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Extraction of Rutin from the Leaf of Male *Carica papaya* Linn. using Microwave-Assisted and Ultrasound-Assisted Extractive Methods

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Abstract. The extraction yield of rutin from the male Carica papaya Linn leaf using Microwave-Assisted Extraction (MAE) and Ultrasound-Assisted Extraction (UAE) methods were investigated and compared. Extraction parameters were analyzed to determine the effects on the yield of rutin. The efficiencies of both extractive methods were also compared. In MAE, the effect and square effect of ethanol mixture concentration, along with its interaction effect with the solid-liquid (S/L) ratio, was found to have significantly influenced the rutin yield. The square effect of particle size was also determined to be important in MAE. In UAE, the effect and square effect of ethanol mixture concentration was found to be crucial to the yield of rutin. The square effect and its interaction effect with extraction time were noticeably significant in UAE. A higher optimized yield of rutin (4.06 ± 0.2 mg/g) was obtained using UAE at an ethanol mixture concentration of 51.5%, sonication time of 70.5 min, the particle size of 355 μm, and S/L ratio of 1:108.6 wt/wt papaya leaf/ethanol mixture despite having longer extraction time and higher energy requirement per gram of rutin than MAE. In contrast, MAE was found to be more efficient by having a higher yield obtained per hour of extraction (27.38 g/h), lower energy consumption (10 W/h), and lower energy required per gram of ruin (3.65 W.h/g). In terms of a greener extraction technique, MAE would be a better fit by consuming lesser extraction solvent and energy to extract rutin from papaya leaf.

Keywords: *Carica papaya*, Male Leaf, Microwave-Assisted Extraction, Response Surface Methodology, Rutin Extraction, and Ultrasound-Assisted Extraction.

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INTRODUCTION

Rutin (also known as rutoside, quercetin-3-*O*-rutinoside, sophorin, or vitamin P), shown in Figure 1, is a natural flavonoid with health benefits that include having antidiabetic, anti-allergic, anti-inflammatory, anticancer, and antioxidant properties. Rutin can be found in more than 70 medicinal plants, such as buckwheat, passionflower, and papaya (Satari, Ghasemi, Habtemariam, Asgharian, & Lorigooini, 2021). *Carica papaya* Linn., part of the Caricaceae family is generally known as papaya. It is widely consumed for its various nutraceutical benefits. Different parts of the papaya plant contain various phytochemicals used to treat illness and prevent diseases, such as papain, vitamins, carotenoids, and polyphenols (Sarker, Khan, & Mohamed, 2021). Studies have also shown that there are differences in the level of active compounds in male and female plants (Rabska, Pers-Kamczyc, Żytkowiak, Adamczyk, & Iszkuło, 2020). The papaya plant generally comes in three genders: male, female, and hermaphrodite. However, previous extraction studies on papaya leaf were conducted without prior identification of the gender of the plant. Hyun et al. reported the 6.30 ppm of rutin in papaya leaf water extract obtained using hot water extraction from papaya leaf (Hyun, Ko, & Hyun, 2021). Next, Maisarah et al. identified 3.33 mg/g of rutin was identified in the extract of papaya leaf (Maisarah, Amira, Asmah, & Fauziah, 2013) by using the maceration extraction method for two hours. 47 mg/g of rutin was determined in the leaf and fruit extract of *Carica papaya* by Khadam et al. (Khadam et al., 2019) using the maceration extraction technique for 24 hours. The present paper focuses on extracting rutin from male papaya leaves. The male papaya plant is often eliminated in the early stage of plant growth as the hermaphrodite plant is generally favored in plantations (Ming, Yu, & Moore, 2007).

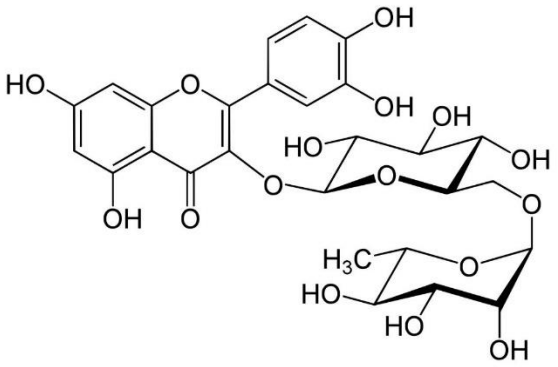

Figure 1: Chemical structure of rutin.

Active compound extraction is a process of separating targeted compounds from material in either solid or liquid form. The criteria for selecting the extraction method and extractive solvent includes the physical and chemical characteristic of the targeted compounds, the efficiency and complexity of the extraction methods, and extraction costs (Zhang, Lin, & Ye, 2018). In this study, green extractive solvents such as water and ethanol are viable solvents for the extraction of rutin. Additionally, ethanol is also a US-FDA (US Food and Drug Administration) approved solvent (De Luna, Ramírez-Garza, & Saldívar, 2020; Nor, Manan, Mustaffa, & Lee, 2017). Rutin can be extracted using different extraction techniques. One common technique is known as maceration. It is a conventional method that involves prolonged extraction time and a high consumption of extraction solvent (Rasul, 2018). Therefore, greener extraction technique is required to preserve nature and resources.

Microwave-assisted extraction (MAE) and Ultrasound-assisted extraction (UAE) are of interest here due to their low operating risks,

simplicity, and efficiency compared to the disadvantages of maceration. MAE has a shorter extraction time ranging from a few seconds to less than an hour. In an MAE process, polar molecules absorb microwave energy to generate heat energy to break the cell walls and release active compounds from plant particles to the surrounding solvent. Over the past few years, academic researchers and industrial professionals have joined hands in trying to scale up microwave-assisted extraction and apply it commercially. Various types of conceptual equipment have arisen for better monitoring and control of the process conditions (Li, Radoiu, Fabiano-Tixier, & Chemat, 2013). A successful scale-up process has also been demonstrated by Radoiu et al. by comparing the result between lab-scale MAE and industrial-scale MAE using a continuous flow process (Radoiu, Splinter, & Popek, 2019). Despite that, new reactor concepts with microwave technology are still required to achieve various goals depending on the nature of targeted compounds, extraction samples, and extraction solvents.

On the other note, UAE utilizes the formation and collapse of bubbles near the plant matrix to break cell walls and allow active compounds to diffuse out of the plant matrix (Ling, Fun, Yeop, Yusoff, & Gimbun, 2019). In this study, process optimization was carried out using Box-Behnken Design (BBD) to investigate the relationship between parameters and yield of rutin. BBD was chosen for its high efficiency, requiring fewer trials that are necessary to form a conclusion for a process (Ferreira et al., 2007). Extraction time, particle size, solvent concentration, and S/L ratio are the parameters focused on in this study. Extraction performance between MAE and UAE is compared at the end of this study.

MATERIALS AND METHODS

Chemicals

HPLC-grade methanol (CH_3OH, 99.9 %) and analytical grade undenatured ethanol (C_2H_5OH, 99.9 %) were procured from Fisher Scientific, rutin hydrate ($C_{27}H_{30}O_{16}{\cdot}xH_2O$, ≥ 94 %) was obtained from Sigma-Aldrich, and ultrapure water from a Milli-Q ultrafiltration system.

Plant materials and preparation

Leaves of male *Carica papaya* Linn were procured from a local plantation in Selangor, Malaysia. Petioles were removed from the leaves. Water was run through the collected leaves to remove noticeable contaminants. The leaves were then placed in an oven and dried at a temperature of 50°C. Dried papaya leaves were grounded to obtain a fine solid sample. Fine leaf samples were then categorized into a different range of sizes between 355 μm and 710 μm (Poureini, Mohammadi, Najafpour, & Nikzad, 2020). The aforementioned leaves samples were stored in a sealed container under 4°C for future use.

Rutin Extraction

Microwave-Assisted Extractive (MAE) Method

In this study, a commercial microwave (Samsung, ME711K, South Korea) with a power range between 100 W to 800 W was utilized. A prefixed ratio of leaves samples and extraction solvent were placed in a Schott bottle to make up a final mass of the sample-solvent mixture of 50 g. The weight of the Schott bottle, leaf sample, solvent, and cap were measured and recorded respectively for the calculation of rutin yield. A separate water bath was prepared to lower the temperature of plant extracts and to condense trapped vapor within the bottle. The parameters

involved in this experiment were ethanol mixture concentration (C), S/L ratio (R), particle size (S), and irradiation time (T_M).

Ultrasound-Assisted Extraction (UAE) Method

Ultrasound-assisted extraction (UAE) was conducted using an ultrasonic water bath (Branson, Bransonic M3800H-E, USA) with a maximum power of 110 watts and an output frequency of 40 kHz. An ultrasonic water bath system was chosen in this study as its indirect contact with the source of the sonication wave will allow for the prevention of plant samples (Machado, Faccio, & Pistón, 2019). Prior to the extraction process, plant samples and extraction solvent were placed into a Schott bottle at a predetermined S/L ratio, making up to a total of 50 g mass of the sample-solvent mixture. The weight of the bottle, plant sample, solvent, and cap were individually recorded for subsequent calculations. Water in the ultrasonic bath was changed after every cycle of the extraction. At the end of the extraction process, the Schott bottle was taken out from the ultrasonic water bath and transferred to a room temperature water bath to reduce the temperature of plant extracts before getting weighed for the second time and transferred into an HPLC vial. The parameters involved in this experiment were ethanol mixture concentration (C), S/L ratio (R), particle size (S), and sonication time (T_U).

Design of Experiment and Statistical Analysis

In the present study, the design of the experiment and construction of the model for the yield of rutin from papaya leaf was carried out using RSM. The correlation between extraction parameters, the response involved, and extraction process optimization was conducted using BBD. A BBD that has four factors with three levels (-1, 0, 1) and five center points was formed based on the variables listed in Table A.1 under supplementary data A. The range of extraction parameters such as extraction time (T_M for irradiation time, T_U for sonication time), S/L ratio (R), ethanol mixture concentration (C), and size of papaya leaf (S) were pre-decided and modified based on an intensive literature review (Chahyadi & Elfahmi, 2020; Martino, Ramaiola, Urbano, Bracco, & Collina, 2006; Poureini et al., 2020). A total of 29 runs of experiments were conducted where the experimental data are tabulated in Table 1 and discussed. Independent variables and responses were formed by fitting experimental data to a quadratic equation. Next, Analysis of Variance (ANOVA) was employed to analyze the effect and significance of experiment parameters and the response. Subsequently, experimental results were used to compare and validate against calculated optimal conditions and responses.

Analytical Methods

HPLC Analysis of Rutin

To identify and quantify the content of rutin, Agilent 1200 series HPLC system was used. Plant extracts were filtered into an HPLC vial using a 0.22 µm syringe filter. HPLC-grade methanol and ultrapure water generated by the Milli-Q ultrafiltration system were selected as the mobile phase. Prior to the analysis, pure methanol was allowed to run through the HPLC system with a purge valve opened for 15 min to get rid of the remaining solvent from the previous user from the HPLC system before entering the analysis column. Subsequently, pure methanol ran through the analysis column for another 15 min to dissolve the remaining compound within a

column and was allowed to remove accordingly. Next, the column was subjected to 5% methanol-water for 15 min to condition the column prior to the start of rutin analysis. The solvent gradient of rutin analysis in this present paper was as follows: 5% methanol-water (0-3 min), 5-100% methanol-water (4-6 min), 100 % methanol (7-13 min), 100-5% methanol-water (14-16 min), 5% methanol-water (17-20 min) at the flow rate of 1.0mL/min. The injection volume of the sample was fixed at 10 µL, and the separation was detected by Ultraviolet-Diode Array Detection (UV-DAD) at the wavelength of 360 nm. The analysis column used in this study was Agilent ZORBAX Eclipse Plus C18, 5 µm, 4.6 x 150 mm, and the operating temperature was set at 25°C. Figures displayed in supplementary data B are the HPLC chromatograms of pure rutin solution (Figure B.1) and papaya leaf extract (Figure B.2). Rutin content in papaya leaf was determined using Eq. (1).

$$\text{Yield of Rutin } \left({}^{\text{mg}}/_{\text{g}}\right) = \frac{\text{Mass of rutin extracted (mg)}}{\text{Mass of papaya leaf(g)}} \quad (1)$$

Surface Morphology Analysis

A desktop SEM (Phenom, Phenom ProX, Netherland) was used to examine the surface morphology of the male leaf sample before and after the extraction process. A double-sided carbon adhesive tape was used to mount oven-dried papaya leaves samples onto a sample stub. It was gently blown by compressed air to ensure all samples were secured before placing them onto a sample holder. The sample holder was then subjected to SEM. A rotary knob and mouse were used to control and adjust the focus, brightness, magnification, and contrasts of the images.

Extraction Efficiencies

In this study, the energy consumption, which is the energy required to produce 1 gram of rutin, and the extraction efficiency for individual MAE and UAE were studied and compared. Extraction efficiency was determined based on the optimized yield of rutin extracted per hour of extraction. The energy consumption of the extraction process was measured using Primera-Line Wattage current meter (PM213E, Hugo Brennenstuhl GmbH & Co. KG, Germany) and calculated according to Eq. (2).

$$Q = P \times t \quad (2)$$

where Q is the energy required (W.h), P is the power dissipated (W), and t is the extraction time (h).

RESULTS AND DISCUSSION

Process Optimization

Experimental results based on 29 runs under different conditions are presented in Table 1. Rutin yields obtained under MAE ranged from 0.84 to 4.12 mg/g, while the yields obtained using UAE ranged between 1.13 and 4.46 mg/g. It can be observed that the range obtained under UAE is generally higher than that of MAE, indicating that UAE could potentially extract rutin in higher quantities for every gram of papaya leaf used. ANOVA analysis and the significance of each experimental variable involved are shown in Table 2. The model generated for rutin extraction using MAE was found to be significant with a p-value of $p<0.05$. The linear and square terms of the extraction variables were also significant ($p<0.05$). The results are further corroborated by the high R^2 (91.2 %) and high adj-R^2 (82.3 %). Generally, a high R^2 value implied a high correlation between

independent variables and response. Additionally, the lack of fit for MAE was found to be insignificant, further emphasizing the significance of the model. The effects of extraction variables on the yield of rutin are detailed in the later part of this paper.

Table 1: The BBD with experimental data of MAE and UAE using male papaya leaves.

MAE						**UAE**					
Parameters				**Yield (mg/g)**		**Parameters**				**Yield (mg/g)**	
T_M (min)	R (wt/wt)	S (µm)	C (%)	Observed	Calculated	T_U (min)	R (wt/wt)	S (µm)	C (%)	Observed	Calculated
5	1:90	500	20	1.90	1.90	70	1:170	500	20	1.31	1.55
5	**1:50**	**500**	**50**	**4.11**	**2.96**	**70**	**1:90**	**500**	**50**	**3.88**	**3.87**
5	1:10	500	20	2.39	1.96	70	1:10	500	20	2.55	2.46
5	**1:50**	**500**	**50**	**4.12**	**2.96**	**70**	**1:90**	**500**	**50**	**4.19**	**3.87**
8	1:10	500	50	3.15	3.20	120	1:10	500	50	3.17	2.30
2	1:10	500	50	2.58	2.53	20	1:10	500	50	3.32	3.52
2	1:50	710	50	2.83	1.80	20	1:90	710	50	2.03	2.18
5	1:90	710	50	2.87	2.25	70	1:170	710	50	1.13	1.44
5	1:10	710	50	2.18	2.34	70	1:10	710	50	2.73	3.22
2	1:50	500	20	1.88	1.50	20	1:90	500	20	3.26	2.61
5	1:10	355	50	2.88	3.02	70	1:10	355	50	3.37	3.15
5	1:10	500	80	1.74	1.32	70	1:10	500	80	1.82	1.78
2	1:90	500	50	3.26	2.42	20	1:170	500	50	1.66	1.72
2	1:50	500	80	1.71	0.57	20	1:90	500	80	2.43	1.86
5	1:50	355	80	2.17	0.97	70	1:90	355	80	3.12	3.13
5	**1:50**	**500**	**50**	**3.42**	**2.96**	**70**	**1:90**	**500**	**50**	**3.81**	**3.87**
5	**1:50**	**500**	**50**	**3.51**	**2.96**	**70**	**1:90**	**500**	**50**	**4.02**	**3.87**
5	1:90	355	50	4.05	2.92	70	1:170	355	50	4.28	3.82
5	1:50	710	20	0.83	0.92	70	1:90	710	20	3.01	2.12
8	1:90	500	50	3.21	3.12	120	1:170	500	50	4.46	3.44
5	**1:50**	**500**	**50**	**3.29**	**2.96**	**70**	**1:90**	**500**	**50**	**3.89**	**3.87**
8	1:50	500	80	2.52	1.48	120	1:90	500	80	2.02	2.77
2	1:50	355	50	3.73	2.36	20	1:90	355	50	3.84	4.06
8	1:50	355	50	3.85	3.13	120	1:90	355	50	3.76	3.72
5	1:50	355	20	2.07	2.08	70	1:90	355	20	2.86	3.07
5	1:90	500	80	2.74	1.19	70	1:170	500	80	1.71	2.02
8	1:50	710	50	2.84	2.36	120	1:90	710	50	3.31	3.30
5	1:50	710	80	1.83	0.80	70	1:90	710	80	2.81	1.78
8	1:50	500	20	2.24	1.96	120	1:90	500	20	1.54	2.21

**Note: Highlighted data are the center points of this study.*

Table 2: ANOVA analysis of MAE and UAE with male papaya leaves.

Terms	MAE			UAE		
	F-Value	***p*-Value**	**Significance**	**F-Value**	***p*-Value**	**Significance**
Model	10.31	0.00	Significant	3.22	0.02	Significant
Linear	4.96	0.01	Significant	2.18	0.13	Insignificant
C (%)	19.59	0.00	Significant	5.33	0.04	Significant
T_M (min)	1.37	0.26	Insignificant		N/A	
T_U (min)		N/A		0.84	0.37	Insignificant
S (μm)	1.53	0.24	Insignificant	0.03	0.86	Insignificant
R (mg/ml)	1.30	0.27	Insignificant	1.76	0.21	Insignificant
Square	26.37	0.00	Significant	6.27	0.00	Significant
C^2	104.89	0.00	Significant	19.57	0.00	Significant
T_M^2	1.75	0.21	Insignificant		N/A	
T_U^2		N/A		1.90	0.19	Insignificant
S^2	4.92	0.04	Significant	0.10	0.76	Insignificant
R^2	4.38	0.06	Insignificant	8.73	0.01	Significant
2-Way Interaction	1.37	0.29	Insignificant	1.90	0.15	Insignificant
$C*T_M$	0.42	0.53	Insignificant		N/A	
$C*T_U$		N/A		0.99	0.34	Insignificant
C*S	2.07	0.17	Insignificant	0.09	0.77	Insignificant
C*R	4.61	0.05	Significant	0.74	0.41	Insignificant
T_M*S	0.09	0.77	Insignificant		N/A	
T_U*S		N/A		1.21	0.29	Insignificant
T_M*R	0.79	0.39	Insignificant		N/A	
T_U*R		N/A		4.94	0.04	Significant
S*R	0.21	0.65	Insignificant	3.44	0.09	Insignificant
Lack-of-Fit	0.67	0.72	Insignificant	27.15	0.00	Significant
R^2		91.2 %			76.3 %	
Adj-R^2		82.3 %			52.6 %	

For UAE, the model was also found to be significant with p = 0.02. However, the linear terms were determined to be insignificant (p>0.05), while the square terms were significant (p<0.05). The R^2 (76.3%) and the adj-R^2 (52.6%) were determined to be lower than those in the MAE. Nevertheless, an R^2 of more than 75% is generally considered acceptable (He, Li, Zhang, Zhang, & Wu, 2016), and the adj-R^2 in a study with a small sample size but having numerous terms could appear to be much smaller than the R^2 value (H.-L. Liu, Lan, & Cheng, 2004). The lack of fit of the UAE model with a p-value of 0.00 was also determined to be significant, demonstrating the possibility of an abnormal result due to unforeseen noises from the surrounding (Açıkel, Erşan, & Sağ Açıkel, 2010). In the present study, the quadratic model was determined to be adequate due to the low p-value and the relatively high R^2 value for the UAE model.

Model Fitting

Quadratic polynomial models were generated for every process in the present study and are expressed by Eq. (3) for MAE and Eq. (4) for UAE. A full second quadratic

model was employed in this paper to determine the effect of the extraction parameters on rutin yield using individual MAE and UAE. Calculated results obtained from Eq. (3) and (4) were compared to observed results and are visualized through diagnostic plots of calculated results versus observed results, shown in Figure 2.

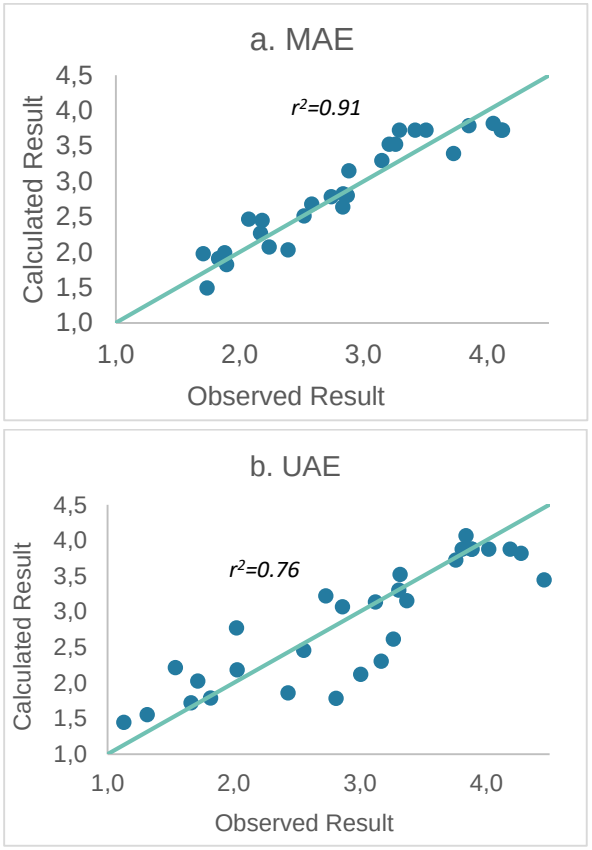

Figure 2: Regression model of rutin extraction from male papaya leaves using MAE (a) and UAE (b).

Figure 2a displays the diagnostic plot of observed versus calculated yields of MAE extraction. A positive, linear trend between calculated and observed results closely surrounded the regression line. The r^2 value of the plot is 0.91, suggesting that this model has 91% of the calculated results correlated with observed results. The standard deviation of the center points for MAE, highlighted in Table 1, is 7.8%, implying an extremely satisfactory result with high accuracy. In Figure 2b, it is noticeable that the data points are bordered along the regression line and possess a positive and linear trend between calculated and observed results for the UAE process. The r^2 value for this process is 0.76, suggesting that 76% of the results are correlated with each other. The standard deviation of center points for this process was found to be 3.8% indicating adequate accuracy, acceptable reproducibility, and tolerable noise from the surrounding environment.

MAE

$$Yield\ (^{mg}/_{g}) = -2.08 + 0.1141\ C + 0.302\ T_M + 0.00691\ S + 0.0210\ R - 0.001560\ C^2 + 0.0201\ T_M^2 - 0.000010\ S^2 - 0.000179\ R^2 + 0.00126\ CT_M + 0.000047\ CS + 0.000312\ CR - 0.000096\ T_M S - 0.00129\ T_M R - 0.000011\ SR \quad (3)$$

UAE

$$Yield\ (^{mg}/_{g}) = 1.35 + 0.1097\ C - 0.0253\ T_U + 0.0019\ S + 0.0222\ R - 0.001279\ C^2 - 0.000144\ T_U^2 - 0.000003\ S^2 - 0.000120\ R^2 + 0.000219\ CT_U - 0.000019\ CS + 0.000119\ CR + 0.000041\ T_U S + 0.000184\ T_U R - 0.000043\ SR \quad (4)$$

The Effect of Extraction Parameters on the Yield of Rutin

Surface and contour plots were constructed using two varying parameters and their corresponding response while keeping the other two parameters constant at 0 levels. Figures 3 and 4 demonstrate the connection between extraction parameters on rutin yield using MAE and UAE.

Effect of Extraction Time on the Yield of Rutin

The influence of extraction time on the yield of rutin appeared to be insignificant in both MAE and UAE based on the high p-value ($p>0.05$) shown in Table 2. While extraction time is statistically insignificant, longer irradiation time is noticeably more favorable in the MAE process (Figures 3d and 3e). For UAE, the 2-way interaction between sonication time and S/L ratio was found to be significant ($p<0.05$). The interrelation between these parameters is further demonstrated in Figure 4e. For a higher rutin yield at a low S/L ratio, a shorter sonication

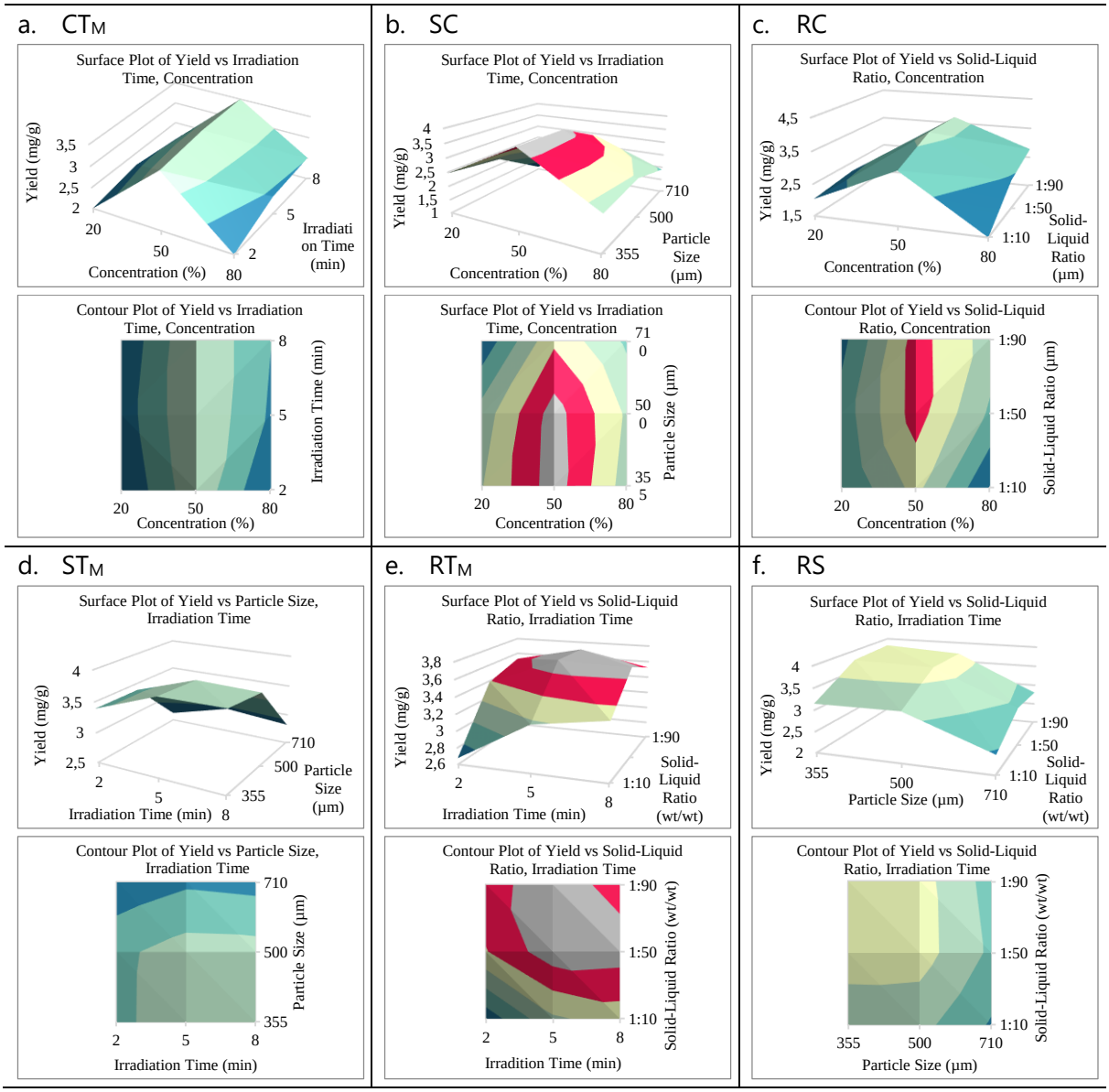

Figure 3: The surface and contour plots for rutin yield with MAE using male papaya leaves under the influence of (a) irradiation time and ethanol mixture concentration, (b) size of plant matrix and ethanol mixture concentration, (c) solid-liquid ratio and ethanol mixture concentration, (d) particle size and irradiation time, (e) solid-liquid ratio and irradiation time, and (f) solid-liquid ratio and size of plant matrix.

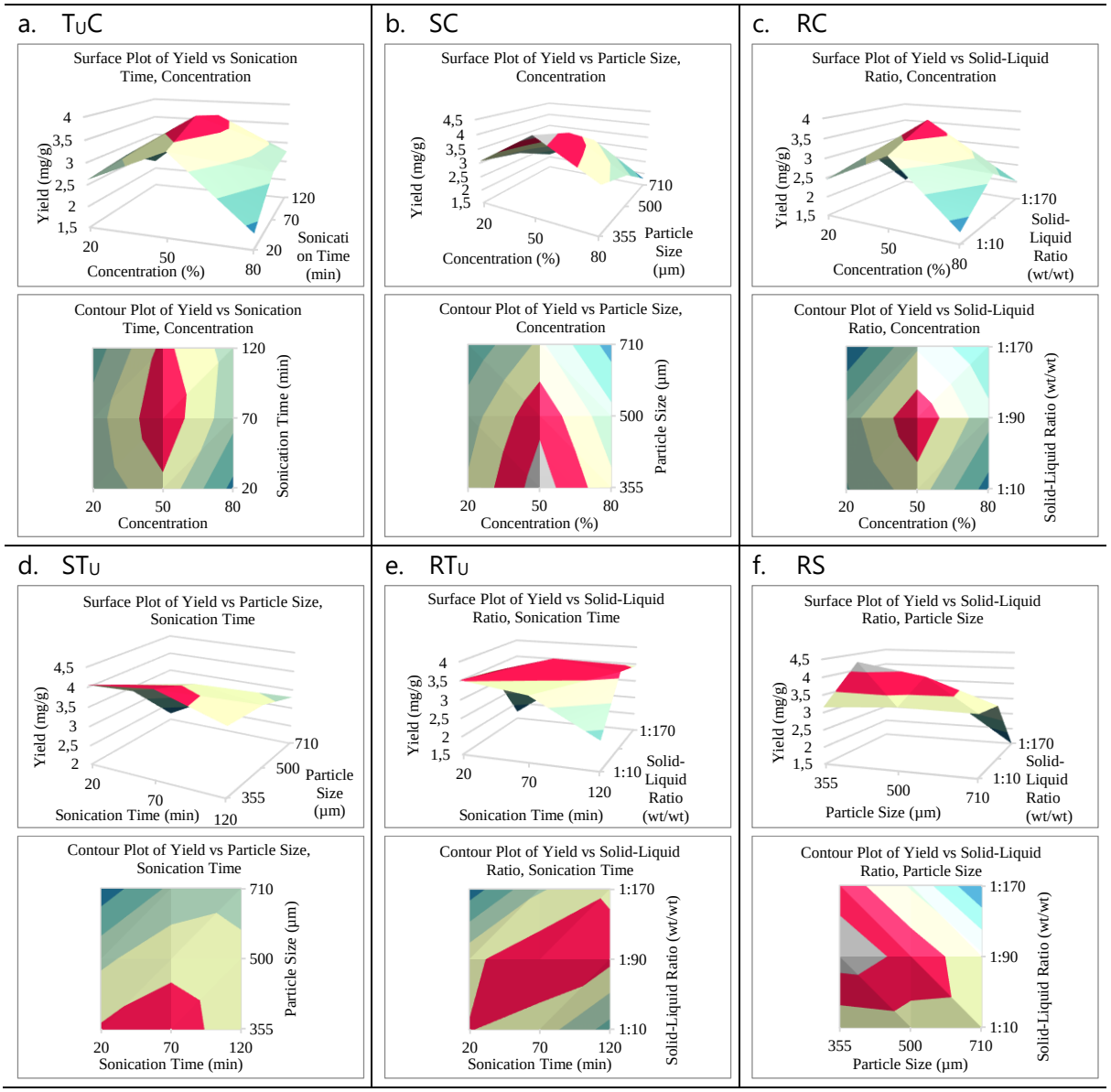

Figure 4: The surface and contour plots for rutin yield with UAE using male papaya leaves under the influence of (a) sonication time and ethanol mixture concentration, (b) size of plant matrix and ethanol mixture concentration, (c) solid-liquid ratio and ethanol mixture concentration, (d) particle size and sonication time, (e) solid-liquid ratio and sonication time, and solid-liquid ratio and (f) size of plant matrix.

time was required, whereas, at a high S/L ratio, a longer sonication time was required. For a combination of sonication time with the S/L ratio, the highest yield was obtained at the mid-points of the S/L ratio and the sonication time. Such observations may be explained as follows. During extraction at a low S/L ratio, the solvent may become too concentrated, resulting in a low mass transfer rate. In addition, the disparity between a longer sonication time and a decrease in yield may be due to the leaching of impurities from solvent, causing low permeability of solvents to the cell walls and therefore resulting in low mass transfer between rutin and aqueous ethanol (Latiff et al., 2021). The required

longer extraction time at a higher S/L ratio could be due to a decrease in the distribution of ultrasonic energy density as UAE power and frequency were kept constant while the S/L ratio increased (Y. Liu, Wei, & Liao, 2013). Another reasonable explanation for this observation is that a high ratio of extraction solvent to plant matrix prolonged the diffusion distance towards the interior tissue, and thus, the yield increased slowly when the S/L ratio increased from low to high (Ying, Han, & Li, 2011). The observation of a higher yield of rutin with the increased S/L ratio and extraction time was similar to the oil extraction from *Moringa peregrina* seeds. (Mohammadpour, Sadrameli, Eslami, & Asoodeh, 2019)

Effect of Ethanol Mixture Concentration on Yield of Rutin

As shown in Table 2, the linear and square effect of ethanol mixture concentration on rutin yield is observed to be the most significant ($p<0.05$) among other parameters in MAE. A higher yield of rutin was noticeable at a 50% ethanol mixture (Figures 3a to 3c). For UAE, the squared effect of ethanol mixture concentration on rutin yield was found to be more significant ($p<0.05$) compared to other parameters. The higher yield of rutin at 50% ethanol mixture (Figures 4a to 4c) further suggested that 50% is a suitable concentration of ethanol mixture in rutin extraction from male papaya leaf. This observation is consistent with the result reported in "Microwave-assisted extraction of phenolic acids and flavonoids from Physalis and Angulata," where the yield of rutin was highest at 50% ethanol-water mixture and decreased gradually after 50% (Carniel et al., 2017).

Effect of Particle Size on the Yield of Rutin

In general, small particle sizes tend to influence the extraction of compounds positively. Smaller particles generally provide a larger surface area for compound extraction and thus boost the transfusion rate of targeted compounds between plant particles and extraction solvent (Oreopoulou, Tsimogiannis, & Oreopoulou, 2019). The square effect of particle size with its corresponding low p-value ($p<0.05$) suggested that this parameter is crucial to the MAE process. This is demonstrated in Figures 3b, 3d, and 3f, where smaller particle size is observed to produce a higher yield. However, the effect of particle size in UAE is determined to be less important than it is in MAE ($p>0.05$). The smaller particle size of the plant matrix is still favorable for a higher yield of rutin in UAE (Figures 4b, 4d, and 4f).

Effect of Solid-Liquid Ration on the Yield of Rutin

In this study, the interaction effect of the S/L ratio and ethanol mixture concentration is found to be significant ($p<0.05$) in MAE. The connection between these two parameters can be discussed in the illustration in Figure 3c. As depicted in Figure 3c, rutin yield is seen to be the highest at a high S/L ratio. This phenomenon is believed to be due to a steep concentration gradient between solid particles and ethanol mixture solvent, prompting the mass transfer between rutin and the surrounding ethanol mixture (Lu et al., 2017). A similar effect of the S/L ratio on the yield of rutin using MAE can be seen in Figures 3e and 3f, where a high S/L ratio was preferable for a higher yield of rutin. Likewise, the square effect of the same parameter and its interaction effect with sonication time is also observed to be significant in UAE, as suggested by the low p-value ($p<0.05$). A

similar trend can be observed in Figures 4c and 4f, where the yield of rutin is highest at the S/L ratio of around 1:90 wt/wt. The S/L ratio range for rutin extraction using UAE was increased from 1:90 wt/wt to 1:170 wt/wt as it was determined to be insufficient.

Validation of Optimal Conditions

In the present work, optimization was carried out to learn the optimum condition for rutin extraction from male papaya leaf using individual MAE and UAE. Obtained optimum conditions were tabulated in Table 3. Under these conditions, the maximum yield of rutin is expected to be 3.88 mg/g with a desirability of 0.93, whereas the maximum yield of rutin is anticipated to be 4.34 mg/g with a desirability of 0.96. This pointed to UAE being possibly a better extraction method for rutin from papaya leaf.

The results from the optimization were verified experimentally and are also tabulated in Table 3. Calculations for the deviations between optimized experimental yield and optimized calculated yield were based on Eq. (5)

$$\text{Deviation } (\%) = \left|\frac{y-\hat{y}}{\hat{y}}\right| \times 100\,\% \qquad (5)$$

where y is the observed yield of rutin and $\hat{y}$ is the calculated yield of rutin from RSM.

As shown in Table 3, the observed optimized yield of rutin for MAE is 3.79 mg/g, with a deviation of 2.31% from the calculated yield. The optimized yield of rutin obtained experimentally for UAE was found to be 4.06 mg/g with a deviation of 6.39%. These results again showed the high accuracy of the RSM models in predicting the results for the present study.

Effect of Different Extraction Methods on the Surface Morphology of Male Papaya Leaves

The changes in surface morphology on male papaya leaf powder before and after the extraction processes under optimal conditions were observed using SEM and compared in Figure 5. Generally, all three samples showed similar patterns under the scale of 100 µm and magnification of 500x. The differences in observation between these three samples were clearer under a larger scale and higher magnification. Under the scale of 30 µm and magnification of 2000x, raw papaya leaf after oven dried process (Figure 5a) was found to have a smoother surface morphology compared to post-

Table 3: Optimized yield of rutin extracted from male papaya leaves under different extraction methods.

No.	Sample	T_m (min)	T_u (min)	C (%)	S (µm)	R (wt/wt)	Calculated Yield (mg/g)	Observed Yield (mg/g)	Deviation (%)
1	MAE	5.9	N/A	52.1	398	1:69.8	3.88	3.79 ± 0.06	2.31 %
2.	UAE	N/A	70.5	51.5	355	1:108.6	4.34	4.06 ± 0.20	6.39 %

a. Before Extraction	b. After MAE Treatment	c. After UAE Treatment
30 µm and 2000 x		

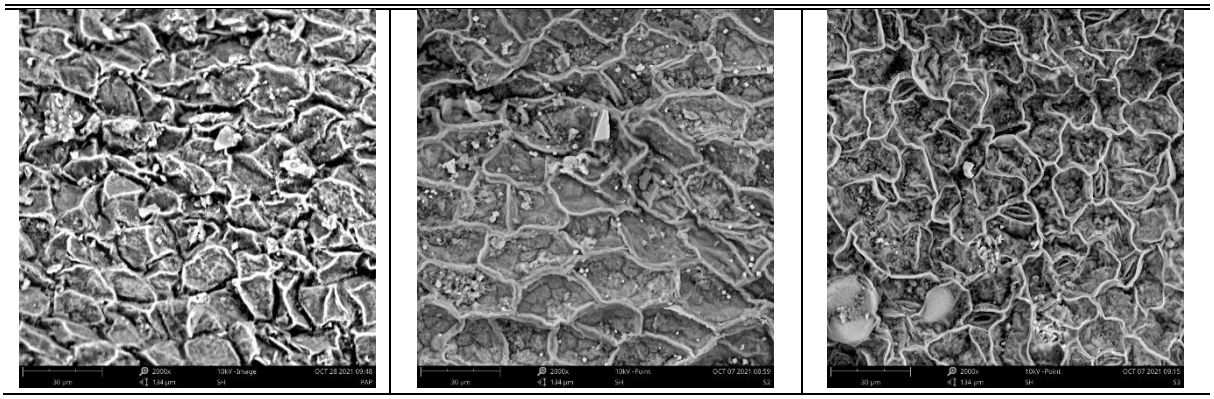

Figure 5: SEM scan of male papaya leaves (a) before the extraction process, (b) after the MAE process, and (c) after the UAE process under the scale of 30 μm and a magnification level of 2000x.

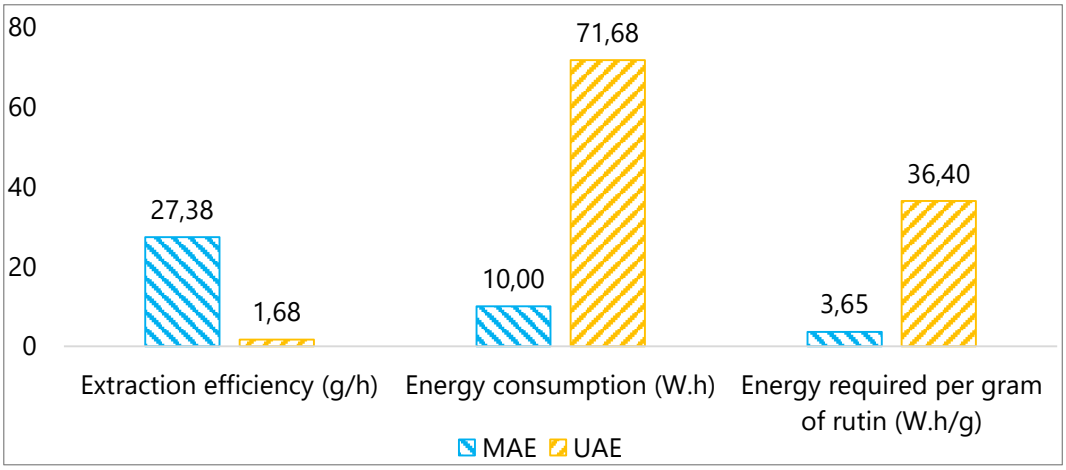

Figure 6: Extraction efficiency, energy consumption, and the energy required to produce 1 gram of rutin using MAE and UAE.

treated papaya leaf (post-MAE and UAE extraction) (Figures 5b and 5c).

Papaya leaf samples after MAE treatment have drought-like cracks and smooth surfaces on their surface (Figure 5b). This observation could be due to solvent evaporation from the heat energy created due to the dipole rotation of liquid molecules. During heating, microwave energy was continuously absorbed by the liquid molecules within the particle and solvent and rotated among themselves. This caused pressure build-up, which led to the disruption of hydrogen bonds within cell walls, rupturing them and resulting in the temperature raised of solvent. Similar observations were observed and reported (See et al., 2016).

In the UAE-treated SEM image, cavities and shrunk-crumpled surfaces were observed. The cavitation phenomenon is assumed to be the cause of the crumpled and shrunk surfaces of the papaya leaf after UAE treatment (Figure 5c). With the introduction of sonication waves in UAE, bubbles formed and collapsed within solvents near the surface of the plant matrix. This helped the solvent penetrate into the plant matrix, dissolving rutin into an ethanol mixture and extracting it out of the leaves (Ling et al., 2019).

Extraction Efficiency and Energy Consumption

The efficiency and energy consumption of the MAE and UAE methods were compared and illustrated in Figure 6. MAE was found to be very efficient in contrast to UAE in terms of the yield obtained per hour of extraction. However, UAE was found to be able to extract a higher amount of rutin per gram of leaf used (as discussed in section 3.1). In terms of energy consumption, MAE consumed less energy and had a lower energy requirement for every 1 gram of rutin extracted. The higher energy consumption in UAE could be partly due to the longer extraction time.

CONCLUSION

An RSM with BBD was employed in the present study to determine the effect of extraction time, particle size, S/L ratio, and ethanol mixture concentration on the yield of rutin from male papaya leaves using MAE and UAE. In MAE, the effect and square effect of ethanol mixture concentration and its interaction effect with the S/L ratio appeared to be significant. A high yield of rutin was observed using a 50% ethanol mixture at a high S/L ratio, where the concentration gradient between plant particles and ethanol mixture was steep. In addition, the square effect of particle size was determined to be important under MAE. In UAE, the effect and square effect of ethanol mixture concentration were also significant towards the yield of rutin.

Interestingly, unlike MAE, the square effect of the S/L ratio and its interaction effect with extraction time was more crucial in UAE. A high yield of rutin was achieved with the increased S/L ratio and extraction time. Although UAE was able to extract a higher yield of rutin from papaya leaf per gram of leaf extracted, MAE has a shorter extraction time compared to UAE. MAE was also determined to have higher efficiency in terms of the amount of rutin extracted per hour of extraction and utilizing lesser energy per gram of rutin extracted. Therefore, MAE is considered a greener extraction technique for rutin from male papaya leaf as it requires a lower amount of extraction solvent and energy during the extraction.

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