Agriculture and Natural Resources xxx (2016) 1–7

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original Article

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

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ARTICLE INFO

Article history:
Received 5 May 2015
Accepted 29 April 2016
Available online xxx

Keywords:
Aril
β-carotene
Gac fruit
Harvesting stage
Lycopene
Prediction

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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http://dx.doi.org/10.1016/j.anres.2016.04.003

Please cite this article in press as: Bhumsaidon, A., Chamchong, M., Variation of lycopene and beta-carotene contents after harvesting of gac fruit and its prediction, Agriculture and Natural Resources (2016), http://dx.doi.org/10.1016/j.anres.2016.04.003

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 $24 \pm 1\%$ 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

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