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Postharvest quality alteration of gac fruit harvested at different maturities and coated with chitosan

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Abstract

Local Thai gac fruit conducting short shelf life after harvest were investigated for changes of fruit attributes during fruit growth and the postharvest. Gac fruit took 9 weeks after fruit setting to be reaching at full ripe stage. The changes of fresh weight and volume of developing fruit showed a single sigmoid curve and the firmness was declined after 5 weeks of the fruit development. Moreover, gac fruit exhibited an ethylene rise pattern during fruit development. Fruit harvested at four different maturity stages of mature green, yellow, orange, and fully red were stored at room conditions of 25°C and 65-70% RH. Fruit at yellow stage reached a normal ripe stage at the end of storage of 12 days when green fruit was unrippened and red fruit become rotten. The highest amount of phenolics was observed in aril mainly in the yellow stage at 6 DOS, followed by the peel and pulp and then decreased in all parts of fruit during storage. Subsequently, harvested fruit at yellow stage were dipped in 0, 0.5, and 1.0% chitosan and then stored at at 10°C, 90-95% RH. Although fruit of all treatments showed no signifant differences in respration rate, firmness, and some other quality attributes, coating with 0.5 and 1.0% chitosan retarded fungal infection and improved fruit visual appearance.

Introduction

Gac fruit (*Momordica cochinchinensis* Spreng), one of members of the family Cucurbitaceae, is originated from Southeast Asia and can be easily grown in many tropical countries (Iwamoto *et al*., 1985). The fruit can be consumed in different purposes upon development stage of fruit (Vuong, 2000; Ishida *et al*., 2004; Kubola and Siriamornpun, 2011). Colorful gac fruit, containing high content of antioxidants and nutritional values, is one of the lesser-known fruits lacking postharvest information (Vuong *et al*., 2006). The major problem of gac fruit after harvest is suddenly declined in firmness and incidences of fungal diseases during storage. The gac fruit growers in Thailand use the external fruit color as the harvesting index, and normally harvest at red ripening stage which is suitable for delivering to industrial factories for processed food and juice. However, recently there have been demands of fresh gac fruit as a health fruit from aboard especially in Japan. Thus, it is needed to know proper harvest maturity index and the postharvest technology for storing gac fruit.

Development of postharvest handling techniques in order to regulate ripening and curtail losses from postharvest disorders and diseases is crucial to increase commercialization of gac fruit. Long term storage management almost exclusively involves the management of temperature and gas composition in the storage room and coating with edible materials (Kader, 1995). Our previous study suggested that 10°C was best for extending storage life of gac fruit (Soe Win *et al*., 2015). For long storage of fruits, appropriate physiological maturity at harvest is important to obtain for proper quality and shelf-life. Furthermore, edible coating is among postharvest technology adapting internal gaseous atmosphere inside the fruit which is improving storage quality of fresh produce. Coating procedure generally creates modified atmospheres inside the treated fruit (Chun *et al*., 1998; Cisneros-Zevallos and Krochta, 2002). These gaseous stress conditions reduce metabolic changes and delay fruit ripening (Sozzi *et al*., 1999). Apart from keeping in modified atmosphere packaging, fruit covered with coating materials can be selected individually and conveniently by customers. Chitosan has been used as effective edible coating for fresh produce (Hernández-Muñoz *et al*., 2008; Reuck *et al*., 2009; Aider, 2010; Xing

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et al., 2010). Therefore, the present study was to investigate changes of fruit characteristics of gac fruit during growth and development. The changes in postharvest quality of gac fruit harvested at different maturity stages and the fruit coated with chitosan and stored low temperature storage were also monitored.

Materials and Methods

Material preparation

Thai gac fruit were tagged after hand pollination during July 2013 to January 2014 from 3 years-old plants at a commercial orchard in Nakorn-Pathom province (latitude: 14º 01' 16.08" N; longitude: 99º 58' 53.63" E), Western Thailand. The average day and night temperatures, and annual rainfall during the study time were 29ºC, 26ºC and 1490 mm.y^{-1}, respectively. Developing fruit starting from fruit setting to ripening were randomly sampled from the mother plants every 7 day interval to measure the growth pattern and physiological changes.

Experimental design

Gac fruit at four maturity groups of mature green fruit (completely green; 5 weeks after fruit setting (WAS)), yellow fruit (6 weeks), orange fruit (7 WAS) and red fruit (8 WAS; red color covering more than 2/3 of the whole fruit skin) (Figure 1) were harvested. Fruit were transported to the Postharvest Technology laboratory, King Mongkut's University of Technology Thonburi in Bangkok at the day of harvest. Fruit were washed and dipped in 200 mg·L^{-1} sodium hypochlorite and then 500 mg·L^{-1} imidazole. Fruit were dried at room temperature and selected for uniformity of size (about 400 to 500 g per fruit) and color. Fruit were then stored under room conditions (25±2ºC and relative humidity at 65-70%).

In subsequent experiment, gac fruit at yellow peel maturity (6 WAS; 40-42 days after fruit setting) were harvested. Fruit were cleaned and selected for uniformity as mentioned above. Fruit were then dipped in 0, 0.5, and 1.0% chitosan (low molecular weight, Sigma-Aldrich Co, Ltd.) for 1 min and air-dried. All treatments were stored at 10°C, 90-95% RH.

Measurement of fruit firmness

Firmness of whole fruit was measured in the middle on two opposite sides of each fruit using a texture analyzer (TA-XT2, Stable Micro Systems Ltd., UK) with a 5-mm diameter plunger and a constant moving rate of 10 mm·min^{-1} for a 10 mm depth. The mean values for maximum force were reported in Newtons (N).

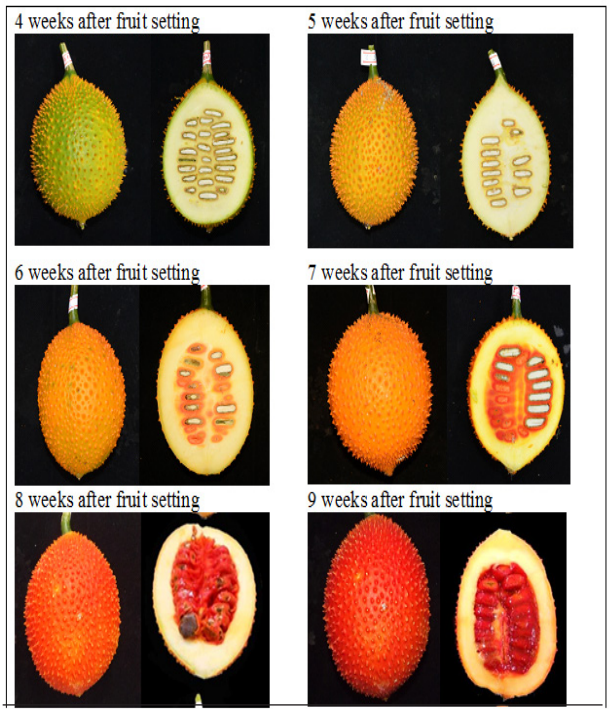

Figure 1. External and internal appearance of fruit maturation of gac fruit

Fruit color changes

Colors of peel and pulp of gac fruit were measured using a colorimeter (Model RC-300, Minolta Co. Ltd., Osaka, Japan). Colors were collected as L* (lightness), a* (red) Hunter scales, and chroma values (color saturation/color purity).

Measurement of ethylene production and respiration

Gac fruit was kept in a 1.8 L airtight container for 1 h under the storage temperature regimes to monitor fruit respiration rate and ethylene production. One mL of the headspace gas was injected into the inlet of gas chromatograph (GC). Ethylene production was measured using a Shimadzu GC Model 14B (Kyoto, Japan) equipped with a 60/80 mesh Propak Q column (set to 80ºC) and a flame ionization detector (FID), and nitrogen was used for the carrier gas at a flow rate of 35 mL·min^{-1} equipped with a stainless steel column packed with Porapak Q 80/100 mesh and a flame ionized detector. Helium was used as carrier gas. Carbon dioxide production as fruit respiration was determined using a Shimadzu GC Model 8A (Kyoto, Japan) equipped with a stainless steel column packed with Porapak Q 80/100 mesh (set to 80ºC) and a thermal conductivity detector. Helium was used as carrier gas at a flow rate of 30 mL·min^{-1}.

Measurement of total phenolics and antioxidant activity

Samples of peel, pulp, and aril were extracted using the method of Abu Bakar *et al*. (2009). One

gram of sample was extracted for 2 h with 10 mL of 80% methanol at room temperature on an orbital shaker set at 180 rpm. The mixture was centrifuged at 1400 g for 20 min and the supernatant was used for analyzing the total phenolic content and antioxidant activity.

Phenolic content in the extracts was determined spectrophotometrically according to the Folin–Ciocalteu method (Singleton *et al.*, 1999) and expressed based on the gallic acid standard curve. Antioxidant activities were detected by Ferric Reducing Antioxidant Power (FRAP) method (Benzie and Strain, 1996). The FRAP reagent containing 25 mL of a 10 mmol·L^{-1} TPTZ (2,4,6- tripyridy-s-triazine, Sigma) solution in 40 mmol·L^{-1} HCl plus 25 mL of 20 mmol·L^{-1} $FeCl_3$ and 250 mL of 0.3 mol·L^{-1} acetate buffer, pH 3.6 was prepared. A volume of 150 µL of fruit extracts was mixed with 2850 µL of the fresh working FRAP solution. After incubation for 30 min, the absorbance was read at 593 nm using a Shimadzu spectrophotometer (Kyoto, Japan).

Statistical analysis

The experiments were conducted following a completely randomized design (CRD) with five replications (one fruit/ replication). Data were analyzed using ANOVA in SPSS program (Version 11.0) when 'ns' in figures were indicated as non-significance between treatments and asterisk (*) were indicated as significance at $P \leq 0.05$. The difference among means was compared using the Duncan multiple range test (DMRT).

Results and Discussion

Changes in fruit growth and development

From evaluating changes in fruit weight and volume, the growth patterns of gac fruit exhibited a single sigmoidal, requiring 9 weeks after fruit setting (WAS) (ca. 63-65 days) to reach full ripe stage from the time when the female flower was pollinated and got fruit setting (Figure 2A). The fruit firmness increased sharply at young immature stages and reached the peak (145. 94 N) at 5 WAS and after that it dramatically declined when ripened (below 20 N) (Figure 2B). Fruit developed to yellow stage in 6-7 WAS and then, within next 2-3 weeks it became ripen (red color stage) (Figure 1). Tiusses around the seeds called 'aril' developed in fruit after 5 WAS by transforming and accumulating intense red color of lycopene and β-carotene (Aoki *et al*., 2002; Vuong *et al.*, 2006) until 9 WAS. The texture of red aril became soft, juicy, and gummy. Furthermore, we found that gac fruit showed an ethylene rise pattern when it

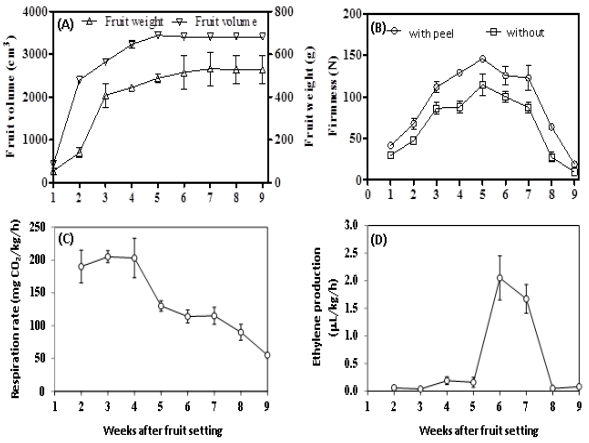

Figure 2. Changes in fresh weight and fruit volume (A), fruit firmness (B), respiration rate (C), and ethylene production rate (D) during fruit growth and development of gac fruit. Data represent means ± SE of five replications

produced the peak (2.03 µL·kg^{-1}·h^{-1}) at 6 WAS and then sharply dropped (Figure 2D). The respiration rate of developing fruit was extreamly high in young stages at 2-4 WAS and then dramatically declined. However, there was no obvious peak of climacteric respiration during fruit maturation, although there would apparently be the respiratory peak at 7 WAS (Figure 2C). Because the fruit respiration was roughly detected in a week interval followed by fruit development, so the respiratory climacteric rise might be taking place during days between the 6^{th} and 7^{th} week. However the climacteric classification of gac fruit needs more evidences to be further supported and clarified.

Changes in postharvest quality of gac fruit harvested at different maturities

Changes in peel and pulp color of gac fruit harvested at four different maturity stages and incubated at 25°C are presented in Figure 3. Peel L^* values of yellow, orange and red fruit decreased gradually during the storage time whereas that of mature green fruit was stable (Figure 3A). As a^* value (+) indicates red color and the ^{a*} value of fully ripened gac fruit were about 40-45 which changed very little during storage. Peel a^* values of yellow and orange fruit increased quickly during storage and reached normal ripening at the end of storage, whereas the a^* value of green fruit remained stable (Figure 3B). On the other hand, pulp L^* values of orange and red fruit slightly decreased whereas L^* of yellow was stable in first 6 days of storage (DOS) and then sharply decreased until day 12. According to pulp a^* value of red fruit stable at 11-13 during storage, the a^* of orange fruit reached at the red fruit level on day 6 whereas that of yellow fruit highly increased after 6 DOS. However, pulp ^{L*} and a^* values of mature green

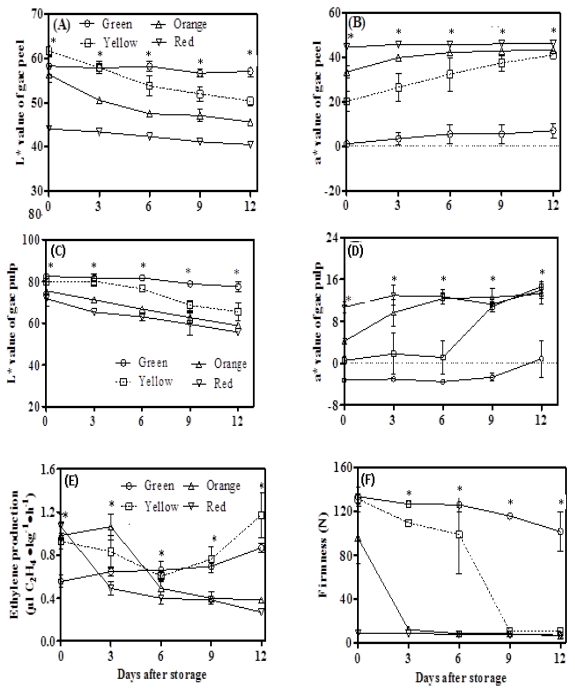

Figure 3. Changes in peel L* (A), peel a*, pulp L* (C), pulp a* (D), ethylene production rates (E), and firmness (F) of gac fruit harvested at different maturity stages during 25°C storage. Data represent means ± SE of five replications

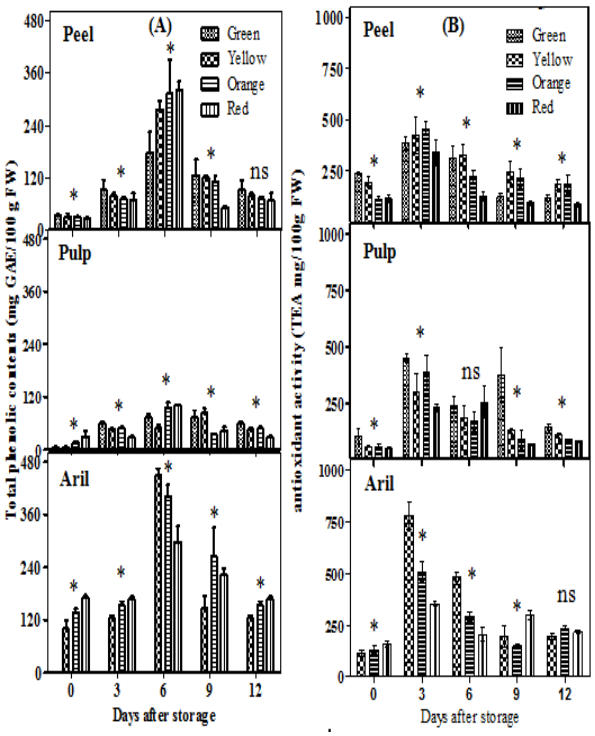

Figure 4. Changes in total phenolic contents (A) and antioxidant activities (B) in peel, pulp, and aril of gac fruit harvested at different maturity stages during 25°C storage. Data represent means ± SE of five replications

fruit were stable throughout the storage (Figure 3C, D).

Ethylene production of fully ripe red stage fruit obviously decreased during storage. In the orange stage, the ethylene production stayed high (0.94-1.16 $\mu L \cdot kg^{-1} \cdot h^{-1}$) at 3 DOS which coincided with the time of ripening and then declined. The ethylene production of yellow stage fruit sharply increased at 6 DOS until the end of storage whereas that of green fruit was low at initial and slightly increased afterward (Figure 3E). At the initial day of harvest, the firmness of the four developmental stages differed significantly. Green and yellow stage of fruit had the highest firmness and the red one showed the lowest firmness (Figure 3F). During storage, green and red fruit changed very little in their firmness values. Orange fruit suddenly declined in its firmness at 3 DOS, while yellow fruit maintained its firmness until 6 DAS. At the end of storage, the highest value of firmness was observed in green fruit due to being unable to ripe. During the ripening, the fruit became softened by the breakdown of the cell walls as a result of increasing metabolism (Kashmire and Kader, 1978) and the activity of softening enzymes (Chuni *et al*., 2010). The firmness of gac fruit in this result indicates that the orange stage fruit and the yellow stage fruit reached their ripeness at 3 and 9 DOS. From changes in peel and pulp color and fruit firmness, gac fruit at yellow and orange stages ripened normally while mature green fruit were unable to reach a fully ripe state during storage. This is similar to the maturation of mangosteen which fruit at the red pad stage (first red shed shining on fruit), which is the first recommended stage for harvesting, is starting to ripen normally. The pre-climacteric stage occurs at the mature green mangosteen when is not proper for harvesting because pericarp color and flavor of harvested fruit cannot be fully developed due to very low level of ethylene concentration (Wongs-Aree and Noichinda, 2014). As results, yellow stage of gac fruit is suitable for the remote markets and logistics.

At the initial day of storage, the highest amount of phenolics was observed in aril mainly in the yellow stage at 6 DOS, followed by the peel and pulp. The total phenolics contents increased at 6 DOS and then decreased in all parts of fruits during storage (Figure 4A). The antioxidant activity in different parts of gac at different maturity stages increased at 3 DOS and then declined gradually during storage (Figure 4B). The initial measurement of antioxidant activity showed that the highest antioxidant activity was found in gac fruit peel, followed by aril and pulp. A similar result was observed by Palafox-Carlos *et al.* (2012) in mango. The storage time decreased the level of antioxidant activity and the maturity stages affected the changes of antioxidant activity at the later days of

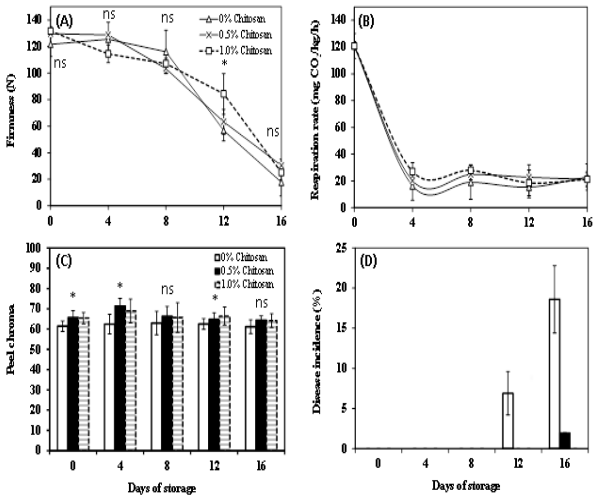

Figure 5. Changes in firmness (A), respiration rates (B), peel chroma, and disease incidence (D) of gac fruit during 10°C storage. Data represent means ± SE of five replications

storage. Maiani *et al.* (2009) reported that difference in ripeness can be contributed to the variation of phytochemical contents in fruits, and to ensure the highest fruit quality at the end of long storage, fruits must be harvested when mature at specific maturity index, but not when fully ripe (Kader, 1995). The positive correlation between phenolic contents and antioxidant activity in gac fruit was observed at 6 DOS and the later stage of storage.

Changes in storage quality of gac fruit coated with chitosan

Respiration rates of all fruit hugely reduced after storage at 10°C and remained stable from 4 DOS until the end without significant difference between treatments (Figure 5B). Firmness of fruit started at the average of 124.37 N, gradually decreased in first 8 DOS, and then increasingly declined to the average of 22.71 (Figure 5A). There were no significant differences in general fruit quality attributes between control and chitosan treated fruit (data not shown). However, the present results found that chitosan coating increasing peel color saturation by increasing chroma values (Figure 5C). This might be due to changes in light refraction on fruit after coated by chitosan material. Furthermore, chitosan treatments showed effective effect on inhibition of postharvest fungal growth on gac fruit during storage when control fruit started decay on day 9 (Figure 5D). Chitosan treatments have been reported to be effective on inhibition of postharvest disease infection and growth in particular to fungi on fresh produce (El Ghaouth *et al*., 1992; Bautista-Banos *et al.*, 2006; Liu *et al*., 2007; Munoz *et al.*, 2009). Consequently, the present benefits of chitosan coating of gac fruit included both improvement of the fruit visual quality and enhancement of fungal infection retardant during storage.

Conclusions

Fruit grwoth and development of gac was in the pattern of a sigmoid curve when the fruit growth inclined to the hightest point at 5 weeks after fruit setting. Gac fruit reached full ripening stage at 9 weeks after fruit setting and showed an ethylene rise pattern during maturation. Fruit harvest at yellow and orange stages reached a normal ripe stage to red fruit when green fruit was unable to get normal ripening. Gac fruit harvested at yellow stage and dipped in 0.5, and 1.0% chitosan were less in fungal infection during low temperature storage but were high in chroma values of the peel.

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