International Food Research Journal 21(4): 1667-1674 (2014)
Journal homepage: *http://www.ifrj.upm.edu.my*

Browning assessment methods and polyphenol oxidase in UV-C irradiated Berangan banana fruit

*Phebe Ding and Yap Shi Ling

Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Article history

Received: 13 December 2013
Received in revised form: 29 January 2014
Accepted: 30 January 2014

Keywords

Browning index
Browning score
Colour
PPO
Ripening

Abstract

Ultraviolet (UV) light especially UV-C has been used to sterilize fruits and vegetables. However, overdose of UV-C irradiation could cause brownish-red colouration to products such as banana fruit. Therefore, the objectives of this study were to: (1) examine the effect of UV-C irradiation at different doses on the surface colour of Berangan banana fruit during ripening; (2) determine polyphenol oxidase (PPO) activity after irradiated with different doses of UV-C, and (3) examine the effectiveness of three browning assessment methods (subjective score, browning index derived from Lab colour space and optical density of 420 nm) in response to PPO activity of UV-C irradiated Berangan banana fruit. Mature green Berangan banana fruit were irradiated with 0, 0.01, 0.02, 0.03 and 0.04 kJ/m^2 UV-C. After irradiation, the fruit were initiated to ripening using 1 mL/L ethylene for 24 h. Then, the fruit were allowed to ripen in 27^{o}C and fruit of day 0, 1, 3 and 5 were sampled for peel colour (L^*, a^*, b^*, C^* and h^o), browning assessment (three methods) and PPO assay. The peel colour, browning assessment using subjective score and optical density, and PPO activity of Berangan banana fruit were affected significantly ($P \leq 0.05$) by interaction of radiation dose x ripening day. The values of L^*, b^*, C^* and h^o decrease while a^* values increase as fruit irradiated with 0.03 and 0.04 kJ/m^2 UV-C indicating brownish-red has occurred. Fruit irradiated with 0.04 kJ/m^2 UV-C discoloured by ripening day 3 while those irradiated with 0.03 kJ/m^2 discolored by day 5. Similar result was obtained when fruit assessed for its browning using subjective score and optical density. A contrary result was obtained in PPO activity where UV-C irradiation has inhibited Berangan banana fruit PPO activity by ripening day 5. Correlation analysis showed that browning index that derived from colour space is highly related to PPO activity with coefficients of 0.93. As conclusion, the lethal dose causing browning for Berangan banana fruit is 0.03 kJ/m^2 and browning index that derived from colour space is most effective to correlate browning with PPO activity.

Introduction

Ultraviolet (UV) light is a kind of electromagnetic spectrum ranges from 100 to 400 nm and UV-C which has wavelengths in the range 200 – 280 nm has long been used as germicide to treat drinking water since 1910 in Marseille (Hijnen *et al*., 2006). Later, UV-C was used as air disinfection and surface decontamination in hospitals, laboratories and food packaging area (Maharaj and Mohammed, 2012). Recently consumers demand for safe and environmental friendly products has urged postharvest scientists to look for green technologies to treat horticultural produce. Since then, literatures on UV-C as postharvest treatment have increased. There are a lot of studies have proven UV-C can maintain quality of fruits (D'hallewin *et al*., 2000; Gonzalez-Aguillar *et al*., 2001; Vicente *et al*., 2005). However, overdose of UV-C treatment enhance phenolic compound in tomato fruit (Liu *et al*., 2012). Oxidation of phenolic compound by polyphenol oxidase (PPO) causes browning (Waliszewski *et al*., 2007). Browning not only reduces the visual quality of fruits but also results in undesirable change in flavor and nutrition loss (Luo and Barbosa, 1997). When 'Dwarf Cavendish' banana were irradiated with 30 W UV-C from a distance of 45 cm for 40 min, brownish-red colouration was observed on the peel (Singh, 1972). A similar observation was also reported in 'Williams Cavendish' banana that irradiated with 1.2 W/m^2 UV-C from a distance of 18 cm for 10 min (Wade *et al*., 1993). However, both reports did not indicate lethal dose that cause banana browning. Information on lethal dose is necessary before application on a commercial scale.

Berangan banana fruit is the most popular dessert bananas in Malaysia. The fruit is medium to large size with an attractive orang-yellow peel and light orange pulp when ripe. It is most favorite dessert banana among local. The genome make up of Berangan and

*Corresponding author.
Email: *phebe@upm.edu.my*
Tel: +603 8947 4829
Fax : +603 8940 8445

Cavendish bananas is the same, that is AAA, but Berangan banana fruit is able to degreen at tropical temperature of 27°C (Ding *et al.*, 2007a). Similar to Cavendish banana fruit, Berangan banana fruit also encountered browning after UV-C irradiation. There are several methods that have been used to measure browning. Judging browning on fruit surface using naked eye is often being used where visible brown area was rated using a scale. This subjective method seems to utilize personal skill that can vary from person to person. Objective measurements using Lab colour space has been used to indicate browning of chopped Indian gooseberry (Ruangchakpet and Sajjaanantakul, 2007). Browning index derived from L^*, a^* and b^* values showed significant increase values concomitant with browning after Indian gooseberry placed at 25°C for 0, 4, 6 and 8 h. In addition, the reading of absorbance from spectrophotometer at optical density of 410 or 420 nm has also been used to assess browning in lychee (Jiang, 2000) and fresh cut wax apple (Supapvanich *et al*., 2011), respectively.

Several researchers have used one of these browning assessment methods to study its relationship with other browning parameters in order to correlate browning behavior of fruit. Scaling browning of fresh cut carambola using naked eye showed positive correlation with PPO activity (Ding *et al.*, 2007b). For lychee pericarp browning, peel browning index derived from browned area showed positive correlation with the absorbance at 410 nm extracted using fruit pericarp (Jiang, 2000). In Indian gooseberry browning index that derived from colour space showed high positive correlation with total phenolic (Ruangchakpet and Sajjaanantakul, 2007). Although there are several methods have been reported to assess fruits browning, no report had shown which method is the most reliable and efficient to determine browning.

Therefore this study was conducted with the aims to: (1) examine the effect of UV-C irradiation at different doses on the surface colour of Berangan banana fruit during ripening; (2) determine PPO activity after irradiated with different doses of UV-C, and (3) examine the effectiveness of three browning assessment methods (subjective score, browning index and optical density) in response to enzymatic browning of UV-C irradiated Berangan banana fruit.

Materials and Methods

Fruit sources and UV-C irradiation

The second and third hands of mature green Berangan banana fruit were purchased from Puchong Wholesale Market, Selangor, Malaysia. Fruits that free from any form of mechanical injury, insect and pathogen damages were selected for the experiments. After that, fruits were illuminated with ultra-violet C (UV-C) light (VER bright, 30 watt, 90 cm length) from a distance of 30 cm for 0, 30, 60, 90 and 120 s in an enclosed chamber. The radiation intensity of respective exposure time was equivalent to 0, 0.01, 0.02, 0.03 and 0.04 kJ/m^2 were then measured by a digital radiometer (UVC-254, Lutron light meter). After irradiation, the banana was initiated to ripening by using 5 mL/L of ethylene for 24 h at 27°C. After 24 h of ripening initiation, the fruits were placed in 27°C/75% relative humidity room for ripening process. Three banana fingers from each irradiation dose at ripening day 0 (before ripening initiation), 1, 3 and 5 (after ripening initiation) were selected randomly for analysis.

Determination of colour

Changes of the colour of banana fruit peel were measured using a Minolta CR- 400 Chroma Meter (Minolta Corp., Osaka, Japan). The values of L^*, a^*, b^*, C^* and h° were taken randomly at three position of a fruit. L^* value indicates lightness of the colour, which range from 0 (dark) to 100 (white). The positive value of a^* indicates red colour, while negative value of a^* indicates green colour. The positive value of b^* indicates yellow colour, while negative value of b^* indicates blue colour. The C^* values indicate the saturation of the colour. The ho values of 0°, 90°, 180° and 270° corresponding to the red, yellow, green and blue colour, respectively.

Determination of browning

The browning of Berangan banana fruit was assessed using three different methods. The first method of browning assessment was by visualizing the total brown area of each fruit surface using following scale: 1 = no browning, 2 = <20% of the peel surface, 3 = 20–40% of the peel surface, 4 = 40–60% of the peel surface and, 5 = >60% of the peel surface, according to Lichaporn *et al.* (2009) method. The second method of browning assessment was carried out by calculating formula as follow: Browning Index (BI) = [100 (x - 0.31)] / 0.17, where x = $(a^* + 1.75L^*) / (5.645L^* + a^* - 0.3012b^*)$, according to Ruangchakpet and Sajjaanantakul (2007). The third method of browning assessment was modified from Supapvanich *et al.* (2011) where 1 g of freeze dried banana peel was extracted using 20 mL of 65% (v/v) ethanol. The mixture was leave in a room of 27°C for 30 min. Then, the extract was filtered using cotton. Absorbance of extract was measured at 420 nm using a spectrophotometer (Biowave 2, Denville,

UK).

Samples extraction and analysis of PPO activity

After frozen in liquid nitrogen, the banana peel was then ground and stored in -20°C until analysis. PPO was extracted according to Ding *et al.* (2007b) with slight modification. Five gram of banana peel sample was homogenized in 50 mL cold 0.2 M sodium phosphate buffer of pH 6.8, 0.5% (v/v) Trition X-100 and 1.5% (w/v) insoluble polyvinylpyrolidone (molecular weight 44 000). The homogenate was centrifuged for 15 min at 12,000 g and 4°C using a refrigerated centrifuge (Avanti J-26 XPI, Beckman Coulter, US). The pellet was discarded and the supernatant that contained the enzyme extract was used to assay PPO activity.

The enzyme extract was assayed for PPO activity according to the modified procedure of Galeazzi *et al.* (1981) with catechol as substrate. 0.05 M catechol, 0.2 M phosphate buffer, pH 6.8 and enzyme extract were placed in a water bath to maintain a temperature at 30°C. After that, 1 mL of 0.05 M catechol, 2 mL of phosphate buffer (0.2 M, pH 6.8) and 0.5 mL of enzyme extract were mixed in a test tube by a vortex mixer (Grant-bio PV-1, UK) and immediately poured in a cuvette for PPO assay. PPO enzyme activity was determined by a spectrophotometer (Biowave 2, Denville, UK) as the increase in absorbance at 410 nm per 10 s. The initial linear portion of the activity curve was used to express the enzyme activity (U/min/s). One unit of PPO activity (U) was defined as the amount of the enzyme that increases the absorbance by 0.001/min under the conditions of the assay (Galeazzi *et al.*, 1981).

Statistical analysis

The experimental design was a randomized complete block design with a factorial arrangement of treatments (5 levels of radiation intensity and 4 days of ripening) and replicated thrice. The collected data was analyzed by means of analysis of variance and correlation. Log transformation was done for browning score by log(x+1), where x is the browning score of Berangan banana in the experiment. Separation of means was carried out using least significant difference (LSD) at $P \leq 0.05$. All statistical analysis was carried out using SAS 9.1.

Results and Discussion

Peel colour

There was highly significant difference at $P \leq 0.05$ between the interaction of radiation dose x ripening day on Berangan banana fruit peel colour (L^*, a^*, b^*, C^* and h^o values) (Table 1). At day 0 and 1, the average L^* values for all treatment is about 57 (Figure 1). As ripening progressed, the L^* values of fruit irradiated with 0 (control), 0.01, 0.02 and 0.03 kJ/m^2 UV-C was higher than 57 and achieved highest value by ripening day 5. In contrast, L^* values of fruit irradiated with 0.04 kJ/m^2 UV-C was lower than 57 when ripening progressed to day 3 and 5. Low L^* values indicated

Table 1. Effect of radiation dose and ripening day on L^*, a^*, b^*, C^* and h° values of Berangan banana fruit peel

Factor	L^*	a^*	b^*	C^*	h°
Radiation dose (R), kJ/m^2					
0	64.40 a[z]	-12.70 d	33.32 ab	36.45 a	113.35 a
0.01	65.41 a	-10.63 bc	34.65 a	36.38 a	111.70 a
0.02	62.97 b	-11.47 cd	32.58 b	35.63 a	111.32 a
0.03	59.27 c	-9.22 b	29.83 c	31.74 b	108.37 b
0.04	54.04 d	-7.26 a	24.00 d	26.53 c	103.66 c
Ripening day (D)					
0	57.17 c	-16.51 c	23.79 c	28.97 c	124.77 a
1	58.09 c	-16.21 c	24.87 c	29.96 c	124.20 a
3	63.24 b	-9.58 b	35.02 b	36.60 b	104.43 b
5	66.36 a	1.30 a	39.82 a	37.85 a	85.32 c
F value					
R	147.98**	12.47**	65.58**	83.51**	16.39**
D	162.53**	247.06**	280.54**	117.01**	497.42**
R x D	49.87**	6.20**	25.05**	32.96**	8.81**

n = 60

[z]Means within columns and factors followed by the same letter are not significantly different based on LSD at $P \leq 0.05$.

**Highly significant at $P \leq 0.05$.

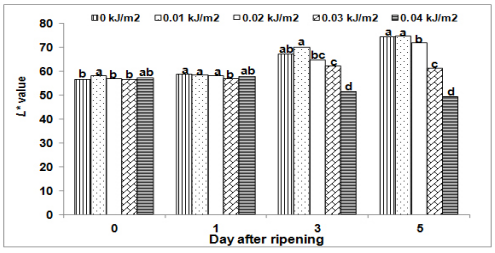

Figure 1. Effect of radiation dose and ripening day on L^* value of Berangan banana fruit peel. Different letters above bars in the same day indicate significant differences between means using LSD test ($P \leq 0.05$).

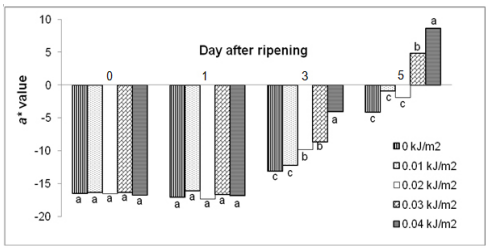

Figure 2. Effect of radiation dose and ripening day on a^* value of Berangan banana fruit peel. Different letters above bars in the same day indicate significant differences between means using LSD test ($P \leq 0.05$).

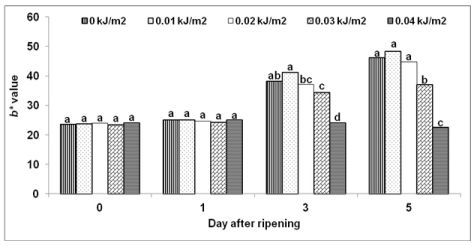

Figure 3. Effect of radiation dose and ripening day on b^* value of Berangan banana fruit peel. Different letters above bars in the same day indicate significant differences between means using LSD test ($P \leq 0.05$).

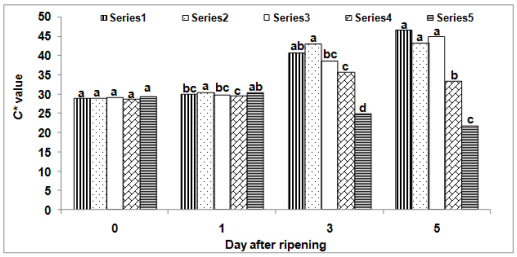

Figure 4. Effect of radiation dose and ripening day on C* value of Berangan banana fruit peel. Different letters above bars in the same day indicate significant differences between means using LSD test ($P \leq 0.05$)

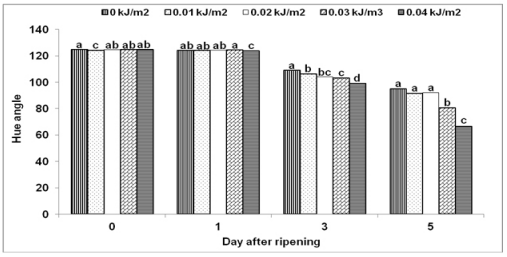

Figure 5. Effect of radiation dose and ripening day on h° value of Berangan banana fruit peel. Different letters above bars in the same day indicate significant differences between means using LSD test ($P \leq 0.05$).

0.04 kJ/m^2 UV-C has caused Berangan banana fruit lose its lightness when ripening took place.

Irrespective of treatment, a* values of Berangan banana fruit peel did not show any variation during first two days of ripening with average value of -16.5 (Figure 2). As fruit ripened to day 3, there was significant increase in a* values towards positive and this behavior was more pronounced in fruit irradiated with 0.02, 0.03 and 0.04 kJ/m^2 UV-C. By ripening day 5, fruit irradiated with 0.03 and 0.04 kJ/m^2 UV-C achieved positive a* values which reflected red colour. Similar to a* values, there was no significance difference in b* values of banana fruit peel among treatments at ripening day 0 and 1 (Figure 3). As ripening advanced to day 3 and 5, b* values of all fruits were higher than those in previous ripening days except fruit irradiated with 0.04 kJ/m^2 UV-C. The low b* values (less positive) of 0.04 kJ/m^2 UV-C irradiated fruit indicated it has less yellow as compared to other treatment of banana which has higher b* values (more positive).

At the beginning of ripening, there was not much of variation in peel C* values among Berangan banana fruit irradiated with different dose of UV-C (Figure 4). When fruit ripened to day 3, C* values of all banana fruits increased and higher than those at day 0 and 1 except for fruit irradiated with 0.04 kJ/m^2 UV-C which showed lower C* values. By day 5, there were no significant difference in C* values among 0, 0.01 and 0.02 kJ/m^2 UV-C irradiated fruit. Fruit irradiated with 0.03 and 0.04 kJ/m^2 UV-C showed significant lower C* value than other treatments. Again, 0.04 kJ/m^2 UV-C irradiated fruit has the lowest C* value among treatments and the value was lower than those at ripening day 0 and 1. Low C* value indicated less vivid in fruit colour.

As observed in other colour components, h° values of banana fruit peel at initial stage of ripening did not show much variation in values among treatment (Figure 5). When ripening progressed to day 3, a variation occurred among treatment where 0.04 kJ/m^2 irradiated fruit showed significant lowest h° values. By day 5, h° values of fruit irradiated with 0.03 and 0.04 kJ/m^2 UV-C were 80 and 67°, respectively, while the rest of banana was more than 90°. Hue angle of 90° indicates yellow colour in Rastali banana fruit peel (Tee *et al.*, 2012). The closer the h° value to 0° indicates redder colour. This show that high dose of UV-C has affected banana fruit peel colour during ripening.

Changes in colour parameters have been reported by several researchers to indicate fruit browning. Cavendish banana fruit irradiated with UV-C showed brownish-red pigment in epidermal and adjacent hypodermal cells (Wade *et al.*, 1993). This banana has lower L* and C* values, and higher a* values as compared to control. Gomez *et al.* (2010) reported that after fresh cut apple disc being irradiated with high dose of UV-C, browning appeared and lower L* values and higher a* values was obtained as compared to control. Monsalve-Gonzalez *et al.* (1993) also stated that an increase in a* values is an indicator for browning. Low values of b* has been associated with browning development in fresh cut nectarines (Bernado *et al.*, 2011) and mango fruits (Montero-Calderon *et al.*, 2008). Bernardo *et al.* (2011) also reported reduction in C* and h° values of fresh cut nectarines had the browning appearance on the fruits surface.

The decrease of L*, b*, C* and h° values with increase of a* values in Berangan banana fruit irradiated with 0.03 and 0.04 kJ/m^2 UV-C indicated that the peel has turned brownish-red. By comparing 0.03 and 0.04 kJ/m^2 UV-C irradiation, fruit treated with 0.04 kJ/m^2 UV-C has shown discolouration by day 3 while fruit irradiated with 0.03 kJ/m^2 UV-C discoloured by day 5. No browning was found after 24 h (ripening day 1) of irradiation in these two irradiation doses. This indicated appearance of browning did not occur immediately after irradiation and became prominent as ripening day advanced. Similar finding was also reported in UV-C irradiated grapes where browning became evident on the third day of storage at 22°C and progressed throughout storage (Gonzalez-Barrio and Salmenkallio-Marttila,

Table 2. Effect of radiation dose and ripening day on browning index, browning score, browning as determined using optical density at 420 nm (OD_{420}) and PPO activity of Berangan banana fruit

Factor	Browning score[z]	Browning index	OD_{420}	PPO activity (U/min/s)
Radiation dose (R), kJ/m^2				
0	0.00 c[y]	52.49 ab	0.135 d	0.48 c
0.01	0.00 c	56.74 a	0.142 d	0.49 bc
0.02	0.00 c	55.05 a	0.151 c	0.52 a
0.03	1.50 b	53.69 ab	0.164 b	0.49 bc
0.04	2.50 a	45.39 c	0.180 a	0.51 ab
Ripening day (D)				
0	0.00 b	27.15 c	0.110 d	0.18 d
1	0.00 b	29.91 c	0.136 c	0.22 c
3	1.60 a	63.44 b	0.172 b	0.72 b
5	1.60 a	87.51 a	0.198 a	0.89 a
F value				
R	1.63 x 10^{16}**	3.2*	34.17**	3.29*
D	1.88 x 10^{16}**	254.44**	210.17**	1812.81**
R x D	6.28 x 10^{15}**	1.18^{ns}	9.77**	9.39**

n = 60

[z]Data were log (x+1) transformed prior to analysis, non-transformed means are shown.

[y]Means within columns and factors followed by the same letter are not significantly different based on LSD at $P \leq 0.05$.

ns, *,**non significant or significant or highly significant at $P \leq 0.05$ respectively.

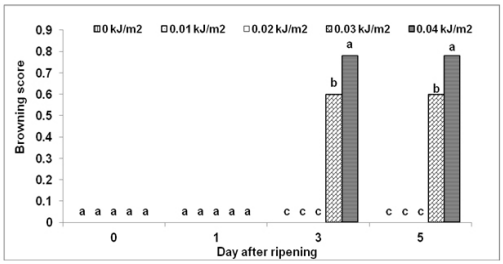

Figure 6. Effect of radiation dose and ripening day on browning score of Berangan banana fruit. Different letters above bars in the same day indicate significant differences between means using LSD test ($P \leq 0.05$).

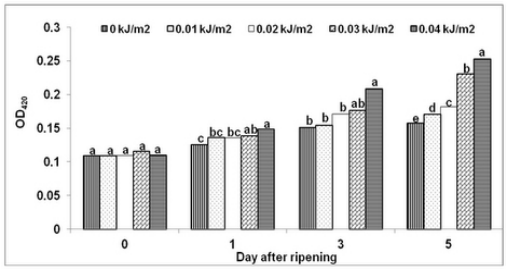

Figure 7. Effect of radiation dose and ripening day on browning browningas determined using optical density at 420 nm (OD_{420}) of Berangan banana fruit. Different letters above bars in the same day indicate significant differences between means using LSD test ($P \leq 0.05$).

2005).

The browning appeared earlier when Berangan banana fruit irradiated with higher dose of UV-C i.e. 0.04 kJ/m^2 in this study. The brownish-red colouration on the peel surface of Berangan banana fruit was believed due to oxidative browning reactions where PPO oxidizes phenolic compounds into melanins, a brown pigment. Tannins bodies can be found in the peel of Rastali banana fruit as early as first week after emergence of the first hand (Tee *et al*., 2011) and the concentration decreased as fruit matured (Tee and Ding, 2010). It has been reported that cells of mature Berangan banana fruit peel also contained tannin bodies (Ding *et al.*, 2007c). Tannin is water soluble phenolic compounds which contribute to astringency when interacting with salivary proteins and glycoproteins in banana (Tee and Ding, 2010). High dose of UV-C treatment could probably enhance tannin or triggered PPO activity in banana fruit peel earlier than low dose UV-C. This would accelerate substrate-enzyme contact and finally leads to earlier occurrence in tissue browning.

Browning assessment

There was highly significant difference at $P \leq 0.05$ in the interaction between radiation dose x ripening day on Berangan banana fruit peel for browning score that assessed subjectively (Table 2). At ripening day 0 and 1, no browning was found on banana peel in all treatments (Figure 6). As ripening advanced to day 3, browning became evident on fruit peel that irradiated with 0.03 and 0.04 kJ/m^2 UV-C. Fruit that irradiated with 0.04 kJ/m^2 UV-C has higher score than those irradiated with 0.03 kJ/m^2 UV-C. Fruit that irradiated with UV-C lower than 0.03 kJ/m^2 did not show any browning. This phenomenon continued until ripening day 5.

Browning index that derived from Lab colour space did not show any significant interaction between radiation dose x ripening day (Table 2). However, main effects of UV-C radiation dose and ripening days affected the browning index of Berangan banana fruit. There was no significant difference in browning index among radiation dose of 0, 0.01, 0.02 and 0.03 kJ/m^2. However, fruit treated with 0.04 kJ/m^2 of UV-C showed significant lower of browning index than others. This finding is contradicted to the subjective browning score and study by Ruangchakpet and Sajjaanantakul (2007) where browning index increase with browning. The inverse browning index could probably due to decreasing L^* values as irradiation dose increase which affect the calculation of browning index. Considering effect of ripening day, browning index of fruit increased tremendously especially when fruit ripened from day 1 to 3 by 112% (Table 2).

There was significant difference at $P \leq 0.05$ in the interaction between radiation dose x ripening day on Berangan banana fruit peel browning assessed using optical density of 420 nm (OD_{420}) (Table 2). At day 0, there was no significant difference in OD_{420} among fruit (Figure 7). After a day of ripening, fruit irradiated with 0.03 and 0.04 kJ/m^2 UV-C has significant higher OD_{420} than control. By ripening day 3, fruit irradiated with 0.04 kJ/m^2 UV-C showed significant higher OD_{420} than those irradiated with 0,

Table 3. Correlation between L^*, a^*, b^*, C^*, h^o, browning score (BS), browning index (BI), browning as determined using optical density at 420 nm (OD_{420}) and polyphenol oxidase activity (PPO) of Berangan banana fruit.

	L^*	a^*	b^*	C^*	h^o	BS	BI	OD_{420}	PPO
L^*	-								
a^*	0.18	-							
b^*	0.91**	0.45**	-						
C^*	0.95**	0.15	0.93**	-					
h^o	-0.24	-0.97**	-0.52**	-0.24	-				
BS	0.48**	0.66**	-0.37*	-0.37*	-0.65**	-			
BI	0.58**	0.84**	0.84**	0.67**	-0.88**	0.37*	-		
OD_{420}	0.01	0.87**	0.30*	0.037	-0.89**	0.78**	0.71**	-	
PPO	0.52**	0.83**	0.76**	0.57**	-0.90**	0.48**	0.93**	0.79**	-

*,** Significant or highly significant at $P \leq 0.05$.

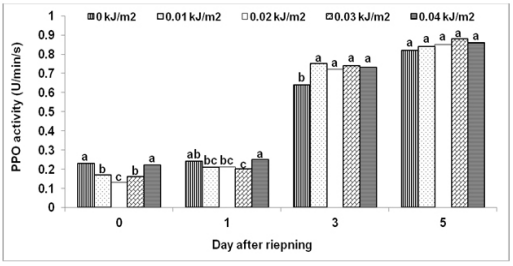

Figure 8. Effect of radiation dose and ripening day on PPO activity of Berangan banana fruit. Different letters above bars in the same day indicate significant differences between means using LSD test ($P \leq 0.05$).

0.01 and 0.02 kJ/m^2 UV-C. When fruits are palatable for consumption at ripening day 5, the OD_{420} among fruits differ significantly from each other with highest value found in 0.04 kJ/m^2 UV-C irradiated fruit. This followed by 0.03, 0.02, 0.01 and 0 kJ/m^2 UV-C irradiated fruit.

There was significant interaction between radiation dose x ripening day on PPO activity of Berangan banana fruit peel (Table 2). PPO activity of Berangan banana fruit was low at the beginning of ripening (Figure 8). When fruit ripened from day 1 to 3, a sudden increase in PPO activity occurred regardless of UV-C radiation dose applied. At this point, control showed significant lower PPO activity than UV-C treated fruit. When fruit ripened to day 5, no differences were observed among fruit, indicating UV-C irradiation inhibit PPO activity in Berangan banana fruit peel. In contrast, a study using UV-C irradiation to reduce Cavendish banana fruit chilling injury found that PPO activity in UV-C irradiated fruit was lower than control (Pongprasert *et al.*, 2011). Most probably the chilling temperature causes variation in PPO activity of these two banana varieties when irradiated with UV-C.

In the present study, the finding of PPO activity was not in accordance with browning occurrence as reported earlier. This indicated no apparent direct association between PPO activity and the observed browning development in irradiated fruit. Similar finding was also reported by Gonzalez-Barrio and Salmenkallio-Martitila (2005) in UV-C irradiated table grapes where development of browning was not associated with PPO activity. These researchers were then investigated phenolic composition (flavonols, hydroxycinnamic acids derivatives, flavan-3-ols, and total phenols) of control and UV-C irradiated grapes. They found out browning development in UV-C irradiated grapes also did not closely relate to changes in the phenolic composition. Although phenolic composition of Berangan banana fruit was not investigated in this study, UV-C irradiated Cavendish banana fruit has higher levels of total phenolic compounds in peel as compared to control (Pongprasert *et al.*, 2011). The high levels of total phenolic compounds in banana peel after UV-C irradiation could most probably explain browning in Berangan banana fruit even though PPO activity was not activated. However, cellular structure study is needed to understand banana browning better. This is because PPO enzyme has been reported to be located in internal thylakoid of chloroplast membranes (Nicolas *et al.*, 1994), while phenolic compounds are located mainly in the vacuole (Walker and Ferrar, 1998). The microscopic observations of UV-C irradiated fresh cut apple indicated breakage of cellular membranes (plasmalemma and tonoplast) (Gomez *et al*., 2010). The loss of functional cell compartmentalization increase enzyme-substrate contact which would then increase apple tissue browning.

Correlation analysis

The relationship between colour spaces, three different methods of browning assessment and PPO activity was investigated using SAS analysis. Although PPO activity was not triggered by UV-C irradiation, it is an important metabolic enzyme involves in banana fruit browning (Nguyen *et al*., 2003). The results given in Table 3 show that browning index that derived from colour space is highly related to PPO activity with coefficients of 0.93. Although there was positive correlation between PPO activity and browning assessment using subjective score (r = 0.48) and optical density (r = 0.79), the coefficients was weak as compared to browning index (r = 0.93). This indicated browning index that derived from Lab colour space is a more reliable assessment method in comparison to the other two methods.

Browning index of Berangan banana fruit decreased as UV-C dose increased (Table 2). This can be explained from Table 3 where L^* value showed weak correlation with browning index (r = 0.58) as compared to a^* (r = 0.84) and b^* (r = 0.84) values. Indeed h^o value showed a stronger correlation with browning index (r = -0.88) than a^* and b^* values. Among colour spaces, h^o value was strongly

correlated to PPO activity with coefficients of -0.90. This followed by a^* value which has coefficients of 0.83 with PPO activity. This phenomenon is expected as more positive a^* value and closer h^o value to zero indicate red colour. Both h^o and a^* values also showed strong relationship with browning assessed using optical density (OD_{420}) with correlation coefficient of -0.89 and 0.87, respectively. From these findings it seems h^o and a^* values are good indicator too to correlate banana browning.

Conclusion

The higher UV-C irradiation dose caused earlier occurrence of browning in Berangan banana fruit where 0.04 kJ/m^2 UV-C cause fruit browning by ripening day 3 while browning in 0.03 kJ/m^2 UV-C irradiated fruit appeared by ripening day 5. The browning became prominent as ripening day progressed. Thus, the lethal dose causing browning for Berangan banana fruit is 0.03 kJ/m^2. PPO activity of Berangan banana fruit was inhibited by UV-C however the activity increased as ripening day progressed. Among three browning assessment methods, browning index that derived from colour space is most effective to correlate with PPO activity. However, in order to obtain browning index, one must have colourimeter which is an expensive device. Therefore, an alternative method is measuring optical density at 420 nm. The least efficient method to correlate browning with PPO activity is subjective browning score. Nonetheless, it is easiest and cheapest methods compared to the other two methods.

Acknowledgements

The authors gratefully acknowledge the research facilities in Universiti Putra Malaysia.

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