Research article

Optimization of chlorophyll extraction from pineapple plantation waste

Siti Hajar Abd Rahim [a], Norazwina Zainol [a,b,*], Kamaliah Abdul Samad [c]

[a] College of Engineering, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia
[b] Earth Resources and Sustainability Centre (ERAS), Universiti Malaysia Pahang, 26300 Gambang, Kuantan, Pahang, Malaysia
[c] Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia

ABSTRACT

The abundance of pineapple waste generated in Malaysia has becomes a serious issue as it puts our environment in grave danger. The presence of chlorophyll in pineapple waste has attracted many researchers to produce a natural dye from chlorophyll as a sensitizer for solar cells. Natural dyes can be considered a promising substitute for synthetic dyes as they are more environmentally friendly and cost less when compared to the synthetic dyes that are currently available on the market. This research aims to optimize the conditions of chlorophyll extraction from pineapple leaves using a mechanical extraction method. A response surface methodology was used to design an experimental design table using a central composite design based on two significant factors. The extraction cycle and the storage time varied between 1 and 5 cycles and 7 and 9 h, respectively, were studied. From the study, the maximum concentration of total chlorophyll (TC) was 693.292 mg/L. The optimum conditions for extraction cycle and storage time were at 3 cycles and 8 h, respectively. Among those two factors, it has been discovered that the storage time has a smaller p-value ($p = 0.1106$) than the extraction cycle, indicating that this factor was the critical factor affecting the TC concentration. The value of R^2 for the analysis was 0.8673, making the analysis a well-fitted model. Thus, it has been established that the models are suitable for optimizing the extraction conditions in order to maximize the concentration of TC using the mechanical method.

1. Introduction

Renewable energy technologies have been popular recently among researchers and developers. The availability of fossil fuel will eventually run out with an escalating population rate in the near future, which has triggered researchers and developers to invent technologies from renewable sources [1]. Biomass is the best green energy option for meeting the growing demand for renewable energy due to the availability of biowastes. With rising global demand for pineapple, the abundance of waste generated in Malaysia poses a threat to environmental issues [2]. To overcome this problem, the application of dye-sensitized solar cell (DSSC) was introduced to be an alternative to a conventional solar cell [3].

The dye sensitizer in the DSSC plays an important role as it absorbs light and transforms solar energy into electrical energy. Most studies on chlorophyll DSSC have been conducted using an extraction process from natural pigments. Wormwood as a chlorophyll dye for DSSC produced an open circuit voltage of 0.585V, a current short circuit of approximately 1.96 mA, a conversion efficiency of 0.538%, and a fill factor (FF) of approximately 47% [4]. According to Jin et al. [5], the efficiency of using

wormwood plants as sensitizer is 0.9%, compared to 0.7% and 0.4% for bamboo and maple, respectively. The DSSC device performance from the chlorophyll in the leaves of *Syngonium Podophyllum* Schott shows 0.308% efficiency, 0.86V of open circuit, 0.6 mA in a current short circuit, and a fill factor (FF) of approximately 44.69% [6]. Another study used shisonin mixed with chlorophyll extracted from shiso leaves as a dye for a solid state DSSC. The open circuit voltage (mV), current density (mA/cm2), FF, and efficiency (%) were discovered to be 432 Mv, 3.52, 0.39, and 0.59, respectively, with the highest efficiency being 1.31%. According to Kushwaha et al. [7], the leaves of teak (*Tectona grandis*) showed the best photosensitization effect with a broad range absorption of the visible region spectrum between 470 nm and 662 nm. These studies show that the natural dye chlorophyll has a high potential, particularly in the application of DSCC. As a result, extensive studies have been conducted on the extraction of chlorophyll pigment from waste using various extraction methods.

The extraction of chlorophyll can be done using three methods, which are aqueous extraction, chemical extraction, enzymatic extraction, and supercritical or subcritical extraction [8]. Most studies performed by various researchers have used chemical extraction. This

method is less time-consuming and high efficiency, but not preferable due to the toxicity of the chemical to the environment, especially when a large amount of solvent is required [9]. Meanwhile, enzymatic extraction was not feasible to be used at the industrial level due to the sensitivity of the behavior of enzymes [10]. Mechanical extraction is the best option for the extraction of chlorophyll as it is easier to handle, does not involve chemicals in the process, and takes the least amount of time to extract. Thus, in this study, chlorophyll was extracted mechanically using a sugarcane press machine. A recent study found that the best conditions to get the highest chlorophyll content using the mechanical method were storage time and extraction cycle. However, no further study was conducted to identify the optimum condition. Hence, this study would focus on optimization to get a higher yield of TC from pineapple leaves.

The optimization process refers to improving the system's performance in order to identify the conditions under which a procedure can produce the best possible response. Conventional optimization carried out only one factor at a time can be time-consuming and costly, especially when there are many independent variables [11]. Furthermore, conventional optimization may overlook the interaction of factors, making the true optimum point impossible to achieve [12]. Response surface methodology (RSM) was the most relevant technique for the optimization process because it provides analysis of the independent variable effects and their interactions on dependent variable. RSM is a statistical modelling approach that is ideal for optimization processes that combines design of experiment with interpolation using a first or second polynomial equation. The experimental design used in this study was central composite design (CCD). Many research conducted using CCD as an experimental design for instance to optimize biogas production from poultry manure wastewater [13], ferulic acid extraction from banana stem [14], and ultrasonic extraction for marine *Spirulina maxima* [15]. Hence, this study was performed to identify the optimum conditions to produce the highest yield of TC from pineapple leaves using mechanical method through the application of RSM with CCD.

2. Material and methods

2.1. Collection of samples

Pineapple leaf samples for this research were acquired from Pekan Pina Sdn. Bhd. in Pahang, Malaysia (pineapple plantation industry). The samples weighed about 10 kg per experiment. Figure 1 depicts a sample of pineapple leaves. In this study, the matured leaves were chosen for further investigation to ensure consistency throughout all experimental runs and to prevent bias. The leaves were also cut to an identical length of 10 cm before being used.

Figure 1. Sample of pineapple leaves.

2.2. Preparation of the sample

The pineapple leaves were first cleaned with tap water to remove any contaminants and soil that could interfere with the extraction later on. The cleaned samples were dried with a paper towel before being stored in the chiller overnight.

2.3. Extraction of chlorophyll from pineapple leaves using a sugarcane press machine

The prepared samples of pineapple leaves were extracted by using a sugarcane press machine in the Faculty of Chemical & Natural Resources Engineering (FKKSA) laboratory. The sample leaves were extracted multiple times using the machine at different extraction cycles. The juice extracted from the extracted samples was filtered and collected from the pipe. The chlorophyll juice was then collected and placed in a centrifuge tube before being measured with an Ultraviolet–visible (UV-VIS) spectrophotometer for chlorophyll concentration. Then, the samples were stored in a freezer at -20 °C to investigate the effect of storage time on chlorophyll concentration. Lastly, samples from different storage times were removed from the freezer, thawed, and measured to determine their final chlorophyll concentration.

2.4. Determination of chlorophyll contents

The chlorophyll concentration in pineapple leaves was identified using a UV-VIS spectrophotometer (Genesys 50, Thermo Scientific). Chlorophyll *a* and *b* are the two forms of chlorophyll present in pineapple juice samples. To identify the peak of chlorophyll *a* and *b*, a wavelength range of 350–800 nm was used for scanning (Suppl. Figure 1). According to the calibration graph of standard chlorophyll, the wavelengths specified for chlorophyll *a* and *b* were 662 nm and 646 nm, respectively. After setting the wavelength, the samples were tested in the UV-VIS spectrophotometer. The wavelength value of both chlorophylls was then read and recorded. The quantity of chlorophyll a and b is calculated using Eqs. (1), (2), and (3) [16]:

$$Chl\ a\ (mg/L) = \frac{A_{662} - 0.0014}{0.0922} \tag{1}$$

$$Chl\ b\ (mg/L) = \frac{A_{646} - 0.0011}{0.043} \tag{2}$$

$$Total\ chlorophyll\ (TC)\ (mg/L) = Chl\ a + Chl\ b \tag{3}$$

2.5. Preliminary experiment setup

In this preliminary study, the experiment was conducted based on their best condition from the previous study [16]. Table 1 shows the four factors with their best conditions that affected the chlorophyll extracted.

2.6. Experimental setup for optimization of chlorophyll extraction

Based on the preliminary research, the two most important factors in obtaining the optimum TC concentration (mg/L) were further optimized [16]. The factors investigated were the extraction cycle and storage time

Table 1. Experimental table for a preliminary experiment.

Factor	Condition
Type of waste	Leaves only
Pre-heat treatment	No
Extraction cycle	3 cycles
Storage time	8 h

(hours). In this study, the design of the experiment (DOE) for optimization was determined using response surface methodology (RSM), which was generated by CCD in Design Expert (DE) software (Ver 7.1.6). A total of 13 experimental runs, including center points were accomplished. Table 2 shows the ranges of selected factors. In the experimental design, low and high factors were coded as -1 and +1, respectively, while the midpoint was coded as 0 and α value was set as 2. ANOVA evaluated the statistical validity of the model at a 95% confidence level using the p-value. A second-order model was then fitted to it in order to link the response variable to the independent variables. Experiments were performed in triplicate to ensure data accuracy.

2.7. Validation of experiment

After obtaining the optimum conditions suggested by CCD for extraction of pineapple leaves that achieves a maximum yield of TC, validation of the model was tested. The optimum conditions were determined to be 3 times of extraction cycle and 8 h of storage time, with a predicted value was 693.292 mg/L. To validate the model, the percentage error between actual and predicted values was calculated using Eq. (4) [16].

$$Error\ (\%) = \frac{predicted\ value - actual\ value}{predicted\ value} \times 100\% \qquad (4)$$

3. Results and discussions

3.1. Preliminary result

Table 3 illustrates the chlorophyll extraction results based on the best conditions. As the result shows, the highest TC concentration was 242.68 mg/L under this condition. Thus, it was proven that the best conditions for chlorophyll extraction to achieve the highest TC yield were using the leaves only with extraction cycle of 3 without pre-heat treatment with a storage time of 8 h.

3.2. Overall result

Table 4 reports the TC content of 13 sets of experiments with varying point of factors: 1–5 of extraction cycle and 7–9 h of storage time. According to the results, the TC concentration of the samples vary from 259.534 mg/L to 693.292 mg/L. The greatest TC yield (693.292 mg/L) was gained with an extraction cycle of 3 and an 8-hours of storage time, whereas the lowest yield was 259.534 mg/L with an extraction cycle of one and an 8-hours of storage time. The results demonstrated that the higher extraction cycle is useful for chlorophyll extraction from leaves. However, exceeding the optimal extraction point could reduce the amount of chlorophyll extracted due to a decrease in juice extraction from the leaves.

3.3. Analysis of variance (ANOVA)

The analysis of variance (ANOVA) as shown in Table 5 was performed to calculate the model coefficients (p-value), determine the significance of each parameter of a regression equation (F-value), and indicate the strength of each parameter's interaction. A "Prob > F" <0.05 implies that the model terms are significant. Model significance was confirmed by an F-value of 9.15 found in this research. A "Prob > F" value of 0.0056

Table 2. Selected factors and corresponding range in CCD.

Independent variable	Range and level				
	2.00 (-α)	-1	0	+1	2.00 (+α)
Extraction cycle	1	2	3	4	5
Storage time (hour)	7	7.5	8.0	8.5	9

Table 3. Result for a preliminary experiment to the best condition from the previous study.

	Chl *a*	Chl *b*	TC (mg/L)
Sample 1	78.0109	163.0907	241.10
Sample 2	70.7223	146.6256	217.35
Sample 3	79.3124	163.3698	242.68

Table 4. Experimental data and response value for optimization.

Std	Factor 1	Factor 2	Response 1 Chl *a* (mg/L)	Response 2 Chl *b* (mg/L)	Response 3 TC (mg/L)
	A	B			
1	7.5	2	180.039	385.858	565.897
2	8.5	2	124.193	243.416	367.609
3	7.5	4	180.657	228.509	409.167
4	8.5	4	183.965	274.347	458.312
5	7.0	3	148.293	278.812	427.104
6	9.0	3	88.9436	183.695	272.639
7	8.0	1	86.2104	173.323	259.534
8	8.0	5	157.306	231.137	388.443
9	8.0	3	178.423	321.323	499.746
10	8.0	3	325.364	367.928	693.292
11	8.0	3	325.364	348.114	673.478
12	8.0	3	276.384	345.788	622.172
13	8.0	3	325.364	367.928	693.292

A: Storage time (hours), B: Extraction cycle.

Table 5. ANOVA for optimization of TC contents from pineapple leaves, response total chlorophyll yield (mg/L).

Source	Sum of Squares	Mean Square	F-value	p-value Prob > F	
Model	240000	47990.43	9.15	0.0056	significant
A	17485.96	17485.96	3.33	0.1106	
B	3065.30	3065.30	0.58	0.4696	
AB	15305.78	15305.78	2.92	0.1314	
A^2	119600	119600	22.81	0.0020	
B^2	142000	142000	27.07	0.0012	
Residual	36721.59	5245.94			
Lack of fit	9996.63	3332.21	0.50	0.7029	not significant
Pure Error	26724.96	6681.24			
Cor Total	276700				
R-squared	0.8673				
Adj- R^2	0.7725				
Pred- R^2	0.5356				
Adeq Precision	7.426				

indicates that there was only a 0.56% probability that a model F-value this large could occur due to noise. The model with the $p < 0.05$ indicates this model is statistically significant, indicating that it was a good fit for this study. In this study, the p-values for storage time and extraction cycle were 0.1106 and 0.4696, respectively. According to McLeod [17] the smaller the p-values, the stronger the evidence to favor the alternative hypothesis, which means the variable studied does influence the response variable. Since storage time has a smaller p-value compared to extraction cycle, this factor was a crucial factor affecting the TC yield. For the interaction of storage time and extraction cycle (AB), the p-value was 0.1314. The result interprets that the interaction has simultaneous changes in the levels of both factors to the output response [18]. This

means that the amount of TC yield cannot be estimated solely on the basis of the individual effects of the various changes in this model due to the interaction effect.

The regression coefficient (R^2) from the ANOVA was 0.8673. According to previous research, an R^2 value greater than 0.75 indicates a highly accurate and satisfactory regression model [19]. The R^2 for these response variables was higher than 0.7, indicating that the regression model was a good fit. Meanwhile, the values of adj-R^2 and pred-R^2 were 0.7725 and 0.5356, respectively. However, the pred-R^2 was not as close to the Adj-R^2 with R^2 as one might normally expect. But since both values were acceptable close to value R^2, this proved that the regression model obtained in this study can determine the optimum parameter precisely [14]. Pred-R^2 was used to determine how well a regression model predicts to provide a good fit, while adj-R^2 was used to compare the goodness of fit for the regression model, and "Adeq Precision" measures the signal-to-noise ratio, with a ratio >4.0 is preferable [20, 21]. For this study, a signal to the noise ratio of 7.426 indicates an adequate signal. As a result, this confirmed that each model in this study can be used to navigate the design space.

The ANOVA also revealed a not significant lack of fit (*p*-value > 0.050). The lack of fit is the inverse of the whole-model test, which determines if all the model terms are significant with respect to the output response. Lack of fit tests also specify if the regression model was a good or poor model of data based on the experimental factors chosen. In this study, the lack of fit p-value was 0.7029, which implies that the lack of fit was not significant relative to the pure error. According to Sivamani [22] if $p > 0.050$ (not significant) means that the model fits well. Hence, the model for this study was well fitted with a good choice of variables to determine the optimum condition for achieving the highest TC yield.

Eq. (5) is the quadratic equation in coded terms that explains the relationship between the yield of TC content and variables:

$$\text{Total Chlorophyll} \ (mg/L) = -15048.51647 + 4177.08280A$$
$$- 501.37057B + 123.71654AB - 289.03613A2 - 78.72987B2 \quad (5)$$

In Eq. (5), A is storage time (hours) and B is extraction cycle. Factors A and B are referred as the main effects, while AB, A^2, and B^2 are the interaction effects in the process.

Figure 2a presents the correlation of actual conversions and values predicted by the model. The predicted values of TC concentration computed from Eq. (5). The presence of a linear distribution implies that the model fits well. The predicted values estimated from Eq. (5) were close to the actual TC concentration values. The normal probability plot presented in Figure 2b suggests that the residuals (difference between predicted and actual values) are normally distributed and develop a nearly straight line.

3.4. Main and interaction effects

Figure 3 displays the influence of two independent factors on TC concentration. The storage time plays an important role in chlorophyll extraction from pineapple leaves. The TC content seems to be affected by storage time more profoundly than extraction cycle. Based on the graph in Figure 3a, the concentration of TC increased with increasing storage time from 7 h to 8 h with 427.10 mg/L and 693.29 mg/L, respectively. However, upon increasing the storage time from 8 h to 8.5 h, TC content seemed to level off. This was due to the sample oxidizing in the environment with a longer storage time [16]. Wasmund [23] reported in their studies that chlorophyll content of vegetables changes or loses color during storage due to chlorophyll degradation. Thus, this result indicates that the longer storage time of chlorophyll extracted did not appear to have a good effect on TC.

On the other hand, for factor B the yield of TC showed an upward trend when the extraction cycle increased from one cycle to three cycles as shown in Figure 3b. However, the tailing of a growing trend started to decrease after the extraction cycles from four to five cycles. This result indicates that excessive extraction applied to the sample using the mechanical method did not influence the TC content. This indicates that three cycles of the extraction cycle were sufficient to produce the highest concentration of TC. It was confirmed by Cano et al. [24] and Bahçeci et al. [25] who stated that samples need to be extracted between one and three cycles in order to achieve total extraction. The chlorophyll concentration of the juice could also not be increased beyond one to three extraction cycles. This was due to the samples were being completely crushed under high compression of sugarcane press machine after three cycles. Olaoye [26] also highlighted that the amount of extracted juice would decrease if the size of the sample decreased. Thus, it was proven that after maximum condition, the machine would totally collapse the cell wall of plant fiber and consequently, the amount of juice extracted from pineapple leaves decreases.

The effects of process parameters and response may also be observed through 3D-response surface and contour plots in Figures 3c and 3d, respectively. The curvature in Figure 3c reveals that the parameters of extraction cycle and storage time factors had a quadratic influence on chlorophyll concentration, indicating an optimum response near the center point. Meanwhile, the elliptical contour plot shown in Figure 3d demonstrates a significant interaction between the parameters, with the peak falling within the parameter ranges indicated by the red area near the center point. Both figures show that chlorophyll concentration improved as extraction cycle and storage time increased and started to decrease as the extraction cycle and storage time became higher. The effect of storage time was more significant at 8 h while the extraction cycle was at three cycles. At this point, the concentration of TC from pineapple leaves was at its maximum yield. As a result, the optimum extraction chlorophyll condition could be achieved using this design.

3.5. Validation of experiment

This extraction procedure is carried out using the numerical optimization approach through the DE software. To maximize the desirability, the extraction cycle and storage time factors were both adjusted within the range, while the yield of TC was adjusted to the highest.
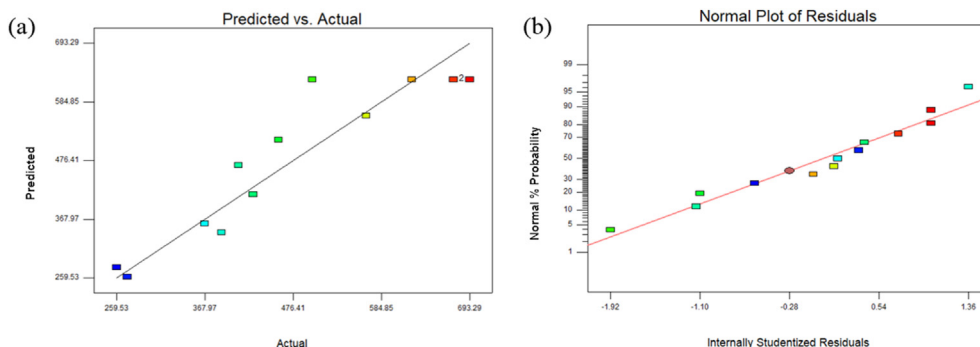

Figure 2. (a) Correlation of actual conversions and values predicted by the model (b) and normal probability of residuals.
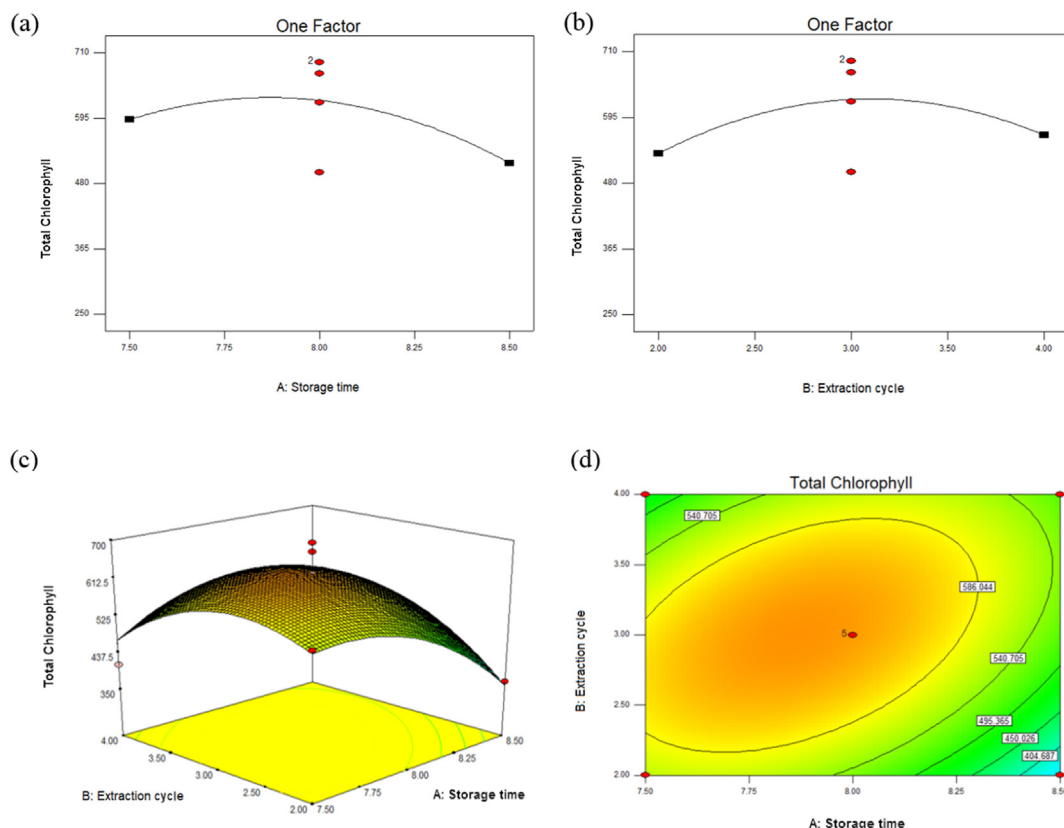
Figure 3. Effect of two individual parameters (a) storage time and (b) extraction cycle on TC concentration. Graphs of (c) 3D-response surface curve and (d) contour plot showing TC concentration as a function of storage time and extraction cycle.

As depicted in Figure 4, the software only recommended one solution: three extraction cycles and 7.87 h of storage time. However, a rounded condition value to 8 h was done in the actual experiment to make it precise. This design has a desirability value of 0.857, indicating that it is appropriate for use. However, the selected conditions can only achieve 631.392 mg/L, although the maximum TC concentration measured was 693.292 mg/L. Therefore, 693.292 mg/L was selected as the predicted value. In validation studies, the discrepancy between predicted and actual values is measured as a percentage error (Table 6). It is shown that the error was within an appropriate range, with a satisfactory error percentage of less than 30% as a rule of thumb. The actual concentration of TC gained under the recommended optimum conditions was 663.500 mg/L, which was the highest of the three validation studies. Therefore, the proposed condition at three cycles of extraction cycle with 8 h storage

time was an acceptable optimum condition for chlorophyll extraction to achieve maximum TC concentration.

In contrast to other past investigations, Wu et al. [27] obtained 4.80 mg TC per gram of chlorophyll from moso bamboo culm using an ultrasonic-assisted extraction. This method is simple, less time-consuming, and reliable for chlorophyll extraction, but this method is not preferable compared to other methods. The proper optimization in ultrasound frequency, propagation of cycle, input power, and nominal power of the device must be determined to ensure that the structure of the value molecules is not damaged during the extraction process [9]. The lack of bath temperature and adequate power control would affect the efficiency of the extraction. In addition, the solvent was also included in this method even in minimal amounts, this was still considered costly and toxic for the environment.

Figure 4. Solution of optimum parameters for maximum TC yield suggested by CCD.

Table 6. The error between the predicted and actual value of all experiments.

Factor 1: Storage time	Factor 2: Extraction cycle	TC yield (mg/L)		Error (%)
		Predicted	Actual	
8 h	3 cycles	693.292	663.500	4.30
8 h	3 cycles	693.292	566.134	18.34
8 h	3 cycles	693.292	500.848	27.76

Table 7. Comparison of TC contents using different methods from the previous study.

Sample	Method	TC yield	Author
Pineapple Leaves	Mechanical extraction	663.5 mg/L	This research
A. sessilis	Solvent extraction	920.5 mg/L	[8]
Moso bamboo culm	Ultrasonification method	4.8 mg/g	[27]
Mature white pine (*Pinus Sylvestris*)	Solvent extraction	2.6 mg/g	[28]
Conocarpus lancifolius	Solvent extraction	59.64 mg/g	[29]

Solvent extraction or leaching method is a very common method used by researchers due to easy operation, low energy consumption, and could extract a higher yield of TC. Jinasena et al. [8] investigated the recovery of TC from *A. sessilis* and gained 920.5 mg/L of TC content using solvent extraction. Another study by Hiscox and Israelstam [28] showed a 2.6 mg/g of TC content also by solvent extraction. Similarly, the research of Jassim [29] did the extraction of chlorophyll from *Conocarpus lancifolius* using solvent extraction and obtained 59.64 mg/g TC. However, this method is costly, particularly when a large amount of chemical is required in the extraction process, and results in increased chemical waste generation, which may be harmful to the environment. These comparisons are listed in Table 7. Therefore, a low-cost and environmentally friendly single step of extraction of chlorophyll, as conducted in this study using mechanical extraction, was a more efficient and feasible method of extracting chlorophyll from pineapple leaves.

4. Conclusion

A CCD was used to determine the optimum conditions for the extraction of TC content from pineapple leaves. Based on *F*-value, it was discovered that the two process variables studied had a significant effect on the TC yield. The *p*-value from the ANOVA test shows that the model terms of storage time and extraction cycle were not statistically significant. But as compared to the p-value, storage time was smaller than the extraction cycle, thus this factor was the strongest factor that influenced the TC contents. Meanwhile, the interaction among the process variables studied shows an interaction effect where there is a simultaneous change in the levels of both factors to the output response. The lack of fit value in this study showed a non-significant lack of fit, indicating that this model was geared toward perfect fitness. Besides that, the quadratic model proposed in this work demonstrates a strong connection between the experimental and predicted concentration of TC. Response surface analysis was discovered to be an effective method for estimating the optimum condition for achieving maximum concentration of TC. The optimal conditions determined were 8 h of storage time and three cycles of extraction cycle. Under this condition, 663.500 mg/L of TC yield was obtained. This accounted for a 4.30% error from predicted models. Therefore, it was suggested the model obtained was favorable for optimizing the extraction of chlorophyll from pineapple leaves using a mechanical method. It is recommended to further research under this condition by using a difference type of mechanical method that may give a higher yield of TC concentration.

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