

Physicochemical quality, antioxidant compounds and activity of MD-2 pineapple fruit at five ripening stages

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Abstract

Although Malaysia is one of the important pineapple fruit producing and exporting country, the production of MD-2 pineapple fruit only started in 2009. Pineapple fruit has been harvested at different ripening stages for different markets. The information on Malaysian grown MD-2 pineapple fruit quality is lacking. Therefore this work was carried out with the aim to determine physicochemical quality, antioxidant compounds and activity of MD-2 pineapple fruit at five ripening stages. Ripening stage affected physicochemical quality of MD-2 pineapple fruit. Soluble solids concentration of MD-2 pineapple fruit increased from 15.41 to 18.02%SSC when fruit ripened from stage 1 to 4 and no significant difference was found in fruit between stage 4 and 5. The ascorbic acid content decreased while total carotenoids content increased as ripening stage advanced. The total phenolic content of both 80% methanol and water extraction solvents increased significantly as fruit ripened from stage 1 to 3 and reduced as fruit ripened to stage 5. The antioxidant activity of MD-2 pineapple fruit as assayed using DPPH, FRAP and ABTS showed similar trend as total phenolic content. These results suggest that ripening stage affect MD-2 pineapple fruit quality and nutritional values.

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Keywords

Ascorbic acid

ABTS

Colour

DPPH

FRAP

Introduction

Pineapple fruit is the third most important tropical and subtropical fruits in the international trade after bananas and citrus. There are more than 100 varieties of pineapple, but the world market is only dominated by six to eight varieties. In 1996, when the Del Monte Corporation introduced hybrid 'MD-2' from a cross between the PRI hybrids 58-1184 and 59-443, the world's pineapple fresh fruit industry went through a transformation (Greig, 2004). The consumption of fresh pineapple quadrupled in the North America and EU markets (Loeillet *et al.*, 2011). As a result, MD-2 pineapple becomes the top breed in the pineapple industry. MD-2 pineapple fruit has glossy and green-golden shell colour with a uniform and attractive cylindrical shape. The fruit is high in vitamin C while the flesh is more yellow, less acidic, sweeter, more aromatic and slightly firmer than other varieties. This leads to higher consumer preferences towards MD-2 pineapple fruit as compared to other varieties (Syahrin, 2011).

Pineapple is a non-climacteric fruit and harvesting at optimum ripening stage is able to ensure satisfying fruit quality. The physicochemical changes that occur during ripening and ripening are important dietary considerations as pineapple fruit is

often being marketed at different stages of ripening, depending on market preference. It has been reported that pineapple fruit harvested at different ripening stages varies in physical properties and chemical composition (Pauziah *et al.*, 2013). The volatiles composition of green-ripe MD-2 pineapple is differed from fruit that harvested at full ripening (Steingass *et al.*, 2014). Malaysia is one of the important pineapple fruit producing and exporting country. However, the production of MD-2 pineapple fruit only started in 2009. It is essential to determine fruit quality where the findings is able to develop the suitable ripening stage of MD-2 pineapple fruit for fresh consumption. Therefore, this study was carried out with the aim to determine physicochemical quality, antioxidant compounds as well as antioxidant activity of Malaysian grown MD-2 pineapple fruit harvested at five ripening stages.

Materials and Methods

Plant materials

MD-2 pineapple (*Ananas comosus* L.) fruits were collected from Ulu Tiram Estate, JTP Trading Sdn. Bhd., Johor, Malaysia, at five different ripening stages. The ripening stages were defined according to the peel colour as follow: stage 1 = mature green (137

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± 3 days after flowering); stage 2 = 25% yellow (147 ± 3 days after flowering); stage 3 = 50% yellow (157 ± 3 days after flowering); stage 4 = 75% yellow (167 ± 3 days after flowering); stage 5 = 100% yellow (177 ± 3 days after flowering). The fruit were harvested at the same time in the morning and transported to the laboratory using an air-conditioned vehicle which took 5 h of journey. Only fruit that free from defects with uniform size (1.3 – 1.8 kg/fruit) and stage of ripening determined by external colour were used. Upon arriving, the fruit was stored in 15°C/RH85% until following morning where the analysis began. There were five fruits in each ripening stage.

Determination of physicochemical quality characteristics

Peel colour was determined using a Minolta CR-300 chroma meter (Minolta Camera Co., Osaka, Japan) and results were expressed as lightness (L^*), chroma (C^*) and hue angle (h°). The L^* value is ranging from 0 = black to 100 = white. The h° is an angle in a colour wheel of 360°, with 0°, 90°, 180° and 270° representing the hues red, yellow, green and blue, respectively, while C^* is the intensity or purity of the hue. A total of nine measurements was carried out at the crown end, equatorial and slip end of an individual fruit and the mean value was obtained.

Flesh firmness was measured as penetration force using a texture analyser, Instron Universal Testing Machine (Model 5543, Instron Co., USA), with Merlin Software version M12-13664-EN. The instrument was equipped with a 6-mm-diameter flat probe that was programmed to penetrate in a normal direction at a crosshead speed of 20 mm/min. The penetration force was expressed in newtons (N). Soluble solids concentration (SSC) was determined using juice extracted from flesh samples using a digital refractometer PAL-1 (Atago Co., Ltd., Tokyo, Japan) to obtain the %SSC. The remainder of the juice from the SSC determination was used to measure pH of juice by using a glass electrode pH meter (model Micro pH 2000, Crison Instruments, Spain). Acidity was determined using a 10 g aliquot of puree in 40 mL of distilled water and titrating with 0.1 mol/L NaOH to a pink solution. Titratable acidity (TA) was expressed as % citric acid according to Ding and Tee (2011) methods.

Determination of ascorbic acid and total carotenoid content

Ascorbic acid content was measured using dye, 2,6-dichlorophenol-indophenol titration method according to Ranganna (1977) and expressed as mg/100 g fruit fresh weight. Total carotenoid

content of MD-2 pineapple fruits was determined spectrophotometrically (Rodriguez-Amaya and Kimura, 2004). Three gram of freeze-dried samples was mixed with 10 mL dH_2O for 30 min followed by 20 mL cold acetone and allowed to stand for 15 min. After filtration, the solid was ground well with 50 mL cold acetone. Then, 1/3 of the extracts was taken and mixed with 20 mL petroleum ether. Distilled water (300 mL) was added for the separation of phases and upper phase was collected. The content of total carotenoid in the petroleum ether extract was determined at the wavelength of 450 nm using a UV-VIS spectrophotometer (GS-UV12, General Scientific Ltd., UK). Then the total carotenoid content was calculated as

$$\text{Total carotenoid content} = (A \times d \times V) / (\epsilon \times w)$$

where A is the absorbance obtained, d is the dilution factor, V is the extract volume (mL), ϵ is the absorption coefficient (2592 for petroleum ether), and w is the weight of sample (g). Results were expressed as $\mu\text{g}/100$ g fruit dry weight (FDW).

Extraction

Three gram of the freeze-dried samples was suspended in 80% methanol (30 mL). After 2 h of continuous shaking, the extract was centrifuged at 3500 rpm for 30 min. The supernatant was collected and residue was reextracted. The extracts were pooled and filtered. The same procedure was followed for the water extraction samples. The extracts were used to explore their total phenolic content (TPC) and antioxidant activity.

Determination of TPC

The TPC of MD-2 pineapple was determined using Folin-Ciocalteu (FC) reagent according to the method of Allothman *et al.* (2009). Extract solution (300 μL) that mixed with 10% FC (1.8 mL) and 7.5% Na_2CO_3 (1.2 mL) was incubated in dark for 1 h. The mixture was then homogenized and the absorbance was measured at 765 nm using a UV-VIS spectrophotometer (GS-UV12, General Scientific Ltd., UK). The measurement was repeated thrice. The concentration of TPC in the extracts was expressed as mg gallic acid equivalent (GAE)/g FDW.

Determination of antioxidant activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was carried out according to Allothman *et al.* (2009) method with some modifications. The extracts (100 μL) were reacted with DPPH methanolic solution (250 μL) and 80% methanol (2 mL). After 30 min

incubation at 25°C, the absorbance was read at 517 nm using a UV/VIS spectrophotometer (GS-UV12, General Scientific Ltd., UK). Trolox was used as the standard curve and antioxidant activity of the extracts was expressed as $\mu\text{mole trolox equivalent (TE)}/\text{g FDW}$.

The 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was performed based on Huang *et al.* (2005) and Shan *et al.* (2005) procedures with some modifications. Equal volume of ABTS (7 mM) and $\text{K}_2\text{S}_2\text{O}_8$ (2.45 mM) solution was mixed and incubated for 16 h in the dark at 25°C to generate ABTS⁺ radical. Then, the ABTS⁺ solution was diluted in dH_2O to give absorbance 0.7 ± 0.02 at 734 nm. The extracts (0.1 mL) was reacted with ABTS⁺ solution (3.9 mL). After 6 min, the absorbance was measured at 734 nm using a UV/VIS spectrophotometer (GS-UV12, General Scientific Ltd., UK). Trolox was used as the standard curve and the antioxidant activity of the extracts determined by ABTS assay was expressed in $\mu\text{M TE}/\text{g FDW}$.

The ferric reducing antioxidant power (FRAP) assay was determined according to Allothman *et al.* (2009) and Benzie and Strain (1996) with some modifications. The FRAP working solution (3 mL) was added with extracts (40 μL) and incubated in the dark at 37°C. After 1 h, the absorbance was measured at 593 nm using a UV/VIS spectrophotometer (GS-UV12, General Scientific Ltd., UK). Trolox was used as the standard curve and antioxidant activity of the extracts was expressed in $\mu\text{mole TE}/\text{g FDW}$.

Experimental design and statistical analysis

The experimental design was randomized complete block design and replicate for three times. Data was analyzed by using ANOVA and LSD was used to separate the means when F-values showing significance at 5%.

Results and Discussion

Physicochemical fruit characterisation

The ripening stage had a significant effect in shell and pulp colours of MD-2 pineapple fruit (Table 1). The L^* values of shell increased significantly as fruit ripened indicated the lightening of the shell colour from dark to bright has took place. Contrast to shell colour, the L^* values of pulp decreased with darker colour as fruit ripened. As ripening progressed from stage 1 to 5, C^* values of both shell and pulp colour increased significantly which indicate fruit colour became more intense. However, the increase of pineapple fruit colour intensity was greater in shell (198%) as compared to pulp (22%). Both h°

values of shell and pulp pineapple fruit decreased as ripening advanced with shell declined sharply by 31% from green to yellow. This indicated that the colour changes of MD-2 pineapple fruit shell was more obvious than pulp. Shell colour is an important external factor to determine fruits ripening stages as it reflects the internal quality by which affecting consumer preference of a product, and often accounting for 40% of the criteria for acceptance (Baardseth *et al.*, 1988).

The pulp firmness of pineapple fruit decreased significantly by 36% as fruit ripened from stage 1 to 5 (Table 2). Contrary to firmness, the SSC of fruit increased significantly by 17% when fruit ripened from stage 1 to 4. These two parameters are important in determining fruit palatability. Firmness affects fruit texture and it is affected by its cell wall chemical composition. Vidal-Valverde *et al.* (1982) reported that the main cell wall component of pineapple fruit is hemicellulose (41.8%) followed by cellulose (33.6%) and pectin (21.2%). High SSC is perceived as sweetness and pineapple fruit pulp contains higher sucrose than fructose and glucose (Hong *et al.*, 2013; Nadzirah *et al.*, 2013). The SSC of Malaysian grown MD-2 at ripening stage 4 (three-quarters of the shell colour is yellow) was 18%SSC and it was higher than Ghana grown MD-2 pineapple fruit (14.50%SSC) (Wardy *et al.*, 2009). This indicated growing location and agronomic practices could affect fruit quality.

The TA of MD-2 pineapple fruit decreased by 28% while pH increased by 6.7% as fruit ripened from stage 1 to 5 (Table 2). Increase pH attributed to more palatable fruit with less acidity when pineapple fruit reaching advanced stage of ripening. A decrease in acidity during pineapple fruit ripening might due to rapid utilization of acids as substrate during respiration where it has been converted to sugars as found in Mauritius pineapple fruit (Fernando and Silva, 2000). pH is a logarithmic measure of hydrogen ion concentration. As TA decreased, the hydrogen ion concentration in cell saps also reduced and thus pH value increases. The TA and pH of Malaysian grown MD-2 pineapple fruit was lower than those grown in Ghana (Wardy *et al.*, 2009), indicating growing conditions of plant is crucial in determining fruit quality. TA reflects organic acid content of fruits. Organic acid content is able to affect fruit quality in terms of flavour. Citric and malic acids are the two major organic acids in pineapple fruit, with a ratio of about 2-3 to 1 (Chan *et al.*, 1973). Saradhuldhat and Paull (2007) found out that the change in TA of both PRI#36-21 and PRI#63-535 pineapple clones was paralleled with the pattern changes of citric acid.

Table 1. Effect of five ripening stages on shell and flesh colour (L*, C* and h°) colours of MD-2 pineapple fruit

Ripening stage	Shell colour			Flesh colour		
	L*	C*	h°	L*	C*	h°
1	34.17 e ^z	11.81 e	119.37 a	77.43 a	31.70 c	98.05 a
2	38.27 d	19.46 d	106.95 b	75.84 a	36.44 b	95.81 b
3	42.83 c	24.85 c	101.71 c	73.38 b	36.29 b	94.87 b
4	45.55 b	27.80 b	92.34 d	71.74 bc	39.13 a	93.41 c
5	51.48 a	35.24 a	82.07 e	71.03 c	38.74 a	93.57 c
F value	20.89**	20.39**	0.99*	2.06*	6.98**	21.44**

^z Means followed by the same letter in the column are not significantly different by LSD at P ≤ 0.05.

Significant difference at *P ≤ 0.05 or **P ≤ 0.01.

n = 15.

L* = lightness, C* = chroma, h° = hue angle.

Table 2. Effect of five ripening stages on firmness, soluble solids concentration, titratable acidity, pH, ascorbic acid content, total and total carotenoids content of MD-2 pineapple fruit

Ripening stage	Firmness (N)	Soluble solids concentration (%SSC)	Titratable acidity (%citric acid)	pH	Ascorbic acid content (mg/100 g FFW)	Total carotenoids content (µg/100 g FDW)
1	8.95 a ^z	15.41 c	0.78 a	3.87 b	63.17 a	291.26 e
2	7.71 b	16.41 b	0.70 b	3.89 b	62.31 ab	450.30 d
3	6.95 c	16.96 b	0.65 bc	4.07 a	57.25 ab	689.78 c
4	6.37 c	18.02 a	0.61 cd	4.11 a	54.63 b	893.85 b
5	5.73 d	17.35 ab	0.56 d	4.13 a	46.11 c	1118.33 a
F value	28.28**	8.60**	0.52*	1.20*	10.08**	102.40**

^z Means followed by the same letter in the column are not significantly different by LSD at P ≤ 0.05.

Significant difference at *P ≤ 0.05 or **P ≤ 0.01.

n = 15.

FFW = fruit fresh weight, FDW = fruit dry weight.

Antioxidant compounds

The ascorbic acid content of MD-2 pineapple fruit decreased by 27% as ripening took place with lowest found in ripening stage 5 (Table 2). In agreement to this study, 'Maspine' pineapple fruit also showed decreasing ascorbic acid content with advancement of ripening stages (Pauziah *et al.*, 2013). Conversely, total carotenoids content of pineapple fruit pulp increased significantly by 284% as fruit ripened. A similar finding was also reported in Smooth Cayenne pineapple fruit grown in Thailand where the pulp carotenoids content increased during ripening (Joomwong, 2006).

Both ascorbic acid and total carotenoids are important antioxidant compounds in fruit. MD-2 pineapple fruit is well-known for its high ascorbic acid content as compared to other cultivar of pineapples (Deka *et al.*, 2005; Raimbault *et al.*, 2011; Pauziah *et al.*, 2013). Ascorbic acid is a main biological form of vitamin C while total carotenoids of pineapple fruit contains provitamin A carotenoids. The primary provitamin A carotenoids found in

pineapple fruit is β-carotene (Freitas *et al.*, 2014). This antioxidant vitamins, which acts as free radical scavengers, making MD-2 pineapple fruit essential to human health.

As shown in Table 3, the TPC of MD-2 pineapple fruit increased from ripening stage 1 to 3, and thereafter decreased at stage 5 for both extraction solvents. The increase of TPC as fruit ripened could due to new biosynthesis of polyphenols while the decrease in TPC at later stage of ripening may attribute by the oxidation of polyphenols by polyphenol oxidase (PPO). It has been reported that the activity of PPO in pineapple fruit varied according to its ripening stage but higher activity were found in the ripe fruit (Soares *et al.*, 2005). With the higher PPO activity, higher chances of polyphenols would be oxidized. This may explain the lower TPC when MD-2 pineapple fruit reached ripening stages 5 in this study.

Although both extraction solvents gave similar trend of TPC changes, lower TPC was found in water solvent (Table 3). The recovery of phenolics

Table 3. Effects of five ripening stages on total phenolic content and antioxidant activity (DPPH, FRAP and ABTS) of MD-2 pineapple fruit extracted using 80% methanol and distilled water

Ripening stage	Total phenolic content (mg GAE/g FDW)		DPPH ($\mu\text{mol TE/g FDW}$)		FRAP ($\mu\text{mol TE/g FDW}$)		ABTS ($\mu\text{mol TE/g FDW}$)	
	methanol	Water	methanol	Water	methanol	Water	methanol	Water
1	4.78 b ^z	3.01 c	31.28 b	23.99 c	15.91 d	15.30 c	22.08 c	21.5 c
2	4.81 b	3.13 bc	31.3 b	24.94 bc	16.04 cd	16.00 c	22.25 bc	21.83 b
3	5.38 a	3.63 a	34.68 a	31.11 a	22.84 a	19.30 a	22.78 a	22.21 a
4	5.00 ab	3.38 ab	33.01 ab	26.53 b	20.00 b	18.18 b	22.39 b	22.06 a
5	4.86 b	3.16 bc	32.09 b	25.07bc	17.21 c	16.31 c	22.27 bc	21.88 b
F value	16.58**	41.97**	38.06**	206.14**	243.79**	165.39**	1.16*	2.58*

^z Means followed by the same letter in the column are not significantly different by LSD at $P \leq 0.05$.

Significant difference at * $P \leq 0.05$ or ** $P \leq 0.01$.

n = 15.

ABTS = 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), DPPH = 1,1-diphenyl-2-picrylhydrazyl, FDW = fruit dry weight, FRAP = ferric reducing antioxidant power, GAE = gallic acid equivalent, TE = trolox equivalent.

content is influenced by the polarity of extracting solvents and its solubility in the solvent used for the extraction processes. Solvent polarity plays a key role in increasing phenolics solubility (Hassas-Roudsari *et al.*, 2009). Methanol solvent is used for determining hydrophilic and lipophilic antioxidant activities (Arnao *et al.*, 2001). It is an efficient and widely used solvent to extract natural antioxidant compound especially phenolics from plant materials. This may due to the methanol-water mixture has high polarity, thus, it contributes to greater efficacy in extracting polar antioxidant compounds (Siddiq *et al.*, 2005). Therefore, the selection of an appropriate solvent system is crucial in optimizing the recovery of TPC and other antioxidants. Present study showed that 80% methanol is a better solvent as compared to water in extracting pineapple fruit phenolics compound.

Phenolic compounds are secondary plant metabolites which considered to be the most important antioxidants. Phenolics content plays a number of roles in biological activities but the most important is antioxidant activity which is associated with a reduce risk of cancers (Czeczot, 2000). The antioxidant properties of phenolics is due to its redox properties where it acts as reducing agent, hydrogen donators and singlet oxygen quenchers (Rice-Evans *et al.*, 1997). This result showed that TPC varied significantly during fruit ripening, which in line with other researchers (Chiniros *et al.*, 2010; Pineli *et al.*, 2011; Tlili *et al.*, 2014). In contrast, TPC of jujube (Zozio *et al.*, 2014), acai (Gordon *et al.*, 2012) and mango (Palafox-Carlos *et al.*, 2012) fruits decreased with ripening while TPC of papaya fruit increased with ripening (Zuhair *et al.*, 2013). The TPC of four cultivars icebox watermelon fruits differed from each other during fruit development and ripening (Soumya and Ramana, 2014). The considerable differences of

TPC in these fruits during ripening indicated genetic and physiological state could affect the degree of phenolic compounds biosynthetic pathway.

Antioxidant activities

Plant tissues contain complex chemicals and different assays are required to assess their antioxidant activity. Antioxidant activity has been determined using a number of methods based on both the free radical scavenging and the redox mechanisms. In this study, the antioxidant activities of MD-2 pineapple fruit were examined using DPPH, FRAP and ABTS assays (Table 3). Similar to TPC, the activities increased as fruit ripened from stage 1 to 3, then decreased as stages advanced for both extraction solvents. Ripening stage is one of the factors that could affect the antioxidant activity of fruits (Fawole and Opara, 2013). The increased level of antioxidant activity during ripening might be a self-defensive response against the effects of oxidative stress (Smirnoff, 1995). Since antioxidants can scavenge reactive oxygen species, the tissue exhibiting high antioxidant activities would better resist oxidative stress than tissue with lower antioxidant potential (Lester, 2008).

The antioxidant activity of present study increased and then decreased in corresponding to its TPC when fruit ripening stage advanced. The reduction in antioxidant activity may associate with apparent decrease in quantity of polyphenols in the fruit during ripening process (Fischer *et al.*, 2011). However, Gordon *et al.* (2012) mentioned that there is no consistent trend derivable for the antioxidant activity at different ripening stage of acai fruits in corresponding to its TPC. According to Gruz *et al.* (2011), the over-ripe medlar fruit lost its functional properties and thus, had lower antioxidant activity compared to the ripe fruit. As such fruit ripening stage

is an important factor when evaluating its antioxidant potential.

Conclusion

In conclusion, ripening stage affected the physicochemical quality and antioxidant compounds as well as antioxidant activity in MD-2 pineapple fruit. Fruit at ripening stages 3 and 4 retained higher content of nutritional value than other stages. These findings could be used by consumers to choose for suitable stage of MD-2 pineapple in their daily diet requirements.

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