Quality and Biochemical Changes of ‘Grand Nain’ Bananas during Shelf Life

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Abstract. Quality attributes, antioxidant compounds, free radical scavenging capacity (FRSC, DPPH IC₅₀ value) and enzymes activities of ‘Grand Nain’ bananas were investigated during shelf life at 20±2°C and 60-70% RH for 7 days. Weight loss, peel color and browning indices increased while, pulp firmness, titratable acidity (TA), pH and membrane stability index (MSI) decreased during shelf life. Total soluble solid (TSS) concentration increased up to day five then remained stable whereas, vitamin C increased ranged from 5.8 to 8.2 mg 100g⁻¹ fresh weight (FW) during the first two days and then decreased. Total phenols concentration in both peel and pulp increased ranged from 0.19 to 1.64 gkg⁻¹ and 0.17 to 0.59 gkg⁻¹ FW, respectively during shelf life. Total flavonoids firstly increased, remained stable until day five then, decreased in both peel and pulp. It ranged from 0.05 to 0.21 gkg⁻¹ FW and 0.03 to 0.19 gkg⁻¹ FW in peel and pulp, respectively. Both total phenols and flavonoids concentrations were higher in peel than pulp. FRSC in peel remained stable until day five then, sharply decreased (higher DPPH IC₅₀ values) and ranged from 3.83 to 19.43 IC₅₀ values. While, in pulp it decreased (higher DPPH IC₅₀ values) during shelf life and ranged from 4.1 to 13.3 IC₅₀ values. Polyphenoloxidase (PPO), peroxidase (POD), polygalacturonase (PG), xylanase and α -amylase activities in peel and pulp greatly varied during shelf life.

Keywords. Banana, Quality, Shelf life, Antioxidants, Antioxidants Capacity, Phenols, Enzymes.

1. Introduction

Bananas (*Musa* spp.) are among few fresh fruit which are available to the consumers throughout the year worldwide (Pereira and Maraschin, 2015). Bananas are considered as functional fruit due to their high nutritional and healthiness properties. It comprises nearly all of the essential nutrients including oligosaccharides, minerals and health beneficial bioactive compounds such as polyphenols (phenolic acid, flavonols, flavanones, flavanols and flavanols) and vitamins (such as vitamin C, A and E) that contribute to their high antioxidant activity

(Ummarat *et al.*, 2011; Pereira and Maraschin, 2015). Generally, bananas are harvested at mature hard green stage and thereafter ripening is triggered, in most cultivars, by exposing to ethylene at a certain dose and duration. However, the climacteric nature of bananas leads to rapid ripening process and a relatively shorter shelf life at ambient conditions following ethylene treatment (Zhang *et al.*, 2010). During ripening, several physical and biochemical changes occurs that include an increase in weight loss, peel chlorophyll degradation and browning, and sugar content of pulp while, others decreased such as pulp firmness and TA (Waliszewski *et*

al., 2007; Baez-Sanudo *et al.*, 2009; Maqbool *et al.*, 2011; Fernando *et al.*, 2014; Wang *et al.*, 2014). Antioxidant compounds such as phenols, flavonoids and vitamin C which largely contributes to the antioxidant activity of fruit varies upon banana cultivar and ripening stage (Kondo *et al.*, 2005; Fernando *et al.*, 2014). Youryon and Supapvanich (2016) reported that antioxidant compounds of 'Kluai Khai' bananas increased during storage at 13 °C whilst, that of 'Kluai Nam Wa' decreased. Most of biochemical changes in bananas during shelf life were associated with the hydrolytic enzymes PG and xylanase (Medina-Suárez *et al.*, 1997; Srivastava and Dwivedi, 2000). However, peel browning during shelf life was accompanied with the antioxidant enzymes POD and PPO (Zhang *et al.*, 2010; Huang *et al.*, 2013). Despite, bananas peel is not edible, it is considered as a natural source for antioxidants (Someya *et al.*, 2002; Pereira and Maraschin, 2015) and frequently have been used as medicine, cattle feeds, blacking of leathers, fillers in rubber (Arawande and Komolafe, 2010). Furthermore, the biochemical changes in peel reflect the physiological and ripening status of fruit. However, most of available literatures describe physico-chemical changes of bananas pulp during ripening, but relatively little is known about such changes in peel. The aim of the present study was, therefore, to characterize the physico-chemical changes during shelf life in both peel and pulp of 'Grand Nain' bananas growing in Saudi Arabia. In this study, the changes in regular quality attributes, antioxidant compounds, antioxidants capacity, and hydrolytic and oxidative enzymes activities were investigated.

2. Materials and Methods

2.1 Plant Materials and Experimental Procedure

This experiment was performed on bananas (Musa AAA group, cv. 'Grand Nain')

collected from a private farm located in Jizan, Saudi Arabia. Fruits were harvested, packed as hands in polyethylene film in perforated cardbox (about 30 kg) and transported from Jizan to Jeddah within 12 h at 15 oC. Bananas (at the ripening stage 1, according to a color chart index) were directly pre-treated with ethylene gas (about 0.01% by volume in air) at 18 oC and 85% RH for 24 h for ripening induction at a commercial airtight ground warehouses with a great deal of bananas. Such practice is critical for ripening induction of 'Grand Nain' banana cultivar. Then, uniform hands were randomly selected at the warehouse and rapidly transported to the postharvest physiology laboratory of King Abdulaziz University in Jeddah. Bananas (at the ripening stage 2) carefully prepared in small uniform hands (about 5 fingers each, free of visual defects and with similar weight and size) were selected. A completely randomized design with three replicates (six hands each)/time was used. All replicates were weighted and stored at 20±2 oC and 60–70% (RH) in perforated cardboard cartons. At the beginning of shelf life, additional three samples (10 fingers of each) were randomly collected for initial quality and biochemical analyses as described below. After 2, 5 and 7 days of shelf life, weight loss and peel color stage were recorded for each replicate as described below. After 2, 5 and 7 days, samples (10 fingers) per replicate were randomly taken for quality and biochemical analyses. Then, these fruit samples were peeled and the peel tissue was sliced and mixed. Random portion of this peel was used for electrolyte leakage measurement and the remaining peel was kept at –80 oC for later enzyme, total flavonoids and phenols and antioxidant activity analysis. Pulp firmness was measured in each sample directly following peeling. The pulp tissue sliced and mixed and a random portion of it was used for TSS, TA, pH, and vitamin C determinations.

While, the other portion was kept at -80°C for later enzyme, total phenols and flavonoids and antioxidant activity analysis.

2.2 Weight loss Determination

The total fruit weight loss was calculated on initial weight basis and expressed in percentage.

2.3 Peel Color Change Estimation

2.3.1 Color chart index

Peel color stage was visually recorded for each sample (10 individual fingers of each) using a banana color chart index (scale 1 to 7, refers to dark green to full yellow color) (Soltani *et al.* 2010).

2.3.2 Minolta Chroma meter index

Ten bananas (from each replicate) from each shelf life period (0, 2, 5, and 7 d) were randomly selected for color measurement of banana peel using a Minolta Chroma Meter CR-410 (Minolta Camera Co. Ltd., Osaka, Japan). The colorimeter was warmed up for 20 min and calibrated on the Hunter lab color space system using a standard white plate (Minolta calibration plate, $Y=84.8$, $x=0.3164$, $y=0.3237$). The values of L^* , a^* and b^* were measured in the middle of each of the ten fruit/sample. L^* value indicates lightness of the color, which range from 0 (dark) to 100 (white). The positive value of a^* indicates red color, while negative value of a^* indicates green color. The positive value of b^* indicates yellow color, while negative value of b^* indicates blue color. $\text{Chroma} = (a^{*2} + b^{*2})^{1/2}$ which represented the hypotenuse of a right triangle with values ranging from 0 = least intense to 60 = most intense according to Baez-Sanudo *et al.* (2009). The chroma values indicate the saturation of the color.

2.3.3 Browning index

Peel browning of fruit was assessed 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 (Ding and Ling, 2014).

2.4 Firmness, TSS, TA, pH and Vitamin C Determinations of Fruit Pulp

Fruit pulp firmness was measured independently in 10 fingers (in the middle of each finger) per replicate by a digital basic force gauge, model BFG 50N (Mecmesin, Sterling, Virginia, USA) supplemented with a probe of 11 mm diameter and the results were expressed as Newton. A homogeneous sample was prepared from these 10 fingers per replicate for measuring TSS, TA, pH and vitamin C. TSS concentration was measured as a percentage in fruit pulp juice with a digital refractometer (Pocket Refractometer PAL 3, ATAGO, Japan). TA was measured in pulp juice diluted in water at a ratio 1 : 2 by titrating with 0.1N sodium hydroxide up to pH 8.2, by automatic titrator (HI 902, HANNA Instrument, USA) and expressed as malic acid percentage. Fruit juice pH was measured by a pH meter (WTW 82382, Weilheim, Germany). Vitamin C was determined by the oxidation of ascorbic acid with 2,6-dichlorophenol endophenol dye and the results expressed as mg 100g⁻¹ on a fresh weight (FW) basis.

2.5 Leakage of Ions From Fruit Peel

Leakage of ions from peel disks was measured according to Sairam *et al.* (1997) with some modifications and was expressed as membrane stability index percentage (MSI %). Three grams of peel disks per replicate was randomly taken and placed in 30 ml of deionized water at ambient temperature for 4 h in a shaker. Conductivity before boiling (C1) was measured with an electrical conductivity digital meter (Orion 150A+, Thermo Electron Corporation, USA). The same disks were kept in a boiling water bath (100°C) for 30 min to

release all electrolytes, cooled to $22 \pm 2^\circ\text{C}$ with running water, and conductivity after boiling was recorded (C2). MSI was expressed in percentage using the formula: $[1 - (C1/C2)] \times 100$.

2.6 Methanol Extract Preparation

Two grams (randomly collected from 10 fingers/replicate) each from peel and pulp were extracted by shaking at 150 rpm for 12 h with 20 ml methanol (80%) and filtered through filter paper No. 1. The filtrate designated as methanol extract that will be used for total phenols, total flavonoids and antioxidant activity estimations.

2.7 Total phenols Estimation

Total phenols concentration was estimated according to Hoff and Singleton (1977). Fifty μl of the methanol extract was mixed with 100 μl Folin-Ciocalteu reagent, 850 μl of methanol and allowed to stand for 5 min at ambient temperature. A 500 μl of 20% sodium carbonate was added and allowed to react for 30 min. Absorbance was measured at 750 nm. Total phenols was quantified from a calibration curve obtained by measuring the absorbance of known concentrations of gallic acid and the results expressed as g Kg⁻¹ FW gallic acid equivalent.

2.8 Total Flavonoids Estimation

Total flavonoids level was estimated by a colorimetric method as described by Zhishen *et al.* (1999). Methanol extract or standard solution (250 μl) was mixed with distilled water (1.25 ml) and 5 % NaNO₂ solution (75 μl). After standing for 6 min, the mixture was combined with 10% AlCl₃ solution (150 μl), 1 M NaOH (0.5 ml) and distilled water (275 μl) were added to the mixture 5 min later. The absorbance of the solutions at 510 nm was then measured. Total flavonoids was quantified from a calibration curve obtained by measuring the absorbance of known

concentrations of catechin and the results expressed as g Kg⁻¹ FW catechin equivalent.

2.9 DPPH Radical Scavenging Assay

Free radical scavenging activity of methanol extract was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method as described in Awad *et al.* (2017).

2.10 Enzymes Measurements of Peel and Pulp

2.10.1 Crude extract

Two grams (randomly collected from 10 fingers/replicate) each from peel and pulp were homogenized with 20 mM Tris-HCl buffer, pH 7.2. The homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant (crude extract) was stored at -20 °C for POD, PPO, PG, xylanase and α -amylase assay.

2.10.2 POD assay

The activity of POD (EC 1.11.1.7) was measured as described by Miranda *et al.* (1995). The reaction mixture containing in one ml: 0.008 ml of 0.97 M H₂O₂, 0.08 ml of 0.5 M guaiacol, 0.25 ml of 0.2 M sodium acetate buffer, pH 5.5 and least amount of enzyme preparation. The change in absorbance at 470 nm due to guaiacol oxidation was followed for 1 min using a spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme which increases the O.D. 1.0 per min under standard assay conditions.

2.10.3 PPO assay

PPO (EC 1.14.18.1) activity was measured with catechol as a substrate according to the spectrophotometric procedure of Jiang *et al.* (2002). The extract (0.2 ml) was rapidly added to 2.8 ml of 20 mM catechol solution prepared in 0.01 M sodium phosphate buffer (pH 6.8). The increase in absorbance at 400 nm was recorded for 3 min using a spectrophotometer. One unit of enzyme

activity was defined as the amount of the enzyme that causes a change of 0.1 in absorbance per min.

2.10.4 PG, α -amylase and xylanase assays

PG (EC 3.2.1.15), α -amylase (EC 3.2.1.1) and xylanase (EC 3.2.1.8) activities were assayed by determining the liberated reducing end products using galacturonic acid, maltose and xylose, respectively as standards (Miller, 1959). The reaction mixture (0.5 ml) containing 5 mg substrate, 0.25 ml of 0.2 M sodium acetate buffer pH 5.5 and a suitable amount of crude extract. Assays were carried out at 37°C for 1 h. Then 0.5 ml dinitrosalicylic acid reagent was added to each tube and heated in a boiling water bath for 10 min. After cooling to room temperature, the absorbance was measured at 560 nm. Substrates used were polygalacturonic acid, starch and xylane for PG, α -amylase and xylanase, respectively. One unit of enzyme activity was defined as the amount of enzyme which liberated 1 μ M of reducing sugar per min under standard assay conditions.

2.11 Statistical Analysis

The data were statistically analyzed as a completely randomized design with three replicates by analysis of variance (ANOVA) using the statistical package software SAS (SAS Institute Inc., 2000, Cary, NC., USA). Comparisons between means were made by the Duncan's multiple range test at $P \leq 5\%$.

3. Results

Fruit weight loss gradually increased during shelf life reaching 7.8% at the end of shelf life (Fig. 1A). Peel color (estimated by color chart) gradually and significantly increased during shelf life (Fig. 1B). Browning index gradually increased until day five and then sharply increased to a value of 3.8 at the end of shelf life (Fig. 1C). Membrane stability index (MSI) of peel tissues gradually

decreased during fruit ripening (Fig. 1D). L^* , b^* and chroma values showed a highest level at day two, but progressively decreased to a lowest level at the end of shelf life (Fig. 2). While, a^* value showed linear increase throughout the shelf life period. Pulp firmness sharply decreased (from 14.5 to 6.3 N) during the first two days then slightly decreased to a lowest level (4.1 N) at the end of shelf life (Fig. 3A). TSS concentration sharply increased until day five reaching a maximum value of 16.4% and remained relatively stable afterwards (Fig. 3B). TA concentration was significantly lower after five and seven days of shelf life than initial (Fig. 3C). TSS/acid ratio sharply increased from initial up to day five (from 15.7 to 63.8) and then slightly increased to a maximum value (66.0) at the end of shelf life (Fig. 3D). pH was significantly lower after 5 and 7 days of shelf life than initial (Fig. 3E). Total phenols concentration in the peel slightly increased during the first five days of shelf life followed by a sharp increase thereafter reaching 1.64 g Kg⁻¹ while, it gradually increased in the pulp during ripening reaching 0.59 g Kg⁻¹ (Fig. 4A). However, in both peel and pulp, total flavonoids concentration sharply increased during the first two days, remained relatively stable until day five, and then rapidly decreased at the end of shelf life reaching 0.13 and 0.03 g Kg⁻¹ for peel and pulp, respectively (Fig. 4 B). Both total phenols and flavonoids concentrations were higher in peel than pulp (Fig. 4A and B). Vitamin C concentration increased to a peak (8.2 mg 100g⁻¹ FW) during the first two days of shelf life and then sharply decreased thereafter to a level similar to initial (Fig. 4C). FRSC (IC₅₀ values) in both peel and pulp ranged from 3.8 to 19.4 and 4.1 to 13.3 μ g phenolics concentration respectively during shelf life (Fig. 4D). FRSC in peel remained relatively stable until day five and then sharply decreased (higher IC₅₀ value), while in pulp, FRSC slightly decreased (higher IC₅₀ value)

from initial to day five and then sharply decreased (higher IC50 value) afterwards. PPO activity in peel sharply increased during the first two days to a peak and then sharply decreased thereafter to a level that was significantly higher than initial. While, in pulp, PPO activity sharply increased during the first two days to a peak, decreased at day five and then increased at the end of shelf life (Fig. 5A). POD activity in peel gradually increased from initial to day five and then decreased to a level similar to initial. While, in pulp, POD activity rapidly decreased during the first two days, slightly increased at day five followed by a sharp increase at the end of shelf life (Fig. 5B). PG activity in peel sharply decreased during the first two days and then increased afterwards to a level similar to initial. While, in pulp, PG activity remained stable during the first two days followed by a gradual increase from day two to the end of shelf life (Fig. 5C). Xylanase activity in peel increased during the first two days and remained stable thereafter during shelf life. While, in pulp, xylanase activity increased to a peak at day two and then decreased to a level that was higher than initial (Fig. 5D). α -amylase activity in both peel and pulp sharply increased during the first two days of shelf life to a peak and then rapidly decreased afterwards to a level similar to initial (Fig. 5E).

4. Discussion

The gradual increase in weight loss of 'Grand Nain' banana during shelf life (Fig. 1) is attributed mainly to loss of water via peel by transpiration process and also to respiration (Baez-Sanudo *et al.*, 2009; Maqbool *et al.*, 2011). Also, the decrease in peel tissue integrity as reflected by lower MSI values during ripening (Fig. 1D) possibly allow more water loss. Similar results were reported by others (Baez-Sanudo *et al.*, 2009; Maqbool *et al.*, 2011) who reported a gradual increase in weight loss during ripening of different banana

cultivars. A shift from green to yellow color reflects the advances of banana ripening during shelf life (Li *et al.*, 1997). Such change is mainly due to chlorophyll degradation by the action of chlorophyllase, as well as to the accumulation of carotene and xanthophylls (Salvador *et al.*, 2007; Zhang *et al.*, 2010; Pereira and Maraschin, 2015). The observed gradual decrease in membrane stability index of peel tissues during shelf life (Fig. 1D) confirm those of Wang *et al.* (2014) on 'Brazil' and Mirshekari *et al.* (2015) on 'Berangan' bananas. Electrolyte leakage is often used as an indicator of plasma membrane rupture, as ions leak out from the peel cells (Kamdee *et al.*, 2009). The increase in minolta L*, b* and chroma values during the first two days and the decrease thereafter is possibly due to browning development in peel after two days of shelf life (Fig. 2). In confirmation, Baez-Sanudo *et al.* (2009) showed that chroma value increased during the first four days of shelf life then gradually decreased afterwards. The decrease in L*, b* and chroma values during shelf life (Fig. 2) indicate a reduction in lightness, yellowness and vivid in color of banana peel, respectively. While, the gradual increase of a* value during shelf life (Fig. 2) might be due to the chlorophyll degradation which lead to decrease of greenness and/or increasing redness (Chen and Ramaswamy, 2002; Gomes *et al.*, 2013; Ding and Ling, 2014). Peel browning, that reduces visual quality, flavor and nutrition value of fruit, is mainly due to oxidation of various orthodiphenols by PPO to the corresponding o-quinones that polymerize non-enzymatically to form the brown melanin pigment (Waliszewski *et al.*, 2007). Interestingly, PPO activity reached a maximum level at 2 days of shelf life (Fig. 5A) coincident with the beginning of browning development (Fig. 1C), confirming the involvement of PPO in this process. The observed decrease in pulp firmness is possibly due to an increase in the hydrolysis enzymes

PG, xylanase and α -amylase activities, which are triggered by ethylene during shelf life (Fig. 3A, 5C and D) (Medina-Suárez *et al.*, 1997; Shiga *et al.*, 2011). Similar results were reported by Imsabai *et al.* (2006) and Youryon and Supapvanich (2016) who found a significant decrease in firmness of 'Kluai Nam Wa' and 'Kluai Khai' bananas during storage at ambient conditions. The increase in TSS concentration is a typical characteristic of banana ripening due to starch conversion into sugar. The increase in TSS concentration during the first 5 days of shelf life (Fig. 3B) might be due to the increase in the activity of the hydrolytic enzymes, especially α -amylase that convert starch into sugar (Fig. 5 C, D and E). In confirmation, Fernando *et al.* (2014) reported that TSS concentration in 'Khai' banana increased during the first eight days then decreased while, in 'Hom Thong' banana it increased during the first 4 days then decreased up to ten days of ripening at 25 °C. The decrease in TA after five and seven days of shelf life compared to initial during shelf life (Fig. 3C) confirm those of Maqbool *et al.* (2011), who reported a gradual decreased in TA in 'Pisang Berangan' bananas during storage at 13 oC for 28 days followed by five days at 25 oC. In the current study, the pattern of vitamin C concentration changes (Fig. 4C) is in agreement with those of Fernando *et al.* (2014) who reported that vitamin C concentration of 'Hom Thong' and 'Khai' bananas slightly increased during shelf life and decreased at the start of senescence. In the present experiment, the increase in total phenols level during shelf life in both peel and pulp of 'Grand Nain' bananas (Fig. 4A) partially confirmation those of Fernando *et al.* (2014) who found that total phenols concentration in 'Khai' banana pulp increased from day two to day eight, but decreased at senescence stage. However, they noted in 'Hom Thong' bananas that total phenols concentration decreased after two days at the

onset of ripening, remained rather stable until day eight, and then decreased at senescence stage. Newilah *et al.* (2010) reported similar results in hybrid banana cultivars in which total phenols concentration increased during ripening before decreasing at the full ripe stage. Also, total phenols concentration in peel of 'Brazil' bananas slightly increased during the first ten days of cold storage at 7 oC and then decreased afterwards (Wang *et al.*, 2014). The higher level of both phenolics and flavonoids in peel than pulp of 'Grand Nain' bananas (Fig. 4 A and B) confirm those of Someya *et al.* (2002) and Pongprasert *et al.* (2011) in Cavendish bananas. The higher flavonoids concentration in peel than pulp (Fig. 4B) is in agreement with those of Tsama *et al.* (2016) who found that flavonol glycosides was higher in peel than pulp of nine plantain cultivars. The observed increase in total flavonoids both in peel and pulp confirm those of Ummarat *et al.* (2011) who recorded an increase in total flavonoids in 'Kluai Hom Thong' bananas during ripening at 25 oC for ten days. The observed relative stability of FRSC in both peel and pulp during the first 2 to 5 days of shelf life followed by a sharp decrease (higher IC₅₀ values) at full ripe stage partly confirm those of Fernando *et al.* (2014) who found that, in 'Hom Thong' banana pulp, DPPH activity (mmol TE/100g FW) slightly increased during ripening while, FRAP activity more than doubled after 4 days of storage and then decreased during ripening at 25°C for ten days. However, Kondo *et al.* (2005) found that IC₅₀ values of superoxide (O₂⁻) and DPPH of 'Namwa' banana peel stored at 6 and 12 oC increased (lower IC₅₀ values) during the first two days and then gradually decreased (higher IC₅₀ values) during the following eight days of storage. The decrease in FRSC with the increase in antioxidant compounds (total phenols and flavonoids and vitamin C) concentrations during shelf life might suggest qualitative

changes in phenolic classes toward lower antioxidant potential. Fernando *et al.* (2014) found no significant correlation between the total phenols concentration and antioxidant activity measured by DPPH and FRAP, except for vitamin C concentration and FRAP in 'Khai' banana pulp. However, Sulaiman *et al.* (2011) obtained only minor to moderate correlation between phenolic concentration and antioxidant activities in nine Malaysian banana cultivars. These results imply that antioxidant compounds other than phenolics and vitamin C might also be involved. In this regards, carotenoids and xanthophylls have been detected in banana and may contribute to the total antioxidant activity (Kondo *et al.*, 2005). Also, it was reported that the antioxidant capacity of phenolics possibly has a concentration saturation limit above which the activity could not increase further with the concentration (Dani *et al.*, 2012). Moreover, differences in sensitivity/potential among individual antioxidant compounds such as phenols, carotenoids and vitamins toward a specific antioxidant assay have been reported (Ciz *et al.*, 2010; Alrashdi *et al.*, 2017). Thus, parallel different assays should be applied to investigate the principles of antioxidant/oxidation activity of a certain horticultural commodity. In both peel and pulp, PPO activity sharply increased to a peak during the first two days and then decreased thereafter during shelf life with fluctuations, especially in pulp, but remained at a higher level than initial (Fig. 5A). These results confirm those of Huang *et al.* (2013) on 'Brazil' and Ding and Ling (2014) on 'Berangan' bananas peel during ripening at ambient conditions as well as those of Nguyen *et al.* (2003) on 'Kluai Khai' and 'Kluai Hom Thong' bananas peel during storage at 6 and 10 °C. POD is an antioxidant enzyme that plays a role in controlling the level of reactive oxygen species in plant cells. The increase in POD activity in peel up to day five and in pulp

after day two of shelf life (Fig. 4A, 5B) partially confirm those of Wang *et al.* (2014) who reported that POD activity gradually increased in 'Brazil' bananas peel during 20 days of storage at 7 °C. However, other antioxidant enzymes such as superoxide dismutase (SOD) activity decreased in 'Namwa' bananas peel during ten days of storage at 6 and 12 °C (Kondo *et al.*, 2005). In the current study, the gradual increase of PG activity during shelf life, especially in pulp, suggest a role for PG in 'Grand Nain' banana softening. Our results confirm those of Srivastava and Dwivedi (2000) on 'Hari chhal', Zhang *et al.* (2010) on 'Brazil' and Mirshekari *et al.* (2015) on 'Berangan' bananas in which PG activity in pulp increased during ripening. The increase in xylanase activity during early stage of ripening and remain at appreciable level suggest a role for xylanase in polysaccharides hydrolysis during 'Grand Nain' banana ripening (Fig. 5D). A similar trend for xylanase activity has been reported by Srivastava and Dwivedi (2000) in pulp of 'Hari chhal' bananas during ripening. Also, our result is in agreement with the findings of Phanayingphaisal *et al.* (2006) who reported that xylanase activity in 'Namwa' banana pulp was highest at early stage of ripening then gradually decreased afterwards. α -amylase activity in both peel and pulp increased to a peak during the first two days of shelf life, and then decreased to a level similar to initial (Fig. 5E). Similar results were reported in which α -amylase attained their highest activity at climacteric peak and then decreased when most of starch had already disappeared (Adewale *et al.*, 2013). In conclusion, great physical and biochemical changes occurred in both peel and pulp during shelf life of 'Grand Nain' bananas. The peel of banana fruit could be considered as a good source for natural phenolics. Such information might be beneficial for postharvest technologist, nutritionists and consumers.

Fig. 1. Changes in fruit weight loss (A), peel color index (B), browning index (C) and peel membrane stability index (MSI) (D) of 'Grand Nain' bananas during shelf life. Vertical bar shows standard deviations (n=3) and values followed by different letters are significantly different according to Duncan's multiple range test at $P \leq 0.05$.

Fig. 2. Changes in L^* (□), a^* (■), b^* (△) and chroma ($(a^{*2}+b^{*2})^{1/2}$) (▲) value of peel of 'Grand Nain' bananas during shelf life. Vertical bar shows standard deviations ($n=3$) and values followed by different letters are significantly different according to Duncan's multiple range test at $P \leq 0.05$.

Fig. 3. Changes in pulp firmness (A), TSS content (B), titratable acidity (C), TSS/acid ratio (D) and pH (E) of 'Grand Nain' bananas during shelf life. Vertical bar shows standard deviations (n=3) and values followed by different letters are significantly different according to Duncan's multiple range test at $P \leq 0.05$.

Fig. 4. Changes in total phenols (A), flavonoids (B), vitamin C concentration (C) and free radical scavenging capacity (FRSC, DPPH IC₅₀ value) (D) of peel and pulp of 'Grand Nain' bananas during shelf life. Vertical bar shows standard deviations (n=3) and values followed by different letters are significantly different according to Duncan's multiple range test at $P \leq 0.05$.

Fig. 5. Changes in hydrolytic and antioxidant enzymes activities (U min g FW) of polyphenoloxidase (PPO) (A), peroxidase (POD) (B), polygalacturonase (PG) (C), xylanase (D), and α -amylase (E) of peel and pulp of 'Grand Nain' bananas during shelf life. Vertical bar shows standard deviations (n=3) and values followed by different letters are significantly different according to Duncan's multiple range test at $P \leq 0.05$.

5. Conclusion

Several biochemical and physiological changes occurred in 'Grand Nain' bananas during shelf life. Weight loss, TSS and browning increased while, firmness, TA and MSI decreased during shelf life. Total phenols increased during shelf life in both peel and pulp. Total flavonoids firstly increased, remained stable until day five then, decreased whereas, vitamin C increased during the first two days of shelf life and then decreased. However, FRSC (DPPH IC₅₀) in both peel and pulp slightly changed at first and then decreased during shelf life. Hydrolytic (PG, xylanase and α -amylase) and antioxidant enzymes (PPO and POD) activities in peel and pulp greatly varied during shelf life. Both peel and pulp of banana fruit could be considered as a good source for natural phenolics. Such information might be beneficial for postharvest technologist, nutritionists and consumers. Further investigations are needed to explore the polyphenolic profile of 'Grand Nain' bananas. Applying other assays than DPPH to estimated total antioxidant capacity are also suggested for better characterization of the relationship between antioxidants level and antioxidant capacity of fruit.

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الجودة والتغيرات البيوكيميائية في صنف الموز "جراندنان" أثناء فترة العرض

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المستخلص. تمت دراسة صفات الجودة ومضادات الأكسدة وقدرة كسح الشقوق الحرة (قيم FRSC, DPPH IC₅₀) والنشاط الإنزيمي لصنف الموز "جراندنان" أثناء فترة العرض على درجة حرارة 20±2 °C ورطوبة نسبية 60-70% RH لمدة 7 أيام. كان هناك زيادة في الفقد في الوزن ولون القشرة ومؤشر اللون البني، في حين حدث نقص في صلابة اللب والحموضة الكلية، والرقم الهيدروجيني، ومعامل ثبات الأغشية الخلوية لقشرة الثمار أثناء فترة العرض. وكانت هناك زيادة مستمرة في تركيز المواد الصلبة الذائبة الكلية حتى اليوم الخامس، ثم بقيت ثابتة بعد ذلك، بينما زاد تركيز فيتامين ج (تراوح بين ٥,٨ إلى ٨,٢ ملجم/١٠٠ جم وزن طازج) في أول يومين ثم انخفض بعد ذلك. زاد تركيز الفينولات الكلية في كل من القشرة واللب أثناء فترة العرض وتراوح بين ٠,١٩ إلى ١,٦٤ و ٠,١٧ إلى ٠,٩٥ جم/كجم وزن طازج على الترتيب. تراوح تركيز الفلافونيدات الكلية في القشرة بين ٠,٠٥ إلى ٠,٢١ جم/كجم وفي اللب بين ٠,٠٣ إلى ٠,١٩ جم/كجم وزن طازج، وكان هناك زيادة في البداية، ثم ظلت ثابتة حتى اليوم الخامس ثم انخفضت بعد ذلك. كان تركيز كل من الفينولات الكلية والفلافونيدات الكلية أعلى في القشرة عنه في لب الثمار. وقدرة كسح الشقوق الحرة في القشرة (تراوحت قيم الـ DPPH IC₅₀ 3.83 to 19.43) ظلت ثابتة حتى اليوم الخامس قبل أن تنخفض بشده بعد ذلك، بينما في اللب (تراوحت قيم الـ DPPH IC₅₀ بين 4.1 to 13.3) انخفضت خلال فترة العرض. كان هناك تباين كبير في نشاط إنزيمات البولي فينول أوكسيداز (PPO) والأوكسيداز (POD) والبولي جلاكتونيز (PG) والألفا أميليز في كل من القشرة واللب أثناء فترة العرض.

الكلمات المفتاحية: موز، الجودة، فترة العرض، مضادات الأكسدة، وقدرة كسح الشقوق الحرة، الفينولات، الإنزيمات.