ACTA
PHYSIOLOGIAE
PLANTARUM
Vol. 23. No. 3. 2001: 357-362

Compositional and enzymatic changes in guava (*Psidium guajava* L.) fruits during ripening

Nisha Jain, Kamal Dhawan, Sarla P Malhotra, Saleem Siddiqui** and Randhir Singh*

Plant Biochemistry and Molecular Biology Laboratory, Department of Biochemistry,
**Department of Horticulture, CCS Haryana Agricultural University, Hisar-125 004, India
*Corresponding author: tel. 01662-37720-26-4500, fax. 01662-34952, e-mail hau @ hau.ren.nic.in

Key words: Guava, *Psidium guajava*, starch, cell wall polysaccharides, hydrolytic enzymes.

Abstract

Changes in chemical composition and hydrolytic enzyme activities in guava fruits cv. Lucknow-49 have been reported at four different stages of maturity, *viz.*, mature green (MG), color turning (CT), ripe (R) and over ripe (OR). Chlorophyll content decreased, while carotenoid content increased with advancement of ripening. Starch content decreased with concomitant increase in alcohol soluble sugars. The cell wall constituents *viz.*, cellulose, hemicellulose, and lignin decreased up to R stage, while the pectin content decreased throughout up to OR stage. Among the cell wall hydrolyzing enzymes, polygalacturonase (PG) and cellulase exhibited progressive increase in activity throughout ripening, while pectin methyl esterase (PME) activity increased up to CT stage and then decreased up to OR stage. The maximum increase in the activities of cell wall hydrolysing enzymes was observed between MG and CT stages. The activities of starch hydrolyzing enzymes, α-amylase and β-amylase decreased significantly with advancement of ripening . These changes in the activities of hydrolyzing enzymes could be considered good indicators of ripening in guava.

List of abbreviations: **MG**, mature green; **CT**, colour turning; **R**, ripe; **OR**, over ripe; **PG**, polygalacturonase; **PME**, pectin methyl esterase; **Chl**, chlorophyll.

Introduction

Guava (*Psidium guajava* L.) is grown all over India and is known to be the most popular fruit like mango, banana, citrus and papaya. Nutrient composition (Paull and Goo 1983) and gelling properties (Wilson 1980) of guava with easy cultivation have made it important in traditional trade as well as in domestic economy of most tropical countries (Samson 1986). Ripe fruits being very soft, get bruised easily and damaged during handling, transit and storage, while unripe fruits can withstand more stress during transport. Thus to increase the shelf life of guava fruit, it is important to understand the ripening process which involves complex metabolic and cellular changes including the synthesis of new proteins and enzymes. Regulation of enzyme activities in order to slow down ripening process with the help of chemicals and/ or through environmental manipulation has been studied in detail (Yang *et al.* 1990). Modification of fruit ripening by suppressing expression of specific enzymes has been tried in tomatoes (Gray *et al.* 1992, Theologis *et al.* 1992). Before we try similar approaches in guava, detailed studies on compositional and enzymatic changes involved in cell wall hydrolysis *etc.* are needed. Guava, being a climac-

teric fruit, exhibits a typical increase in respiration and ethylene production during ripening. This leads to early softening and spoilage. However, unripe guava fruits are very hard and could be transported to long distance without much of the damage. Thus, increase in shelf-life of such fruits would lead to their better use and bring greater profits to farmers. This is possible through regulation of ripening process which as stated above entails detailed information on biochemistry of fruit ripening. Such information is available for fruits like tomato (Richardson and Hobson 1987), mango (Pandey *et al.* 1998), apple (Selvaraj and Kumar 1989) and banana (Mustaffa *et al.* 1998) *etc.* However, very little is known about the biochemical changes occurring during ripening of guava fruits. The present paper reports the biochemical and enzymatic changes occurring during ripening of guava fruit cv. Lucknow-49. The information obtained could be useful to identify the stage at which the fruits should be picked up from the fields to transport them to distant places.

Material and Methods

Guava fruits cv. Lucknow-49 were obtained from four years old trees from the Experimental Orchard of the Department of Horticulture, CCS, Haryana Agricultural University, Hisar. Based on visual observations of texture and pigmentation, they were collected at four different stages, *i.e.* mature green (MG), colour turning (CT), ripe (R) and over-ripe (OR). Fruit pulp was oven dried at 60 °C to a constant weight and used for further analysis. For enzymatic studies, fruits were dipped in liquid N_2 and stored in an ultrafreezer maintained at -80 °C.

Chemical analysis

Chlorophyll and carotenoids were extracted in 80 % aqueous acetone and determined by the method of Arnon (1949) as modified by Wellburn (1994). Total sugars were extracted in 80 % ethanol and estimated according to Yemm and Willis (1954) using anthrone (0.2 % in 70 % H_2SO_4). Reducing sugars were measured using arsenomolybdate reagent by the method of Nelson (1944) modified by Somogyi (1952). Non-reducing sugars were determined by difference. Starch was extracted from sugar free residue by 52 % perchloric acid (Clegg 1956) and was estimated by anthrone taking glucose as the standard. Starch content was calculated by multiplying glucose concentration by a factor of 0.9. Cell wall polysaccharides *viz.*, hemicellulose, cellulose and lignin were determined according to Singh and Pradhan (1980). Total pectin was extracted by the method of Ahmed and Labavitch (1977) and determined as uronic acid by following the procedure of Blumekrantz and Asboe-Hansen (1973).

Enzyme extraction and assay

Preliminary experiments were conducted to standardize the extraction conditions with respect to buffer components, molarity and pH of buffer and concentration of stabilizing agents *etc.* Assay methods were standardized to get linearity between enzyme activity *vs.* enzyme concentration and incubation time.

Polygalacturonase (PG; EC 3.2.1.15) and cellulase (EC 3.2.1.4) were extracted from the fruit pulp (1.0 g) in a medium containing 0.1 M sodium acetate buffer (pH 5.2), 0.02 M sodium metabisulfite and 10 % (w/v) sodium chloride by homogenising in a pre chilled pestle and mortar. The homogenate was centrifuged at 10,000 x g for 30 min. The supernatant obtained was dialysed against 0.01 M sodium acetate buffer (pH 5.2) (Singh and Singh 1993). The assay mixture contained 0.2 ml of enzyme extract, 0.2 ml sodium acetate buffer (0.1 M, pH 5.2), and 125 µg each of chloramphenicol and cycloheximide. The substrate (0.5 ml) for polygalacturonase was 0.3 % polygalacturonic acid and for cellulase 0.5 % carboxymethyl cellulose (Ahmed and Labavitch 1980). The reaction mixture in each case was incubated at 37 °C for 20 h and the reaction terminated by heating in a boiling water bath for 10 min. Reducing sugars thus liberated were determined by the procedure of Nelson (1944) modified by Somogyi (1952). One unit of polygalactouronase activity is defined as the amount of enzyme required to release 10 µg of galacturonic acid per 20 h at 37 °C, whereas one unit of cellulase activity is equivalent to release of 10 µg of glucose per 20 h at 37 °C. Pectin methyl esterase (PME, EC 3.1.1.11) was extracted from fruit pulp (4.0 g) in 10 ml of 0.4 M sodium chloride (pH 7.5). After 1 h, the mixture was centrifuged at 6,000 x g for 20 min at 4

°C. The enzyme was assayed by the method of Hobson (1963). Five ml of 0.5% apple pectin in 0.1 M NaCl (pH 7.5) was incubated with 3 ml of enzyme extract for 30 min at 25 °C. The carboxyl groups liberated by the action of PME were estimated by titrating with 0.01 N NaOH using 0.1% methyl red as indicator. Milliequivalents of carboxyl groups was calculated by multiplying titer value with normality of alkali used. One enzyme unit is defined as the amount of enzyme required to release one microequivalent of carboxyl group·h^{-1}·g^{-1} fresh weight. α- and ß-amylases were extracted from fruit pulp (1 g) by using citrate phosphate buffer (0.1 M; pH 5.7) containing 1 mM EDTA, 6 mM ß-mercaptoethanol and 10 mM $MgCl_2$. After centrifugation at 10,000 x g for 15 min at 4 °C, the supernatant was dialysed against 0.01 M citrate phosphate buffer (pH 5.7) for 4 h. α-amylase was assayed by incubating the enzyme extract (1.0 ml) with 1% starch solution. In case of a-amylase, starch solution was prepared in 0.2 M phosphate buffer (pH 6.9) containing 6.7 mM NaCl, while for ß-amylase, starch was dissolved in 16 mM acetate buffer (pH 4.8). The reaction was terminated by addition of 2 ml of dinitrosalicylic acid reagent (1 g dinitrosalicylic acid dissolved in 20 ml of 2 N NaOH containing 30 g Rochelle salt and volume made to 100 ml). The mixture was heated for 5 min in boiling water bath and 2.0 ml of distilled water was added. The absorbance was read at 540 nm. The amount of maltose released was calculated from a standard curve prepared by using maltose (0.2-2 mg). One unit of enzyme is defined as the amount of enzyme required to release 10 µg of maltose under assay conditions.

Results and discussion

The content of total chlorophyll, chlorophyll a and chlorophyll b decreased while that of carotenoid increased during ripening of fruits (Fig. 1), indicating the chlorophyll content to be a good parameter to determine the age of guava fruit. The mature green fruits were dark green in colour with chlorophyll content of 1.45 mg/100 cm^2, which decreased to 0.33 mg/100 cm^2 at over ripe stage. Chlorophyll a and chlorophyll b similarly decreased during ripening. However, the amount of chlorophyll b was more than that of chlorophyll a at all the stages of

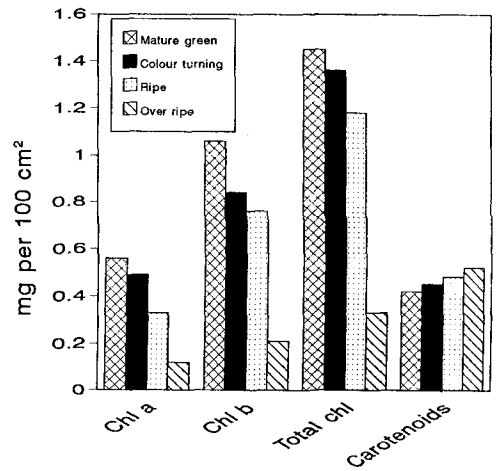

Fig. 1. Chlorophyll and carotenoid contents of guava at different stages of ripening. (C.D. at 5 % for chl a, chl b, total chl and total carotenoids was 0.02, 0.03, 0.09 and 0.02 respectively).

fruit ripening. These results are in close agreement with those reported earlier for apple (Schubret and Dathe 1973), and ber (Meel *et al.* 1991). The loss in chlorophyll during ripening is due to increased activities of chlorophyll degrading enzymes including chlorophyllase (Looney and Patterson 1967), chlorophyll oxidase and peroxidase (Martinoida *et al.* 1982, Abeles *et al.* 1988). This decrease in chlorophyll content was accompanied by increase in carotenoid content, which reached to a level of 0.52 mg/100 cm^2 in over ripe fruits. The increase in carotenoid content has also been reported in ripening mango (Mann *et al.* 1974) and ber fruits (Bal *et al.* 1978), while Sharma (1996) observed no signifi-

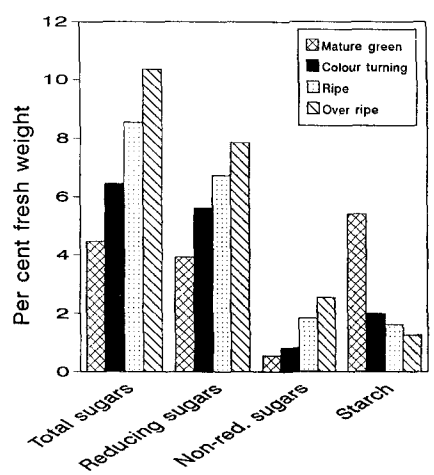

Fig. 2. Sugar and starch content of guava at different stages of ripening. (C.D. at 5 % for total, reducing, non-reducing sugars and starch was 0.67, 0.54, 0.41 and 0.39 respectively).

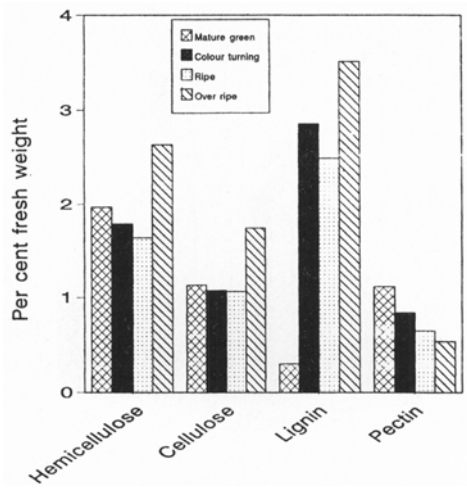

Fig. 3. Hemicellulose, cellulose, lignin and total pectin contents of guava at different stages of ripening. (C.D. at 5 % was 0.14, 0.10, 0.14 and 0.08 respectively).

cant difference in carotenoids during ripening of ber.

The ripening fruits are known to store starch transiently, which is ultimately converted to sugars leading to sweet taste of ripe fruits. In ripening guava, starch content decreased progressively, while the soluble sugars increased approximately two fold (Fig. 2). Total and reducing sugars increased from 4.5 and 4.0 % at MG to 10.4 and 7.8 % at OR stage, respectively. Banana fruits have been shown to exhibit a ten fold increase in soluble sugars from 1.8 % (unripe) to 18.6 % (ripe) during ripening (Prabha and Bhagyalakshmi 1998). This increase in sugar content was attributed to the conversion of starch into soluble sugars (Prabha and Bhagyalakshmi 1998) and to the breakdown of hemicellulose and pectins (Cheng and Huber 1996, 1997). Similar increase in total soluble sugars has been reported in ripening mango (Pandey *et al.* 1998), tomato (Singh *et al.* 2000), papaya (Firmin 1997), and banana (Mustaffa *et al.* 1998).

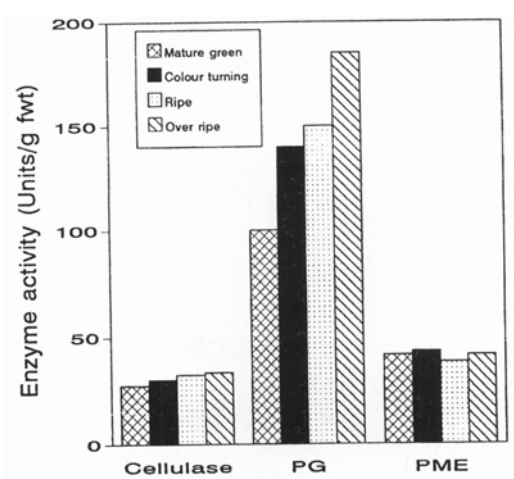

Fig. 4. Cellulase, polygalacturonase and pectin methyl esterase activities of guava at different stages of ripening. (C.D. at 5 % was 10.50, 10.18 and 1.54 respectively).

Cell wall composition plays an important role in determining fruit firmness. Data on the components of cell wall polysaccharides are presented in Fig. 3. The cell wall components *viz.*, hemicellulose, cellulose and lignin decreased significantly to 1.64, 1.20 and 2.48 %, respectively during ripening of fruits and thereafter increased to 2.63, 1.74 and 3.51 % respectively in over-ripe fruits. The decrease in cell wall components may be due to the increase in the activity of cell wall hydrolyzing enzymes during ripening (Prabha and Bhagyalakshmi 1998). However, increase in cell wall components at OR stage may either be due to loss in moisture or due to depolymerization of hemicelluloses (Lazen *et al.* 1995) or to utilization of reserve material for fruit respiration resulting in the softening of the fruits. In the latter process, proportion of stone cells which have highly lignified secondary cell walls increases (Marcelin *et al.* 1993). The decrease in hemicellulose content in mango (Mitcham and Mc Donald 1992) and in cellulose content in ber (Sharma 1996) has also been reported. Total pectin was found to be more abundant in mature green fruits (1.11 %) which decreased continuously up to over ripe stage, suggesting that mature green fruits could be good source for preparing commercial pectin.

Activities of cell wall degrading enzymes *viz.*, cellulase, polygalacturonase and pectin methyl esterase are shown in Fig. 4. PG activity increased significantly throughout ripening and the increase was found to be maximum between MG and CT stages *i.e.*, 100 and 140 units·g^{-1} fresh weight, respectively. Increase in PG activity during fruit ripening has also been reported in several other fruits including banana (Prabha and Bhagyalakshmi 1998). The activity of cellulase also increased throughout ripening, however, the increase was less as compared to PG at all stages of ripening. This suggests that cellulase has a minor role in softening of the fruits. The increase in cellulase activity has also been observed in other fruits like mango

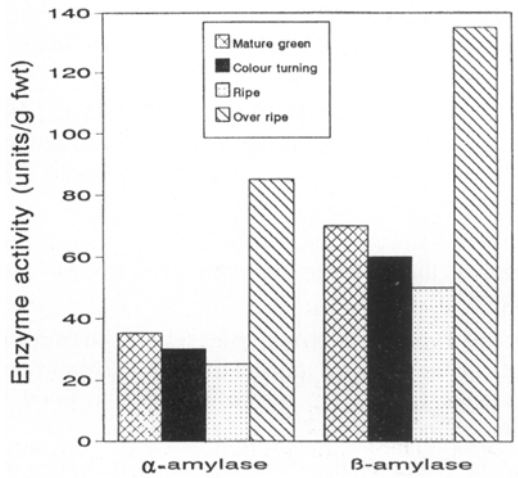

Fig. 5. α-amylase and ß-amylase activities of guava at different stages of ripening. (C.D. at 5 % was 4.52 and 15.34 respectively).

(El-Zoghbi 1994) and cherimoya (Sanchez *et al.* 1998). The activity of PME was almost constant throughout ripening except a slight increase at CT stage, which suggested that the PME plays a little role (Priya Sethu *et al.* 1996) after formation of pectic substances for PG. Similar observations were made earlier by Chin *et al.* (1994) in guava.

The activities of starch hydrolyzing enzymes, *i.e.* α- and ß-amylase decreased initially and then increased abruptly at over ripe stage (Fig. 5). The activity of amylase though decreased during the ripening, the inherent activity in fruits was perhaps sufficient to hydrolyze starch because starch is being hydrolyzed to sugars in the present study. The decrease in the activity of amylases during fruit ripening has also been observed in papaya (Pal and Selvaraj 1987) and ber (Sharma 1996). However, Prabha and Bhagyalakshmi (1998) found an increase in amylases during ripening of banana fruits.

From the results discussed above, it could be concluded that maximum metabolic changes in ripening guava occur during transition from MG to CT stage. Thus for desired post-harvest fruit handling or suppressing expression of specific enzymes, the phase from MG to CT stage need to be exploited.

References

Abeles F.B., L.J. Dunn, P. Morgan, A. Callahan, R.E. Dinterman, R.E. Schmidt and J. Schmidt, 1988. Induction of 33 KD and 60 KD peroxidase during ethylene induced senescence of cucumber cotyledons. Plant Physiol. 87: 609-615.

Ahmed A.E. and J.M. Labavitch, 1977. A simplified method for accurate determination of cell wall uronide content. J. Food Biochem. 1: 361-365.

Ahmed A.E.R. and J.M. Labavitch, 1980. Cell wall metabolism in ripening fruit. II. Changes in carbohydrate degrading enzymes in ripening barlett pears. Plant Physiol. 65: 1014-1016.

Arnon D.L., 1949. Copper enzyme in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 24: 1-5.

Bal J.S., P. Singh and S.S. Mann, 1978. Changes in total yellow pigment, protein and total carbohydrates during ripening of ber fruits. Prog. Hortic. 10(1): 73-75.

Blumekrantz N. and G. Asboe-Hansen, 1973. New method for quantitative determination of uronide acids. Analytical Biochem. 54: 484-489.

Cheng G.W. and D.J. Huber, 1996. Alteration in structural polysaccharides during liquifaction of tomato locule tissue. Plant Physiol. **111**: 447-457.

Cheng G.W. and D.J. Huber, 1997. Carbohydrate solubilization of tomato locule tissue liquifaction during ripening. Physiol. Plant. 101: 51-58.

Chin L.H., Z.M. Ali and H. Lazan, 1994. Comparative softening of guava fruits. Solubilization and depolymerization of cell wall carbohydrates during ripening. Proc. Malayasian Biochem. Soc. Confer. 19: 147-150.

Clegg K.M., 1956. The application of the anthrone reagent to the estimation of starch in cereals. J. Sci. Food Agric. 1: 40-44.

El-Zoghbi M., 1994. Biochemical changes in some tropical fruits during ripening. Food Chem. 49: 33-37.

Firmin A., 1997. Physico-chemical changes in papaya during storage. Tropical Sci. 37(1): 49-51.

Gray J.E., S. Picton, J. Shabbeer, W. Schuch and D. Gricrson, 1992. Molecular biology of fruit ripening and its manipulation with antisense genes. Plant Mol. Biol. 19: 69-87.

Hobson A.M., 1963. Pectin esterases in normal and abnormal tomato fruit. J. Biochem. 86: 358-361.

Lazan H., M.K. Selamat and Z.M. Ali, 1995. ß-galactosidase, polygalacturonase and pectinesterase in different softening and cell wall modification during papaya fruit ripening. Physiol. Plant. 95: 106-112.

Looney N.E. and M.E. Patterson, 1967. Chlorophyllase activity in apples and bananas during climacteric phase. Nature 214: 245-246.

Mann S.S., P.N. Singh and R.M. Pandey, 1974. Maturity studies in Dashehari and Langra cvs. Of mango (*Mangifera indica* L.). Haryana J. Hortic. Sci. 3: 97-105.

Marcelin O., P. William and J.M. Brillouet, 1993. Isolation and characterization of the two main cell types from guava (*Psidium guajava* L.) pulp. Carb. Res. 240: 233-243.

Martinoida E., M.J. Dalling and P. Matile, 1982. Catabolism of chlorophyll : Demonstration of chloroplast – localized peroxidative and oxidative activities. Z. Pflanzen Physiol. 107: 269-279.

Meel O.P., A.S. Charia, R. Kumar and M.S. Joon, 1991. Physico-chemical changes in the fruits of Sandhura Narnaul ber during growth and development. Haryana J. Hortic. Sci. 20: 65-72.

Mitcham E.J. and R.E. McDonald, 1992. Cell wall modification during ripening of Keitt and Tommy Atkins mango fruits. J. Amer. Soc. Hortic. Sci. 17: 919-924.

Mustaffa R., A. Osman, S. Yusof, and S. Mohamed, 1998. Physico-chemical changes in Cavendish banana (*Musa cavendishii* L. var. Montel) at different positions within a bunch during development and maturation. J. Sci. Food Agric. 78(2): 201-207.

Nelson N.J., 1944. A photometric adaptation of the Somogyi method for determination of glucose. J. Biol. Chem. 153: 375-380.

Pal D.K. and Y. Selvaraj, 1987. Biochemistry of papaya fruit ripening changes in RNA : DNA, protein and enzymes of mitochondrial, carbohydrate, respiratory and phosphate metabolism. J. Hortic. Sci. 62: 117-124.

Pandey M., G.C. Srivastava and N.K. Prasad, 1998. Physiological changes associated with ripening in two mango varieties. Indian J. Plant Physiol. 3:94-96.

Paull R.E. and T. Goo, 1983. Relationship of guava (*Psidium guajava* L.) fruit detachment force to the stage of fruit development and chemical composition. Hort. Sci. 18: 65-67.

Prabha T.N. and N. Bhagyalakshmi, 1998. Carbohydrate metabolism in ripening banana fruit. Phytochem. 48: 915-919.

Priya Sethu K.M., T.N. Prabha and R.N. Tharanathan, 1996. Post-harvest biochemical changes associated with the softening-phenomenon in *Capsicum annuum* fruits. Phytochem. 42(4): 961-966.

Richardson C. and G.E. Hobson, 1987. Composition changes in normal and mutant tomato fruit during ripening and storage. J. Sci. Food Agric., 40: 245-252.

Samson J.A., 1986. Tropical Fruits, Second edition. Longman Scientific and Technol, Longman Group UK Ltd.

Sanchez J.A., J.P. Zamorano and R. Alique, 1998. Polygalacturonase, cellulase and invertase activities during cherimoya fruit ripening. J. Hortic. Sci. Biotechnol. 73: 87-92.

Schubret Et. and E.M. Dathe, 1973. Experiments to determine the maturity of apples for storing. Arch. Cartenb. 21: 353-359.

Selvaraj Y. and R. Kumar, 1989. Studies on fruits softening enzymes and polyphenol oxidase activity in ripening mango (*Mangifera indica* L.) fruit. J. Food Sci. Technol. 26: 218-222.

Sharma R.K., 1996. Physiological and biochemical studies during ripening in ber fruits on tree and in storage. Ph.D. Thesis, CCS Haryana Agric. Univ., Hisar.

Singh A. and M. Singh, 1993. Cell wall degrading enzymes in *Orobanche aegyptiaca* and its host *Brassica campestris*. Physiol. Plant. 89: 177-181.

Singh M., K. Dhawan and S. Malhotra, 2000. Carbohydrate metabolism in Tomato (*Lycopersicon esculentum* L. Mill) fruits during ripening. J. Food Sci. Technol. 37: 222-226.

Singh R. and K. Pradhan, 1980. Forage evaluation. Allied Publishers, New Delhi.

Somogyi M., 1952. Notes on sugar determination. J. Biol. Chem. 195: 19-23.

Theologis A., T.L. Zarembenski, P.W. Oeller, Z. Liang and S. Abel, 1992. Modification of fruit ripening by suppressing gene expression. Plant Physiol. 100: 549-551.

Wellburn A.R., 1994. The spectral determination of chlorophylls a and b as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144: 307-313.

Wilson C.W., 1980. Guava. In: Nagy, S. and Shaw, P.E. (eds.). Tropical and Subtropical Fruits : Composition, Properties and Uses. AVI Publ. Inc., Westport Connecticut.

Yang R.F., T.S. Cheng and R.L. Shewfelt, 1990. The effect of high temperature and ethylene treatment on the ripening of tomatoes. J. Plant Physiol, 136: 368-372.

Yemm E.W. and A.J. Willis, 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57: 508-514.

Received September 19, 2000; accepted March 19, 2001