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Determination of Suitable Solvent and Extraction Conditions for Lycopene Isolation from Gac Fruit (*Momordica Cochinchinensis Spreng*.)

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Abstract

This study investigated the extraction of lycopene from the Gac arils. The selection of suitable extraction solvent was firstly predicted using Hansen solubility parameters prediction. The suitable extraction conditions including Gac aril size powder, solvent to solid ratio, and extraction time were then determined. The prediction results revealed that the most suitable extraction solvent was ethyl acetate following by methyl acetate, ethanol, and methanol, respectively. The validation results also confirmed that ethyl acetate gave the highest lycopene yield (0.272 mg/g dried aril powder) compared with the others (0.250, 0.235, and 0.069 mg/g dried aril powder for methyl acetate, ethanol, and methanol, respectively.). The suitable extraction conditions were found to be 40 mesh of Gac aril size powder, 30:1(ml/g) of solvent to solid ratio, and 120 minutes of extraction time, giving lycopene content of 0.448 mg/g dried aril powder.

Keywords: Suitable Extraction Condition, Gac Fruit, Hansen Solubility Parameters, Lycopene.

1. INTRODUCTION

Nowadays, food supplementary or nutraceutical products from natural sources are increasing interested, making the continuing growth in market share. The products normally contain some bioactive compounds that have specific health-beneficial effects. Ones of the most important bioactive compounds are carotenoids which have many health benefits such as anti-inflammatory activity, antioxidants, including sun protection, improved heart health, and a lower risk of certain types of cancer (Do et al., 2019; Le et al., 2018).

Lycopene classified as carotenoids has been widely focused on by many groups of researchers (Saini et al., 2020; Costa-Rodrigues et al., 2017; Gerster, 1997). The well-known source of lycopene in tomatoes. However, Gac fruit (*Momordica cochinchinensis* Spreng.) is the plant that originates in Vietnam (United States Department of Agriculture, 2019) and is found in other countries in South East Asia including Thailand, was found to contain an 8 times higher amount of lycopene compared to those amounts found in tomato (Chuyen et al., 2014). The Gac fruit composes of peel, fruit, aril, and seed but has the highest amount of lycopene in aril (seed membrane) (Shida et al., 2004). Therefore, there were many studies about lycopene extraction from Gac aril using various solid-liquid extraction techniques such as supercritical CO_2 and maceration with an organic solvent (Akkarachaneeyakorn et al., 2016; Kubola et al., 2013)

To achieve the highest efficiency of solid-liquid extraction, the screening of suitable extraction solvent is the crucial step. Hansen solubility parameters (HSPs), the parameters describing the solubility behavior by three parameters of dispersion energy or London force (δ_D), polar interaction energy (δ_P), and hydrogen-bond break energy (δ_H) are recently used as the prediction method for screening the suitable extraction solvent. The three parameters can be used to estimate the relative energy difference between any solvents and target solute. The HSPs were reported as a good prediction for the selection of the solvent for solid-liquid extraction. For example, M. Aissou et al. (2017) reported the good agreement with prediction results and HSPs and experimental results in which D-limonene was reported as the most suitable solvent for β-carotene extraction from dried carrot. Yara-Varón et al. (2016) also reported the good agreement between experimental data and HSPs prediction of the screening suitable solvent (cyclopentyl methyl ether) for carotenoids extraction from dried carrot.

This work, therefore, aimed to study the extraction of lycopene from Gac aril. The HSPs were firstly used to predict the suitable extraction solvent. Ethyl acetate, methyl acetate, ethanol, and methanol were selected for this study due to their availability and low toxicity. The prediction result was validated with the experiment result by Soxhlet extraction. The effects of extraction conditions including the size of aril powder, solvent to solid ratio (ml/g), and extraction time on the amount of lycopene were then investigated. The statistically analyzed use t-test: paired two sample for means method.

2. Materials and Method

2.1 Materials and Chemicals

Fresh Gac fruits were purchased from entrepreneurs of Ban Chok Na Sam Sub-District, Prasat District, Surin Province, which were separated arils from Gac seeds. The arils were dried in the hot air oven at 60 ° C for 5 hours. The dried arils with less than 10 percent moisture were obtained and then grounded at the desired particle size (5, 10, and 40 mesh or 4, 2, and 0.4 mm, respectively). The dried aril powder was kept in an incubator to obtain the sample with <5% moisture. Solvents used for extraction including ethanol (99.8%), methanol (99.8%), ethyl acetate (99.8%), and methyl acetate (99.8%) were purchased from Sigma Aldrich, Singapore.

2.2 Screening of suitable extraction solvent

2.2.1 Hansen solubility parameters prediction

The Hansen solubility parameters (HSPs) (Charles, 2007) is starting from the basic theory is “like dissolves like”. Values for describing a substance “like” or “unlike” consist of three components from the total energy of vaporization nonpolar or dispersion (atomic) forces (E_D), the permanent dipole–permanent dipole (molecular) forces (E_P), and hydrogen bonding (molecular) forces (E_H).

$$E = E_D + E_P + E_H \tag{1}$$

Dividing the individual cohesive energy terms by the molar volume (V) gives Equation (2)

$$\frac{E}{V} = \frac{E_D}{V} + \frac{E_P}{V} + \frac{E_H}{V} \tag{2}$$

$$\delta_T^2 = \delta_D^2 + \delta_P^2 + \delta_H^2 \tag{3}$$

δ_D, δ_P, and δ_H are the HSP for the dispersion, polar, and hydrogen bonding interactions, respectively. δ_T is the Hildebrand solubility parameter, $(E/V)^{1/2}$. It might be noted that the value of a solubility parameter in $MPa^{1/2}$ is 2.0455 times larger than in the often used $(cal/cm^3)^{1/2}$ units.

These 3 parameters will used calculated the distance of substances properties as shown in Equation (4).

$$R_a = \sqrt{4(\delta_{D1} - \delta_{D2})^2 + (\delta_{P1} - \delta_{P2})^2 + (\delta_{H1} - \delta_{H2})^2} \tag{4}$$

Subscript 1 refers to HSPs of solvent, 2 refers to HSPs of solute and R_a is the energy distance between two molecules, lower R_a means substances more soluble.

However, the values of HSPs of a number of bioactive compounds were not reported, but they could be estimate by contribution method as shown in Equations (5)-(7).

$$\delta_D = \frac{\sum F_{Di}}{Vm} \tag{5}$$

$$\delta_P = \frac{\sqrt{\sum F_{Pi}^2}}{Vm} \tag{6}$$

$$\delta_H = \sqrt{\frac{\sum E_{Hi}}{Vm}} \tag{7}$$

where F_{Di}, F_{Pi}, and E_{Hi} are the group contributions of type i to the dispersion component, polar component and hydrogen-bonding energy per structural group, respectively and these terms can be taken from the Hansen Handbook by (Charles, 2007). The molar volume values (Vm) were obtained from Van Krevelen and Hoftyzer (1976).

2.2.2 Validation method

10 grams of Gac aril powder (particle size = 10 mesh) was placed into a cellulose extraction thimble which then placed in Soxhlet apparatus. The exact amount of 200 ml of extraction solvent (ethyl acetate, methyl acetate, ethanol, and methanol) was then added to Soxhlet apparatus. The extraction was carried out at solvent to solid (dried Gac aril powder) ratio 20:1 (ml/g) for 60 minutes.

2.3 Study of suitable extraction conditions

To study suitable extraction conditions, the extraction was carried out using Soxhlet apparatus which the detail was described in section 2.2.2. In this section, Gac aril powder size, solvent to solid ratio and extraction time were varied from 5 to 40 mesh, 20:1 to 40:1 (ml/g) and 60 to 180 minutes, respectively.

2.4 Lycopene analysis

The lycopene in the extracts was analyzed by a UV-visible spectrophotometer at wavelength 470 nm. The absorbance obtained (A) was used to calculate the lycopene concentration (C_{st}) in the extract by substituting it into the Equation (8).

$$C_{st} = \frac{A \times 10{,}000}{3.450} \tag{8}$$

where C_{st} is the concentration of lycopene in the spectrophotometer (mg/ml), A is the absorbance value, 3.450 is the specific absorbance of all lycopene in hexane and 10,000 is the conversion factor to get the concentration in milligrams per liter (Akkarachaneeyakorn et al., 2016).

The results were reported as total lycopene content (mg lycopene to g of dried Gac aril powder) which can be calculated by multiply the concentration of lycopene obtained from Equation (8) with dilution volume and then divide by dilution weight as shown in Equation (9).

$$Total\ lycopene\ content = \frac{C_{st} \times v}{w} \times 10 \tag{9}$$

where w is milligram of dilution extract weight (5-10 mg), v is dilution volume in lycopene analysis (5 ml), and 10 is gram of dried Gac aril powder.

2.5 Statistical analysis

The experiments were carried out in triplicate. The results were expressed as mean ± SD and assessed for statistical significance using t-test: paired two sample for means method (using Microsoft Excel 2016). The differences between means, correlations and regressions were considered statistically significant at $\alpha < 0.05$.

3. Results and Discussion

3.1 Screening of suitable extraction solvent

3.1.1 Prediction suitable solvent by HSPs

As shown in Figure 1, lycopene structure consists of CH_3, CH_2, =C< and -CH= groups which frequency of the groups are 10, 4 ,8, 18, respectively. The frequency of group contribution was used to calculate ΣF_{Di}, ΣF_{Pi}, and ΣE_{Hi} which the values are shown in Table 1. The Hansen solubility parameters (δ_D, δ_P, and δ_H) of lycopene were then estimated by using Equations (5)-(7).

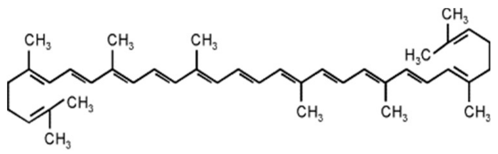

Figure 1 Lycopene structure

Table 1 List of group contributions of lycopene

Group	Freq.	ΣF_{Di} $(MJ/m^3)^{1/2}$/mol	ΣF_{Pi} $(MJ/m^3)^{1/2}$/mol	ΣE_{Hi} (J/mol)	V_m (cm^3/mol)
-CH3	10	4200	0	0	335
-CH2-	4	1080	0	0	64.4
=C<	8	560	0	0	-44
-CH=	18	3600	0	0	243
Sum	-	9440	0	0	598.4

Table 2 Energy distance (R_a) of lycopene and any solvent

Substances		δ_D	δ_P	δ_H	R_a
Solute	Lycopene	15.78	0.00	0.00	-
Solvent	Methanol	15.10	12.30	22.30	25.50
	Ethanol	15.80	8.80	19.40	21.30
	Methyl acetate	15.50	7.20	7.60	10.48
	Ethyl acetate	15.80	5.30	7.20	8.94

The Hansen solubility parameters prediction results are shown in Table 2. Hansen solubility parameters of methanol, ethanol. Methyl acetate, and ethyl acetate were obtained from Hansen's handbook. The results suggested that ethyl acetate was the most suitable solvent for extraction of lycopene, indicated by the lowest value of Ra (8.94). Compared to other solvents, the dispersion term (δ_D) of ethyl acetate was comparable to methanol, ethanol, and methyl acetate. However, the differences were showed in values of polarity term (δ_P) and H-bonding term (δ_H). The lowest δ_P and δ_H of ethyl acetate implied that the low polarity of the solvent which is described the good solubility for lycopene (non-polar substance (Zuorro, 2017)). It is worth to be noted that the using of lower polarity solvent such as propyl acetate might give the lower value of Ra compared with ethyl acetate. However, ethyl acetate was more attractive solvent in term of its availability and lower toxicity.

3.1.2 Validation of HSPs prediction by experiments.

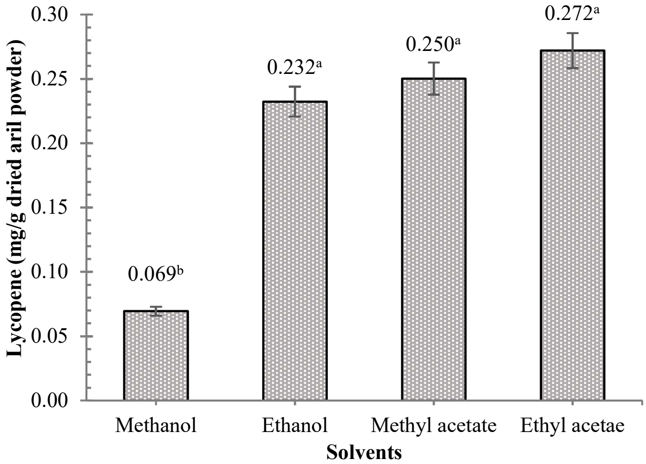

Figure 2 Extraction yield of lycopene using 4 different solvents: methanol, ethanol, methyl acetate, and ethyl acetate.

The validation results were shown in Figure 2 which the experimental results were in good agreement with HSPs prediction. Ethyl acetate was the most suitable solvent for lycopene extraction from Gac arils in this study based on the highest lycopene yield (0.272 mg/g dried Gac aril powder). The slightly lower of lycopene yields were observed by using methyl acetate and ethanol as solvent (0.250 and 0.232 mg/g dried Gac aril powder). Methanol was found to be the lowest performance for lycopene extraction since it gave the significantly lower of lycopene yield (0.069 mg/g dried Gac aril powder).

3.2 Study of suitable extraction conditions

3.2.1 Effect of size of Gac aril powder

For this experiment, ethyl acetate as the most suitable solvent was used in extraction at the solvent to solid ratio (mL/g) of 20:1 for 60 minutes. The Gac aril powder size was varied from 5 to 40 mesh. As shown in Figure 3, the results indicated that the decrease in Gac aril powder size from 5 to 10 mesh resulted in the significantly increase in lycopene yield from 0.039 to 0.272 mg/g dried Gac aril powder. The lycopene yield was found to be slightly increased from 0.272 to 0.311 mg/g dried Gac aril powder by decrease in Gac aril powder size from 10 to 40 mesh. These results might be explained by the increased in contact surface area of the powder and solvent when decreasing powder size. The 40 mesh of Gac aril powder size was therefore selected as the most suitable condition for the next study. These results were also observed by Yuliani et al. (2019) who studied effect of particle size on daidzein yield extracted from tempeh. The results showed that the decrease in sample particle size from 1.7 to 1.2 mm resulted in higher yield of daidzein from 8.13 to 8.64 mg.

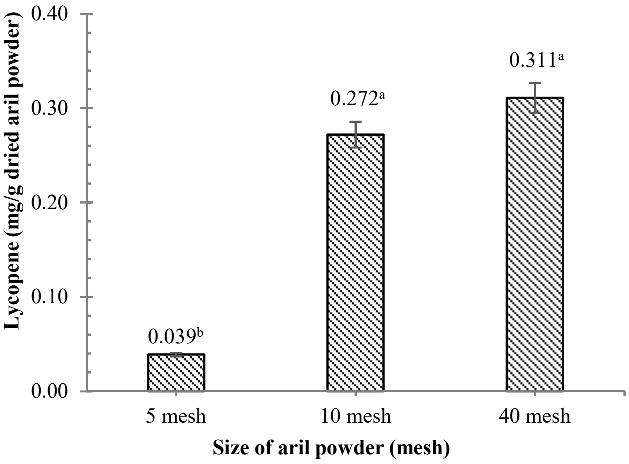

Figure 3 Extraction yield of lycopene with any size of aril powder at 5, 10, and 40 mesh

3.2.2 Effect of solvent to aril powder ratio

The extraction in this section was carried out using 40 mesh Gac aril powder for 60 minutes of extraction time. The solvent to solid ratio was varied from 20:1 to 40:1 (mL/g). From Figure 4, the increase in solvent to solid ratio from 20:1 to 30:1 (ml/g) resulted in the significantly increased lycopene yield from 0.311 to 0.412 mg/g dried Gac aril powder. However, lycopene yield was found to be insignificant different at the solvent to solid ratio higher than 30:1 (ml/g). In general, increased amounts of solvent can increase the extraction yield but the extraction was limited by the solubility of the solute in the solvent (Machmudah et al., 2012), causing the constant extraction yield at the higher solvent to solid ratios.

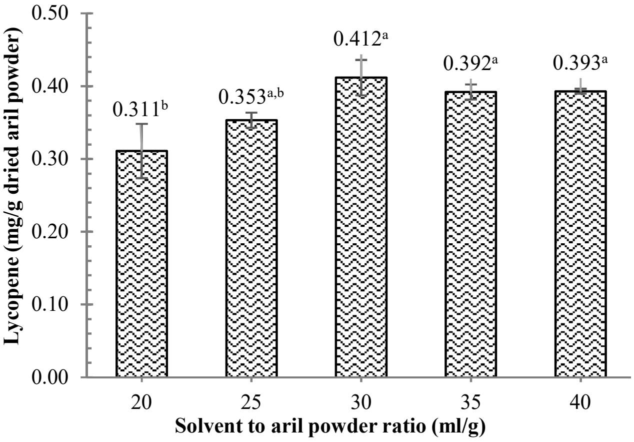

Figure 4 Extraction of lycopene yield with any solvent to aril powder ratio at 20:1, 25:1, 30:1, and 40:1

3.2.3 Effect of extraction time

In this section, the extraction was conducted using 40 mesh of Gac aril powder at solvent to solid ratio of 30:1 (mL/g). The extraction time was varied from 60 to 180 minutes as shown in Figure 5, the increase in extraction time from 60 to 120 minutes caused the increase in lycopene yield from 0.412 to 0.448 mg/g dried Gac aril powder. However, the insignificant different results can be observed at the extraction time higher than 120 minutes. The results could also be explained by extraction equilibrium. In addition, the amount of highest lycopene content obtained in this study (0.448 mg/g dried Gac aril powder) were in the range that reported by other research groups (0.38 – 0.45 mg/g of dried Gac seed aril powder) (Aoki et al., 2002; Tran et al., 2016). The differences of lycopene content might be related to the variety of species, harvesting methods, and cultivation regions.

Moreover, it is worth to be noted that lycopene has high thermal stability at the temperature of 90 °C for 3 hours (Xianquan et al., 2005). In our study, the extractions were carried out for 1 hr with extraction temperature lower than 90 °C, relating to the boiling point temperature of solvents (56.9, 64.6, 77, and 78.5 °C for ethanol, methanol, ethyl acetate, and methyl acetate, respectively). Therefore, the degradation of lycopene might be neglectable at these extraction conditions.

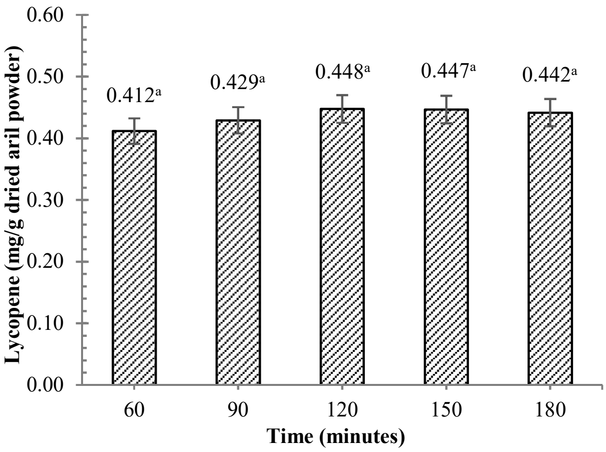

Figure 5 Extraction yield of lycopene with any extraction time at 60, 90, 120, 150, and 180 minutes

4. CONCLUSION

In this study, Hansen solubility parameters (HSPs) showed the reasonable prediction results of suitable solvent for lycopene extraction from Gac aril powder. The prediction suggested that ethyl acetate was the most suitable solvent following by methyl acetate, ethanol, and methanol, respectively. The prediction was in good agreement with validation results which ethyl acetate gave highest lycopene yield. These results confirmed that HSPs prediction might help decrease experiment number, time, and cost. The most suitable extraction conditions were then study using ethyl acetate as solvent and the results revealed that 40 mesh of aril size powder, 30:1 (mL/g) of solvent to solid ratio and 120 minutes of extraction time were the suitable extraction conditions, giving 0.448 mg/g dried Gac aril powder.

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