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Application of Ultrasound for Lycopen and Beta-Caroten Extraction in Gac Fruit *Momordica Cochinchinensis* Spreng.)

Nguyen Phuoc Minh[1,*], Tan Thanh Vo[2], Truong Nghia Trong[3], Dang Van Son[4], Luong Nguyet Chau[5], Nguyen Thuy Ha[6]
[1]*Faculty of Chemical Engineering and Food Technology, Nguyen Tat Thanh University, Ho Chi Minh, Vietnam*
[2]*NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam*
[3]*Can Tho University, Can Tho City, Vietnam*
[4]*An Giang University, An Giang Province, Vietnam*
[5]*Tien Giang University, Tien Giang Province, Vietnam*
[6]*Bac Lieu University, Bac Lieu Province, Vietnam*

Abstract.
Gac fruit (*Momordica cochinchinensis* Spreng.) has a rich source of bioactive compounds including lycopen and beta-caroten. Various parameters influencing to lycopen and beta-carote extraction were examined including blanching, solvent, solvent: solid, extraction time and temperature under ultrasound, carrier and temperature for drying. Optimal results were clearly shown that the Gac pulp should be blanched under 95°C in 10 seconds; extraction should be conducted by ethanol 90%, ethanol: solid (2.0:1.0), 60°C, 8 minutes; vaccum drying should be executed at 60°C with the support of carrier (0.5 maltodextrin: 0.5 gelatin: 1.0 Gac). Ultrasonic had positive effect on lycopen and beta-caroten extraction and this method could be utilized to optain the most lycopen and beta-caroten powder.
Keywords: *Gac, lycopen, beta-caroten, ultrasonic, blanching, extraction, carrier, drying*

I. INTRODUCTION

Natural colorants have the potential to be used as acceptable additives in foods as they are natural and they also have potential health benefits. However, natural pigment compounds usually have poor stability as compared to artificial food colorants, which hinders their usage. Carotenoids are red, orange, or yellow colored oil-soluble terpenoid compounds located in the chloroplasts and chromoplasts of plants and they have many functions in plants, including light-harvest and photoprotection. When ingested, carotenoids have vitamin A activity, and they have antioxidative properties related to many health benefits, as reviewed by, for example, Krinsky (1989), Kiokias & Gordon (2004) and Jomova & Valko (2013). Oxidation of carotenoids can be initiated by many oxidizing agents and the oxidation may process via different mechanisms. In food products, carotenoids are very sensitive to heat so processes involving heat treatments should be kept at minimum both for temperature and time, and storage in frozen temperatures is advised for maximum carotenoid stability (Abdel-Aal et al. 2010, Cerón-García et al. 2010, Rubio-Diaz et al. 2010, Wenzel et al. 2010, Cervantes-Paz et al. 2014).

Momordica cochinchinensis Spreng., is called "gac", and the seed membrane (seed pulp or aril) of the ripe fruit is widely used as a rice colorant due to its intense red color from its high carotenoid content. Total carotenoid concentrations (±standard deviation) were 497 (±154) μg/g fresh material with lycopene dominating and exceeding beta-carotene concentrations by a factor of approximately five (408 μg/g versus 83 μg/g). The alpha-tocopherol concentration in the pulp was 76 μg/g (Le ThuyVuong et al., 2006). Carotenoids from gac fruit aril (*Momordica cochinchinensis*[Lour.] Spreng.) are more bioaccessible than those from carrot root and tomato fruit (JudithMüller-Maatsch et al., 2017). Fruit stored at 4ºC showed both external and internal chilling injury symptoms during storage (Soe Win et al., 2015). The aril of the Gac fruit is processed and the peel is discarded although it contains high levels of carotenoids and phenolic compounds, which could be extracted for commercial use (Hoang V. Chuyen et al., 2017).

Dang Thi Tuyet Nhung et al., (2009) observed changes in carotenoid contents (lycopene and beta carotene) in gac aril. Carotenoid concentrations in the aril remained stable after 1 week but sharply declined after 2 weeks of storage.

Le Khac Lam Dien et al., (2013) had a study to develop an understanding of suitable conditions for the processing of Gac fruit, with three pretreatment methods: blanching, blanching in citric acid solution and steaming; as well as to investigate the different ratioes of carrier material to find out which one is the adequate ratio to protect carotene in Gac powder.The result shows that steaming in 6 minutes is the best pretreatment method for the protection and maintenance of total carotenoid content in gac powder; and the most appropriate ratio of carrier: Gac is 1: 1 (dry matter) in which the ratio of maltodextrin: gelatin is 0.5: 0.5 (w/w). Le Khac Lam Dien et al., (2014) investigated the changes of total carotenoids content of Gac powder product in accelerated temperature to find out the appropriate temperature and shelf-life of product storage. The result shows that total carotenoid content maintains at 70% in comparing with the beginning content in three months at 10°C or five months at 5°C in condition of absent oxygen and light. Carotenoids concentration of gac (*Momordica cochinchinensis* Spreng.) fruit oil was produced using cross-flow filtration technology (Huynh Cang Mai et al., 2014).

Gac fruit pulp was dried by different drying methods including tray drying (40–60 °C), heat pump-assisted dehumidified drying (40–60 °C), microwave drying (450–900 W), mixed-mode solar drying and freeze drying. The Modified Henderson model presented the best fit of desorption isotherms. New model proposed was the best drying model. Quality evaluation by β-carotene, lycopene, lutein, total phenolics and antioxidant activity revealed that heat pump-assisted dehumidified drying at 60 °C provided the highest lutein, total phenolics and antioxidant activity and could reduce drying time by 25 % and increased lutein, total phenolics and antioxidant activity by 12.6 %, 32.0 % and 0.3 %, respectively and is more promising drying method for gac fruit pulp (Wittawat Trirattanapikul, Singhanat Phoungchandang, 2016). Extraction of lycopene from gac fruit (*Momordica cochinchinensis* spreng) and preparation of nanolycopene was investigated. The results showed that the suitable drying temperature for Gac aril was 60-70 ^{o}C. The suitable solvents for lycopene extraction were dichloromethane or chloroform. The lycopene content in dried Gac aril is about 0.28-0.46 %. Nanolycopene was prepared successfully by freeze-dried method, with relatively small particles size of 40-60 nm. Nanolycopene is relatively stable in the inert environment in the presence of antioxidants such as butylated hydroxytoluene (BHT) (Ho Thi Oanh et al., 2017).

In the present study, we examined different parameters influencing to lycopen and beta-caroten extraction such as blanching, solvent, solvent: solid, extraction time and temperature under ultrasound, carrier and temperature for drying.

II. MATERIALS AND METHOD

2.1 Material

We collected Gac fruit in Mekong river delta, Vietnam. They must be cultivated following VietGAP to ensure food safety. After harvesting, they must be conveyed to laboratory within 8 hours for experiments. Fruits was washed thoroughly under turbulent washing to remove dirt, dust and adhered unwanted material. Besides Gac fruits we also used other materials during the research such as ethanol, acetone, ethyl acetate, maltodextrin, gelatin, DMSO. Lab utensils and equipments included oven, ultrasonicator, vaccum dryer, centrifugator, water bath.

Figure 1. Gac (*Momordica cochinchinensis*)

2.2 Researching procedure

2.2.1 Effect of blanching on lycopen and beta-caroten recovery (%)

Different blanching conditions were performed (100^{o}C, 5 sconds; 95^{o}C, 10 seconds; 90^{o}C, 15 seconds; 85^{o}C, 20 seconds) to verify the effectiveness (% recovery) of blanching to lycopen and β-caroten extraction.

2.2.2 Effect of different solvents on lycopen and beta-caroten recovery (%)

Different solvents (water, ethanol, acetone, ethyl acetate) were used to examine the effectiveness (% recovery) of lycopen and β-caroten extraction.

2.2.3 Effect of solvent ratio: material on lycopen and beta-caroten recovery (%)

Different ratios of solvent: solid (1.0:1.0, 1.5:1.0; 2.0:1.0; 2.5:1.0; 3.0:1.0) were verified to demonstrate the effect of ratio between solvent and material on the effectiveness (% recovery) of lycopen and β-caroten extraction.

2.2.4 Effect of extraction temperature by ultrasonic combination with solvent on lycopen and beta-caroten recovery (%)

Different temperature conditions (30^{o}C, 40^{o}C, 50^{o}C, 60^{o}C, 70^{o}C) under ultrasonic combined solvent were examined to prove the effectiveness (% recovery) of lycopen and β-caroten extraction.

2.2.5 Effect of extraction interval by ultrasonic combination with solvent on lycopen and beta-caroten recovery (%)

Different time interval of ultrasonic treatment (2 minutes, 4 minutes, 6 minutes, 8 minutes, 10 minutes) were investigated to verify the effectiveness (% recovery) of lycopen and β-caroten extraction.

2.2.6 Effect of ratio of carrier for drying

Different ratios of carrier: gac (1 maltodextrin: 1 gac; 1 gelatin: 1 gac; 0.5 maltodextrin: 0.5 gelatin: 1 gac) were demonstrated to show the effectiveness of drying to lycopen and β-caroten recovery (%).

2.2.7 Effect of drying temperature

Vaccum drying was applied at different temperature (50^{o}C, 55^{o}C, 60^{o}C, 65^{o}C, 70^{o}C) to the final moisture content of dried powder 6 ± 1%.

2.3 Physico-chemical and biological analysis

Lycopene and β-carotene (μg/g) analysis was performed by Apinya Bhumsaidon, Montip Chamchong (2016). 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.

2.4 Statistical analysis

The experiments were run in triplicate with three different lots of samples. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT). Statistical analysis was performed by the Statgraphics Centurion XVI.

III. RESULT & DISCUSSION

3.1 Phytochemical composition in Gac (*Momordica cochinchinensis*)

Phytochemical composition such as lycopen and beta-caroten in fresh Gac pulp was analyzed. Result was clearly depicted in table 1 representing that Gac pulp was suitable for utilization to collect this healthy pigment.

Table 1. Phytochemical composition in Gac (*Momordica cochinchinensis*)

Major composition	Lycopen	β-caroten
Value (μg/g)	415.29±0.03	94.45±0.02

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

3.2 Effect of blanching on lycopen and beta-caroten recovery (%)

Different blanching conditions were performed (100°C, 5 seconds; 95°C, 10 seconds; 90°C, 15 seconds; 85°C, 20 seconds) to verify the effectiveness (% recovery) of blanching to lycopen and beta-caroten. Results were depicted in table 2.

Haecheon Ahn, Eunok Choe (2015) examined the effects of blanching and drying on pigments and antioxidants of daraesoon. Blanching caused a significant (p<0.05) increase in the β-carotene content to 4,953.4 mg/kg from 4,590.5 mg/kg, compared with controls.

3.3 Effect of different solvents on on lycopen and beta-caroten recovery (%)

Different solvents (water, ethanol 90%, acetone, ethyl acetate) were used to examine the effectiveness (% recovery) of lycopen and beta-caroten extraction. Results were elaborated in table 3.

Shurook Mohammad Kadhim Saadedin et al., (2017) investigated three different organic solvents mixture, Ethanol\ethyl acetate (6:4), Hexane\ ethyl acetate\ Ethanol (2:1:1), Hexane\acetone\ Ethanol (2:1:1) and 100% Hexane to find the solvents that extracts the highest lycopene and β-carotene from Gac aril fruit powders. Result showed that the combination of ethanol / ethyl acetate (6:4) had the best extraction efficiency for lycopene (40640 μg/g) while Hexane\acetone\Ethanol (2:1:1) had the best extraction efficiency for β-carotene (2912 μg/g).

3.4 Effect of solvent ratio: material on on lycopen and beta-caroten recovery (%)

Different ratios of solvent: solid (1.0:1.0, 1.5:1.0; 2.0:1.0; 2.5:1.0; 3.0:1.0) were verified to demonstrate the effect of ratio between solvent and material on the effectiveness (% recovery) of lycopen and beta-caroten extraction. Results were elaborated in table 4.

Dipen Pandya et al., (2017) carried out the optimization of the solvent extraction process for the maximum recovery of lycopene from tomato pomace by selecting the suitable solvent system, temperature-time combination and feed to solvent ratio, i.e. Acetone: Ethyl acetate (1:1), 40°C/5hr and 1:30(w/v). The maximum lycopene was extracted using optimized solvent extraction process and had the lycopene content 611.105mg/100g, refractive index1.37604 and colour value 5.59L*, 8.00a*, 6.14b*. The storage of lycopene extract at refrigerated condition was found safe for maintaining its lycopene content. There was just 2.6% reduction in lycopene content after 60 days of storage at refrigerated condition.

Table 2. Effect of blanching to lycopen and beta-caroten extraction recovery (%)

Blanching	100°C, 5 seconds	95°C, 10 seconds	90°C, 15 seconds	85°C, 20 seconds
Lycopen extraction recovery (%)	70.21÷0.01[b]	73.30÷0.03[a]	69.11÷0.01[c]	66.19÷0.02[d]
Beta-caroten extraction recovery (%)	70.06÷0.03[b]	73.47÷0.01[a]	68.96÷0.02[c]	65.38÷0.01[d]

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 3. Effect of different solvents on lycopen and beta-caroten extraction recovery (%)

Solvent	Water	Ethanol 90%	Acetone	Ethyl acetate
Lycopen extraction recovery (%)	44.12÷0.01[d]	78.10÷0.02[a]	73.96÷0.01[c]	75.35÷0.02[b]
Beta-caroten extraction recovery (%)	44.58÷0.03[d]	78.75÷0.01[a]	73.65÷0.02[c]	75.11÷0.01[b]

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 4. Effect of solvent ratio: material on lycopen and beta-caroten extraction recovery (%)

Solvent: solid	1.0: 1.0	1.5: 1.0	2.0: 1.0	2.5: 1.0	3.0: 1.0
Lycopen extraction recovery (%)	64.98÷0.02[e]	67.79÷0.01[d]	80.27÷0.01[a]	78.11÷0.03[b]	70.34÷0.03[c]
Beta-caroten extraction recovery (%)	64.24÷0.01[e]	67.24÷0.02[d]	80.49÷0.04[a]	78.69÷0.01[b]	70.02÷0.01[c]

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 5. Effect of extraction temperature by ultrasonic combination with solvent on lycopen and beta-caroten recovery (%)

Extraction temperature (°C)	30°C	40°C	50°C	60°C	70°C
Lycopen extraction recovery (%)	67.45÷0.01[e]	73.48÷0.02[d]	77.04÷0.01[c]	81.94÷0.02[a]	80.17÷0.02[b]
Beta-caroten extraction recovery (%)	65.78÷0.01[e]	71.05÷0.03[d]	75.39÷0.02[c]	80.11÷0.01[a]	78.29÷0.01[b]

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 6. Effect of extraction interval by ultrasonic combination with solvent on lycopen and beta-caroten **recovery (%)**

Extraction time (minutes)	**2**	**4**	**6**	**8**	**10**
Lycopen extraction recovery (%)	72.19÷0.02[d]	76.05÷0.02[c]	81.07÷0.02[b]	84.18÷0.03[a]	84.41÷0.02[a]
Beta-caroten extraction recovery (%)	70.36÷0.03[d]	74.29÷0.01[c]	80.28÷0.01[b]	83.39÷0.01[a]	83.15÷0.01[a]

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 7. Effect of ratio of carrier for drying

Carrier: Gac	**1 maltodextrin: 1 gac**	**1 gelatin: 1 gac**	**0.5 maltodextrin: 0.5 gelatin: 1 gac**
Lycopen drying recovery (%)	77.03÷0.01[c]	80.14÷0.02[b]	85.49÷0.02[a]
Beta-caroten drying recovery (%)	76.48÷0.02[c]	89.27÷0.01[b]	84.75÷0.01[a]

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 8. Effect of drying temperature on lycopen and beta-caroten recovery (%)

Drying temperature (°C)	**50**	**55**	**60**	**65**	**70**
Lycopen drying recovery (%)	83.04÷0.02[d]	86.12÷0.03[b]	88.95÷0.01[a]	84.20÷0.02[c]	81.11÷0.02[e]
Beta-caroten drying recovery (%)	80.29÷0.01[d]	83.44÷0.03[b]	85.74÷0.02[a]	82.05÷0.02[c]	78.29÷0.01[e]

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

3.5 Effect of extraction temperature by ultrasonic combination with solvent on on lycopen and beta-caroten recovery (%)

Different temperature conditions (30°C, 40°C, 50°C, 60°C, 70°C) under ultrasonic (37 kHz) combined solvent were examined to prove the effectiveness (% recovery) of lycopen and beta-caroten extraction. Results were elaborated in table 5.

Seher Kumcuoglu et al., (2014) studied the extraction yield of lycopene from tomato paste processing wastes by application of ultrasound assisted extraction (UAE) was compared with conventional organic solvent extraction (COSE) method. BHT (butylated hydroxytoluene) 0.05 % (w/v) added hexane:acetone:ethanol (2:1:1) mixture was used as solvent. Three different solvent solid ratios; 50:1, 35:1 and 20:1, (v/w) were used in both COSE and UAE. COSE experiments were performed at 20 °C, 40 °C and 60 °C for 10, 20, 30 and 40 min. 50, 65 and 90 W of ultrasonic power were applied in UAE for 1, 2, 5, 10, 15, 20 and 30 min. Lycopene contents of the samples were determined by spectrophotometric method. The effects of different factors, including the temperature, solvent solid ratio and ultrasonic power on lycopene yield were investigated. It was determined that the most efficient application for COSE was extracting samples by 50:1 solvent solid ratio at 60 °C for 40 min run, for UAE, 35:1 (v/w) solvent solid ratio, 90 W ultrasonic power for 30 min run. It was showed that UAE of lycopene requires less time, lower temperature and lower solvent than COSE.

3.6 Effect of extraction interval by ultrasonic combination with solvent on on lycopen and beta-caroten recovery (%)

Different time intervals of ultrasonic treatment (2 minutes, 4 minutes, 6 minutes, 8 minutes, 10 minutes) were investigated to verify the effectiveness (% recovery) of lycopen and beta-caroten extraction. Results were elaborated in table 6.

T. Yilmaz et al., (2016) investigated the ultrasound-assisted extraction (UAE) of lycopene and β-carotene from tomato-processing wastes. Hexane: acetone: ethanol (2:1:1 v/v/v) including 0.05% (w/v) butylated hydroxyl toluene (BHT) was used as a solvent, with 1:35 w/v solid liquid ratio at 15±5°C. Ultrasonic power (50, 65, 90W) was applied in UAE for 1-30 min. Conventional organic solvent extraction (COSE) was applied under the same solvent and temperature conditions for 10-40 min. UAE was more effective and required a shorter time than COSE. Maximum lycopene and β-carotene yields were obtained using 90W ultrasonic power for 30 and 15 min, respectively.

3.7 Effect of ratio of carrier (maltodextrin) for drying

Different ratios of carrier: Gac (1 maltodextrin: 1 gac; 1 gelatin: 1 gac; 0.5 maltodextrin: 0.5 gelatin: 1 gac) were demonstrated to show the effectiveness of drying to lycopen and beta-caroten recovery (%). Results were elaborated in table 7.

Haecheon Ahn, Eunok Choe (2015) examined the effects of blanching and drying on pigments and antioxidants of daraesoon. Drying of blanched *daraesoon* caused a significant (p<0.05) loss of chlorophylls and carotenoids, and carotenoids were more affected by light than chlorophylls

3.8 Effect of drying temperature and time

Vaccum drying was applied at different temperature (50°C, 55°C, 60°C, 65°C, 70°C) to the final moisture content of dried powder 6 ± 1%. Results were elaborated in table 8.

Andrea Mendelová et al., (2013) evaluated the effect of drying temperature on changes of the content of lycopene in selected varieties of tomato. Drying was performed at 45

°C, 70 °C and 90 °C. The content of lycopene in fresh tomatoes ranged from 63.378 mg 100 g-1 of dry matter (Orange) to 302.37 mg 100 g-1 of dry matter (Šejk F1). After drying at all temperatures, content of lycopene decreased on average of 64.48 to 68.3%. The most significant changes were found at temperature of 90 °C. Based on the results the most appropriate temperature for drying among the monitored temperatures was at 70 °C, in this case the content of lycopene in the product ranged from 12.839 mg 100 g-1 dry matter (Orange) to 115.9 mg 100 g-1 of dry matter.

IV. CONCLUSION

Gac (Momordica cochinchinesis, Spreng) is a popular fruit in Vietnam. Studies on physiological effects of lycopene and beta carotene have reported that the consumption of lycopene and beta carotene can reduce the incidence of several diseases, including eye diseases, heart diseases and cancers. We have successfully optimized different aspects affecting to lycopen and beta-caroten extraction such as blanching, solvent, solvent: solid, extraction time and temperature under ultrasound, carrier and temperature for drying. Natural lycopen and beta-caroten colorant has the potential to be used as acceptable additive in foods as natural and potential health benefits.

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