

Response of gac fruit (*Momordica cochinchinensis* Spreng) to postharvest treatments with storage temperature and 1-MCP

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Abstract

The effects of various low temperatures and 1-methylcyclopropene (1-MCP) pre-treatments on changes in physical, physiological, and phytochemical attributes, and in antioxidant activity of gac fruit were evaluated during storage. Low temperature regimes, including 4, 10, and 13°C had the greatest impacts on ripening, color changes, and bioactive compound content of gac fruit, compared to control at 25°C. However, fruit stored at 4°C showed both external and internal chilling injury symptoms starting from day 25 of the storage. Low temperature greatly preserved the reduction of phenolics and antioxidant capacity detected by FRAP (Ferric Reducing Antioxidant Power) during storage. 1-MCP fumigation at 500 nL·L⁻¹ showed slight effect on retarding an increase of ethylene production and on delaying fruit softening during 10°C storage. Nevertheless 1-MCP had no effect on changes of peel color, phytochemicals and antioxidant activity of gac fruit.

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Introduction

Gac (*Momordica cochinchinensis* Spreng), one of the family members of Cucurbitaceae, is an indigenous plant to Southeast Asia and a popular fruit in Vietnam (Vuong *et al.*, 2006). It has recently emerged as a prominent vegetable fruit for health, showing colorful fruit with high content of antioxidants and nutritional value. Whereas in Vietnam the bright red seed pulp (aril) of the ripe fruit is primarily used in the preparation of “*xoi gac*” (red rice) (Vuong, 2000), the fruit is consumed for different purposes at different developmental stages in Thailand. Immature gac fruit (green) is consumed as vegetable while the fruit meat (pulp) which has a flavor similar to papaya and is cooked or boiled, is consumed with chili paste or used in a curry. The aril of ripe gac is used as raw material for juice or ice cream processing (Kubola and Siriamornpun, 2011). Furthermore, gac pulp and aril are not only consumed for dietary purposes, but for medicinal functions (Iwamoto *et al.*, 1985; Vuong *et al.*, 2006). The fruit can make significant contributions of disease prevention, particularly by reducing vitamin A deficiency. Vuong *et al.* (2002) found that daily consumption of “*xoi gac*” significantly improved plasma levels of retinol, α - and β -carotenes, and lycopene in pre-school children. As rich sources of

phytochemicals such as carotenoids, phenolics, and antioxidants, foods made from gac fruit play a role in cancer prevention by scavenging reactive oxygen species and preventing intracellular damage (Nunes *et al.*, 2012).

For local use for processing as a food and other products, the gac fruit growers normally harvest the fruit at full ripening stage (red color peel) which has only 7 days of shelf life at ambient conditions (Soe Win *et al.*, 2015). The previous study on changes in postharvest quality and bioactive compounds of gac fruit at different maturity stages showed that the yellow stage of gac fruit was suitable for long term storage, while fully ripened fruit had a range of physiological changes and produced high respiration rate of 102.42 mg CO₂·kg⁻¹ h⁻¹ and ethylene production rate of 1.04 μ L·kg⁻¹ h⁻¹ (Soe Win *et al.*, 2015). There is currently a high demand from both domestic and export markets for fresh fruit for consumption but little information is available about postharvest and storage techniques. Its short postharvest life, high susceptibility to mechanical damage and pathogens limit the distribution to domestic markets. The rapid softening of fruit leads to losses in the supply chain due to bruising and over-ripeness. Therefore, development of postharvest handling techniques aiming at regulating ripening and curtail losses from postharvest disorders and diseases is crucial to

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increase commercialization of gac fruit.

Long term storage management almost exclusively involves the management of temperature and gas composition in the storage room (controlled atmosphere storage) and coating with edible materials (Kader, 1995). Storage temperature is one of the most important factors affecting the quality and maintenance of the shelf-life of fresh produce (Roura *et al.*, 2000). The lower storage temperature is, the longer storage life extension of fresh produce by reducing respiration rate and senescence will be, accompanied by a reduction of growth of spoilage microorganisms (Watada *et al.*, 1996; Roura *et al.*, 2000), in spite of a limitation due to a minimum temperature required by tropical fruits. Every fresh produce has an optimum storage temperature depending on geographic origin of the products. Although low temperature has been widely used to store fruits and vegetables given the beneficial effects of low temperature in delaying senescence and maintaining quality, there is little information regarding the antioxidant activity of different parts of gac fruit, and no information concerning the changes of phytochemicals and antioxidant activity of gac fruit during low temperature storage. Besides the storage temperature, 1-MCP has been successfully used in extending shelf-life and maintaining quality of fruits and vegetables. The use of 1-MCP is increasing in postharvest storage to extend the commercial life of fruits and vegetables. 1-MCP is now registered in many countries, though its first commercial applications date back only a few years (Blankenship and Dole, 2003). 1-MCP has a non-toxic mode of action, leaves negligible residues and is active at very low concentrations (Watkins, 2006). The action mechanism of 1-MCP involves competition with ethylene receptors for the binding site (Sisler and Serek, 1997), and it usually affects respiration rate and weight loss, and the onset of ethylene-dependent quality traits and perception (Dal Cin *et al.*, 2006; Watkins, 2006). The successful application of 1-MCP to whole fruit is strongly dependent on many factors, among them the species, cultivar and harvest maturity stage, the concentration applied, exposure time, temperature and frequency of application (single or cyclic), its application alone or in combination with other gases, and the subsequent postharvest conditions of temperature, relative humidity (RH), atmosphere, and storage duration (Watkins, 2006; Sozzi and Beaudry, 2007). Therefore, this experiment was conducted to select the optimum storage temperature combined with 1-MCP to maintain quality, extend the shelf-life and investigate the changes of phytochemicals and

antioxidant activity of gac fruit.

Materials and Methods

Material preparation

Gac fruit at the yellow peel maturity stage (40 days after fruit setting) were harvested from 3 years-old plants at a commercial orchard in Nakorn-Pathom province, Thailand (latitude: 14° 01' 16.08" N; longitude: 99° 58' 53.63" E) between March and August 2013. The average day and night temperatures, and annual rainfall during the study time were 29°C, 26°C and 1490 mm·y⁻¹, respectively. The fruit were transported to the Postharvest Technology laboratory at King Mongkut's University of Technology Thonburi, Bangkok within 2 h after harvest. After arrival, 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 500 to 600 g per fruit) and color.

Experimental design

Gac fruit harvested in March 2013 were randomly divided into four groups and stored at different temperature regimes of 4, 10, 13°C (90 – 95% RH) or 25°C (65 – 70% RH) for a month. In each storage temperature, four replications were assigned to a completely randomized design (CRD) in which two fruit for each replication were sampled. During storage, samples were taken at 5-days interval to measure the changes of physical (weight loss percentage, firmness, and color changes), physiological (respiration rate and ethylene production), and phytochemical characters (total phenolics contents, and antioxidant activity).

For a subsequent experiment of 1-MCP treatment, fruit harvested in August 2013 were pretreated by fumigated with 0 (control), 500, and 1000 nL·L⁻¹ 1-MCP (AnsiP-G, Taipei and Taiwan or EthylBloc™ Sachet) in a 43-L glass chamber at 25°C for 12 h. The experiment was designed as a CRD with four replications (one fruit per replication). After treatments, the fruit were ventilated to remove residual 1-MCP and transferred to the 10°C (90 – 95% RH) storage room. The same parameters measured in storage temperature experiment were analyzed at 5-days interval.

Measurement of weight loss and fruit firmness

The fresh weight of gac fruit was determined after treatment and weight loss was calculated as a percentage of the initial weight. 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).

Fruit color changes

Peel color was measured from the middle of the gac fruit in three different places of each fruit by a colorimeter (Model RC-300, Minolta Co. Ltd., Osaka, Japan). Colors were collected as lightness (L^*), green and red (a^*), and blue and yellow (b^*). Numerical values of a^* and b^* were calculated into hue angles ($H^\circ = \tan^{-1} b^*/a^*$) (Francis, 1980). A change in hue indicates fruit ripening from green to yellow or red, where 0 = red, 90 = yellow, and 180 = green. The color change (ΔE = a metric of the difference or distance between two colors) was computed by using the following formula:

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2}$$

where, L_0^* , a_0^* and b_0^* = color values of samples on initial day of the experiment, and L^* , a^* and b^* = the color values of samples at 5 days interval.

Measurement of respiration rate and ethylene evolution

Gac fruit was kept in an airtight container (1.8 L) for 1 – 2 h (closed system) under indicated storage temperature regimes to monitor the respiration rate and ethylene (C₂H₄) concentration. One mL gas sample was withdrawn from the headspace of the chamber using a one mL plastic syringe with a 1-inch (2.54 cm) long needle. Carbon dioxide production was determined by gas chromatography using Shimadzu Model 8A (Kyoto, Japan) equipped with a stainless steel column packed with Porapak Q 80/100 mesh (set to 80°C) and a thermal conductivity detector. Helium was used as carrier gas at a flow rate of 30 mL·min⁻¹. Ethylene production was measured by gas chromatography using Shimadzu Model 14B (Kyoto, Japan) equipped with a 60/80 mesh Propak Q column (set to 80°C) 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.

Extraction and determination of total phenolic content and antioxidant activity

The freeze-dried samples of peel, pulp and aril

from gac fruit were extracted using the method modified from Abu Bakar *et al.* (2009). The samples prepared from the frozen fruit (1 g) were 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 decanted into a 30 mL vial. The pellet was re-extracted under identical conditions. The supernatant was combined and used for the total antioxidant activity and total phenolics content.

Total phenolics content in the extracts was determined spectrophotometrically according to the Folin–Ciocalteu method (Abu Bakar *et al.*, 2009). Briefly, 300 µL of extract were mixed with 2.25 mL of Folin–Ciocalteu reagent diluted (1:10) in distilled water and allowed to stand at room temperature for 5 min; 2.25 mL of sodium carbonate (60 g·L⁻¹) solution was added to the mixture. After 90 min at room temperature, the absorbance was measured at 725 nm using a spectrophotometer. The total phenolics content of the extracts was calculated and expressed as gallic acid equivalents per 100 gram of fresh weight (mg GAE·100 g⁻¹ FW) based on the gallic acid standard curve.

The principle of Ferric Reducing Antioxidant Power (FRAP) method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous, colored form in the presence of antioxidants. The total reducing capacity was determined using the FRAP assay (Benzie and Strain, 1996). The FRAP reagent contained 25 mL of a 10 mmol·L⁻¹ TPTZ (2,4,6- tripyridyl-s-triazine, Sigma) solution in 40 mmol·L⁻¹ HCl plus 25 mL of 20 mmol·L⁻¹ FeCl₃ and 250 mL of 0.3 mol·L⁻¹ acetate buffer, pH 3.6 and the fresh working solution was prepared and heated to 37°C before using. The 150 µL of fruit extracts were allowed to react with 2850 µL of the fresh working FRAP solution. After incubation for 30 min, the absorbance was read at 593 nm using a spectrophotometer. A standard curve was prepared by plotting the percentage (%) of free radical scavenging activity of Trolox versus its concentration.

Statistical analysis

The experiments were conducted following a completely randomized design (CRD). Data were analyzed using ANOVA in SPSS program (Version 11.0) and difference among means was compared using the least significant difference (LSD) test at P ≤ 0.05.

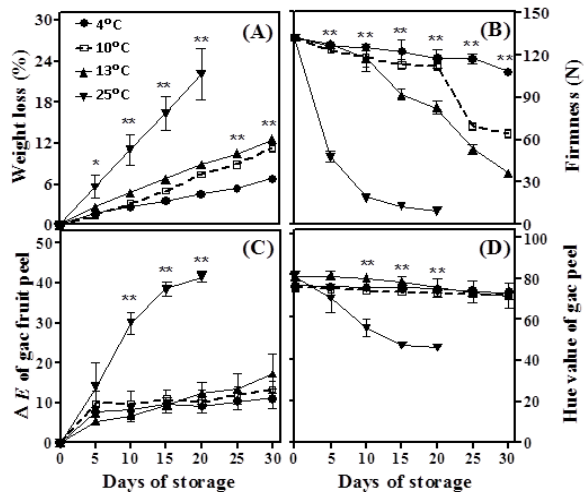


Figure 1. Effect of different storage temperatures on weight loss (A), firmness (B), color changes (ΔE values) (C) and hue value (D) of gac fruit. Data represent means \pm SE of four replications

Results and Discussion

Effects of low temperature on physical changes and disorders of gac fruit

The weight loss percentage of gac fruit was significantly affected by storage temperature. Its weight change was positively related to the storage temperature, and weight loss of gac fruit increased with storage time which was more prominent at 25°C (Figure 1A). The highest weight loss percentage was observed in gac fruit stored at 25°C, when about 22.03% of its initial weight was lost on 20th day of storage (DOS). Fruit stored at 25 °C and low relative humidity of 65-70% showed slight peel shriveling on day 15 and were mostly infected by fungus and then rotted after 20 DOS. Due to this infection, the weight loss percentage of gac stored at 25°C increased more rapidly than in other treatments. Fruit held at 4°C had the lowest weight loss (only 6.77% of their initial weight) followed by fruit held at 10 and 13°C (11.19% and 12.50%, respectively) at 30th DOS. Although the fruit stored at 4°C had the lowest weight loss percentage, some abnormal features of chilling injury were observed at the end of storage. Postharvest weight loss in fruit is usually due to the loss of water through transpiration. Water loss during storage is a major cause of fruit deterioration. Reduction in turgidity as a result of water loss causes shriveling and faster depletion of nutrients. Weight loss can lead to shriveling which reduces both the market value and consumer acceptability. De Castro *et al.* (2006), who tested the effects of different storage temperatures in tomato, demonstrated that weight loss was proportional to the storage period and storage temperature. High temperature increases the

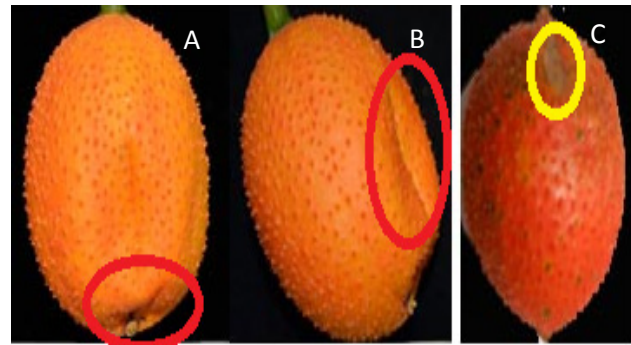


Plate 1. The chilling symptoms of gac fruit stored at 4°C; the shriveling of fruit (A and B) and water soaked area on the peel of fruit (C)

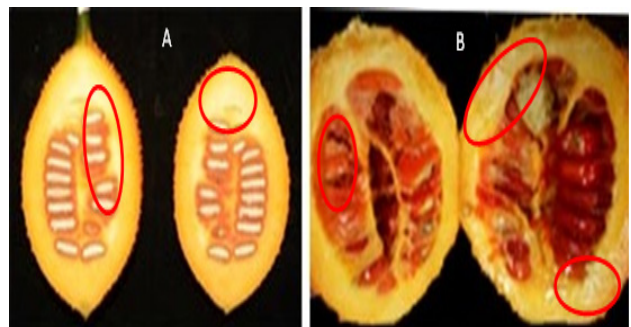


Plate 2. The internal disorders of gac stored at 4°C; the abnormal ripening of mesocarp and aril (A) and the water leathery like mesocarp and abnormal aril (B)

rates of respiration and other metabolic processes that cause depletion of substrates like sugars and proteins resulting in further weight loss (Nyanjage *et al.*, 2005). At higher temperatures, vapor pressure deficit increases, resulting in increased water loss which mainly accounts for weight loss. Lower weight loss that coincided with a decrease in storage temperature is in agreement with the findings of Ramana Rao *et al.* (2011).

Fruit firmness is the main important character of gac subjected to mechanical damage during transportation and storage. The decrease in fruit firmness is an indication of the level of softening of fruit. The firmness of gac fruit kept at 25°C on 5th DOS declined more rapidly than that of fruit stored at 4, 10, and 13°C, which coincided with the rise in respiration and ethylene production. The firmness of gac stored at 25°C suddenly decreased from 132.8 N to 19.57N within 10 DOS, whereas that of the fruit stored at 4, 10, and 13°C decreased gradually. The lowest decrease in firmness was observed in fruit stored at 4°C (Figure 1B). During ripening, the fruit tended to soften either as result of the cells losing water and becoming less turgid or by the breakdown of the cell walls as a result of increasing metabolism (Kashmire and Kader, 1978). Loss of fruit firmness in fruit was due to the softening of the cell wall, which may be due to the activity of softening enzymes

Table 1. Effect of different storage temperatures on mean of total phenolics content in peel, pulp and aril of gac fruit

Treatments	Total Phenolics Content (mg GAE·100 ⁻¹ g FW) ^{1/}						
	Peel						
	D0	D5	D10	D15	D20	D25	D30
4°C	266.50	154.83a	136.83ab	113.50	105.83a	101.83ab	95.50
10°C	266.50	156.83a	148.17a	114.17	109.83a	103.17a	86.17
13°C	266.50	147.83b	102.17bc	84.83	78.83b	85.17b	81.50
25°C	266.50	113.50b	69.83c	76.17	68.50c	-	-
F-test	1	*	*	ns	**	*	ns
CV%	13.67	16.74	19.77	35.12	10.62	11.39	16.35
	Pulp						
4°C	55.17	52.17b	100.83a	56.50b	60.83	194.50a	166.50a
10°C	55.17	67.50a	71.17ab	85.83a	68.17	129.17b	123.50b
13°C	55.17	60.17b	56.17b	48.17b	61.17	143.83b	111.83c
25°C	55.17	70.83a	54.50b	49.50b	47.17	-	-
F-test	ns	*	*	*	ns	**	**
CV%	30.13	23.64	29.06	24.61	28.36	8.8	2.63
	Aril						
4°C	276.83	174.17	102.50c	109.50a	106.83b	98.17	94.17ab
10°C	276.83	179.83	148.83a	116.83a	100.17a	96.50	122.17a
13°C	276.83	182.83	98.83ab	93.50b	97.50b	92.17	74.83b
25°C	276.83	149.50	78.17c	53.83b	58.50c	-	-
F-test	ns	ns	**	**	**	ns	*
CV%	12.73	12.76	21.79	43.47	18.71	21.52	15.96

^{1/} Means (n=4) with different lower case letters within the same column are significantly different.

ns: non significant, * Significant difference at $P \leq 0.05$, ** Significant difference at $P \leq 0.01$

(Chuni *et al.*, 2010). Low temperature treatment was an effective way to maintain fruit firmness and to delay fruit softening, resulting in extending the storage life of fruit.

The natural color of gac peel change from yellow to red. The color changes of gac fruit peel were affected by the storage temperature and the changes increased as the storage time progressed. Among temperature treatments, 25°C storage hastened the color changes of gac peel. The color changes of gac stored at 4, 10, and 13°C were not significantly different during storage (Figure 1C). Fruit stored at 25°C turned from yellow to red in 10 days and the decrease of peel hue value was very pronounced whereas there was only a slight change of hue value of gac fruit peel stored at 4, 10, and 13°C (Figure 1D). The color change in fruit was due to a decrease in chlorophyll and an increase in carotenoid synthesis reflecting the transformation of chloroplasts to chromoplasts (Pretel *et al.*, 1995). The low temperatures would retard the carotenoid biosynthesis since fruit stored at 4, 10, and 13°C just turned orange at the end of storage.

Although low temperature may prolong storage life, excessively low temperature can cause chilling injury and result in loss of quality and shelf-life. On 25th DOS, we found that the fruit stored at 4°C showed several external chilling injury symptoms including improper ripening and shriveling (Plate 1A), tissue collapse (Plate 1B) and water soaked area

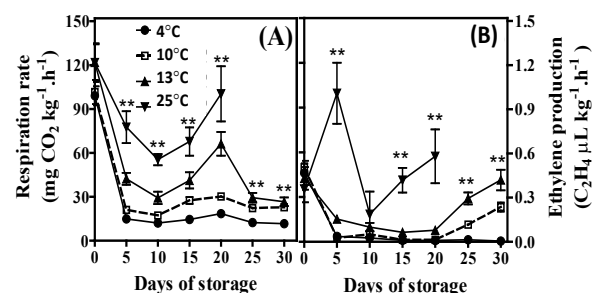


Figure 2. Effect of different storage temperatures on respiration rate (A) and ethylene production (B) of gac fruit. Data represent means \pm SE of four replications was found on the peel (Plate 1C). Moreover, when the internal pulp and aril were examined, the aril was not showing its normal color i.e. it was pale red (Plate 2A) and the pulp (mesocarp) showed leathery like meat (Plate 2B) that was not edible.

Effect of low temperature on physiological characters

The respiration rates of gac fruit decreased on 10th DOS and increased on 20th DOS (Figure 2A). Among three storage temperature regimes, the fruit stored at 4°C showed the lowest respiration rate while the highest one was observed in those stored at 25°C, due to the fungal infection on 20th DOS. The same results were reported by Bhande *et al.* (2008) in bananas and Ding *et al.* (1998) in loquat fruit. On the 1st day after storage, a high rate of respiration was found that was due to abiotic stress such as transportation shock and mechanical injury during

Table 2. Effect of different storage temperatures on mean value of antioxidant activity (measured by FRAP assays) in different fractions (peel, pulp and aril) of gac fruit

Treatments	Antioxidant activity (TEA mg·100g ⁻¹ FW) ^{1/}						
	Peel						
	D0	D5	D10	D15	D20	D25	D30
4°C	380.83	347.00b	428.83b	333.00b	283.67ab	240.50	237.67a
10°C	380.83	367.33a	560.33a	399.33a	415.83a	240.00	257.50a
13°C	380.83	487.17b	335.17c	266.00c	318.33ab	282.83	121.33b
25°C	380.83	365.50b	239.83d	323.83bc	189.83b	-	-
F-test	ns	**	**	**	**	ns	**
CV%	16.93	9.02	9.21	9.99	24.76	11.23	16.77
	Pulp						
4°C	181.50	149.00	151.33a	170.83b	160.83a	85.00a	78.83a
10°C	181.50	158.67	168.17a	200.50a	178.83a	71.00a	63.17a
13°C	181.50	151.00	138.00b	259.00a	184.83a	44.00b	34.17b
25°C	181.50	145.17	121.83b	135.67b	106.00b	-	-
F-test	ns	ns	**	**	**	**	**
CV%	69.8	28.98	21.18	17.63	17.61	24.91	24.91
	Aril						
4°C	265.00	236.33	427.50	404.33	329.33	307.17a	105.83
10°C	265.00	250.83	427.50	350.83	327.33	272.00ab	128.50
13°C	265.00	242.00	393.00	323.17	311.17	265.50b	127.00
25°C	265.00	230.00	399.50	248.83	251.67	-	-
F-test	ns	ns	ns	ns	ns	**	ns
CV%	24.58	51.86	11.78	31.91	26.8	17.14	15.43

^{1/} Means (n=4) with different lower case letters within the same column are significantly different. ns: non significant, * Significant difference at $P \leq 0.05$, ** Significant difference at $P \leq 0.01$

transport. The diminution of enzymatic activities occurring at in general low temperature reduces the rate of respiration. Thus, fruit at low temperatures had lower rates of respiration than those kept at high temperature. The ethylene production of gac fruit stored at 25°C suddenly increased on 5th DOS, decreased thereafter and then increased again. At 4, 10, and 13°C storage temperatures, the ethylene production of gac gradually decreased till 20 DOS, and thereafter, the level increased in fruit kept at 13°C (Figure 2B). The peak of ethylene production in gac coincided with the time of ripening, indicating that gac could be classified as a climacteric fruit. Biale *et al.* (1954) reported the findings on the role of ethylene in ripening fruits on the basis on measurements of ethylene produced by the fruit. However, the measurements were not sufficiently sensitive at threshold concentrations needed for physiological activity. After storage of gac at 25°C, the increase of ethylene production might be explained by biotic stress such as fungal attacks. The ethylene production of gac kept at 4°C showed no peak during the storage.

Effect of low temperature on phenolics content and antioxidant activity

The contents of phytochemicals in different fractions of gac fruit may be changed by many postharvest factors. Although gac aril of ripen fruit is mainly processed for juice and the pulp is used to cook a curry or food, the peel which is rarely used

for consuming was measured in the present study to accumulate high amount of phenolics and antioxidant capacity. Nowadays, many different kinds of products such as soap, shampoo, even cosmetics are made from not only aril and pulp of gac but also from the peel. In general, total phenolics content in peel and aril of gac was gradually decreasing during storage while the phenolic contents in pulp increased when the fruit ripen. Among the three parts of fruit (peel, pulp, and aril) the highest contents of total phenolics was found in aril (276.83 mg GAE·100⁻¹ g FW) followed by peel (266.50 mg GAE·100⁻¹ g FW), and pulp (55.17 mg GAE·100⁻¹ g FW) on the first day of storage (Table 1). Total phenolics content is associated with cultivar, pre- and postharvest handling factors. The storage temperature affected the loss of phenolics content in different fractions of fruit. The high temperature decreased the phenolics content and the highest value of phenolics content was observed in fruit stored at 10°C at the end of storage. Gajewski *et al.* (2009) reported on eggplants stating that the total phenolics content in fruit skin increased during storage, but the content in fruit flesh was not affected. Storage at 25°C caused the highest reduction of phenolics content for almost all storage periods. Similar results were obtained by Rivera-Pastrana *et al.* (2010) in 'Maradol' papaya in which phenolics of 'Maradol' papaya were influenced by postharvest storage temperature and tended to decrease during ripening at 25°C. However, the result

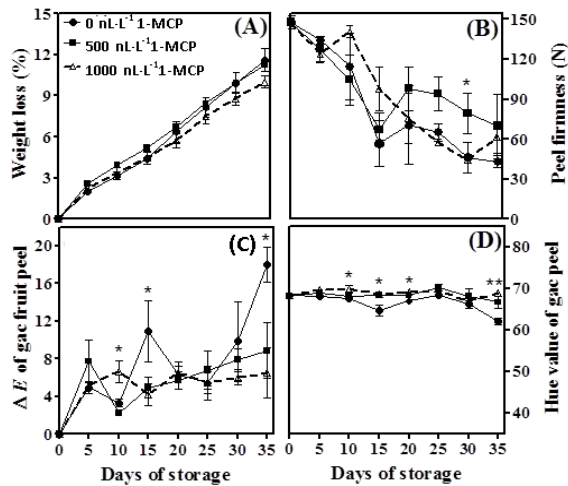


Figure 3. Effect of different dosages of 1-MCP on weight loss (A), firmness (B) color changes (ΔE value) (C) and hue value (D) of gac fruit storage at 10°C. Data represent means \pm SE of four replications

of the present study was opposite to that reported by Ayala-Zavala *et al.* (2004) in strawberries. They found that total phenolics content increased continuously in berries stored at 10°C and 5°C during storage time. In the present study at the lowest temperature (4°C and 10°C) the total phenolics content remained unchanged. This result agrees with the findings of Tomas-Barberan and Espin (2001) who reported that phenylalanine ammonia lyase (PAL) activity was higher at lower temperatures. At the same time, the activity of enzymes responsible for polyphenol degradation, polyphenoloxidase, are inhibited by lower temperatures. Leja *et al.* (2003) stated that an increase in phenol content could be due to lower activity of polyphenoloxidase at low temperatures, so that oxidation processes were minimised. Increases in polyphenol content during shelf life can be explained by a higher ethylene production which results in a stimulation of PAL (Napolitano *et al.*, 2004).

Total antioxidant activities of gac fruit were measured with FRAP assay. Among the three fractions of gac the highest antioxidant activity was observed in peel (380.83 TEA mg·100g⁻¹ FW) followed by aril (265.00 TEA mg·100g⁻¹ FW), and the pulp (181.50 TEA mg·100g⁻¹ FW) on the first day of storage (Table 2). The magnitudes of antioxidant activity measured by FRAP in gac peel was nearly constant during the storage and the high temperature decreased the antioxidant activity. Among the three parts of fruit (peel, pulp, and aril), the pulp had the lowest antioxidant activity and its values were affected by storage temperatures. Fruit antioxidants protect tissues against stresses and disease. Pinelo *et al.* (2004) reported that several food components,

i.e. carotenoids, vitamin C, vitamin E, phenolic compounds and their interactions contribute to the overall antioxidant activity of foods, and thus, it is difficult to measure total antioxidant activity on the basis of individual active components. In general, the highest values of antioxidant activity in pulp were found in fruit kept at low temperature. In aril the antioxidant activity decreased on 5th DOS and then increased till 25 DOS. The antioxidant activity in aril did not significantly differ between treatments except at 25°C. The highest amount of antioxidant activity was observed in fruit stored at 4°C followed by that of 10°C. Overall, the fruit stored at low temperature maintained its the high antioxidant activity than those in high temperature. The decrease in the antioxidant activity may be due to the decreasing content of phenolic compounds in storage at high temperature. Similar result was found in blood orange (*Citrus sinensis* cv. Tarocco) by Hamedani *et al.* (2012) who reported that an increase in temperature and storage time reduced reduced the antioxidant capacity.

Among different storage temperature, 10°C was an optimum storage temperature for gac fruit. Gac fruit stored at 10°C had longer shelf life about 30 days without chilling injury symptoms while the shelf life of fruit at room temperature was about 15 days. Moreover, the fruit stored at 10°C showed lower weight loss and maintained the fruit quality, in particular high fruit firmness, which is an important factor for transportation and logistics. Although the fruit stored at 4°C had the highest total phenolics content and antioxidant activity, it could not ripen normally.

Effects of 1-MCP on physical responses of gac fruit

In previous study, the gac fruit showed an increase of ethylene production during ripening and thus, the gac fruit were treated with 1-MCP (0, 500, and 1000 nL·L⁻¹). There was no significant difference between treatments with regard to weight loss or fruit firmness (Figure 3A and B). There was a gradual weight loss increase and firmness decrease during the storage time and the 1000 nL·L⁻¹ 1-MCP caused the lowest weight loss of fruit. The similar findings of no effect of 1-MCP on weight loss of oranges and tomatoes were stated by Porat *et al.* (1999) and Wills and Ku (2002), respectively. Jeong *et al.* (2003) stated that 1-MCP delay the weight loss in avocado. Massolo *et al.* (2011) reported that weight loss in eggplant treated with 1-MCP was significantly lower than control fruit because water exchange in eggplant occurred mostly through the calyx, and it is possible to associate the decrease in softening, weight loss and dehydration with the maintenance of

Table 3. Effect of different dosages of 1-MCP on changes in total phenolics content in different fractions (peel, pulp, and aril) of gac fruit stored at 10°C

Treatments	Total phenolics content (mg GAE·100 ⁻¹ g FW) ^{1/}				
	Peel				
	D0	D5	D15	D25	D35
0 nL·L ⁻¹ (control)	275.75	277.92	264.08	241.92	233.42
500 nL·L ⁻¹	275.75	256.25	241.42	237.75	235.92
1000 nL·L ⁻¹	275.75	251.92	247.58	245.42	237.42
F-test	ns	ns	ns	ns	ns
CV%	3.77	9.15	5.52	3.06	1.85
	Pulp				
0 nL·L ⁻¹ (control)	61.83	66.50	48.50	41.833a	23.92
500 nL·L ⁻¹	61.83	47.17	41.83	20.83b	20.92
1000 nL·L ⁻¹	61.83	65.17	36.17	17.83b	16.08
F-test	ns	ns	ns	*	ns
CV%	43.77	38.97	12.31	41.96	30.94
	Aril				
0 nL·L ⁻¹ (control)	298.75	313.92	299.25	297.58a	231.25a
500 nL·L ⁻¹	298.75	327.95	282.25	288.58b	238.25a
1000 nL·L ⁻¹	298.75	304.08	276.92	279.75a	218.92b
F-test	ns	ns	ns	**	**
CV%	9.06	10.81	6.85	3.59	1.78

^{1/} Means (n=4) with different lower case letters within the same column are significantly different.

ns: non significant, * Significant difference at $P \leq 0.05$, ** Significant difference at $P \leq 0.01$

calyx integrity in 1-MCP treated fruit, which might then result in higher resistance to water vapor flux. Weight loss of produce is mainly due to transpiration and evaporation of water inside the produces through surface. Moreover, cell wall degradation may affect water loss from produce, membrane permeability, and electrolytic leakage also participate in water loss from inside. Blankenship and Dole (2003) reported that the effective concentrations of 1-MCP vary with respect to species, cultivars, treatment time, and temperature.

The firmness of fruit treated by 1-MCP was not significantly different during the storage except on 30th DOS in which the highest firmness was found in 500 nL·L⁻¹ 1-MCP treated fruit. Fan *et al.* (1999) and Vilaplana and Valentines (2006) studied apples in which 1-MCP delays the ripening-related changes that influence quality and especially reduces loss of firmness. In the present study on gac fruit, the lack of effect of 1-MCP on weight loss and firmness was due to low dosages and short duration of treatment. A similar result was obtained by Gutierrez *et al.* (2008) in goldenberry fruit in which treatment with 5 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP tended to delay goldenberry softening at all stages though differences with control fruit were not significant, probably due to a relatively high variability. Tassoni *et al.* (2006) found that

application of 5 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP brought about little effect on firmness in tomato and another solanaceous species. Further studies are needed to determine if concentrations higher than those tested in this experiment will maintain firmness.

The gac fruit was harvested at the yellow stage and its peel color changed to red when ripe. The change of peel color (shown as ΔE value) was gradually increased during storage in all treatments (Figure 3C). No significant difference in peel color changes between treatments was observed in gac fruit during storage except on 10th and 35th DOS. At the end of storage (35 DOS), the highest peel color change was observed in control fruit and the lowest was obtained from 1000 nL·L⁻¹ 1-MCP treated fruit. The hue values of gac peel treated with 1-MCP are shown in Figure 3D. Its values gradually decreased during storage, however, there was no significant difference between treatments during storage except on 10th, 15th, and 20th DOS. On those days the lowest hue value was observed in 500 nL·L⁻¹ 1-MCP treated fruit. Gutierrez *et al.* (2008) stated that the hue angle was higher in 1-MCP-treated goldenberries fruit than in control at yellow stage. Moretti *et al.* (2002) stated that pigment synthesis and expression appeared to be delayed when 1-MCP was applied at early stages of maturity and in tomato, later maturities were

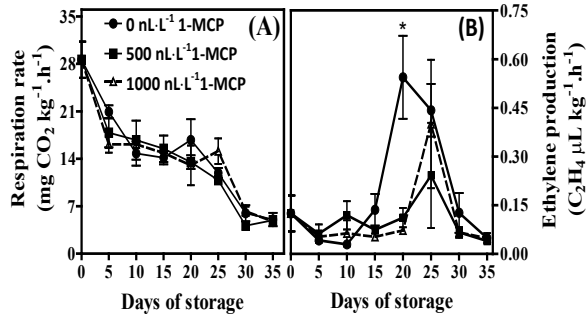


Figure 4. Changes in respiration rate (A) and ethylene production (B) of gac fruit treated with different dosages of 1-MCP and stored at 10°C. Data represent means \pm SE of four replications

demonstrated to be less affected by 1-MCP treatment (Ergun *et al.*, 2006).

Effects of 1-MCP on physiological responses of gac fruit

The respiration rate of gac fruit treated with 1-MCP was the highest on the first day of storage and it gradually decreased during storage period. Although CO₂ production was not consistently affected by 1-MCP at the yellow stage throughout the experimental period, 1-MCP-treated fruit showed an average respiration rate lower than that in untreated fruit. A slight increase of CO₂ from gac was observed when the fruit started to ripen. The 1-MCP delayed the increase of respiration rate, however, the magnitude of CO₂ was not significant among all treatments (Figure 4A). The 1-MCP treatments in most products decreased or delayed the respiration rate, decrease or delay in especially in climacteric fruits. In contrast, 1-MCP had no effect on nectarine and apricot (Dong *et al.*, 2001; 2002) and arazá fruit (Carrillo *et al.*, 2011). The respiration, however, appeared to be inhibited in 1-MCP treated broccoli as a function of its concentration (Fan and Mattheis, 2000). The ethylene production of gac fruit treated with 1-MCP is shown in Figure 4B. The ethylene production decreased in all treatment during the first 10 DOS and then gradually increased and reached the maximum on 20th DOS in control fruit and 25th DOS in fruit treated with 1-MCP when the fruit became ripe. The magnitude of ethylene production of gac fruit treated with 1000 nL.L⁻¹ 1-MCP was higher than that in 500 nL.L⁻¹ 1-MCP on 25th DOS. It has been suggested that the competitive binding of 1-MCP to the ethylene binding site may suppress an auto-inhibitory or other feedback mechanism, allowing ethylene production to increase. Effect of 1-MCP on the ethylene production may be increased or decreased depending on types of produce (Watkins, 2006). 1-MCP lowered ethylene production in strawberry (Jiang *et al.*, 2001), slowed

ethylene production in apricots and plums (Dong *et al.*, 2002), and inhibited ethylene production in Fuji apple (Fan and Mattheis, 1999). In pineapple, 1-MCP treated fruit produced higher ethylene than the control (Selvarajah *et al.*, 2001). The effect of 1-MCP depends on time and concentration. The dosage and duration of treatment of 1-MCP in this experiment was not sufficient to penetrate inside the gac fruit. Thus, the effect to 1-MCP on ethylene production was not pronounced and showed no significant difference between treatments. However, the concentration of 500 nL.L⁻¹ 1-MCP resulted in a lower ethylene production than that in any other treatment and it should be applied for further study.

Effects of 1-MCP on phenolics content and antioxidant activity of gac fruit

Effect of different dosages of 1-MCP on total phenolics content in different fractions (peel, pulp, and aril) of gac fruit stored at 10°C is shown in Table 3. The highest amount of total phenolic contents was observed in aril of gac fruit followed by peel and pulp. The total phenolic contents in peel and aril of gac fruit increased 5 days after storage and then gradually decreased during the storage and there was no significant difference between treatments except at 25 and 35 DOS in aril. Cheng *et al.* (2012) observed that all Nangou pear fruit treated with 1-MCP showed a relatively lower total phenolics content than that in control.

Effects of different dosages of 1-MCP on antioxidant activity measured by FRAP assay in different fractions (peel, pulp, and aril) of gac fruit stored at 10°C are presented in Table 4. The highest value of antioxidant activity was observed in peel of gac fruit followed by aril and pulp. The antioxidant activity in gac increased at 5 and 15 DOS and then decreased. There was not significant difference in antioxidant activity between treatments. The highest value of antioxidant activity was found in 1000 nL.L⁻¹ 1-MCP treated fruit. The findings of the present study are consistent with those of Vilaplana and Valentines (2006) in which no significant differences in total antioxidant activity were found between the control and the 1-MCP-treated apple fruit. Vilaplana and Valentines (2006) reported that 1-MCP did not detrimentally affect the antioxidant potential of the Golden Smoothie apple fruit and provided evidence to support the hypothesis that the beneficial effects of 1-MCP on ripening are not exclusively limited to its effect on ethylene. The dosage and duration of 1-MCP treatments in the present study did not affect the changes in postharvest characters, probably due to low concentration and short duration of 1-MCP or

Table 4. Effect of different dosages of 1-MCP on changes in antioxidant activity (measured by FRAP assays) in different fractions (peel, pulp, and aril) of gac fruit stored at 10°C

Treatments	Antioxidant activity (TEA mg·100 g ⁻¹ FW) ^{1/}				
	Peel				
	D0	D5	D15	D25	D35
0 nL·L ⁻¹ (control)	447.62	323.81	365.08a	239.37b	133.97
500 nL·L ⁻¹	447.62	292.06	250.79b	196.51b	118.73
1000 nL·L ⁻¹	447.62	425.40	371.43a	262.86a	243.49
F-test	ns	ns	*	**	ns
CV%	9.25	36.61	13.87	9.84	46.12
	Pulp				
0 nL·L ⁻¹ (control)	164.13	174.57	121.90	98.73	93.17
500 nL·L ⁻¹	164.13	164.29	109.68	97.14	93.02
1000 nL·L ⁻¹	164.13	133.49	93.17	73.97	52.06
F-test	ns	ns	ns	ns	ns
CV%	41.43	38.04	31.60	39.32	63.84
	Aril				
0 nL·L ⁻¹ (control)	325.40	335.08	381.25	255.87	180.95
500 nL·L ⁻¹	325.40	421.27	438.25	265.71	261.27
1000 nL·L ⁻¹	325.40	418.73	425.24	240.95	232.38
F-test	ns	ns	ns	ns	ns
CV%	34.98	16.81	19.68	63.94	36.54

^{1/} Means (n=4) with different lower case letters within the same column are significantly different.

ns: non significant, * Significant difference at $P \leq 0.05$, ** Significant difference at $P \leq 0.01$

even the maturity of gac fruit used in this study.

Conclusion

In conclusion, the optimum conditions for storage of gac fruit are 10°C, 90-95% RH, as these conditions had the greatest impacts on gac fruit ripening and fruit quality, especially the fruit firmness. Fruit stored at 10 and 13°C had a shelf life of 30 days, whereas storage at room temperature of 25°C resulted in an acceptable quality of the fruit for 15 days. Although fruit stored at 4°C showed acceptable characteristics considering their physical and physiological aspects, the fruit exhibited chilling injury symptoms such as peel shrinkage and collapse as well as leathery pulp at the end of the storage time. Gac fruit at yellow peel stage fumigated with 1-MCP treatments prior to 10°C storage, showed no significant differences in fruit quality during storage. Phenolics and FRAP antioxidant activity were high in peel and aril, and lower in pulp of gac fruit. 1-MCP treatments and storage at low temperature had no effect on maintaining the bioactive components that became markedly reduced during storage.

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