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Efficient production of fatty acids and lycopene from Gac aril by flash extraction

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Abstract

The aim of the study was to develop an efficient and cost-effective method for fatty acids and lycopene separation from Gac aril by using flash extraction. The extraction efficiency (EE) of fatty acids and lycopene was investigated. When 22 mL of anhydrous ethanol per gram of solid was agitated at 3,000 rpm (revolutions per minute) for 100 s, the highest EE of fatty acids reached 88.48%, and then 98.27% was achieved after saponification refining. After fatty acid extraction, lycopene was extracted from the residues of the Gac arils, and the maximum EE of lycopene was 94.86% by using 24 mL of petroleum ether per gram of solid with agitation rate at 4,000 rpm for 40 s. 97.20% was achieved after crystallization purification. In conclusion, flash extraction is a promising approach that will enhance the utilization of Gac fruit from Gac processing for use in the food and pharmaceutical industries.

Keywords: flash extraction; gac aril; fatty acid; lycopene; extraction efficiency.

Practical Application: Production of active ingredients from Gac aril.

1 INTRODUCTION

Gac fruit (*Momordica cochinchinensis Spreng*) contains high levels of bioactive compounds, especially essential fatty acids, β-carotene, and lycopene, that are well known to be beneficial for human health (Kubola and Siriamornpun, 2011; Vuong et al., 2006). Aril of Gac fruit has high fatty acid and lycopene contents, at levels that surpass those of other main dietary plant sources. The main fatty acids (about 70%) in the Gac aril are unsaturated, and 50% of these are polyunsaturated (Vuong, 2000). Moreover, the lycopene from Gac aril emerged as one of the best lycopene compared to other well-known fruits and vegetables due to its cis structure, which is more conducive to nutrient absorption. Therefore, it is very important to exploit and enhance these effective phytochemical resources, process them for extraction, and preserve the bioactive compounds.

Previously, many studies have been made to separate bioactive compounds from Gac aril. In general, three approaches were employed. Mechanical pressing (Kha et al., 2013a; Kha et al., 2013b; Vuong, 2000; Vuong & King, 2003), supercritical carbon dioxide extraction (Akkarachaneeyakorn et al., 2017; Kha et al., 2014b; Martins et al., 2015; Tai & Kim, 2014), and organic solvent (Soxhlet method) extraction, including microwave-assisted (Honda et al., 2018; Kha et al., 2014a; Le et al., 2018; Xiang et al., 2018), enzyme-assisted (Mai et al., 2013; Nhi et al., 2016), with ultrasound-assisted (Kha et al., 2015), and without ultrasound-assisted (Aamir & Jittanit, 2017; Kubola et al., 2013; Thuat et al., 2010) extractions, were conducted. However, the products separated by these methods were all mixtures, and the separation of fatty acids and lycopene was not achieved, which limited the application of active substances in Gac aril and reduced its application value. Therefore, it is highly desirable to establish a high-efficiency alternative method for separation of fatty acids and lycopene to meet yield and quality requirements.

Flash extraction, as a type of physical breaking process, is an efficient method widely used in the pharmaceutical and food industries, benefiting from its low cost and convenience (Liu et al., 2012; Song et al., 2019). High-speed rotation of the cutter head helps break up the plant cell wall as well as generate solvent pressure to extract the bioactive molecules from the plants in a short time. Flash extraction offers a number of advantages, including shorter extraction times, ambient temperature operation, non-solvent residues, higher extraction yields, and better retention of nutritional and valuable bioactive compounds (Meng et al., 2013).

The goal of this study was to develop and optimize a process for isolation of fatty acids and lycopene from Gac aril. To determine a suitable extraction method for fatty acids and lycopene to yield the maximum extraction efficiency (EE) and cost-effectively, fatty acids and lycopene separation processes, including type of solvent, ethanol concentration, petroleum concentration, liquid-solid ratio, agitation rate, extraction time, and refining process, were investigated. This study has the potential to be applied to the inexpensive and industrial-scale production of fatty acids and lycopene.

2 MATERIALS AND METHODS

2.1 Materials

Dried Gac aril was purchased from the Guangxi Province in China (Guangxi, China); the total fatty acid and lycopene

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contents were 255.20 and 2.87 mg/g of fruit, respectively. Lycopene and methyl oleate were purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Oleic acid was purchased from Xilong Science Co., Ltd. (Guangdong, China). Linoleic acid was purchased from Shanghai McLean Biotechnology Co., Ltd. (Shanghai, China). Stearic acid was purchased from Wuhan Yuqing Jiaheng Pharmaceutical Co., Ltd. (Shanghai, China). Palmitic acid was purchased from Fande Biotechnology Co., Ltd. (Beijing, China). All the other reagents were of analytical grade and purchased from Beijing Chemical Plant (Beijing, China).

2.2 Fatty acid separation

Fatty acids are extracted from dried Gac aril with a flash extractor (JHBE-50T, Henan Zhijing Biological Technology Co., Ltd., Henan, China). For each experiment, 5 g of Gac aril were loaded, and ethanol was used at concentration ranging from 60 to 100%, liquid-solid ratio from 16:1 to 24:1 (mL/g, v:w), agitation rate from 2000 to 6,000 rpm (revolutions per minute), and extraction time from 20 to 120 s. The Gac oil was analyzed by gas chromatography-flame ionization detector (GC-FID).

2.3 Lycopene separation

Lycopene is extracted from the residues of the Gac arils, followed by fatty acid extraction. For each experiment, 5 g of the Gac aril residues were loaded, and petroleum ether was used at liquid-solid ratio from 16:1 to 24:1 (mL/g, v:w), agitation rate from 2,000 to 5,000 rpm, and extraction time from 30 to 70 s. The concentration of lycopene was analyzed by an ultraviolet spectrophotometer.

2.4 Analytical methods

2.4.1 Analysis method of fatty acid

Fatty acid concentration was determined by GC-FID (Shimadzu GC-2010, Kyoto, Japan) with a DB-FFAP (30 m × 0.32 mm, 0.5 μm; Agilent Technologies) capillary column. High-purity nitrogen was used as the carrier gas at a flow rate of 20 mL/min. The heating program was set as follows: the column temperature rises from 50 to 170°C at a rate of 20°C/min, holds for 1 min; the temperature rises from 170 to 200°C at a rate of 5°C/min, holds for 5 min; and then from 200 to 230°C at a rate of 15°C/min, holds for 5 min (Zhai et al., 2017). The detector temperature was set at 250°C. Each sample (over the range of 5–150 μg/mL) was filtered through 0.45 μm micro-membrane, and a 1.0 μL of the filtrate was loaded into the GC system for a single run. Each sample was analyzed in triplicate.

2.4.2 Analysis method of lycopene

The concentration of lycopene was determined by an ultraviolet spectrophotometer (UV2000, Unico (Shanghai) Instrument Co., Ltd., Shanghai, China). Absorbance detection wavelength of lycopene was set at 502 nm (Roldán-Gutiérrez & Luque de Castro, 2007).

3 RESULTS AND DISCUSSION

3.1 Effects of fatty acid separation

The fatty acids in the Gac aril were identified by GC-MS at the Center of Analysis, Beijing University of Chemical Technology (Beijing, China), and the results are shown in Table 1. As can be seen from the table, Gac aril has a high concentration of linoleic acid and omega-6 and omega-3 fatty acids. Linoleic acid, oleic acid, palmitic acid, and stearic acid contributed 91.68% of the total fatty acids and accounted for 27.15% of the content of the aril of the dried Gac aril. Additionally, the concentrations of each fatty acid were calculated from a standard curve made by fatty acid reference standards. The regression lines for linoleic acid, oleic acid, palmitic acid, and stearic acid were $Y_{linoleic}$=300427.8678$X_{linoleic}$+903.5712 (R^2=0.9998), Y_{oleic}=247222.1858X_{oleic}+515.4075 (R^2=0.9999), $Y_{palmitic}$=307626.0465$X_{palmitic}$+586.6831 (R^2=0.9996), and $Y_{stearic}$=509092.5260$X_{stearic}$-147.2623 (R^2=0.9956), respectively, where Y and X are the peak area in chromatograms and the concentration of fatty acids (μg/mL), respectively.

Concentrations of fatty acids and lycopene in extracted oil depend on the solvent used in the process. Considering product safety, it is necessary to select extraction solvents that can be used as food processing aids in order to minimize the impact of the production processes (Parjikolaei et al., 2015). For this reason, n-hexane, ethyl acetate, petroleum ether, and anhydrous ethanol were selected as extraction solvents. The EE of fatty acids and lycopene on different solvents is shown in Table 2. Results (Table 2) indicate that EE of fatty acids obtained from four solvents is almost the same. Compared with the EE of lycopene, n-hexane, ethyl acetate, and petroleum ether, they are significantly higher than anhydrous ethanol. This is because lycopene is very hydrophobic and has limited solubility in ethanol. Therefore, anhydrous ethanol could be used as a solvent to carry out the fatty acids in fruit aril first. Then, the residue can be further extracted with petroleum ether for lycopene to achieve efficient separation for both compounds.

Table 1. Fatty acid composition and content in Gac aril.

FAME	No. 1 (%)	No. 2 (%)	No. 3 (%)	Avg (%)
Myristic acid	0.30	0.36	0.48	0.38
Palmitic acid	25.62	20.38	22.13	22.71
Daturic acid	0.32	0.32	0.21	0.28
Stearic acid	3.70	3.46	3.39	3.52
Oleic acid	40.40	40.24	34.14	38.26
Linoleic acid	21.65	31.56	28.36	27.19
Linolenic acid	0.74	1.03	0.93	0.90
Arachidic acid	0	0.23	0.29	0.17
Docosanoic acid	0	0.32	0.32	0.21

Table 2. Effects of different extraction solvents on fatty acids and lycopene.

Extraction efficiency (%)	N-hexane	Ethyl acetate	Petroleum ether	Anhydrous ethanol
Fatty acid	80.83	86.43	87.24	83.52
Lycopene	81.93	83.71	85.76	6.39

The concentration of ethanol is an important factor that influences the extraction yields of target compounds due to its polarity. Different concentrations of ethanol (60–100%) were tested, and the results are shown in Figure 1. In Figure 1, the concentration of ethanol has the most significant influence on the yield of fatty acids. The higher the concentration of ethanol used as a solvent, the higher the yield of fatty acids will be achieved. The maximum EE of fatty acids was 85.25% at an ethanol concentration of 100%. The reason is that ethanol contains water and easily produces emulsification with the fatty acids in the process of high-speed rotation, which would cause part of the fatty acids not to be able to dissolve in the solution and lead to poor yields. In this case, anhydrous ethanol was selected for the fatty acid extraction method.

To investigate the influence of liquid-solid ratio on EE of fatty acids, 16:1–24:1 (mL/g, v:w) liquid-solid ratios on EE were evaluated. The results (Figure 2) indicated that the EE of target compounds was markedly increased with increasing liquid-solid ratio and reached a maximum EE of 84.90% at 20:1 (mL/g, v:w). However, further increases in the liquid-solid ratio led to a clear decrease in EE. Too much extraction liquid in the system requires a longer mix time and higher mixing speed to reach the balance of extraction, which leads to a lower EE. The 20:1 (mL/g, v:w) liquid-solid ratio was selected as the optimal ratio.

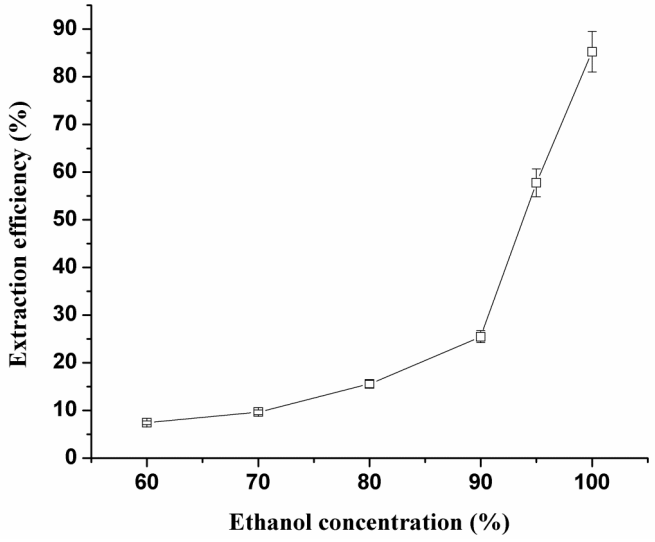

Figure 1. Effect of different ethanol concentrations on extraction of fatty acid.

In flash extraction, agitation rate plays an important role in cavitation, turbulence, and mass transfer. The effect of agitation rate on fatty acid extraction was examined, and the result is shown in Figure 3. The results (Figure 3) showed that increasing the agitation rate from 2,000 to 4,000 rpm caused an accelerated EE and reached a maximum EE of 85.80% at a speed of 4,000 rpm. Additional increments in the agitation rate, however, led to poor EE. This can be explained by the fact that a high agitation rate will form a vortex in the solution, which is unfavorable for mass transfer between the fruit and solvent. The optimal agitation rate for the fatty acid extraction was selected at 4,000 rpm since it provided enough driving force to break down the plant cell and improve mass transfer.

It is important to prolong the contact of the solvent with the aril in order to maximize the yields of fatty acids. The effect of extraction time on EE is illustrated in Figure 4. As can be seen in Figure 4, the EE of fatty acids increased rapidly with the increase in extraction time from 20 to 100 s. The maximum EE of fatty acids obtained was approximately 84.51% at the extraction time of 100 s. Further increases in extraction time did not enhance the EE significantly. A 100 s extraction time was chosen for the process.

According to the results of the single factorial experiment, three levels of solid-liquid ratio (A), ethanol concentration (B), extraction time (C), and agitation speed (D) were selected and evaluated, and three levels of each factor that had a great influence on the extraction yield of fatty acids were selected to carry out L9 (3^4) orthogonal experiment optimization. The specific experimental scheme is shown in Table 3. According to the experimental results (Table 4), the order of factors affecting

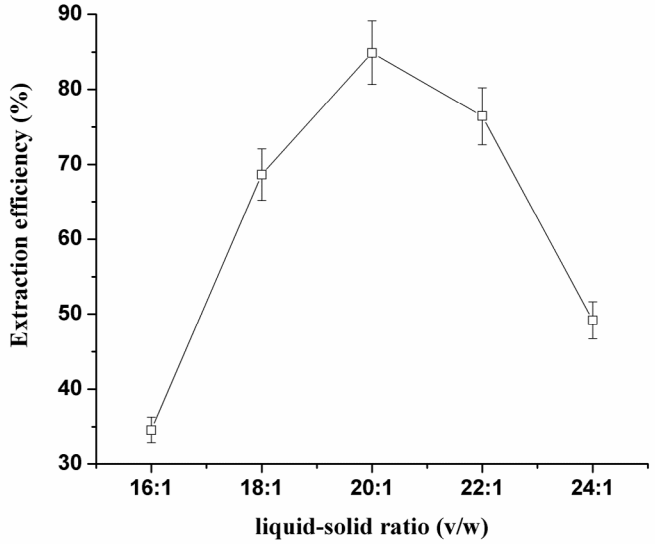

Figure 2. Effects of different liquid-solid ratios on extraction of fatty acid.

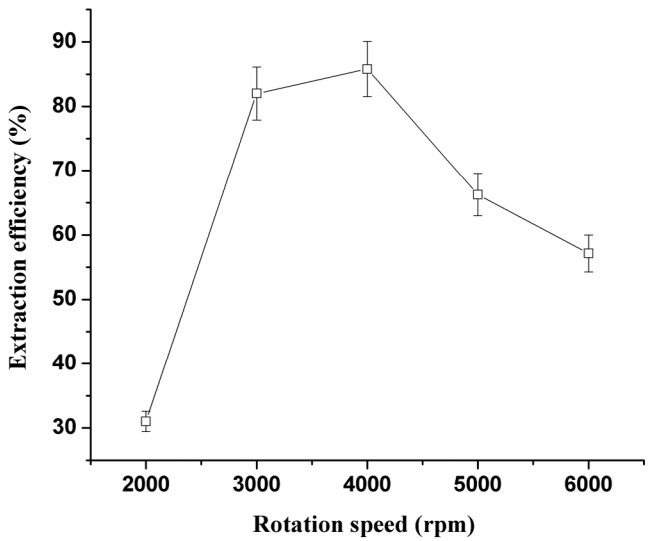

Figure 3. Effect of different agitation rates on extraction of fatty acid.

the extraction of fatty acids was B>D>C>A. Within the range of experimental design, the best combination of extraction conditions was $A_3B_3C_2D_1$, namely, solid-liquid ratio 1:22, absolute ethanol, extraction time 100 s, and extraction speed 3,000 rpm. Based on the optimized conditions, the extraction yield of fatty acid was 227.70 mg/g aril, and the extraction rate was as high as 88.48%, which was higher than the best single factorial experiment result (85.80%).

To obtain high-purity fatty acid products, the extracted fatty acid from Gac aril was purified by saponification. The crude fatty acid was added to a 6% (w:w) KOH-ethanol solution according to the solid-liquid ratio of 1 g to 4 mL, and the solution was sonicated at 50°C for 30 min. The solution was dried to obtain solid saponification. Then, an appropriate amount of water was added to dissolve the saponification; dilute hydrochloric acid was used to adjust the pH to 2–3; shake well; and further extracted by ethyl acetate. Finally, the upper oil phase was obtained and washed to neutrality with distilled water. An appropriate amount of anhydrous sodium sulfate was added to the oil phase to remove the water, and then the filtrate was collected. After the filtrate was dried, the purity of the fatty acid reached 98.27%.

3.2 Effects of lycopene separation

The concentration of lycopene in the Gac aril was determined by an ultraviolet spectrophotometer. The regression line for lycopene was $Y_{lycopene}$ =0.2354$X_{lycopene}$-0.0486 (R^2=0.9989), where *Y* and *X* are the absorbance and the concentration of lycopene (μg/mL), respectively.

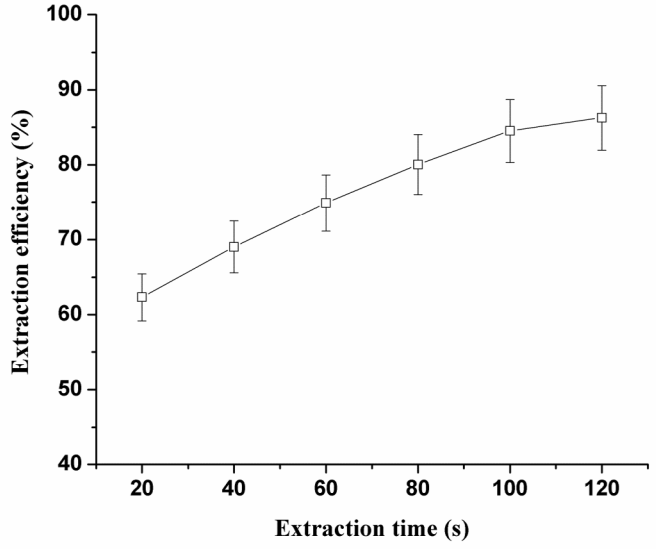

Figure 4. Effect of different extraction times on extraction of fatty acid.

Table 3. Orthogonal experiment design of fatty acid extraction.

Levels	A (liquid-solid ratio, mL/g)	B (ethanol concentration, %)	C (extraction time, s)	D (agitation speed, rpm)
1	18:1	90	90	3,000
2	20:1	95	100	4,000
3	22:1	100	110	5,000

From the results of Table 2, petroleum ether was the best solvent for lycopene extraction. The effect of 16:1–24:1 (mL/g, v:w) liquid-solid ratios on the EE of lycopene was examined. As shown in Figure 5, the EE of lycopene increased as the liquid-solid ratio increased from 16:1 to 22:1 (mL/g, v:w) and reached a maximum EE of 92.10% at 22:1 (mL/g, v:w), indicating higher liquid-solid ratios are advantageous to lycopene extraction. However, a significant decrease in lycopene extraction was observed when the liquid-solid ratio increased from 22:1 to 24:1 (mL/g, v:w), suggesting that 22:1 (mL/g, v:w) was the optimal liquid-solid ratio for lycopene extraction.

The effect of the agitation rate on EE is shown in Figure 6. The results (Figure 6) showed that increasing the agitation rate from 2,000 to 3,000 rpm caused an accelerated EE and reached a maximum EE of 88.02% at a speed of 3,000 rpm. However, further increasing the extraction agitation rate led to a considerable decrease in EE of lycopene. Compared to fatty acid extraction,

Table 4. Results of orthogonal experiment for fatty acid extraction.

	A (mL/g)	B (%)	C (s)	D (rpm)	Yield (mg/g)
1	1	1	1	1	142.3
2	1	2	2	2	77.7
3	1	3	3	3	213.2
4	2	1	2	3	79.3
5	2	2	3	1	164.9
6	2	3	1	2	222.8
7	3	1	3	2	105.5
8	3	2	1	3	132.4
9	3	3	2	1	225.8
K_1	433.2	327.1	497.5	533.0	
K_2	467.0	375.0	382.8	406.0	
K_3	463.7	661.8	483.6	424.9	
$\overline{K}_1$	144.400	109.033	165.833	177.667	
$\overline{K}_2$	155.667	125.000	127.600	135.333	
$\overline{K}_3$	154.567	220.600	161.200	141.633	
R	11.267	111.567	38.233	42.334	

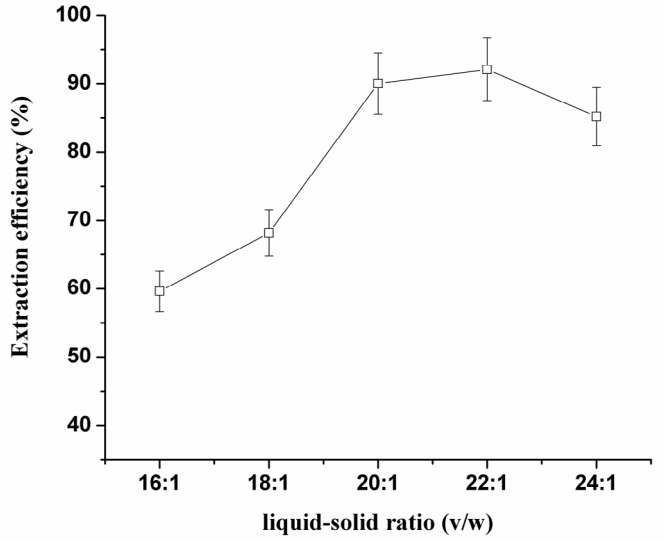

Figure 5. Effect of different liquid-solid ratios on extraction of lycopene.

lycopene extraction requires less agitation rate. In the previous fatty acid extraction process, aril had been crushed into fine particles, so the lycopene could be lifted up with only a small agitation rate during the lycopene extraction process. Excessive agitation will generate high temperature, which cause the loss of lycopene. The agitation rate of 3,000 rpm was selected as the most suitable condition for lycopene extraction.

Generally, increasing extraction time leads to more completed product yield. The effect of extraction time on the lycopene EE was tested. As can be seen in Figure 7, an increase in extraction time up to 50 s boosted the EE of lycopene, and the maximum EE of lycopene reached 93.25%. However, further increases in extraction time led to a remarkable decrease in lycopene extraction. Extensive extraction time in the system will lead to a higher temperature in the system, which causes damage to the lycopene. Compared to fatty acid extraction, lycopene extraction requires less extraction time. As we mentioned previously, smaller particle size and cell membrane rupture by fatty acid extraction process may promote a faster rate of solvent diffusion, which assist reduced lycopene extraction time. 50 s was selected as the most optimal extraction time for the lycopene extraction.

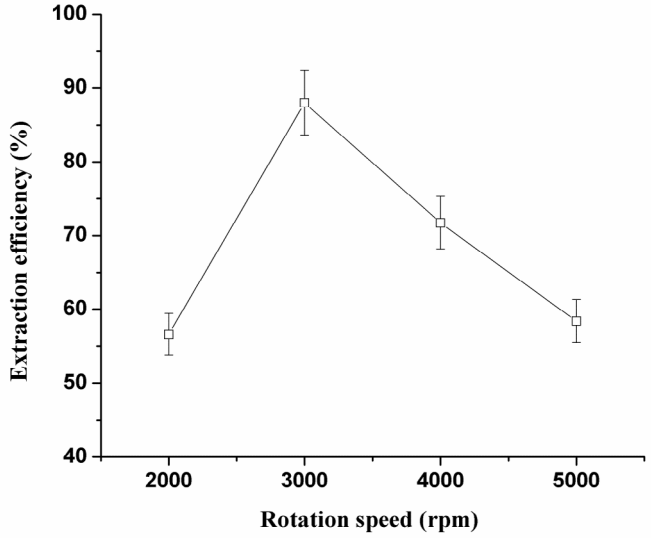

Figure 6. Effects of different agitation rates on the extraction of lycopene.

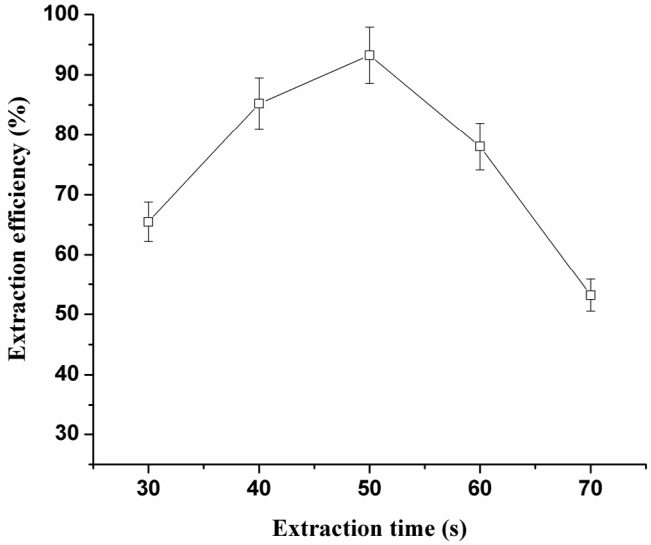

Figure 7. Effect of different extraction times on extraction of lycopene.

To obtain the optimal extraction process of lycopene, according to the results of the single factor experiment, the orthogonal experiment of three factors and three levels L9 (3^3), including solid-liquid ratio (A), extraction time (B), and extraction speed (C), was evaluated. The specific experimental scheme is shown in Table 5. According to the experimental data (Table 6), the order of factors affecting lycopene extraction was A>C>B. Within the range of experimental design, the optimal extraction condition combination is $A_3B_1C_3$, which indicates a solid-liquid ratio of 1:24, an extraction time of 40 s, and an agitation speed of 4,000 rpm. Based on the optimized conditions obtained by the orthogonal experiment, the extraction amount of lycopene was 2.7224 mg/g aril, and the extraction yield reached 94.86%, which was higher than the best single factorial experiment result (93.25%).

Lycopene petroleum ether extract was concentrated by a rotatory evaporator to one-third of the volume of the extracted liquid. After cooling under -18°C overnight, lycopene crystal powder was extracted and filtered. The crystalline powder was obtained after vacuum freeze-drying for 3 h with the purity of lycopene up to 97.20%.

3.3 Comparison of different extraction methods for fatty acids and lycopene

At present, all the methods were used to separate bioactive compounds from Gac aril (Aamir & Jittanit, 2017;

Table 5. Orthogonal experiment design of lycopene extraction.

Levels	A (liquid-solid ratio, mL/g)	B (extraction time, s)	C (agitation speed, rpm)
1	1:20	40	2,000
2	1:22	50	3,000
3	1:24	60	4,000

Table 6. Results of orthogonal experiment for lycopene extraction.

	A (mL/g)	B (s)	C (rpm)	Yield (mg/g)
1	1	1	1	1.4608
2	1	2	2	1.3874
3	1	3	3	1.9074
4	2	1	2	2.4591
5	2	2	3	2.3946
6	2	3	1	2.2983
7	3	1	3	2.7224
8	3	2	1	2.2359
9	3	3	2	2.6158
K_1	4.7556	6.6423	5.9950	
K_2	7.1520	6.0179	6.4623	
K_3	7.5741	6.8215	7.0244	
$\overline{K}_1$	1.585	2.214	1.998	
$\overline{K}_2$	2.384	2.006	2.154	
$\overline{K}_3$	2.525	2.274	2.341	
R	0.940	0.268	0.343	

Akkarachaneeyakorn et al., 2017; Honda et al., 2018; Kha et al., 2013a; Kha et al., 2013b; Kha et al., 2014a; Kha et al., 2014b; Kha et al., 2015; Kubola et al., 2013; Le et al., 2018; Mai et al., 2013; Martins et al., 2015; Nhi et al., 2016; Tai & Kim, 2014; Thuat et al., 2010; Vuong, 2000; Vuong & King, 2003; Xiang et al., 2018), the final products were obtained as mixtures.

There is limited information in the published literature on the effects of processing parameters on fatty acids and lycopene extraction from Gac aril. By comparing these extraction methods, the flash extraction used in our study has three advantages. High EE of fatty acids (88.48%) and lycopene (94.86%) from Gac aril can be achieved by simple operation, which lays the foundation for the high-value utilization of Gac aril. Higher efficiency was achieved with less time consumption (less than 100 s) than Soxhlet extraction, which means a short production period and low production cost. More low-carbon and environmental-protection operation processes were obtained on ambient temperature extraction with less toxic solvents such as anhydrous ethanol and petroleum ether. That means the product's quality is improved due to mild operating conditions.

Therefore, innovative the use of flash extraction technology as the extraction method for fatty acids and lycopene in Gac aril has the advantages of high EE, low production cost, low carbon, environmental protection, and good product quality. Additionally, flash extraction operations are simple and require less equipment investment.

4 CONCLUSION

This study shows that flash extraction has the potential to provide an efficient and cost-effective method for fatty acid and lycopene production from Gac aril. Anhydrous ethanol and petroleum ether were selected as the most suitable solvents for the extraction of fatty acids and lycopene, respectively. Under optimal extraction conditions, the highest EE of fatty acids and lycopene reached 88.48 and 94.86%, respectively. After saponification refining and concentrated crystallization refining, the purity of fatty acids and lycopene reached 98.27 and 97.20%, respectively. In conclusion, flash extraction is very promising from an industrial perspective for fatty acid and lycopene production from Gac aril and would provide a reference for other similar separation systems.

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