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Antioxidant activity and physicochemical properties changes of papaya (*Carica papaya* L. cv. Hongkong) during different ripening stage

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Abstract

Papaya (*Carica papaya* L. cv. Hongkong) is an economically important fruit crop grown in Malaysia. During its ripening stages, (*C. papaya* L.) exhibits different physicochemical properties, antioxidant capacities, and sensory quality results. The objective of this study was to elucidate in detail the antioxidant capacity of *C. papaya* as determined by total phenol content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP),2,2-diphenyl-1-picrylhydrazyl (DPPH) and scavenging systemand (ABTS). The study also aimed to study physicochemical changes of papaya fruits based on measured pH, titratable acidity (TA), total soluble solids (TSS), moisture and fruit color at five different stages of ripening. The fruits were harvested at five different, stages RS1, RS2, RS3, RS4, and RS5 corresponding to 12, 14, 16, 18, and 20 weeks after anthesis, respectively. Significant differences were found at different stages of ripening. The pH of the fruit decreased significantly ($P < 0.05$), whereas TA, moisture, and TSS increased significantly ($P < 0.05$) during the ripening process. The redness (a^*) and yellowness (b^*) values of fruit color both increased significantly ($P < 0.05$), whereas lightness (L^*) varied. The total phenol content TPC, TFC, FRAP, DPPH and ABTS values increased significantly ($P < 0.05$) with the ripening process. Sensory evaluation based on the color, sweetness, sourness, flavor, and overall acceptance for the last three maturity stages was also performed. RS5 had a better score than RS3 or RS4. The results showed the important role of the ripening stage in increasing the antioxidant content of papaya fruits.

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Introduction

Carica papaya L. is part of Caricaceae family, and a variety of Caricaceae types have medicinal properties and have been used against diseases for many years (Munoz *et al.*, 2000; Mello *et al.*, 2008). Papaya has been ranked one of the top for nutritional scores among 38 common fruits (Ming *et al.*, 2008). Practically every part of the fruit is used in a variety medical purposes (Hewitt *et al*., 2000; Da Silva *et al*., 2010). It has been argued by scientists that all parts of papaya, including seeds, roots, rinds, and fruits have positive effects on general health preventing diseases (Seigler *et al.*, 2002). Oxidation can be delayed by antioxidants of a few substrates in a chain reaction. Thus, antioxidants has a crucial role in diseases prevention, and can be used in increasing doses as technological and economic advancement are obtained (Halliwell *et al*., 1992). The decline in morbidity and mortality due to heart disease and cancer are often associated with the consumption of fruits and vegetables (Aruoma, 1998). Papayais the main source of many vitamins, such as vitamin C containing also vitamin E, pectin, and carotenoids. In terms of digestion the fruits' latex, and juice aidin dyspepsia, intestinal irritation, habitual constipation, and chronic diarrhea and coupled with them, bleeding piles, as well as enlarged spleen and liver can also be improved. Antioxidants have been revealed in a larger number of agricultural products, including fruits, vegetables and grains and medical plants (Abas *et al*., 2006). May be an important property of plants medicines related with the treatment of many of ill-fated diseases including diabetes and cancer. It must be noted that the capacity of fruits in terms of antioxidant differs based on their genetic properties for example time of harvest, season of harvest, postharvest and processing elements (Kevers *et al*., 2007). Papaya quality is affected by the ripening process (Chonhenchob and Singh, 2005). Findings of research show that the antioxidant capacity of fruits is vital as a consequence of their short postharvest shelf life. The aim of this study was to determine the effect of different ripening stages on the total phenol content, total flavonoids content, and physicochemical properties (pH, titratable acidity, moisture, total soluble solid and color). Furthermore, we aimed at determining the physicochemical properties, sensory quality and evaluating the antioxidant activity of papaya fruits growing in Malaysia.

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Materials and Methods

Samples collection

Papaya fruits (*Carica papaya* L. cv. Hongkong) were collected from farms at semenyih during season 2011. Samples were transfer on the same date to University Kebangsaan Malaysia (UKM) Food Laboratory, Bangi. The fruits were selected with different ripening stages of papaya (RS1 as fruits at stage 1, 12 weeks after anthesis, RS2 as fruits at stage 2, 14 weeks after anthesis, RS3 as fruits at stage 3, 16 weeks after anthesis, RS4 as fruits at stage 4, 18 weeks after anthesis and RS5 as fruits at stage 1, 20 weeks after anthesis). For the purposes of this study approximately 20 fruits from each stage of maturity.

Extraction of antioxidant

Papaya were peeled, cut into 1 cm slices and crushed in a food processor to produce uniform slurries by using (Waring blender, HGBZWTS3). The slurry was prepared fresh to preserve the extracted antioxidant compounds. In the extraction process, about 1 g of papaya slurries were weighed in universal bottles and 10 ml solvent was added. Solvents used were 50% methanol; samples (papaya slurries with solvents) were then homogenized using homogenizer (T 250, IKA, Germany) at 24,000 rpm for 1 min. All extracted samples were centrifuged by using table top centrifuge (MLX 210, Thermo-line, China) at 4750 g for 10 min. The supernatants were collected for further analysis.

Total phenol content (TPC)

The determination of antioxidant activity through TPC was carried out according to the method of Musa *et al.* (2011). About 100 µL papaya extracts was added with 0.4 mL distilled water and 0.5 mL diluted Folin-Ciocalteu reagent. The samples (papaya extracts with Folin-Ciocalteu reagent) were left for 5 min before 1 mL 7.5% sodium carbonate (w/v) was added. The absorbances were taken at 765 nm wavelength with spectrophotometer after 2 hours. Calibration curve of gallic acid was set up to estimate the activity capacity of samples. The result was expressed as mg of gallic acid equivalents per 100 g of fresh sample (mg GA/100 g of FW).

Total flavonoid content (TFC)

The TF content was determined by the colorimetric method as described by Abu Bakar *et al.* (2009). A total 0.5 ml of the extract was mixed with 2.25 ml of distilled water in a test tube, followed by the addition of 0.15 mL of 5% (w/v) $NaNO_2$ solution. After 6 min, 0.3 ml of a 10% $AlCl_3 \cdot 6H_2O$ solution was added, and the reaction was allowed to stand for another 5 min before 1.0 ml of 1 M NaOH was added. The mixture was mixed well by vortexing, and the absorbance was measured immediately at 510 nm using a spectrophotometer (Epoch, Biotek, USA). The results were expressed as milligrams of quercetin equivalents (QE) per 100 g of fresh sample (mg QE/100 g of FW).

Ferric reducing antioxidant power (FRAP)

The determination of antioxidant activity through FRAP was carried out according to the method of Musa *et al.*, (2011). FRAP reagent was prepared fresh as using 300 mM acetate buffer, pH 3.6 (3.1 g sodium acetate trihydrate, plus 16 mL glacial acid made up to 1:1 with distilled water); 10 mM TPTZ (2,4,6-tris (2-pyridyl)-s-triazine), in 40 mM HCLl; and 20 mM $FeCl_3 \cdot 6H_2O$ in the ratio of 10:1:1 to give the working reagent. About 1 ml FRAP reagent was added to 100 µL papaya extracts and the absorbances were taken at 595 nm wavelength with spectrophotometer after 30 minutes. Calibration curve of Trolox was set up to estimate the activity capacity of samples. The result was expressed as mg of Trolox equivalents per 100 g of fresh sample (mg TE/100 g of FW).

DPPH radical scavenging activity

The determination of antioxidant activity through 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging system was carried out according to the method of Musa *et al.*, (2011). Stock solution was prepared by dissolving 40 mg DPPH in 100 ml methanol and kept at -20°C until used. About 350 mL stock solution was mixed with 350 ml methanol to obtain the absorbance of 0.70 ± 0.01 unit at 516 nm wavelength by using spectrophotometer (Epoch, Biotek, USA). About 100 µL papaya extracts with 1 ml methanolic DPPH solution prepared were kept overnight for scavenging reaction in the dark. Percentage of DPPH scavenging activity was determined as follow: DPPH scavenging activity (%) = $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$. Where A is the absorbance.

ABTS assay

The ABTS radical cation (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) was generated by the interaction of ABTS (250 µM) and $K_2S_2O_8$ (40 µM). After the addition of 990 µL of ABTS solution to 10 ml of fruit extract, the absorbance at 734 nm was monitored. The percentage decrease of the absorbance was calculated and plotted as afunction of the concentration of the extracts and Trolox for the standard reference data Özgen *et al.*, (2006). The following formula was used: Percentage (%) of

reduction power = $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$. Where A is the absorbance.

Physiochemical properties of fruits

Moisture content was measured by drying sample at 105°C overnight in Memmert Oven (Germany). Titratable acidity (TA) was determined from 10 ml of sample diluted with 50 ml of water, titrated with 0.1 N NaOH and calculated as percent citric acid. Total soluble solids (TSS) were measured with an abbe refractometer at 20°C and pH was determined using pH meter using juice extracted directly from pulp. The pulp color was longitudinally determined on four points of each flat side of the fruit using a Minolta CR-300 colorimeter. The (L^*) value represented the luminosity of the fruit, where 0 = black and 100 = white but the (a^*) value ranged from the negative (green) to the positive (red) scale and the (b^*) value ranged from negative (blue) to positive (yellow), AOAC (1998).

Sensory evaluation

All papaya fruit samples at three maturity stages (stage 3, 16 weeks after anthesis; stage 4, 18 weeks after anthesis and stage 5, 20 weeks after anthesis) were subjected to sensory evaluation. A hedonic test was carried using 30 student panelists from the Faculty of Science and Technology of the University Kebangsaan Malaysia (UKM). Testing was performed in the sensory laboratory with six individual taste booths under fluorescent lighting equal to daylight. Fresh papaya fruit samples were served in small plastic cups labeled with random digit codes. Panelists were asked to taste the sample and evaluate it for each attribute in a specific scale provided. Distilled water was provided to rinse the mouth between evaluations. The hedonic scale comprised scores of 1 to 7, where 1 indicated "disliked extremely" and 7 indicated "liked extremely (Aminah 2004). The sensory attributes evaluated color, flavor, sweetness, sourness, and overall acceptance.

Statistical analysis

Data were expressed as the means of three independent experiments. Statistical comparisons of the results were performed byone-way ANOVA using SPSS ver.19. Significant differences ($P < 0.05$) among the ripening stages were analyzed by Duncan' triplicates range test Bryman and Cramer (2012).

Results and Discussion

Antioxidant capacity assays

In the human body, phenols and flavonoids relate to bioactive compounds involving a decrement of differing deteriorative processes. It is because of its capacity to lessen free radical build up as well as to scavenge free radicals (Gokturk Baydar *et al.*, 2007). Table (2) depicts total phenol and flavonoid dynamics in papaya fruit at five RS. The obvious observation was RS was affected significantly ($P < 0.05$) of the entire phenolic composition in the papaya. This increased at different RS which increased with fruit maturity, the highest values being in RS5 (42.97 mg GAE/100 g FW) and the lowest in RS1 (11.13 mg GAE/100 g FW). It was reported that fresh papaya cultivated in Thailand had phenolic content of 54 mg GAE/100 g FW of papaya, (Patthamakanokporn *et al.*, 2008), while according to Lim *et al*., (2007), the TPC contents in papaya fruitwas 28 ± 6 mg GA/100 g FW. It must be known that papaya fruit is one of the major dietary sources of flavonoids for Malaysian, (Lee *et al*., 1995). RS significantly affected ($P < 0.05$) the TFC of papaya fruit, having highest values of quercetin equivalents in RS 5 (36.26 mg QE/100 g FW) followed by RS4, RS3, RS2 and RS1with (31.72, 25.96, 23.46 and 18.45 QE/100 g FW), respectively Table (2). Papaya fruit at RS4 had about 55% lower flavonoids than the RS1. Alothman *et al.* (2009) reported that total flavonoid content (mg /100 g fresh samples) of papaya fruit was 57.80 mg rutin /100 g dry samples.

Table 2 shows the FRAP of tested papaya showing variations in papaya fruits at five RS. In general RS affected significantly ($P < 0.05$) the FRAP content in papaya fruit the FRAP content of papaya at different RS increased with fruit ripening, the highest values were recorded in RS5 (114.71 mg GAE/100 g FW) and the lowest in RS1 (19.27 mg GAE/100 g FW). It is important to note that the entire antioxidant capacity of the samples was derived by measuring their capacity to reduce Fe^{3+} to Fe^{2+} using FRAP test. The differences of the results obtained from different tropical fruits may be the result of the increase in FRAP ability because of hydroxyls is present in the fruits 106 ± 28 mg TE/100 g FW in papaya (Lim *et al.*, 2007), (26-1393 mg TE/100 g FW in banana (Sulaiman *et al*., 2011), 160-180 mg TE/100 g FW in mango (Palafox-Carlosa *et al*., 2012).

The scavenging activity on DPPH radical was related to the ripening stage, the activity increased as a result of increasing ripening stage for each stage (Table 2). The scavenging effect of extracts from five stages of papaya fruits on the DPPH radical followed the order: RS5> RS4> RS3>RS2>RS1 and was 41.06%, 35.74%, 30.02%, 22.42% and 12.25% respectively. The results correspond with most research (Alothman *et al.*, 2009; Choo *et al.*, 2010) which showed that

Table 1. Description of the selected ripening stages for sample collection of papaya fruit

Ripening stages (weeks)	Skin colour	Flesh colour	Seed colour
12	Green	White	White
14	Green	Pale yellow	Brown
16	Green	Yellow	Pale black
18	Trace of yellow	Orange	Black
20	More yellow	Reddish orange	Black

Table 2. Mean (n = 3) antioxidant activity of papaya fruits estimated by TPC, TFC, FRAP, DPPH and ABTS

Ripening stage	TPC mg/100 g FW	TFC mg/100 g FW	FRAP mg/100 g FW	DPPH %	ABTS %
RS1	11.13 ± 0.17 ^{e}	18.45 ± 0.46 ^{e}	19.27 ± 0.20 ^{e}	12.25 ± 0.45 ^{e}	24.01 ± 0.02 ^{e}
RS2	21.48 ± 0.31 ^{d}	23.46 ± 0.40 ^{d}	58.84 ± 0.05 ^{d}	22.42 ± 0.46 ^{d}	35.59 ± 0.01 ^{d}
RS3	33.38 ± 0.99 ^{c}	25.96 ± 0.61 ^{c}	85.57 ± 0.16 ^{c}	30.02 ± 0.21 ^{c}	43.11 ± 0.01 ^{c}
RS4	38.33 ± 0.63 ^{b}	31.72 ± 1.88 ^{b}	96.85 ± 0.40 ^{b}	35.74 ± 0.20 ^{b}	49.34 ± 0.01 ^{b}
RS5	42.97 ± 0.21 ^{a}	36.26 ± 0.58 ^{a}	114.71 ± 0.13 ^{a}	41.06 ± 0.67 ^{a}	62.22 ± 0.02 ^{a}

$^{a-e}$Mean with different letters within each column are significantly different (P > 0.05).

Table 3. Correlation coefficients of the antioxidant activities using different assays

	TPC	TFC	FRAP	DPPH	ABTS
TPC	*	*	*	*	*
TFC	0.95	*	*	*	*
FRAP	0.99	0.95	*	*	*
DPPH	0.99	0.97	0.99	*	*
ATBS	0.96	0.98	0.97	0.98	*

Table 4. Effect of ripening stages on the flesh color of papaya fruit

Ripening stages	Flesh color		
	L*	a*	b*
RS 1	62.43 ± 1.51 ^{a}	- 10.93 ± 0.64 ^{e}	11.01 ± 0.83 ^{e}
RS 2	57.06 ± 0.21 ^{b}	- 3.87 ± 0.07 ^{d}	16.41 ± 3.55 ^{d}
RS 3	55.81 ± 0.53 ^{c}	12.96 ± 2.38 ^{c}	21.15 ± 0.76 ^{c}
RS 4	51.87 ± 2.50 ^{d}	21.72 ± 0.79 ^{b}	24.43 ± 3.17 ^{b}
RS 5	47.97 ± 0.89 ^{e}	26.80 ± 1.08 ^{a}	30.02 ± 0.92 ^{a}

$^{a-e}$Mean with different letters within each column are significantly different (P < 0.05).

DPPH scavenging operations differed significantly (P < 0.05) among the entire ripening stages RS1, RS2, RS3, RS4, and RS5. Hence, the DPPH scavenging activity was sequenced as; RS5> RS4> RS3> RS2> RS1. In papaya fruit, DPPH radical scavenging activity also showed an increasing trend from green to ripe maturity (ripe papaya cv. Red Lady 65.1%, green papaya cv. Red Lady, 29.7%, green papaya cv. Exp. 15, 10.4% (Mahattanatawee *et al*., 2006).

Also, the ABTS radical has been used to confirm results obtained with DPPH, because both possess similar antioxidant mechanisms. It has been reported that phenolic compounds or ascorbic acids react vigorously with ABTS, while lipophilic compounds make them weaker (Perez-Jimenez *et al*., 2008). The reducing potential of papaya fruit was significantly affected by the level of ripening stage in the order of RS5> RS4> RS3> RS2> RS1, 62.22%, 49.34%, 43.11%, 35.59% and 24.01%, respectively. Based on these findings, the indication is that the antioxidant activity increases corresponding to progress of the plant's maturity stages as observed in a previous study (Thaipong *et al.*, 2006). Grant *et al.* (2009) reported that ABTS radical of papaya fruit was 25.6% unripe and 34.4% ripe. The different results obtained from the previous studies may be attributed to different cultivars, growing conditions, maturity stage at harvest, or the storage conditions and time elapsed before the fruits were analyzed. Sample preparation method may also influence the results.

Correlations among the antioxidant assays

A linear between the TPC and antioxidant activity by different methods in fruits and vegetables was shown in a number of researches (Mahattanatawee *et al*., 2006; Corral-Aguayo *et al*., 2008). Findings of researches of correlation analyses among TPC, TFC, and antioxidant activities (FRAP, DPPH, and ABTS) are high (Table 3). There have been significant effects on the antioxidant activities of papaya fruit based on the maturity stage.

Physicochemical properties of fruits

The possible of explanation for the color change in papaya fruit was related with the enzymatic degradation of chlorophyll as fruit maturity increased (Ding *et al*., 2007). The pulp of papaya fruit developed an orange–red color with decreasing L^* and increasing a^* and b^* values. The result of physicochemical of bitter melon based on ripening stages showed at Table 4. The RS5 was the highest a^* (redness) and b^* (yellowness), 26.80 and 30.02, respectively and showed significant difference ($p < 0.05$) compared other RS. Value of a^* (redness) of fruit showed significant difference among all the ripening stages at ($p < 0.05$). Marin *et al*., (2004) reported that changes in the fruit flesh color of tamarillo fruit were well pronounced with 44% of decreased in L^* values as fruit ripeness progressed. In a study it was shown that pulp colour of papaya fruits were in the range of L^* 69 - 48, a^* -8.5 to +22, b^* +20 to +39 (Felipe *et al*., 2009).

The measurements of papaya fruit for pH and TA showed at Table 5. The ranges of pH on stage papaya fruit were 6.20 -5.70. The samples showed significant difference at ($p < 0.05$). RS2 and RS3 were similar, 6.10 and 6.08, respectively and showed no significant difference at ($p < 0.05$). Sanudo Barajas *et al.* (2008), performs a study in green "Maradol" papaya, this study defines that the pH value changes depending on the variety and ripening stage of the fruit. The TA on RS4 and RS5 were higher than other samples. Both

Table 5. Effect of ripening stages on the physicochemical properties of papaya fruits

Ripping stages	pH	Titratable acidity (%)	Moisture (%)	TSS (Brix)
RS1	6.20 ± 0.05 [a]	0.05 ± 0.11 [e]	84.29 ± 0.87 [c]	6.60 ± 0.57 [d]
RS2	6.10 ± 0.04 [c]	0.07 ± 0.02 [d]	84.60 ± 0.96 [c]	6.60 ± 0.57 [d]
RS3	6.08 ± 0.01 [b]	0.08 ± 0.00 [c]	85.93 ± 1.06 [bc]	7.00 ± 1.0 [c]
RS4	5.80 ± 0.02 [b]	0.12 ± 0.00 [b]	86.37 ± 1.41 [b]	9.00 ± 1.0 [b]
RS5	5.70 ± 0.03 [d]	0.17 ± 0.00 [a]	89.39 ± 0.07 [a]	11.50 ± 0.50 [a]

[a-e] Mean with different letters within each column are significantly different (P < 0.05).

the samples showed significant difference (p < 0.05). More ripening the papaya fruit showed increased of mean on TA value 0.17. In contrast, other researchers depicted TA at the maximum as the fruit changes color to yellow entirely (Wills and Widjanarko, 1995). However, this result was higher than reported Bron *et al.* (2006) ranges of TA on fresh Golden papaya fruits were 0.09 – 0.12%. In addition to, Lazan *et al*., (1989) concluded that the TA increases with fruit ripening until approximately 75% of yellow skin decreasing thereafter.

The total soluble solids (TSS) of the papaya fruit increased continuously from 6.60 (RS1) to 11.50 (RS5), with fruit ripening advancing. The increase in TSS with advancing fruit maturity (RS1 to RS5) might be explained by the hydrolysis of starch to sugars as fruit advances in maturation (Kulkami and Aradhya, 2005). The TSS found in fully ripened fruits 11.50 was higher than 11.00 reported by Felipe *et al*., (2009) for the Maradol cultivar. There was also a gradual increase in the moisture content 12, 14, 16,18 and 20 weeks after anthesis (84.29%, 84.60%, 85.93%, 86.37% and 89.39% respectively) in papaya fruit as shown in Table 4. These results indicated that this rate was very high, and may be caused by the ability of papaya to keep its moisture content longer than other plants. This finding was in agreement with Mia *et al.* (2010) who found that moisturecontent in ripe papaya was 89.42%.

Sensory evaluation

Observable differences in the general characters investigated is present in the ripening stage of papaya fruit (Bron and Jacomino, 2006). However, in the sensory descriptors between fruits harvested at unripe RS3, half-ripe RS4 and full-ripe RS5 a significant difference (P < 0.05) was observed. From the sensory evaluation panel significant differences (P < 0.05) in the color, sweetness, sureness, flavor and the entire acceptance were detected. The findings of the sensory evaluation showed that the overall acceptance increased corresponding to the progress of ripening stages of papaya based on color, flavor sweetness, and sourness. The sweetness, sourness and flavor produced from full-ripe papaya fruits RS5 were found to be the most preferred in comparison to those from the half-ripe RS4 and unripe RS3 papaya. It must be noted that as starch in fruits is changed into sugars during ripening process, the taste changes to sweet as a result of increased sugar composition contributing to the taste of the fruit (Kader 2008). Overall differences acceptability of papaya fruit from the unripe RS3, half-ripe RS4 and full-ripe RS5 were significant (P < 0.05). The declining acceptance of papaya fruit with ripening may be because of the softening of the pulp, high moisture and high total soluble solids composition when the fruit is completely ripe. This result corresponds with a previous study (Bender *et al.*, 2000).

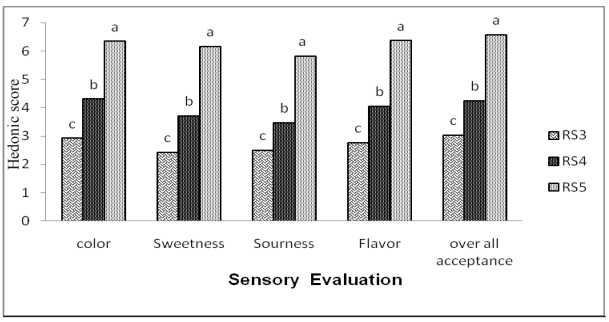

Figure 1. Mean (n = 50) of hedonic scores of stages of ripening (stages 3, 4, and 5) fresh papaya fruit sample

[a-c]Mean with different letters within each column are significantly different (p < 0.05)

Conclusion

As compared to other total phenolic content, TFC, FRAP, DPPH and ABTS of RS5 were highest RS and showed significant difference (P < 0.05). According to the results, more ripened stages of papaya fruit brought about higher physicochemical characteristics, higher antioxidant activity, higher total phenolic content and total flavonoid content. In addition, more ripened papaya fruit showed more redness and yellowness. Different physicochemical properties, including pH, TA, TSS, moisture and fruit color, depended on different ripening stages. The result of sensory evaluation revealed that the overall acceptance increased with the progress of ripening stages of papaya fruit depending on color, flavor, sweetness, and sourness.

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