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Rutin extraction from female C*arica papaya* Linn. using ultrasound and microwave-assisted extractive methods: Optimization and extraction efficiencies

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ABSTRACT

Green extractive methods accompanied by resource conservation through process optimization are important in working towards sustainable processes. In the present paper, rutin was extracted from the leaf of female *Carica papaya* Linn using microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), sequential microwave ultrasound-assisted extraction (MUAE), and sequential ultrasound microwave-assisted extraction (UMAE) methods. Subsequently, the effect of extraction parameters on rutin yield were analyzed and compared. In addition, the extraction efficiency and energy consumption of the extraction processes were measured and discussed. In the present study, solid-liquid (S/L) ratio was determined to be the most significant extraction variable. Under optimized conditions, MUAE and UMAE were determined to yield the highest amount of rutin extracted at 18.46 ± 0.64 mg/g and 18.43 ± 0.81 mg/g, respectively. However, MUAE was determined to be the least resource efficient method as it consumed the highest amount of energy due to its relatively long extraction time. UAE was determined to be the most efficient in resource utilization as it required the least amount of energy for every mg/g of yield extracted, while the yield obtained was, nonetheless, comparatively high. The optimal condition obtained for UAE was 20 min of ultrasonic extraction time (T_U), 20 % of ethanol mixture concentration (C), 710 μm of particle size (S), and 1:650 wt/wt of solid-liquid (S/L) ratio (R).

1. Introduction

Carica papaya Linn. (family: Caricaceae) or commonly known as papaya, is popular for its various medicinal benefits [1]. There are three sexes of the papaya plants, which are male, female, and hermaphrodite. Hermaphrodites are most favored by growers as hermaphrodite fruits command a higher selling price, while male and female papaya plants are needed for cross-pollination. However, the ratio of male to female is generally kept at 1:10 as the male papaya plant does not bear any fruits [2]. Typically, excessive male and female plants are removed once the gender of the plant is identified to reduce plantation costs [3]. In moving towards a circular

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economy, various materials from the undesired papaya plants can be extracted and turned into products to maximize their benefits [4]. Phytochemicals such as phenolic acids, rutin, coumarin and flavonoids are metabolites that offer many health advantages and can be found in different parts of the papaya plants [5]. Rutin, a flavonoid that can be found in the papaya plant, offers diverse benefits that include anti-inflammatory, antioxidant and antifungal benefits, as well as having anti-aging effects on the skin [6,7]. In addition, it also has antimicrobial and anti-allergic properties while preventing neurodegenerative disorders, skin cancer and cardiovascular diseases [8]. It has been reported that the consumption of rutin could effectively reduce the growth of human leukemia HL-60 tumors in a xenograft mouse model [9] and rutin could also be used to reduce high blood pressure [10].

By and large, a number of extractive studies had been conducted on the papaya plant to extract beneficial bioactive components. However, in these studies, the gender of the papaya leaf was often excluded; invariably affecting the validity of the outcomes, since the content of bioactive materials differ with gender [11]. In addition to the possibility of creating other valuable by-products from undesired parts of the papaya plant, this study also aimed to optimize and compare the extraction process and extraction efficiencies of rutin from female *Carica* papaya Linn. using different extractive methods. As the world trudges toward greater sustainability goals, conservation of materials and energy are essential. Hence, the efficiency of an extractive method is not only dependent upon its yield, but also, dependent upon the resources required to obtain the necessary yield.

Microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) are two of the intensified extraction techniques commonly employed in extracting bioactive compounds due to their low complexity, efficiency, and low operating risks [12]. MAE and UAE are capable of performing the extraction process with lesser amount of solvents compared to conventional methods and, therefore, regarded as more environmental friendly [13]. Under MAE, microwave energy is absorbed by polar molecules; generating heat energy that results in the rupture of cell walls and allowing the transfusion of active compounds between plant sample and solvent [14]. For UAE, the process involved the formation and collapsing of bubbles near plant samples under the influence of sonication waves that resulted in pressure built-up within cell walls of the plant. The built-up pressure is followed by the rupture of cell walls; subsequently allowing for the transfusion of active compounds from the plant sample into the solvent [15]. While sole MAE and sole UAE are advantageous in their own rights, the possible sequential combinations of MAE followed by UAE (known as MUAE) or UAE followed by MAE (known as UMAE) are also receiving more attention over the years [16]. For MUAE, the initial step used microwave power as a pre-treatment phase for the plant sample, prior to being subjected to the ultrasonic extraction. Likewise, for UMAE, the ultrasound energy was used as a pre-treatment stage prior to the microwave extraction. It is believed that the cell structure of a plant sample would be damaged in the pre-treatment process; leading to easier transfusion of active compound between plant sample and solvent in the later process [17].

In the present study, a Box Behnken Design (BBD) is utilized for process optimization and to determine the relationship between variables. BBD is advantageous for its high efficacy in processing the response of a process with fewer trials required to form a conclusion [18]. Extraction variables such as time, S/L ratio, particle size, solvent concentration and microwave power are considered crucial in maximizing yield with several studies emphasizing the importance of these parameters [19–22]. Poureini et al. [23] reported that smaller particle sized samples with larger surface area typically accelerated the rate of extraction. However, smaller particles also tend to remain on the surface of the extraction solvent due to its lightness, and therefore, caused a lower extraction yield [23]. An increased in extraction time may have a positive effect on extraction yield. However, a longer extraction time risked having thermal degradation on the desired compounds [22].

In a mixture of solvents, the composition of the solvents and the ratio between the solid sample and the solvent liquid ratio were also reported to have significant effect on extraction yield [21,22]. The extractability of a bioactive compound is partly dependent on the solubility of the compound in the extractive solvents. Solubility depends largely on solvent polarity that changes with varying composition of the extractive solvents [24]. A balanced ratio of solid particle and extraction solvent is also essential to ensure complete immersion of sample particle. An insufficient solvent volume may result in an incomplete extraction of the desired compound, while an excessive solvent volume could lead to decreased extraction yields and unnecessary solvent wastage [22]. Extraction yields generally increase with microwave power, up to an optimum point. However, excessively high microwave power could cause thermal degradation of a bioactive compound, leading to low extraction yield. To avoid thermal degradation, a combination of low microwave power with longer exposure time can be applied [25]. In the present study, microwave power is kept constant at 100 W to prevent overheating and compound degradation, while varying compositions of water and ethanol are used as extractive solvents. These two solvents are considered green with ethanol being approved by the US FDA (US Food and Drug Administration) and reported to be a more suitable solvent for rutin extraction [26–28]. Subsequently, a comparative extraction yield performance was made among the four extractive methods. In the present study, extraction efficiencies in terms of energy and resource utilizations are also considered. Novel methods of determining energy efficiency through a yield-to-energy ratio (YER) and evaluating efficiency in resource utilization through a yield-to-resource ratio (YRR), are introduced in the present paper. Hence, further comparative studies were made based upon YER and YRR in determining the most efficient extractive method.

2. Materials and methods

2.1. Chemicals

Analytical grade, undenatured ethanol, and high-performance liquid chromatography (HPLC) grade methanol were purchased from Fisher Scientific, female papaya leaves were supplied by a papaya plantation in Malaysia, rutin hydrate (purity $\geq$ 94%) was purchased from Sigma-Aldrich, and ultrapure water was obtained through Mili-Q ultrafiltration system.

2.2. Plant materials and preparation

Female *C. papaya* leaves of the Sekaki variety of about 1 year old were purchased and gathered from a local farm in Selangor, Malaysia around April of the year 2021. The petioles were removed from the leaves and disposed. Collected leaves were washed under running water to remove visible impurities from its surface. Next, the leaves were oven dried at 50 °C for 72 h to remove all moisture content within the papaya leaves. Dried papaya leaves were grounded using commercial juice blender to obtain fine powder and further categorized into different range of sizes. Categorized leaves samples were stored in an air-tight container and under the temperature of 4 °C for future use.

2.3. Extraction of rutin

The extraction methods involved in this study are MAE, UAE, and the sequential MUAE and UMAE. Parameters involved in this study are concentration of ethanol in water, sample particle size, extraction time, and S/L ratio. The extraction process of MAE, UAE, sequential MUAE, and sequential UMAE are described in the following sections. For every extractive method utilized, the individual weights of Schott bottle, leaf sample, ethanol mixture and cap, pre-and post-processing, were recorded for calculation purposes.

2.3.1. Microwave-assisted extraction (MAE)

In this study, commercial microwave oven (Samsung, ME711K, South Korea) with a range between 100 W and 800 W was employed to carry out the extraction using MAE. The MAE procedure was adapted from the work of Chan et al. [29]. Female papaya leaf sample and ethanol mixture were placed in a Schott bottle at a predetermined S/L ratio and the bottle was capped before subjecting to microwave extraction. A water bath at room temperature between 26 and 27 °C was prepared by the side of microwave oven to lower the temperature of the extraction mixture after each extraction cycle. The provision of the water bath also allowed for that the condensation of trapped vapour within the bottle to prevent any loss of solvent during the extraction process. Subsequently, the cooled mixture was filtered into a HPLC vial for further analysis.

2.3.2. Ultrasound-assisted extraction (UAE)

An ultrasonic water bath (Branson, Bransonic M3800H-E, USA) with an output frequency of 40 kHz was used in this study. An ultrasonic water bath was used to prevent sample detexturation through direct contact with the source of sonication wave [30]. The following UAE procedure was adapted from the work Savic et al. [31]. The leaf sample and ethanol mixture were placed in a Schott bottle at a prefixed S/L ratio and the bottle was capped before being placed in an ultrasonic bath. A maximum of 4 bottles were placed in the ultrasonic bath at a time. Subsequently, a water bath at room temperature between 26 and 27 °C was prepared by the side of the ultrasonic bath to lower the temperature of extraction mixture after the extraction process before filtering the solution into a HPLC vial for further analysis. The water in the ultrasonic bath was also replaced after each extraction to ensure accuracy and consistency of each experiment.

2.3.3. Sequential microwave ultrasonic-assisted extraction (MUAE)

In the conduct of the sequential MUAE method, MAE was first employed prior to UAE. The same equipment stated in section 2.3.1 for MAE and 2.3.2 for UAE were utilized for the MUAE method. A prefixed amount of leaf sample and ethanol mixture were placed in a Schott bottle and subsequently put into the microwave oven. In addition, a water bath at room temperature between 26 and 27 °C was prepared by the side of the microwave oven and ultrasonic bath to cool down the temperature of extraction mixture after each extraction process. The MUAE procedure was modified and adapted from the work of Gorgani et al. [17].

2.3.4. Sequential ultrasonic microwave-assisted extraction (UMAE)

In this sequential UMAE method, UAE was first employed followed by MAE using the equipment utilized in section 2.3.1 and 2.3.2. A predetermined amount of leaf sample and ethanol mixture were placed in a Schott bottle before placing in the ultrasonic bath. A water bath at room temperature between 26 and 27 °C for cooling the mixture post-UMAE was prepared prior to filtering into a HPLC vial for further analysis. The UMAE procedure was referred from the work of Liew et al. [16].

Table 1
Extraction parameters of MAE, UAE, MUAE, and UMAE from female papaya leaf.

No.	Factors	Extractive methods	Level		
			−1	0	1
1	Microwave extraction time, T_M (min)	MAE, MUAE, UMAE	0.5	5	9.5
2	Ultrasonic extraction time, T_U (min)	UAE, MUAE, UMAE	20	190	360
3	Solid-liquid ratio, R (wt/wt)	MAE	1:10	1:90	1:170
		UAE, MUAE, UMAE	1:10	1:330	1:650
4	Particle size, S (μm)		355	500	710
5	Ethanol mixture concentration, C (%)		20	50	80

2.4. Design of experiments and statistical analysis

In this study, Response Surface Methodology (RSM) by Design Expert ® software version 11, Minneapolis was employed for experimental design to form a constructive model for the yield of rutin from female papaya leaf. Four-factors, three levels (−1, 0, 1) BBD with five centre points were created for MAE and UAE while five-factors, three levels BBD with eight centre points were created for UMAE and MUAE, based on the parameters displayed in Table 1.

The high and low range for ethanol mixture concentration (C) and particle size (S) were predetermined based on literature review, with particle size also being subjected to the physical limits of the equipment used. The range displayed for extraction time (T_M for MAE, T_U for UAE) and S/L ratio (R) were determined based on preliminary studies, shown under Supplementary Data A and B. A total of 29 runs were conducted for MAE and UAE and the results are tabulated in Table 2 while 48 runs were conducted for MUAE and UMAE with the results tabulated in Table 3. Subsequently, these results are discussed in section 3.0 where the experimental data were fitted to quadratic equations to form independent variables and responses. The equation can be expressed as Eq. (1). Effect and significance of experimental variables and responses were statistically and graphically analyzed with the use of Analysis of Variance (ANOVA). Optimal conditions and responses were validated using triplicates experimental results.

$$Y = \beta_0 + \sum_{i=1}^{j} \beta_i x_i + \sum_{i=1}^{j} \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

where Y is the response, which is yield of rutin in this current paper; i and j are the linear and quadratic coefficients, respectively; x_i and x_j are the uncoded independent parameters; k represents the number of studied and optimized variables in this study; β_0 is the constant coefficient; β_i, β_{ii}, and β_{ij} are the interaction coefficients of linear, quadratic and second-order terms, respectively; and lastly, ε is the error occurred.

2.5. Analytical methods

Identification and quantification of rutin were analyzed with HPLC while the surface morphologies of papaya leaf before, and after, the extraction process were observed using a Scanning Electron Microscopy (SEM). The procedure for HPLC analysis and surface morphology analysis was described under 2.5.1 and 2.5.2. Extraction efficiencies of four different extraction methods were analyzed

Table 2
Yield of rutin from female papaya leave using MAE and UAE.

No	MAE						UAE					
	Parameters				Yield		Parameters				Yield	
	T_M (min)	R (wt/wt)	S (μm)	C (%)	Observed (mg/g)	Calculated (mg/g)	T_U (min)	R (wt/wt)	S (μm)	C (%)	Observed (mg/g)	Calculated (mg/g)
1	0.5	1:90	355	50	3.43	3.48	20	1:330	355	50	10.39	9.96
2	0.5	1:90	500	20	3.42	3.59	20	1:10	500	50	1.50	1.53
3	0.5	1:10	500	50	1.31	1.05	20	1:330	500	20	10.85	10.60
4	0.5	1:170	500	50	5.67	5.69	20	1:330	500	80	8.21	8.41
5	0.5	1:90	500	80	2.68	2.75	20	1:650	500	50	16.60	16.48
6	0.5	1:90	710	50	3.52	3.89	20	1:330	710	50	9.84	9.81
7	5	1:90	355	20	4.19	4.11	190	1:10	355	50	1.62	2.45
8	5	1:10	355	50	1.52	2.01	190	1:330	355	20	9.51	9.29
9	5	1:170	355	50	5.28	5.5	190	1:330	355	80	10.23	9.36
10	5	1:90	355	80	3.24	3.00	190	1:650	355	50	14.38	15.19
1	5	1:10	500	20	1.44	1.37	190	1:10	500	80	1.17	1.02
12	5	1:170	500	20	6.59	6.56	190	1:10	500	20	1.35	1.07
13	5	1:90	500	50	3.32	3.74	190	1:330	500	50	9.90	10.20
14	5	1:90	500	50	3.51	3.74	190	1:330	500	50	9.22	10.20
15	5	1:90	500	50	4.1	3.74	190	1:330	500	50	12.97	10.20
16	5	1:90	500	50	4.07	3.74	190	1:330	500	50	9.41	10.20
17	5	1:90	500	50	3.39	3.74	190	1:330	500	50	9.83	10.20
18	5	1:10	500	80	0.87	1.05	190	1:650	500	80	14.33	14.20
19	5	1:170	500	80	4.43	4.66	190	1:650	500	20	16.98	16.70
20	5	1:90	710	20	4.08	4.44	190	1:10	710	50	1.37	−0.04
21	5	1:10	710	50	1.35	1.23	190	1:330	710	80	6.49	7.26
22	5	1:170	710	50	7.22	6.93	190	1:330	710	20	9.28	10.49
23	5	1:90	710	80	3.08	3.32	190	1:650	710	50	18.20	16.78
24	9.5	1:90	355	50	4.21	3.97	360	1:330	355	50	10.61	10.20
25	9.5	1:90	500	20	4.23	4.28	360	1:10	500	50	1.53	2.07
26	9.5	1:10	500	50	1.61	1.71	360	1:330	500	80	9.59	9.32
27	9.5	1:170	500	50	5.5	5.88	360	1:330	500	20	10.41	9.69
28	9.5	1:90	500	80	2.96	2.9	360	1:650	500	50	15.55	15.93
29	9.5	1:90	710	50	4.04	4.21	360	1:330	710	50	9.42	9.45

Table 3
Yield of rutin from female papaya leave using MUAE and UMAE.

No	MUAE							UMAE						
	Parameters					Yield		Parameters					Yield	
	T_M (min)	T_U (min)	R (wt/wt)	S (μm)	C (%)	Observed (mg/g)	Calculated (mg/g)	T_U (min)	T_M (min)	R (wt/wt)	S (μm)	C (%)	Observed (mg/g)	Calculated (mg/g)
1	0.5	20	330	500	50	8.80	9.29	20	0.5	1:330	500	50	9.73	8.60
2	0.5	190	330	500	20	9.56	9.42	190	0.5	1:330	355	50	9.55	11.04
3	0.5	190	10	500	50	1.59	2.29	190	0.5	1:330	500	20	9.19	9.10
4	0.5	190	330	355	50	11.15	10.37	190	0.5	1:10	500	50	1.61	3.65
5	0.5	190	330	710	50	9.04	8.45	190	0.5	1:650	500	50	17.86	16.39
6	0.5	190	650	500	50	12.95	14.21	190	0.5	1:330	500	80	7.52	7.98
7	0.5	190	330	500	80	8.96	8.33	190	0.5	1:330	710	50	8.98	7.85
8	0.5	360	330	500	50	9.05	8.85	360	0.5	1:330	500	50	8.24	7.50
9	5	20	330	500	20	9.53	9.76	20	5	1:330	355	50	9.57	11.08
10	5	20	10	500	50	1.54	1.87	20	5	1:330	500	20	10.01	9.84
11	5	20	330	355	50	9.52	9.19	20	5	1:10	500	50	1.60	3.29
12	5	20	330	710	50	9.66	9.78	20	5	1:650	500	50	17.12	17.74
13	5	20	650	500	50	14.20	14.31	20	5	1:330	500	80	9.93	8.23
14	5	20	330	500	80	8.79	7.67	20	5	1:330	710	50	9.21	9.01
15	5	190	10	500	20	1.43	0.38	190	5	1:330	355	20	9.05	11.85
16	5	190	330	355	20	9.61	9.96	190	5	1:10	355	50	20.46	9.17
17	5	190	330	710	20	9.14	9.38	190	5	1:650	355	50	17.32	17.11
18	5	190	650	500	20	16.87	16.40	190	5	1:330	355	80	9.47	11.47
19	5	190	10	355	50	1.68	2.04	190	5	1:10	500	20	1.72	3.25
20	5	190	10	710	50	1.49	1.79	190	5	1:650	500	20	22.56	19.95
21	5	190	330	500	50	8.68	8.84	190	5	1:330	500	50	9.41	8.77
22	5	190	330	500	50	9.60	8.84	190	5	1:330	500	50	9.06	8.77
23	5	190	330	500	50	8.55	8.84	190	5	1:330	500	50	8.16	8.77
24	5	190	330	500	50	8.75	8.84	190	5	1:330	500	50	8.94	8.77
25	5	190	330	500	50	9.14	8.84	190	5	1:330	500	50	7.71	8.77
26	5	190	330	500	50	7.94	8.84	190	5	1:330	500	50	9.59	8.77
27	5	190	330	500	50	8.49	8.84	190	5	1:330	500	50	8.67	8.77
28	5	190	330	500	50	9.59	8.84	190	5	1:330	500	50	9.12	8.77
29	5	190	650	355	50	16.79	15.99	190	5	1:10	500	80	1.31	4.61
30	5	190	650	710	50	14.47	13.92	190	5	1:650	500	80	16.11	15.28
31	5	190	10	500	80	1.23	2.08	190	5	1:330	710	20	11.47	10.55
32	5	190	330	355	80	9.02	9.32	190	5	1:10	710	50	1.45	−0.73
33	5	190	330	710	80	7.38	7.58	190	5	1:650	710	50	15.73	21.29
34	5	190	650	500	80	11.02	12.46	190	5	1:330	710	80	8.52	7.06
35	5	360	330	500	20	7.84	8.65	360	5	1:330	355	50	11.29	11.25
36	5	360	10	500	50	1.62	0.98	360	5	1:330	500	20	9.02	9.41
37	5	360	330	355	50	9.90	10.48	360	5	1:10	500	50	1.65	3.58
38	5	360	330	710	50	6.88	7.57	360	5	1:650	500	50	15.54	16.50
39	5	360	650	500	50	15.90	14.94	360	5	1:330	500	80	8.87	7.71
40	5	360	330	500	80	9.06	8.52	360	5	1:330	710	50	8.89	7.61
41	9.5	20	330	500	50	8.45	9.08	20	9.5	1:330	500	50	9.88	8.71
42	9.5	190	330	500	20	9.46	9.55	190	9.5	1:330	355	50	7.90	11.51
43	9.5	190	10	500	50	1.64	1.11	190	9.5	1:330	500	20	11.94	10.37
44	9.5	190	330	355	50	9.65	9.85	190	9.5	1:10	500	50	1.64	3.44
45	9.5	190	330	710	50	9.36	9.45	190	9.5	1:650	500	50	19.82	18.08
46	9.5	190	650	500	50	15.56	15.59	190	9.5	1:330	500	80	9.21	8.18
47	9.5	190	330	500	80	8.81	8.41	190	9.5	1:330	710	50	8.65	8.99
48	9.5	360	330	500	50	9.31	9.26	360	9.5	1:330	500	50	9.63	8.86

and compared using calculation described in section 2.5.3.

2.5.1. HPLC analysis

An Agilent 1200 series HPLC system was utilized in this study for identification and quantification of rutin from papaya leaf extracts. A 0.22 μm syringe filter was used to filter plant extracts into a HPLC vial whereas HPLC grade methanol and ultrapure water produced by Milli-Q ultrafiltration system were employed as the mobile phases of this analysis. To identify and quantify rutin in leaf extract, pure methanol was allowed to flow through the HPLC system with the purge valve opened for 15 min to remove the remaining solvent from previous users of the system. Next, pure methanol was allowed to flow through an analytical column for 15 min to remove any remaining compound within the column. The column was then subjected to 5 % methanol in water to condition the column to ensure that the analysis started with 5% methanol in water. The solvent gradient of rutin analysis was as follow: 5 % (0–3 min), 5 %–100 % (4–6 min), 100 % (7–13 min), 100 %–5 % (14–16 min), 5 % (17–20 min) at the flow rate of 1.0 mL/min. The injection volume of sample was set at 10 μL and the separation were detected by Ultraviolet-Diode Array Detection (UV-DAD) at the wavelength of 360 nm. The results obtained were compared against standard calibration curve of pure rutin solution to determine the rutin concentration of plant extract. The analytical column utilized in this study was an Agilent ZORBAX Eclipse Plus C18, 5 μm, 4.6 × 150 mm and the operating temperature fixed at 25 °C. This method has been modified and adapted based on those employed in Ref. [32]. Eq. (2) was utilized to calculate the yield of rutin of individual extracts.

$$\text{Yield of Rutin}\left(\text{mg}/\text{g}\right) = \frac{\text{Mass of rutin extracted (mg)}}{\text{Mass of papaya leaf(g)}} \tag{2}$$

2.5.2. Surface morphology analysis

A desktop SEM (Phenom, Phenom ProX, Netherland) was used in this study to examine the change in the surface morphology of the female papaya leaf before, and after, the extraction process. Dried papaya leaf was attached onto a sample stub using a double-sided carbon adhesive tape. Compressed air was then used to gently blow on the leaf samples to ensure all samples were secured. Next, the sample stub was placed on the sample holder and subjected to SEM for analysis purposes. The brightness, contrast, focus, and magnification were then controlled and adjusted using the rotary knob and mouse attached with it. The scale and magnification level of 30 μm and 2000 x were selected, best images were taken and compared in the later part of the present paper.

2.5.3. Extraction efficiencies

In this present paper, the rate of extraction, energy consumption, yield-to-energy ratio and yield-to-resource ratio were compared based on the optimized results of the four extractive methods. Extraction rate was calculated based on the optimized yield of rutin obtained per hour of extraction and is given by $\left[\frac{\text{mg of rutin extracted}}{\text{g of leaf•h of extraction time}}\right]$. Energy consumption for each of the extraction process was

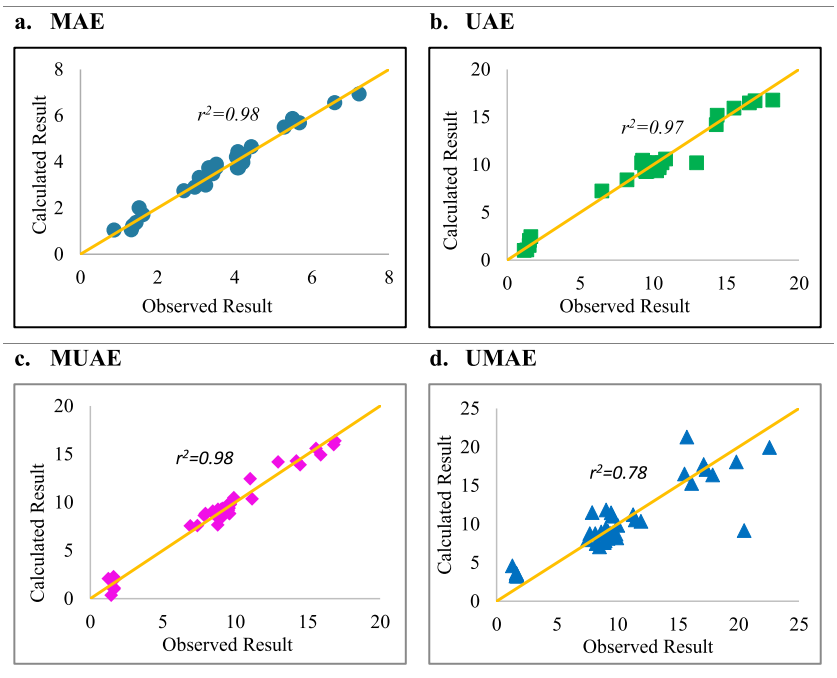

Fig. 1. Calculated and observed result of (a) MAE, (b) UAE, (c) MUAE, and (d) UMAE whereas the yellow line represented the best fit line of the plots.

Table 4
ANOVA analysis of MAE, UAE, MUAE, and UMAE of female papaya leaf.

Term	MAE			UAE			MUAE			UMAE		
	F-Value	*p*-Value	Significance	F-Value	*p*-Value	Significance	F-Value	*p*-Value	Significance	F-Value	*p*-Value	Significance
Model	40.66	0.00	Significant	34.81	0.00	Significant	58.03	0.000	Significant	4.88	0.00	Significant
Linear	137.51	0.00	Significant	117.09	0.00	Significant	12.00	0.000	Significant	0.89	0.50	Insignificant
T_M	4.16	0.06	Significant		N/A		2.44	0.130	Insignificant	0.06	0.81	Insignificant
T_U		N/A		0.02	0.90	Insignificant	0.43	0.519	Insignificant	0.00	0.97	Insignificant
C	30.70	0.00	Significant	5.35	0.04	Significant	1.27	0.269	Insignificant	0.15	0.71	Insignificant
S	1.45	0.25	Insignificant	0.28	0.60	Insignificant	1.41	0.246	Insignificant	2.88	0.10	Insignificant
R	513.72	0.00	Significant	462.72	0.00	Significant	48.09	0.000	Significant	0.51	0.48	Insignificant
Square	2.05	0.14	Insignificant	2.18	0.12	Insignificant	4.01	0.008	Significant	1.06	0.40	Insignificant
T_M^2	0.47	0.50	Insignificant		N/A		1.16	0.292	Insignificant	0.05	0.83	Insignificant
T_U^2	N/A			0.01	0.94	Insignificant	0.00	0.997	Insignificant	0.02	0.90	Insignificant
C^2	3.92	0.07	Significant	2.50	0.14	Insignificant	0.57	0.458	Insignificant	0.07	0.80	Insignificant
S^2	2.34	0.15	Insignificant	0.49	0.50	Insignificant	3.82	0.06	Insignificant	2.17	0.15	Insignificant
R^2	0.25	0.62	Insignificant	6.88	0.02	Significant	10.61	0.00	Significant	3.04	0.09	Significant
2-Way Interaction	2.86	0.05	Significant	1.19	0.37	Insignificant	2.55	0.03	Significant	0.69	0.72	Insignificant
T_M*T_U		N/A			N/A		0.15	0.70	Insignificant	0.04	0.84	Insignificant
T_M*C	0.60	0.45	Insignificant		N/A		0.00	0.98	Insignificant	0.00	0.99	Insignificant
T_M*S	0.07	0.80	Insignificant		N/A		0.93	0.34	Insignificant	0.07	0.80	Insignificant
T_M*R	0.48	0.50	Insignificant		N/A		2.58	0.12	Insignificant	0.07	0.79	Insignificant
T_U*C		N/A		0.61	0.45	Insignificant	1.52	0.23	Insignificant	0.03	0.87	Insignificant
T_U*S		N/A		0.08	0.79	Insignificant	4.87	0.04	Significant	0.01	0.91	Insignificant
T_U*R		N/A		0.21	0.65	Insignificant	1.02	0.32	Insignificant	0.10	0.76	Insignificant
C*S	0.00	1.00	Insignificant	2.05	0.17	Insignificant	0.54	0.47	Insignificant	0.27	0.61	Insignificant
C*R	5.38	0.04	Significant	1.12	0.31	Insignificant	12.61	0.00	Significant	0.97	0.33	Insignificant
S*R	10.61	0.01	Significant	3.08	0.10	Insignificant	1.28	0.27	Insignificant	5.38	0.03	Significant
Lack-of-Fit	0.75	0.68	Insignificant	0.41	0.89	Insignificant	2.29	0.13	Insignificant	31.54	0.00	Significant
R^2		97.60 %			97.21 %			97.73 %			78.35 %	
R^2 (adj)		95.20 %			94.42 %			96.04 %			62.31 %	

* Note: **T_M** represents irradiation time, **T_U** represents sonication time, **C** represents concentration of extraction solvent, **S** represents size of plant matrix, and **R** represents solid-liquid ratio.

measured with a Primera-Line Wattage current meter (PM213E, Hugo Brennenstuhl GmbH & Co. KG, Germany) and calculated based on Eq. (3):

$$Q = P \times t \tag{3}$$

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

Yield-to-energy ratio (YER) is introduced here as the degree by which rutin yield was obtained for every W·h of energy utilized, given by $\left[\frac{\text{mg of rutin extracted / g of leaf}}{\text{energy consumed in W•h}}\right]$. The yield-to-resource ratio (YRR) is also introduced in the present work as the degree of efficiency in resource utilization, given by $\left[\frac{\text{mg of rutin extracted / g of leaf}}{\text{g of solvent•energy consumed in W.h}}\right]$. As the calculation for energy consumed was based on both dissipated power and extraction time, YRR can best provide an overview of the conservation of resource utilization (energy, time and raw materials) for the four extractive methods. A higher YRR would indicate a better use of resources.

3. Results and discussion

3.1. Process optimization

Table 2 displays the 29 runs of the calculated and observed yields of rutin extracted using MAE and UAE. The yields of rutin obtained from MAE ranged between 0.87 mg/g to 7.22 mg/g and in contrast, were found to be lower than the range obtained in the UAE method (1.17 mg/g to 18.20 mg/g). Hence, UAE is comparatively better than MAE at extracting rutin from female papaya leaf. Table 3 lists the 48 runs of the calculated and observed yields of rutin obtained using sequential MUAE and UMAE. The range of yields obtained using MUAE is between 1.23 mg/g and 16.87 mg/g while those obtained from UMAE ranged from 1.31 mg/g to 22.56 mg/g. The generally higher yields obtained in UMAE, in contrast to MUAE, UAE and MAE, point to UMAE being the most promising extractive method. Regression models generated for rutin extraction using different extraction approaches are expressed in quadratic equations with Eq. (4) determined for MAE, Eq. (5) for UAE, Eq. (6) for MUAE and Eq. (7) for UMAE. Calculated yields generated from equations (4)–(7) and presented in Tables 2 and 3 were compared against observed yields to construct Fig. 1a – d to determine the accuracy between the predicted versus observed yields.

The ANOVA analysis generated for MAE, UAE, MUAE, and UMAE are shown in Table 4, with the respective design models found to be significant ($p < 0.05$); indicating that the models generated were well established to predict the response of the study. In MAE, the linear and interactive effects of the extraction parameters were determined to be significant ($p < 0.05$). The high determination coefficient (R^2) value and adjusted-R^2 value (97.6 % and 95.2 %) of the model again indicated high goodness of fit for the model. In addition, a high lack of fit in the p-value further pointed to a high accuracy of the model. This is supported by high correlation coefficient (r^2) of the graph (0.98) plotted using calculated and observed yields as shown in Fig. 1a. On the other hand, linear effect of the extraction term was found to be significant ($p < 0.05$) in UAE. High R^2 and adjusted-R^2 value (97.2 % and 94.4 %) of the model also showed that the model has a high goodness of fit, and is able to predict accurate response based on the extraction conditions provided. Furthermore, a high lack of fit in the p-value (0.10), again, pointed to a high certainty of the model. High r^2 (0.97) shown in Fig. 1b further indicated high accuracy of the regression model for UAE.

For MUAE, the linear, square, and interactive effects of the extraction parameters were found to be significant ($p < 0.05$). The high R^2 and adjusted-R^2 value (97.93 % and 96.04 %) point to the model having a high goodness of fit. In addition, a high lack of fit of the model further indicated a high accuracy of the model which is backed by a high r^2 (0.98), as illustrated in Fig. 1c. The UMAE process showed insignificant linear, square, and interactive effects of the extraction variables ($p > 0.05$) toward the yield of rutin. The R^2 value (78.35 %) and adjusted-R^2 value (62.31 %) indicated adequate accuracy of the model due to its significantly low p-value ($p < 0.05$). The significant lack of fit ($p < 0.05$) suggested unforeseen abnormality or unaccountable variables during the design of experiment [33, 34]. A slight lower r^2 (0.78) shown in Fig. 1d, further indicated the adequate accuracy of the model.

$$\begin{aligned} Yield\left(mg/g\right) = {} & 3.03 + 0.0307\,C + 0.198\,T_M - 0.00978\,S - 0.000295C^2 - 0.00455\,T_M^2 + 0.000007\,S^2 - 0.000010\,R^2 - 0.00098\,C{*}T_M \\ & - 0.000000\,C{*}S - 0.000165\,C{*}R - 0.000054\,T_M{*}S - 0.000328\,T_M{*}R + 0.000039\,S{*}R \end{aligned} \tag{4}$$

$$\begin{aligned} Yield\left(mg/g\right) = {} & -3.82 + 0.1411\,C - 0.0007\,T_U + 0.0132\,S + 0.02558\,R - 0.000807\,C^2 + 0.000001\,T_U^2 - 0.000011\,S^2 - 0.000012\,R^2 \\ & + 0.000089\,C{*}T_U - 0.000155\,C{*}S - 0.000064\,C{*}R - 0.000005\,T_U{*}S - 0.000005\,T_U{*}R + 0.000018\,S{*}R \end{aligned} \tag{5}$$

$$\begin{aligned} Yield\left(mg/g\right) = {} & 3.94 - 0.542\,T_M + 0.00600\,T_U + 0.0606\,C - 0.0129\,S + 0.03371\,R + 0.0136\,T_M^2 + 0.000000\,T_U^2 - 0.000215\,C^2 \\ & + 0.000017\,S^2 - 0.000008\,R^2 + 0.000200\,T_M{*}T_U - 0.00008\,T_M{*}C + 0.000474\,T_M{*}S + 0.000444\,T_M{*}R \\ & + 0.000096\,T_U{*}C - 0.000029\,T_U{*}S + 0.000007\,T_U{*}R - 0.000054\,C{*}S - 0.000147\,C{*}R - 0.0000\,S{*}R \end{aligned} \tag{6}$$

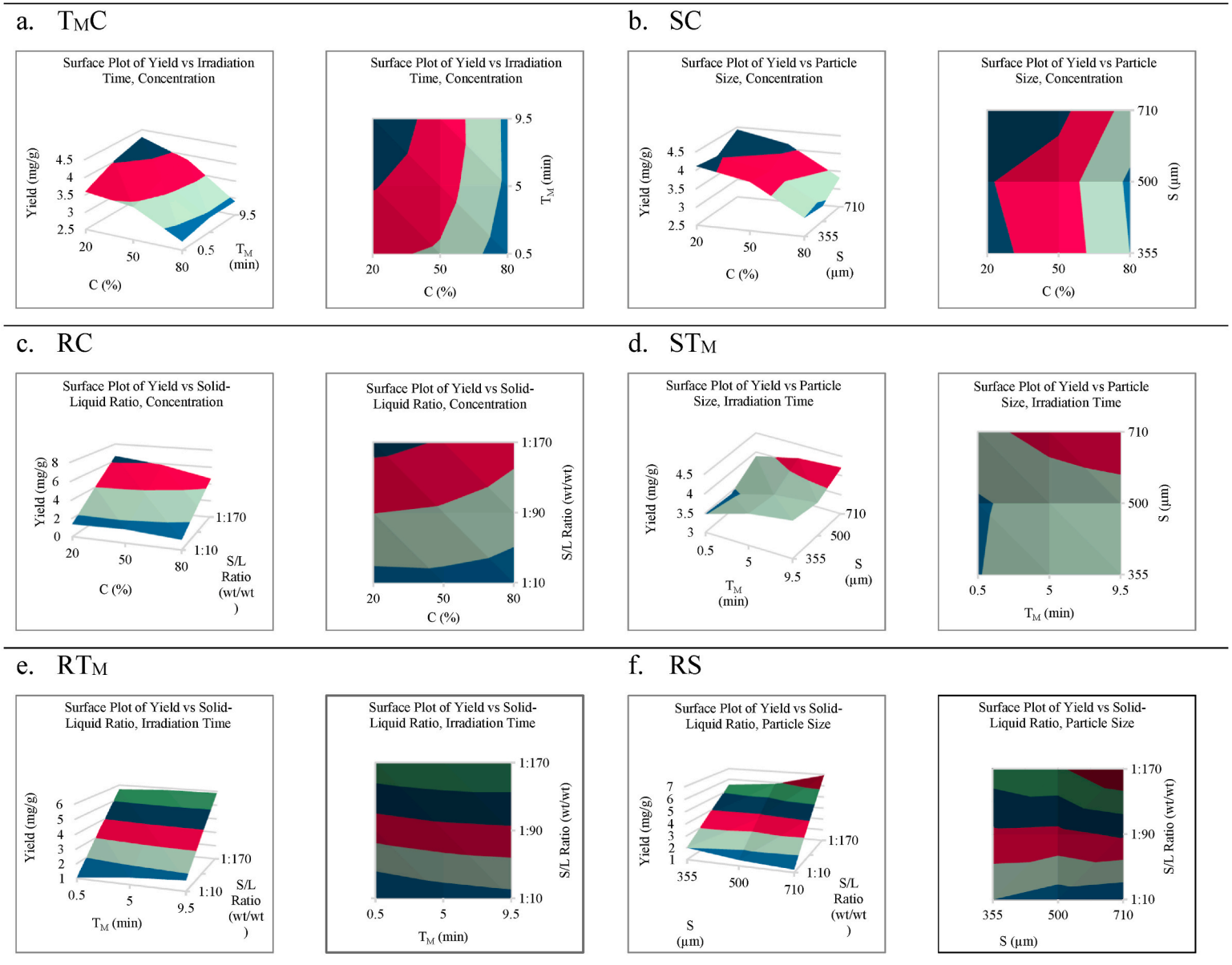

Fig. 2. Surface and contour plots of MAE using female papaya leaf.

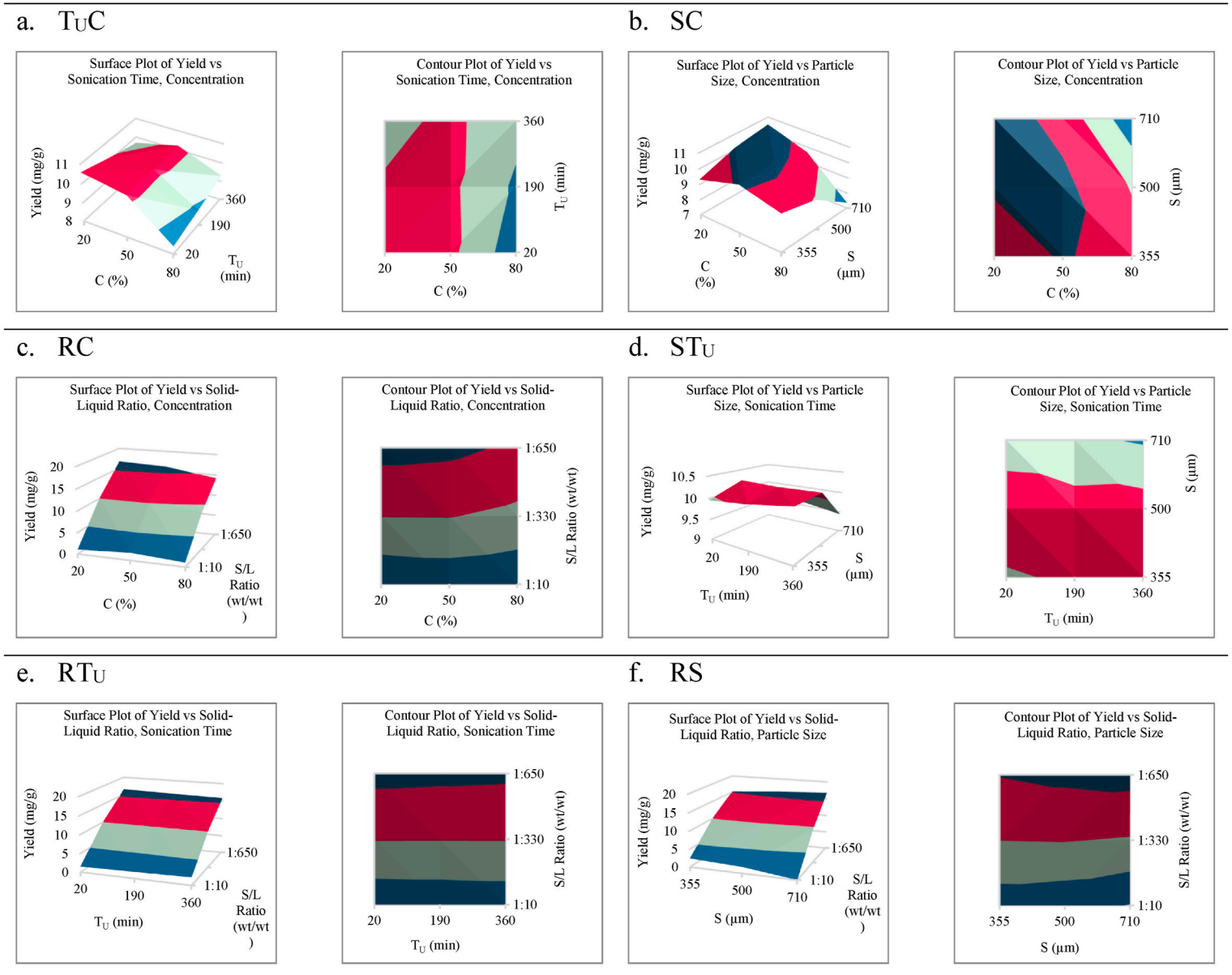

Fig. 3. Surface and contour plots of UAE using female papaya leaf.

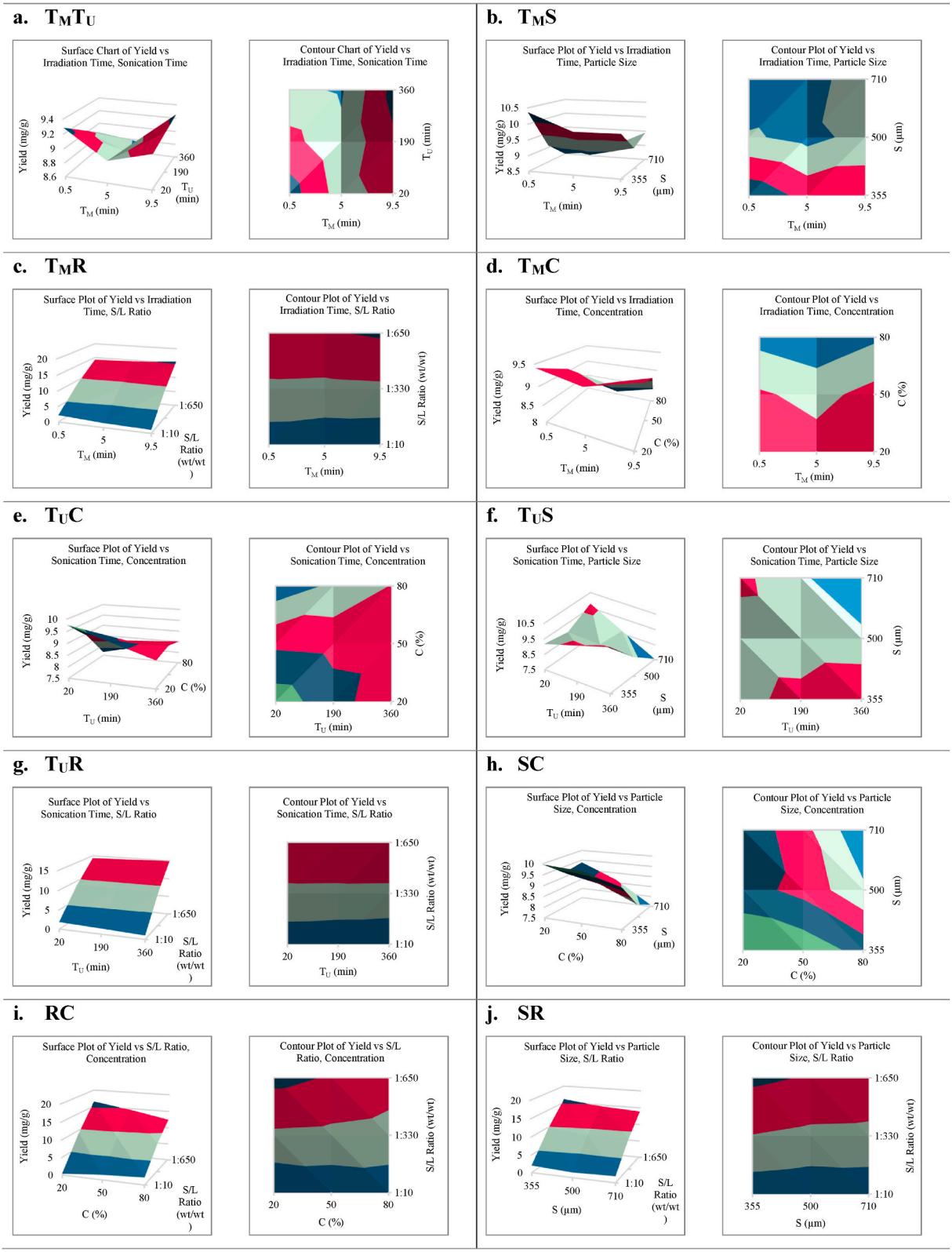

Fig. 4. Surface and contour plots of MUAE using female papaya leaf.

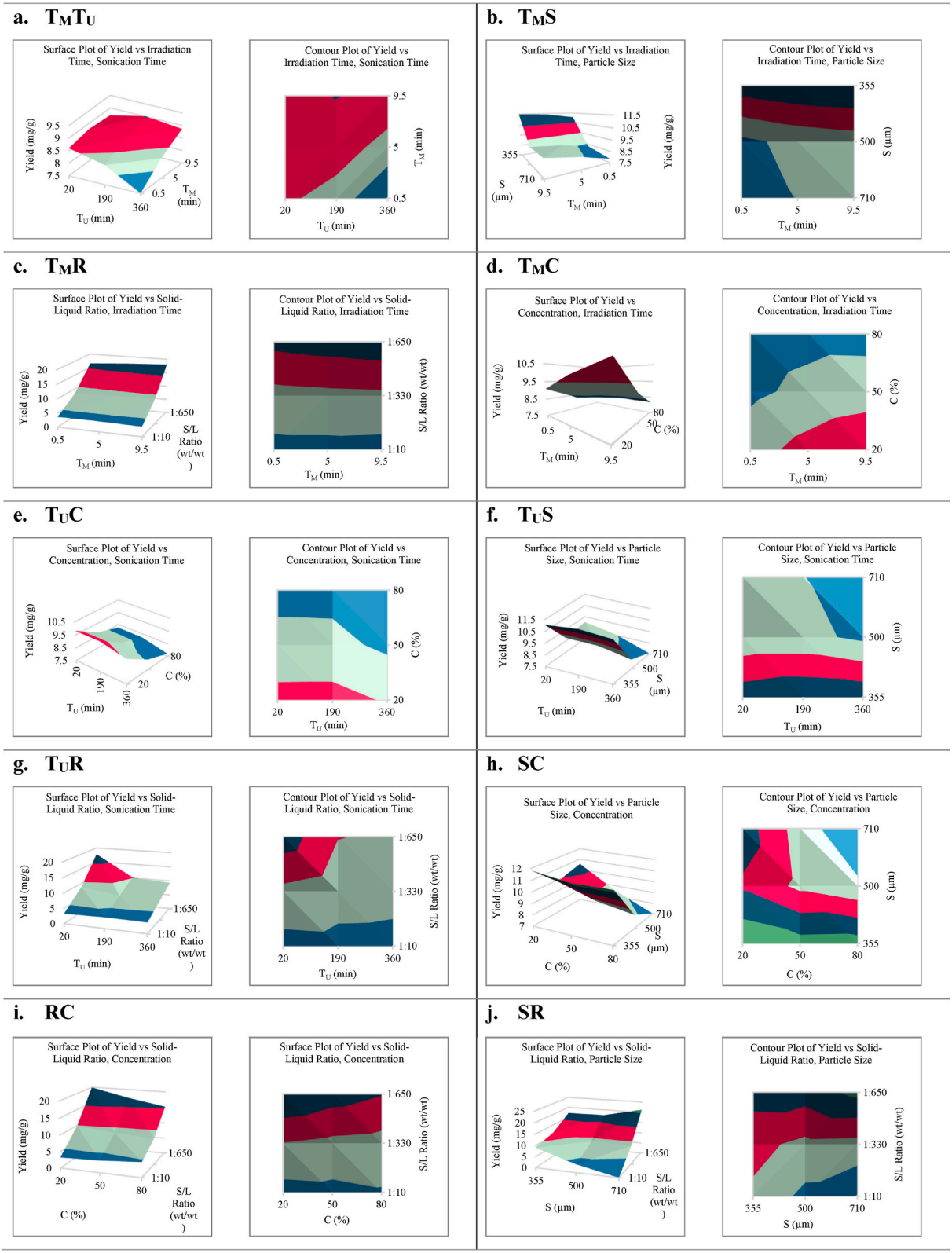

Fig. 5. Surface and contour plots of UMAE using female papaya leaf.

$$Yield\left(mg/g\right) = 25.9 + 0.0086\,T_U - 0.05\,T_M + 0.079\,C - 0.0709\,S - 0.0133\,R - 0.00000\,T_U^2 - 0.0060\,T_M^2 + 0.00029\,C^2 + 0.000048\,S^2 + 0.000017\,R^2 + 0.00041\,T_U{*}T_M - 0.000004\,T_U{*}C - 0.000013\,T_U{*}S - 0.000007\,T_U{*}R - 0.0020\,T_M{*}C + 0.00021\,T_M{*}S + 0.00033\,T_M{*}R - 0.000146\,C{*}S - 0.000157\,C{*}R + 0.000062\,S{*}R \quad (7)$$

3.2. Effect of extraction parameters on rutin yield

The surface and contour plots are shown in Fig. 2 (a – f) for MAE, Fig. 3 (a – f) for UAE, Fig. 4 (a – j) for MUAE and Fig. 5 (a – j) for UMAE. For MAE, MUAE and UMAE, the interactive effects of irradiation time with ethanol concentration are depicted in Figs. 2a, 4d and 5d, with particle size in Figs. 2d, 4b and 5b, and the interactive effects between irradiation time with S/L ratio in Fig. 2e, 4c and 5c. For UAE, MUAE and UMAE, the interactive effects of sonication time with ethanol concentration are shown in Fig. 3a, 4e and 5e, with particle size in Fig. 3d, 4f and 5f, and with S/L ratio in Fig. 3e, 4g and 5g. In addition, the interacting effects of particle size and ethanol concentration are shown in Fig. 2b, 3b and 4h and 5h for the MAE, UAE, MUAE and UMAE, respectively. The effects of S//L ratio with ethanol mixture concentration are shown in Fig. 2c, 3c and 4i and 5i, while S/L ratio with particle size shown in Fig. 2f, 3f and 4j and 5j. For both MUAE and UMAE, the interactive effects of irradiation time and sonication time on extraction yield are illustrated in Figs. 4a and 5a. The effects of each extraction parameter on rutin yield are discussed further in subsequent sections.

3.2.1. Effect of extraction time on Yield of rutin

In general, the effect of extraction time was observed to be less significant ($p > 0.05$) for all four processes studied in the present work. The interactive effect of ultrasonic extraction time and particle size was determined to be significant ($p < 0.05$) in the MUAE process. The relationship between these two variables is illustrated in Fig. 4f. High yields of rutin were determined to be at shorter ultrasonic extraction time with larger particle size, and at longer ultrasonic extraction time with smaller plant particle. As smaller particles have a tendency of staying on the surface of the extracting solvent, the interaction between the sonication wave of the solvent with the plant samples was poorer. Thus, longer ultrasonic extraction time is required to induce a higher rutin yield for smaller particles [35]. In contrast, larger particles are heavier and have a tendency to sink below the surface of the extracting solvent; allowing for better contact between the plant particle and the sonication wave. Hence, a shorter time frame is required during sonication for a larger plant particle.

3.2.2. Effect of ethanol mixture concentration on Yield of rutin

For the ethanol-water mixture, a low ethanol concentration between 20 % and 50 %, was generally observed to be more promising in this study. The significant linear effect of the ethanol concentration ($p < 0.05$) on the yield of rutin was noticeable in MAE and UAE. The interactive effect between ethanol concentration and the S/L ratio was also observed to be significant ($p < 0.05$). The relationship between ethanol concentration and S/L ratio in MAE is illustrated in Fig. 2c whereas the interactive effect between ethanol concentration and S/L ratio is demonstrated in Fig. 4i. In Figs. 2c and 4i, a high yield of rutin was achieved at 20 % ethanol mixture and high S/L ratio of papaya leaf/ethanol mixture at 1:170 wt/wt and 1:650 wt/wt.

3.2.3. Effect of particle size on Yield of rutin

In active compound extraction from medicinal plants, samples with smaller particle sizes are generally believed to be more advantageous [36]. In this study, a majority of the results showed positive impact of smaller size of particle on the yield of rutin. Nevertheless, a significant interactive effect between particle size and S/L ratio ($p < 0.05$) was observed for MAE and UMAE, with the relationship illustrated in Figs. 2f and 5j. These two figures showed that a higher yield of rutin was obtained for a larger particle size with a high S/L ratio. This could be due to smaller particles having a tendency of staying on the surface of the solvent that leads to poor interactions between process energy and the plant sample, and thus, lowering rutin yield [35].

3.2.4. Effect of S/L ratio on Yield of rutin

The linear effect of S/L ratio on the yield of rutin was found to be significant ($p < 0.05$) for MAE, UAE, and MUAE. With reference to Table 2, the square effect of S/L ratio on yield of rutin was also determined to be significant ($p < 0.05$) in UAE and MUAE. Additionally, a significant interaction effect between concentration and S/L ratio ($p < 0.05$) towards the yield of rutin was observed for MAE and MUAE with the interrelationship presented in Figs. 2c and 4i. From these two figures, a high yield of rutin was determined at 20 % ethanol mixture and at a high S/L ratio. It is believed that due to the high S/L ratio, the concentration gradient between the leaf sample and the ethanol mixture is much steeper; leading to an easier diffusion of rutin from the plant matrix into the surrounding solvent [37]. Nevertheless, a high S/L ratio is generally observed to have a positive impact on rutin yield.

The significant effect ($p < 0.05$) of either linear, square or interactive effect of every extraction parameter was demonstrated in Table 4. With reference to Table 4, the linear effect of microwave time, ethanol mixture concentration, and S/L ratio were observed to be significant for MAE. Square effect of ethanol mixture concentration and interactive effect between S/L ratio and ethanol mixture concentration, and S/L ratio and particle size, were also noted to be significant. For UAE method, linear effect of ethanol mixture concentration and S/L ratio were reported to be significant along with the square effect of S/L ratio on yield of rutin. Additionally, the linear effect and square effect of S/L ratio were noticed to be important in MUAE. Moreover, the interactive effect between ultrasonic extraction time and particle size, and ethanol mixture concentration and S/L ratio, were noted to be significant in MUAE. The square effect of S/L ratio and interactive effect of particle size and S/L ratio was determined to be significant for UMAE procedure. Hence, S/L

ratio appeared to be the most influential parameters to MAE, UAE, MUAE, and UMAE extraction techniques.

3.3. Validation of optimal conditions

The data obtained in this study were optimized with the objective of extracting the highest yield of rutin under the optimum conditions. Extraction based on the optimum conditions were then conducted in triplicates and the corresponding observed results are presented in Table 5. Subsequently, the percentage of error between calculated and observed results are compared and discussed.

In the present work, the optimized extraction yields were determined to be the highest under the MUAE and UMAE methods. Extraction performance in terms of optimized yield of rutin per gram of leaf for MUAE and UMAE showed an increased improvement of 225 % compared to MAE. However, the difference between MUAE and UMAE was insignificant, with optimized rutin yield for MUAE being only 0.16 % higher than UMAE. Meanwhile, MUAE and UMAE demonstrated a mere 10 % improvement when compared to UAE. By contrasting the extraction performance of MAE and UAE in terms of the optimized yield of rutin, it was determined that UAE yielded 195 % higher than that of MAE.

The significantly higher deviation between observed and calculated yields for UMAE could be due to the regression model being a weaker correlative model for UMAE (see Fig. 1d). This is in contrast to the better representation by the regression model for MUAE, shown in Fig. 1c. For UMAE, the disparity between having high yields but the model bearing the lowest r^2 value also indicate a need to revisit the variables used in the present study as there could be potential critical parameters that are undetermined at this point in time.

A comparison of the rutin yield was also conducted among the four extraction methods utilized in the present paper with other studies from the literature. The comparison has determined that the obtained optimized yields of rutin from papaya leaf, obtained in the present work, were generally higher than those extracted using maceration (3.33 mg/g) [38] and extracted using hot water under 65 °C for 4 h (0.063 mg/g of papaya leaf water) [39]. This suggested the extraction methods selected in this study to be more promising.

3.4. Effect of different extraction methods on the surface morphology of female papaya leaves

The changes in the surface morphology of female papaya leaf before and after each extraction process were observed under 30 μm and 2000 x magnification and are shown in Fig. 6. The pre-extraction dried leaf sample was observed to have irregular edges and smoother surface morphology (Fig. 6a) compared to other post-extracted papaya leaf samples (Fig. 6b–e). The surface morphology of the papaya leaf post-MAE was noted to have crumpled surfaces under the SEM scan (Fig. 6b). This observation could be due to the sudden rise in temperature and internal pressure, caused by the heat generated from the dipole rotation of the liquid molecules. During MAE, the liquid molecules within the plant sample and solvent constantly absorbed microwave energy, and the vibration of the molecules within the cell wall cause a rise in temperature and the subsequent pressure build-up. This leads to a disruption in the hydrogen bonds within cell walls, and consequently, the cell walls break down. Similar observations were also noted in Ref. [40]. In contrast, cavities and shrunk-crumpled surfaces were observed in post-UAE treated leaf sample. This is believed to be caused by the formation and collapsing of the bubbles near leaf sample under the influences of sonication waves. During the formation and collapsing of bubbles, pressure constantly built up near the leaf sample causing the cell walls to break down and allowing the transfusion of rutin between sample and ethanol mixture [41].

For post-MUAE leaf sample, the influence of both MAE and UAE can be observed under the lenses of SEM in Fig. 6d. Both the drought-like surfaces of MAE, and the shrunk-crumpled surfaces and cavities from UAE, were observed in this SEM image for MUAE in Fig. 6d. For post-UMAE sample, the impacts from both MAE and UAE were also noticeable, as shown in Fig. 6e. Nevertheless, the impact from MAE was clearly more significant compared to the impact from UAE within the UMAE process, as this could be due to a prolonged irradiation time during the MAE stage and a minimum sonication time during the UAE stage.

3.5. Extraction efficiencies

In this study, four intensifying extracting techniques were optimized and evaluated in which UMAE and MUAE were devised based on synergistic potential of MAE and UAE. The underlying process intensification principles that improve process efficiency and safety, reduce costs, resources and wastes can be realized through the possible approaches of spatial (structure), temporal (time), functional (synergy) and thermodynamic (energy) [42,43]. Optimized yield of rutin, rate of extraction, energy consumption, the yield-to-energy ratio and yield-to-resource ratio are shown in Fig. 7. Among these four extraction methods, UAE was observed to have the highest extraction rate followed by UMAE due to the relatively shorter extraction time for both methods (Fig. 7c). The extraction rate for

Table 5
Optimum conditions of rutin extraction from female papaya leaf using MAE, UAE, MUAE, and UMAE.

Extraction Method	Parameters					Calculated Yield (mg/g)	Observed Yield (mg/g)	% Error
	Tm (min)	Tu (min)	C (%)	S (μm)	R (wt/wt)			
MAE	9.3	N/A	20	710	1:170	7.56	5.67 ± 0.16	10.25
UAE	N/A	20	20	710	1:650	19.45	16.73 ± 2.84	14.99
MUAE	9.5	360	20	355	1:650	18.97	18.46 ± 0.64	6.44
UMAE	9.5	20	20	710	1:650	26.60	18.43 ± 0.81	30.7

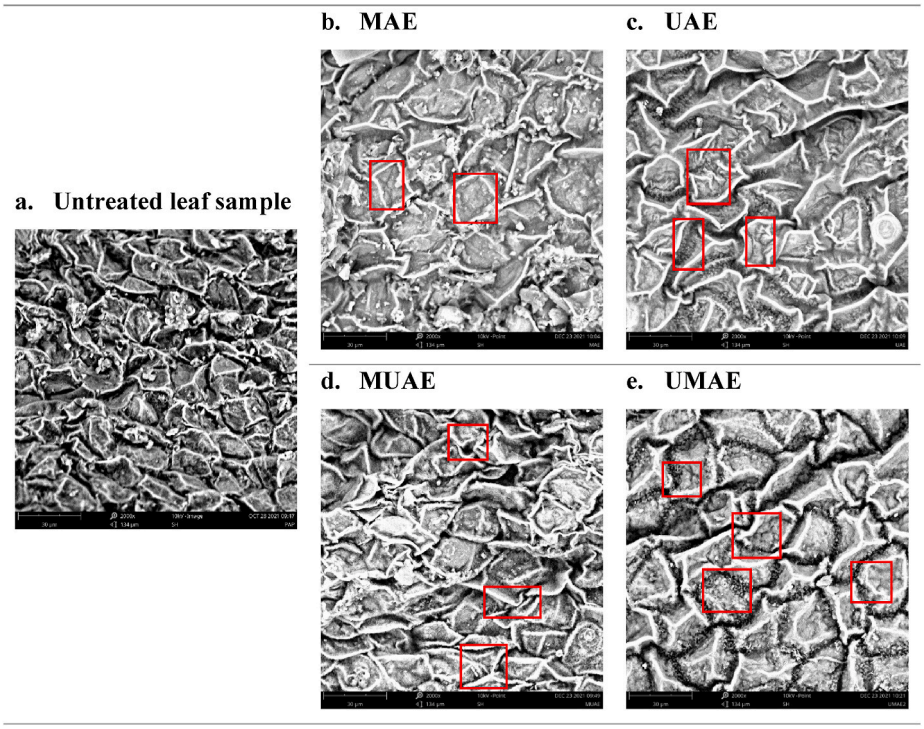

Fig. 6. SEM scan of female papaya leaves (a) before extraction, (b) after MAE, (c) after UAE, (d) after MUAE, and (e) after UMAE under the scale of 30 μm and under the magnification level of 2000×.

MUAE was very low due to its prolonged extraction time. In terms of energy consumption, MUAE was significantly higher compared to the other three methods and therefore, the least energy efficient technique (Fig. 7b).

A comparison of the yield-to-energy ratio (YER) for the four extractive methods ascertained that UAE was the most energy efficient approach with the highest amount of rutin extracted for every W-h of energy consumed (Fig. 7d). In comparing the yield-to-resource ratio (YRR) for each extraction, MAE was determined to be the most resource efficient due to the relatively low solvent utilization; almost 4 times lower than the other three methods (Fig. 7e). However, the yield obtained through MAE was the lowest which was approximately 3 times lower than all three methods; indicating an inefficiency of extracting rutin from the female papaya leaf (Fig. 7a). Based on the considerations in terms of extraction yield, extraction rate, YER and YRR, the most favorable extraction technique for rutin from female papaya leaf is determined to be the UAE approach. UAE generated a relatively high yield of rutin, with the highest rate of extraction and the most energy efficient approach. While UAE is secondary to MAE in the YRR category due to the higher solvent utilization, this setback can be mitigated by introducing solvent recycling to the extraction process. It is also of interest here that the synergistic MUAE and UMAE methods both generated the highest yields. However, when extraction rate, YER and YRR are taken into consideration, both MUAE and UMAE pale in comparison to UAE. Hence, it can be seen that approximate additional 10 % yield was not sufficient to cover for the additional resources required to extract them out.

4. Conclusion

Rutin extraction was carried out on female papaya leaf using MAE, UAE, MUAE, and UMAE approaches. The optimized yields of rutin and the performance of extraction methods were compared and discussed. Of the extraction parameters investigated, the S/L ratio was determined to have the most significant effect on all four extractive methods utlized in this study. The most efficient extractive method determined in this study was UAE as it extracted the most rutin for every gram of leaf and hour of extraction. In addition, UAE was also determined to be the most efficient in its energy consumption for every mg/g of rutin extracted. Comparatively, MUAE was observed to obtain the highest yield of rutin but also commanding the highest energy consumption. Hence, with every factor being considered, UAE is determined to be the more superior extractive method for rutin from the leaf of female *C. papaya* Linn.

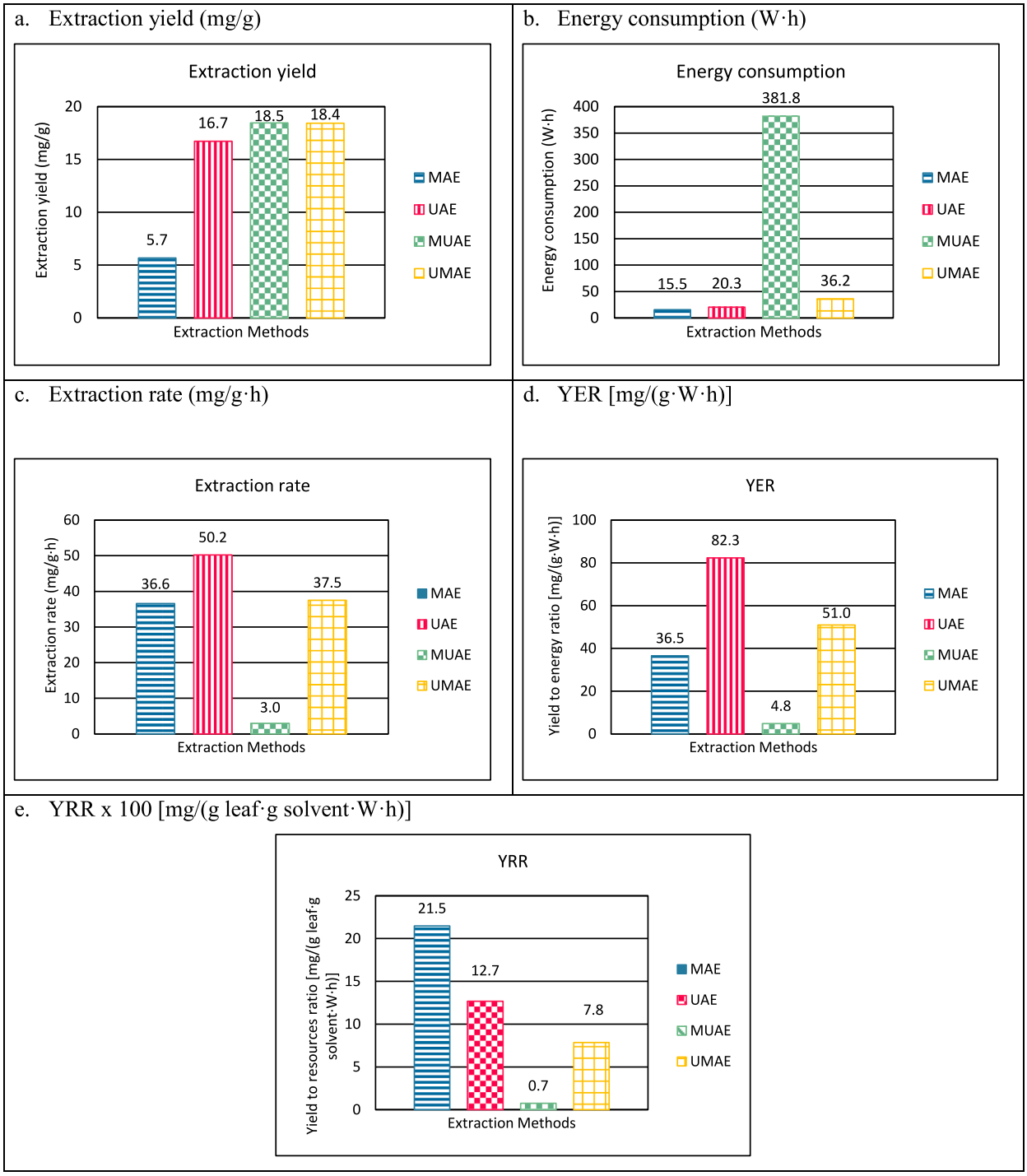

Fig. 7. (a) Extraction yield, (b) energy consumption, (c) the rate of extraction, (d) yield-to-energy ratio (YER) and (e) yield-to-resource ratio (YRR) for MAE, UAE, MUAE and UMAE based on optimized conditions.

Author contribution statement

See Khai Chew: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Wen Hui Teoh: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sok Lai Hong: Analyzed and interpreted the data.
Rozita Yusoff: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20260.

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