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Effect of Different Extraction Methods on Physicochemical Properties of Gac Fruit (*Momordica Cochinchinensis*) Pulp and Peel Oil

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ABSTRACT

Gac fruit (*Momordica cochinchinensis*) is an underutilized fruit where its pulp and peel are often discarded. Fewer studies were conducted on its pulp and peel than on its arils and seeds. This study provides insights into the comparison of crude oil yield and compositions of palmitic and stearic acid of the crude oil extracted using three different extractions, including maceration, ultrasound-assisted extraction (UAE), and enzymatic-assisted extraction (EAE). The pH, refractive index, color measurement, total phenolic content (TPC), DPPH radical scavenging activity, and iron-reducing antioxidant power of FRAP assay were also studied. Results showed that different extraction methods might have significant differences in the yield of crude oil, palmitic and stearic acid compositions, pH, refractive index, color measurement, TPC, DPPH, and FRAP values at $p<0.05$. Crude oil samples for pulp and peel extracted using the UAE method have the highest yield at 7.81±1.84% and 4.24±0.20%, respectively, as compared to other methods. As for concentrations of palmitic in the sample, crude pulp oil extracted using UAE showed the highest concentration (0.792±0.102 ppm) in comparison to other methods. However, no significant difference was observed in stearic acid concentration. Crude oil extracted using UAE method also showed a significant difference in pH for pulp (6.24±0.05), refractive index for pulp (20.27±0.15) and peel (18.50±0.35), TPC for pulp (6.51±0.04 mg GAE/g) and peel (6.65±0.07 mg GAE/g) and percentage of DPPH radical scavenging from pulp (41.09±0.24%) and peel (68.75±0.09%). In conclusion, UAE was a more efficient extraction method for extracting the highest oil yield, free fatty acid content, and antioxidant activity. In contrast, the physicochemical properties of pulp and peel are comparable.

INTRODUCTION

Gac fruit (*Momordica cochinchinesis*) is a less-known fruit in Malaysia that originates from Southeast Asia. The morphology of Gac fruit is peel (exocarp), pulp (mesocarp), aril, and seed. Gac fruit is a bright-red, short-spined fruit rich in fatty acids such as linoleic acid and oleic acid and antioxidants such as β-carotene and lycopene. Due to this, Gac fruit has been highly sought in many industries in recent decades, especially in the pharmaceuticals, food, and cosmetic industries [1]. Fatty acids are important to human health as they provide energy, assist in fat-soluble vitamin absorption, support cell growth, and act as messengers in protein synthesis. On the other hand, antioxidants help scavenge free radicals that harm and

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damage the cells in the body. Even though Gac fruit has gained popularity due to health awareness, this fruit is highly underutilized. Usually, the arils and seeds will be extracted to obtain antioxidant-rich oil to be encapsulated and marketed [2,3]. Other than that, Gac arils have also been incorporated with dairy products as added nutrients. Gac fruit is seasonal, and studies on preserving it to make it available throughout all seasons are actively being investigated. In the Gac oil-producing industry, mechanical pressing and steam distillation are normally used to extract the oil. However, these methods lead to the loss of desirable components in Gac fruit, especially antioxidants, and cannot fully extract the oils in the fruit [4,5]. Other extraction methods, such as solvent and enzymatic, are used to overcome mechanical extraction and steam distillation limitations.

Extraction is a process of separating substances from the matrix. Extraction can be approached by several methods, including mechanical extraction, solubility in solvents, and pre-treatment process. In the case of Gac oil extraction, while there has been significant exploration, more comprehensive studies need to focus on other parts, such as pulp and peel. These gaps hinder a holistic understanding of the phenomenon and limit the applicability of existing knowledge. Therefore, this research aims to bridge these gaps by delving deeper into the extraction of Gac pulp and peel oils, providing novel insights, and contributing a more comprehensive understanding of their physicochemical properties. Three types of extraction were focused on extracting Gac pulp and peel oil.

Maceration is one of the oldest traditional extraction techniques that can be conducted at room temperature. The main principle of maceration involves soaking powdered or coarse materials in solvent for extended periods [6]. The soaking time is often accompanied by an agitation process to increase the efficiency of the extraction process. The mechanism involves: i) solvent diffusion into materials' cell walls through agitation, ii) extended soaking time to promote cell wall destruction to extract components of interest into the solvent. After extraction, a filtration process will be followed to recover the bioactive compounds. The factors contributing to its efficiency include the types of solvents, particle size, extraction time, and solid-to-solvent ratio [7]. This extraction process has its advantages and disadvantages. As for the advantages, maceration is a simple, low-cost extraction process compatible with a wide range of solvents. It does not require heat, thus making it suitable for recovering heat-sensitive materials. Meanwhile, the disadvantages of maceration include long extraction time and low yield, as well as the fact that it can be a source of microbial growth and lead to fermentation, which is a major drawback to the food industry.

Ultrasonic-assisted extraction is a technique that applies high-frequency sound waves (exceeding 20 kHz) to extract compounds from various sources. The UAE has been used in many studies to extract oil, including rapeseed flakes [8], mahua seed oil [9], sweet passion fruit seeds [10], macauba pulp [11], and Gac fruit aril [12]. The UAE is considered a better alternative than conventional extraction techniques as it can shorten the extraction time, use less solvent, and operate at low temperatures. High-pressure and low-pressure alternates within the liquid created by the sound waves, forming tiny bubbles identified as cavitation bubbles that become unstable and collapse [13]. According to Das [13], the bubbles' molecular motion frequency and speed increase during cavitation, making them unstable and collapse. The disruption of bubbles creates high temperatures, pressure, and sheer forces, creating shockwaves called hot spots. These hot spots create micro-streaming and turbulence effects, which then break down the cell walls, increase solvent penetration into the matrix, and enhance the mass transfer of the solid matrix to the solvents. Thus, extraction of materials can be performed.

Lastly is the enzymatic-assisted extraction. Using enzymes to recover oil from a matrix eliminates the use of solvents that can be harmful to the environment as it contributes to emissions of volatile organic compounds (VOCs), increasing the greenhouse effect. Other than that, enzymatic extraction can lower

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investment costs and energy consumption. However, due to economic reasons, obtaining the enzymes can be problematic due to high operational costs. Thus, the enzymatic extraction method is suitable for large-scale applications, such as at the industrial level. Enzymes that can be used to assist the oil extraction are protease, cellulase, pectinase, and α-amylase or combinations of these enzymes. It is found that a mix of these four enzymes can extract about 82% of Gac aril oil [14]. As the enzymatic reactions increase, the maximum oil yield can also be obtained at 58°C. As the temperature increases, the reaction rate starts to decrease, and eventually, the enzymes become denatured.

EXPERIMENTAL

Materials and Pre-Treatment of Gac Fruit Pulp and Peel

Gac fruits were purchased from a local seller from Johor, Malaysia, at maturity 4-5 (fully ripe) [15]. Before conducting the extractions, the Gac fruit pulp and peel were separated from other parts of the fruit, including the seeds and arils. Then, the pulp and peel were dried at 60℃ for 8 h using a cabinet dryer. Then, the dried pulp and peel were reduced in size using a lab blender and sieved using a 0.5 mm sieve. The dried powder was stored in sealed bags at 5℃ until further use.

Maceration

Oil was extracted according to the method described by Vald *et al.* [16]. One hundred and five milliliters of 75% ethanolic solution was added to 7 g of dried powder in a 250 mL conical flask. Then, the mixture was mixed using an incubator shaker for 4 h at room temperature. The mixture was centrifuged at 2200 rpm for 15 min, and the first supernatant layer was collected. Then, 15 mL of ethanol was added to the residue and shaken for another 2 h. Next, the mixture was centrifuged again, and the second supernatant layer was collected. The solution was evaporated with a Buchi Rotary Evaporator R-215 under reduced pressure at 50℃ until constant weight was achieved. The weight of the oil in the flask was recorded to calculate the oil yield. Lastly, the crude oil was transferred into dark sealed vials and stored at 5℃ until further analysis.

Ultrasound-Assisted Extraction (UAE)

The UAE was conducted using the Rodrigues *et al.* [11] method with modifications. About 7 g of dried Gac powder was placed in an Erlenmeyer flask (250 mL). About 70 mL ethanol was added to the flask and covered. The UAE probe was immersed into the flask. The test was conducted at 40 kHz and an extraction time of 30 min. After that, the sample was filtered, and the excess filtrate was evaporated into a Buchi Rotary Evaporator R-215 under reduced pressure at 50℃ until a constant weight was achieved. The weight of the oil in the flask was recorded to calculate the oil yield. Lastly, the crude oil was transferred into dark sealed vials and stored at 5℃ until further analysis.

Enzymatic-Assisted Extraction (EAE)

This extraction method was conducted as described by Mai *et al.* [14] and Song *et al.* [17] with slight improvisations. About 7 g of Gac powder was mixed with 49 mL of ethanol (Gac pulp powder : Ethanol, 1:7). The powder was mixed with deionized water and mixed thoroughly using a magnetic stirrer in a 1000 mL graduated beaker. The stirring speed was set at 150 rpm with an incubation temperature of 57℃. When the temperature reached 50℃, the solution was adjusted to pH 4.5 using 1 M HCl solution. Pectinase was added to the solution with about 0.15% w/w of powder plus water and incubated for 1 h. After incubation,

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the temperature was raised to 60℃, ethanol was added, then the pH was raised to 9.0 using 1 M NaOH solution and stirred for 1 h. The incubation temperature must not exceed 65℃ as it can decrease the activities of pectinase or denature it.

Upon completion, the mixture was added to distilled water and stirred vigorously to separate the oil from the residue. After that, the sample was centrifuged (Kubota, Model 5420) at 2200 rpm for 30 min. Then, the sample was filtered, and the excess filtrate was evaporated using a Buchi Rotary Evaporator R-215 under reduced pressure at 50℃ until a constant weight was achieved. The experiment was carried out in triplicate. The weight of the oil was recorded to calculate the oil yield. Lastly, the crude oil was transferred into dark sealed vials and stored at 5℃ until further analysis.

Oil Yield (%)

The oil extracted by each method was weighed using an analytical balance as described by Rodrigues *et al.* [11]. The oil yield must be expressed in terms of mass percentage as in equation 1:

$$\text{Oil yield } (\%) = \frac{mass\ of\ extracted\ oil\ (g)}{mass\ of\ sample\ (g)} \times 100\ \% \qquad \text{Equation 1}$$

Determination of Free Fatty Acids Composition

Fatty acids composition was determined using fatty acid methyl esters (FAME) in a GC with Flame Ionization Detector (FID) as described by Thilakarathna *et al.* [9] with slight modification. The crude oil was measured into a 15 mL centrifuge tube. Two millimeters of petroleum ether was added, followed by 1 mL of 2 M methanolic KOH. The tube was closed, and the tube was shaken vigorously for 30 seconds, and then centrifuged. The petroleum ether layer (containing FAME) was retrieved and transferred into a GC vial. About 1 μL of FAME was injected into the capillary column for analysis. Palmitic acid and stearic acid were used as standards for peak identification. The GC was prepared as below (Table 1):

Table 1: GC-FID settings

Injector and detector temperature	250℃
Oven temperature	Hold at 60℃ (2 min), increase up to 200℃ at rate of 10℃/min, increase from 200 to 240℃ at rate of 5℃/min, and hold for 18 min
Carrier gas	Nitrogen

Refractive Index (RI)

The RI was measured using the method described by Pereira *et al.* [11]. The extracted oil was measured using an Abbe refractometer at 25℃. The secondary prism was opened, and 2-3 drops of oil sample were dropped onto the center of the surface of the main prism. The prism was closed gently. The measuring knob was slowly turned until a boundary line can be obtained. The RI was recorded.

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Color Measurement

The color measurement was conducted using a chromameter (Konica Minolta, Model CR-400, Japan) as described by Thumthanaruk *et al.* [18]. The chromameter was calibrated using a standard white tile. Then, the crude oil was poured onto a glass container, and the color was measured in terms of Hunter color values of ***L****, ***a****, and ***b****.

pH

The pH measurement was conducted using a pH meter (Hanna Instrument, Model 2215-02) as described by Thumthanaruk *et al.* [18]. The pH is calibrated using the pH buffer solution of 4 and 7. About 5 mL was transferred into a small beaker, and the pH probe was dipped in the crude oil sample. The pH reading was recorded.

Total Phenolic Content (TPC)

According to Thilakarathna *et al.* [9], with slight modifications, TPC was performed by mixing 100 μL of the oil with 0.5 mL of the Folin-Ciocalteu, followed by 7.9 mL distilled water and 1.5 mL sodium carbonate (Na_2CO_3). Then, the oil was left dark for 2 h, and the absorbance was read at 765 nm. Gallic acid was used as reference standard and expressed as milligrams of Gallic Acid Equivalent per gram of sample (mg GAE/g sample).

DPPH Radical-Scavenging Assay

The DPPH radical-scavenging assay was conducted based on Abdulqader *et al.* [19] and Kubola & Siriamornpun [15] with slight improvisations. The DPPH working solution was prepared at a concentration of 0.001 M DPPH in absolute ethanol. About 1.2 mL crude oil was added to 9 mL DPPH solution and vortexed. Then, it was incubated for 20 min in dark conditions. The 1000 ppm ascorbic acid was used as a positive control, while ethanol was used as the blank. Next, the absorbance was measured at 517 nm using a UV/Vis spectrophotometer. The percentage of scavenging activity was calculated using the formula equation 2:

$$\%\ scavenging\ activity = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100\% \qquad \text{Equation 2}$$

Where, A_0 = absorbance of control, A_1 = absorbance of the crude oil with DPPH

Ferric Reducing Antioxidant Power (FRAP) Assay

The assay was performed based on the method described by Tinrat *et al.* [20]. The FRAP reagent was prepared by combining the 100 mL of 300 mM acetate buffer with 20 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) solution prepared in 0.33 mL of 40 mM HCl and 10 mL of 20 mM iron (III) chloride-6-hydrate ($FeCl_2.6H_2O$) solution. The fresh working solution was warmed at 37℃ for 10 min. In a test tube, about 300 μL of the crude oil was added to 2.7 mL of FRAP reagent and mixed well using a vortex mixer for 10 seconds. Then, the test tube was placed in a water bath at 50℃ for 1 h. Next, the absorbance was measured at 596 nm using a UV/Vis spectrophotometer. A standard calibration curve of Trolox of different concentrations (0.02, 0.04, 0.06, 0.08, and 0.10 mg/mL) was prepared. The results were expressed as milligrams of Trolox equivalent per gram of crude oil (mg TE/g).

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Statistical Analysis

All analyses were performed with triplicate analysis. The result was expressed as mean ± standard deviation. One-way ANOVA and Tukey tests were performed to determine the significant difference at $p < 0.05$.

RESULTS AND DISCUSSION

Oil Yield

The yield of crude pulp and peel oil extracted using maceration, UAE, and EAE was presented in Table 2. Gac fruit pulp and peel oil extracted using ultrasound have higher yields and were shown to have a significant difference ($p<0.05$) than the other two methods. This shows that factors like extraction methods can influence the oil yield obtained. Gac aril oil extracted using a mechanical press and solvent methods was found to have a higher oil yield using the solvent method than mechanically pressed Gac aril oil [21]. Another study by Tu *et al.* [22] shows that the ultrasound extraction method can enhance the yield obtained from defatted pumpkin seed powder. Ultrasounds utilize cavitation, which destroys the double bond in a structure; thus, the extraction method can be done effectively. UAE can also decrease the extraction time by 72-fold as compared to the maceration process [23]. Based on the extraction methods above, the extraction time of UAE and maceration took about 30 min and 6 h, respectively. The decrease in extraction time by UAE is aligned with a study conducted by Osorio-Tobón [23].

Table 2: Percentage of oil extracted

Methods	Yield (Pulp) (%)	Yield (Peel) (%)
Maceration	4.44 ± 0.55^{b}	3.43 ± 0.66^{b}
UAE	7.81 ± 1.84^{a}	4.24 ± 0.20^{a}
EAE	4.06 ± 0.20^{b}	4.15 ± 0.15^{a}

Values are expressed as mean ± SD of triplicate measurement. Numbers on the same column with differing superscripts are significantly different at $p < 0.05$. UAE, ultrasound-assisted extraction. EAE, enzymatic-assisted extraction.

Free Fatty Acids Composition

The concentration of fatty acids present in all extracted crude oil was measured using GC-FID (Table 3). According to Ishida *et al*. [24], the three main fatty acids of fats in Gac arils were palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2). Meanwhile, according to Aamir & Jittanit [21], the highest amount of fatty acids in Gac aril were palmitic acid, stearic acid (C18:0), oleic acid and linoleic acid. In this study, two main saturated fatty acids, palmitic and stearic acid, were studied.

Extraction using the UAE method was shown to have a significant difference ($p<0.05$) between EAE and maceration, as it can extract more palmitic acid in terms of concentration. However, the stearic acid concentration showed no significant difference ($p>0.05$) between the three extraction methods, as stearic acid concentration was relatively the same amount. A study by Kha *et al.* [12] shows that UAE has recovered Gac aril oil better than Soxhlet extraction. Fatty acids were not quantified for gac peels crude oil due to the instrument's detection limit. This means the fatty acids in the sample are present in trace amounts. Therefore, the fatty acids cannot be quantified accurately.

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Table 3: Concentration of free fatty acids

Methods	Palmitic acid, C 16:0 (ppm)	Stearic acid, C 18:0 (ppm)
Maceration (pulp)	0.238 ± 0.098^{b}	0.017 ± 0.001^{a}
UAE (pulp)	0.792 ± 0.102^{a}	0.032 ± 0.013^{a}
EAE (pulp)	0.254 ± 0.122^{b}	0.006 ± 0.001^{a}
Maceration (peel)	N/D	N/D
UAE (peel)	N/D	N/D
EAE (peel)	N/D	N/D

Values are expressed as mean ± SD of triplicate measurement. Numbers on the same column with differing superscripts are significantly different at $p < 0.05$. UAE, ultrasound-assisted extraction. EAE, enzymatic-assisted extraction. ppm, part per million. **N/D**: not detected.

Refractive Index

The refractive index (RI) of crude pulp and peel oil extracted from different extraction methods is presented in Table 4. The Gac arils oil extracted was reported to have a RI of 72% at 25℃ [14]. Compared to the data obtained, the Gac pulp oil extracted from all three methods has a much lower RI than Gac aril oil. Among the three methods, UAE showed a higher RI than oil extracted from maceration and EAE. Brix value directly correlates with dissolved components in a sample. In the food industry, Brix can be used to signify the ripeness and quality of a product. Compared to other edible oils such as palm oil (1.45-1.46 nD) and sunflower oil (1.46 nD), Gac pulp oil obtained has a slightly lower reading in RI than palm oil and sunflower oil. The RI also signifies the quality of the oil. For example, a RI is used to grade and standardize the edible oil to ensure the oil meets specific quality index requirements. The RI also shows different components, such as fatty acids, esters, and other organic compounds, that influence the chemical composition of the oil. A high RI shows a higher degree of unsaturation, molecular weight, and chain length of fatty acids. For example, a study by Rahman *et al.* [25] showed that the RI is influenced by the degree of unsaturation, molecular weight, chain length of fatty acids, and different extraction methods.

Table 4: Refractive index of extracted oil

Methods	Pulp	Peel
Maceration	16.30 ± 0.26^{b}	13.30 ± 0.17^{b}
UAE	20.27 ± 0.15^{a}	18.50 ± 0.35^{a}
EAE	19.97 ± 0.23^{a}	18.03 ± 0.06^{a}

Values are expressed as mean ± SD of triplicate measurement. Numbers on the same column with differing superscripts are significantly different at $p < 0.05$. UAE, ultrasound-assisted extraction. EAE, enzymatic-assisted extraction.

Color Measurement

Color measurements of crude pulp and peel oil using a chromameter were evaluated in terms of lightness (L^*), redness (a^*), and yellowness (b^*) (Table 5). Based on Table 5, macerated crude pulp oil had a lighter color than EAE and UAE crude oil. Although using maceration will produce a lower yield of components of interest, the maceration process uses a lower temperature for extraction (room temperature) than UAE and EAE, making it a gentle extraction method and suitable for heat-sensitive materials. This statement is aligned with a study conducted by Reiter *et al.* [26], where it was observed that there was an increase in lightness in enzymatic macerated carrot fruit oil.

Table 5: Colour measurement of extracted oil

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Methods	*L**	*a**	*b**
Maceration (pulp)	31.24±0.03[a]	-0.29±0.02[c]	12.93±0.03[b]
UAE (pulp)	30.38±0.02[c]	2.02±0.04[b]	16.17±0.07[a]
EAE (pulp)	30.52±0.01[b]	3.40±0.05[a]	16.05±0.05[a]
Maceration (peel)	28.73±0.03[a]	0.80±0.05[a]	12.59±0.03[a]
UAE (peel)	27.03±0.02[b]	-0.44±0.11[b]	11.24±0.03[b]
EAE (peel)	27.06±0.02[b]	-0.50±0.10[b]	11.34±0.06[b]

Values are expressed as mean ± SD of triplicate measurement. Numbers on the same column with differing superscripts are significantly different at $p < 0.05$. UAE, ultrasound-assisted extraction. EAE, enzymatic-assisted extraction. *L**, lightness. *a**, (+) red / (-) green. *b**, (+) yellow / (-) blue

pH

Table 6 shows the pH of crude pulp and peel oil extracted from three different extracts measured using a pH meter. The oil extracted by the maceration method has a slightly acidic pH, while UAE and EAE oil have an almost neutral pH. According to Thumthanaruk *et al.* [18], the pH of Gac aril oil ranges from 5.5 to 5.63, while another source reports that Gac fruit oil has a pH range of 5.5 to 6.0 [27]. The pH of UAE crude oil increases due to the degradation of materials such as proteins, polysaccharides, and others that might contribute to the release of alkaline substances. The cavitation effects of UAE also generate high temperatures and pressure, which lead to the breakdown of water molecules and the release of OH ions that can increase the pH of crude oil. Similar results were observed, as evidenced by the increase of pH near 6, which is almost neutral in a study conducted by Elshreef *et al.* [28].

Another study by Kumar *et al.* [29] showed an increase in pH due to the recovery of bioactive compounds from fruits and vegetables processing by-products using UAE. An increase in pH during ultrasound extraction can positively influence the extraction of bioactive compounds, including those with antioxidant properties, and contribute to enhanced radical scavenging activities. As for EAE, adding enzymes could be a factor in increasing the pH of crude oil. The interaction between the enzyme and substrate (the fruit materials) can affect the overall pH dynamic of the extraction mixture.

Table 6: pH of extracted oil

Methods	Pulp	Peel
Maceration	5.15±0.02[b]	5.95±0.03[c]
UAE	6.24±0.05[a]	6.17±0.02[b]
EAE	6.11±0.01[a]	6.34±0.01[a]

Values are expressed as mean ± SD of triplicate measurement. Numbers on the same column with differing superscripts are significantly different at $p < 0.05$. UAE, ultrasound-assisted extraction. EAE, enzymatic-assisted extraction.

Total Phenolic Content

The total phenolic content (TPC) of crude pulp and peel oil extracted using three different methods is presented in Table 7. A study by Kubola *et al.* [15] shows the antioxidant activity of Thailand Gac fruit from various parts (arils, seeds, pulp, and peel). According to them, Gac fruit pulp has about 1.52 mg GAE/g from red pulp and 2.60 mg GAE/g from yellow pulp. This shows that as the maturity stage of the fruit increases, the antioxidant activity decreases. The data obtained from the three extraction methods (Table 7) were higher than that of Kubola's findings, especially the oil extracted from the UAE method. Another

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study by Abdulqader *et al*. [19] shows that the pulp has about 28.9 mg GAE/g of dry sample. Also, it was noted that the study found that the total phenolics in pulp, peel, and arils are present at similar levels, but with arils having a higher level of phenolic content. These results aligned with a study by Zhang *et al*. [30], where UAE can extract a higher yield of phenolic content, while maceration has the lowest phenolic compounds extracted.

Table 7: Total phenolic content of extracted oil

Methods	Pulp (mg GAE/g)	Peel (mg GAE/g)
Maceration	5.43 ± 0.03^{c}	5.54 ± 0.03^{c}
UAE	6.51 ± 0.04^{a}	6.65 ± 0.07^{a}
EAE	5.58 ± 0.01^{b}	5.76 ± 0.08^{b}

Values are expressed as mean ± SD of triplicate measurement. Numbers on the same column with differing superscripts are significantly different at $p < 0.05$. UAE, ultrasound-assisted extraction. EAE, enzymatic-assisted extraction.

DPPH Radical Scavenging Activity

Table 8 shows the percentage of radical scavenging of DPPH in crude pulp and peel oil. UAE's crude pulp and peel oil showed significant differences ($p<0.05$), whereby the crude oils could scavenge more free radicals of DPPH as compared to other methods. However, maceration's crude pulp oil has no significant difference from UAE's crude pulp oil. A study by Saini *et al.* [31] showed that the UAE technique for the extraction of polyphenols from peels of different citrus cultivars (kinnow mandarin and mousambi peels) is more efficient, which were 48.23% and 39.73%, respectively, in contrast to maceration technique, which was 42.96% and 22.46%, respectively. This study indicates the possibility of UAE extraction methods over maceration regarding the total antioxidant capacity of citrus peels extracted. Table 8 also shows that EAE's crude pulp and peel oil have a lower percentage of DPPH radical scavenging than UAE and maceration. According to Gómez-García *et al*. [32], phenolics released depend on the type of enzyme, period of enzyme treatment, particle size, and solvent extraction type. Pectinase might not be effective in recovering polyphenol compounds.

Table 8: Percentage of radical scavenging activity of DPPH.

Methods	Pulp (%)	Peel (%)
Maceration	40.56 ± 0.35^{a}	66.06 ± 0.67^{b}
UAE	41.09 ± 0.24^{a}	68.75 ± 0.09^{a}
EAE	38.63 ± 0.03^{b}	66.63 ± 0.28^{b}

Values are expressed as mean ± SD of triplicate measurement. Numbers on the same column with differing superscripts are significantly different at $p < 0.05$. UAE, ultrasound-assisted extraction. EAE, enzymatic-assisted extraction.

Ferric Reducing Antioxidant Power (FRAP) Assay

Results in Table 9 showed there is no significant difference ($p>0.05$) between methods and fruit fractions (pulp and peel). A study conducted by Tinrat *et al*. [20] shows a similar result when there is no significant difference ($p>0.05$) in FRAP values between pulp and peel. Another study by Pereira *et al*. [10] showed that using UAE to extract sweet passion fruit oil obtained a higher FRAP value (288 μM TEAC/g oil) than Soxhlet and subcritical propane extraction. The factors that influence variations in results may be

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due to the presence of different antioxidants where there are different types of antioxidants in the sample and owing to different fruits used in research due to climate, planting techniques, and others [33]. According to Chuyen *et al.* [34], a study of optimization of extraction conditions Gac peel for carotenoid recovery and antioxidant activity showed insignificant iron-reducing activities using UAE. Chuyen's study aligned with the results obtained in this study, where low FRAP values were determined for all extraction methods for crude pulp and peel oil.

Table 9: Iron-reducing power of FRAP assay

Methods	**Pulp (mg TE/g)**	**Peel (mg TE/g)**
Maceration	0.09 ± 0.00^{a}	0.08 ± 0.00^{a}
UAE	0.09 ± 0.00^{a}	0.09 ± 0.00^{a}
EAE	0.09 ± 0.00^{a}	0.08 ± 0.00^{a}

Values are expressed as mean ± SD of triplicate measurement. Numbers on the same column with differing superscripts are significantly different at $p < 0.05$. UAE, ultrasound-assisted extraction. EAE, enzymatic-assisted extraction. TE, Trolox equivalent.

LIMITATIONS OF STUDY

One of the directions of research to be carried out to further this study is to search for other effective extraction methods for extracting pulp and peel oil. Not only that, in terms of the physicochemical properties of oils, the determination of iodine value and peroxide value, as well as quantification of other fatty acids, can also be investigated. Lastly, food application from the crude oil extracted can be conducted to study the effect of pulp and peel oil on food products.

CONCLUSION

Gac pulp and peel oil that was extracted using maceration, ultrasound-assisted extraction, and enzymatic-assisted extraction were analyzed in terms of percentage of yield, fatty acids composition, refractive index, color measurement, total phenolic content, DPPH scavenging activity, and FRAP assay. Using UAE, a higher yield of crude oil with a higher composition of palmitic acid, refractive index, total phenolic content, and total antioxidant capacity was observed. In contrast, using maceration produced a lighter color of crude oil with lower fatty acid values as compared to UAE and EAE. At the same time, using the EAE method, it is able to produce significant amounts of oils.

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AUTHOR'S CONTRIBUTION

Siti Anis Habibah carried out the research and wrote and revised the article. Eng-Keng Seow conceptualized the central research idea, provided the theoretical framework, designed the research, supervised the research progress, anchored the review and revisions, and approved the article submission.

CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted without any self-benefits, commercial, or financial conflicts and declare the absence of conflicting interests with the funders.

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