Original Research Article

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Physico-chemical Properties Comparison Between Released Varieties and Local Germplasm of Sapota (*Manilkara Zapota*)

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ABSTRACT

A study on physico-chemical properties comparison between released varieties and local germplasm of Sapota was conducted with a view to selecting the superior germplasm in respect of nutritional quality. Samples were collected from selected ten (10) Sapota plants at different homestead of Dumki Upazila, Germplasm Center, PSTU and Bangladesh Agricultural Research Institute, Gazipur and chemical analysis was done at Plant Biotechnology Lab and Postharvest Lab, Patuakhali Science and Technology University. The highest fruit length (4.85cm), width (4.93 cm), weight (115.33 g), edible portion (92.33%), phenolic content (2.537 mg/100gm), anthocyanin content (1.807µg/100gm) were exhibited in G₃ and carotenoid content (5.320µg/100gm) and (5.173µg/100gm) were found in G₃ and G₁. G₁ exhibited the highest vitamin-C (11.42 mg/100g) content, G₂ exhibited the highest percentage of carbohydrate (22.99%), V₁₀ exhibited the highest percentage of TSS (21.28%) & highest peel weight (6.80 g) and the highest percentage of antioxidant (95.80mg/100gm) was exhibited in V₉. Based on the most of all physicochemical properties G₃ was better than the other germplasm/varities. G₁, G₂, V₉ and V₁₀ are also a good source of important compositions than the others. The G3 germplasm might be better for eating fresh fruit as well as processing than the other germplasms/varities.

Keywords: Physico-chemical properties; Released Varieties; Local Germplasm; Sapota

1. INTRODUCTION

Sapodilla (Manilkara Zapota L.) which belongs to the family Sapotaceae, is a tropical fruit 27 commonly known as "Sapota". Generally in Bengali it is known by the people as Sofeda. 28 Immature fruits are hard, gummy and rich in tannin, while the ripe fruits are soft and juicy 29 with good source of nutrient and a sweet taste, which makes them wonderful dessert fruit 30 [1]. From late October to late November it is usually available in our country. It grows well 31 throughout the country, as we are in the tropical environment. It yields fruits two times a 32 33 year. Sapota is an important minor fruit crop and can be considered as one of the healthy fruits because of the presence of various nutritious components in it. Sapota fruit contains 34 sugar, acids, protein, amino acid, phenolics, gallic acid, catechin, chlorogenic acid and 35 Leucopelargonidin, carotenoids, ascorbic acids, and minerals like potassium, calcium and 36 37 iron [2]. Fruits contain carbohydrate (50.49 g/100 g), protein (0.7 g /100g), fat (1.1 g /100g), 38 fiber (2.6g /100g), and minerals nutrient such as calcium (28mg /100g), iron (2.0mg /100g), phosphorus (27mg /100g), ascorbic acid (6.0mg /100g) [3]. People of Bangladesh are 39 40 generally poorly nourished. Most of the people suffer from malnutrition and resultant 41 diseases. It is needed to improve the nutritional status and to increase the food security, particularly for the rural poor. If the minor fruits can be utilized, they may help to contribute in 42 43 food security, nutrition, health, and income generation [4]. In this study an attempt has been made to assess the physico-chemical properties of local germplasm and released varieties 44 and proper categorization of the released varieties and local germplasm of Sapota according 45 46 to their nutritional quality which help in selection of superior germplasm with higher nutritional qualities from among the existing local germplasm. 47

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MATERIALS AND METHODS

1.1 Duration and location

- The study was conducted during March, 2018 to February, 2019 at the Plant Biotechnology
- Lab and Postharvest Lab, Department of Horticulture, Patuakhali Science and Technology
- 53 University, Bangladesh.

54 1.2 Sample collection

- With a view to selecting the local germplasm and released varieties of mature Sapota (120)
- days from fruit setting), four local germplasms were collected from different homestead of
- 57 Dumki upazila and six released varieties were collected from Germplasm Center,
- 58 Department of Horticulture, PSTU and Bangladesh Agricultural Research Institute, Gazipur
- 59 (BARI). Samples were named as G_1 , G_2 , G_3 , G_4 = Germplasm, V_5 = BAU-1, V_6 = BAU-2, V_7 =
- BAU-3, V_8 = BARI-1, V_9 =BARI-2 and V_{10} = BARI-3 were used for Study.

1.3 Treatments and their combinations

- 62 Each selected plant was considered as a treatment. Each treatment was replicated for 4
- times by selecting 4 branches randomly. So, the experiment was comprised of 10 treatments
- with 4 replications. Therefore, treatment combination was 40.

65 1.4 Design and layout of the experiment

The laboratory experiment was done in Completely Randomized Design (CRD) with 4 replications.

1.5 Experimental observations

1.5.1 <u>Physical Characteristics</u>

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- 71 2.5.1.1 Fruit shape: The fruit shape was observed through eye estimation.
- 72 2.5.1.2 Fruit weight measurement: Fully matured 40 (1 fruits x 4 branches x 10 plants) fruits were
- 73 gradually collected to find out the mean weight and other measurement of fruits. The weight was
- taken in gram with the help of an electrical balance.

- 75 *2.5.1.3 No. of seeds per fruit:* Number of seeds per fruit was manually counted after the fruit's ripeness. Ripe fruits were used to calculate the number of seeds per fruit.
- 77 2.5.1.4 Seed weight measurement: Fully ripe and soft fruit was used to collect the seeds. Seeds were
- 78 detached from pulp and wash away thoroughly with distilled water. Then the attached water was
- removed with the help of tissue paper. After that the weight of seeds was taken in gram with the help
- of a balance sensitive to 10 g.
- 81 2.5.1.5 Peel weight measurement: Peel weight data was measured from (fruit weight seed weight+
- 82 pulp weight).
- 83 2.5.1.6 Colour of pulp: Colour of pulp observed through eye estimation.
- 84 2.5.1.7 Fruit length measurement: Length of the fruits was measured from basal to polar by using
- 85 slide calipers and a total of 40 (1 fruits x 4 branches x 10 plants) matured fruits were used to
- determine the length of fruits in cm.
- 87 2.5.1.8 Fruit width measurement: Diameter of the fruits was measured by using slide calipers and a
- 88 total of 40 (1 fruits x 4 branches x 10 plants) fully matured fruits were used to determine the width of
- 89 fruits in cm.
- 90 2.5.1.9 Percentage of edible portion: The percentage of edible (pulp) portion was measured by using
- 91 the following formula [5],
- 92 Weight of edible parts
- 93 Percent of edible portion = ———×100
- 94 Weight of whole fruit
- 2.5.1.10 Weight loss after 7 days: The percentage of weight loss after 7 days was measured by using
 the following formula:
- 97 Weight loss (%) = $\frac{W_1 W_1}{W_1} \times 100$
- Where, W_1 = Initial weight of Sapota fruit
- $W_n = Weight of Sapota fruit after 7 days$

100 1.5.2 Chemical Characteristics

- For chemical evaluation, 9 different chemical characteristics (TSS, TA, Vitamin C, pH, Carbohydrate,
- antioxidant, phenol, Anthocyanin, Carotenoid) were observed.
- 103 2.5.2.1 Determination of Total Soluble Solids (TSS): The TSS of Sapota pulp was determined by
- using a digital refractometer (BOECO, Germany). The remaining of the filtrated juice from TA
- determination was used to measure the TSS of the fruit pulp. Before measurement, the refractometer
- was calibrated with distilled water to give a 0% reading. About 1-2 drops of the filtrate was placed on the prism glass of the refractometer to obtain the % TSS reading. The readings were multiplied by
- dilution factor to obtain an original % TSS of the pulp tissues. Since difference in sample temperature
- could affect the measurement of TSS, each of the reading was standardized to a temperature of 20°C
- 110 by adding 0.28% to obtain % TSS at $26 \pm 1^{\circ}$ C.
- 2.5.2.2 Determination of Titratable Acidity (TA): Titratable acidity (TA) was determined according to a
- 112 standard method [6].

- 2.5.2.3 Determination of ascorbic acid (Vitamin C): Ascorbic acid content was determined according
- to a standard method [6].
- 2.5.2.4 Determination of pH: The remainder of the filtrated juice from TA determination was used to
- 116 measure the pH of the fruit pulp. The pH was determined by using a glass electrode pH meter (GLP
- 21, Crison, Barcelona, EEC). The pH meter was calibrated with buffers at pH 4.0 followed by pH 7.0.
- 118 After that, the glass electrode was kept into the filtrate solution to measure the pH and stabilized
- reading was noted. For correctness of the reading, the glass electrode was washed with distilled
- water after each reading and dry with tissue paper.
- 2.5.2.5 Determination of total soluble carbohydrates: Total soluble carbohydrates were estimated by
- 123 phenol sulphuric acid method [7].
- 124 2.5.2.6 Determination of phenolic content and Antioxidant

- 125 Sample preparation: At first 6 gm sample was taken in Petridis and kept in oven at 3 hours in 60 °C
- temperature. Then 2 gm sample were dissolved in 50 mL methanol in a falcon tube to prepare a stock
- solution. The solutions were vortexes and solicited for several minutes (20-30 minute). The stock
- solution were preserved in room temperature and diluted to necessary concentration when needed.
- 129 Chemical and reagents: Folin-Ciocalteau reagent, Gallic cacid, Rutin Hydrate, TPTZ, ferric chloride,
- 130 sodium hydroxide and methanol were purchased from Merck, Germany, 2, 2-diphenyl-l-picrylhydrazyl
- 131 (DPPH) from Sigma Aldrich Co. Ascorbic acid and NBT were purchased from BDH Co. and Ferrozine
- from Loba India. All the chemicals and reagents were analytical grade.
- 2.5.2.7 Determination of total phenolic content: Phenols, sometimes called phenolic are one of the
- main secondary metabolites present in the plant kingdom
- Reagents: Folin-Ciocalteu reagent (0.5 N), saturated sodium carbonate (20%), Gallic acid (10 µg/mL)
- Procedure: The amount of total phenolic content was determined following the established method [8]
- with some modifications. A 0.5 mL of extract (concentration of extract is 1.0 mg/ mL) and 0.5 mL of
- Folin Ciocalteu reagent (0.5 N) were mixed and incubated at room temperature for 5 minutes. Then
- 2.0 mL saturated sodium carbonate was added and further incubated for 30 minutes at room
- 140 temperature.
- 2.5.2.8 Determination of total antioxidant capacity: Determination of total antioxidant activity was done
- as per as the phosphor molybdenum method with some modification [9]. The basic principle of this
- determination is centered on the reduction of Mo (VI) to Mo (V) by the extract and subsequent
- development of a green phosphate Mo (V) complex at acidic pH.
- Reagent: 6M sulfuric acid, 28mM sodium phosphate, 4mM ammonium molybdate
- Procedure: 1 mL extract was combined with a mixture of 3 mL of reagent solution (0.6M sulfuric acid,
- 147 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction
- solution were then capped and incubated at 95°C for 90 minutes. After the samples had cooled to
- room temperature, the absorbance of the solution was then measured at 695 run against blank.
- Methanol (1 mL) in the place of extract is used as the blank. The total antioxidant content was
- calculated from a calibration curve y = 256.11x 12.645, R2 = 0.9974, where x is absorbance and y is
- 152 concentration of Gallic acid. The antioxidant activity is expressed as the mg of equivalents of Gallic
- 153 acid.
- 2.5.2.9 Determination of total anthocyanin: Total anthocyanin content of leather was determined by a
- 155 standard method [10].
- 2.5.2.10 Determination of total carotenoids: The total carotenoids of the leather pulp were determined
- by a standard method [10].
- 158 **2.6 Statistical analysis**
- The collected data on various parameters under this study were compiled and tabulated in
- proper form for statistical analysis. Analysis of variance was done with the help of MSTAT-C
- 161 computer package program. The mean differences among the treatments were calculated
- with the help of Duncan's Multiple Range Test (DMRT) at 1% and 5% levels of probability
- 163 **[11]**.
- 164 3 RESULTS AND DISCUSSION:
- 165 3.1 Physical Characteristics
- 166 Significant variation was observed among the selected local germplasm and released
- varieties in respect of fruit length. The longest fruit length was obtained from local
- germplasm G₃ and varieties V₉ and V₇ (4.87, 4.85 and 4.60 cm) whereas the shortest fruit
- 169 was found in G₄ (3.13 cm) (Table 1).
- 170 Statistically significant variation was observed among the selected local germplasm and
- 171 released varieties in respect of fruit width. The highest fruit width was obtained from local
- 172 germplasm G₃ (4.93 cm) whereas the lowest fruit width was recorded from variety V₁₀ (3.60
- 173 cm) (Table 1).
- 174 Significant difference was observed in respect of fruits weight among the selected varieties
- and local germplasm of Sapota. The highest fruit weight was recorded in local germplasm G₃

176 (115.33 g) whereas the lowest fruit weight was recorded from the local germplasm G_4 (61.33 g) which was statistically identical with V_7 (63.17 g) (Table 1).

Significant difference was observed in respect of peel weight among the selected local germplasm and released varieties. The highest peel weight was recorded in germplasm G_1 and variety V_9 (6.80 and 6.76 g) whereas the lowest peel weight was recorded from the variety V_7 (5.10 g) which was statistically identical with G_2 (5.20 g) (Table 1).

Significant variation was observed among the selected released varieties and local germplasm of Sapota in respect of edible portion. The highest pulp edible portion was exhibited in G_3 and V_{10} (92.33 and 92.00 g) whereas the lowest edible portion was recorded in variety V_6 (56.00 g) (Table 1).

Table 1: Length of fruit, width of fruit, weight of fruit, weight of peel and edible portion of selected local germplasm and released varieties of Sapota

SI. No.	Length of fruit (cm)	Width of fruit (cm)	Weight of fruit (g)	Weight of peel (g)	Edible portion (%)
G ₁	4.10 b	4.60 d	87.33 d	6.80 a	88.67 b
G_2	3.97 b	4.40 e	96.00 c	5.20ef	91.00 ab
G_3	4.87 a	4.93 a	115.33 a	6.47 b	92.33 a
G_4	3.13 d	3.88 f	61.33i	5.77 c	62.00 f
V_5	4.13 b	4.85 b	72.00 f	5.60 d	65.33 e
V_6	3.55 c	4.40 e	67.83 g	5.30 e	56.00 g
V_7	4.60 a	4.75 c	63.17 hi	5.10 f	64.33ef
V_8	3.50 c	4.85 b	77.50 e	6.46 b	84.67 c
V_9	4.85 a	4.77bc	65. 00 h	6.76 a	81.00 d
V_{10}	4.17 b	3.60 g	112.67 b	6.50 b	92.00 a
Level of Sig.	**	**	**	**	**
CV (%)	4.10	1.07	1.69	1.48	2.04

Means in a column followed by the same letter (s) do not differ significantly by DMRT.

Here, G1, G2, G3, G4= Local germplasm, V5= BAU-1, V6= BAU-2, V7= BAU-3, V8= BARI-1, V9=BARI-2 and V10= BARI-3

There was no significant difference found in case of number of seed, weight of seed, weight loss after 7 days and fruit shape of selected Sapota germplasm and varieties.

Table 2: Number of seed, weight of seed, weight loss after 7 days and fruit shape of selected germplasm and released varieties of Sapota

SI. No.	Number of seed	Weight of seed (g)	Weight loss after 7 days (%)	Fruit shape
G ₁	3.00	1.26	25.44	Round
G_2	2.67	1.60	29.86	Round
G_3	2.67	1.63	25.33	Round
G_4	3.33	1.67	21.67	Round
V_5	2.67	1.56	24.33	Round
V_6	3.00	1.66	25.07	Round

^{**} Significant at 1% level of probability

V_7	3.33	1.96	26.00	Round
V_8	2.67	1.40	25.00	Round
V_9	2.67	1.53	21.67	Round
V_{10}	3.33	1.77	27.33	Round
Level of Sig.	NS	NS	NS	
CV (%)	14.96	14.95	13.07	

Means in a column followed by the same letter (s) do not differ significantly

By DMRT,

NS= Non significant

Here, G1, G2, G3, G4= Local germplasm, V5= BAU-1, V6= BAU-2, V7= BAU3, V8= BARI-1, V9=BARI-2 and V10= BARI-3

3.2 Chemical Characteristics

3.2.1 pH: pH of sapota increased from 5.9 to 6.67. But pH value decreases when it over ripened ,the pH value decreases due to turning sour to fermentation sugar [12] [13]. No significant variation was observed in case of pH content among the local germplasm and released varieties of Sapota.

3.2.2 Titratable acidity (%): Acidity of sapota decreased from 1.92 to .74 [13]. The TA decrease because of soluble sugar which is increase during the course of ripening [14]. No significant variation was observed in case of TA content among the local germplasm and released varieties of Sapota.

3.2.3 Total soluble solids (%): TSS concentration significantly from the level at zeroth day 19% to 24%. The total soluble solids increases in all fruits as the fruit ripens [14]. The percentage of total soluble solids (TSS) showed significant variation among different released varieties and local germplasm of Sapota. The highest percentage of TSS was found in V_{10} variety (21.28%) while the lowest percentage was found in local germplasm G_1 (7.53%) (Figure 1).

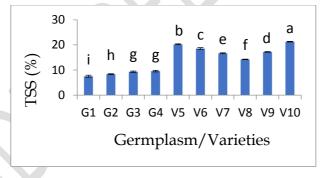


Figure 1: TSS content of selected Sapota fruits

Vertical bar represent error bar with standard deviation

Here, V1= Local verity, V2= Local verity, V3= Local verity, V4= Local verity, V5= BAU-1, V6= BAU-2, V7= BAU-3, V8= BAR-1, V9=BARI-2 and V10= BARI-3

3.2.4 Vitamin-C (mg/100g): Vitamin-C of sapota decrease from 13.2-5.34 in control. This decrease trend of Vitamin-C occurs due to increase in total soluble sugar present in the fruit. [12] [13]. The ascorbic acid content of fruits varied significantly among the released varieties and local germplasm of Sapota. It was found that, the vitamin-C content was higher in local germplasm G_1 and G_2 (11.42 and 11.33 mg/100g) whereas the lower vitamin-C content was found in V_7 variety (3.38 mg/100g) (Figure 2).

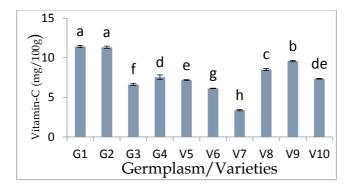


Figure 2: Vitamin-C content of selected Sapota fruits

Vertical bar represent error bar with standard deviation

Here, V1= Local verity, V2= Local verity, V3= Local verity, V4= Local verity, V5= BAU-1, V6= BAU-2, V7= BAU-3, V8= BARI-1, V9=BARI-2 and V10= BARI-3

3.2.5 Carbohydrate content (%): In fresh Sapota fruit the percentage of carbohydrate per 100 gm is about 19,9 g,Percentage of RDA 15% [15]. Significant variation was observed in case of carbohydrate content among the selected local germplasm and released varieties. It was found that, the carbohydrate content was higher in local germplasm G_2 (22.99%) whereas the lower carbohydrate content was counted in V_5 variety (11.68%) (Figure 3).

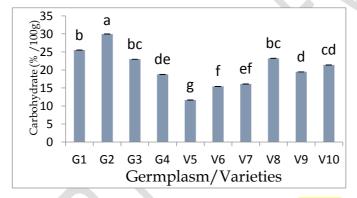


Figure 3: Carbohydrate content of selected Sapota fruits

Vertical bar represent error bar with standard deviation

Here, V1= Local verity, V2= Local verity, V3= Local verity, V4= Local verity, V5= BAU-1, V6= BAU-2, V7= BAU-3, V8= BARI-1, V9=BARI-2 and V10= BARI-3

3.2.6 Phenolic content: Total phenolic contents assay is known to overestimate the content of phenolic compounds, because other agents present in food, such as carotenoids, amino acids, sugars and vitamin C, can interfere [16] [17]. Significant variation was observed in case of total phenolic content among the selected Sapota germplasm and released varieties. It was found that, the phenolic content was higher in local germplasm G_3 (2.537 mg\100gm), whereas the lower phenolic content was found in local germplasm G_4 (1.033mg\100gm) (Figure 4).

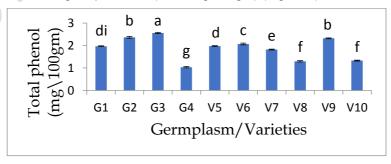


Figure 4: Total Phenolic content of selected Sapota fruits

Vertical bar represent error bar with standard deviation

3.2.7 Antioxidants content: Fruit and vegetable are sources of nutrients and antioxidants that may be ingested in form of edible films made from them [17]. Significant variation was observed in case of total antioxidants content among the selected germplasm and released varieties. It was found that, the antioxidant was higher in V_9 variety (95.80 mg\100gm), whereas the lower antioxidant content was found in local germplasm G_4 (80.64mg\100gm) (Figure 5).

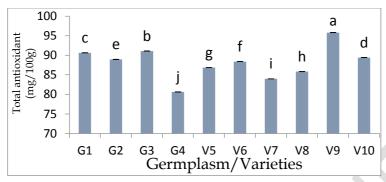


Figure 5: Total antioxidant content of selected Sapota fruits

Vertical bar represent error bar with standard deviation

Here, V1= Local verity, V2= Local verity, V3= Local verity, V4= Local verity, V5= BAU-1, V6= BAU-2, V7= BAU-3, V8= BARI-1, V9=BARI-2 and V10= BARI-3

3.2.8 Anthocyanin content: Anthocyanin is well known because of their antioxidant properties and their Pigmenting power that makes them attractive to be used as food colorants [18]. Significant variation was observed in case of anthocyanin content among the selected Sapota germplasm and released varieties. It was found that, the anthocyanin content was higher in germplasm G_3 (1.807µg\100gm), whereas the lower anthocyanin content was found in V_6 (.6667µg\100gm), G_4 (.6100µg\100gm), and V_7 (.5867µg\100gm) (Figure 6).

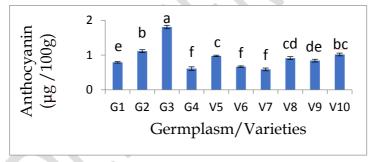


Figure 6: Anthocyanin content of selected Sapota fruits

Vertical bar represent error bar with standard deviation

Here, V1= Local verity, V2= Local verity, V3= Local verity, V4= Local verity, V5= BAU-1, V6= BAU-2, V7= BAU-3, V8= BARI-1, V9=BARI-2 and V10= BARI-3

3.2.9 Carotenoid content: Carotenoids are widely distributed in nature and they are liposoluble antioxidants [18]. Significant variation was observed in case of carotenoids content among the selected Sapota germplasm and released varieties (Figure 13). It was found that, the carotenoids content was higher in germplasm G_1 (5.173 μ g\100gm) and G_3 (5.320 μ g\100gm), whereas the lower carotenoids content was found in V_5 variety (1.277 μ g\100gm) (Figure 7).

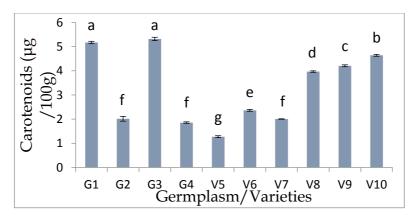


Figure 7: carotenoids content of selected Sapota fruits

Vertical bar represent error bar with standard deviation

Here, V1= Local verity, V2= Local verity, V3= Local verity, V4= Local verity, V5= BAU-1, V6= BAU-2, V7= BAU-3, V8= BARI-1, V9=BARI-2 and V10= BARI-3

4. SUMMARY AND CONCLUSION:

From the above study nutritional contents varied among the studied germplasm. The G₃ germplasm might be superior for eating fresh fruit as well as processing than the other germplasms/varities. If farmer produce this high quality Safoda more, people will get a good source of nutrient and the farmer will also be benefited. Further study is needed for production, processing and preservation of nutritive Safoda and good quality Safoda based products.

282 **COMPETING INTERESTS**

283 Authors have declared that no competing interests exist.

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APPENDIX

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Appendix 1: Analysis of variance (ANOVA) of physical characteristics of Sapota

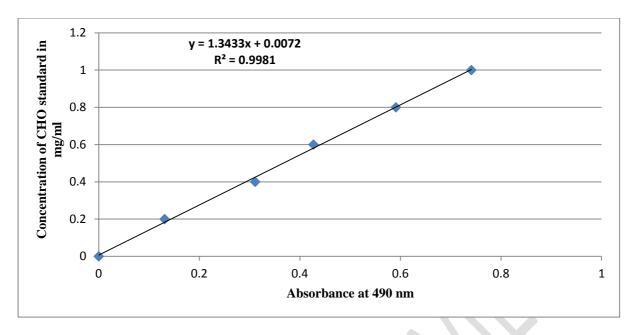
SI .No.	Length of	Width of fruit(cm)	Weight of	Weight of peel	Edible portion	Number of seed	Weight of	Weight loss after 7 days
.NO.	fruit(cm)	Truit(CIII)	fruit(g)	of peel (g)	(%)	or seed	seed (g)	(%)
Repli cation	0.061	0.016	1.633	0.012	4.933	0.933	0.137	1017.023
Facto r A	1.006**	2.382**	1222.4 71**	1.356**	608.05 9**	0.281 ^{NS}	0.109 NS	17.607 ^{NS}
Error	0.028	0.009	1.902	0.008	2.526	0.0193	0.058	10.819

Appendix 2: Analysis of variance (ANOVA) of chemical characteristics of Sapota

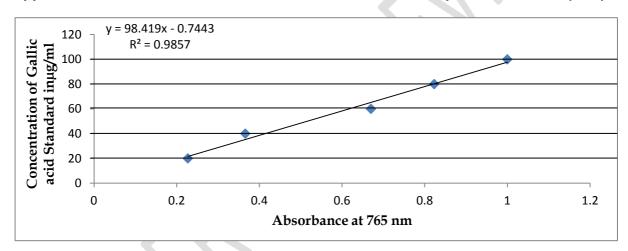
	,	- ,		
Sl. No.	pН	Titratable	Total Soluble	Vitamin-C
		Acidity	Solid	
Between	0.082 ^{NS}	0.017 ^{NS}	81.564**	17.806**
Within	0.037	0.016	0.056	0.018

Appendix 3: Analysis of variance (ANOVA) of chemical characteristics of Sapota

Sl. No.	Carbohydrate	Anthocyanine	carotenoids	Phenolic Contents	Antioxidants
Between	85.426**	.376**	7.009	.765	52.139
Within	2.715	.004	0.010	0.001	0.001



Appendix 5: Calibration curve of Gallic acid standard for total phenolic content (TPC)



Appendix 6: Calibration curve of Gallic acid standard for total antioxidant capacity determination

