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Influence of aril browning on biochemical properties of pomegranate (*Punica granatum* L.)

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ABSTRACT

Aril browning (AB) is one of the major physiological problems of pomegranate, resulting in diminution of quality and commercial value of the fruits. The present study was taken up to investigate the biochemical changes in pulp and seed of aril browning affected fruit in comparison with healthy fruits. Biochemical studies revealed that AB affected aril had higher total sugars (84.45 mg g^{-1} fresh weight of tissue), reducing sugar (53.40 mg g^{-1} fresh weight of tissue), TSS (16.3%) and starch (194.96 mg g^{-1} of tissue dry weight) as compared to healthy aril. Whereas, protein content was lower in AB affected seeds (6.92 mg g^{-1} FW of tissue) as compared to healthy seeds (7.832 mg g^{-1} FW of tissue). There was a gradual decrease in anthocyanin (from 0.53 to 0.33 mg 100^{-1} g) and total phenol content (from 141.25 to 109.70 mg 100^{-1} g) with the increase in intensity of browning. AB affected seeds showed reduced activities of enzyme like amylase (7.36 mg maltose liberated h^{-1} g^{-1} of protein) and total dehydrogenase (1.44 ΔA_{485} g^{-1} fresh weight of tissue), in contrast to this there was increased activity of enzyme polyphenol oxidase (0.0063 $\Delta A_{412} mg^{-1}$ protein min^{-1}) as compared to seed of healthy aril.

***Keywords*:** Aril browning, browning intensity, pomegranate

Pomegranate cultivation is getting an increased attention due to its excellent health promoting effect and wide use in food and processing industry. The edible part of the fruit is the arils which constitute 52% by weight of fruit, comprising 78% juice and 22% seeds. The fresh juice contains 85.4% moisture and considerable amounts of total soluble solids (TSS), total sugars, reducing sugars, anthocyanins, phenolics, ascorbic acid and proteins and it is also reported to be a rich source of antioxidants. The anthocyanins from pomegranate fruit have been shown to have higher antioxidant activity than vitamin E (α-tocopherol), vitamin C (ascorbic acid) or β -carotene (Shukla *et al*., 2008). Moreover, commercial pomegranate juice has been shown to have three times higher the antioxidant activity of green tea and red wine (Gil *et al*., 2000). Apart from the demand for fresh fruits and juice, the processed products like wine and candy are also gaining importance in world trade. The fast increase in demand of the fruit in the international market has widened the scope for earning higher dividend from this crop. India exports only 2.55% of its total production (APEDA, 2006).One of the major cause behind this hurdle is lack of export quality of fruit. In order to meet growing demand, there is a need to maintain high quality of the fruit. The high incidence of physiological disorder called Aril Browning (AB) has threatened the popularity of pomegranate fruit. Aril browning in pomegranate is a physiological disorder wherein, the brown flattened and soft arils are noticed when fruit is cut open. The browning of aril starts with a dark dot and later on spreads to the entire aril and many of them have a streaked appearance due to fine white lines radiating from the seeds. Affected arils are soft, light creamy – brown to dark blackish – brown, deformed and possess unacceptable off-flavour and unfit for consumption and exhibit poor dessert quality. Development of aril browning in pomegranate is a complex process. Hence there is need to understand the biochemical mechanism of browning during development of the disorder. Present study was undertaken with an objective to study effect of aril browning on biochemical properties of pomegranate.

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MATERIALS AND METHODS

Sample collection

Pomegranate fruits of cv. Bhagwa were collected from orchard located in the Sira district, Karnataka. Fruits were harvested on the 126th day from fruit set when they had attained 90% maturity ripened at room temperature (26 ± 2°C) and relative humidity (70 ± 5%) for 4–10 days. Ripe fruits were cut open and the AB-affected arils were separated out from each fruit.

Estimation of total moisture content

The moisture content of the pulp and seed sample was analyzed by the gravimetric procedure. 5g of each sample in three replications was taken and dried at 70 ^{o}C in a hot air oven for 72 hours. The weight of the sample before and after drying was recorded and the moisture percentage was calculated as.

$$\text{Moisture (\%)} = \frac{\text{(Fresh weight-Dry weight)}}{\text{Fresh weight}} \times 100$$

Estimation of biochemical parameters

Total sugar, reducing sugar and starch of seed and pulp (juice) of fruit were analyzed using the dinitrosalicylic acid method (Selvaraj and Lodh, 1973). Total soluble solid (TSS) of juice was determined with refractometer and results were reported in degree Brix. Estimation of total soluble protein was done by following the method described by Lowry *et al.* (1951).Total anthocyanin content of the fruit juice was determined by measuring absorbance at 540nm (Fuleki,1969). Total phenol content was estimated by spectrophotometric method described by Malick and Singh, 1980.

Enzyme assay

Amylase activity was assayed according to DNS method and activity in the sample was expressed as mg maltose liberated h^{-1} g^{-1} of protein (Bernfeild, 1955), whereas, total dehydrogenase and polyphenol oxidase (PPO) activity of the seed were determined by TTC (2,3,5,- triphenyl tetrazolium chloride) test (Sung and Chen, 1988) and by method described by Esterbaner (1977) respectively. Intensity of aril browning is measured as follows:

Low intensity (LI) represents occurrence of small whitish to grayish dots of the size of a pin head on the aril. Big spot (BS) or medium intensity (MI): represents browned spots on the aril with a diameter ranging from 1-2 mm. High intensity (HI) represents incidence of aril browning where more than 50% area of the aril was affected by browning some of which were shriveled also.

The data was statistically analyzed by adopting the paired t-test and critical difference values were compared at 1% levels of significance and wherever found significant, treatment means were compared.

RESULTS AND DISCUSSION

Moisture changes in healthy and AB affected aril

Moisture changes in healthy and AB affected pulp and seed are presented in fig.1. The results showed that the moisture content in pulp of AB affected aril was lower (82.33%) compared to the moisture content in healthy pulp of the same fruit (83.56%). Seed also showed a considerable difference in moisture content between healthy (48.11%) and affected aril (46.22%). The decrease in moisture content of both pulp and seed of affected aril indicated the mobilization of water away from the aril. Similar results of decrease in moisture content of spongy tissue affected mango fruits mesocarp was observed by Shivashankar *et al.* (2007).

Total soluble sugars, reducing sugars, TSS and starch

Total soluble sugars and reducing sugars were higher in both AB affected juice (pulp) and seed as compared to healthy (Table 1).

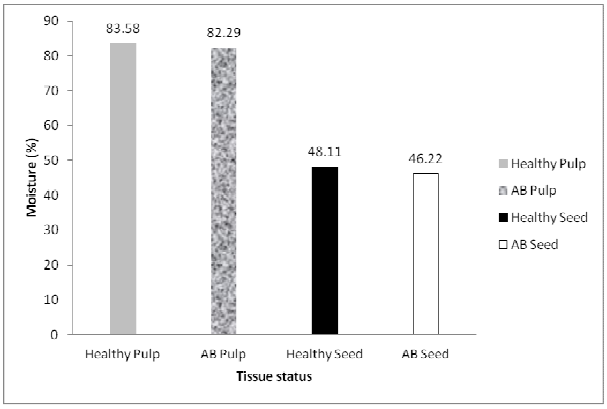

Fig.1: Moisture content in pulp and seed of healthy and AB affected aril

A significant increase in TSS was recorded in browning affected aril (16.3%) as compared to healthy one being, 14.4% (Table 3). The increase in total sugars, reducing sugars and TSS in browning affected aril could be due to decreased moisture content in browning affected aril as compared to healthy. Tabar *et al.* (2009) reported that the peel percent, dry matter of juice, acidity, total soluble solids and total sugars increased faster in case of disorder fruit (aril browned fruit) than those in intact fruit(healthy fruit). It is observed from table1 there was significant variation in the starch content of aril browning affected aril compared to healthy. Seed of healthy aril showed lower starch content (110.75 mg g^{-1} dry weight of tissue) compared to affected seed (194.96 mg g^{-1} dry weight of tissue). Shivashankara *et al.* (2004) suggested that the browning of arils in pomegranate resulted in lower starch and acid metabolism.

Total protein content

Results presented in table- 2 showed there was higher total protein content in the seed of healthy aril than from affected aril. Gupta *et al.* (1985) and Shivashankar *et al.* (2007) observed difference in total protein content in affected and healthy mesocarp of spongy tissue affected fruit.

Changes in anthocyanin content

Table-3 shows a gradual decrease in anthocyanin content with the increased intensity of AB. Decrease in anthocyanin content of the aril could be explained on the basis of browning mechanism in other fruits like litchi. Post harvest browning of litchi was thought to be caused by a rapid degradation of the red pigment by polyphenol oxidase (PPO), producing brown-coloured by-product (Akamine, 1960; Huang *et al*., 1990). Recently Jiang (2000) reported that litchi PPO cannot oxidize anthocyanin, but the anthocyanin might be degraded rapidly in an anthocyanin –PPO-phenol system and thus, suggested that it may be the presence of the sugar moiety which caused steric hindrance

Table1: Changes in content of total soluble sugars, reducing sugars and starch in pomegranate fruit

Fruit status	Total soluble sugars (mg g^{-1} FW of pulp)	Total soluble sugars (mg g^{-1} FW of seed)	Reducing sugars (mg g^{-1} FW of pulp)	Reducing sugars (mgg^{-1} FW of seed)	Starch (mg g^{-1} FW of seed)
Healthy	66.79	10.52	41.79	6.50	110.75
AB	84.45	29.89	53.40	21.75	194.96
T test	**	**	**	**	**
T-value	8.09	20.37	6.29	6.85	4.97

Table2: Changes in protein, amylase, PPO and TDH activities of affected seed as compared to healthy seed

Fruit status	Protein (mg g^{-1} FW of seed)	Amylase activity (mg maltose h^{-1} g^{-1})	PPO activity (ΔA_{412}mg^{-1} protein min^{-1})	TDH activity (ΔA_{485} g^{-1} tissue FW)
Healthy	7.832	7.36	0.0063	2.21
AB	6.92	1.64	0.0170	1.44
T test	NS	**	**	**
T-value	1.182	5.64	4.984	5.632

Note: ** Significant at 5% level of significance, NS: non significant, FW: fresh weight, AB: aril browning affected tissue, PPO: polyphenol oxidase, TDH: total dehydrogenase

Table 3: Changes in TSS, total phenol and anthocyanin with increasing intensity of AB in fruit juice

Tissue status	TSS (°Brix)	Total phenolics (mg 100^{-1} g of aril)	Anthocyanin (ΔA_{540} $g^{-1}$of aril)
Healthy arils	14.40	141.25	0.53
LI of AB affected aril	15.26	131.99	0.49
MI of AB affected aril	15.90	124.90	0.42
HI of AB affected arils	16.30	109.76	0.33
SEm (±)	**0.54**	**1.49**	**0.01**
LSD (0.01)	**1.76**	**7.08**	**0.07**

Since anthocyanins were unstable they could be degraded nonenzymatically or enzymatically. This was further supported by results of PPO activity which showed an increase in PPO activity in seed of AB affected aril as compared to healthy (Table 2).

Changes in total phenol

There was significant decrease in total phenol content with increase in intensity of browning from 141.25 mg 100^{-1} g of aril for healthy to 109.76 mg 100^{-1} g for high intensity of browning affected aril (Table 3). This may happen due to higher activity of PPO which causes oxidation of phenols.

Amylase activity

The amylase activity in seed from AB affected aril was less than from healthy (Table 2). It was observed that amylase activity was almost four times higher in seed of healthy aril (7.36 mg maltose liberated h^{-1} g^{-1} of protein) as compared to affected aril (1.64 mg maltose liberated h^{-1} g^{-1} of protein). Decrease in amylase activity might be the reason for the higher starch (194.96 mg g^{-1} dry weight of tissue) content of browning affected seed as against healthy seed (110.75 mg g^{-1} dry weight of tissue).

Polyphenol oxidase activity

There was a significant increase in polyphenol oxidase activity in seed of AB affected aril (0.0170 ΔA_{412}mg^{-1} protein min^{-1}) as compared to the healthy seed (0.0063 ΔA_{412}mg^{-1} protein min^{-1}) results are indicated in table- 2. This data supports the decrease in total phenol content of affected seed as PPO may cause oxidation of phenol.

Total dehydrogenase activity

Total dehydrogenase activity was higher in seed of healthy aril (2.21 ΔA_{485} g^{-1} fresh weight of tissue) than affected aril (1.44 ΔA_{485} g^{-1} fresh weight of tissue) as indicated in table-2. It is likely that the lower dehydrogenase activity in seed of affected aril could be the result of decreased moisture content of affected seed as against the healthy. Shivashankar *et al*. (2007) also suggested role of seed in development of spongy tissue. They found there were significant changes in biochemical parameters of seed during the development of spongy tissue in mango fruit.

Pomegranate fruits with aril browning did not exhibit any external characteristic differences as compared to healthier one. There was a distinct significant difference in physical and biochemical properties of fruit. It can be inferred from the study that aril browning is a complex process. Enzymes like PPO and total dehydrogenase are playing important role in development of the disorder but exact cause behind this disorder is not yet understood. Further investigations are needed to improve our understanding on mechanism of development of browning to overcome this disorder.

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