Research Article Antioxidant Activity and Physicochemical Properties of Mature Papaya Fruit (*Carica papaya* L. cv. Eksotika)

Zuhair Radhi Addai, Aminah Abdullah, Sahilah Abd. Mutalib, Khalid Hamid Musa and Eqbal M.A. Douqan

School of Chemical Science and Food Technology, Faculty of Science and Technology, University Kebangsaan Malaysia, 43600 Bangi Selangor, Malaysia

Abstract: Papaya (*Carica papaya* L. cv. Eksotika) is one of the most commonly consumed tropical fruits by humans, especially Malaysians. The objective of this study was to evaluate the effect of the maturity stage (12, 14, 16, 18 and 20 weeks after anthesis) of papaya fruit on its physicochemical properties, antioxidant capacity and sensory characteristics. Papaya fruits were selected and classified based on their visual maturity, i.e., stages 1 to 5. The activities of several antioxidants were tested, including the Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Ferric Reducing Antioxidant Power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS). The physicochemical changes were measured in terms of the pH, Titratable Acidity (TA), moisture, Total Soluble Solids (TSS) and pulp color of the papaya fruits at the five ripening stages. Significant differences (p<0.05) were found in different degrees of ripening. The pH of the fruit decreased significantly (p<0.05), whereas the TA, moisture and TSS all increased significantly (p<0.05) with maturity. The redness (a^*) and yellowness (b^*) values of the fruit color both increased significantly, whereas the lightness (L^*) of the color fluctuated. The TPC, TFC, FRAP, DPPH and ABTS values also increased significantly (p<0.05) with ripening. Sensory evaluation based on the color, sweetness, sourness, flavor and overall acceptance for the last three maturity stages was also performed. Stage 5 had a better score than stages 3 or 4. The results showed the important role of the ripening stage in increasing the antioxidant content of papaya fruits.

Keywords: Antioxidant activity, maturity stage, papaya fruit, physicochemical properties, sensory evaluation

INTRODUCTION

Papaya fruit (Carica papaya L. cv. Eksotika) belongs to the family of Caricaceae and several species of Caricaceae have been used as remedy against a variety of diseases (Mello et al., 2008; Munoz et al., 2000). Papaya was originally derived from the southern part of Mexico; papaya is a perennial plant which is distributed over the whole tropical and subtropical area. It is one of the most consumed fruits. The interior flesh of the fruit goes through color changes from green (immature) to yellow (ripe) and when it is to overripe (McGrath and Karahadian, 1994). A climacteric fruit exhibits a sudden burst of high respiration causing the fruit to ripen within a few days (Zhu and Zhou, 2007). That Papaya is comparing with other fruit such as the banana or the apple, it has been found a good natural source of macronutrients (carbohydrates and proteins) and macronutrients (vitamin A and vitamin C) (Peterson et al., 1982). There is also a strong relationship between the intake of these antioxidantcontaining plants and reduced mortality of the

aforementioned diseases (Halliwell et al., 1992). Indeed, natural antioxidants warrant further scientific scrutiny given their activity against free radicals, which contribute to chronic degenerative diseases (Bray, 2000). Medicinal plants play important roles in preventing various diseases and have received much attention from many researchers over the last few decades. Studies on the antioxidant contents of fruits and vegetables are increasing because natural antioxidant consumption has been found to be related with decreased risk for cancer and heart diseases (Temple, 2000). Harvest time is essential to get a high quality fruit with storage potential. Some physiological properties can be affected by cultivar; agronomic condition and maturity stage (Kevers et al., 2007). Harvest date has also influence on fruit sensorial quality. Papaya harvested at more advanced maturity stages had better consumer acceptance. The present study aimed to obtain the maximum concentration of target compounds and the highest antioxidant activity of papaya fruit extracts, as well as their physicochemical properties at different maturity stages.

Corresponding Author: Zuhair Radhi Addai, School of Chemical Science and Food Technology, Faculty of Science and Technology, University Kebangsaan Malaysia, 43600 Bangi Selangor, Malaysia, Tel.: +60129804268 This work is licensed under a Creative Commons Attribution 4.0 International License (URL: http://creativecommons.org/licenses/by/4.0/).

MATERIALS AND METHODS

Samples collection: Papaya fruits (*Carica papaya* L. cv. Eksotika) were collected from farms at semenyih during season 2011 (May, June and July). After fruits selection, each fresh fruit was washed under running tap water, followed by washing with distilled water to remove surface impurities. The fruits were selected with different maturity stages of papaya 12, 14, 16, 18 and 20 weeks after anthesis.

Extraction of antioxidant: Papaya were peeled, cut into 1 cm slices and crushed in a food processor to produce uniform slurries. 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 tabletop 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 μ L 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 h. 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/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) NaNO₂ solution. After 6 min, 0.3 mL of a 10% AlCl₃·6H₂O 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 acetatebuffer, pH3.6 (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 mMHCLl; and 20 mMFeCl3•6H2O in the ratio of 10:1:1 to give the working reagent. About 1 mL FRAP reagent was added to 100 μ L papaya extracts and the absorbances were taken at 595 nm wave length with spectrophotometer after 30 min. 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 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 solutions 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 μ L 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 $_{blank}$ × 100.

where, A is the absorbance.

ABTS assay: The ABTS radical cation was generated by the interaction of ABTS (250 μ M) and K₂S₂O₈ (40 μ M). After the addition of 990 μ L of ABTS solution to 10 mL of fruit extract or Trolox standard (final concentration of 0-20 μ M) in methanol or 20 mM acetate buffer (pH 4.5), 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}×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) was 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 Universiti Kebangsaan Malaysia. 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. A sample of the form is shown in Appendix C. 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 were 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 by one-way ANOVA using SPSS ver.19. Significant differences (p<0.05) among the maturity stages were analyzed by Duncan 'triplicates range test (Bryman and Cramer, 1997).

RESULTS AND DISCUSSION

Antioxidant capacity assays: Several food components such as carotenoids, vitamin C, vitamin E and phenolic compounds and their interactions contribute to the overall antioxidant activity of foods. Hence, the total antioxidant activity is difficult to measure based on individual active components (Pinelo *et al.*, 2004). Ripening process from green, white, yellow to orange in papaya fruits increased antioxidant capacity and total phenols from mature to immature papaya fruits.

Total Phenol Content (TPC): The antioxidant capacity of the fruits was evaluated by five different methods, including DPPH radical-scavenging, FRAP, TPC, ABTS and TF content. The papaya fruit extracts had different antioxidant capacities in relation to the method of estimation. The antioxidant capacities of the fruit extracts and their order for each assay are shown in Table 1. The TPC was determined because of its strong correlation with the antioxidant activity in various kinds

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

of C. papava (Gorinstein et al., 2004; Sellappan et al., 2002). Based on DPPH and FRAP estimations, stage 5 papaya had the highest percentage of antioxidant activity and stage 1 had the lowest. There was no significant difference (p<0.05) between the TPC of stage 1 (unripe) papaya and the Eksotika variety, i.e., stage 1 also had the lowest activity and stage 5 had the highest However, Eksotika had a higher activity of approximately 60.40 mg/100 g. The differences among the antioxidant activities of the fruits can be attributed their differences in phenolic contents and to compositions as well as to other non-phenolic antioxidants present in the samples likewise the discrepant antioxidant values between the present and previous studies may be caused by the differences in the vitamin C content and TPC. Such differences in the results of TPC compared with other researchers may be linked to different varieties of fruits and the varying antioxidant extraction methods used. Moreover, factors such as fruit maturity, agro climate and post-harvest storage conditions are known to affect the content of polyphenols in fruits (Mahattanatawee et al., 2006).

Total flavonoid content (TFC): Flavonoids are class of (poly) phenolic and poly phenolic derivative compounds present in various fruits and vegetables (Sun et al., 2002). The amount of flavonoids extracted from the papaya pulp was significantly affected by the level of maturity stage of the fruit in the order of stage 1, stage 2, stage 3, stage 4 and stage 5 The present study showed that the TF content was very high 12 weeks after anthesis 22.53 mg/100g FW. However, this rate increased slowly and reached 38.12 mg/100g FW, 20 weeks after anthesis stage 5. Eksotika fruit pulps had relatively higher total flavonoids 22.53 mg/100g FW than other medicinal plants such as Alceakurdica, Stachyslavandulifolium, Valerianaofficinalis, Lavand ulaofficinalis and Melissa officinal (0.22-10 mg CE/g DW), (Marinova et al., 2005; Bouayed et al., 2007) reported that TFs in Eksotika fruit are also higher than in other fruits and vegetables, including apple, raspberry, peach, broccoli, celery and tomato (2.5-190.3 mg CE/100g FW) and they showed that the total flavonoid content in the beal fruit is 15.20 mg, which was lower than that of papaya in the present study.

Ferric Reducing Antioxidant Power (FRAP): The mechanisms of assay play an important role in evolution of antioxidant activity of papaya fruits. The

significant increase in antioxidant values that we showed during ripening stage of papaya fruits was apparent in the FRAP, DPPH, ABTS, TPC and TF. Antioxidants assays revealed significant increases (p<0.05) in antioxidants capacity during papaya fruit ripening the ripe fruits had the highest amounts of antioxidant capacity and bioactive compounds (Khanavi et al., 2009). Ferric Reducing/Antioxidant Power (FRAP) showed that durian at the ripe stage had higher antioxidant activity than the other samples. Table 1 shows that the highest antioxidant activity was in stage 5 of maturity with a value of approximately 180.28 mg/100g FW. These values were higher than the activity estimated for DPPH. Stage 1 had the lowest activity, followed by moderate activity in stages 2 and 3 and the highest activity occurred in stage 5. The antioxidant activity estimated by the five methods (TPC, TF, FRAP, DPPH and ABTS) were almost the same in C. papaya. The FRAP antioxidant activity and DPPH, ABTS, TPC and TF determined in different stages of maturity showed that the ripe papaya showed higher values than the green ones (Mahattanatawee et al., 2006; Tabart et al., 2009).

DPPH radical scavenging activity: The bleaching of DPPH absorption by a test compound is representative of its capacity to scavenge free radicals generated independently of any enzymatic or transition metalbased system. This method is widely used to evaluate antioxidant activities within a relatively short time compared with other methods. Antioxidants react with DPPH, a stable free radical and convert it to 1, 1diphenyl-2-(2, 4, 6-trinitrophenyl) hydrazine. The degree of discoloration indicates the scavenging potential of an antioxidant compound. As shown in the Table 1, the highest activity was in stage 5, followed by moderate activities in stages 3 and 4 and the lowest activity in stage 1 (unripe fruit). A comparison of the antioxidant activity values in the present investigation with literature data was problematic due to the large variability and lack of standardization of the assay methods. Therefore, the antioxidant capacities were ranked instead. The highest antioxidant activity was observed in different types of C. papaya. C. papaya is an important dietary source of vitamin C, minerals and amino acids and also contains phenolic compounds and tannins. Hence, the high antioxidant activity observed for *C. papaya* in the present investigation may be due to the high concentrations of vitamin C and other antioxidant compounds. (Leong and Shui, 2002) have observed the highest activity using DPPH, although their antioxidant activity values are lower than those in the present study. The most possible reason may be the lower quality of the fruits used and the different maturity stages of the fruit samples. The majority of the antioxidant activity of fruits is known contributed by polyphenols, vitamin C, vitamin E, β-carotene and lycopene (Gancel et al., 2008; Tabart et al., 2009).

Table 2: Mean $(n = 2)$	effect of the maturity stage on color pa	paya
fruit		

	Flesh color		
Maturity stage	 L*	a*	 b*
1	64.45±2.46 ^a	$-7.27\pm0.14^{\circ}$	$16.27\pm0.84^{\circ}$
2	56.43±0.19 ^b	-3.73 ± 0.62^{d}	18.09 ± 2.18^{d}
3	51.96±0.83°	12.44±0.19°	24.42±0.72°
4	48.93±1.01 ^d	16.20±0.78 ^b	28.15±1.22 ^b
5	46.56±0.98 ^e	19.30±0.57 ^a	34.00 ± 0.89^{a}

 a^{-e} : Different letters within each column indicate significant difference (p<0.05)

ABTS assay: Papaya fruits showed antioxidant capacity and total flavonoids and total phenols contents significantly higher than those of unripe green papaya fruit (stage 1). The level of antioxidant activity by ABTS was high and close to that by TPC. Starting with a value of 19.95 % (12 weeks after anthesis) this percentage was similar to FRAP at the same stage. However, the antioxidant activity of Eksotika increased slowly from 19.95 % in stage 1 to 68.10 % in stage 5. The ABTS values of Eksotika were lower than those obtained from the FRAP and DPPH assays (Pérez-Jiménez et al., 2008). However, other studies have shown that phospholipids had the highest rate of antioxidant activity by ABTS (Jastrzebski et al., 2007). These results were similar to those of others (Cai et al., 2004), i.e., 58% for antioxidant activity and 60.40 for TPC.

where, A is the absorbance.

Physiochemical properties of fruits: There were many pulp color changes observed in the L^* ranging from 64.45 at maturity stage 1 and 46.56 at maturity stage. The results showed that the pulp color intensity of papaya fruit increased to different levels in stages 1 and 5, from a^* (-7.27 to 19.30), b^* (16.27 to 34.00) at stage 5 of maturity These fruits were in good condition and had no apparent mechanical damage to their surface. The color intensity and uniformity affect the fruit quality (López, 2003). In many fruits, these changes involve the loss of chlorophyll, synthesis of new pigments such as carotenoids and unmasking of other pigments previously formed during fruit development (Ferreira et al., 2002). The initial changes in the papaya fruit appearance observed in the present study were caused by decreased luminosity (L^*) 14 weeks after anthesis. There were changes in the values of yellow and green among maturity stages. The (b^*) value better indicated the middle maturity stages because it can distinguish between the luminosity of fruits and those undergoing the ripening processes (Table 2). The results study showed a strong relationship between the color and maturity stages; with increased maturity stage, C. papaya changes from green to luminous to red.

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Table 3: Mean (n = 3) effect of the maturity stage on the physicochemical properties of papaya fruit

Maturity stage	pH	Titratable acidity (%)	Moisture (%)	TSS
1	$6.07{\pm}0.02^{a}$	0.04±0.01 ^e	84.57±1.20 °	4.33±0.29 ^d
2	6.06±0.02 ^a	0.07 ± 0.02^{d}	85.52±1.18 ^{bc}	5.66±0.58°
3	$6.05{\pm}0.02^{ab}$	$0.09 \pm 0.02^{\circ}$	86.04±0.61 ^b	8.33±0.29 ^b
4	5.77±0.02°	0.11 ± 0.02^{b}	88.05±1.14 ^a	9.00 ± 1.00^{b}
5	5.62±0.01 ^{cd}	0.12 ± 0.0^{a}	90.05±0.67ª	12.56±0.81ª

^{a-e}: Different letters within each column indicate significant difference (p < 0.05)

Table 4: Mean (n = 3) antioxidant activities of papaya fruit estimated by TPC, TFC, FRAP, DPPH and ABTS assays

	TPC	TFC	FRAP		
Maturity stages	(mg/100 g FW)	(mg/100 g FW)	(mg/100 g FW)	DPPH (%)	ABTS (%)
1	11.20±0.27 ^e	22.53±0.56 ^e	19.93±0.26 ^e	10.48±0.85 ^e	19.95±0.01 ^e
2	17.43±0.14 ^d	24.11 ± 0.70^{d}	31.94 ± 0.01^{d}	16.37 ± 0.50^{d}	22.30±0.05 ^d
3	35.56±0.26°	31.04±0.23°	95.88±0.25°	36.85±0.20°	46.43±0.03°
4	39.28±0.17°	33.23±0.60°	124.19±0.06 ^b	45.02±0.33 ^b	55.72±0.01 ^b
5	60.40±0.45 ^a	38.12±0.98 ^a	180.28±0.04 ^a	72.19±0.10 ^a	68.10±0.0 ^a

^{a-e}: Different letters within each column indicate significant difference (p < 0.05)

The maturity stage of papaya significantly affected the pH, especially in C. papaya (Eksotika). Table 2 shows decreased pH values from stage 1 to 5. The pH values showed significant changes in the different maturity stages of papava fruit. The pH values in C. papaya (Eksotika) were higher 12 weeks after anthesis, but decreased with the maturity stage especially 20 weeks after anthesis (5.62), which agreed with the finding of (Bari et al., 2006). In the present study, TA increased with the maturity stage, similar to the results of Wills and Widjanarko (1995), who have found that the TA values increase with the maturity stage starting from 0.04 to 0.14. However, Lazan et al. (2006) have shown that the TA increases with fruit ripening to about 75% and decreases thereafter. However, the present study shows that the TA increases with the maturity stage. The TSS in fruits from plantation 1 rapidly increased between the green fruit stage and stage 1 and then increased more gradually in stages 4 and 5, reaching values of up to 12.56 TSS. Fruit color changes are widely used as a visual maturity index in many fruits (Adil, 2002). The TA was 0.12 in ripe Eksotika in the current study (Table 2), which was very high compared with that previously found (Paul et al., 1981) and very low compared with other fruits. Other researchers have shown that the TA reaches the maximum when the fruit color becomes completely yellow (Wills and Widjanarko, 1995). Table 3 shows that in C. papaya (Eksotika), the changes in the moisture content depend on the maturity stage. The moisture content 20 weeks after anthesis (stage 5) was significantly (p<0.05) higher 90.05. There was also a gradual increase in the moisture content 12, 14, 16, 18 and 20 weeks after anthesis in C. papaya (Eksotika), 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.

Sensory evaluation: The sensory evaluation results of maturity stages are presented. The results revealed in

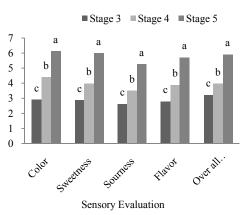


Fig. 1: Mean (n = 50) hedonic scores of the maturity stage (stages 3 to 5) of fresh papaya; ^{a-c}: Different letters within each column indicate significant difference (p<0.05)

Fig. 1 showed significant differences (p < 0.05) in pulp color, sweetness, sourness, flavor and overall acceptance. There were significant differences (p < 0.05) in the fruits with the different maturity stages. The maturity stage of papaya significantly affects the sensory responses of humans. For example, from stages 3 to 5, the general acceptance was ratio 3.00 to 5.90, respectively. The fruits with stage 5 was rated excellent also had a good overall appearance because the pulp was not only sweet and pleasant, but also possessed a characteristic aroma. The sensory evaluation revealed that with the advancement of maturity stage, the acidity decrease and maximum TSS of 12.56% but the flavor and sweetness increased. These results were similar with those of Bron and Jacomino (2006). The results of sensory evaluation showed that stage 5 was the most acceptable with the highest sensory rating for colour, sweetness, sourness and flavor. There was a significant increased (p<0.05) in sensory rating with increased stages of maturity. This disagrees with the results reported by Kittur and et al. (2001).

CONCLUSION

The result of this study showed that papaya (*Carica papaya* L. cv. Eksotika) is most acceptable at maturity stages of 5 with pH and TSS of 5.72 and 12.56% respectively. It was rated the highest acceptance for color, sweetness, sourness and flavor compare to stage 3 or stage 4. Likewise it has the highest antioxidant activity and has the highest capacity to scavenge free radicals. Thus by consumed papaya stage 5 the papaya fruit is at maximum nutritional quality and contained the highest antioxidant activity.

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