PERGAMON

Phytochemistry 62 (2003) 13–19

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Biochemical characterization of sap (latex) of a few Indian mango varieties

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Received 23 May 2002; received in revised form 20 August 2002

Abstract

Mango sap (latex) from four Indian varieties was studied for its composition. Sap was separated into non-aqueous and aqueous phases. Earlier, we reported that the non-aqueous phase contained mainly mono-terpenes having raw mango aroma (Phytochemistry **52** (1999) 891). In the present study biochemical composition of the aqueous phase was studied. Aqueous phase contained little amount of protein (2.0–3.5 mg/ml) but showed high polyphenol oxidase (147–214 U/mg protein) and peroxidase (401–561 U/mg protein) activities. It contained low amounts of polyphenols and protease activities. On native PAGE, all the major protein bands exhibited both polyphenol oxidase and peroxidase activities. Both polyphenol oxidase and peroxidase activities were found to be stable in the aqueous phase of sap at 4 °C. Sap contained large amount of non-dialyzable and non-starchy carbohydrate (260–343 mg/ml sap) which may be responsible for maintaining a considerable pressure of fluid in the ducts. Thus, the mango sap could be a valuable by-product in the mango industry as it contains some of the valuable enzymes and aroma components.

Keywords: Mango; Sap; Polyphenol oxidase; Peroxidase; Carbohydrate; Polyphenols; Protein

1. Introduction

Latexes from different plants contain various biologically active compounds. The latex of the rubber tree (*Hevea brasiliensis*) contains proteins, resins, sugars, glucosides, tannins, alkaloids and mineral salts (Purseglove, 1968). Papaya (*Carica papaya*) latex contains papain, multiple forms of chymopapain A, peroxidase (POD) and peptidase A (Park et al., 1979; Brockehurst and Salih, 1985). Recently, Saby John et al. (1999) reported that 100 kg of mango fruit yield 100–250 ml of sap (latex) depending on the variety and they further reported that non-aqueous phase of saps from Indian mango varieties contain mainly terpenoids like ocimene, or β-myrcene or limonene. However, the composition of the aqueous phase has not been studied in detail. The presence of enzyme laccase/polyphenol oxidase (PPO) in sap has been reported earlier (Joel et al., 1978; Robinson et al., 1993).

During harvest of mangoes, the sap spurts out and is frequently deposited on the surface of the same or other fruits causing sap-injury. Sap-injury is characterized as darkening or browning of the peel due to contact with the sap. De-sapping of mangoes is one of the methods practised to control sap-injury and the sap thus obtained is currently being wasted.

Therefore, in the present study, the composition of the aqueous phase of mango sap with respect to the protein, enzymes, carbohydrate and polyphenol content was determined.

2. Results and discussion

Saps collected from different mango varieties were found to be viscous in nature and were separable by centrifugation into an upper, non-aqueous phase and a lower, aqueous phase. The non-aqueous phase has a raw mango aroma and consists of terpenoids (Saby John et al., 1999). The volume of aqueous phase present in sap varied from variety to variety (Saby John et al., 1999). The pH of the aqueous phase was found to be around 4.0 in all varieties. The aqueous phase was analyzed for protein, carbohydrate, a few enzyme activities and polyphenol content.

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0031-9422/02/$ - see front matter
PII: S0031-9422(02)00441-7

2.1. Protein

The protein content in sap was found to be the highest in the *Totapuri* variety (3.5 mg/ml) and lowest in the *Banganapalli* variety (2.0 mg/ml; Table 1). Native-polyacrylamide gel electrophoresis (PAGE) of the aqueous phase of sap from different mango varieties was carried out. One major protein band was observed in the sap (Fig. 1A). A significant difference was noted in the case of *Totapuri* sap proteins. In this variety the sap proteins were separated into three bands with similar intensities. Thus, the protein patterns exhibited varietal differences. Native-PAGE profiles of the sap indicated that there were very few proteins in mango sap. Most of the bands visible on native gels with Coomassie blue also stained positive for POD and PPO activities (Fig. 1B,C) suggesting that these enzymes may form a major part of the sap proteins. The same bands stained positive for both PPO and POD activities, indicating either that these two enzymes are either co-migrating on the gel or that the same proteins are exhibiting two different activities.

2.2. Polyphenol oxidase

PPO activity was found to be the highest in sap of the mature *Badami* mango (214 U/mg protein, Table 1). The PPO activity in mango sap is much higher than in most fruits and vegetables reported so far. The specific activity of PPO in banana was reported to be 15.4 U/mg protein (Galeazzi et al., 1981), in pine apple 0.73 U/mg protein (Das et al., 1997), in potato 0.21 U/mg protein (Cho and Ahn, 1999).

PPOs have gained attention primarily due to their role in browning of tissues subsequent to bruising, or during the preparation of many fruit/vegetables for processing (Vamos-Vigyazo, 1981). The browning is the result of enzymatic oxidation of phenols and eventual non-enzymatic polymerisation of the quinones formed into tannins or "melanins". However, studies have indicated that this enzyme may play a vital role in plant senescence. It also plays a defensive role against attack by insects and microorganism (Hoffman et al., 1970). The role of this enzyme in sap is not known. However, in mango industry during harvest, the regions of mango that come in contact with the sap turn brown in colour which is generally referred to as sap-injury. It is shown that terpenoids present in the sap are mainly involved in the sap-injury; however, involvement of sap PPO was also suggested by Loveys et al. (1992). Presence of PPO/laccase activity in sap was reported earlier (Joel et al., 1978; Robinson et al., 1993). PPO activity has been observed in the latexes of other members of the Anacardiaceae, including *Shinus molle*, *Rhus* and *Mangifera* (Joel et al., 1978).

2.3. Peroxidase

The activity of POD in the sap of mature mango was found to be the highest in *Totapuri* (561 U/mg protein; Table 1). There are reports of POD activity in many of the common fruits (Vamos-Vigyazo, 1981). However, the presence of POD activity in the sap of mango has not been reported so far. In the presence of H_2O_2, POD catalyses the oxidation of a wide variety of substrates such as phenols, aromatic-, primary-, secondary- and tertiary-amines, leuco dyes, ascorbic acid and indole. Despite its ubiquity and extensive study, the physiological role of POD is not clear. PODs have been implicated in plant senescence, fruit ripening, physiological breakdown of fruits and vegetables, and in the deteriorative changes in flavour, texture, colour, and nutritional value in processed fruits and vegetables (Vamos-Vigyazo, 1981; Yang, 1967). Similar to PPO, POD also has been involved in protein cross-linking through di- and tri-tyrosine linkages or by Michael addition of −SH and amino groups to benzoquinone (Matheis and Whitaker, 1987).

2.4. Protease

The sap from all the mango varieties studied exhibited both serine protease and cysteine protease activities. The serine protease activity in the sap of mature mango ranged from 2.4 to 4.3 U/mg protein (Table 1). It was the highest in *Seedling* followed by *Badami* and the

Table 1
Protein, carbohydrate content, activities of PPO, POD, serine and cysteine proteases in sap aqueous phase of mature mango[a]

Variety	Content (mg/ml of aqueous phase)			Specific activity of enzymes (U / mg protein)			
						Protease	
	Total phenolics	Carbohydrate	Protein	PPO	POD	Serine	Cysteine
Badami	0.127±0.010c	302±6.8b	3.1±0.10b	214±13.7c	456±19.0b	4.0±0.174b	0.38±0.003c
Seedling	0.049±0.009a	343±11.2c	2.9±0.09b	170±11.3b	451±25.5b	4.3±0.199b	0.22±0.007b
Totapuri	0.049±0.010a	260±7.4a	3.5±0.08c	166±9.4b	561±22.8c	2.6±0.100a	0.22±0.009b
Banganapalli	0.108±0.013b	314±8.8b	2.0±0.09a	147±8.4a	401±16.2a	2.4±0.108a	0.12±0.010a

[a] Values shown in the table are mean±SD of four values. Different letters shown in the same column differ significantly at level $P<0.05$.

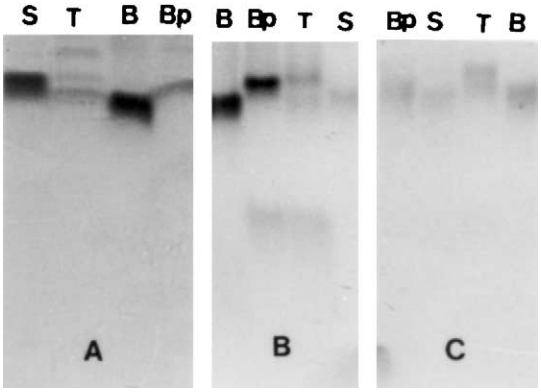

Fig. 1. Electrophoresis of aqueous phase of sap from different mango varieties on native polyacrylamide gel (7.5%). A. Protein stain; B, Peroxidase stain; C. Polyphenol oxidase stain. Lanes B, *Badami*; S, *Seedling*; T, *Totapuri*; Bp, *Banganapalli*.

varieties *Totapuri, Banganapalli had* similar activities (around 2.5 U/mg protein). Differences were also noticed in the activities of cysteine proteases in sap from different mango varieties. It was found to be the highest in *Badami* and lowest in *Banganapalli* (Table 1). The presence of protein-hydrolysing enzymes in fruit is well documented and the properties of some proteases, for example, papain from papaya latex, have been extensively studied (Park et al., 1979; Brockehurst and Salih, 1985). Protease activity has been reported to play an important role in protein turnover and particularly during the final stages of senescence when protein catabolism is responsible for increases in free amino acids and amides (Smith and Kimmel, 1960).

2.5. Activities of other enzymes

Very low activities of lipoxygenase (0.037–0.048 U/mg protein), amylase (2.5 U/mg to 4.3 U/mg protein) and polygalacturonase (1.1–1.3 μmol/h/mg protein) were detected and catalase and pectin methyl esterase activities were found to be absent in mango sap.

2.6. Carbohydrate

The total carbohydrate content in the sap aqueous phase of mature mango ranged from 260 mg/ml in *Totapuri* to 343 mg/ml in *Seedling*. In the other varieties it was about 300 mg/ml sap. The carbohydrate content in sap did not decrease upon dialysis indicating that it has a high molecular weight in nature. It had a very small amount of free sugar (0.45 mg glucose equivalents/ml). This carbohydrate showed negative to an iodine test indicating that it is non-starchy in nature. On the other hand the pulp portion of the mango was reported to be rich in starch (Morga et al., 1979). Thus the sap carbohydrate was found to be a non-starchy high molecular weight polysaccharide. The physiological role of this carbohydrate in sap is not known. It is likely that it provides the colloidal nature to the sap to draw water from the fruit into the duct system for maintaining considerable pressure of fluid in the ducts.

2.7. Total phenols

The aqueous phase of saps was analysed for total phenolics content. It ranged from 0.049 mg/ml to 0.127 mg/ml (Table 1) indicating that the total phenols content in sap is low. Phenolic compounds are widely distributed in the plant kingdom and are particularly prominent in fruits where they are important in determining colour and flavour. Plant phenolics have long been suspected of playing a role in the disease resistance of plants, and this function has been accounted in a number of ways. The oxidized phenolics are more potent anti-microbial agents than the non-oxidized phenolics, and may also play a role in the hypersensitivity reaction which consists of rapid death of a few cells resulting in the confinement of the pathogen to a restricted area of high PPO and oxidized phenol content (Cruickshank and Perkin, 1964).

2.8. Changes in protein, carbohydrate contents and enzyme activities at different stages of fruit development

The protein and carbohydrate contents of mango sap were found to decrease as the fruit matured, in all varieties studied. The decrease in protein content from stage I to stage III was around 35% in all varieties. The decrease in carbohydrate content from stage I to stage III ranged from 8% in *Banganapalli* to 25% in *Badami*,*Totapuri* and *Seedling* varieties (Fig. 2). In the case of PPO and POD enzyme activities, the general trend was towards a decrease in activity as the fruit matured (Fig. 2). With respect to PPO the decrease in specific activity in saps from stage I to stage III ranged between 12% in *Totapuri* and 38% in *Banganapalli*. In *Seedling* and *Badami*, it was 27 and 19% respectively. In the case of POD, the decrease in specific activity from stage I to stage III ranged between 19% in *Banganapalli* and 54% in *Badami*. In *Seedling,* the decrease was about 37% and in *Totapuri*, 21%.

There are no reports regarding PPO and POD activities in mango fruit at different stages of development. However, in apple and grape from tender stage to mature fruit stage a decrease of 17 and 5% respectively were reported (Marques et al., 1995). Coseteng and Lee (1987) also reported a decrease in PPO activity in apple during fruit maturity. However, there are reports in mango regarding changes in the enzyme activities during ripening after the harvest. An increase in PPO, POD and catalase activities were reported in mango during ripening of mangoes after harvesting (Singh and Chundawat, 1991).

The serine protease activity was higher at the mature stage compared to the initial tender stage and the

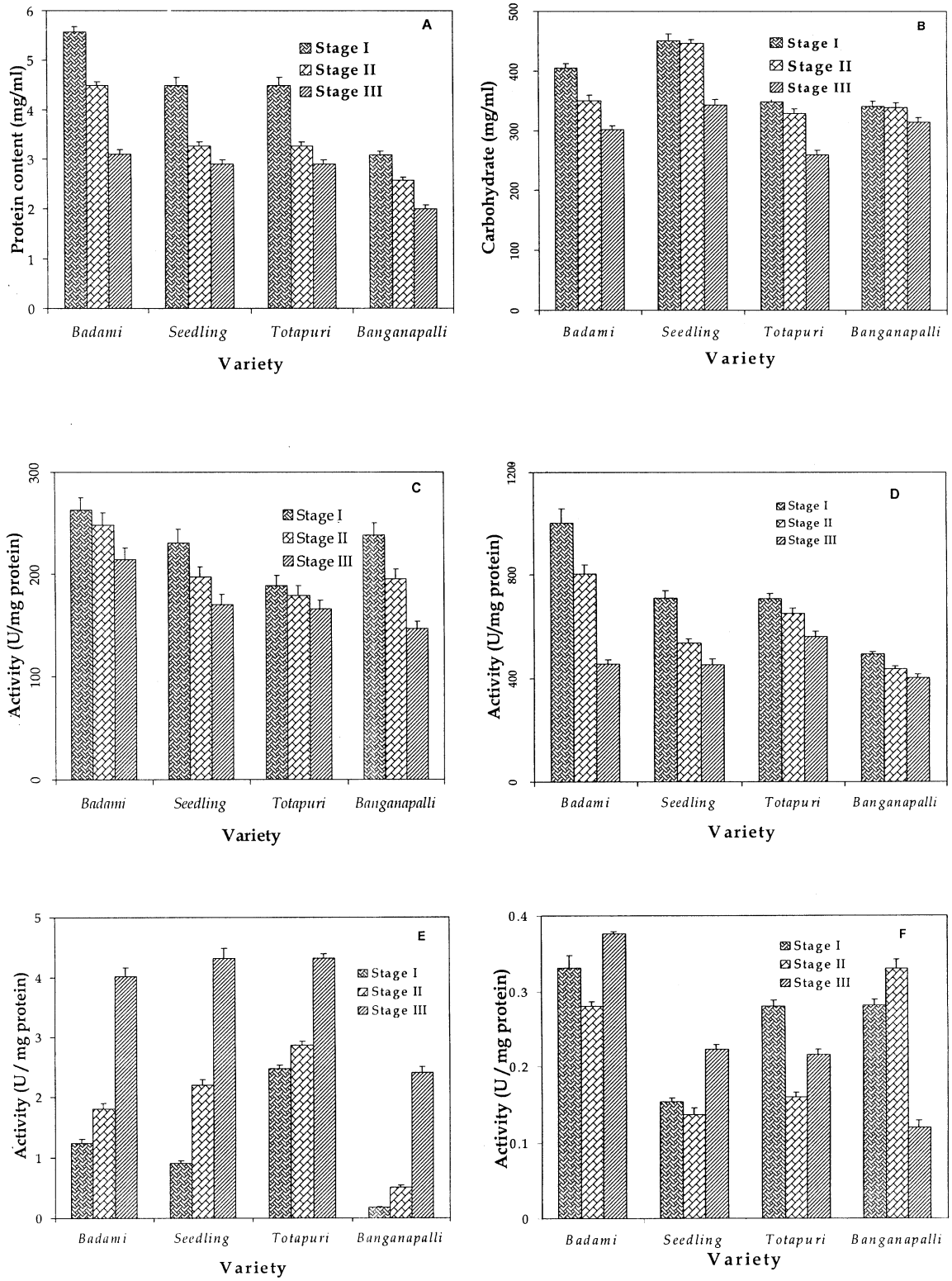

Fig. 2. Changes in composition of sap components at different stages mango fruit development. A, Protein; B, Carbohydrate; C, PPO; D, POD; E, Serine protease; F, Cysteine protease activity.

increase in activities in different varieties from stage I to stage III ranged from 14% in *Totapuri* to 94% in *Banganapalli* (Fig. 2). In the case of cysteine protease activity, no definite trend was observed.

2.9. Stability of enzymes

The stability of PPO and POD in sap stored at room temperature and at 4 °C was studied. Both PPO and

POD stored at room temperature showed good activity with a half life of 3–7.5 months (Table 2). Sap stored at 4 °C had better enzyme activities with a half-life of 8 months to more than a year (Table 2). This may be having an important bearing on the use of aqueous phase as a source of enzyme for various applications.

In summary, the present study indicated that the aqueous phase of the mango sap contains little protein, which may be associated with the activities of oxidative enzymes mainly PPO and POD. Sap is also rich in carbohydrate (260–343 mg/ml sap), which probably gives a viscous nature to the sap. The high content of carbohydrate may also give the sap a colloidal nature thus enabling it to draw water from the peel and pulp into the duct system and helping to maintain the sap in the lactiferous system at considerable pressure. Recently, we have reported that some of the components of mango sap showed antimicrobial activity (Negi et al., 2002). Since the mango sap is present at considerable pressure in the lactiferous system the infected or damaged area may be oozed with the sap as a protective measure to localize the damage and immobilize the pathogen.

Mango sap is a rich source of PPO (147–214 U/mg protein) and POD (401–561 U/mg protein) enzymes having several commercial applications (Matheis and Whitaker, 1987). Earlier, we reported that the non-aqueous phase of mango sap is rich in terpenoids like ocimene, β-myrcene, limonene which impart raw mango aroma (Saby John et al., 1999) and that it can be used as a food preservative as it possess antimicrobial properties (Negi et al., 2002). Therefore, mango sap which is generally collected systematically from the harvested mangoes to avoid sap-injury could be a valuable by-product.

3. Experimental

3.1. Plant materials

Mango varieties *Badami* and *Seedling* grown on the campus of C.F.T.R.I., Mysore, Karnataka, *Totapuri* obtained from the Ramakrishna Vidyashala, Mysore, Karnataka, and *Banganapalli* variety grown in Chirala, Andhra Pradesh, were used in this study.

3.2. Collection of mango sap and separation into aqueous and non-aqueous phases

As described previously (Saby John et al., 1999) mango fruits were harvested with pedicels intact. Subsequently, the pedicels were detached from the fruit at the abscission zone and the sap was collected into glass tubes for about 1 min. The sap was collected at three different stages of fruit development and these stages were defined based on the time period from initiation of flowering; stage I–3 months; stage II–4 months; stage III—harvest maturity. Mango sap was separated into aqueous and non-aqueous phases by centrifugation at 3000 × *g* at room temperature (Saby John et al., 1999) and the aqueous phase thus obtained used for various studies.

Table 2
Storage stability of PPO and POD of sap from different varieties

Variety	Enzyme	Half-life stability (months)	
		4 °C	Room temperature
Badami	PPO	8.5	6.0
	POD	9.0	3.5
Banganapalli	PPO	8.0	7.0
	POD	10.0	7.0
Totapuri	PPO	9.5	7.5
	POD	13.0	3.0
Seedling	PPO	11.5	7.5
	POD	14.5	6.0

3.3. Protein, carbohydrate, total phenolics contents in mango

Protein content in aqueous phase of sap was determined using Bradford's method (Bradford, 1976). The total carbohydrate content in aqueous phase of sap was determined using phenol sulfuric acid method using glucose as standard (Dubois et al., 1956). To determine the free sugars content in aqueous sap, the aqueous sap was subjected to 70% alcohol precipitation at 4 °C for 3 h with occasional shaking, centrifuged at 2000 × *g*, and the free sugar content in supernatant was estimated by phenol sulfuric acid method using glucose as standard (Dubois et al., 1956). The starch content was determined using the iodometric method of Hassid et al. (1964). To find out the dialyzable carbohydrate content, sap was dialysed against water for one day (three changes) using 12,000 molecular weight cut off dialysis tube and the carbohydrate content in the sap was estimated before and after dialysis using phenol sulfuric acid method (Dubois et al., 1956). The content of total phenolics in aqueous phase of sap was determined using Folin–Ciocalteu reagent and the absorbance of the colour developed was measured at 675 nm by the method of Swain and Hillis (1959). Gallic acid in 80% ethanol was used as a standard.

3.4. Enzyme assays

Peroxidase (POD) activity in aqueous phase of sap was measured using 100 μl of 1% hydrogen peroxide and 100 μl of 8 mM ortho-dianisidine as substrates in 1 ml of reaction mixture at its optimum pH 6.0, in 50 mM sodium acetate buffer (Aparicio-Cuesta et al., 1992).

One unit of activity was defined as that amount of enzyme which produced an increase of one absorbance per minute at 460 nm. Polyphenol oxidase (PPO) activity was assayed using 100 μl of 0.5 M catechol as substrate in 1 ml of reaction mixture at its optimum pH 6.0, in 50 mM sodium acetate buffer (Coseteg and Lee, 1987). One unit of polyphenol oxidase activity was defined as that amount of enzyme, which produces an increase in absorbance of one per minute at 420 nm in 1 ml reaction mixture. Protease content was estimated using azocasein as substate (Sarath et al., 1989). In the case of the serine protease assay 50 mM Tris–HCl (pH 8.0) buffer and in case of cysteine protease assay, buffer containing sodium phosphate (50 mM)-cysteine (35 mM)-disodium EDTA (38 mM) (pH 6.0) was used (AOAC, 1975). An increase in absorbance of one per minute was regarded as 1 unit of activity in one ml of reaction mixture. Amylase was assayed using 1% gelatinized soluble starch solution as substrate at pH 6.0 (Bernfield, 1955). One unit of activity was defined as that amount of enzyme which catalyses the liberation of reducing sugar equivalent to 1 μmole per minute. Polygalacturonase was assayed according to the procedures described by Luchsinger and Cornesky (1962), Pressey and Avants (1976), using the reaction mixture consisting of 0.2 ml enzyme in 0.15 M NaCl, 0.2 ml of 0.2 M Tris-acetate buffer (pH 4.5), 0.1 ml of 0.01 M CaCl2 and 0.5 ml of 1% polygalacturonate. One unit of activity was defined as the amount which catalyses the formation of 1 μmol of reducing group per hour. Pectin methyl esterase (PME) was assayed according to the method described by Priya Sethu et al. (1996). One unit of PME activity was defined as the amount of enzyme capable of catalyzing the consumption of 1 mmol of base per hour under the assay conditions. Lipoxygenase activity in the non-aqueous phase of sap was assayed according to the method described by Shiiba et al. (1991) using linoleic acid as substrate. Enzyme activity was expressed in terms of conjugated diene hydroperoxide formed (μmol/min). Catalase assay was performed according to the method of Aebi (1984) in which the decomposition of H_2O_2 was followed directly by the absorbance at 240 nm. One unit of activity was defined as the degradation of 1 μmol of H_2O_2 per min.

3.5. Stability of polyphenol oxidase and peroxidase in mango sap

Mango sap aqueous phase was kept separately at 4 °C and room temperature, aliquots were drawn, and assayed for polyphenol oxidase and peroxidase activities at regular intervals.

3.6. Polyacrylamide gel electrophoresis

The aqueous phase of sap was mixed with equal volume of 2x sample buffer, centrifuged at 8000 × *g* and 50 μl of supernatant was applied on 7.5% native gel. The gel composition, running buffer composition, staining and destaining procedures were followed according to the method described by Laemmli (1970) without SDS and β-mercaptoethanol. The gel was cut into three parts: one part was subjected to protein staining, another was for polyphenol oxidase staining and the third was for peroxidase staining. Proteins were stained with Coomassie brilliant blue R-250, polyphenol oxidase was detected in the gel using catechol and p-phenylenediamine as described by Lee (1991), and peroxidase was detected using 3,3′-diaminobenzidine as described by Hoffman et al. (1970).

3.7. Statistical analysis

Statistical analysis was done using the method of Steel and Torrie (1980).

Acknowledgements

Ms. K. Saby John thanks council of Scientific and Industrial Research, New Delhi, India, for the award of Senior Research fellowship. The authors also thank Sri Ramakrishna Vidyasala, Mysore, for permission to collect the mangoes from their orchards.

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