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Original Article

# Variation of lycopene and beta-carotene contents after harvesting of gac fruit and its prediction

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ABSTRACT

The effects were investigated of three different stages of harvesting, the storage time and sample preparation methods before extraction using a Waring blender (WBM) or ball mill (BMM) on the lycopene and β-carotene contents from the gac aril. It was found that after harvesting and being stored at 26 ± 1 °C and 24 ± 1% RH for 15 d, the lycopene contents from the color break, medium ripe and fully ripe stages of gac fruits grown in Thailand were in the ranges 0.11–8.99 mg/100 g fresh weight (FW), 3.88–22.94 mg/100 g FW and 18.95–50.11 mg/100 g FW, respectively, while the β-carotene contents were in the ranges 0.002–4.82 mg/100 g FW, 0.31–13.59 mg/100 g FW and 22.68–39.16 mg/100 g FW, respectively. In addition, neither the WBM nor the BMM sample preparation method had any significant ($p > 0.05$) effect on the analysis of these phytonutrients. Gac fruit at the fully ripe stage after 6 d of storage provided the highest lycopene content of 50.11 ± 1.59 mg/100 g FW, while the β-carotene was found highest (39.16 ± 1.29 mg/100 g FW) from fully ripe stage fruit after 15 d storage or when they had spoiled. Without classifying the fruits according to harvesting stages, equations for mixed ripe fruit were able to predict the lycopene and β-carotene contents in the aril with coefficients of determination of 0.77 and 0.89 with standard errors of the estimate of 16.09 and 6.39, respectively.



## Introduction

Fruit from gac (*Momordica cochinchinensis* (Lour.) Spreng) originated in East and Southeast Asia (Iwamoto et al., 1985) and contains very high amounts of phytonutrients, especially lycopene and β-carotene (Kubola and Siriamornpun, 2011). The Vietnamese like to mix seed membranes (the gac aril) and pulp in cooking rice (Aoki et al., 2002). Moreover, gac fruit can be used medicinally to treat dry eye symptoms as well as to promote healthy vision and increase the plasma level in blood (Vuong et al., 2002). Seeds of gac fruits also are used by the Chinese in a traditional medicinal treatment called “mubiezi” (Burke et al., 2005). However, gac fruit in Thailand is known as “Fakkao” and is grown as a backyard vegetable or on small farms and has been popularly processed for healthy foods and drinks; traditionally, young tips and fruit pulp are blanched first and served with chili sauce or cooked in a Thai spicy mixed vegetable curry, but ripe fruit are not used (Klungsupya et al., 2012). Since the gac aril is known to be an excellent source of phytonutrients, many healthy food and drink products as well as cosmetics in Thai niche markets are composed of this part of the plant (Bootprom et al., 2012).

Ripe gac fruit is rich in pigments of carotenoids, the colors of which are yellow, orange and orange-red and fruits continued to ripen after they are harvested and an ethylene peak in the least mature fruit may reflect a climacteric behavior; furthermore, these pigments are found mainly in the red gac aril and very little is in the gac pulp (Tran et al., 2016). Some researchers have reported on the amount of lycopene and β-carotene contents in the gac aril but with large variations in their data. The lycopene and β-carotene contents in the gac aril from fruit cultivated in Vietnam were in the ranges 38–373 mg/100 g fresh weight (FW) and 8–84 mg/100 g FW, respectively (Vuong et al., 2006; Aoki et al., 2002; Ishida et al., 2004; Nhung et al., 2010). In contrast, the lycopene content from fruit cultivated in Thailand varied from 70 mg/100 g FW to 116 mg/100 g FW while the β-carotene content was about 26 mg/100 g FW (Kubola and Siriamornpun, 2011; Wihong et al., 2014). The average weight of fruit in Vietnam is higher (about 710 g with 125 g (18%) in the aril) than in Thailand where it is only about 438 g but with a higher percentage of aril (21% or 110 g) according to Banchong et al. (2010). Moreover, the lycopene content in the gac aril has been

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recognized as high as 70 times that in tomatoes (Burke et al., 2005) which are the major source of lycopene in the Western diet.

The molecular structures of lycopene and β-carotene are arranged in many forms which have conjugated double bonds in the chain resulting in powerful antioxidant performance (Basuny, 2012). Oxidation reactions in the human body are caused by free radicals and reactive oxygen species (Lobo et al., 2010). However, while the protection mechanism in the human body is able to build up its own antioxidants, under certain conditions, the amount of free radicals may exceed the level that the human system can control which leads to extensive cellular damage, autoimmune disease, cancer or finally, aging (Pham-Huy et al., 2008). Thus, food consumption containing high level of antioxidants can enhance the protection system in the body (Lobo et al., 2010). The gac aril provides an acceptable and natural food source of high levels of valuable antioxidants that are inexpensive and bio-available (Tien et al., 2005; Kubola and Siriamornpun, 2011).

The large variation in the lycopene and β-carotene contents in the gac aril could be caused by many factors such as the plant variety, climate or harvesting season, harvesting stage, stage of maturity, growing location or geographic site and even by the fertilizer used (Maiani et al., 2009). Therefore, it is possible to obtain different levels of lycopene and β-carotene content in the different countries where it is cultivated. Moreover, little information has been published about the change in the lycopene and β-carotene contents in the gac aril after harvesting or during storage. Nhung et al. (2010) conducted research on lycopene and β-carotene content analyses from gac fruit cultivated in Vietnam with 2 weeks storage time after harvesting. However the analyses were conducted 1 week apart and this would appear to be too long a period to satisfactorily predict the possible change which could occur within a week.

Due to the very high contents of lycopene and β-carotene in the gac aril, attempts have been made to find a suitable preparation method to extract the lycopene and β-carotene with the least variation. One of the factors that might affect the lycopene and β-carotene contents during extraction is the method used in the sample preparation which can provide different degrees of fineness. Grinding a sample using a Waring blender (the WB method or WBM) is easier when the gac aril is in the fully ripe stage and has a soft texture but the tough fiber can cause difficulties. Thus, the assumption should be investigated that such samples could be ground into finer particles with a ball mill (the BM method or BMM) after the sample has been frozen using liquid nitrogen. The objectives of this research were to study the effect of three different harvesting stages of gac fruit, the storage time and the sample preparation method before extraction on the lycopene and β-carotene contents in the gac aril from different varieties of gac fruit cultivated in central Thailand.

## Materials and methods

### *Plant material and sample preparation*

A sample of 46 kg of gac fruits, with an average weight of 0.77 kg, were selected and purchased from a garden in Plug Mai Lai, Tungkoung sub-district, Kamphaeng Saen district, Nakhon Pathom province, Thailand in February 2012. The fruits were classified into three groups according to harvest stage—color break, medium ripe and fully ripe. The color break fruit were an overall light green while the medium ripe fruit had a yellow or orange skin over less than one-third to two-thirds of each fruit's surface, respectively (Fig. 1). A fully ripe fruit had more than two-thirds of the fruit already red. After being harvested, the fruits were stored at 26 ± 1 °C and 24 ± 1% RH in an air conditioned room. Each fruit was measured for weight, the number of seeds and the weight of the arils before undergoing lycopene and β-carotene analysis.

All gac fruits were stored for 15 d with sampling carried out on days 0, 3, 6, 9, 12 and 15 storage. At each sampling time, three fruits were randomly taken from each harvested group to provide three replications for each analysis. Arils were obtained by first cleaning each gac fruit with tap water and leaving to air dry. Then, the seeds were removed and the arils were meticulously separated.

The arils from of all gac seeds in each fruit were divided into two parts. Part I was minced using the WBM with a Waring blender (Maxi Chopper, Tomex T-1128; China) for 5 min at a power of 750 W. The other part was initially frozen in liquid nitrogen for about 3 min before using the BMM with grinding in a ball mill (Retsch® MM 301; Germany) at a frequency of 30 cycles/s for 30 s.

### *Lycopene and β-carotene analysis*

One gram of minced or ground gac aril was put in a test tube. Then, 10 mL of the mixed solvents of acetone and hexane in the ratio 4:6 (volume per volume) were added and mixed well using a spatula. Two other concentrations of extracted matter were made in the same way by adding 14 mL and 18 mL, respectively, of mixed solvent to 1 g of minced arils. The dilutions were selected to be just below the capability of the absorption range of the UV–visible spectrophotometer (Thermo Spectronic, GENESYS 10 UV–Vis; USA).

Before performing the measurement according to Kimura's method (Nagata and Yamashita, 1992), each dilution of extracted gac arils was homogenized using a homogenizer (Polytron®, PT-MR 2100; Switzerland) at 15,000 rpm for 1 min. Then, the light absorption values ($A$) at 453, 505, 663 and 645 nm wavelength were recorded for the determination of the lycopene and β-carotene contents in each sample. Eqs. (1) and (2) were used to calculate the lycopene and β-carotene contents in milligrams per 100 mL of mixed solvent. The obtained values then were calculated further to be based on 100 g of fresh gac aril (FW).

$$\textit{Lycopene}\ (\text{mg}/100\ \text{mL}) = -0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453} \quad (1)$$

$$\beta - \textit{carotene}\ (\text{mg}/100\ \text{mL}) = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453} \quad (2)$$

The calculated values in Eqs. (1) and (2) obtained from a dilution with more than 10 mL solvent were adjusted back to be based on the concentration of 10 mL solvent.

### *Statistical analysis*

The lycopene and β-carotene contents obtained were subjected to ANOVA from the different harvested stages, storage times and methods of preparation and the mean values were compared using Duncan's new multiple range test (DMRT) at the 95% confidence level as well as being used in multiple linear regression analysis using the SPSS software program version 16 (SPSS Inc., Chicago, IL, USA).

## Results and discussion

### *Preparation and characteristics of gac fruit*

Fig. 1 shows the appearance of half-cut gac arils according to the harvesting stage-color break, medium ripe and fully ripe. Generally the gac aril had a thickness of about 1–3 mm, the color in the color

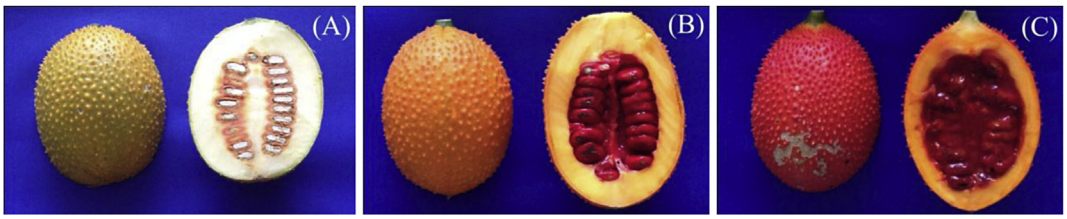


**Fig. 1.** Whole gac fruit and a half section at three harvested stages: (A) color break; (B) medium ripe; (C) fully ripe.

break stage being white and translucent and similar to the color of pulp. Additionally, the seed coat of fruit at this stage was rather soft so that it was not easy to completely separate the gac aril from the pulp and seed coat. However the aril texture was quite different from the pulp and seed coat and could be differentiated visually during separation. At the medium ripe stage, the gac aril was yellow and it seemed easier to separate the yellow aril from the white-colored pulp and seed coat, though the fruit pulp was still firmly attached to the aril. At the fully ripe stage, the gac aril was orange-red and distinguishable from the light yellow pulp. In addition, the seed coat was a soft sheet resulting in complete separation of the aril with no part of the fruit pulp and seed coat mixed in. Therefore, no attempt to determine the weight of the gac aril at each harvest stage was carried out.

*Variation in lycopene content during storage*

*Gac fruit at color break stage*

From Table 1, it was found that for the WBM, the lycopene content slowly increased until 12 d of storage and then rapidly and significantly increased until the end of storage (15 d). The BMM resulted in the lycopene content increasing with a similar trend and the lycopene contents during 0–15 d storage were not significantly different. A comparison between the preparation methods on each storage day showed no significant differences in the lycopene content. Therefore, the BMM seemed unable to extract more lycopene from gac arils than the WBM. This could be explained by the fact that there is a large variation in the lycopene content within each fruit and between fruits (Nhung et al., 2010). In addition, since the WBM used shear force to cut the particles into small pieces, it was also suitable to mince the soft and uncohesive texture of the gac aril at this stage of development (Henderson and Perry, 1955). The BMM, which changed the condition of the sample from soft and uncohesive to a frozen, solid stage before pulverizing, could not provide any advantage over the WBM with gac arils at the color break stage.

When considering the change in the lycopene content from samples prepared by the WBM during the 15 d storage as shown in Fig. 2A, the lycopene content at day 0 initially was very low (0.11 ± 0.02 mg/100 g FW). Then, it increased slowly up to 9 d storage. During this period, the respiration rate of fruit at the color break stage probably gradually increased due to early maturity after harvesting. The fruit which was harvested at early maturity tended to have little metabolic change as the ripening state was not completed and this resulted in low ethylene production to induce the ripening progress as well as improper biochemical change in the fruit (Wills et al., 1998).

During storage from 12 to 15 d, the lycopene content increased rapidly due to the ripening progress and the higher respiration rate in this period even though the fruit had been harvested in the early maturity state. This is a typical characteristic of climacteric fruit such as banana, tomato, mango and papaya (Wills et al., 1998). The chemical composition in fruit such as the lycopene content, sugar content and chlorophyll, as well as the fruit physiology changed substantially with degradation of the chlorophyll and creation of carotenoid (Cantwell and Reid, 1993). Thus, to obtain the highest lycopene content in these fruits at the color break stage, the fruit should be kept in storage for longer than 15 d after harvesting. However, at about 10 d storage, the fruit started to shrivel and show signs of rot on the peel (Fig. 3A).

*Gac fruit at medium ripe stage*

As at the color break stage, no significant difference was found in the lycopene content prepared by the two different methods (WBM versus BMM) during storage for 15 d (Table 1). The pattern of the lycopene content prepared using the WBM, as shown in Fig. 2B, was similar to that found in the gac arils of fruit at the color break stage but at a higher level throughout the profile until day 15. This could have been as a result of the greater stage of maturity when the fruit was harvested. Thus, the lycopene content which was the product of the ripening progress demonstrated the change associated with the respiratory climacteric rate in the more mature state. However, for both these harvesting stages, the maximum lycopene content was not reached within 15 d storage. In addition, a period of 15 d storage was sufficient for gac fruit at the medium ripe stage to show physical symptoms of aging such as shriveling and some rot (Fig. 3B).

**Table 1**
Mean lycopene content ± SE (milligrams/100 g fresh weight) in gac arils at harvesting stage using Waring blender method (WBM) and ball mill method (BMM).

| Storage time (d) | Color break stage | | Medium stage | | Fully ripe stage | |
|---|---|---|---|---|---|---|
| | WBM | BMM | WBM | BMM | WBM | BMM |
| $0^{ns}$ | $0.11 \pm 0.02^{b}$ | $0.33 \pm 0.13^{a}$ | $3.88 \pm 0.55^{d}$ | $6.25 \pm 1.27^{c}$ | $19.74 \pm 1.28^{f}$ | $18.95 \pm 0.80^{e}$ |
| $3^{ns}$ | $0.50 \pm 0.12^{b}$ | $0.39 \pm 0.11^{a}$ | $4.52 \pm 0.99^{d}$ | $6.06 \pm 1.27^{c}$ | $30.24 \pm 1.40^{d}$ | $31.64 \pm 3.52^{c}$ |
| $6^{ns}$ | $2.62 \pm 0.48^{b}$ | $2.01 \pm 0.37^{a}$ | $9.01 \pm 0.51^{c}$ | $9.18 \pm 0.39^{bc}$ | $49.61 \pm 1.36^{a}$ | $50.11 \pm 1.59^{a}$ |
| $9^{ns}$ | $3.35 \pm 1.36^{b}$ | $3.47 \pm 1.42^{a}$ | $9.64 \pm 1.18^{c}$ | $9.45 \pm 0.78^{bc}$ | $43.46 \pm 0.89^{b}$ | $43.70 \pm 1.08^{b}$ |
| $12^{ns}$ | $2.54 \pm 0.67^{b}$ | $2.98 \pm 0.91^{a}$ | $14.26 \pm 1.61^{b}$ | $12.52 \pm 0.97^{b}$ | $33.41 \pm 2.39^{c}$ | $34.38 \pm 1.15^{c}$ |
| $15^{ns}$ | $8.99 \pm 4.33^{a}$ | $4.37 \pm 2.06^{a}$ | $21.05 \pm 2.21^{a}$ | $22.94 \pm 3.92^{a}$ | $25.43 \pm 1.61^{e}$ | $28.00 \pm 2.51^{d}$ |
| Average | $3.02 \pm 0.83$ | $2.26 \pm 0.47$ | $9.77 \pm 0.90$ | $9.79 \pm 0.84$ | $33.85 \pm 1.60$ | $34.63 \pm 1.63$ |

$^{a-f}$ = superscript lowercase letters in columns indicate a significant ($p \leq 0.05$) difference at each storage time using Duncan's new multiple range test.
$^{ns}$ = superscript indicate a non–significant ($p > 0.05$) difference due to preparation method (WBM and BMM) by Student's *t* test.

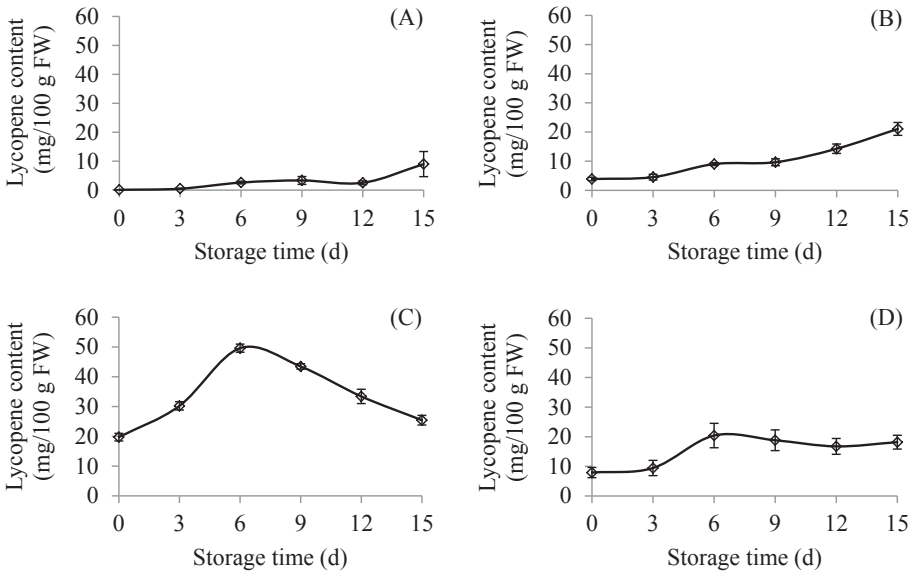


**Fig. 2.** Trend of lycopene content in gac arils prepared by Waring blender method during 15 d storage according to different harvest stages: (A) color break stage; (B) medium ripe stage; (C) fully ripe stage; (D) mixed stage. Values are expressed as mean ± SE; FW = fresh weight.

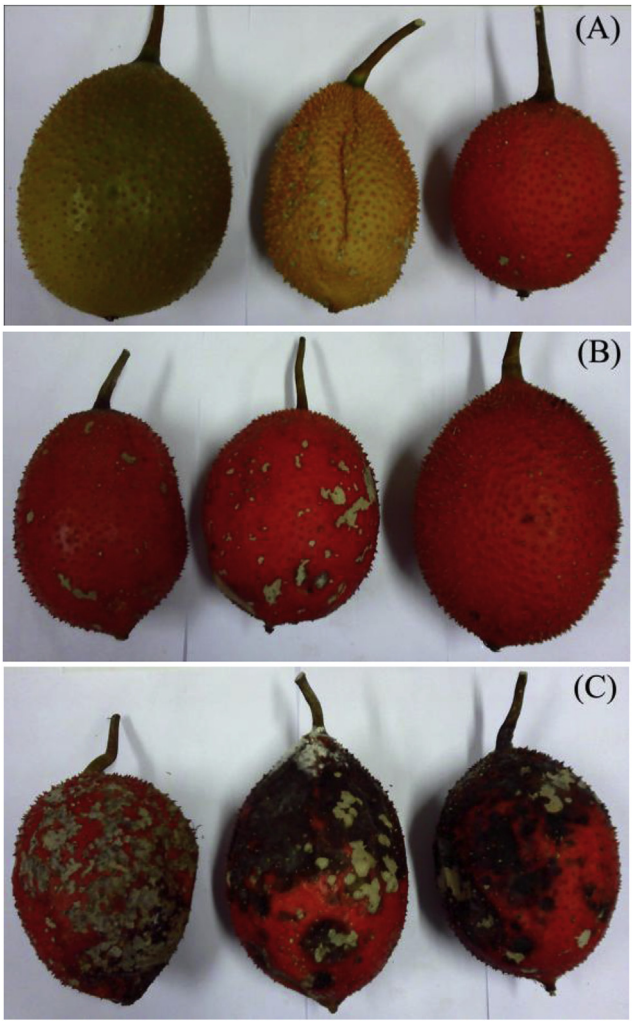


**Fig. 3.** Appearance of gac fruits at storage time on 15 d after storage: (A) color break stage; (B) medium ripe stage; (C) fully ripe stage.

*Gac fruit at fully ripe stage*

From Fig. 2C, the lycopene content of gac arils at the fully ripe stage demonstrated variation corresponding to the climacteric respiratory pattern more clearly than at the two earlier harvesting stages. When fruit was fully matured at harvesting, the physical development of the fruit through cell division and enlargement was complete. Thus, during ripening, it would be usual for the respiration rate to increase sharply to its maximum. Major changes in the fruit were seed maturation, color changes, ethylene production, softening of the texture, protein and organic acid changes as well as the production of flavor volatiles. The disappearance of chlorophyll in the fruit is often associated with the synthesis or revelation of pigments ranging from yellow to red. Many of these pigments are carotenoids which include lycopene (Khoo et al., 2011). Carotenoids are stable compounds and remain intact in the tissue even when extensive senescence has occurred (Wills et al., 1998). Therefore variation in the lycopene content could be one of the outcomes of the biochemical changes in the gac fruit during the maturation to senescence stages. Table 1 shows that the maximum lycopene content was 49.61 ± 1.36 mg/100 g FW and 50.11 ± 1.59 mg/100 g FW on day 6 of storage when prepared by the WBM and BMM, respectively, and it then decreased until day 15 of storage. There was no significant difference in the lycopene content between the two preparation methods throughout the 15 d storage. In addition, on day 10 of storage the fruit started to shrivel, and rot was evident on the peel. Therefore, to obtain the maximum benefit from the lycopene, the fruit should be harvested at this stage and stored at 26 ± 1 °C and 24 ± 1% RH for 6–9 d.

*Lycopene content variation model*

Models of the lycopene content variation associated with 15 d storage were developed using nonlinear regression analysis corresponding to the three harvesting stages (Table 2). It was found that when the harvesting fruits were at the color break and medium ripe stage, exponential models as shown in Eqs. (3) and (4) provided good estimates of the variation in the lycopene content (coefficients of determination value ($R^2$) of 0.84 and 0.97, respectively, and standard error of the estimate (SEE) of 16.21 and 9.87 for validation, respectively). The only suitable model of fruit already at the fully ripe stage was a polynomial as shown in Eq. (5). However

**Table 2**
Predicted equations of lycopene content in gac arils using a Waring blender as preparation method.

| Ripeness group | Equation[a] | Coefficient of determination | Standard error of estimate | Equation number |
|---|---|---|---|---|
| Color break | $y = 0.218e^{0.256x}$ | 0.84 | 16.21 | (3) |
| Medium ripe | $y = 3.733e^{0.114x}$ | 0.97 | 9.87 | (4) |
| Fully ripe | $y = -0.416x^2 + 6.555x + 18.87$ | 0.87 | 35.69 | (5) |
| Mixed ripe | $y = -0.104x^2 + 2.249x + 7.001$ | 0.77 | 16.09 | (6) |

[a] $x$ refers to storage day and $y$ refers to lycopene content (milligrams/100 g fresh weight) after harvesting and $n = 9$ for Eqs. (3)–(5) and $n = 27$ for Eq. (6).

when fruit that could not be classified into a harvesting stage was detached from the tree or was considered to be at a mixed stage of ripening, the polynomial model in Eq. (6) was more appropriate with a moderate $R^2$ value of 0.77 (SEE of 16.09 for validation).

*Variation in β-carotene content during storage*

*Effect of harvesting stage*

From Table 3, the average β-carotene content from gac arils obtained from gac fruit at the color break stage during 15 d storage was the lowest (about 1.20 ± 0.46 mg/100 g FW) and increased at the medium stage and reached its maximum (28.29 ± 1.01 mg/100 g FW) at the fully ripe stage. This was probably due to the fact that when fruits are harvested at a more mature stage, the physical and biochemical changes in cell structure (such as cell division or enlargement) were complete. These results at normal ripening involved symptoms of chlorophyll degradation, pigment accumulation, aroma development, cell wall loosening and texture change.

The β-carotene content in the arils of climacteric fruit like gac after harvesting (day 0) at the color break stage was very low (0.002 ± 0.00 mg/100 g FW) and almost zero. During this time, there was only a little evolution of ethylene. The pigment probably had not developed based on the white color of the flesh (pulp) and the arils in the fruit. However, after storage at 26 ± 1 °C and 24 ± 1% RH for 12 d, there was a very small increment which then increased significantly up to 4.82 ± 2.40 mg/100 g FW on day 15. Thus, ripening at the early maturity of the color break stage resulted in the physical and biochemical changes not being at the maximum climacteric peak. This in turn could result in less pigment of β-carotene and aroma development, incomplete chlorophyll degradation and less red pigment accumulation as well as causing eventual shriveling.

The fruits shown in Fig. 4B were probably harvested early in the maturity stage or in the pre-climacteric stage due to the small amount of β-carotene (0.58 ± 0.15 mg/100 g FW) found at day 0 or after harvesting. The flesh (pulp) and the arils of the fruit in this period were pale yellow. During storage for 12 d, the β-carotene content increased slowly at first and then sharply after day 9 to reach its maximum at day 12. This could be explained by the fact that as in the normal life of the plant, ethylene production is induced during certain stages of growth, including fruit ripening (Saltveit, 1999). The ripening of fruit on day 9 accelerated the production of ethylene. The ethylene production reached a maximum on day 12 and then reduced (Tran et al., 2016). The senescence mechanism probably started after day 12 which resulted in the reduction of β-carotene could be observed by the appearance of shriveled fruit on day 15.

The fruits harvested in the fully ripe stage (Fig. 4C) seemed to have been detached from the tree during the pre-climacteric stage but very close to the occurrence of the climacteric stage on the tree due to the very high amount of β-carotene (30.07 ± 2.84 mg/100 g FW) found on day 0. In addition this high value of β-carotene could be associated with a high level of ethylene production and a high respiration rate as a result of the bruising or wounding, water loss and stress during fruit detachment (Nakano et al., 2003) as well. Then, changes in the chemistry and physiology of the gac fruit continued as the fruit entered the ripening stage and the climacteric peak or maximum respiration rate at day 6. The closer the pre-climacteric fruit is picked to the occurrence of the climacteric stage on the tree, the shorter the time from harvest until ethylene is produced (Kupferman, 1986). The time to the climacteric peak of fully ripe fruit was shorter than that of fruit at either the medium ripe or color break stage. However, the β-carotene content was increased again and was highest on day 15 or during senescence, which was not the case with lycopene, which decreased. This pattern was similar to the change in Cavendish banana (Wills et al., 1998), since when skin spotting on the peel color (stage 7) occurred, the β-carotene content also increased again.

From Table 3, the β-carotene content from gac arils at the fully ripe stage was highest at 38.51 ± 1.42 mg/100 g FW when the fruit exhibited rot on the peel. This value in gac arils was higher than that proposed by Vuong et al. (2002, 2006) and Aoki et al. (2002) but less than that reported by Ishida et al. (2004). This was probably due to different sample pretreatments before extraction and the carotenoid extraction method used in the quantitative analysis apart from differences in the harvesting season and stage as well as the cultivar. Nevertheless, the highest value of β-carotene obtained was close to that reported by Nhung et al. (2010).

*Comparison between sample preparation methods*

The effect of sample preparation on the β-carotene content was investigated based on the different degrees of sample fineness

**Table 3**
Mean β-carotene content ± SE (milligrams/100 g fresh weight) in gac arils at harvesting stage using the Waring blender method (WBM) and ball mill method (BMM).

| Storage time (d) | Color break stage | | Medium stage | | Fully ripe stage | |
|---|---|---|---|---|---|---|
| | WBM | BMM | WBM | BMM | WBM | BMM |
| 0 | $0.002 \pm 0.00^{bA}$ | $0.05 \pm 0.03^{aA}$ | $0.58 \pm 0.15^{dA}$ | $0.31 \pm 0.16^{cA}$ | $30.07 \pm 2.84^{bA}$ | $31.12 \pm 2.97^{bA}$ |
| 3 | $0.02 \pm 0.02^{bA}$ | $0.10 \pm 0.05^{aA}$ | $0.89 \pm 0.24^{dA}$ | $3.17 \pm 0.70^{bB}$ | $26.04 \pm 3.00^{bcA}$ | $24.36 \pm 2.18^{cA}$ |
| 6 | $0.20 \pm 0.18^{bA}$ | $0.30 \pm 0.15^{aA}$ | $3.67 \pm 0.83^{cA}$ | $4.04 \pm 0.91^{bA}$ | $27.56 \pm 0.58^{bcA}$ | $33.74 \pm 1.97^{bB}$ |
| 9 | $1.21 \pm 0.62^{bA}$ | $1.50 \pm 0.75^{aA}$ | $2.46 \pm 1.00^{cdA}$ | $4.97 \pm 1.08^{bA}$ | $22.68 \pm 1.21^{cA}$ | $23.74 \pm 1.23^{cA}$ |
| 12 | $0.94 \pm 0.27^{bA}$ | $0.90 \pm 0.34^{aA}$ | $13.59 \pm 0.89^{aA}$ | $12.59 \pm 0.47^{aA}$ | $24.14 \pm 0.56^{cA}$ | $25.67 \pm 0.69^{cA}$ |
| 15 | $4.82 \pm 2.40^{aA}$ | $1.43 \pm 0.84^{aA}$ | $6.26 \pm 1.00^{bA}$ | $5.62 \pm 1.89^{bA}$ | $38.51 \pm 1.42^{aA}$ | $39.16 \pm 1.29^{aA}$ |
| Average | 1.20 ± 0.46 | 0.71 ± 0.21 | 4.48 ± 0.71 | 5.09 ± 0.64 | 28.29 ± 1.01 | 29.94 ± 1.07 |

$^{a–d}$ = superscript lowercase letters in columns indicate a significant ($p \leq 0.05$) difference at each storage time using Duncan's new multiple range test.
$^{A–B}$ = superscript uppercase letters in rows indicate a significant ($p \leq 0.05$) difference due to preparation method (WBM and BMM) by Student's $t$ test.

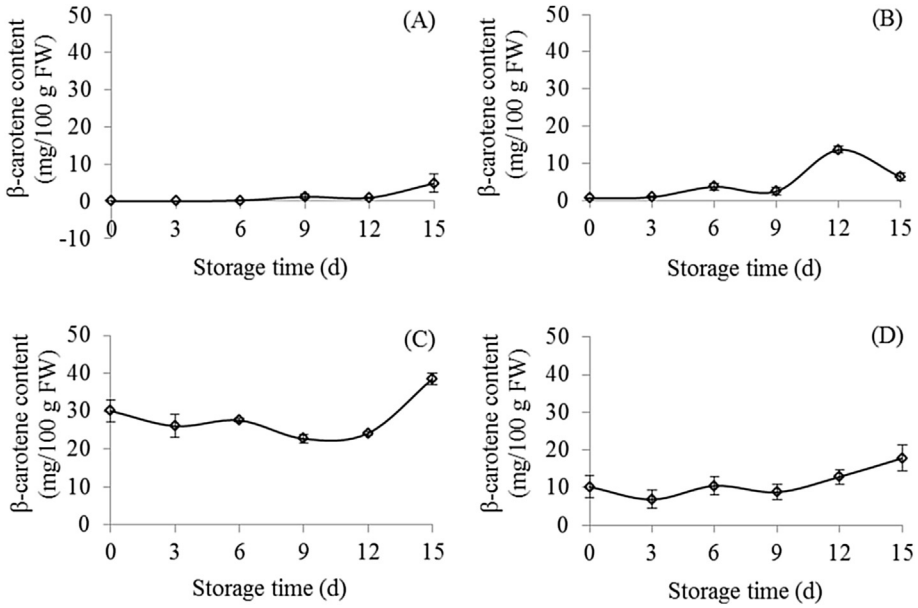


**Fig. 4.** Trends of β-carotene content in gac arils prepared using the Waring blender method during 15 d storage according to different harvest stages: (A) color break stage; (B) medium ripe stage; (C) fully ripe stage; (D) mixed harvesting stage. Values are expressed as mean ± SE; FW = fresh weight.

**Table 4**
Predicted equations of β-carotene contents in gac arils using a Waring blender as preparation method.

| Ripeness group | Equation[a] | Coefficient of determination | Standard error of estimate | Equation number |
|---|---|---|---|---|
| Color break | $y = 0.004e^{0.500x}$ | 0.92 | 6.99 | (7) |
| Medium ripe | $y = 0.665e^{0.187x}$ | 0.79 | 9.83 | (8) |
| Fully ripe | $y = 0.025x^3 - 0.397x^2 + 0.743x + 29.28$ | 0.90 | 12.00 | (9) |
| Mixed ripe | $y = 0.086x^2 - 0.772x + 9.867$ | 0.89 | 6.39 | (10) |

[a] $x$ refers to storage day and $y$ refers to β-carotene content (milligrams/100 g fresh weight) after harvesting and $n = 9$ for Eqs. (3)–(5) and $n = 27$ for Eq. (6).

when using the WBM versus the BMM. From Table 3, it was found that the β-carotene contents in gac arils which had been ground using either of the two methods, when sampled during storage for 15 d were not significantly different, regardless of the harvesting stage. This result could be explained by the fact that during extraction using the mixed solution of hexane and acetone, the extraction performance was enhanced by using minced particles. The degree of fineness of the particles obtained when using the Waring mincer (WBM) for 5 min was sufficient and there was no significant difference in the β-carotene content extracted even when finer samples were used from BMM. Therefore the analysis for β-carotene using the spectrophotometric method was not sensitive to finer particles than those obtained from the WBM at all harvesting stages of the gac fruit.

*Prediction of β-carotene*

Equations to predict β-carotene during storage at 26 ± 1 °C and 24 ± 1% RH according to the harvesting stage are shown in Table 4. They could be used to predict the β-carotene content quite well except for Eq. (8) at the medium ripe stage of fruit since the coefficients of determination were greater than or equal to 0.79. For the mixed harvesting stage fruit, as shown in Fig. 4D and Eq. (10) was able to predict β-carotene as well.

The peak of the lycopene content (50.11 ± 1.59 mg/100 g FW) was found in gac fruit at the fully ripe stage during harvesting after 6 d storage at 26 ± 1 °C and 24 ± 1% RH. However the highest value of β-carotene was from fully ripe fruits which became rotten with spoilage after about 10 d storage. In addition, the grinding methods (WBM and BMM) had no significant effect on these two phytonutrients. Predicted equations for the lycopene and β-carotene contents were obtained when fruits were classified according to harvesting stages with $R^2$ values in the range 0.84–0.97 and 0.79–0.92, respectively.

## Conflict of interest statement

The authors declare that there is no conflict of interest.

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