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Comparative Study of Functional Properties and Nutritive Value of *Manilkara Zapota* (Sapodilla) Flesh and Peel Powder at Different Maturity

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Abstract: Sapodilla fruit was known for various properties which can bring health benefits. However, limited shelf life of fruit and waste of sapodilla peels can lead to food waste and loss of nutrients and excellent biomass. A study has been made to compare the effect of maturity in functional properties and nutritive value from flesh and peel by transforming the raw fruit into powder to increase the shelf life and utilize the health benefits in desired maturity. From the studies, pH value of mature unripe sapodilla peel yields higher acidity in pH 4.97 than sapodilla flesh, pH 5.05 while titratable acidity of sapodilla flesh was 0.20% higher than sapodilla peel as 0.17%. Mature unripe fruit indicates high quality than mature ripe and mature overripe fruit. Higher total soluble solid recorded in sapodilla flesh in mature ripe, 6.10 °Brix indicate the sweetness of sample. Vitamin C content of mature unripe sapodilla peels as high as 12.12 mg/100g than sapodilla flesh yields 9.85 mg/100g. Nutritive values was higher in mature unripe fruit which sapodilla flesh high in citric acid while sapodilla peel high in vitamin C. Mature unripe sapodilla peel inhibit higher antioxidant activity noted in 15.32% than sapodilla flesh, 14.03%. Meanwhile total phenolic content was higher in sapodilla peel, which was recorded as 0.09 mg GAE/g than 0.07 mg GAE/g in sapodilla flesh. Intense peak of O-H stretch and C-O stretch indicate phenolic content at mature unripe sapodilla peel. For the functional properties, mature unripe sapodilla peel possesses higher antioxidant content than sapodilla flesh and other maturity level which potential to be used to develop a new product while reduction of food waste can be avoided in early stages.

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Keywords: Sapodilla, Shelf Life, Maturity, Sapodilla Peel, Sapodilla Flesh, Powder, Functional Properties, Nutritive Value

1. Introduction

Sapodilla is a unique tropical fruit species that comes from the family Sapotaceae which emerged from Southern Mexico or Central America [1]. It consists of heterogeneity of chemical compounds such as carbohydrates, amino acids, minerals, vitamins, phenolic compounds, terpenes, steroids, saponins, fixed oils, and hydrocarbons [2]. From the different health-promoting functional compounds abound in fruit extracts, dietary supplements and functional foods or nutraceuticals can be developed with these components [3]. However, this fruit has a short period of shelf life which also can lead to wastage of overripe fruit. It is also known as a perishable fruit which will rot after two weeks [4]. So, it is important to decide which maturity level of fruit is the best quality as a standard to develop new products. In this study, the peel and flesh turned into powder to increase the shelf life by reducing water activity and removing excessive water content. By that way, the stability and functional properties of the fruit can be preserved before undergoing the analysis [5].

Generally, peel of the fruit being discarded in the food processing thus increases the food waste [6], [7]. However, peel extract of the fruit reported consists of higher antioxidants compared to the flesh [8]. Antioxidants' activity has been shown to reduce the risk of cognitive impairment in the elderly [9]. In order to avoid the loss of nutrients and excellent biomass in the peel extract, this study includes the analysis between peel and flesh to investigate the different nutritive value and functional properties that are abundant in the fruit sample. The analysis of the fruit became a concern among the researcher because every fruit consists of different functional properties and nutritive value.

The nutritional value of fruit can be proven by the existence of total soluble sugars, organic acids, essential fatty acids, amino acids, and some major secondary metabolites [10]. Thus, this study was aimed to transform sapodilla fruit (peel and flesh) into powder form and investigate the effect of maturity that affecting functional properties and nutritive value by comparing the physicochemical characteristics, vitamin C content, antioxidant activity, total phenolic content, and spectral analysis of sapodilla fruit. It involves different storage times from day 3, day 4 and day 8 which represent mature unripe, mature ripe and mature overripe fruit.

2. Materials and Methods

2.1 Sample collection, preparation, and extraction

Sapodilla fruit was collected at unripe stage from the same tree and species, *Manilkara zapota (L.) P. Royen.* from cultivars of C54 according to the Department of Agriculture (DOA). Each fruit is stored for day 3, day 4 and day 8 to reach a different maturity stage and undergo a drying process in an oven dryer at 65°C at 24 hours for peel and 48 hours for flesh [11]. Then ground into powder form, stored in a zip lock bag and desiccator until further studies. The methanolic extract was prepared for radical scavenging activity (RSA) and total phenolic content (TPC) by dissolving 0.1g sample in 10 ml methanol then centrifuged for 15 min at 4200 rpm at 4°C [12].

2.2 Eye observation on colour, texture of flesh and mean weight measurement

The physical analysis from its colour and texture of flesh were determined by eye observation. The weight of sapodilla fruit was measured using an electronic balance for different parts. Weight of peel was calculated as Eq. 1 [13]. The colour of fruit can be identified by the eye observation which indicates that each different maturity indicates different colour of flesh. According to Manilkara & Madani (n.d.) the ripe flesh of sapodilla will be dark-brown while unripe flesh will be light yellow but the overripe will turn to reddish-brown colour [14]. The flesh texture in different levels of maturity would be

different in which the ideal maturity will be soft, very juicy, and sweet with a pleasant flavour while unripe flesh will be coarse, grainy, or smooth [15],[16].

$$\text{Weight of peel} = \text{Whole fruit weight} - [\text{seed weight} + \text{pulp weight}] \qquad Eq.\ 1$$

2.3 Physicochemical analysis

The pH value of the sample was measured using a pH meter by mixed 1:10 ratio with distilled water and calibrated with buffer solution, pH 7 [17]. Total soluble solid (TSS) of the sample measured by digital refractometer by mixing with distilled water as in 1:10 ratio and expressed as °Brix according to the AOAC (2016) [13], [18]. Titratable acidity (TA) was measured by 5 mL of sample diluted with 25mL of distilled water then titrated with 0.1N NaOH up to pH 8 and titre value was recorded [17]. The percentage of acid based on citric acid can be calculated as Eq. 2 [19]:

$$\text{Citric acid (\%)} = \frac{Volume\ of\ NaOH\ used \times 0.1N\ NaOH \times miliequivalent\ factor \times 100}{grams\ of\ sample} \qquad Eq.\ 2$$

2.4 Estimation of Vitamin C content

Ascorbic acid content was determined by 2,6-dichloroindophenol (DCPIP) titration method. The DCPIP dye, which is blue in alkaline solution, is reduced by ascorbic acid to a colourless form. About 5 mL of 3% metaphosphoric acid extract of the sample was titrated with DCPIP dye to a pink colour end point and ascorbic acid can be calculated by Eq. 4. The ascorbic acid content was calculated using the dye factor (Eq. 3), determined by the titration of the standard ascorbic acid solution with DCPIP. All the methods follow according to Lim et al. (2018) [20] with modification.

$$\text{Dye factor} = \frac{0.5}{Titre\ value} \qquad Eq.\ 3$$

$$\text{Ascorbic acid (mg/100g)} = \frac{Dye\ factor \times tire\ value\ (ml) \times Volume\ made\ up \times 100}{Volume\ of\ sample\ (ml) \times Weight\ of\ sample(g)} \qquad Eq.\ 4$$

2.5 Determination of antioxidant activity

The antioxidant activity was determined by the free radical scavenging activity according to Roy et al. (2013) with slight modification [21]. 2,2 – diphenyl-1-picrylhydrazyl (DPPH) solution was made by dissolving 4 mg DPPH reagent in 100 mL methanol in volumetric flask (0.1 mM). 400 µL of sample extract or standard gallic acid was mixed with 2 mL of 0.1 mM of DPPH methanol solution and were vortexed [21]. The solution was left in the dark place for reaction by incubation for 30 minutes [18]. The concentration of remaining DPPH in the solution was analysed by UV-Vis spectrophotometer by the absorbance at 517 nm [22]. The percentage of inhibition was calculated as Eq. 5. [12]:

$$\text{\% inhibition} = \frac{(AControl - ASample) \times 100}{AControl} \qquad Eq.\ 5$$

2.6 Determination of total phenolic content

Total phenolic content was determined using Folin-Ciocalteu reagent. In brief, 0.3 mL of sample was mixed with 2.25mL of Folin-Ciocalteu reagent. After 5 minutes, 2.25 ml of sodium carbonate solution (Na2CO3) were added to the mixture and vortexed. After 90 min stored in the dark place, absorbance was measured at 765 nm against a blank. Total phenolic content was calculated based on the calibration curve of gallic acid and expressed as gallic acid equivalents [23]. A calibration curve ($y = 4.20097x - 4.4263$; $R^2 = 0.9692$) was used to calculate the total phenolic content.

2.7 Spectral analysis

The presence of phenolic content and other bioactive compounds in flesh and peel powder were determined using a Fourier Transform Infra-Red (FTIR) spectrophotometer. The wavelengths were in

the range of 500 to 4000 cm-1 in flesh and peel powder. The FTIR spectrum was expressed in terms of % transmittance.

2.8 Statistical analysis

All the analysis were done in triplicate and were expressed as mean ± standard deviation. The mean, standard deviation, and significant difference ($p<0.05$) were determined using the one – way analysis (ANOVA) by MINITAB 19 Statistical Software.

3. Results and Discussion

3.1 Physical observation on sapodilla

Based on Table 1, the colour of flesh from the eye observation changes from light-brown, dark-brown, and reddish-brown from day 3, 4 and 8. The texture of flesh from coarse, grainy, and smooth on day 3 became very soft, watery, and bruised on day 8. It indicates that maturity level affects the physical appearance of fruit and quality of end products as shown in Table 2. The colour and texture of flesh can be a standard to maturity level of fruit thus post-harvest losses can be avoided. The mean weight of raw fruit shows a decline upon increasing maturity stage. It can be explained by the loss of moisture due to respiration and transpiration during the ripening process during fruit ripening. In that case, the mean weight of sapodilla fruit can be a parameter to develop quality products. From that, the level of maturation in fruit considered in a good state can be known. Unripe fruit indicates low mass in weight while ripe fruit recorded high mass of weight while overripe sapodilla is contributing to less mass due to the ripening [24].

Table 1: Physical observation on colour, texture, mean weight and other measurement

Sample	Colour of flesh	Texture of flesh	Mean weight measurement of raw fruit(g)	Weight powder (g)
CP 3	-	-	178.71	50.24
CP 4	-	-	210.55	58.69
CP 8	-	-	215.06	59.17
CF 3	Light-brown	Coarse, grainy, smooth	1230.30	207.51
CF 4	Dark-brown	Soft, juicy, sweet with pleasant flavour	1058.54	182.24
CF 8	Reddish-brown	Very soft, watery, have bruise	1028.62	166.53

Sapodilla peel is stated as CP and sapodilla flesh as CF for Day 3 (Mature unripe), Day 4 (Mature ripe), Day 8 (Mature overripe). Weight based on 20 samples each maturity level.

Table 2: Colour and texture of flesh in different fruit sample CF 3, CF 4 and CF 8

	CF 3	CF 4	CF 8
Fruit sample			

Colour and texture of flesh			

3.2 Physicochemical analysis

Sapodilla peel shows a rise in pH value from day 3 ($4.97^{c} \pm 0.01$) to $5.05^{c} \pm 0.01$ for day 4 and $5.33^{b} \pm 0.13$ on day 8 as shown in Table 3. As for sapodilla flesh also shows a rise pattern from $5.05^{c} \pm 0.01$, $5.23^{b} \pm 0.01$ and $5.78^{a} \pm 0.03$ at day 3, 4 and 8 respectively. The increasing trend of pH value indicates the acidity of the sample was decreasing upon storage time. Sapodilla peel was more acidic than sapodilla flesh and the highest pH value recorded at day 3 which indicates that the most acidic sample occurred at mature unripe stage. Sapodilla fruit recorded as 5.30 to 6.30 of pH value in increasing ripening stage and it is due to the decreasing acidity affecting ripening of fruit [24].

The highest titratable acidity was identified in sapodilla flesh on day 3 ($0.20^{a} \pm 0.15$). Then, the titratable acidity declines as an increasing maturity stage for day 4 and 8 respectively as in Table 3. It can be justified which the highest value of titratable acidity in sapodilla tend to occur during unripe stage which recorded as high as 5.89% fall into 4.95% during overripe stage based on previous researcher [25]. Titratable acidity and pH are two variables in food analysis that are linked and focus on acidity which can determine the quality of food. The analysis was carried out to analyse the organic acid present in fruit. The most amount of organic acid reported in sapodilla fruit are malic acid, citric acid, and tartaric acid [26].

For TSS, sapodilla flesh perceived higher value at day 4 ($6.10^{a} \pm 0.00$) ºBrix than sapodilla peel at day 4 ($4.77^{d} \pm 0.06$) ºBrix as in Table 4.4. TSS indicate the sweetness of the product which can be used as standard to the quality of the product [27]. For the sugar content, sapodilla flesh was proven sweeter than sapodilla peel. The high amount of the total soluble solid can be explained according to the ripening stage for the product which sugar levels in ripe fruit are high resulting in senescence [28].

Table 3: Physical analysis on pH, titratable acidity and total soluble solid

Sample	pH	Titratable Acidity (% citric acid)	Total Soluble Solid (°Brix)
CP 3	$4.97^{c} \pm 0.01$	$0.17^{ab} \pm 0.15$	$3.77^{f} \pm 0.12$
CP 4	$5.05^{c} \pm 0.01$	$0.14^{bcd} \pm 0.12$	$4.77^{d} \pm 0.06$
CP 8	$5.33^{b} \pm 0.13$	$0.10^{cd} \pm 0.15$	$4.27^{e} \pm 0.06$
CF 3	$5.05^{c} \pm 0.01$	$0.20^{a} \pm 0.15$	$5.53^{b} \pm 0.15$
CF 4	$5.23^{b} \pm 0.01$	$0.14^{bc} \pm 0.10$	$6.10^{a} \pm 0.00$
CF 8	$5.78^{a} \pm 0.03$	$0.09^{d} \pm 0.06$	$5.30^{c} \pm 0.00$

Sapodilla peel is stated as CP and sapodilla flesh as CF for Day 3 (Mature unripe), Day 4 (Mature ripe), Day 8 (Mature overripe).

Values are expressed as mean±SD of triplicates (n = 3). Values with different superscripts within the row are significantly different ($p<0.05$).

3.3 Vitamin C content

The highest amount of ascorbic acid was identified in the sapodilla peel on the unripe stage. Then it starts to decline upon senescence during day 4 and day 8 of storage as in Table 4. CP3 recorded a higher amount of ascorbic acid ($12.12^{a} \pm 0.06$ mg/100g) than CF3 ($9.85^{ab} \pm 0.06$ mg/100g) which are

regarded as having higher antioxidant properties. Sapodilla fruit have high ascorbic acid content in the lowest level of ripening and the overripe fruit is proven as not rich in ascorbic acid [25].

3.4 Antioxidant activity

For DPPH Radical Scavenging Activity (RSA), CP3 recorded as the higher inhibition at $15.32^{b} \pm 0.01$ than CF3 at $14.03^{ab} \pm 0.01$ as shown in Table 4. DPPH assay is one of the most widely used methods for evaluating the antioxidant potential of foods and testing the ability of substances to serve as free radical scavengers or hydrogen donors [29]. From the results, it shows that inhibition of RSA decreases from day 3 until day 8 due to the increase in maturity.

3.5 Total phenolic content (TPC)

The highest TPC content detected in CP3 was recorded as $0.09^{a} \pm 0.0113$ mg GAE/g, followed by $0.07^{ab} \pm 0.0028$ mg GAE/g in CF3. Then the pattern shows a reduction in TPC in day 4 and day 8 upon the senescence of the fruit maturity level as in Table 4. The amount of TPC in each sample was significant at ($p<0.05$). Previous researchers found that the unripe stage was recorded as 2.7 mg GAE/g of phenolic compound then reduced to 1.0 mg GAE/g at the ripe stage [30].

However, the efficiency of antioxidant content and phenolics content from the natural sources depend on the extraction method for instance, solvent concentration, extraction time and temperature [22]. It is explained the low value of DPPH Scavenging Activity and total phenolic content of sapodilla fruit in the research study.

Table 4: Chemical analysis of sapodilla peel and flesh

Sample	Ascorbic acid content (mg/100g)	DPPH Scavenging Activity (%)	Total Phenolic Content (mg GAE/g)
CP 3	$12.12^{a} \pm 0.06$	$15.32^{b} \pm 0.01$	$0.09^{a} \pm 0.01$
CP 4	$7.57^{bc} \pm 0.06$	$13.17^{ab} \pm 0.01$	$0.07^{ab} \pm 0.01$
CP 8	$6.44^{bc} \pm 0.03$	$11.21^{a} \pm 0.03$	$0.07^{ab} \pm 0.01$
CF 3	$9.85^{ab} \pm 0.06$	$14.03^{ab} \pm 0.01$	$0.07^{ab} \pm 0.00$
CF 4	$6.06^{bc} \pm 0.06$	$12.64^{ab} \pm 0.01$	$0.06^{bc} \pm 0.00$
CF 8	$4.54^{c} \pm 0.10$	$11.41^{a} \pm 0.01$	$0.04^{c} \pm 0.00$

Sapodilla peel is stated as CP and sapodilla flesh as CF for Day 3 (Mature unripe), Day 4 (Mature ripe), Day 8 (Mature overripe).

Values are expressed as mean±SD of triplicates (n = 3). Values with different superscripts within the row are significantly different ($p<0.05$).

3.6 Fourier transform infrared (FTIR) analysis

FTIR was utilized to determine the chemical functional group by showing the infrared (IR) spectra of respective compounds. A sharp peak O-H stretch were noted between (3700-3584 cm^{-1}) compound class alcohol at 3669 cm^{-1} for CP3, weak peak at 3835, 3738 cm^{-1} for CP4, sharp peak at 3754 and 3679 cm^{-1} for CP 8, sharp peak at 3823, 3693, 3677, 3654 and 3618 cm^{-1} for CF 4, sharp peak at 3768 for CF 8 and lastly strong and broad peak at 3300-2500 cm^{-1} for carboxylic acid noted at 3306, 3245, 2910 cm^{-1} for CF 3. It was observed that in CF3 consist of carboxylic acid compounds whereas the other was noted to have alcohol compounds. Alcohol compounds were observed having intense sharp peaks in CF4 and CP3. C-O stretch was noted between 1200-1000 cm^{-1} which CP3 was observed having broad and medium peak at 1030 cm^{-1}, broad and high peak in CP4 at 1021 cm^{-1}, broad and medium peak in CP8 at 1022 cm^{-1}, broad and high peak in CF3 at 1017 cm^{-1}, broad and medium peak in CF4 at 1022 cm^{-1} and broad and weak peak in CF8 at 1016 cm^{-1}. The intense and broad peak observed at 1030 cm^{-1} indicates the presence of C-O stretch for the esters and ethers functional group [31]. It can be observed that the highest peak is recorded at CP3 which indicates the hydroxyl group. From the band peak it

shows the presence of phenolic content with the presence of hydroxyl groups from O-H stretch and C-O stretch. Hydroxyl group was identified at all samples but shown in different IR spectra according to Table 5 and the highest peak recorded at CP3.

Table 5: Spectral band peak for each functional group

Functional group/ Vibrations	Alcohol (O-H stretch)	Alkyne (C≡C stretch)	Amine (C-N)	Esters /ethers (C-O stretch)	Carboxylic acid (O-H stretch)	Isothiocyanate (N=C=S stretch)	Carbonyl group (C=O)
			Band peak (cm^{-1}) /				
CP 3	3669	2133	1235	1030	-	-	-
CP 4	3835, 3738	2231	1243	1021	-	-	-
CP 8	3754, 3679	-	1243	1021	-	-	-
CF 3	-	-	1246	1017	3306, 3245, 2910	-	-
CF 4	3823, 3693, 3677, 3654, 3618	-	-	1022	-	2000-2500 2018	-
CF 8	3768	2260-2190 2175	-	1016	-	-	1704

Sapodilla peel is stated as CP and sapodilla flesh as CF for Day 3 (Mature unripe), Day 4 (Mature ripe), Day 8 (Mature overripe).

4. Conclusion

In conclusion, shelf life of sapodilla fruit was extended in powder form. CP3 yields higher acidity in pH 4.97 than CF3 recorded as 5.05 while TA of CF3 was 0.20% higher than CP3 which noted as 0.17%. The quality of food determined which acidity decreased accordingly on day 4 and 8. Higher TSS value recorded in CF4, 6.10 °Brix indicate the sweetness of sample. Vitamin C content of CP3 high as 12.12 mg/100g than CF3 yields 9.85 mg/100g. Nutritive values such as organic acid and Vitamin C were determined higher on day 3 with CF3 high in citric acid while CP3 high in vitamin C. Higher inhibition of DPPH noted in CP3 for 15.32% than CF3, 14.03%. Meanwhile TPC content was higher in CP3 which recorded as 0.09 mg GAE/g than 0.07 mg GAE/g in CF3. For the functional properties, CP3 possesses higher antioxidant content than CF3 and another storage time. O-H stretch and C-O stretch indicate phenolic content with intense peak noted at CP3. Further studies required concerning the isolation of bioactive compounds in sapodilla fruit from different maturity levels by the different extraction conditions for effective identification of nutritive value and functional properties.

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