Research Article

# Increasing the Efficiency of Gac (*Momordica cochinchinensis* Spreng) Aril Oil Extraction by Commercial Pectinase Pretreatment and Microwave Dehydration

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**Abstract**
The objectives of this research were to determine the optimal conditions for using commercial pectinase (Pectinex® Ultra SP-L) and microwave dehydration to increase the efficiency of gac (*Momordica cochinchinensis* Spreng) aril oil extraction by using a screw press, to find mathematical models to predict the yields, extraction efficiencies, and β-carotene and lycopene contents in gac aril oil that varied with the enzyme concentrations, enzyme incubation temperatures, and microwave drying powers, and to validate the mathematical models. A Box-Behnken experimental design for three factors, including enzyme concentrations (0.01, 0.11, and 0.21% w/w), enzyme incubation temperatures (30, 45, and 60 °C), and microwave drying powers and times (450 W for 28 min, 600 W for 20 min, and 800 W for 14 min), was applied to determine the optimal conditions for increasing the efficiency of gac (*Momordica cochinchinensis* Spreng) aril oil extraction using commercial pectinase and microwave dehydration pretreatments before pressing with a screw press. It was found that the optimal conditions for extracting oil from gac aril based on the highest values of yield (12.14%), extraction efficiency (72.92%), and β-carotene and lycopene contents (104.64, and 27.90 mg/100 g oil, respectively) and the lowest of microwave energy consumption (720 kJ) were an enzyme concentration of 0.13% (w/w), enzyme incubation temperature of 45 °C and microwave drying power of 600 W for 20 min. To validate the mathematical models, the predicted yields, extraction efficiencies, and β-carotene and lycopene contents were compared to the experimental data and the deviants were less than 10%, which indicated that the model predictions were reliable.

**Keywords**: Gac aril, Gac aril oil, Microwave dehydration, Pectinase enzyme, Screw press

## 1 Introduction

Gac (*Momordica cochinchinensis* Spreng) fruit belongs to the melon family (*Cucurbitaceae*), is indigenous to Southeast Asia and has high β-carotene and lycopene contents [1]–[5]. Gac aril has a noticeably bright red color and is rich in β-carotene and lycopene. Lycopene has been reported to be associated with reduced risks for certain types of cancer, such as prostate cancer, digestive tract cancers, and lung cancer [6], [7]. β-carotene is converted to vitamin A in the body [7]. Gac aril oil contains high levels of unsaturated fatty acids and carotenoids. Gac aril oil also contains high concentrations of fatty acids (22% w/w), including oleic (29%), palmitic (32%) and linoleic acids (20%) [8]. Moreover, the β-carotene and lycopene concentrations were found in gac oil at levels of 2.6 and 2.4 mg/g, respectively [5]. In gac arils, a concentration of 102 mL/g oil was found, which consists of 69% monounsaturated fatty acids, and 35% polyunsaturated fatty acids, which are beneficial to metabolic processes in the body and help reduce cholesterol in the blood [9].

Screw press oil extraction is one of the most commonly used methods due to its ease, economy and low maintenance. A screw press extracts oil by applying pressure. The produced oil is free from chemicals [10], [11]. The efficiency of screw press extraction

depends on sample preparation and sample humidity. If the samples are very humid, oil yields will decrease. This is due to the low sample friction during pressing [10], [12].

The use of enzymes to extract edible oils is considered to be a clean and environmentally friendly technology. The advantage of using enzymes is the high oil yields, and they also help to improve oil quality [13].

Microwave drying causes the temperature of foods to rise rapidly, water to evaporate, and can rapidly decrease the moisture content of foods. Microwave drying is different from hot air drying. Microwaves penetrate inside pieces of food, and the food is heated directly due to the dielectric properties of the food components. Hot air-drying transfers heat from the outside into food pieces by conduction and/or convection. Therefore, this method requires longer drying times than microwave drying. Microwave drying increases the drying efficiency [14], [15].

To increase the extraction efficiency of gac aril oil with the screw press method, this research used enzymes and microwave-assisted extraction of oil from gac aril. Commercial pectinase was used since pectin is the main component of gac aril. A commercial pectinase is a group of enzymes that are capable of hydrolyzing pectin substances or degrading molecules that are found in the middle lamella and primary cell walls of plants [16]. Pectinex® is a mixture of several types of pectinases that are principally designed for treating fruit and vegetable mashes and for macerating plant tissues. Enzyme preparation can be used to increase oil yields extracted from vegetables [17].

The objectives of this research were to determine the optimal pectinase and microwave drying conditions to optimize the extraction of gac aril oil with a screw press and to examine the optimum conditions based on the highest yields, extraction efficiencies, β-carotene contents, lycopene contents and the lowest microwave energy consumption. We aimed to determine a mathematical model for predicting the yield, extraction efficiency, β-carotene, and lycopene content values that varied with the enzyme concentrations, enzyme incubation temperatures, and microwave drying powers and times and finally to validate the mathematical model with the experimental data.

## 2 Materials and Methods

### 2.1 *Raw materials*

Frozen raped gac aril was obtained from the Yardthip orchard, Ubon Ratchathani, Thailand and commercial pectinase (Pectinex® Ultra SP-L) was obtained from Brenntag Ingredients (Thailand) Public Company Limited, Bangkok, Thailand. Pectinex® Ultra SP-L consists of a blend of pectinases, hemicellulases and beta-glucanases. Its main enzymes are polygalacturonase, pectin lyase, and pectinesterase.

These enzymes exhibit their optimal activity at pH 4.5 and 45–60 °C, stability over wide pH (2.8–6.5) and temperature ranges (15–55 °C), with an activity of 3,800 PGNU/mL (Brenntag Ingredients (Thailand) Public Company Limited, Bangkok, Thailand).

### 2.2 *Study of the effect of the enzymatic incubation temperature on the apparent viscosity of fresh gac aril*

Frozen gac aril was thawed at room temperature (25 ± 2 °C). A total of 2,000 g of gac aril was weighed, and commercial pectinase was added at a concentration of 0.11% (w/w). Then, the pH was adjusted to the optimal pH range of the pectinase enzyme, which was 4.5 ± 0.05 with 0.1 N hydrochloric acid. Next, the container was covered with aluminum foil, incubated at 45 °C or 60 °C for 2 h, inactivated by immersion in a water bath at 90 °C for 5 min, and the solution was immediately cooled with ice to approximately 25 °C. The apparent viscosity of 200 mL of gac aril solution was measured by a Brookfield viscometer (model RVDV-IIL, Brookfield USA) with spindle no. 3 at 140 rpm and at room temperature (25 ± 2 °C). The measurements were performed in nine replicates.

### 2.3 *Study of the optimal conditions for using commercial pectinase and microwave drying-assisted extraction of oil from gac arils by screw pressing*

The optimal condition of using commercial pectinase and microwave drying-assisted oil extraction from gac arils with a screw press was determined. A total of 2,000 g of gac aril was adjusted with hydrochloric acid to a pH of 4.5 ± 0.05, commercial pectinase was added at concentrations of 0.01,

0.11, and 0.21% (w/w), and the mixtures were then incubated at 30, 45, or 60 °C for 2 h. The commercial pectinase was inactivated in a water bath at 90 °C for 5 min. The samples were cooled immediately to preserve the nutrients. A microwave oven (model MG23F301EAS, Samsung, Malaysia) with a frequency of 2,450 MHz (power of 450 W for 28 min, or 600 W for 20 min, or 800 W for 14 min) was used to dry the samples until the moisture levels decreased to 6–8% (wet basis). The samples were ground into powders with a grinder (model WF-04, Thaigrinder, Thailand) and were then steamed with a 25-cm diameter steam cooker (model HGP-10HTG, Hanabishi, Thailand); 400 mL of water was used for steaming at 100 °C for 20 min); the samples were dried, and the oil was extracted with a screw press with a compression distance of 2.02 cm and speed of 14.10 rpm (Kasetsart University, Thailand). The conditions for extracting gac aril oil by screw press extraction were obtained from the study by Akkarachaneeyakorn *et al.* [18], who determined the optimal condition (e.g., optimal rotational speed and compression length) for oil extraction from gac (*Momordica cochinchinensis* Spreng) aril using a screw press to achieve the highest amounts of β-carotene, lycopene, and iodine values, and the lowest values of acidity and peroxide. Steaming gac aril powder prior to pressing with a screw press increased the extraction efficiency and prevented the inhibition of polyphenol oxidase activity and microorganism growth. In addition, under the steaming process, heat can soften and break down the oil-containing cells and decrease the oil viscosity. Hence, the oil is easily released in the subsequent pressing [12]. Kha *et al.* [12] found that moisture contents between 8% and 11% (wet basis) after drying and steaming were the best for hydraulic pressing.

The physicochemical properties of gac aril oil were determined as follows:

*2.3.1 Apparent viscosity*

A 15-mL oil gac aril sample was added to a beaker to measure the apparent viscosity. The apparent viscosity was measured with a Brookfield viscometer (RVDV-II., Brookfield, USA) with spindle no. 27 at 100 rpm at room temperature (25 ± 2 °C). The measurements were performed in nine replicates.

*2.3.2 Color value*

Two milliliters of gac aril oil were added to a sample container for color measurements. These measurements were performed in triplicate with a HunterLab ColorQuest 450 (Color Global Co., Ltd., USA) in a CIE lab system.

*2.3.3 pH*

Ten-milliliter samples of gac aril oil were added to a beaker for pH measurements. The pH levels were determined with a pH meter (CyberScan pH 510, Eutech, USA).

*2.3.4 β-carotene and lycopene contents*

The method used for determining the β-carotene and lycopene contents was modified from that of Nagata and Yamashita [19]. A 0.1-g sample of oil was added to 10 mL of a mixture of hexane:acetone at a 4:6 (v/v) ratio and was vortexed for 1 min. The mixture was then diluted by a factor of 10, and the light absorption levels of the solution were subsequently determined using a UV-visible spectrophotometer at wavelengths of 663, 645, 505, and 453 nm. An acetone:hexane solution at a 4:6 (v/v) ratio was used as a blank. The measured values were used to calculate the β-carotene contents with Equation (1) and lycopene contents with Equation (2)

$$\beta\text{-carotene (mg/100gFW)} = 0.216A_{663} - 1.220A_{645} + 0.304A_{505} - 0.452A_{453} \quad (1)$$

$$\text{Lycopene (mg/100gFW)} = -0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453} \quad (2)$$

*2.3.5 Extraction efficiency percentages*

The oil extraction efficiencies were determined from the ratios of the weight of the obtained oil extract after subtracting the moisture content and the weight of the oil extract, as determined with a Soxhlet extractor, as shown in Equation (3). The total oil sample obtained from gac aril was treated in a microwave oven before extraction and was then analyzed using a BÜCHI extraction system (B-8118, BÜCHI, Switzerland).

Extraction efficiency (%) = (weight of oil extract – moisture content)/weight of oil extract using a Soxhlet extractor (3)

The moisture contents were measured using the Ca 2e-55 method AOCS [20].

*2.3.6 Yield*

The oil yields were calculated with Equation (4).

Yield (%) = weight of oil extract/weight of fresh gac aril (4)

*2.3.7 Calculation of the energy consumption of the microwave oven*

The energy consumption of the microwave oven was calculated from Equation (5)

$E_{mw}$ (kJ) = mw power input (kW) × mw heating time (s) (5)

## 2.4 *Statistical analysis*

A completely randomized design was used to study the effects of the incubation temperature of commercial pectinase on the apparent viscosity of fresh gac aril. The differences between treatments were analyzed using Fisher's least significant difference (LSD) method at a 95% confidence level using Minitab version 16.2.2. The Box-Behnken design method with 3 factors, including enzyme concentrations, incubation temperatures, and microwave powers and drying times was applied to determine the optimal conditions for the commercial pectinase and microwave drying-assisted extraction of gac aril oil by screw pressing. The advantages of the Box-Behnken design include its flexibility for regulation because the design employs only 3 factor levels (–1, 0, and 1), as shown in Table 1. The design also allows for the addition of more factors by using previously obtained experimental data [21]. The independent variables were the enzyme concentrations ($X_1$), enzyme incubation temperatures ($X_2$), and microwave drying powers and times ($X_3$). The dependent variables were the yields ($Y_1$), extraction efficiencies ($Y_2$), β-carotene contents ($Y_3$), and lycopene contents ($Y_4$).

**Table 1**: The factors and levels of the experimental design

| Factors | Code/Real Values | | |
|---|---|---|---|
| | –1 | 0 | +1 |
| Enzyme concentration (% w/w) | 0.01 | 0.11 | 0.21 |
| Incubation temperature (°C) | 30 | 45 | 60 |
| Microwave power (W), time (min) | 450, 28 | 600, 20 | 800, 14 |

The design included 15 experiments with 3 replications of the center point, as shown in Table 2. The experiments were carried out in random order. The levels for each parameter in the Box-Behnken design were determined from preliminary experiments. The quadratic model was used to predict each response (Yk) in all experimental regions, as shown in Equation (6). All measurements were performed in triplicate. The data were analyzed using Minitab version 16.2.2 at a 95% confidence level.

$$Y_k = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{11}X_1^2 + B_{22}X_2^2 + B_{33}X_3^2 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 \quad (6)$$

**Table 2**: Box-Behnken design with three independent variables (real level) for the commercial pectinase and microwave drying-assisted extraction of oil from gac aril by a screw press

| Run No. | Enzyme Concentration (% w/w) | Incubation Temperature (°C) | Microwave Power and Time (W, min) |
|---|---|---|---|
| 1 | 0.11 | 45 | 600, 20 |
| 2 | 0.11 | 30 | 450, 28 |
| 3 | 0.11 | 45 | 600, 20 |
| 4 | 0.11 | 60 | 450, 28 |
| 5 | 0.21 | 60 | 600, 20 |
| 6 | 0.11 | 30 | 800, 14 |
| 7 | 0.01 | 30 | 600, 20 |
| 8 | 0.21 | 45 | 800, 14 |
| 9 | 0.01 | 60 | 600, 20 |
| 10 | 0.01 | 45 | 800, 14 |
| 11 | 0.11 | 45 | 600, 20 |
| 12 | 0.11 | 60 | 800, 14 |
| 13 | 0.21 | 30 | 600, 20 |
| 14 | 0.01 | 45 | 450, 28 |
| 15 | 0.21 | 45 | 450, 28 |

## 3 Results and Discussion

### 3.1 *Effect of the enzymatic incubation temperature on the apparent viscosity of fresh gac aril*

The mean apparent viscosity of fresh gac aril was 559.23 ± 1.60 cP. It was found that when 0.11% (w/w) of commercial pectinase was used and the incubation temperature was 45 °C for 2 h, these conditions decreased the apparent viscosity by 18% (apparent viscosity 456.67 ± 2.87 cP). This result is consistent with that of Gouvêa *et al.* [22], who studied the effects of commercial pectinase (Pectinex®) concentrations (e.g., 0.10, 0.20 and 0.30% w/w) and incubation temperatures (e.g., 35, 45, 55 and 60 °C) on the apparent viscosity of the fruit pulp (umbu). The apparent viscosity of the fresh pulp (84.8 mPa.s at a 100 $s^{-1}$ shear rate) and a significant, four times lower, apparent viscosity reduction of 18.9 mPa.s were observed under the optimum process conditions (35 °C and at 0.10% w/w) [22]. Commercial pectinase is an enzyme that degrades pectic substances [22]. Pectin substances are polysaccharides that are present in plant cell walls, where they contribute to complex physiological processes such as cell growth and cell differentiation and determine the integrity and rigidity of plant tissues [23]. Pectin substances are present in most vegetable tissues, mainly in ripe fruit [24]. Commercial pectinase digests the polysaccharide structures in cells. This allows more oil to be extracted [25].

The apparent viscosity of the fresh gac aril after incubation with 0.11% (w/w) commercial pectinase at 60 °C was 544.50 ± 2.51 cP, which was statistically higher than that at 45 °C (apparent viscosity 456.67 ± 2.87 cP) ($p < 0.05$). The optimum activity of commercial pectinase occurs at approximately 45 °C, which causes it to be most efficient for breaking down the pectin and has the effect of decreasing the apparent sample viscosity [24]. The enzyme activity levels are affected by factors, such as temperature, pH, and the concentrations of enzymes, substrates and salt [26].

### 3.2 *Results of the study of the optimal conditions for using commercial pectinase and microwave drying-assisted extraction of oil from gac arils by screw pressing*

The optimal conditions for the commercial pectinase and microwave drying-assisted extraction of gac aril oil with a screw press were determined. The experimental plan used a Box-Behnken design with 3 factors. The enzyme concentrations, enzyme incubation temperatures, and microwave drying powers and times based on the maximum yield, extraction efficiency, and β-carotene and lycopene contents and the minimum microwave energy consumption were determined.

#### *3.2.1 Yields and extraction efficiencies of gac aril oil*

Three-dimensional graphs of the yields and extraction efficiencies obtained by screw pressing are shown in Figures 1 and 2.

From Figures 1(a) and 2(a), at a constant microwave drying power of 600 W for 20 min, when the 0.01% (w/w) enzyme concentration was increased to 0.11% (w/w) and the temperature was increased from 30 °C to 45 °C, the oil yield and extraction efficiency increased. Robinson [26] found that when the enzyme concentration was increased by 10%, the enzyme reaction was 10% faster. At an enzyme concentration of 0.11% (w/w), enzyme incubation temperature of 45 °C and microwave drying power of 600 W for 20 min, the highest oil yield (14.70%) was obtained. When the enzyme concentration was increased from 0.11% (w/w) to 0.21% (w/w) and the enzyme incubation temperature was increased from 45 °C to 60 °C, the oil yields and extraction efficiencies decreased [Figures 1(b), 1(c), 2(b), and 2(c)]. The reaction rates of the enzymatic reactions varied with temperature. The effects of temperature on enzyme activity are quite complex and can be regarded as two forces that act simultaneously but in opposite directions. As the temperature is raised, the rate of molecular movement and hence the reaction rate increases, but at the same time, there is a progressive inactivation that is caused by the denaturation of the enzyme. The temperatures at which denaturation becomes significant vary from one enzyme to another. Normally, the denaturation levels are negligible below 30 °C and begin to become significant above 40 °C [26]. If the temperature is too high, the enzymes, which are proteins, denature and result in a loss of the enzyme abilities to catalyze the reaction. Gonzalez and Rosso [24] found that at 75 °C, 2.49% commercial pectinase (Pectinex Ultra SP-L) after 10 min of exposure to heat treatment and after 20 min, complete inactivation

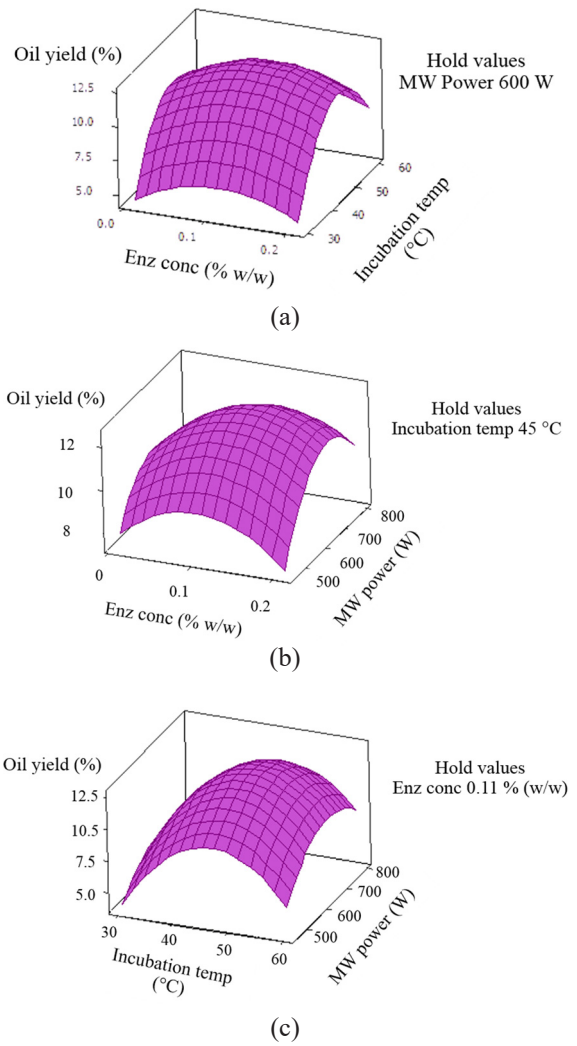


**Figure 1**: Three-dimensional graph of gac aril oil yields using a screw press at various enzyme concentrations ($X_1$) and incubation temperatures ($X_2$) (a) enzyme concentrations ($X_1$) and microwave powers and times ($X_3$) (b) incubation temperatures ($X_2$) and microwave powers and times ($X_3$) (c).

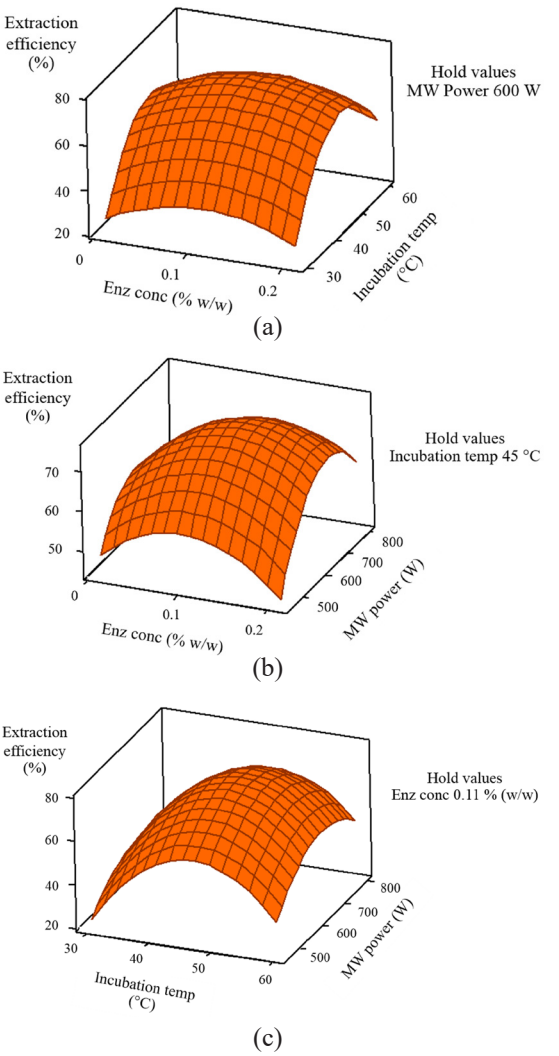


**Figure 2**: Three-dimensional graph of gac aril extraction efficiency by screw press at various enzyme concentrations ($X_1$) and incubation temperatures ($X_2$) (a) enzyme concentrations ($X_1$) and microwave powers and times ($X_3$) (b) incubation temperatures ($X_2$) and microwave powers and times ($X_3$) (c).

occurred. The optimal temperature for Pectinex 100 L plus Panzyn Clears commercial pectinase was 45 °C at pH levels of 4.0 to 4.5. Commercial pectinase was used to induce cell wall disintegration, which led to a greater oil release when pressed with a screw press [27], [28]. Silvamany and Jahim [24] increased the level of palm oil extraction by using a mixture of commercial pectinases that consisted of Cellic CTec2, Cellic HTec2, and Pectinex Ultra SP-L. They found that a maximum oil recovery of 88% was achieved with an enzyme ratio of 0.46:0.34:0.2 (Cellic, CTec2:Cellic, HTec2:Pectinex, and Ultra SP-L) at pH 4.8 after 2 h of incubation at 50 °C. Through enzymatic treatment, the trapped oil was released due to cell wall degradation, which led to higher oil yields. Figures 1(b), 1(c), 2(b), and 2(c) show that microwave drying at 600 W for

20 min influenced the structural changes of gac aril due to the water molecules, and the ions in the gac aril moved and created heat directly inside the gac aril by two mechanisms. Firstly, dipole rotation, in which the water movements in the fresh gac aril were influenced by electrical fields, and positive and negative charges were induced. This caused the movements to change direction to align in an orderly manner when passing through an alternating magnetic field. The positive and negative charges in the water molecules rotated to change their directions to the direction of the alternating electric field. These rotations occur rapidly at a microwave frequency of 2,450 million cycles per second. The second mechanism is ionic polarization, in which the movements of ions, such as potassium chloride, sodium chloride, calcium chloride, and magnesium chloride in fresh gac aril move back and forth in an electromagnetic field based on the microwave frequency, which was 2,450 million cycles per second. The potassium, sodium, calcium, and magnesium contents of the fresh gac aril were 487.40, 71.64, 20.72, and 11.37 g/100 g FW, respectively [29]. Therefore, 2 mechanisms caused friction and generated heat inside the gac aril [30]. The resulting heat caused the gac aril cell walls to disintegrate, which made it easier for the oil to be released when it is extracted by a screw press, which thereby increases the yields and extraction efficiencies. Microwave drying at 800 W for 14 min created excessive amounts of heat. Therefore, heat accumulates and destroyed the tissues, structures, and nutrients in gac aril, which resulted in the lowest yields and extraction efficiencies. Microwave drying at 450 W for 28 min caused the cell walls of gac aril to disintegrate slightly and caused less oil to be released, which resulted in low yields and extraction efficiencies [Figure 1(b), 1(c), 2(b), and 2(c)]. Based on the analysis results, the mathematical models of the oil yields and extraction efficiencies shown in Table 3 have regression coefficients ($R^2$) of 92.10% and 90.79%, respectively. The lack of fit of the P values of the predicted model of oil yields, and the extraction efficiencies were 0.938 and 0.926, respectively, which provided regression coefficients that were greater than 90% and a lack of fit measure for the P-values greater than 0.05, which indicated that the independent variables (e.g., enzyme concentration, enzyme incubation temperature, microwave power and time) and variations in the dependent variables (e.g., oil yield and extraction efficiencies) fit well. The mathematical models in Table 3 can accurately predict the oil yields and extraction efficiencies.

**Table 3**: Models and $R^2$ values of the yields, oil extraction efficiencies, and β-carotene and lycopene values in coded and real units

| Responses | Models | $R^2$ (%) |
|---|---|---|
| Yield (%) | $-1.7170(x_1)^2 - 4.5817(x_2)^2$ $-2.4980\,(x_3)^2 + 0.4666x_1 + 1.4619x^2 +$ $0.5275x_3 + 0.2700x_1x_2 + 0.7219x_1x_3 +$ $0.5169x_2x_3 + 12.419$ and $-171.6670(X_1)^2 - 0.020363\,(X_2)^2$ $- 0.0000815675(X_3)^2 + 8.54949X_1$ $+ 1.78726X_2 + 0.0915752X_3$ $+ 0.1800X_1X_2 + 0.0412525X_1X_3$ $+ 0.000196902X_2X_3 - 60.2727$ | 92.10 |
| Extraction efficiency (%) | $-10.289(x_1)^2 - 27.864(x_2)^2$ $- 14.694(x_3)^2 + 2.736x_1 + 8.630x_2$ $+ 2.837x_3 + 1.515x_1x_2 + 4.734x_1x_3$ $+ 2.622x_2x_3 + 74.379$ and $-1028.9200(X_1)^2 - 0.123841(X_2)^2$ $- 0.00048(X_3)^2 + 39.1899X_1$ $+ 10.9856X_2 + 0.541277X_3$ $+ 1.0100X_1X_2 + 0.270525X_1X_3$ $+ 0.000998923X_2X_3 - 363.6170$ | 90.79 |
| β-carotene (mg/100g oil) | $-5.696(x_1)^2 - 5.777(x_2)^2 - 9.178(x_3)^2$ $+ 0.627x_1 - 3.047x_2 - 5.799x_3$ $- 0.828x_1x_2 + 0.660x_1x_3 - 2.241x_2x_3$ $+ 103.507$ and $-569.6150(X_1)^2 - 0.0256744(X_2)^2$ $- 0.0003(X_3)^2 + 132.8630X_1$ $+ 2.70177X_2 + 0.37575X_3$ $- 0.551967X_1X_2 + 0.0376996X_1X_3$ $- 0.000854X_2X_3 - 67.4301$ | 84.39 |
| β-carotene (mg/100g oil) | $-3.1732(x_1)^2 - 2.7188(x_2)^2$ $- 5.4155(x_3)^2 + 0.0996x_1$ $+ 0.0331x_2 - 1.5290x3$ $- 0.3265x_1x_2 + 0.6086x_1x_3$ $- 0.2581x_2x_3 + 27.8205$ and $-317.3210(X_1)^2 - 0.012084(X_2)^2$ $- 0.000177(X_3)^2 + 58.8675X_1$ $+ 1.17513X_2 + 0.212904X_3$ $- 0.217683X_1X_2 + 0.034776X_1X_3$ $- 0.000098X_2X_3 - 65.7641$ | 68.94 |

Note: $x_1$ = coded value of enzyme concentration, $x_2$ = coded value of incubation temperature, $x_3$ = coded value of microwave power and time, $X_1$ = enzyme concentration (0.01–0.21% w/w), $X_2$ = incubation temperature (30–60 °C), and $X_3$ = microwave power and time (450–800 W).

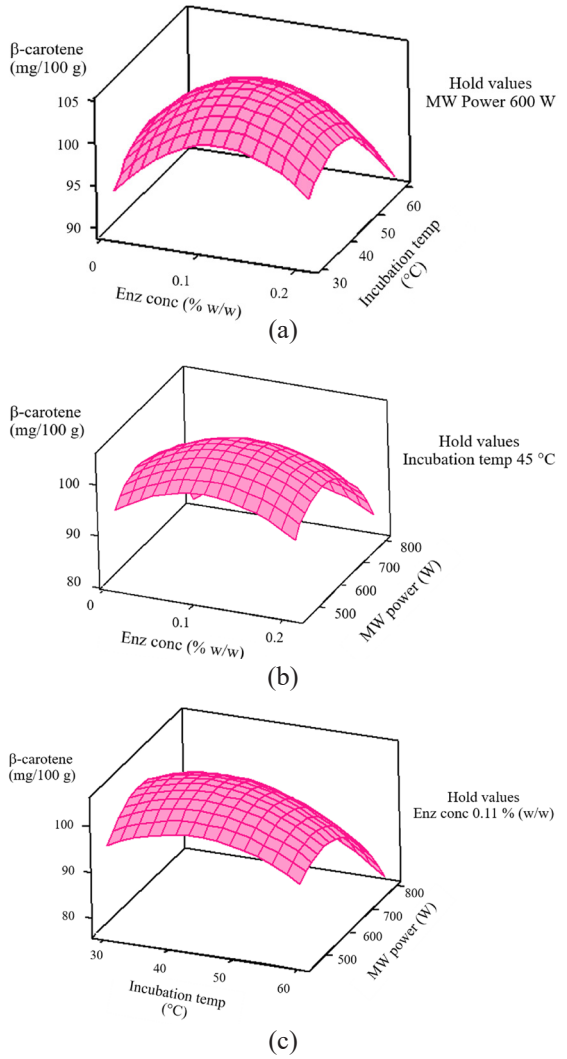


**Figure 3**: Three-dimensional graphs of the β-carotene contents for gac aril oil extraction by a screw press at various enzyme concentrations ($X_1$) and incubation temperatures ($X_2$) (a) enzyme concentrations ($X_1$) and microwave powers and times ($X_3$) (b) incubation temperatures ($X_2$) and microwave powers and times ($X_3$) (c).

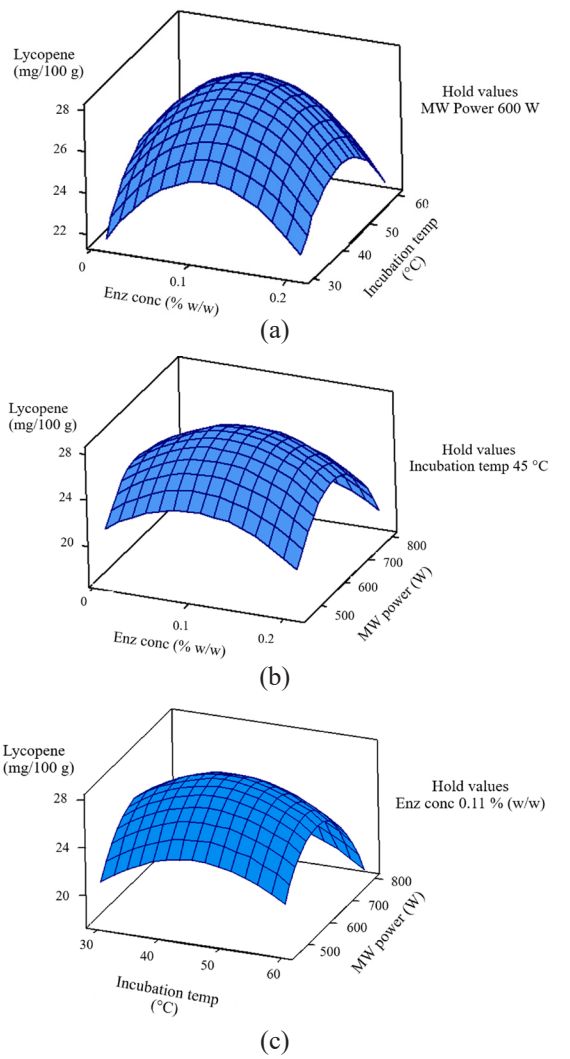


**Figure 4**: Three-dimensional graphs of the lycopene contents for gac aril oil extraction by a screw press at various enzyme concentrations ($X_1$) and incubation temperatures ($X_2$) (a) enzyme concentrations ($X_1$) and microwave powers and times ($X_3$) (b) incubation temperatures ($X_2$) and microwave powers and times ($X_3$) (c).

*3.2.2 β-carotene and lycopene contents*

Figures 3 and 4 show that at an enzyme concentration of 0.11% (w/w), enzyme incubation temperature of 45 °C and microwave drying power of 600 W for 20 min resulted in β-carotene and lycopene contents in gac aril oil of 106.08 and 30.89 mg/100 g, respectively. Microwave drying at 600 W for 20 min caused the cell walls to disintegrate due to heating.

Therefore, during the oil extraction with a screw press, the greater oil release resulted in higher β-carotene and lycopene contents. Kha *et al.* [12] increased the gac aril oil extraction efficiency and β-carotene and lycopene contents by using microwave

drying before hydraulic pressing. When compared with air drying, they found that microwave drying resulted in higher β-carotene and lycopene contents. Microwave drying can reduce the drying time 7–10 times more than hot air drying and helps to maintain quality and preserve nutrients [14], [15], [30]. Microwave drying before pressing gac aril enhances the yields, extraction efficiencies, and β-carotene, and lycopene contents due to the disintegration of gac aril cell walls. Therefore, the trapped oil is released along with valuable components, such as antioxidants, β-carotene, lycopene, and taste flavors [12]. The extraction efficiencies and β-carotene and lycopene contents when using microwave drying before hydraulic pressing of gac aril were 93%, 140, and 414 mg/100 mL, respectively [12]. The extraction efficiencies and β-carotene and lycopene contents when using air drying before pressing were 68%, 55, and 240 mg/100 mL, respectively [12]. Microwave drying at 800 W for 14 min caused heat accumulation in gac aril, which resulted in lower β-carotene and lycopene contents due to oxidative degradation. β-carotene and lycopene are unstable in the presence of oxygen, heat, light, and metals (e.g., $Cu^{2+}$ and $Fe^{3+}$) [31], [32]. When drying by microwave irradiation at 450 W for 28 min, this long drying time resulted in lower β-carotene and lycopene contents due to the oxidation reactions that were caused by β-carotene and lycopene being exposed to oxygen, heat, and light for a long period [12]. When the drying temperature is high or a long drying period is used, these conditions help to accelerate the oxidative degradation reactions of β-carotene and lycopene. The decomposition rates of β-carotene and lycopene take place according to the first-order kinetic reaction rates and activation energies that are in the range of 18−83 kJ/mol, which are dependent on the reaction medium, β-carotene source, and processing conditions [31], [32]. In addition, the decomposition of β-carotene and lycopene in gac arils may be decreased by the reaction of lipoxygenase, which is an enzyme naturally present in fruits and vegetables that promotes the oxidative degradation of β-carotene and lycopene. Lipoxygenases can be thermally inactivated above 60 °C [33]. The mathematical models of the β-carotene and lycopene contents in gac aril oil are shown in Table 3. The $R^2$ values were 84.39 and 68.94%, respectively, which mean that the independent variables (e.g., enzyme concentration, enzyme incubation temperature, and microwave drying power and time) can describe the variations in the dependent variables (β-carotene and lycopene contents in gac aril oil). The mathematical models shown in Table 3 can be used to generate predictive equations to correctly determine the β-carotene and lycopene contents. When considering the P-value of lack of fit, it was found that the lack of fit values were 0.157 and 0.556, which are greater than 0.05, which are insignificant ($p \geq 0.05$) and mean that the β-carotene and lycopene prediction equations, as shown in Table 3, do not indicate a lack of fit; that is, the resulting equation fits the experimental results. The mathematical model for the lycopene contents had lower regression coefficients (68.94%). This might be because the lycopene contents in gac aril oil were determined by a spectrophotometric method. Tran [34] determined the total carotenoids in gac aril powder by using spectrophotometry and high-performance liquid chromatography (HPLC). The total carotenoid content measured by the spectrophotometric method was 5.06 mg/g, which was higher than that measured by the HPLC method (4.01 mg/g). The spectrophotometry method uses a photometer to measure light intensity in the ultraviolet and visible ranges. It is often applied to quantify transition metals or highly conjugated organic compounds in solutions. However, this method also has a major drawback. It cannot detect a separate peak for each carotenoid, and the absorbance at one wavelength consists of the combined absorbance from many carotenoids that have overlapping spectra and possibly the absorbance from other interfering compounds that are not carotenoids [34]. When compared with HPLC, spectrophotometry yields higher mean values and standard deviations, but is quicker, simpler, less expensive and is still reliable for industrial applications due to the use of less toxic solvents [34].

### *3.2.3 Determination of the optimal conditions for extracting gac aril oil using a screw press*

The energy consumption by microwaves was calculated from Equation (5). Microwave drying powers and times of 450 W and 28 min, 600 W for 20 min, and 800 W for 14 min resulted in energy consumption levels that were equal to 756, 720, and 672 kJ, respectively. An increase in the microwave drying power and time resulted in higher energy

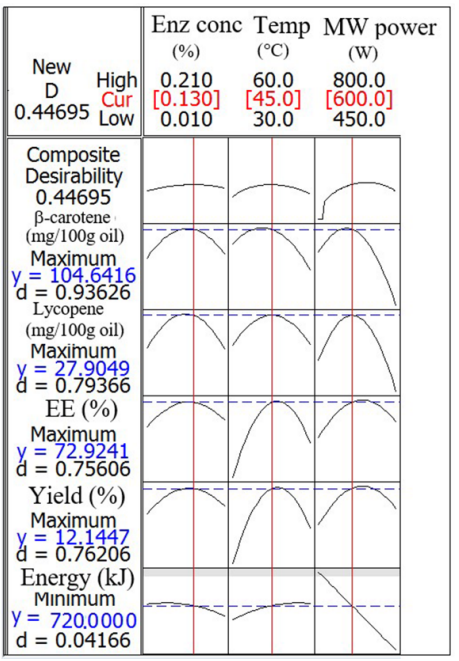


**Figure 5**: Determining the optimal conditions for oil extraction from gac arils using real values.

consumption. The response optimizer function in Minitab 16.2.2 was used to determine the optimal conditions for extracting gac aril oil with a screw press. The optimal conditions to achieve the highest yield, extraction efficiency, β-carotene, and lycopene contents and the lowest of microwave energy consumption are shown in Figure 5. It was found that the highest yield (12.14%), extraction efficiency (72.92%), β-carotene, and lycopene contents (e.g., 104.64 and 27.90 mg/100 g oil, respectively), while the lowest energy consumption (720 kJ) occurred when using commercial pectinase at a concentration of 0.13% (w/w), incubation temperature of 45 °C, and microwave drying power and time of 600 W and 20 min, respectively. Kha *et al.* [13], found that the highest oil yield (34% g/g), extraction efficiency (95%) and β-carotene (83 mg/100 mL oil) and lycopene (508 mg/100 mL oil) contents were obtained when using the commercial enzyme (Pectinex® Ultra SP-L) at a 0.10% (w/w) with a concentration pretreatment before air-drying at a temperature of 50 °C prior to supercritical carbon dioxide extraction at a pressure of 200 bar and extraction temperature of 50 °C. The optimal conditions reported by Kha *et al.* [13] were slightly different from those reported in this study due to the types and concentrations of the substrates, enzymes, pH levels, incubation times, temperatures, salt levels and extraction method [26].

### *3.2.4 Validation of mathematical models*

Validations of the mathematical models for oil yield, extraction efficiency, and β-carotene and lycopene contents of gac aril were conducted. The deviants obtained from the experiments were compared with the values that were obtained from the mathematical model (%D) at the optimum pectinase concentration of 0.13% (w/w), enzyme incubation temperature of 45 °C and microwave drying power of 600 W for 20 min. The %D values of the yield, extraction efficiency, β-carotene, and lycopene content were below 10% (Table 4), which indicated that the model was suitable for the application.

**Table 4**: %D values of oil yield, extraction efficiency, and β-carotene and lycopene contents

| Response | $Y_{predicted}$ | $Y_{experimental}$ | %D |
|---|---|---|---|
| Yield (%) | 12.14 | 12.66 | 4.11 |
| Extraction efficiency (%) | 72.92 | 75.36 | 3.24 |
| β-carotene content | | | |
| (mg/100 g oil) | 104.64 | 105.97 | 1.26 |
| Lycopene content | | | |
| (mg/100 g oil) | 27.90 | 30.63 | 8.91 |

Note:

$$\%D = \frac{\text{abs}(Y_{predict} - Y_{experimental}) \times 100\%}{Y_{experimental}}$$

### *3.2.5 Physicochemical properties of gac aril oil extracted under optimal conditions*

The gac aril oil that was extracted under optimal conditions (enzyme concentration of 0.13% (w/w) at 45 °C and microwave drying power of 600 W for 20 min) had a pH of 4.01, the apparent viscosity of 163.567 cp.s, L* value of 10.92, a* value of 20.41, b* value of 15.98, C* value of 25.96 and h* value of 38.67°. The β-carotene and lycopene contents in the gac aril oil were 105.97 and 30.63 mg/100 g of oil, respectively.

### *3.2.6 Comparison of the oil extraction efficiencies from gac aril with and without commercial pectinase*

We compared the extraction efficiencies and β-carotene

and lycopene contents of gac aril oil extracted under the optimum conditions (enzyme concentration of 0.13 (w/w) at 45 °C and microwave drying power of 600 W for 20 min) that were determined in this study with those of Akkarachaneeyakorn *et al.* [18], who pressed gac aril without enzyme treatment and used microwave drying (720 W for 20 ± 1 min); it was found that extraction efficiencies, β-carotene and lycopene contents from this study were 1.2, 5.7, and 5.6 times higher, respectively than those obtained when not using enzyme treatments (Table 5). Using commercial pectinase helps to degrade fruit cell walls, releases trapped oil and leads to higher oil releases when pressed with a screw press [25].

**Table 5**: Effects of different extraction methods on the yields, extraction efficiencies, and β-carotene, and lycopene contents

| Sample | Yield (%) | Extraction Efficiency (%) | β-carotene (mg/100 g oil) | Lycopene (mg/100 g oil) |
|---|---|---|---|---|
| Pressing with pectinase pretreatment (this study) | 12.14 | 72.92 | 104.64 | 27.90 |
| Pressing without enzyme treatment Akkarachaneeyakorn *et al.* [18] | n/a | 61.82 | 18.22 | 5.02 |

Note: nd = not available

## 4 Conclusions

Compared to pressing without pretreatment with commercial pectinase and microwave drying, it was found that the use of commercial pectinase and microwave drying before pressing gac aril with a screw press can increase the extraction efficiencies and β-carotene and lycopene contents. The optimal conditions for using commercial pectinase and microwave drying-assisted oil extraction from gac arils with a screw press based on the highest yield, extraction efficiency, and β-carotene and lycopene contents and the lowest of microwave energy consumption were achieved with an enzyme concentration of 0.13% (w/w), incubation temperature of 45 °C and microwave drying power and time (600 W and 20 min), respectively.

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