Physical, Biochemical and Mineral Evaluation of Sapota Fruits During Growth, Development and Ripening

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ABSTRACT

Sapota (Manilkara archas Forb.) also called Chickoo or Chiku is one of the major fruit crop in tropical areas. Sapota fruit quality changes during development and ripening. Therefore this study was conducted to evaluate various changes that the fruit undergoes during growth and development. Fruits were harvested at 2, 3, 4 and 5 months after flower opening in order to study their quantity and quality. Then fruits within each treatment (harvest stage) were analysed by their physical properties, biochemical composition (TSS, pH, chlorophyll a, chlorophyll b, carotenoids and total phenol) and Minerals Composition (K, Ca, Na, Mg, Fe, Mn and Zn). Results indicated that weight, diameter and length of fruits increased during development and specially at full-ripe stage. There was a greater increase in length than in the diameter during the change from green to ripe. This classifies the fruit more towards ovoid in shape. TSS percent increased progressively towards the end of ripening stage. Ripening was associated with a reduction in fruit firmness from about 8 kgcm⁻² in early stage to 2.5 kgcm⁻² in ripe fruit. Analysis of the total Carotenoid contents at different stages suggests that loss of carotenoid occurs during ripening. Total phenol content also showed a decreasing trend from first stage to ripening stage. Various mineral contents decreased as fruit matured from the early to the ripe stage of development. Among the minerals studied, potassium was most abundant.

Keywords: Chiku, chlorophyll content, Manilkara archas, maturation, mineral composition, phenol.

INTRODUCTION

Sapota (Manilkara archas Forb.) also called chickoo or Chiku belongs to Sapotaceae family, and is one of the major fruit crops in India, Mexico, Guatemala, Venezuela and many other tropical regions (Mohamed et al., 2003). Sapota fruit is a berry with a scurfy and brown peel. Fruit may be round to oval-shaped or conical, 2 to 4 inches (5-10 cm) in diameter and weigh 75 to 100 g. The pulp is light brown, brownish yellow to reddish brown, with a texture varying from gritty to smooth. The pulp has a sweet to very sweet (19-24° Brix), pleasant flavour making it widely accepted among consumers. Seed number varies from 0 to 12. Seeds are dark brown to black, smooth, flattened and shiny with 1.9 cm long (Gilman and Watson, 1994; Kute and Shete, 1995). Athmaselvi et al. (2014) have reported that average mass, length, breadth, and width of sapota are 48.42 g, 0.45, 0.417, and 0.457 cm, respectively. The surface area of sapota also ranges from 50.16 to 69.3 cm² with the average value being 58.82 cm².

The fruit has many health beneficial ingredients in sufficient quantities such as dietary fibre, fructose, sucrose, vitamins, minerals and antioxidant compounds. Sapota fruit is reported to contain sugars, acids (Shanmugavelu and Srinivasan, 1973), protein, amino acids (Selvaraj and Pal, 1984), phenolics, viz., gallic acid, catechin, chlorogenic acid, leucodelphinidin, leucocyanidin and leucopelargonidin (Mathew and Lakshminarayana, 1969), carotenoids, ascorbic acid, and minerals like potassium, calcium and iron (Selvaraj and Pal, 1984; Lakshminarayana and Rivera, 1979). Shui et al. (2004) have reported the change of antioxidant levels during storage of Manilkara zapota L. Ripening is a biochemical process in fruits, in which physical and chemical characteristics including dramatic bioactive compounds production, such as reducing sugars, organic acids, ascorbic acid, anthocyanin, and pigments for each fruit stages at maturity.

Unripe chiku, are found to possess extremely high antioxidant capacity that that is partly responsible for the antioxidant capacity of many fruits (Shui et al., 2004). Most of the previous research work (Abdul Karim et al., 1987, 1989; Lakshminarayana and Subramanyam, 1966; Sastry, 1970; Ingle et al., 1981, 1982) on Sapota has
mainly focused on physical and chemical parameters changes during fruit ripe and over ripening. Furthermore, there is lack of information on the physical and chemical characteristics of the fruit during first stages of developing. Regarding this issue, the major objective of the present investigation was to study the physico-chemical characteristics of sapota during fruit development and ripening.

**MATERIALS AND METHODS**

**Materials and Treatments:**

The experiment was performed by collecting the sapota fruit samples at different stages from a farm in Agricultural Research Station of Minab, Hormozgan, Iran. To obtain fruits at different maturity stages, flowers of 10 year old trees were tagged randomly at opening. Tagged flowers were allowed to develop and then fruits were harvested at 2, 3, 4 and 5 months after opening. The harvested fruits were brought to the laboratory, sorted and packed. The experiment was designed as a completely randomized block design with three replications. Then fruits within each treatment (harvest stage) were analysed by their physical properties, biochemical and mineral composition.

**Physical Properties:**

1. **Size**

   The length and diameter of the fruit were measured using a Vernier caliper. The measurement of the length was made in the polar axis of the fruit, i.e. between apex and stem. The maximum width of the fruit, measured in the direction perpendicular to the axis was defined as diameter.

2. **Weight**

   Weight (in gram) was determined using an analytical balance.

3. **Firmness**

   Firmness was measured by using a texture analyser fitted with a 3 mm diameter flat probe and firmness was expressed as kgcm⁻².

**Biochemical Composition:**

1. **TSS and pH**

   For the determination of total soluble solids content (TSS, °Brix), five ripe fruits per replication were homogenized and the homogenates was filtered through a cheese cloth to obtain clear juice. Total soluble solids (TSS) percentages were determined using hand refract meter. The pH values were determined by using pH-meter (Metrom, Swiss).

2. **Chlorophyll and Carotenoids**

   In order to measure pigment content of sapota fruits, weight of fruits were recorded and then, fruit samples (0.1g) were extracted with 15 ml of 85% methanol. The extracts were then filtered and centrifuged (Higen21 Herolab, Germany) at 4000g for 10 min and the supernatant was separated. The developed colour was measured at three-wavelength lengths 470, 646 and 663 nm, using UV/VIS spectrophotometer (Varian spectrAA220, Germany). The amounts of pigments were calculated according to Lichtenthaler and Wellburn (1985) with the below mentioned formula.

   - Chlorophyll a=12.25×A663 – 2.79×A646
   - Chlorophyll b=21.21×A646 – 5.1×A663
   - Carotenoid=(1000×A470–1.8×Ca–85.02×Cb)/198

3. **Total Phenol**

   100 g of fruit was extracted with 300 ml methanol–water (4:1, v/v), at room temperature for 5 h using an orbital shaker. The extracts were then filtered and centrifuged (Higen21 Herolab, Germany) at 4000g, for 10 min and the supernatant was concentrated under reduced pressure at 40 °C for 3 h using a rotary evaporator to obtain the methanolic crude extract. The crude extract was kept in dark glass bottles for three days inside the freezer until use. The total phenolic contents of fruit extracts were determined according to the method described by Malik and Singh (1980). Aliquots of the extracts were taken in a 10 ml glass tube and made up to a volume of 3 ml with distilled water. Then 0.5 ml folin-ciocalteau reagent (1:1 with water) and 2 ml Na₂CO₃ (20%) were added sequentially in each tube.

   A blue colour was developed in each tube because the phenols undergo a complex redox reaction with phosphor-molybdic acid in folin-ciocalteau reagent in alkaline medium which resulted in a blue coloured complex, molybdenum blue. The test solutions were warmed for 1 minute, cooled and absorbance was measured at 650 nm against the reagent used as a blank. A standard calibration plot was generated at 650 nm using known concentrations of gallic acid. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg gallic acid equivalent of phenol per g FW.

**Minerals Composition:**

Analysis of the trace minerals was carried out with an Atomic Absorption Spectrophotometer (spectrAA 220 Model). About 1 g of dry, ground fruit was weighed in a crucible and was ashed at 550 °C. The ash was then dissolved in 5 mL of Analar grade hydrochloric acid (20%) and the solution transferred to a 50 mL volumetric flask; the final volume was achieved with distilled water as recommended by AOAC (1990).

**Statistical Analysis:**

Results were analysis by one way ANOVA test of variance. The means were compared by Duncan’s multiple range tests (DMRT). Differences were considered to be significant at p<0.05. All statistical analysis were performed using the Statistical Package for the Social Sciences (SPSS) 11.0.
RESULT AND DISCUSSION

Physical Properties:

It is seen from results that all the growing stages of Sapota fruit had significant influence on physical parameters of the fruit. The average diameter and length of the ripe Sapota fruit were 5.5 and 6.8 cm, respectively, and there was a significant difference in these parameters between the different stages (Table 1). There was a greater increase in length than in the diameter during the change from green to ripe. The fact that the length was greater than the diameter classifies the fruit more towards ovoid in shape. The present findings of increasing trends in length and diameter of fruit are supported by Pawar (1988) in Karonda fruit and Honde (1995) in Sapota. As shown in Table 1 fresh weights of Sapota fruit at the early stage were less than 24 g. However, the weight of them at the ripening stage reached 115 g, which was 4 fold greater than weights of the green stage fruits. The changes in this parameter showed a clear sigmoid pattern for palm fruit growth. These results confirm those Pawar (2010, 2011) and Brito and Narain (2002) for other Sapota cultivars.

Table 1. Physical properties of Sapota fruit during different stages of growth, development and ripening.

<table>
<thead>
<tr>
<th>Fruit stages</th>
<th>Length(cm)</th>
<th>Diameter(cm)</th>
<th>Weight(g)</th>
<th>TSS (%)</th>
<th>pH</th>
<th>L/D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1†</td>
<td>3.9±0.3</td>
<td>3.4±0.3</td>
<td>24±2</td>
<td>14.5±0.4</td>
<td>5.0±0.1</td>
<td>1.14</td>
</tr>
<tr>
<td>Stage 2</td>
<td>4.7±0.4</td>
<td>.0±0.2</td>
<td>37±4</td>
<td>19.5±0.4</td>
<td>5.5±0.2</td>
<td>1.17</td>
</tr>
<tr>
<td>Stage 3</td>
<td>5.5±0.2</td>
<td>4.5±0.3</td>
<td>60±7</td>
<td>21.0±0.5</td>
<td>5.4±0.1</td>
<td>1.22</td>
</tr>
<tr>
<td>Stage 4</td>
<td>6.8±0.4</td>
<td>5.4±0.3</td>
<td>115±9</td>
<td>23.0±0.5</td>
<td>5.0±0.2</td>
<td>1.25</td>
</tr>
</tbody>
</table>

† Stage 1: 2 months after flower opening; Stage 2: 3 months after flower opening; Stage 3: 4 months after flower opening; Stage 4: 5 months after flower opening.

TSS and pH:

The results (Table 1) showed that TSS % increased progressively towards the end of ripening stage. TSS increased gradually from 14.5 in first stage to 23, during fruit development. Sapota ripening was associated with an increase in total fruit soluble solids, which appears linked in increase to cell wall hydrolysing enzyme during ripening as reported in other fruits. Our result fell more or less within the ranges reported by other researchers. Rohani and Halijah (2006) reported that TSS was gradually decreased during development reaching a maximum level at the late stage of Chiku var. ‘Subang’. They showed TSS changed from 12 to 20.

Analysis showed that pH decreased continuously during ripening (Table1), although some changes were not statistically significant. Similar tendencies have been reported by Brito and Narain (2002). He reported that pH reduced significantly at the ripe stage of Sapota fruit. However some others that have studied different stages of Sapota fruit development (mature, ripe, over ripening) have reported an increase in pH from 5.30 to 6.30. They have concluded that this might attributed to the decrease in acidity during ripening. Similar to our result, previous studies (Paralkar, 1985; Sapota and Pawar, 1988; Rohani and Halijah, 2006) have reported pH decrease during fruit ripening

Firmness:

Measurements of fruit firmness during ripening is shown in Fig. 1. Significant differences were found during fruit development stages regarding fruit firmness. Ripening was associated with a reduction in fruit firmness from about 8 kgcm⁻² in early stage to 2.5 kgcm⁻² in ripe fruit. Rohani and Halijah (2006) reported that firmness decrease from 5 to 2 kgcm⁻² during Sapota ripening. It appears that much of softening results from the degradation of the middle lamella in the walls of the cortical parenchyma cells with increased release of pectin. Arenas-Ocam et al. (2003) have reported that polygalactronase plays an important role in the rapid softening of sapodilla fruits during ripening.

Chlorophyll and Carotenoids:

Analysis of the total Carotenoid contents at different stages suggests that loss of carotenoid occurs during ripening (Fig. 2). Dramatic decreases in Carotenoid were observed during the late stages; however, the rate of carotenoid loss diminished considerably during first stages. The results of this investigation are in agreement with the results obtained by Raut (1999) in Sapota fruit. There aren't any data on first stage of fruit growing but our result in ripening stage agree with other search on ripening stage. Kulkarni et al. (2007) and Moo-Huchin et al. (2014) showed that Sapota fruit at ripening stage have 0.92 mg/100g and 1.7 mg/100g carotenoid loss respectively. Although this is much higher than those found in our study, this is probably due to the existing differences between the two samples in variety, environmental and analysis conditions. Carotenoids play a vital role in human health because of their role as a precursor for vitamin A or equivalent.

Chlorophyll showed the same trend as carotenoid, but its value was much more than carotenoid (Fig. 3). During ripening, the
concentrations of both chlorophylls decreased continuously during fruit ripening. Up to our knowledge, there is no published scientific evidence that shows chlorophyll changes during sapota fruit development. Subsequent decrease in chlorophyll content during ripening denotes both decrease in chlorophyll biosynthesis and an increase in chlorophyll catabolism.

Fig. 1. Firmness changes of Sapota fruit during fruit growth, development and ripening.

Fig. 2. Chlorophyll content changes of Sapota fruit during fruit growth, development and ripening.

Fig. 3. Carotenoid content changes of Sapota fruit during fruit growth, development and ripening.

Total Phenol:
Phenol showed a decreasing trend from first stage to ripening stage during Sapota fruit development. There were significant differences in phenol content among fruit development stages. As shown in Fig. 4 the total phenol content that was measured spectrophotometrically, varied considerably from 2.7 to 1 mg in terms of gallic acid equivalents per g FW at the early stage and at the final studied stages, respectively. Torres-Rodriguez et al. (2011) reported that total soluble phenolic content decreased from 2563 to 234 μg GAE g⁻¹ of fresh weight when Mamey Sapote fruits went from unripe to consumption maturity. The polyphenolic content of tropical fruits has shown to be dependent of species, cultivar, and tissue (Rinaldo et al., 2010). In the case of Mamey Sapote, composition can have high variation even among fruits of the same region, as showed by Gaona-Garcia et al. (2008) and Torres-Rodriguez et al. (2011). Differences observed for the same cultivar are mainly due to the climate conditions and harvesting period. Decrease in phenolics is also related to the reduction of primary metabolism in ripe fruit, resulting in a lack of substrates necessary for the biosynthesis of phenolic compounds. The later decrease in phenols throughout the maturation probably occurs by the transformation (polymerization, oxidation and conjugation) of bound phenolic acids.

Fig. 4. Total phenol content changes of Sapota fruit during fruit growth, development and ripening.

Minerals Composition:
As shown in Fig. 5 and 6, various mineral contents decreased as fruit matured from the early to the ripe stage of development. Among the minerals studied, potassium was most abundant with a concentration of 7.2 mg g DW⁻¹, a little significant difference existed in sodium, calcium, potassium and magnesium minerals during different stages (p>0.05). Similar to our results, Sulladmath (1983) reported that potassium had higher concentration rather than other minerals. According to our result micro element reached the minimum level at late stage of ripening. Our results showed a similar trend to those found in other fruit. Our result (at late stage) fell more or less within the ranges reported by other researchers. These differences may be due to the geographic origin of the cultivars, environmental conditions and use of different analytical methods. Hamza et al. (2013) showed that fruits of Manilkara zapota have higher
concentration of Fe (14 μg g⁻¹) among the other trace metals. He showed elements of edible part of the fruit were Fe (14.17 μg g⁻¹), Mn (1.49 μg g⁻¹), Cu (1.70 μg g⁻¹) and Zn (1.02 μg g⁻¹).

Sapota juice was also found to be a rich source of calcium, potassium, zinc, iron which are most essential mineral requirements for the normal functioning of a biological system (Kulkarni et al., 2007). Morton et al. (1987) showed that different variety of Sapota fruit have 28.2-121.0 mg per 100 g calcium, 22.9-33.1 mg per 100 g phosphorus, 0.52-2.62 mg per 100 g iron. Sapota juice is reported to be a good source of the three important transition metals, viz., iron, copper and zinc, which strengthen important nutritional quality Sapota fruit juice beyond doubt (Rao, 1979; Avilan et al., 1980; Sulladmath, 1983).

CONCLUSION
This study provides an overview of the physico-chemical properties of Sapota fruit during growth and development. Generally it could be concluded from the present study, that except from physical properties, most biochemical characteristics (TSS, pH, Chlorophyll a, Chlorophyll b, Carotenoids and Total Phenol) and minerals composition of this fruit decreases during growth and development in order to give a better taste to consumers. This is while Sapota fruit can serve as an important source of mineral for human beings.

![Fig. 5. Macro element content changes of Sapota fruit during fruit growth, development and ripening.](image1)

![Fig. 6. Micro element content changes of Sapota fruit during fruit growth, development and ripening.](image2)

REFERENCES


