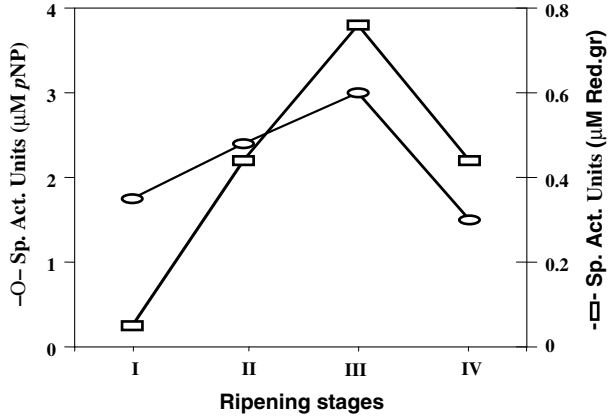

Fig. 3. Activity profiles of mannan degrading enzymes in mango during ripening (–□– β-endomannanase; –◯– α-mannosidase).

with ripeness in some fruits (Prabha & Bhagyalakshmi, 1998). Upon IEC the crude enzyme was resolved into two distinct peaks of activity (isoforms I and II), in a relative ratio of 78% and 22%, respectively (Fig. 4). The two isozymes were further purified by GPC (Figs. 5 and 6), whose molecular weights, based on their elution volume, were 75 and 43 kDa, respectively. Electrophoretic profiles of isoforms I and II revealed a single band, indicating their homogeneity (Fig. 7). The specific activity of 1.03 for the crude extract increased to 10.06 and 1.81 upon IEC, which further increased to 36.43 and 66 upon GPC with a final fold purification of 35 and 64, and a recovery of 7% and 15%, for isoforms I and II, respectively. Table 2 summarizes the purification profile of α-mannosidases of ripe mango. The molecular weight of the two isoforms of mango α-mannosidase was comparable to those of banana

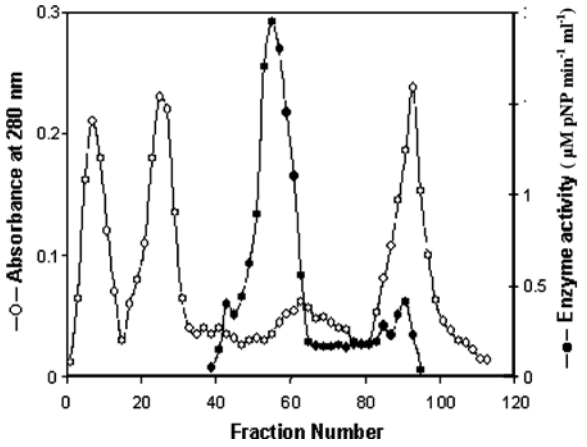

Fig. 4. Chromatography on DEAE-Sephadex A-50 of α-mannosidase from ripening mango.

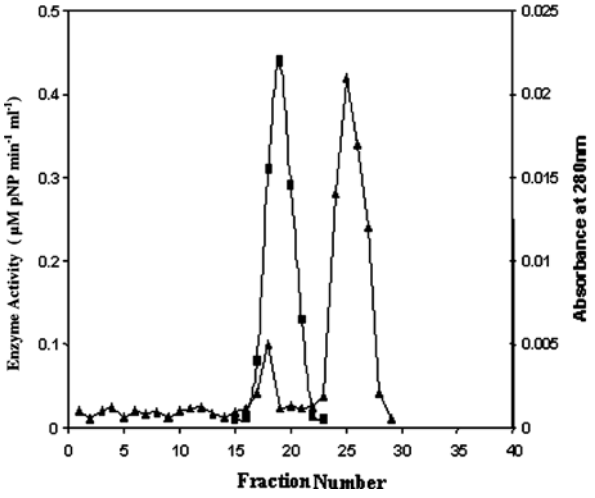

Fig. 5. GPC on Sephadex G-200 of α-mannosidase isoform I from ripening mango.

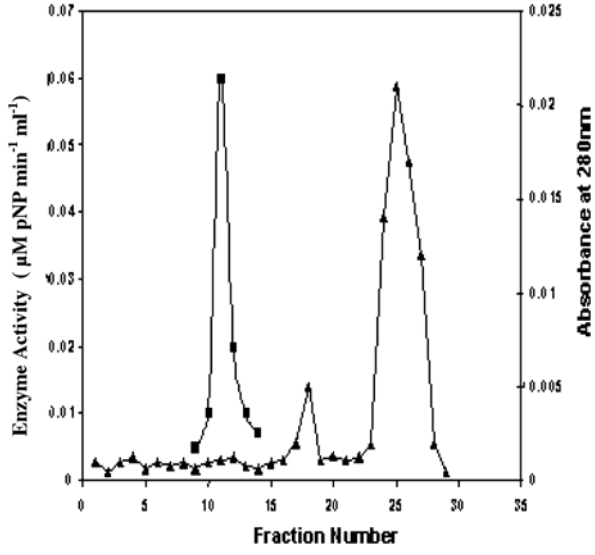

Fig. 6. GPC on Sephadex G-200 of α-mannosidase isoform II from ripening mango.

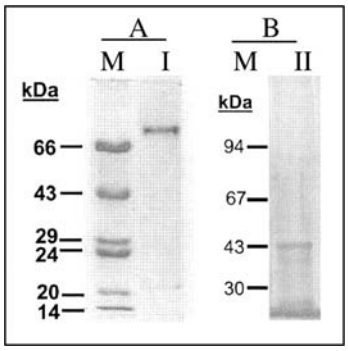

Fig. 7. Gel electrophoregram of GPC purified α-mannosidase from ripening mango, A – 12% gel, M – Mol. wt. markers, I – purified isoform I; B – 8% gel, M – Mol. wt. markers, II – purified isoform II.

Table 2
Summary of purification of α-mannosidases of ripe mango

Fractions	Total activity (U)	Total protein (mg)	Sp. activity (U mg^{-1} protein)[a]	Fold purification
Crude enzyme	151.8	147.50	1.03	1.00
IEC on DEAE-Sephadex A-50				
Isoform I	15.7	1.56	10.06	9.76
Isoform II	5.0	2.76	1.81	1.75
GPC on Sephadex G-200				
Isoform I	10.2	0.28	36.43	35.37
Isoform II	2.0	0.03	66.0	64.0

[a] 1 unit (U) = 1 μM *p*-nitrophenol released min^{-1}.

and tomato, but differed from that of capsicum (Prabha & Bhagyalakshmi, 1998; Suvarnalatha & Prabha, 1999).

In Table 3 are summarized the properties of the two α-mannosidases. Their pH optimum, 4.8 was same and temperature optima were 65 °C and 75 °C for isoforms I and II, respectively. The enzymes were thermally stable for 15 min at these temperatures. The K_m values for *p*NP α-mannopyranoside were 1.4 and 0.7 mM for isoforms I and II, respectively, indicating a higher enzyme – substrate affinity for isoform II (the minor enzyme). Among the metal ions tested Mg^{2+}, Zn^{2+} and Hg^{2+} at 1 mM concentration exhibited ~40% inhibition of isoform II, which was considerably lower for isoform I (see Table 4). There

Table 3
Properties of α-mannosidases I and II of ripe mango

Properties	Isoform I	Isoform II
Elution on IEC	0.15 M NaCl	0.2 M NaCl
Abundance	78% (Major)	22% (Minor)
K_m with *p*NP (mM)	1.40	0.70
pH optimum	4.80	4.80
Temperature optimum (°C)	75	65
Thermal stability (15 min) (°C)	75	65
Molecular weight (Da)	~75,000	~43,000
Ability to degrade β-endomannan	No	No
Inhibition by		
Metal ions	Weak	Weak
Structural analogues	No	No

Table 4
Effect of various inhibitors on α-mannosidases I and II of ripe mango

Inhibitors	% Inhibition over control	
Metal ions (1 mM conc.)	Isoform I	Isoform II
Mn^{2+}	11.2	9.5
Mg^{2+}	19.4	36.1
Zn^{2+}	23.0	40.0
Hg^{2+}	20.6	43.0
Cu^{2+}	23.0	00.0
Fe^{2+}	20.0	7.8
EDTA (10 mM conc.)	54.0	53.0
Activity on structural analogues (13 mM conc.)		
*p*NP-α-mannopyranoside	+	+
*p*NP-β-mannopyranoside	–	–
*p*NP-α or β-glucopyranoside	–	–
*p*NP-α or β-galactopyranoside	–	–
Glucose (upto 50 mM)	–	–
Galactose (upto 50 mM)	–	–
Mannose (upto 50 mM)	–	–

was ~20% inhibition of isoform I by Ca^{2+}, while isoform II was not inhibited. The isoforms of α-mannosidase from capsicum (Prabha & Bhagyalakshmi, 1998) were strongly inhibited by Fe^{2+} and Ca^{2+}, whereas those of tomato and banana (Suvarnalatha & Prabha, 1999) were inhibited by Hg^{2+} and Ca^{2+}. Mannose did not inhibit the enzyme indicating no feed back inhibition. Both the isoforms lacked endomannanase activity.

Mango α-mannosidases showed highest activity towards synthetic substrates, whereas on galactomannans (guar gum and locust bean gum) and glucomannan (konjack) they showed no activity, probably because of the presence of mannose in the β form. A similar observation has been made with regard to α-mannosidases from tomato (Suvarnalatha & Prabha, 1999) and capsicum (Priya Sethu et al., 1996). As far as the endogenous substrates are concerned, the hot water soluble and hemicellulose fractions of unripe mango fruits (Yashoda et al., 2006) showed considerable hydrolytic activity (Table 5), thus it can be inferred that in vivo, it also can occur.

Table 5
Activity of α-mannosidase isoforms of mango on natural and endogenous substrates

Substrates	Activity[a]	
	Isoform I	Isoform II
Natural substrates		
Galactomannan	+	+
Endogenous substrates		
Cold water soluble (CWS) polysaccharide – unripe [0.15 M $(NH_4)_2CO_3$]	21.04	21.04
CWS polysaccharide – ripe [0.15 M $(NH_4)_2CO_3$]	16.10	21.04
Hot water soluble (HWS) polysaccharide – unripe [0.15 M $(NH_4)_2CO_3$]	8.66	14.85
HWS polysaccharide – unripe (0.15 M NaOH)	33.41	49.50
Hemicellulosic polysaccharide – unripe (0.1 M NaOH)	32.20	42.10

[a] One unit of enzyme activity is defined as the amount of 1 μM *p*NP released min^{-1}.

Since α-mannosidase is implied in the textural softening of several fruit systems, showing a climacteric rise in activity during ripening, its specific role in the context of fruit ripening is worth further analysis. α-Mannosidase may have a vital role in signal transduction, by way of deglycosylation, and also in some of the membrane functions since membranes are rich in mannose-containing macromolecules. Although the presence of α – mannan in cell walls may not be evident, it is quite likely that the mannose residues as α – linked side chains to other hemicellulosic polymers and/or glycoconjugates may facilitate the activity of α-mannosidase in ripe mango fruits.

Acknowledgement

Thanks are due to Council of Scientific and Industrial Research, New Delhi, for the award of senior research fellowship to H.M.Y.

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