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Phenolic changes during different stages of fruit ripening of climacteric fruit of mango (*Mangifera indica* L.) and non-climacteric fruit of cashew apple (*Anacardium occidentale* L.)

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R. Rooban*, M. Shanmugam, T. Venkatesan and C. Tamilmani

Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India

Abstract

The present investigation was made to study the ripening behaviour of climacteric fruit of mango (*Mangifera indica* L.) and a non–climacteric fruit of cashew apple (*Anacardium occidentale* L.) The different stages of fruits are immature, mature, quarter ripen, half ripen, full ripen and over ripen fruits. The total phenol content and the activities of peroxidase, polyphenoloxidase and catalase of peel and pulp tissues of mango, pericarp tissues of cashew apple fruits were estimated. The phenol content was high at immature stage and low in over ripen stage. On the other hand peroxidase, polyphenoloxidase and catalase were low in immature stage and high in over ripen stage. The phenol content was gradually decreased while peroxidase, polyphenoloxidase and catalase activities were gradually increased both in mango and cashew apple fruits.

Keywords: Mango, Cashew apple, Phenol, Peroxidase, Polyphenoloxidase and Catalase.

INTRODUCTION

Fruit ripening involves dramatic changes in the colour, texture, flavour, and aroma, characteristics which are highly attractive to humans. In addition, fresh and processed fruits are important components of the human diet as they provide sugars, fiber, vitamins, minerals and antioxidants (Barry *et al.,* 2005).

Fruits can be divided into two groups according to the regulatory mechanisms underlying the ripening process. Climacteric fruits, such as tomato, apple, pear and melon are characterized on the basis of ripening-associated processes such as increase in respiration and ethylene production. On the contrary, non-climacteric fruits, such as orange, grape and pineapple are characterized by the lack of ethylene-associated respiratory peak. At the onset of climacteric fruit there is a peak in respiration, and a concomitant burst of ethylene production. The relationship existing between the climacteric respiration and fruit ripening has been disputed on the basis of th reports which prove that the ripening of fruits could occur without any evidence of associated increase in respiratin (Salveit, 1993; Shellie and Salveit, 1993).

Polyphenols are a large group of organic compounds found in plants, characterized by the presence of phenol units in the structure. Polyphenols are generally divided into two subgroups, hydrolyzable tannins and phenylpropanoids. All phenylpropanoids are biosynthesized from the amino acid phenylalanine. They have a variety of functions including protection from UV light, defense against pathogens or herbivores, as structural components of cell wall, or as pigments and signaling molecules (Weisshaar and Jenkins, 1998; Vogt, 2009).

In fruits, phenylpropanoids constitute one of the most important groups of secondary metabolites. Phenylpropanoid compounds offer protection during the early stages of fruit development and act as pigments serving visual signals for seed dispersers in ripen fruits. Phenylpropanoids have a major role in quality characteristics of ripen fruits and fruit products. They contribute not only to the characteristic flavour and colour but also to unfavourable traits such as browning of fruit tissues via enzymatic oxidation of phenolic compounds by polyphenol oxidases (Macheix *et al.,* 1990). Fruit phenylpropanoids form an important part of the recommended human diet. Phenylpropanoid compounds have long been assumed to be antioxidants that scavenge excessive damaging free radicals, which arise from normal metabolic processes (Stevenson and Hurst, 2007). There is increasing evidence for many potential benefits of phenylpropanoids in the regulation of cellular processes such as inflammation from human trials (Badimon *et al*., 2010). Phenylpropanoid compounds can also have indirect antioxidant effects through the induction of endogenous protective enzymes (Stevenson and Hurst, 2007).

*Corresponding Author :
email: *roobanshankar@gmail.com*

Mango is a climacteric fruit, exhibiting a climacteric pattern of respiration and an increase in ethylene production during ripening (Cua and Lizada, 1990; Reddy and Srivastava, 1999; Lalel *et al*., 2003). The initiation of ethylene production within the fruit triggers and coordinates the changes that occur during ripening. These changes include colour changes in the peel and pulp, softening of the pulp, and development of sweet flavour and aroma. Mangoes can be ripened after harvest when picked at physiological maturity (mature-green), when they are fully sized, but before ripening has been initiated. Maturity indices are chosen to predict fruit quality potential and post harvest behaviour (Peacock *et al*., 1986; Medlicott *et al*., 1988).

Polyphenol oxidase catalyses the oxidation of mono and diphenols into *o*-quinones, which then polymerize to produce brown pigments. PPO activity increases slightly from harvest maturity to the half-ripe stage and then declines in 'Banganapalli', 'Dashehari', 'Fazli' and 'Langra' mangoes, and decreases in 'Alphonso', 'Suvarnarekha' and 'Totapuri' mangoes (Selvaraj and Kumar, 1989). The PPO isolated from 'Haden' mango is active towards the *o*-diphenolic compounds, showing higher activity in the presence of catechol, followed by chlorogenic acid, but not with monophenols (Park *et al*., 1980).

Cell wall degeneration occurs due to the action of hydrolases, including pectolytic enzymes such as polygalacturonase (PG), pectin methylesterase (PME) and pectate lyase (PL) (Brummell and Harpster, 2001). PG, an important hydrolytic enzyme, is the primary enzyme playing a significant role in pectin dissolution *in vivo* which would result in textural softening and loosening of cell structure. PG acts on pectic acid (polygalacturonic acid) and hydrolyses _-1, 4-linked D-galacturonic acid, following de-esterification of pectin by PME(Brownleader *et al*., 1999). On the other hand, PME catalyses the hydrolysis of pectin methylester groups resulting in deesterification (Ren and Kermode, 2000).

In this sense, antioxidant activity of cashew apples (*Anacardium occidentale* L.) is a topic interesting because tropical fruit consumption is increasing on the domestic and international markets due to growing recognition of its nutritional and therapeutic value. Brazil boasts a large number of fruit species interest to the agroindustry and a possible future source of income for the local population.

Ripe cashew apples are a good source of health promoting nutrients, such as ascorbic acid, cashew apples possess a five-fold greater ascorbic acid content (228 mg x 100 g-1) than orange. Besides, ripe cashew apples contain vitamin C and several other compounds with antioxidant potential, including carotenoids (0.40 mg x 100 g-1), yellow flavonoids (63.80 mg x 100 g-1), polyphenols (118 mg x 100 g-1), phenolic acids and oligomeric tannin (29 mg x 100-1) (Rufino *et al.,* 2010; Michodjehoun-Mestre *et al*., 2009).

Evidence suggests that the high content of fiber and antioxidants (e.g., ascorbic acid and polyphenols), of diets rich in fruits and vegetables may decrease the risk of chronic diseases (World Health Organization - WHO, 2003).This beneficial effect is due to the action of antioxidant compounds, which are capable of neutralizing free radicals and reduce oxidative damage in the body (Clifford, 1995). For this reason, the interest in the evaluation of antioxidant activity of fruits and vegetables has substantially increased and numerous studies have been performed (Thili *et al*., 2011; Ilahy *et al*., 2011; Park *et al*., 2011).

The present article deals with the ripening behaviour of climacteric fruit of mango (*Mangifera indic*a L.) and non-climacteric of fruit cashew apple (*Anacardium occidentale* L.), a detailed account of phenolic changes that occur during different stages of ripening and the difference between climacteric and non-climacteric fruits in the process of ripening .

MATERIALS AND METHODS

The detached climacteric fruits (*Mangifera indic*a L.) and non-climacteric fruits (*Anacardium occidentale* L.), belonged to the family Anacardaceace, were selected for the present study. The Mango and Cashew apple fruits were collected from Ramapuram Village in Cuddalore District, Tamil nadu. The fruits were kept at temperature of 28±2 º C with relative humidity of 85 per cent in the laboratory of Botany Department, Annamalai University. The different stages of fruits namely immature, mature, quarter ripen, half ripen, full ripen and over ripen fruits were used for analyses in Mango and Cashew apple fruits. All the experiments were conducted with seven replicates. The peel and pulp tissues of mango and pericarp tissues of cashew apple fruits were used to study the ripening process.

Estimation of total phenols

Total phenols were extracted and estimated, following the method of Chandramohan *et al.* (1973), and quantitative estimation was done based on Bray and Thorpe (1954) method.

Three grams of fruit material was homogenized in 10 ml of 90 per cent ethanol. The homogenate was filtered through a cheese cloth. The residue was extracted with 80 per cent ethanol and the filtrate was made up to 15 ml (1 g of material in a 5 ml of ethanol). To 1 ml of the ethanolic, 1 ml of folin-ciocalteau reagent and 2 ml of 20 per cent sodium carbonate were added. The mixture was kept in boiling water bath for 1 minute. Then it was cooled immediately in running water and the volume was raised to 25 ml. the colour intensity was read at 725 nm in a spectronic-20. The phenolic content was expressed as catechol equivalent.

Estimation of peroxidase

Fruit material weighing about 100 mg was homogenized with 10 ml of 1.0 N phosphate buffer (pH 7.0) in a prechilled mortar and pestle. The homogenate was centrifuged at 24,000 rpm and at 4ºC in a refrigerated centrifuge for 30 minutes, and the aliquot was used as the source of the enzyme.

Peroxidase activity was assayed by the method of Kumar and Khan (1982). Assay mixture of polyphenoloxidase contained 2 ml of 0.1 M phosphate buffer (pH 7.0) 1 ml of 0.01 M pyrogallol and 1 ml of well-dilute enzyme extract. This was incubated for 5 minutes at 25 ºC after which the reaction was stopped by adding 1 ml of 2.5 N H_2SO_4 to the assay mixture. The amount of purpurogalin formed was determined by taking the absorbance at 420 nm. The enzyme activity has been expressed in absorbance units.

Estimation of polyphenoloxidase

Polyphenoloxidase activity was assayed as described in the previous paragraph. Assay mixture of polyphenoloxidase contained 1 ml of 0.1 M phosphate buffer (pH 7.0) 1 ml of 0.01 M pyrogallol and 1 ml of well-dilute enzyme extract. This was incubated for 5 mintes at 25 ºC after which the reaction was stopped by adding 1 ml of 2.5 N H2SO4 TO THE ASSAY mixture. The amount of purpurogalin formed was determined by taking the absorbance at 420 nm. The enzyme activity was expressed in absorbance units.

Estimation of catalase

Catalase activity was assayed 2.5 ml of 0.1 M phosphate buffer (pH 6.4) was taken in a cuvette, and 0.1 ml of 2 per cent H_2O_2 and 0.2 ml of tissue extract was added. The reaction was read using a spectrophotometer at 230 nm for 75 seconds at an interval of 15 seconds, after the addition of peroxidase. The cuvette containing tissue extract and buffer alone was used to adjust the absorbance to zero. The control was maintained using boiled tissue extract.

RESULTS AND DISCUSSION

Mango *(Mangifera indica)* is a climacteric fruit. Cashew apple (*Anacardium occidentale)* is a non-climacteric fruit. The climacteric and non–climacteric fruits considerably differ in their ripening process. The different stages of fruits were used for the analyses namely immature, mature, quarter ripen, half ripen, full ripen and over ripened fruit. The phenolic changes parameters such as total phenol, peroxidase, polyphenoloxidase and catalase were determined. The results of the analyses which were obtained during different stages of fruit ripening are discuseed.

It was found that the phenol content gradually decreased during the course of ripening both in mango and cashew apple fruit s (Table 1). The phenol content was high at immature stage and low in over ripen stage in the peel and pulp of the fruits of both mango and cashew apple. The decrease in phenol content was more in the peel than in the pulp of both mango and cashew apple. It has been reported that the phenolic content of mangoes was high during early development, then decreased and remained fairly steady during ripening (Lakshminarayana and Subramanyam *et al*.,1970). This is associated with loss of astringency (Selvaraj and Kumar, 1989). The peel of mango fruit has a higher phenolic content than the pulp at all stages of fruit development (Jain, 1961; Lakshminarayana and Subramanyam *et al*., 1970). The gradual increase in total soluble phenolics was reported in mangoes, as starch was converted to simple sugars by amylase activity during storage (Gil et al., 2000). Moreover Adou et al. (2012) found that the level of total phenolic content was increased in two types of cashew apple fruits, yellow and red.

The activities of enzyme peroxidase, polyphenoloxidase and catalase were found gradually increased during the course of ripening both in mango and cashew apple fruits (Table 2, 3 and 4). All the three enzyme activites were low in immature stage and high at over ripen stage in the peel and pulp of the fruits of both mango and cashew apple. The increasing activity of enzyme was more in cashew apple followed by mango peel and pulp. The polyphenoloxidase activity was more than that of peroxidase and catalase both in mango and cahew apple fruits. Lopes et al. (2012) evaluated the cashew clones (CCP 09, CCP 76, BRS 265 and BRS 189) during the same seven stages of development and ripening stages showed that four clones were differing in their non-climacteric ripening and exhibited differences in their oxidative behaviour and CCP 09 showed that it was high during ripening, but at stage 7 the CCP 76 clone showed the behaviour higher than other clones. Additionally the analyses showed that the activity of PPO at mature (green) stage was found to be about 3 times lower than that at ripen stage. A positive correlation was observed between the increasing PPO and total PC at ripening stage. PPO is an oxidative enzyme, which catalyzes the oxidation of phenolic substrates mainly due to enzymatic browning (Jiang , 1999). Interestingly Venkatesan and Tamilmani (2010) observed in mango of off-season variety called 'neelum' that the phenolic content gradually decreased while peroxidase, polyphenoloxidase and catalase gradually increased.

CONCLUSION

Among the phenolic changes during different stages of fruit ripening of mango and cashew apple the phenol content was high at immature stage and low in over ripen stage. On the other hand peroxidase, polyphenoloxidase and catalase were low in immature stage and high in over ripen stage. The phenol content

gradually decreased while peroxidase, polyphenoloxidase and catalase gradually increased both in mango and cashew apple fruits.

REFERENCES

Adou marc Kouassi Didier ange, Tetchi fabrice achille and Amani n'guessan georges. 2012. Phenolic profile of cashew apple juice (*Anacardiumoccidentale* L.) from Yamoussoukro and Korhogo(Côte d'Ivoire) *J. Appl. Biosciences,* 49: 3331– 3338 ISSN 1997–5902. 2012

Badimon, L., Vilahur, G., and Pardo, T. 2010. Nutraceuticals and atherosclerosis: human trials. *Cardiovascular Therapeutics*, 28: 202–215.

Barry, C.S., McQuinn, R.P., Thompson, A.J., Seymour, G.B., Grierson, D., Giovannoni, J.J. 2005. Ethylene insensitivity conferred by the green-ripe and never-ripe 2 ripening mutants of tomato. *Plant Physiol.* 138: 267–275, http://dx.doi.org/10. 1104/pp. 104. 057745.

Bray, H.G., and Thorpe, W.V. 1954. Analysis of phenolic compounds of interest metabolism. *Meth. Biochem. Anal.,* 1: 27 – 52.

Brownleader, M. D., Jackson, P., Mobasheri, A., Pantelides, A. T., Sumar, S. and Trevan, M. 1999. Molecular Aspects of Cell Wall Modifications during Fruit Ripening. *Crit. Rev. Food Sci. Nutr.,* 39: 149-164.

Brummell, D. A. and Harpster, M. H. 2001. Cell Wall Metabolism in Fruit Softening and Quality and Its Manipulation in Transgenic Plants. *Plant Mol. Biol.,* 47: 311-340.

Chandramohan, D., Purushothama, D., and Kothandaram, R. 1973. Soil phenols and plant growth inhibition, *Pl. and Soil*. 39: 303 – 308.

Clifford, M.N., 1995. Understanding the biological effects of dietary complex phenols and tannins and their implications for the consumer's health and well being. *Report of the European project FAIR-CT95-0653. European Community Programme for Research, Technological Development and Demonstration in the ûeld of Agriculture and Fisheries.*

Cua, A.U. and Lizada, M.C.C. 1990. Ethylene production in the 'Carabao' mango (*Mangifera indica* L.) fruit during maturation and ripening. *Acta Horticulturae* 269: 169–179.

Gil, A., Duarte, I., Delgadillo, I., Colquhoun, I., Casuscelli, F., Humpfer, E.and Sprauls, M., 2000. Study of the compositional changes of mango during ripening by use of nuclear magnetic resonance spectroscopy. *J. Agric. Food Chem*. 48: 1524–1536.

Ilahy, R. Hidder, C., Lenucci, M.S. 2011 Antioxidant activity and bioactive compounds changes during fruit ripening of highlycopene tomato cultivars. *Journal of Food and Composition and Analysis*, 24: .588-595, .

Jain, N.L. 1961 Chemistry and technology of mango. *Review of Food Technology,* 3: 131–162.

Jiang, Y. M. 1999. Food Chemist. 66-75. http://dx.doi.org/10.1016/S0308-8146(98)00242-8

Kumar, K.B., and Khan, P.A. 1982. Peroxidase and polyphenoloxidase in excised ragi (Elusine carocana Cv PR 202) leaves during senescence. *Ind. J. Exp. Bot*. 20: 412 – 416.

Lakshminarayana, S. and Subramanyam, H. 1970 Carbon dioxide injury and fermentative decarboxylation in mango fruit at low temperature storage. *Journal of Food Science and Technology* 7: 148–152.

Lalel, H.J.D., Singh, Z. and Tan, S.C. 2003 Maturity stage at harvest affects fruit ripening, quality and biosynthesis of aroma volatile compounds in 'Kensington Pride' mango. *Journal of Horticultural Science and Biotechnology* 78: 225–233.

Lopes, M.M.A., Miranda, M.R.A., Moura, C.F.H. and Enéas, J.E. 2012. Bioactive compounds and total antioxidant capacity of cashew apples (*Anacardium occidentale L.*) during the ripening of early dwarf cashew clones. Ciênciae Agrotecnologia, 36:325-332.

Macheix, J.J., Fleuriet, A., and Billot, J. 1990 *Fruit Phenolics*. CRC Press, Boca Raton, FL.

Medlicott, A.P., Reynolds, S.B., New, S.W. and Thompson, A.K. 1988 Harvest maturity effects on mango fruit ripening. *Tropical Agriculture* 65: 153–157.

Michodjehoun-Mestre, L. Souquet, J.M., Fulcrand, H., Bouchut, C., Reynesa, M. and Brillouet, J.M. 2009. Monomeric phenols of cashew apple (*Anacardium occidentale* L.). *Food Chemistry*, Barking, 112:.851-857,

Park, Y.K., Sato, H.H., Almeida, T.D. and Moretti, R.H. 1980 Polyphenoloxidase of mango (*Mangifera indica* var. Haden). *Journal of Food Science* 45: 1619–1621.

Park, Y. S., Leontowicz, H., Leontowicz, M., Namiesnik, J., Suhaj, M., Milena Cvikrova, Olga Martincova, Weisz, M. and Gorinstein, S. 2011. Comparison of the contents of bioactive compounds and the level of antioxidant activity in different kiwifruit cultivars. *Journal of Food and Composition and Analysis*, 24: 963-970,

Peacock, B.C., Murray, C., Kosiyachinda, S., Kasittrakakul, M. and Tansiriyakul, S. 1986. Infl uence of harvest maturity of mangoes on storage potential and ripe fruit quality. *ASEAN Food Journal,* 2: 99–103.

Reddy, Y.V. and Srivastava, G.C. 1999 Ethylene biosynthesis and respiration in mango fruits during ripening. *Indian Journal of Plant Physiology* 4: 32–35.

Ren, C. and Kermode, A. R. 2000. An Increase in Pectin Methylesterase Activity Accompanies Dormancy Breakage and Germination of Yellow Cedar Seeds. *Plant Physiol.,* 124: 231-242.

Rufino, M. S. M., Ricardo, E., Alves, Edu, S. de Brito, Jara Perez Jimenez, Fulgencio Saura-Calixto and Jorge Mancini-Filho. 2010. Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry*, Barking, 121:996- 1002,

Salveit, M.E. Jr. 1993. Internal carbon dioxide and ethylene levels in ripening tomato fruit attached to or detached from the plant. *Physiol Plant,* 89:204–210.

Selvaraj, Y. and Kumar, R. 1989 Studies on fruit softening enzyme and polyphenoloxidase activity in ripening mango (*Mangifera indica* L.) fruit. *Journal of Food Science and Technology* 26: 218–222.

Shellie, K.C. and Salveit, M.E. Jr. 1993. The lack of a respiratory rise in muskmelon fruit ripening on the plant challenges the definition of climacteric behaviour. *J. Exp. Bot.,* 44:1403–1406.

Stevenson, D.E., and Hurst, R.D. 2007 Polyphenolic phytochemicals—just antioxidants or much more? *Cellular and Molecular Life Sciences*, 64: 2900–2916.

Tlili Imen, Chafik Hdider, Marcello Salvatore, Lenucci, C. Ilahy Riadh, Hager Jebari and Giuseppe Dalessamdro. 2011. Bioactive componds and antioxidant activities during fruit ripening of watermelon cultivars. *Journal of Food and Composition and Analysis*, .24: P.923-928.

Venkatesan. T and . Tamilmani, C. 2010 Effect of ethrel on phenolic changes during ripening of offseason fruits of mango (*Mangifera indica* L. var. Neelum) *Curr. Bot.,* 1(1):22-28.

Vogt, T. 2009. Phenylpropanoid biosynthesis. *Molecular Plant*, 3:2–20.

Weisshaar, B., and Jenkins, G.I. 1998 Phenylpropanoid biosynthesis and its regulation. *Current Opinion in Plant Biology*, 1: 251–257.

World Health Organization - Who. Diet, nutrition and the prevention of chronic diseases: report of a joint WHO/ FAO expert consultation. WHO Reports, 2003.