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REGULATION OF FRUIT COLOUR DEVELOPMENT, QUALITY AND STORAGE LIFE OF *HIBISCUS SABDARIFFA* L. AS INFLUENCED BY PLANT GROWTH REGULATORS

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Abstract

The physico-chemical properties of abscisic acid (ABA) and IAA treated roselle calyces stored in different storage temperature was determined in this study. Mature roselle was dipped in distilled water (control), 10^{-4} mol/l of ABA and 10^{-4}mol/l of IAA for 5 minutes. All treated calyces were kept at ambient temperature (23°C) for 4 days, or stored at cold storage (10°C) for 8 days. Low temperature (10°C) can prolong the shelf life of roselle about 4 days longer than ambient storage (23°C). However, plant growth regulator (ABA or IAA) only showed minimal effect on quality and shelf life of roselle. Therefore, the application of higher concentration of ABA or IAA as well as dipping for more than 5 minutes can influence the postharvest quality and shelf life of roselle calyces, respectively.

Introduction

Hibiscus sabdariffa L. is a tropical annual herbaceous shrub of Malvaceae family which is widely grown in tropical and sub-tropical areas of both hemispheres (Norhaizan *et al*. 2010). An estimation of 15,000 ton of roselle entered the world market every year from various countries and the price in the world market is about US$ 1,200 - 3,600/ton depending on the qualities and times of the year (McClintock and El Tahir 2011).

The calyx (sepal of the flower) of roselle has deep and intense maroon colour, rich in antioxidant, anthocyanin and ascorbic acid which make it a valuable food product (Zaharah 2012). Besides that, the calyx has been suggested as a natural food colourant in food industries, emulsifier for carbonated drinks (Duangmal *et al*. 2004). The calyx has also been used in folk medicines to reduce blood viscosity and hypertension (Christian *et al.* 2006), treatment for inflammatory diseases, cancer and reducing serum cholesterol (Lin *et al.* 2007). Nowadays, roselle become popular due to its medicinal value. As fresh roselle wilt rapidly, therefore it is mainly sold whole or dried. However, roselle is better to be freshly consume (Maryani and Kristiana 2010). Therefore, it is beneficial to prolong the shelf life and improve the quality of roselle. Previous studies have found that exogenous application of abscisic acid (ABA) enhance the colour and taste of fruits (Jiang and Joyce 2003, Sun *et al*. 2010). It is also known that application of IAA has been used to prolong shelf life of cut flowers and fruits. Wills *et al*. (1998) mentioned that temperature is the most important factor that affects the fruit storage life. All the physiological processes such as respiration and ethylene production which lead to senescence are controlled by temperature. Hence, the effect on colour development, postharvest qualities and storage life of roselle calyces by using plant growth regulators were determined in this study.

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Materials and Methods

Three concentrations of ABA and IAA (10^{-4}, 10^{-5} and 10^{-6}mol/l) were used to determine fruit colour development and quality of roselle kept at ambient temperature and storage. Parameter of fresh weight, colour, firmness, soluble solids concentration (SSC), titratable acidity (TA) and pH were recorded.

The roselles calyxes were dipped in the optimum concentration of ABA (Sigma Aldrich Co., St. Louis, Missouri, USA) or IAA (Sigma Aldrich Co., St. Louis, Missouri, USA) obtained from the preliminary study, respectively for 5 minutes at ambient temperature (23 ± 1°C). Tween 20 (0.05% v/v) was added in both treatment solution to act as surfactant. For control, roselles calyxes were dipped in distilled water which contains 0.05% v/v Tween 20.

All parameters for roselles stored under ambient temperature or cold storage were collected for four or nine days, respectively. Six calyces for each replication were randomly picked for daily parameter analysis from both storage temperatures

The calyx colour of roselles have been determined by using chromameter (Konica Minolta CR-400 Chroma meter, Minolta Corp., Japan) with CIE 1976 L* a* b* (CIELAB) Space. Roselle seed were removed by hand. The firmness of fresh roselle calyxes have been tested by using Instron (Instron Model 5543 Load Frames, Instron Corp., Massachusetts, USA) in unit of newton (N).

Ten grams of roselle calyxes derived from six fruits and 40 ml of distilled water were macerated and homogenised by using blender. The mixtures were filtered with cheese clothes. A drop of filtrate was then placed on the prism glass of digital hand-held pocket refractometer (Atago PAL-1, Atago Co. Ltd., Tokyo, Japan) to obtain SSC in roselle calyxes in unit of percentage (%).

Titratable acidity is the measurement of total amount of acid contain in the samples. Five ml from the same extracted juice were used to measure TA by titrating with 0.1 N sodium hydroxide (NaOH) (Merck KGaA, Darmstadt, Germany) until the pH expressed as pH 8.3 which is the phenolphthalein endpoint. The pH was determined by using pH meter as high colour pigmentation in roselle is unable to show changes to pink colour indicated by phenolphthalein. The results were calculated as percentage of malic acid of the following formula:

$$\% \text{ malic acid} = \frac{\text{Vol. of NaOH} \times 0.1 \times 67 \times 50 \times 100}{5 \times 10 \times 1000}$$

where; 67 = Equivalent weight of malic acid, 50 = Total volume made up (ml), 5 = Extract juice sample (ml), 10 = Weight of sample (g), and 1000 = Mili conversion.

The experimental data were subjected to one- or two-way (Treatment × day) ANOVA using SAS release 9.2 (SAS Institute Inc., Cary, North Carolina, USA). LSD were calculated following a significant ($p \leq 0.05$) F-test. All the assumptions of ANOVA were checked to ensure validity of statistical analysis.

Results and Discussion

The preliminary study showed significant different soluble solids concentration due to different concentrations of both ABA and IAA treatments. Besides, different IAA treatments showed significant effect on fresh weight of roselle. Higher percentage of SSC in roselle indicates that higher sugar accumulation in fruits resulted in increase of fruit sweetness. In this study, 10^{-4} mol/l ABA treated roselle showed higher SSC as compared with 10^{-5}, 10^{-6} mol/l ABA treated roselle and other treatments. Besides, 10^{-4} mol/l IAA showed significantly higher fresh weight as

compared to control and 10^{-5} and 10^{-6} mol/l IAA. Higher fresh weight indicates higher water content in the 10^{-4} mol/l IAA treated roselle calyxes. Therefore, the storage life of 10^{-4} mol/l IAA treated roselle calyxes were longer compared to control and 10^{-5} and 10^{-6}mol/l IAA treated roselle.

There was a significant ($p \leq 0.001$) decrease in fresh weight in the control and treated roselles with increasing storage duration in both ambient and cold storage (Fig. 1A, B). However, ambient storage roselles showed greater weight loss as compared to cold storage roselles. Calyces treated with IAA treatment resulted in lower weight loss as compared to control on day 1 and 4 during 5 days of storage period at ambient temperature (Fig. 1A).

ABA treated roselles showed greater weight loss as compared to control on day 0 and 2 during storage period (0 to day 4) at ambient temperature (Fig. 1A) and day 0, 2, 3 and 6 during storage period (0 to day 8) at cold storage (Fig. 1B). The mean fresh weight of ABA treated roselles showed one-fold higher than control in ambient storage (Fig. 1A) while, the trend was reversed in cold storage roselles (Fig. 1B). Current study showed that weight loss was affected by storage temperature. Similarly, Mutari and Rees (2011) reported that weight loss was higher at 20°C than 12°C on tomato fruit. Temperatures higher than 20°C would increase respiration rate and resulted to water loss to the surrounding, hence, reduction in weight occurs (Thanh and Acedo 2006).

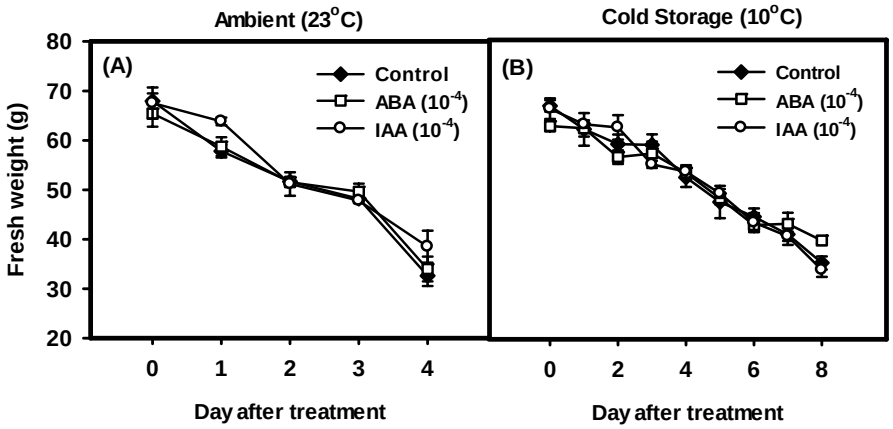

Fig. 1. Fresh weight of roselles stored in ambient (A) and cold storage (B) as influenced by ABA and IAA treatment and storage period. Vertical bar represents SE of means and are invisible when the values are smaller than the symbol.

There was significant decrease in chromaticity L*, a*, b* and C* value with increasing of storage duration in ambient temperature (Figs 2A, C, E, 3A) and cold storage roselle (Figs 2B, D, F, 3B). However, hue angle (h°) significantly ($p \leq 0.001$) increased in ambient (Fig. 3C) and cold storage (Fig. 3D) roselles.

IAA treatment exhibited significantly ($p \leq 0.05$) lower (1.03-fold) in mean of chromaticity L* as compared with control on roselles stored in cold storage (Fig. 2B). IAA and ABA treatments significantly ($p \leq 0.05$) enhance 1.10- and 1.09-folds in mean of hue angle (h°) as compared to control of cold storage roselles (Fig. 3D).

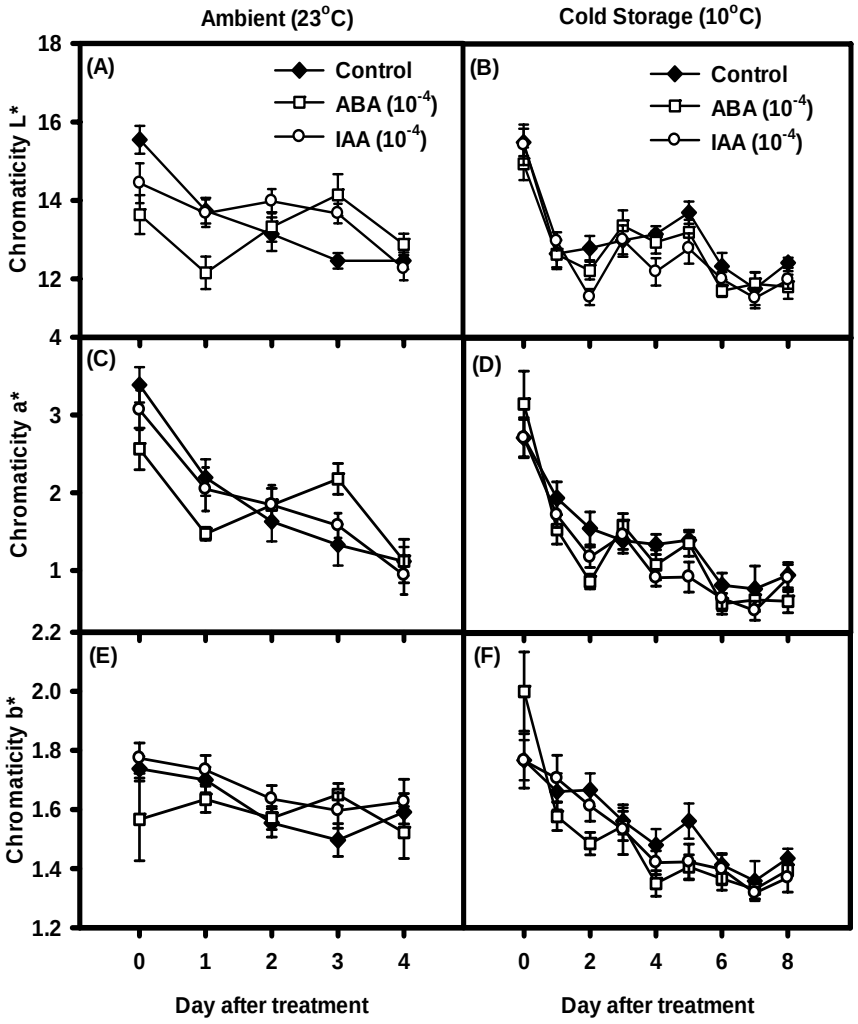

Fig. 2. Chromaticity L* (A and B), chromaticity a* (C and D) and chromaticity b* (E and F) as influenced by ABA and IAA treatment and storage period on roselles stored in ambient and cold storage. Vertical bar represents SE of means and are invisible when the values are smaller than the symbol.

IAA treatment reduce chromaticity L* and enhances hue angle (h°) of the roselle calyxes during storage at cold temperature (Figs 2B, 3D). This result indicated that calyxes colour of IAA treated roselles are darker, less reddish purple compared with control. This were due to IAA begin to enhance senescence and deterioration of cold storage roselle on day 6 after treatment. ABA do not result, to greater red colour development. This result contradict with Jiang and Joyce (2003) who reported that application of ABA on strawberry enhanced greater red colour development.

The firmness of calyxes was at the highest point in the range of 5.84 to 6.13 N at day 0 of experiment. The firmness significantly ($p \leq 0.001$) decreased with increasing of storage time in both ambient and cold storage roselles (Fig. 4). IAA treatment resulted decrease in fruit softening by 19.11% as compared to control during 5 days of storage period at ambient storage (Fig. 4A).

IAA also decreased of fruit softening by 3.88% as compared to control during 9 days of storage period at cold storage (Fig. 4B).

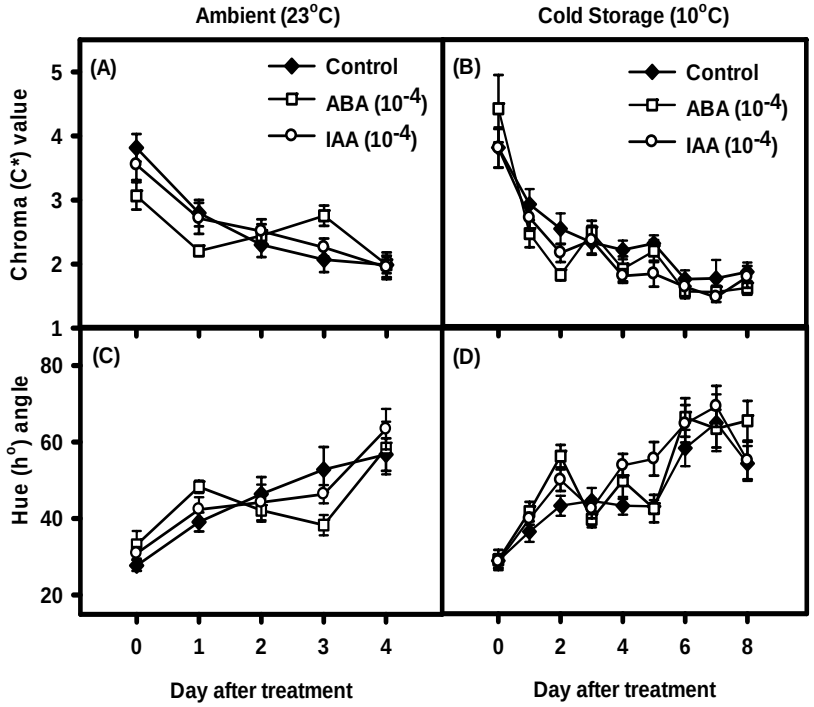

Fig. 3. Chroma value (C*) (A and B) and hue angle (h°) (C and D) as influenced by ABA and IAA treatment and storage period on roselles stored in ambient and cold storage. Vertical bar represents SE of means and are invisible when the values are smaller than the symbol.

The results showed that ABA enhance fruit softening while IAA delay fruit softening however, the differences were not significant. It is found in previous studies that ABA enhances fruit softening on mango (Zaharah *et al*. 2011), strawberry (Jiang and Joyce 2003) and banana (Jiang *et al*. 2000). The exposure to ABA might trigger fruits sensitivity toward ethylene that resulted to enhance senescence. The firmness of roselles decreased throughout the storage period due to ripening and senescence process. Increase in fruit softening also indicate that polymeric carbohydrate breakdown especially pectic substances and hemicelluloses weaken the cell walls and cohesive forces that bind the cell together during senescence. This happened because the rate of pectic substance degradation is directly proportional with the rate of fruit softening (Wills *et al*. 1998). IAA delayed softening from day 1 to day 3 after treatment while it starts to promote softening from day 4 after treatment in cold storage. Hence, the firmness of cold storage IAA treated roselles drastically decreases as compared to control and ABA treated roselles.

Soluble solid concentrations were significantly ($p \leq 0.001$) increased with increase of storage period (Fig. 5A, B). SSC of IAA treated roselles significantly ($p \leq 0.05$) increase 1.09-folds compared with control in cold storage roselles (Fig. 5B). IAA drastically increased SSC higher than ABA and control roselles on day 7 after treatment in cold storage. ABA treated roselles increase 1.02-folds of SSC than control roselles. However, the mean SSC of control and ABA

treated roselles were not significantly different. Fig. 5A showed that SSC of ABA treated roselles was higher than control and IAA treated roselles from day 0 and day 1 after treatment. Control roselles have 1.03- and 1.08-folds higher SSC than ABA treated roselles on day 3 and day 4 after treatment stored at ambient temperature. Mean SSC of control was 1.02- and 1.10-fold higher than ABA and IAA treated roselles in ambient storage. However, the difference between treatments were not significantly affecting SSC in roselles. The interaction between treatment and storage period significantly ($p \leq 0.01$) affected SSC of cold storage roselles but not in ambient storage roselles. The result showed that SSC of roselles significantly increased with the length of storage duration. It was a general tendency due to break down of complex carbohydrates and the soluble solids increase during ripening and senescence (Rab *et al*. 2010). The conversion of organic acids to sugars during fruit ripening and senescence increased the level of SSC of fruits (Wills *et al*. 1998). Besides, the SSC was drastically increased since day 3 and day 6 after treatment in ambient and cold storage roselles. The drastic increase in SSC might be due to low moisture content of fruit as percentage SSC was a function of total dissolved solids and moisture content of the fruit (Rab *et al*. 2010).

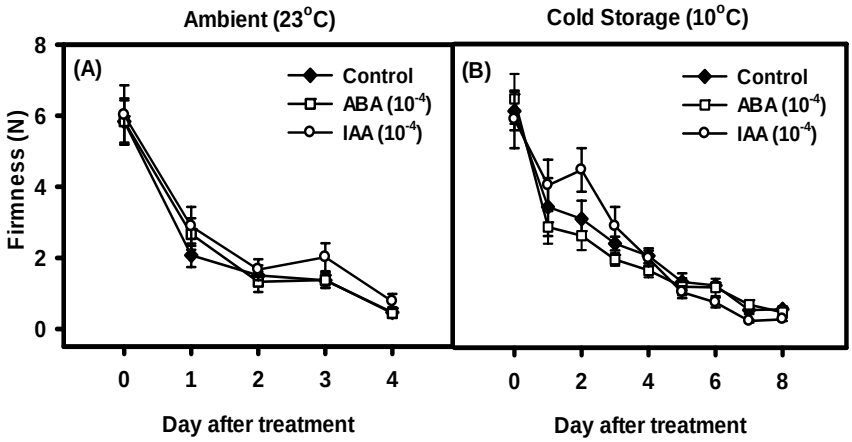

Fig. 4. Fruit firmness as influenced by ABA and IAA treatment and storage period on roselles stored in ambient (A) and cold storage (B). Vertical bar represents SE of means and are invisible when the values are smaller than the symbol.

Titratable acidity of day 4 after treatment were significantly ($p \leq 0.001$) increased 1.3-folds as compared to day 0 after treatment in ambient storage (Fig. 5C). Roselle calyxes kept under cold storage 9 days after treatment were significantly ($p \leq 0.001$) 2.47-folds higher TA as compared to day 0 (Fig. 5D). The TA in IAA treated roselles significantly ($p \leq 0.001$) increased 1.11-folds as compared to control. TA of IAA treated roselles drastically increased since day 6 after treatment (Fig. 5D).

ABA treatment increased the TA of cold storage roselles but the difference was not significant with the control. At ambient storage, ABA and IAA treated roselles showed lower TA value as compared with control from day 0 after treatment until day 3 after treatment (Fig. 5C). However, the mean of TA showed no significant difference between treatment in ambient roselles. The interaction between treatment and storage period significantly ($p \leq 0.01$) affected TA of cold storage roselles but not in ambient storage roselles. TA determination was served as a measurement of fruit acidity. The level in acidity was an important index of sour flavour. IAA treated roselles showed higher content of TA as compared to control. Similarly, tomato treated with different concentrations of IAA showed significant increase in TA (Olaiya *et al.* 2009). This

study showed that TA increased gradually with increase of storage period which contradicted with the result showed on orange kept at room temperature for 75 days (Rab *et al*. 2010). According to Wills *et al*. (1998), organic acids was required in operation of metabolic pathways. Therefore, commonly organic acids will decline during senescence due to respiration and conversion of organic acid to sugars resulted to decrease of TA value. However, the experiment result was in contrast with this statement. This might be due to other organic acids in roselles such as oxalic acid, tartaric acid and citric acid degraded and converted to sugars instead of malic acid which have been used to determine TA value in this study.

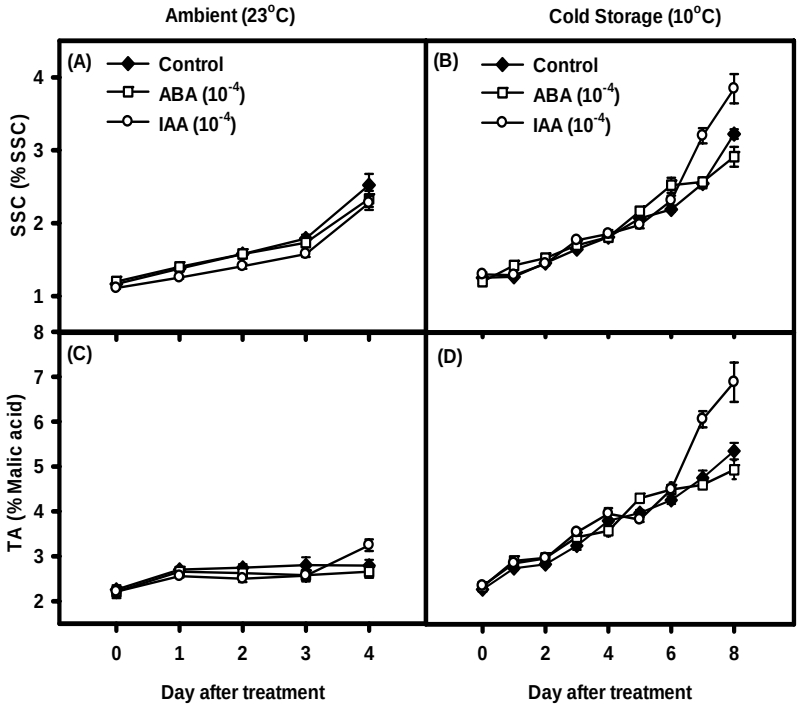

Fig. 5. Soluble solids concentration (SSC) (A and B) and titratable acidity (TA) (C and D) as influenced by ABA and IAA treatment and storage period on roselles stored in ambient and cold storage. Vertical bar represents SE of means and are invisible when the values are smaller than the symbol.

In conclusion, results showed ambient storage of roselles causes greater weight loss, colour deterioration and calyxes softening as compared to cold storage roselles. Cold storage of roselles decreased the rate of weight loss, colour deterioration and calyxes softening of roselles. Therefore, roselles were better to keep under cold storage because low temperature prolongs the shelf life of roselle about 4 days longer than ambient temperature storage. However, the application of ABA and IAA only showed minimal effect on quality and shelf life of roselle. Besides, results of preliminary study and experiment on fresh weight and SSC of 10^{-4} mol/l ABA and IAA treated roselle stored in ambient temperature are slightly different. It showed that 10^{-4} mol/l ABA and IAA were unstable in regulating the physiological processes of roselle calyxes during storage. Higher concentration of ABA and IAA and increased treatment duration might hasten or delay their quality changes and shelf life of roselle calyxes, respectively.

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References

Christian KR, Nair MG and Jackson JC 2006. Antioxidant and cyclooxygenase inhibitory activity of sorrel (*Hibiscus sabdariffa*). J. Food Compos. Anal. **19**: 778-783.

Duangmal K, Saicheua B and Sueeprasan S 2004. Roselle anthocyanins as a natural food colourant and improvement of its colour stability. Proceeding of AIC Color and Paints, Interim Meeting of the International Color Association. pp. 155-158.

Jiang Y and Joyce DC 2003. ABA effect on ethylene production, PAL activity, anthocyanin and phenolic contents of strawberry fruit. Journal Plant Growth Regulation **39**: 171-174.

Jiang Y, Joyce DC and Macnish AJ 2000. Effect of abscisic acid on banana fruit ripening in regulation to the role of ethylene. Journal Plant Growth Regulation **19**: 106-111.

Lin TL, Lin HH, Chen CC and Lin MC 2007. *Hibiscus sabdariffa* extract reduces serum cholesterol in men and women. Nutrition Research **27**: 140-145.

Maryani H and Kristiana L 2010. Khasiat dan manfaat roselle. Kuala Lumpur: Synergy Media.

McClintock NC and El Tahir IM 2011. *Hibiscus sabdariffa* L. [Internet] Record from PROTA4U. In *Hibiscus sabdariffa* L. [Internet] Record from PROTA4U., eds. M. Brink and E.G. Achigan-Dako, PROTA (Plant Resources of Tropical Africe). Wageningen, Netherlands. Retrieved 8 December 2011.

Mutari A and Rees D 2011. The effects of postharvest handling and storage temperature on the quality and shelf of tomato. African Journal of Food Science **5**: 446-452.

Norhaizan M, Fong SH, Amin I and Chew LY 2010. Antioxidant activity in different parts of roselle (*Hibiscus sabdariffa* L.) extracts and potential exploitation of the seeds. Food Chemistry **122**:1055-1060.

Olaiya CO, Soetan KO and Ogunkolade NS 2009. Evaluation of the biochemical effects of auxins on nutritional quality of tomato (*Solanum lycopersicon*), genotype JM 94/47. African Journal of Food Science **4**: 41-45.

Rab A, Haq S, Khalil SA and Ali SG 2010. Fruit quality and senescence related changes in sweet orange cultivar blood red uni-packed in different packing materials. Sarhad Journal of Agriculture **26**: 221-227.

Sun L, Zhang M, Ren J, Qi J, Zhang G and Leng P 2010. Reciprocity between abscisic acid and ethylene at the onset of berry ripening and after harvest. BMC Plant Biology **10**: 257.

Thanh CD and Acedo JAL 2006. Causes of quality losses and technological concerns for fresh and processed tomato and chilli. *In:* Causes of quality losses and technological concerns for fresh and processed tomato and chilli. RETA 6208 Postharvest Technology Training and Development of Training Master Plan 17-20 Oct. Lao PDR.

Wills R, McGlasson B, Graham D and Joyce DC 1998. An Introduction to the Physiology and Handling of Fruit, Vegetables and Ornamentals. Australia: UNSW Press.

Zaharah SS 2012. Unique Features. *In:* Nature's yield and wonders of art (Nyawa 12: Fruit), eds. Q.Z. Faridah, B. Nasir and M.T. Osman, 94-103. Serdang, Selangor: Universiti Putra Malaysia Press.

Zaharah SS, Singh Z, Symons GM and Reid JB 2011. Role of brassinosteroids, ethylene, abscisic acid, and indole-3-acetic acid in mango fruit ripening. Journal of Plant Growth Regulation **31**: 363-372.

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