MJFAS MALAYSIAN JOURNAL OF FUNDAMENTAL AND APPLIED SCIENCES
PRINT ISSN: 2289-5981 | ONLINE ISSN: 2289-599X

RESEARCH ARTICLE

Effect of seedling size and flowering time on fruit quality, secondary metabolite production and bioactivity of pineapple [*Ananas comosus* (L.) Merr. var. 'Yankee'] fruits

Siti Zubaidah Husin [a], Mawiyah Mahmud [a], Sujatha Ramasamy [a], Rashidi Othman [c], Jamilah Syafawati Yaacob [a, b,*]

[a] *Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[b] *Centre for Research in Biotechnology for Agriculture (CEBAR), Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[c] *International Institute for Halal Research and Training (INHART), Herbarium Unit, Department of Landscape Architecture, Kulliyyah of Architecture and Environment Design, International Islamic University Malaysia, 53100 Kuala Lumpur, Malaysia*

* *Corresponding author: jamilahsyafawati@um.edu.my*

Article history
Submitted 24 November 2017
Revised 19 January 2018
Accepted 29 January 2018
Published Online 15 Mac 2018

Abstract

Recently, antioxidants derived from natural sources have gained wide interest worldwide due to their high medicinal values and industrial applications. Various factors have been reported to affect the antioxidant content in plants. This study aimed to analyze the effect of seedling size and flowering time on quality attributes and bioactivity of pineapple fruits, *Ananas comosus* L. var. Yankee. Free radical scavenging activities of the fruits produced from seedlings of different sizes (grades A, B and C), produced either through natural flowering or artificially induced flowering were investigated using DPPH, ABTS and FRAP assays. The methanolic extract of fruits from grade A seedlings showed the lowest IC_{50} value of ABTS radical and the highest FRAP value, indicating good scavenging activity. However, DPPH assays showed that fruits from grade C seedlings (either naturally produced or artificially induced) exhibited the highest scavenging activity against DPPH, compared to fruits from other seedling grades. Moreover, fruits from grade B seedlings produced from natural flowering showed significantly better antioxidant potential than fruits that were artificially induced. Other quality attributes such as fruit weight and length, total titratable acidity (TTA), amount of total soluble solid (TSS) and pH were also observed to be not significantly different among fruits produced from different seedling sizes, and their phytochemical constituents were also similar. These results suggested that *A. comosus* L. var. Yankee fruits contain various pharmacologically important phytoconstituents which can be further exploited for various uses.

Keywords: Antioxidant, bioactivity, physical attributes, physicochemical analysis, secondary metabolites

INTRODUCTION

Ananas comosus (L.) Merr. or commonly known as pineapple is cultivated mostly in tropical and subtropical countries as well as in several mild climate regions. It is recognized as the third most important tropical crop after banana and mango (Ogata *et al.*, 2016). Pineapple is a rich source of proteolytic enzyme known as bromelain which is found either in the stem or fruit of the plant and is widely used in food industry, such as for baking, to avoid browning of apples (Kaur *et al.*, 2015), as meat tenderizer (Carlier *et al.*, 2007, Kaur *et al.*, 2015) and has also been used in the textile industry to improve dyeing properties of protein fibers (Kaur *et al.*, 2015, Koh *et al.*, 2006). Pineapple is also reported to have therapeutic effects such as anti-inflammatory and analgesic activity (Sudjarwo, 2005), anti-cancer (Chobotova *et al.*, 2010) and promote wound healing (Rosenberg *et al.*, 2004), thus making it widely used in medical and pharmaceutical industries (Hebbar *et al.*, 2008).

Pineapple is a perennial, herbaceous monocotyledon (d'Eeckenbrugge *et al.*, 2011) which is mainly vegetatively propagated, by stem suckers, peduncle slips or fruit crowns (Carlier *et al.*, 2007). The sucker is the buds at the axils of leaves that will elongate to form lateral branches whereas slips are the grow-out buds in the axils of short, modified leaves below the inflorescence (Carr, 2014). Slips are the shoots produced on the peduncle at the base of the fruits whereas crowns are usually produced at the top of the fruits (Hepton, 2003). Seed size is one the main criteria of seed selection, which will affect the seed growth vigor (Shirin *et al.*, 2008), crops performance (Adebisi *et al.*, 2011), seed germination and emergence (Kaydan and Yagmur, 2008). These factors have therefore rendered seed size to be directly related to agronomical aspects in the farming of diverse crop species (Kaydan and Yagmur, 2008). Besides, seed size is widely accepted as a measure of seed quality. An increase in yield and vigorous seedling growth were reported in corn when bigger seed size was used (Enayat Gholizadeh *et al.*, 2014). It was proposed that plants derived from larger seeds produced better yield due to

better vigor and the seeds' ability to acquire a bigger proportion of plant growth factors than smaller seeds. Larger pineapple slips were observed to have better vegetative and productive performances compared to small slips, as well as showing more vigorous growth and producing higher yields (Reinhardt *et al.*, 2003).

Flowering in pineapple can occur naturally in relation to environmental factors such as the cooled temperature at night added with shortened daytime (Janick and Paull, 2008) and water content (Charrier, 2001) which stimulate the floral induction in a pineapple plant. The flowering of pineapple, however, can also be induced by growth regulators (Janick and Paull, 2008) under commercial cropping conditions. Since *Ananas comosus* is a strongly self-incompatible species, pollination is unnecessary and their vegetative propagules are distributed either by animal or human or extreme environmental events such as flooding (d'Eeckenbrugge *et al.*, 2011). Bromeliaceae species can be induced to flower by external treatments with ethylene, thus this practice is followed by commercial pineapple growers and farmers worldwide to achieve a synchronized flower and fruit development. This step is an important cultivation practice in pineapple farming as the fruit is of non-climacteric nature. Natural flowering which can occur before external ethylene-induced flowering produced unsynchronized flower and consequently fruit development. Thus, natural flowering is among the major agronomic problems encountered in pineapple cultivation, leading to yield losses besides suppressing the market supply (Van de Poel *et al.*, 2009, Wang *et al.*, 2007).

The tropical climates are the most suitable for cultivation of pineapple, mainly in the regions with low water availability (Cushman, 2005), thus causing Malaysia to become one of the most suitable countries for pineapple cultivation since the temperature of its lowlands ranges from 22°C to 33°C with the average daily temperature of 26.5°C (Mekhilef *et al.*, 2012). In Malaysia, there are nine cultivars that are available in the market where Mauritius, Sarawak, Gandul, Maspine, N36 and Josapine are the mostly planted pineapple cultivars. Moris, Sarawak and Josapine are cultivated mainly for the local fresh fruit consumption whereas MD2 and N36 are usually exported since it has a longer shelf life. At present, other pineapple variety such the 'Yankee' is growing in demand due to its very sweet taste and less-fibrous texture. In this study, the effect of seedling size on the occurrence of natural flowering in *Ananas comosus* (L.) Merr. var. Yankee was evaluated. Its effects on fruit quality attributes, bioactivity and phytochemical properties of the extracts were also investigated.

EXPERIMENTAL

Plant materials

The pineapple plant, *A. comosus* var. Yankee used in this study were grown at Glami Lemi Biotechnology Research Centre, Jelebu, Negeri Sembilan, Malaysia using three different seedling sizes with planting density of 90 cm × 60 cm × 30 cm. The seedling sizes of the pineapple crops are divided into three grades (Grades A, B, and C) as described by Malaysian Pineapple Industrial Board (MPIB) (MPIB, 2016) (Table 1).

Table 1 Grade of pineapple seedlings based on size and measurements.

Grade	Size	Measurement (cm)	Measurement (inch)
A	Big	60	24
B	Medium	45	18
C	Small	30	12

*Diameter of the seedlings core is between 10-15 cm.

The plants were treated with two types of fertilizers; NPK (N – Nitrogen, P – Phosphorus, K – Potassium) granules and foliar sprays. NPK (15:15:15) fertilizer granules were applied at the rate of 20 g per plant at 1, 3, and 6 months after planting. A foliar fertilizer mix was sprayed two times at 1.5 months (640 g hydrated lime, 42 g copper sulphate, 42 g zinc sulphate and 21 g ferrous sulphate in 18 L water) and 4.5 months (added with 640 g urea in 18 L water) after planting. 50 – 100 ml of foliar fertilizer mix was sprayed to each plant.

The fruits produced from natural induction were harvested from June 2015 to August 2015 and stored at -80 °C until further analysis. Then, the pineapple plants were artificially induced to flower twice by using hormone ethephon on December 2015 and January 2016, according to MPIB guidelines (MPIB, 2016). After 3 – 4 months, mature fruits were harvested from March 2016 to April 2016 and kept in -80 °C until further analysis.

Physical and physicochemical analysis

The samples were randomly collected from the pineapple field and used for both physical and physicochemical analysis. The weight of fruit with the crown, crown and fruit (separate) were measured using an electronic balance. The diameter and length of the fruits were measured using a vernier calliper, while the diameter of the pineapple core was measured using a ruler. For physicochemical analysis, the pineapple flesh of fruits at Index 6 of ripening stage was used (MPIB, 2016). Sample preparation for the physicochemical analysis was prepared according to standard procedure (Appiah *et al.*, 2012), with some modifications. Briefly, 30 g of fresh fruit pulp and core of pineapple from three grades (A, B and C) were macerated in 90 mL distilled water by using laboratory blender for 2 min and filtered. The pH, TTA and TSS of the filtrate were measured according to the standard procedure described (Dadzie and Orchard, 1997, Appiah *et al.*, 2012).

Sample preparation

The flesh of fruits for each grade (A, B and C) was cut and freeze-dried. The freeze-dried fruits were subjected to solvent extraction using methanol. Briefly, 2 g of freeze-dried fruits were soaked in 60 ml absolute methanol and ground using a mortar. Then, the sample mixture in solvent was kept on a rotary shaker at 100 rpm for 24 hours at room temperature (RT) followed by filtration using filter paper. The whole extraction procedure was repeated with the residue previously obtained. The filtrates were pooled and evaporated to dryness by using a rotary evaporator (45°C) (Kalaiselvi *et al.*, 2012) to yield methanolic extract. The concentrated extract was adjusted to a concentration of 20 mg/ ml using absolute methanol before stored at -20 °C until further analysis.

For analysis of carotenoid content, 1.0 g of freeze-dried samples were rehydrated with 1.0 ml distilled water and soaked overnight at RT in 5 ml of acetone:methanol (7:3). Then, the sample mixture was vortexed and centrifuged at 13500 *g* for 2 min, where the supernatant was then transferred into a 50 ml graduated polypropylene centrifuge tubes covered with foil. The supernatant was centrifuged again at 13500 *g* for 5 min to remove fine particulates. The supernatant was then collected and stored at 4 °C in the dark, prior to analysis. For extraction of carotenoids, 1:1 ratio of hexane and distilled water was added to the sample mixture, vortexed and centrifuged at 13500 *g* for 1 min. The upper carotenoids layer was collected and dried under O_2-free nitrogen gas. Then, the vials were immediately capped, sealed with parafilm and stored at -80 °C until subsequent analysis.

Total phenolic content

Total phenolic content (TPC) of the extracts were quantified using Folin-Ciocalteu's method according to the method described by Sulaiman and Ooi (2012). Folin-Ciocalteu's reagent (25 µL) was added to 10 µL of methanolic extract (at six concentrations) in the well of a 96-well plate and incubated for 5 min at RT. Then, 25 µL of 20% (w/v) Na_2CO_3 was added to the mixture, and the final volume was adjusted to 200 µL per well using distilled water. The absorbance was read at 760 nm against a blank (methanol) using a microplate reader after 30 min incubation at RT. The standard curve was plotted using gallic acid. Measurements were carried out in triplicate and the TPC was calculated based on the calibration curve obtained with the gallic acid standard. The TPC of the samples was expressed as mg of gallic acid equivalents (GAE) per g sample dry weight (DW).

Total flavonoid content

Total flavonoid content (TFC) of the fruit extracts were estimated using the aluminium chloride colorimetric method. Fruit methanolic extract (30 µL) was mixed with 180 µL distilled water, followed by

addition of 10 µL 5% $NaNO_3$ and incubated at RT for 6 min. Then, 20 µL of 10% $AlCl_3$ was added, mixed well, and left to stand for further 6 min. After that, 60 µL of 4% NaOH was added to each extract and incubated for 15 min at RT. The absorbance of the solution was read at 510 nm using a microplate reader. Measurements were carried out in triplicate and the TFC was calculated based on the calibration curve obtained with the gallic acid standard. The TFC of the samples was expressed as mg of gallic acid equivalents (GAE) per g sample dry weight (DW).

Carotenoid content

Quantification of carotenoid content was conducted using HPLC, on an Agilent 1200 series model that is comprised of micro vacuum degassers, a binary pump with autosampler injector, thermostatted column compartment and a diode array detector. Separation was done using HPLC column ZORBAX SB-C_{18} end capped (5 µm, 4.6 × 250 mm) reverse phase column (Agilent Technologies, USA). The eluents used were; (A) acetonitrile:water (9:1 v/v) and (B) ethyl acetate. The solvent gradient used was as followed: 0-40% solvent B (0-20 min), 40-60% solvent B (20-25 min), 60-100% solvent B (25-25.1 min), 100% solvent B (25.1-35 min) and 100-0% solvent B (35-35.1 min) at a flow rate of 1.0 ml min^{-1}. The column was allowed to re-equilibrate in 100% solvent A for 10 min prior to the next injection. The injection volume was 10 µL. The temperature of the column was kept at 20ºC. Carotenoid peaks were detected between the range of 350 to 550 nm. In this study, the fruit extracts were screened for 8 types of carotenoid; neoxanthin, violaxanthin, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, lycopene and lutein.

Antioxidant capacities

DPPH (2, 2-diphenyl-1-picrylhydrazyl)

DPPH radical scavenging activity of the methanolic extracts was measured according to the method described by Sulaiman and Ooi (2012). Briefly, 150 µL DPPH solution (60 mM) was added to 50 µL of extract (at various concentration) in each well of the 96-well plate before incubation for 30 minutes at RT. As for blank, 50 µL of methanol was added to DPPH solution. The absorbance value was taken at 515 nm using a microplate reader at the end of the incubation period. All of the extracts were assayed in triplicate. The antioxidant capacity of the test extracts was expressed as $IC_{50,}$ which is the concentration necessary for 50% reduction of DPPH free radicals.

ABTS (2, 2-azino-bis (3-ethylbenzotiazoline-6-sulfonic acid)

ABTS scavenging activity assay was performed using the colorimetric method described by Chen *et al.* (2014) with slight modifications. ABTS radical cation was prepared by mixing 10 ml of 7.4 mM ABTS solution with 10 ml 2.6 mM of $K_2S_2O_4$ solution. This mixture was stored at RT in the dark room for 12-16 h before use. Prior to the assay, this mixture was diluted with double distilled water (ddH_2O) until the absorbance was adjusted to 0.70 + 0.2 at 734 mM. A volume of 200 µL ABTS solution was added to 20 µL of sample. After incubation at RT for 30 minutes, the absorbance was measured at 734 nm. The assay was repeated in triplicate and the percentage of inhibition was calculated according to the formula:

$$\% \text{ inhibition} = [(A_{blank} - A_{sample})/ (A_{blank})] \times 100$$

The antioxidant capacity of the test extracts was expressed as $IC_{50,}$ which is the concentration necessary for 50% reduction of ABTS (Gülçin *et al.*, 2011).

FRAP (ferric reducing antioxidant power)

The FRAP assay was conducted based on standard protocol (Benzie and Strain, 1996, Kong *et al.*, 2012), with modifications. Three reagents were prepared prior to analysis; 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6,tripyridyl-s-triazine (TPTZ) in 40 mM hydrochloric acid (HCl) and 20 mM iron chloride ($FeCl_3$). FRAP reagent was prepared by mixing acetate buffer, TPTZ solution in 40 mM HCL and 20 mM $FeCl_3$ at a ratio 10:11 (v/ v/ v), respectively. Extract (10 µL) was added to 300 µL of FRAP reagent prior to 30 min incubation at 37°C. Subsequently, the absorbance was measured at 593 nm. The FRAP values were calculated by constructing a calibration curve with aqueous solutions of known ferrous ion concentration ($FeSO_4.7H_2O$) and expressed as mmol Fe^{2+}/100g sample (Müller *et al.*, 2010). The antioxidant ability of the extract to reduce Fe (II)-2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) complex to Fe (II)-TPTZ resulting in intense blue colour is being linearly related to the amount of the antioxidant present (Gardner *et al.*, 2000, Benzie and Strain, 1996).

Phytochemical analysis

The screening of other phytochemical constituents such as tannins and alkaloids in the fruit methanolic extracts was conducted according to standard method previously described (Solihah *et al.*, 2012).

Sensory analysis

30 untrained panelists were involved to evaluate the organoleptic qualities of the 'Yankee' pineapple fruits produced from seedlings of different grades (A, B and C). Questionnaires were distributed to each of the panelists. Data analysis using Just-about-right (JAR) was used to analyze the preference of the panelists towards the samples tested, by calculating the frequencies of each attribute and combining frequencies in scale 1 with scale 2 and assuming it as the lowest preference, scale 3 as the middle preference, and combining frequencies of scale 4 with scale 5, assuming it as the highest preference by panelists. The data of frequencies is expressed in percentage of intensities of each attribute. A hedonic scale of 1-5 was used in the analysis to assess the panelists' preference in 4 sensory attributes; flesh colour, flavor, firmness and level of acceptability.

Statistical analysis

All analyses were carried out in triplicate and the data was expressed as means $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) was used to determine the significance of the differences between the means. Correlations were obtained by Pearson's correlation coefficient (*r*) in bivariate linear correlation.

RESULTS

Physical and physicochemical analysis

The effects of the seedling size on the physical characteristics of pineapple *A. comosus* var. Yankee fruits are depicted in Table 2. Seedling sizes significantly influence the physical characteristics of pineapple fruits except for crown weight. Based on Table 2, both fruits from Grade A seedlings exhibited the best quality attributes in terms of fruit weight, fruit length and also fruit width. However, the fruits produced by artificial means gave the best quality attributes in terms of fruit weight, fruit length and fruit width compared to the fruits produced through natural flowering.

The fruit weight ranged from 200.48 g to 1441.90 g for the three seedling grades after flowering induction, heavier compared to fruit weight from natural flowering which ranged from 144.50 g to 377.33 g. The fruit length ranged from 168.99 mm to 214.56 mm for fruits developed from induced flowering, longer compared to fruit length produced through natural flowering which ranged from 62.01 mm to 111.04 mm. The fruits produced through induced flowering were also bigger with a fruit width ranging from 96.70 mm to 114.64 mm compared to fruits from natural flowering which ranged from 53.08 mm to 78.38 mm.

Table 2 Physical characteristics of pineapple *A. comosus* var. Yankee fruits produced from seedlings of different grades.

Samples		Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Core diameter (mm)	Crown weight (g)
Natural flowering	A	377.33 ± 38.94 [a]	111.04 ± 14.11 [b]	78.38 ± 6.70 [a]	15.50 ± 3.08 [a]	135.17 ± 19.77 [a]
	B	248.25 ± 59.55 [ab]	89. 18 ± 16.18 [b]	68.21 ± 3.65 [b]	11.75 ± 3.77 [a]	82.75 ± 14.23 [a]
	C	144.50 ± 6.36 [b]	62.01 ± 2.12 [a]	53.08 ± 2.54 [c]	8.00 ± 0.00 [b]	143.50 ± 4.95 [a]
Induced flowering	A	1441.90 ± 516.84 [a]	214.56 ± 22.57 [a]	114.64 ± 9.27 [a]	20.99 ± 1.11 [a]	70.27 ± 13.91 [b]
	B	690.28 ± 218.28 [ab]	189.12 ± 34.60 [b]	104.00 ± 13.85 [ab]	19.20 ± 4.6 [a]	96.00 ± 48.03 [ab]
	C	200.48 ± 63.40 [b]	168.99 ± 20.55 [b]	96.70 ± 4.33 [b]	18.40 ± 3.80 [a]	104.80 ± 32.82 [a]

* Data represent means ± SD.
* Means followed by different letters in a column are significantly different at p<0.05, analyzed using ANOVA and Duncan's Multiple Range test.

Table 3 showed the physicochemical attributes of the fruits produced by different seedling sizes. Based on the results, the seedling size did not affect the physicochemical properties of the fruits since there is no significant variation in the data obtained. It was also shown that fruits from natural flowering exhibited higher TSS (total soluble solids) compared to fruits from induced flowering. However, the pH of fruits from natural flowering (for all seedling sizes) was more acidic compared to fruits from induced flowering. The TTA (total titratable acidity) was also higher in fruits from natural flowering than fruits from induced flowering.

Table 3 pH, Total soluble solids (TSS), and titratable acidity (TTA) of pineapple *A comosus* var. Yankee fruits produced from seedlings of different grades.

Samples		pH	TSS (°Brix)	TTA (% citric acid)
Natural flowering	A	3.664 ± 0.297 [a]	9.32 ± 1.12 [b]	1.72 ± 0.34 [a]
	B	3.687 ± 0.447 [a]	10.13 ± 0.85 [ab]	1.36 ± 0.17 [a]
	C	3.548 ± 0.068 [a]	11.70 ± 0.85 [a]	1.58 ± 0.01 [a]
Induced flowering	A	4.203 ± 0.066 [a]	8.80 ± 1.45 [b]	1.08 ± 0.14 [a]
	B	4.253 ± 0.200 [a]	10.61 ± 1.38 [a]	1.16 ± 0.25 [a]
	C	4.040 ± 0.138 [b]	9.58 ± 0.25 [ab]	1.20 ± 0.15 [a]

* Data represent means ± SD.
* Means followed by different letters in a column are significantly different at p<0.05, analyzed using ANOVA and Duncan's Multiple Range test.

Biochemical analysis

Tables 4 and 5 showed the results of TPC, TFC, carotenoid content and antioxidant capacities determined by DPPH, ABTS and FRAP assays of pineapple *A. comosus* var. Yankee fruits extract produced from seedlings of different grades. Based on the results, the TPC, TFC and carotenoid content of fruits from natural flowering are the highest compared to fruits from induced flowering. The TPC of fruits from natural flowering ranged from 4.9319 mg GAE/100 g DW to 6.9969 mg GAE/100 g DW, whereas the TPC of fruits from induced flowering ranged from 3.8229 mg GAE/100 g DW to 4.5046 mg GAE/100 g DW. Similar observations were recorded for TFC, where fruits from natural flowering showed higher TFC (5.5593 mg GAE/100 g DW to 10.4607 mg GAE/100 g DW) compared to fruits from induced flowering (3.6711 mg GAE/100 g DW to 6.5461 mg GAE/100 g DW). The extracts were also assessed for the presence of carotenoid compounds. Interestingly, only β-carotene was found in all the samples (Fig. 1), where fruits produced from Grade B seedlings showed the highest β-carotene in both categories (either from natural or induced flowering).

Table 4 Total phenolic contents (TPC) and total flavonoid contents (TFC) and antioxidant capacities determined by DPPH, ABTS and FRAP assays of pineapple *A. comosus* var. Yankee fruits extract produced from seedlings of different grades.

Samples		TPC value (mg GAE/ 100g DW)	TFC value (mg GAE/ 100g DW)	β-carotene content (µg/g DW)
Natural flowering	A	6.9969 ± 0.0034 [a]	10.4607 ± 0.0097 [a]	3.41 ± 0.69 [ab]
	B	4.9319 ± 0.0030 [c]	6.8751 ± 0.03693 [b]	4.09 ± 0.96 [b]
	C	5.8130 ± 0.0024 [b]	5.5593 ± 0.0231 [b]	2.41 ± 1.04 [ab]
Induced flowering	A	4.0234 ± 0.0002 [b]	6.5461 ± 0.0261 [b]	1.36 ± 0.18 [a]
	B	4.5046 ± 0.0024 [c]	3.6711 ± 0.0356 [a]	2.45 ± 0.30 [ab]
	C	3.8229 ± 0.0023 [a]	6.1185 ± 0.0167 [ab]	1.17 ± 0.29 [a]

*Data represent means ± SD.
*Means followed by different letters in a column are significantly different at p<0.05, analyzed using ANOVA and Duncan's Multiple Range test.

Table 5 Total phenolic contents (TPC) and total flavonoid contents (TFC) and antioxidant capacities determined by DPPH, ABTS and FRAP assays of pineapple *A. comosus* var. Yankee fruits extract produced from seedlings of different grades.

Samples		DPPH, IC_{50} (mg/ mL)	ABTS, IC_{50} (mg/ mL)	FRAP (mmol FE/ 100g DW)
Natural flowering	A	3.2428 ± 0.3740 [b]	3.4034 ± 0.0760 [b]	5.3634 ± 0.4235 [a]
	B	5.1950 ± 0.1281 [a]	5.4971 ± 0.0906 [a]	0.5119 ± 0.0139 [b]
	C	1.4162 ± 0.0768 [c]	4.7769 ± 0.1193 [b]	0.4493 ± 0.0227 [b]
Induced flowering	A	3.7116 ± 0.2132 [b]	7.2423 ± 0.5589 [a]	0.2598 ± 0.0306 [b]
	B	5.4810 ± 0.6115 [a]	7.3309 ± 0.0229 [a]	0.6708 ± 0.2141 [a]
	C	2.7249 ± 0.0844 [c]	6.6261 ± 0.4203 [a]	0.4666 ± 0.1447 [ab]

*Data represent means ± SD.
*Means followed by different letters in a column are significantly different at p<0.05, analyzed using ANOVA and Duncan's Multiple Range test.

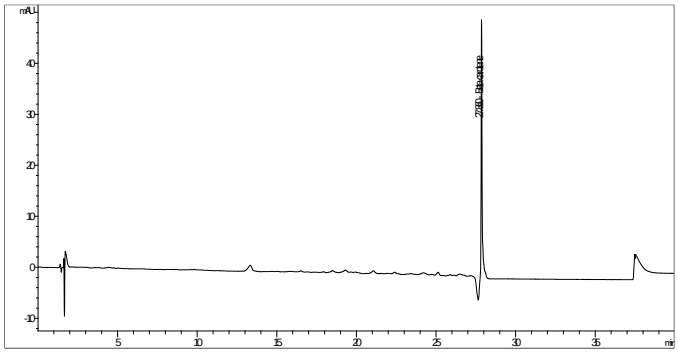

Fig. 1 An example of HPLC chromatogram showing the presence of β-carotenoid in methanolic extract of pineapple fruit produced from Grade A seedlings.

Fruits produced from natural flowering exhibited lower IC_{50} than fruits from induced flowering, indicating better scavenging capacity against DPPH radical. Similarly, ABTS assay also showed lower IC_{50} values in fruits from natural flowering compared to fruits from induced flowering. On the other hand, the FRAP assay determine the ability of the compounds present in the extracts to reduce Fe^{3+} to Fe^{2+}. The FRAP values ranged from to 0.4493 mmol FE/100 g DW to 5.3634 mmol FE/100 g DW for fruits from natural flowering and ranged from 0.2598 mmol FE/100 g DW to 0.6708 mmol FE/100 g DW for fruits from induced flowering. The antioxidant capacity determined by FRAP assay in decreasing order is as followed: Grade A (NF) > Grade B (IF) > Grade B (NF) > Grade C (IF) > Grade C (IF) > Grade A (IF).

In order to establish the influence of TPC, TFC, carotenoid content and seedling size on individual antioxidant activity, correlation studies were also carried out. From Table 6, correlation studies based on Pearson's correlation coefficients between the variables showed that, TPC and TFC were strongly correlated with ABTS (r = 0.924 and r = 0.701), where increasing TPC and TFC in the fruits will significantly decrease the ABTS IC_{50} value ($p<0.01$). A strong correlation between TPC and TFC with FRAP (r = 0.802 and r = 0.731 respectively) was also observed, where increasing TPC and TFC in the fruits will significantly increase the FRAP values ($p<0.01$). However, the correlation between TPC and TFC with DPPH is not significant.

Table 6 Pearson's correlation coefficients between the variables.

Variables	Seedling size	TPC	TFC	Carotenoid content
Seedling size	N/A	0.256	0.472*	0.172
DPPH, IC_{50}	0.404	-0.268	-0.180	0.251
ABTS, IC_{50}	-0.108	-0.924**	-0.701**	-0.448
FRAP	0.524*	0.802**	0.731**	0.339

Strength indicator: Weak (0.1 to 0.3), Moderate (0.3 to 0.5), Strong (0.5 to 1.0)
** Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.
N/A not available.

On the other hand, seedling size was found to significantly affect TFC. In this study, a moderate correlation was observed between seedling size and total flavonoid content, where the increase in seedling size will significantly increase TFC of the fruits ($p<0.05$). Moreover, a strong correlation between seedling size and FRAP value was also observed, where the increase in seedling size will significantly increase the FRAP value of the fruits ($p<0.05$). However, no significant correlation was found between the carotenoid content and antioxidant activities of the fruits.

Sensory analysis

Fig. 2 shows the preference percentage of various sensory attributes of fruits derived from Grade A, Grade B and Grade C seedlings as evaluated by 30 panelists. Based on the results, the overall acceptability of pineapple fruits derived from Grade A seedlings is the highest (93.33%), compared to by Grade B (86.67%) and Grade C (60%). Similarly, fruits from Grade A seedlings also recorded the highest percentage of preference in terms of its flavor (90%) and firmness (90%), followed by Grade B (flavor: 83.33%, firmness: 90%) and Grade C (flavor: 36.67%, firmness: 80%). However, in terms of the colour of the fruit flesh, 100% of the respondents liked the colour of fruits from Grade A and Grade C seedlings, followed by Grade B (93.33%). Therefore, in general, the quality of fruits produced from seedlings of all grades is good and acceptable, as shown by the high percentage of preference and acceptability percentage recorded in the sensory analysis on the random panelists.

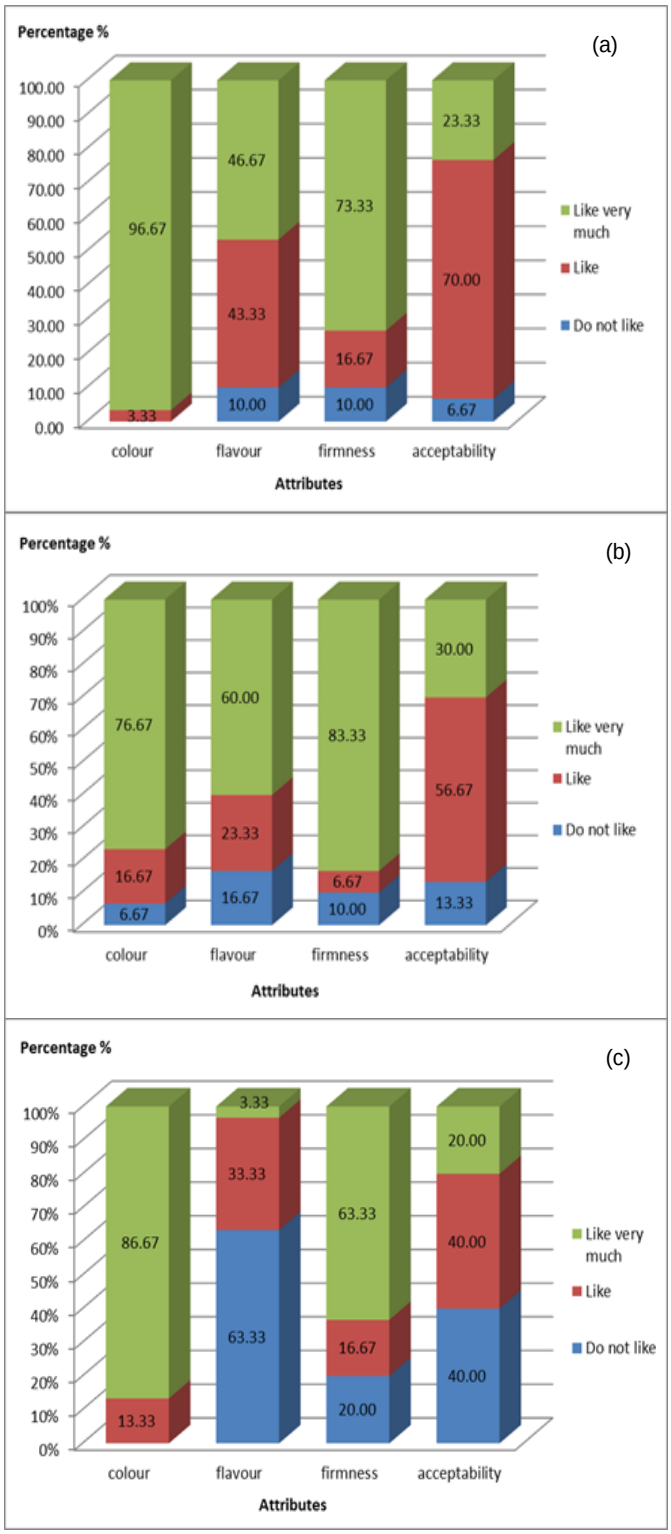

Fig. 2 Percentage of sensory attributes of fruits from seedlings of various sizes; (a) grade A seedlings, (b) grade B seedlings and (c) grade C seedlings.

DISCUSSION

Based on data analysis, seedling size was found to significantly influence the physical characteristics of pineapple *A. comosus* var 'Yankee' fruits except the crown weight. Fruits from grade A seedlings exhibited the best quality attributes in terms of fruit weight, fruit length and also fruit width. These results are in accordance to the data reported by Fassinou Hotegni *et al.* (2015), where the weight of the planting material influenced the average pineapple fruit weight and uniformity of the fruit quality. Fruits from heavy planting materials were heavier, have longer infructescence, shorter and smaller crowns compared to light planting materials. In this study, we also found that the fruits produced by artificial means yielded the best quality attributes in terms of fruit weight, fruit length and fruit width compared to fruits produced through natural flowering. A similar finding was obtained by Fassinou Hotegni *et al.* (2015), where flowering induction at optimum time was found to increase the yield and proportion of pineapple fruits to meet the standard of export to Europe.

Seedling size gives no significant effect on the physicochemical properties of the fruits since there is no significant variation in the data obtained. These findings are in accordance with the study by Cunha *et al.* (1993), (Reinhardt *et al.*, 2003) in which they found size of slips had no significant effect on the qualitative fruit quality (e. TSS and TTA contents) of 'Perola' pineapple juice. Hotegni *et al.* (2015) also reported that there was no correlation between seedling size and TSS in artificially induced pineapple fruits. Besides that, based on Pearson's analysis, a significant moderate correlation was also found between seedling size and total flavonoid content of the fruits, indicating that fruits produced from bigger seedlings have higher amounts of flavonoids. The amount of polyphenols in fruits and vegetables are affected by various factors such as plant variety and species, environmental and growing conditions, maturity stages and harvesting factors, as well as stresses and tissue localization (Tiwari *et al.*, 2013) and (Rice-Evans and Packer, 2003).

Pineapple is a very rich source of carotenoids, one of the most widespread pigments that give fruits and vegetables their colour, i.e. red, yellow and orange (Siddiq *et al.*, 2012). Carotenoids can be divided into carotenes or xantophylls based on their chemical composition. In this study, only β-carotene is detected in the fruit samples. Based on Pearson's correlation analysis, a strong correlation was observed between ABTS IC_{50} and carotenoid content, but the correlation was not significant. (Ding and Syazwani, 2015) reported that carotenoids and ascorbic acids contribute to antioxidant activity of MD2 pineapple fruits. (Kongsuwan *et al.*, 2009) also reported similar observations, where the 'Phulae' and 'Nanglae' pineapple fruits were found to contain high levels of *β*-carotene, vitamin C and total phenolic.

Other than carotenoids, phenolic such as flavonoids, phenolic acids and other polyphenolic compounds have been reported to be directly associated with the antioxidant activity of vegetables and fruits (Lu *et al.*, 2014). They have attracted much interest due to their potential as antioxidants, which serves as an important indicator of health promoters (Meng *et al.*, 2012). The TPC and TFC obtained in this study were comparatively lower compared to the TPC obtained by Hossain and Rahman (2011) where the TPC and TFC of pineapple methanolic extracts were 51.1 ± 0.2 mg caffeic acid equivalent /g FW and 55.2 ± 0.2 mg catechin equivalent/ g FW respectively. The variation reported in the TPC and TFC contents in fruit extracts may be due to chilling stress. A study by Shofian *et al.* (2011) reported that freeze-drying has the possibility of affecting the composition of some antioxidant components and antioxidant activity of the fruits. de Torres *et al.* (2010) also found 35% drop of the flavonol compounds, 35% drop of anthocyanins beside reduction in acids amount in Carmenere grape skin after freeze-drying.

The present study also compares the physicochemical and phytochemical properties of the fruits produced from natural flowering and induced flowering. The initiation of flowering in pineapple depends on the physiological state and nutritional reserve of the plant, besides day length and temperature. A minimum difference between day and night temperatures however is necessary to stimulate natural flowering (Farahani, 2016). Flowering initiation occurs at the terminal axis of the stem and typically, the fruit size will be highly correlated with plant size during flowering induction (Carr, 2014). A plant mush reach a minimum size before natural flowering can occur or in order for a plant to be easily 'forced' to flower using a growth regulator (Carr, 2014). Natural flowering is not preferred in commercial cultivation of pineapple because it can cause unsynchronized and unpredictable fruit yield. Therefore, floral inducers such as etephon, calcium carbide and acetylene has been used to induce flowering of pineapple to ensure a more controlled and synchronized fruiting throughout the years (Cunha, 2005).

However, according to Hotegni *et al.* (2015), despite the advantages provided by artificial flowering induction, it could also be the source of poor fruit quality compared to natural flowering. For example, Hotegni *et al.* (2015) reported that artificially induced pineapple plants produced fruits with reduced TSS compared to fruits from natural flowering. Similar results were also obtained in this study whereby it was also found that naturally produced fruits exhibited higher TSS (9.32% to 11.70%) compared to TSS of fruits from induced flowering (8.80% to 10.61%). Other pre-harvest factors may also influence the TSS content, such as solar radiation, temperature, day length, water availability, irrigation, pruning techniques, fertilization regime and soil mineral content (Dorais *et al.*, 2008). Moreover, the antioxidant capacity (expressed in IC_{50}) evaluated by DPPH-radical scavenging analysis and ABTS assay showed that fruits from natural flowering have significantly lower IC_{50} values, indicating better scavenging capacity than fruits from induced flowering. However, other factors may also affect the amount of bioactive compounds, such as cultivars, natural variation of fruit, climatic conditions or soil, fertilizer and geographical origin (Charoensiri *et al.*, 2009, Kongsuwan *et al.*, 2009).

CONCLUSION

Based on data analysis, it could be concluded that seedling size and flowering time significantly affects the physical properties of 'Yankee' pineapple fruits, its TSS content and antioxidant potential.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

ACKNOWLEDGEMENT

The authors thank the University of Malaya, Malaysia for experimental facilities and financial support (Grant No. RP015B-14AFR) provided.

REFERENCES

Adebisi, M., Kehinde, T., Ajala, M., Olowu, E. and Rasaki, S. (2011). Assessment of seed quality and potential longevity in elite tropical soybean (Glycine Max L.) Merrill grown in Southwestern Nigeria. *Nigeria Agricultural Journal*, Vol. 42 pp. 94-103.

Appiah, F., Kumah, P. and Oppong, D. (2012). Predicting the consumer acceptability of dried MD2 and smooth Cayenne Pineapple Pulps from chemical composition. *Journal of Food Research*, Vol. 1 No. 2, pp. 210.

Benzie, I. F. F. and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry.* Vol. 239 No. 1, pp. 70-6.

Carlier, J. D., d'Eeckenbrugge, G. C. and Leitão, J. M. (2007). Pineapple. In Kole, C. (Ed.) *Fruits and Nuts.* Berlin: Springer Berlin Heidelberg, pp. 331-42.

Carr, M. K. V. (2014), *Advances in Irrigation Agronomy: Fruit Crops*, England: Cambridge University Press.

Charoensiri, R., Kongkachuichai, R., Suknicom, S. and Sungpuag, P. (2009), Beta-carotene, lycopene, and alpha-tocopherol contents of selected Thai fruits. *Food Chemistry*, Vol. 113 No. 1, pp. 202-207.

Charrier, A. (2001), *Tropical Plant Breeding*, CIRAD. United Kingdom: Science Publishers Inch.

Chen, L., Hwang, J.-E., Choi, B., Gu, K. M., Park, Y. and Kang, Y.-H. (2014). Antioxidant capacities and cytostatic effect of Korean red pepper

(Capsicum annuum L): a screening and in vitro study. *Journal of the Korean Society for Applied Biological Chemistry*, Vol. 57 No. 1, pp. 43-52.

Chobotova, K., Vernallis, A. B. and Majid, F. A. A. (2010). Bromelain's activity and potential as an anti-cancer agent: current evidence and perspectives. *Cancer Letters*, Vol. 290 No. 2, pp. 148-56.

Cunha, G. A. P. d. (2005). Applied aspects of pineapple flowering. *Bragantia*, Vol. 64 No. 4, pp. 499-516.

Cunha, G. A. P. d., Reinhardt, D. and Caldas, R. (1993), *Effect of planting season, moult size and plant age on floral induction on the yield of 'Perla' pineapple in Bahia*, EMBRAPA/CNPMF.

Cushman, J. C. (2005). Crassulacean acid metabolism: recent advances and future opportunities. *Functional Plant Biology*, Vol. 32 No. 5, pp. 375-80.

d'Eeckenbrugge, G. C., Sanewski, G. M., Smith, M. K., Duval, M.-F. and Leal, F. (2011). Ananas. In Kole, C. (Ed.) *Wild Crop Relatives: Genomic and Breeding Resources: Tropical and Subtropical Fruits*, Berlin: Springer Berlin Heidelberg, pp. 21-41.

Dadzie, B. K. and Orchard, J. E. (1997). *Routine post-harvest screening of banana/plantain hybrids: criteria and methods.* International Plant Genetic Resources Institute (IPGRI).

de Torres, C., Díaz-Maroto, M.C., Hermosín-Gutiérrez, I. and Pérez-Coello, M.S. (2010). Effect of freeze-drying and oven-drying on volatiles and phenolics composition of grape skin. *Analytica Chimica Acta*, Vol. 660 No. 1–2, pp. 177-82.

Ding, P. and Syazwani, S. (2015). Physicochemical quality, antioxidant compounds and activity of MD-2 pineapple fruit at five ripening stages. *International Food Research Journal*, Vol. 23 No. 2, pp. 549-555.

Dorais, M., Ehret, D. L. and Papadopoulos, A. P. (2008). Tomato (Solanum lycopersicum) health components: from the seed to the consumer. *Phytochemistry Reviews*, Vol. 7 No. 2, pp. 231-50.

Enayat Gholizadeh, M., Bakhshandeh, A., Shoar, M. D., Ghaineh, M., Saeid, K. A. and Sharafizadeh, M. (2014). Effect of source and seed size on yield component of corn S. C704 in Khuzestan. *African Journal of Biotechnology*, Vol. 11 No. 12, pp. 2938-44.

Farahani, F. (2016). Growth, flowering and fruiting in vitro pineapple (Ananas comosus L.) in greenhouse conditions. *African Journal of Biotechnology*, Vol. 12 No. 15, pp. 1774-1781.

Fassinou Hotegni, V. N., Lommen, W. J. M., Agbossou, E. K. and Struik, P. C. (2015). Influence of weight and type of planting material on fruit quality and its heterogeneity in pineapple [*Ananas comosus* (L.) Merrill]. *Frontiers in Plant Science*, Vol. 5, Article 798.

Gardner, P. T., White, T. A., McPhail, D. B. and Duthie, G. G. (2000). The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chemistry*, Vol. 68 No. 4, pp. 471-474.

Gülçin, İ., Topal, F., Çakmakçı, R., Bilsel, M., Gören, A.C. and Erdogan, U. (2011). Pomological features, nutritional quality, polyphenol content analysis, and antioxidant properties of domesticated and 3 wild ecotype forms of raspberries (Rubus idaeus L.). *Journal of Food Science*, Vol. 76 No. 4, pp. C585-C93.

Hebbar, H. U., Sumana, B. and Raghavarao, K. (2008). Use of reverse micellar systems for the extraction and purification of bromelain from pineapple wastes. *Bioresource Technology*, Vol. 99 No. 11, pp. 4896-4902.

Hepton, A. (2003). Cultural system. In Bartholomew, D. P., Paull, R. E. and Rohrbach, K. G. (Eds.), *The Pineapple: Botany, Production, and Uses.* New York: CAB International, pp. 109-42.

Hossain, M.A. and Rahman, S.M.M. (2011), "Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple", *Food Research International*, Vol. 44 No. 3, pp. 672-6.

Hotegni, V. N. F., Lommen, W. J., Agbossou, E. K. and Struik, P. C. (2015). Trade-Offs of Flowering and Maturity Synchronisation for Pineapple Quality, *PloS one*, Vol. 10 No. 11, p. e0143290.

Janick, J. and Paull, R. E. (2008), *The Encyclopedia of Fruit and Nuts.* North American Office: CABI

Kalaiselvi, M., Ravikumar, G., Gomathi, D. and Uma, C. (2012). In vitro free radical scavenging activity of Ananus comosus (l.) Merrill peel. *International Journal of Pharmacy Pharmaceutical Sciences*, Vol. 4 No. 2, pp. 604-9.

Kaur, T., Kaur, A. and Grewal, R. K. (2015). Kinetics studies with fruit bromelain (Ananas comosus) in the presence of cysteine and divalent ions. *Journal of Food Science and Technology*, Vol. 52 No. 9, pp. 5954-60.

Kaydan, D. and Yagmur, M. (2008). Germination, seedling growth and relative water content of shoot in different seed sizes of triticale under osmotic stress of water and NaCl. *African Journal of Biotechnology*, Vol. 7 No. 16, p. 2862 - 2868.

Koh, J., Kang, S.-M., Kim, S.-J., Cha, M.-K. and Kwon, Y.-J. (2006). Effect of pineapple protease on the characteristics of protein fibers. *Fibers and Polymers*, Vol. 7 No. 2, pp. 180-185.

Kong, K. W., Mat-Junit, S., Aminudin, N., Ismail, A. and Abdul-Aziz, A. (2012). Antioxidant activities and polyphenolics from the shoots of Barringtonia racemosa (L.) Spreng in a polar to apolar medium system. *Food Chemistry*, Vol. 134 No. 1, pp. 324-332.

Kongsuwan, A., Suthiluk, P., Theppakorn, T., Srilaong, V. and Setha, S. (2009). Bioactive compounds and antioxidant capacities of phulae and nanglae pineapple. *Asian Journal of Food & Agro-Industry*, No. S44-S50.

Lu, X.-H., Sun, D.-Q., Wu, Q.-S., Liu, S.-H. and Sun, G.-M. (2014). Physico-chemical properties, antioxidant activity and mineral contents of pineapple genotypes grown in China. *Molecules*, Vol. 19 No. 6, pp. 8518-8532.

Mekhilef, S., Safari, A., Mustaffa, W. E. S., Saidur, R., Omar, R. and Younis, M. A. A. (2012). Solar energy in Malaysia: Current state and prospects. *Renewable and Sustainable Energy Reviews*, Vol. 16 No. 1, pp. 386-396.

Meng, J.-F., Fang, Y.-L., Qin, M.-Y., Zhuang, X.-F. and Zhang, Z.-W. (2012). Varietal differences among the phenolic profiles and antioxidant properties of four cultivars of spine grape (Vitis davidii Foex) in Chongyi County (China). *Food Chemistry*, Vol. 134 No. 4, pp. 2049-2056.

MPIB, M.P.I.B. (2016), "Manual Pengurusan Tanaman Nanas", p. 2. Retrieved from http://www.mpib.gov.my/

Müller, L., Gnoyke, S., Popken, A. M. and Böhm, V. (2010). Antioxidant capacity and related parameters of different fruit formulations. *LWT - Food Science and Technology*, Vol. 43 No. 6, pp. 992-999.

Ogata, T., Yamanaka, S., Shoda, M., Urasaki, N. and Yamamoto, T. (2016). Current status of tropical fruit breeding and genetics for three tropical fruit species cultivated in Japan: pineapple, mango, and papaya. *Breeding Science*, Vol. 66 No. 1, pp. 69-81.

Reinhardt, D. H., Souza, A. P. M., Caldas, R. C., Alcântara, J. d. P. and Almeida, A. A. d. (2003). Management of slips and its effect on growth and production of 'Pérola' pineapple plants. *Revista Brasileira de Fruticultura*, Vol. 25 pp. 248-252.

Rice-Evans, C.A. and Packer, L. (2003), *Flavonoids in Health and Disease, Second Edition.* United Kingdom: Taylor & Francis.

Rosenberg, L., Lapid, O., Bogdanov-Berezovsky, A., Glesinger, R., Krieger, Y., Silberstein, E., Sagi, A., Judkins, K. and Singer, A. J. (2004). Safety and efficacy of a proteolytic enzyme for enzymatic burn debridement: a preliminary report. *Burns*, Vol. 30 No. 8, pp. 843-850.

Shirin, M., Enayatgholizadeh, M., Siadat, E. and Fathi, G. (2008). Comparison of suitable seed vigour of hybrid Zea Maize (CV. SC. 704) in the field condition of Ahvaz. 10th congeres of Agronomy Plant breeding. *Karaj*, Vol. 330, pp. 2938-2944.

Shofian, N. M., Hamid, A. A., Osman, A., Saari, N., Anwar, F., Dek, M. S. P. and Hairuddin, M. R. (2011). Effect of freeze-drying on the antioxidant compounds and antioxidant activity of selected tropical fruits. *International Journal of Molecular Sciences*, Vol. 12 No. 7, pp. 4678-4692.

Siddiq, M., Ahmed, J., Lobo, M. G. and Ozadali, F. (2012). *Tropical and Subtropical Fruits: Postharvest Physiology, Processing and Packaging.* USA: John Wiley & Sons.

Solihah, M. A., Rosli, W. W. I. and Nurhanan, A. R. (2012). Phytochemicals screening and total phenolic content of Malaysian Zea mays hair extracts. *International Food Research Journal*, Vol. 19 No. 4, pp. 1533-1538.

Sudjarwo, S. A. (2005). Anti-inflammatory and analgesic effect of bromelain in mice and rats. *Universa Medicina*, Vol. 24 No. 4, pp. 155-160.

Sulaiman, S. F. and Ooi, K. L. (2012). Polyphenolic and vitamin C contents and antioxidant activities of aqueous extracts from mature-green and ripe fruit fleshes of Mangifera sp. *Journal of Agricultural and Food Chemistry*, Vol. 60 No. 47, pp. 11832-11838.

Tiwari, B. K., Brunton, N. P. and Brennan, C. (2013). *Handbook of Plant Food Phytochemicals: Sources, Stability and Extraction.* USA: John Wiley & Sons.

Van de Poel, B., Ceusters, J. and De Proft, M. P. (2009). Determination of pineapple (Ananas comosus, MD-2 hybrid cultivar) plant maturity, the efficiency of flowering induction agents and the use of activated carbon. *Scientia Horticulturae*, Vol. 120 No. 1, pp. 58-63.

Wang, R.-H., Hsu, Y.-M., Bartholomew, D. P., Maruthasalam, S. and Lin, C.-H. (2007). Delaying natural flowering in pineapple through foliar application of aviglycine, an inhibitor of ethylene biosynthesis. *HortScience*, Vol. 42 No. 5, pp. 1188-1191.