RESEARCH ARTICLE

STUDIES ON ANTIOXIDANT ACTIVITY IN PULP AND PEEL OF SAPOTA (*MANILKARA ZAPOTA* L.) FRUITS IN DIFFERENT STAGES OF RIPENING

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Abstract: Fruits are major source of antioxidant enzymes. So, in this study the antioxidant activity and its related enzymes have been discussed in the peel and pulp of sapota during the three stages of ripening i.e. mature, half ripe and full ripe. Activity of all antioxidant and its related enzymes viz. superoxide dismutase, ascorbate peroxidase, peroxidase and glutathione reductase decreased during ripening from mature to full ripe stage. Mature fruits have highest content of ascorbic acid and all antioxidant enzymes. Peel of the fruit had higher activity of all antioxidant and its related enzymes as compared to pulp.

Keywords: Sapota, Pulp, Ripening stages, Antioxidant enzymes

INTRODUCTION

Fruits constitute a commercially important and nutritionally indispensable food commodity for human beings. Fruits are generally not part of staple diet but are helpful as a part of diet for providing essential minerals and protection from various diseases. Sapota commonly known as chiku in hindi, is rich sources of various vitamins, minerals, fibres and has a high calorific value. This fruit is used as antipyretic (Ganguly *et al*., 2013), antioxidant (Kulkarni *et al*., 2007), antibacterial (Chanda & Nagani, 2010), antimicrobial activity (Osman *et al*., 2011) and also has analgesic effect (Jain *et al*. (2011). Even the latex of fruit is helpful for filling the tooth cavities. Sapota is rich in antioxidant, because of various enzymes such as high activity of superoxide dismutase, glutathione reductase, peroxidase and catalase which are helpful to increase the antioxidant activity. However, fruits are diverse in antioxidant composition and antioxidant activity and those with high antioxidant activity generally contain more antioxidants (Guo *et al*., 1997). Sapota is one of the fruit which is consumed with peel and without peel. As peel of sapota is not having astringent value so it can be consumed along with peel. It is very well reported in literature that peel of fruit is having higher antioxidant activity, ascorbic acid and various antioxidant enzymes than pulp. However, there is no studies available in literature about these value in peel as well as pulp at various stages of ripening. So, present study was undertaken to compare the various antioxidant and its related enzymes at different stages of ripening in pulp and peel.

MATERIAL AND METHOD

Present experiment was conducted in the laboratory of department of Botany & Plant Physiology, CCS, Haryana Agricultural University, Hisar. The experiment was designed in completely randomized design. Fully mature fruits of sapota were harvested from the orchard of department of Horticulture, CCS, Haryana Agricultural University, Hisar with the secateurs keeping small intact pedicel with each fruits. Fruits were harvested and divided into 3 lots. Sapota fruits were categorized into mature, half ripe (50%) and fully ripe. The peels of Sapota fruits of uniform thickness were removed by fruit peeler from the pulp part and both pulp and peel were used for biochemical estimation separately. Ascorbic acid was determined by the titration method of AOAC (1990). The antioxidant activity of the fruit (peel and pulp) extracts in all three stages was evaluated by DPPH free radical scavenging method according to the method of Shimada *et al.* (1988). The activity of superoxide dismutase was assayed by the procedure of Beauchamp & Fridovich (1971). Ascorbate peroxidase was assayed by the procedure of Nakano & Asada (1981). Peroxidase was assayed according to the method described by Dias & Costa (1983). Glutathione reductase was analysed by the method of Halliwell & Foyer (1978).

RESULT AND DISCUSSION

Ascorbic acid content decreased with the ripening stages from 17.84 mg/100g in mature to 8.60 mg/100g in full ripe stage, when considered irrespective of peel and pulp. The reduction in ascorbic acid content in fruits may be due to oxidation of ascorbic acid into dehydro-ascorbic acid by ascorbic acid oxidase enzyme (Nayak *et al*., 2011). These results of decrease in ascorbic acid are in conformity with the previous findings of Iloki *et al.* (2013) in Noni (*Morinda citrifolia* L.) and Kamol *et al*. (2014) in pineapple. Peel of fruits at all three stages had higher content of ascorbic acid i.e. 19.27 mg/100g, 15.10 mg/100g and 9.77 mg/100g as compared to pulp of fruits i.e. 16.4 mg/100g, 13.44 mg/100g and 7.42 mg/100g in mature, half ripe and

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full ripe fruits respectively. The higher content of ascorbic acid in peel perhaps might have protected the fruit from infection of micro-flora from outer surface. These results are in agreement with the earlier reports of Abdrabba & Hussein (2015) in red Grape. Less ascorbic acid content indicated that the peel of the fruits should be consumed along with the pulp for antioxidant activity. Higher activity was observed in mature fruits i.e. 17.84 mg/100g whereas minimum in full ripe fruits i.e. 8.60 mg/100g. This indicates that for maximum antioxidant activity, fruits should be consumed in mature stage. However, mature fruits are not in good in taste. So, it is better that fruits should be consumed in half ripe stage.

Table 1. Ascorbic acid (mg/100g) and free radical scavenging activity (%) in Sapota fruit parts during different stages of ripening

Stages (S)	Ascorbic acid (mg/100g)			Free radical scavenging activity (%)		
	Fruit parts (P)			Fruit parts (P)		
	Pulp	Peel	Mean	Pulp	Peel	Mean
Mature	16.40	19.27	**17.84**	61.77	99.15	**80.46**
Half ripe	13.44	15.10	**14.27**	49.25	93.60	**71.43**
Full ripe	7.42	9.77	**8.60**	22.63	67.13	**44.88**
Mean	**12.42**	**14.72**		**44.55**	**86.63**	
CD at 5 %	**S=0.28 P=0.23 S×P=0.39**			**S=1.26 P=1.03 S×P=1.79**		

Free radical scavenging activity measured the efficiency of fruits to remove the free radical formed within the fruit. Higher is the activity of free radical scavenging activity more antioxidant it will be. Free radical scavenging activity decreased with the ripening stages from mature (80.46%) to full ripe stage (44.88%), when considered irrespective of peel and pulp. This might be due to high ascorbic acid present in fruits which might have been responsible for the higher activity of free radical scavenging activity. As there was direct correlation between ascorbic acid and free radical scavenging activity in half ripe and full ripe fruits also. As in half ripe and full ripe stage the content of ascorbic acid decreased which might be correlated with decreased in activity of free radical scavenging activity. This decrease in free radical scavenging activity results are also in conformity with the results of Iloki *et al.* (2013) in Noni (*Morinda citrifolia* L.). Peel of fruits at all three stages had higher content of free radical scavenging activity i.e. 99.15%, 93.60% and 67.13% as compared to pulp of fruits i.e. 61.77%, 49.25% and 22.63% in mature, half ripe and full ripe fruits respectively. This may be because ascorbic acid content was more in peel part of the fruit (Someya *et al*., 2002). The decrease in free radical scavenging activity results are in conformity with the results of Barros *et al*. (2012) in Citrus, and Woo *et al*. (2013) in Sapodilla.

Table 2. Superoxide dismutase activity (nmol/g fw) and ascorbate peroxidase activity (nmol/min/g fw) in Sapota fruit parts during different stages of ripening

Stages (S)	Superoxide dismutase (nmol/g fw)			Ascorbate peroxidase (nmol/min/g fw)		
	Fruit parts (P)			Fruit parts (P)		
	Pulp	Peel	Mean	Pulp	Peel	Mean
Mature	458.30	706.13	**582.22**	680.90	986.48	**833.69**
Half ripe	280.84	460.59	**370.72**	365.09	733.58	**549.34**
Full ripe	255.58	420.32	**337.95**	350.76	694.34	**522.55**
Mean	**331.57**	**529.01**		**465.58**	**804.80**	
CD at 5 %	**S=0.96 P=0.78 S×P=1.35**			**S=2.60 P=2.12 S×P=3.67**		

Superoxide dismutase (SOD) enzyme is a metaloprotein that catalyzes the dismutation of superoxide to H_2O_2 and molecular oxygen (Allen, 1995) (Table 2). It has been assumed that SOD had a central role in defense system against oxidative stress (Alscher *et al*., 2002). The SOD activity decreased from mature stage (582.22 nmol) to full ripe (337.95 nmol) fruit during ripening. This may be due to the reason that defense system decreases with the process of ripening. Similar decrease in SOD activity during ripening has also been reported in Guava (Goyal, 2010). The activity of enzyme has higher in the peel of the fruits 529.01 nmol as compared to 331.57 nmol in the pulp of fruits. Higher activity of SOD in the peel again indicates that peel is having better defense system than the pulp. Similar results of higher activity of SOD in peel of the fruit have also been reported by Abbasi *et al*. (2010) in 'Pink Lady' Apple fruit.

Table 3. Peroxidase activity (nmol/min/g fw) and glutathione reductase acitivity (nmol/min/g/ fw) in Sapota fruit parts during different stages of ripening

Stages (S)	Peroxidase (nmol/min/g fw)			Gtutathione reductase (nmol/min/g fw)		
	Fruit parts (P)			Fruit parts (P)		
	Pulp	Peel	Mean	Pulp	Peel	Mean
Mature	317.57	523.28	420.43	423.83	623.27	**523.55**
Half ripe	242.59	366.36	304.48	283.57	541.56	**412.57**
Full ripe	179.82	230.82	205.32	192.89	392.75	**292.82**
Mean	246.66	373.49		**300.10**	**519.20**	
CD at 5 %	**S=0.70 P=0.57 S×P0.99**			**S=0.78 P=0.63 S×P=1.10**		

Ascorbate peroxidase (APX) plays a pivotal role in eliminating hydrogen peroxide from plant cell (Madhusudhan *et al*., 2003). The activity of APX (Table 2) was found to decrease from 833.69 nmol in mature fruits to 522.55 nmol in full ripe stage when considered irrespective of pulp and peel. Decrease in activity of ascorbate peroxidase during ripening may be due to the reason that production of H_2O_2 decreases during ripening. The decrease in APX activity during ripening may also be either due to substrate being limited or the enzyme being inactivated. Highest APX activity at mature green stage followed by a continuos decrease during ripening has also been reported in Guava (Ram, 2007). Peel of the fruits (804.8 nmol) had higher activity of APX than pulp (465.58 nmol). The higher activity of enzyme ascorbate peroxidase in the peel indicates that more amount of H_2O_2 is produced in the peel than pulp. Similar observation of higher activity of APX in the peel has also been reported by Lata *et al*. (2005) who observed ascorbate peroxidase activity in peel and pulp of Apple cv. Elise.

The activity of peroxidase enzyme during ripening of Sapota is presented in table 3. Peroxidase activity is responsible for destroying H_2O_2 produced in fruits. Higher is the activity of peroxidase enzyme better antioxidant present in it. Peroxidase activity decreased from 420.43 nmol in mature stage to 205.32 nmol in full ripe stage when considered irrespective of pulp and peel. This may be due to reason that metabolic activity decreased from mature to full ripe stage. So, less H_2O_2 produced and as a results of which the activity of peroxidise enzyme also decreased. Similar decrease of peroxidase during ripening has also been observed by Goyal (2010) in Guava, Praduman (2010) in Ber fruits. Peel of the fruits has higher activity of the peroxidase enzyme i.e. 373.49 nmol as compared to pulp (246.66 nmol). The less activity of peroxidase in the pulp indicates that less metabolic rate in tissue of the pulp.

Glutathione reducatase (GR) activity of Sapota fruit was highest at mature stage (523.55 nmol) and declined to fully ripe stage (292.82 nmol) (Table 3) when considered irrespective of pulp and peel. As already reported in peroxidase enzyme, GR enzyme is also responsible for destroying of H_2O_2. The activity of which decreased during the process of ripening. This may also be because of reason that during the process of ripening the rate of various metabolic processes decreased which reduced the activity of this enzyme also. Decrease in GR activity during ripening has also been observed in Guava (Mondal *et al*., 2009) and Pradumen (2010) in Ber. Peel of the fruit had higher activity of GR (519.20 nmol) as compared to pulp part (300.10 nmol). This again indicate that metabolic activity are faster in peel part than the pulp. Similar finding of higher GR activity in peel has also been reported by Lata *et al*. (2005) in cv. Elise of Apple fruit.

CONCLUSION

From above all the studies, it is clear that, the peel of fruit is a good source of antioxidant and its related enzymes which are really required. Among the different studies, mature fruit had a higher content of antioxidant and its related enzymes and their activity decreased with the process of ripening. However, because of astringent taste fruits are not fit for consumption at mature stage. So, it is concluded from the studies fruits of half ripe stage are fit for consumption.

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