

ULTRASOUND-ASSISTED EXTRACTION OF STARCH FROM OIL PALM TRUNK: AN OPTIMIZATION BY RESPONSE SURFACE METHODOLOGY

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Article history

Received

13 August 2022

Received in revised form

07 March 2023

Accepted

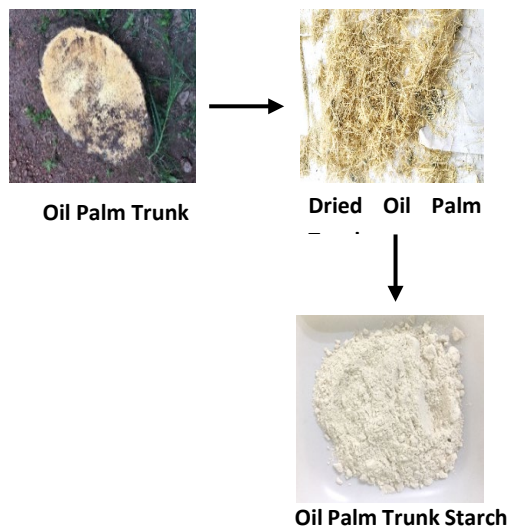
27 March 2023

Published online

30 November 2023

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Graphical abstract



Abstract

Higher production of palm oil in Malaysia has resulted in a significant amount of oil palm biomass including oil palm trunk (OPT). OPT has been studied as an alternative starch source. To date, reports on the extraction of starch from OPT using ultrasound-assisted extraction (UAE) are limited. The application of UAE is considered to be a simpler and effective way in starch extraction compared to conventional extraction methods. This study aims to investigate the potential of ultrasound-assisted extraction to extract starch from OPT, and optimization using response surface methodology. A three-factor central composite design (CCD) was designed to determine the effects of variables on response. The three variables involved in the study, namely: sonication temperature (45-65°C), sonication time (30-60 min) and sodium metabisulfite concentration (0.5-1.5% w/v). All of the data were analysed using Analysis of Variance (ANOVA). The R^2 value (95.6%) and model ($p < 0.0001$), indicating the acceptability of the model and significance in the interaction between independent variables. At the optimum conditions of 52.84°C temperature, 30.66 min exposure time and 0.58% w/v sodium metabisulfite concentration, the starch yield achieved its optimum value by 34.21%. Therefore, this study proved the potential of UAE in extracting higher OPT starch.

Keywords: oil palm trunk, optimization, response surface methodology, starch, ultrasound-assisted extraction

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1.0 INTRODUCTION

Oil palm is one of the valuable plants in Malaysia, Thailand and Indonesia. Malaysia has become a major producer and exporter of palm oil. Felda, Sime Darby Plantation Berhad, IOI Corporation Berhad and Genting Plantations Berhad are among the agencies responsible for managing Malaysia's palm oil industries. High demands of palm oil resulted to generation of large amount of waste, including oil palm fronds (OFB), empty fruit bunch (EFB), oil

palm trunk (OPT) and palm oil mill effluents (POME) and [1,2]. OPT is a solid waste produced during the replanting activities, where the wastes are mostly unutilized in Malaysia. Due to global concerns of environmental problems, dumping and burning are no longer allowed to dispose the waste. It is reported that million tonnes of OPT in the form of felled trunks were left behind after replanting activities every year [3]. Thus, it is expected that the aim of extracting the starch from OPT is very feasible to be explored in order to increase the added value of the waste. The extraction of

starch from the trunks will not only benefit the economy, but will also broaden the application of starch-free fibres into a number of valuable uses

Starch is made up of two primary polysaccharides; amylopectin and amylose. Amylose is a linear chain polymer in which anhydroglucose units are linked by α -1,4 glycosides bonds. Generally, amylose contains 20% to 30% of common starch and has low molecular weight [4]. Meanwhile, amylopectin is a short chain polymer that has higher molecular weight than amylose and contains about 70% of common starch [5]. Amylopectin is a major component in starch and contributes to the crystallinity of starch granules. Starch is an abundant material which mainly found in cereals and tubers such as potatoes, corn, wheat, rice, tapioca, cassava and sago. The utilization of starch has increased especially in water and wastewater treatment due to its biodegradability, inexpensive, non-toxicity and environmental friendly. Based on the studies conducted by Hashim et.al [3] and Omar et.al [6], it is stated that the core part of oil palm trunks contain the highest amount of starch. The OPT is composed of three main components, including vascular bundles, parenchyma cells and fibers. The outer part of OPT are surrounded by vascular bundle, where parenchyma cells is stored inside the vascular bundle coarse. Starch are rich in parenchyma cells. Ramle et.al [7] claimed, parenchyma content increased in each part of the core, middle and outer part of OPT. while vascular bundle content decreased. These are aligned with the result reported by Omar et.al [6], where starch content were higher (8.8%) at the core portion of OPT than middle (7.2%) and outer portion (7.15%). Nonetheless, the extraction of starch from OPT is difficult compared to the extraction of starch from corn, wheat and cassava. However, starch extraction from OPT is more complex than the extraction of starch from corn, wheat, and cassava. This is due to the presence of lignin in vascular bundle which inhibits the breakdown of parenchyma cells. Thus, it is essential to use effective extraction method to extract higher amount of starch from OPT.

Common method for starch extraction from OPT is steeping method. Several researchers have conducted studies on starch extraction using steeping method, however, the results showed that this method could only produce starch ranging from 2% to 17% [2-3, 8-9]. Moreover, steeping method required longer times to extract the starch from OPT, which up to 24 hours [2]. Recently, ultrasound-assisted extraction (UAE) has increasingly attracted global attention due to its potential to improve the extraction yields of starch and time consuming. González-Lemus et.al [10] reported that the application of ultrasound in jicama roots increased starch yield by 25%. Park et.al [11] used ultrasonic method in extracting starch from local corn and successfully achieved maximum starch yield of 70%. Pinyo et.al [12] claimed that the highest extraction yield of 71.5% of sago pith starch was obtained using ultrasound. Unlike jicama roots [10], wheat [13], corn [14], tapioca [15], sago pith waste [16] and breadfruit [17], however, studies on the extraction of starch from OPT using ultrasound assisted extraction method are limited. Therefore, the objective of the study is to explore the utilization of UAE to extract higher amount of starch from OPT.

Based on the previous literatures, for the starch extraction conditions, sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$) was mainly used as a solvent in the experiment due to its ability to extract higher amounts of starch. According to Park et.al [11], different concentration of sodium metabisulfite (0.5%, 1%, 2%) was able to extract sorghum starch up to 70%. A study by Chandra et.al [18]

indicated that, 43% of corn starch were yielded with addition of 3mg L^{-1} of sodium metabisulfite concentration. However, starch extraction is difficult due to the starch granules are embedded in a protein matrix. Also, it is noted that sodium metabisulfite is effective in increasing the whiteness of the starch. Chandra et.al [18] stated that there is no requirement for the minimum value of the degree of whiteness of starch, based on ISO standards.

Sonication temperature was also identified as one of the factors affecting the extraction of starch. Determining the optimum temperature is crucial to avoid extensive swelling of starch granules which then resulted to starch gelatinization. Starch gelatinization is a process where starch molecules swell irreversibly. The increase of the temperature leads to the penetration of excessive water into the starch granules, causing the disintegration of starch. Most of the studies stated that the gelatinization occurs when the temperature reaches 60-70 °C, which depends on the starch sources. Moreover, with continued heating and the application of shear, starch granules may lose their crystallinity and birefringence as well as leaching of amylose to the inter-granular space may occur. Further, Liu et.al [19] noted that higher temperature could lowered the amount of starch extracted due to the decreased of amylopectin. Nonetheless, a study by Usman et.al [20] reported that lower temperature of less than 30°C also lead to the lower extraction of starch yield. A similar observation was evidenced by Tay et.al [2], where the lowest starch yield of 0.68% was extracted when temperature of 26°C was applied. Several studies indicate that temperature ranging from 40°C to 60 °C is effective to achieve higher starch yield [2, 20, 21]. This is because when the temperature reaches 40°C, starch granules start absorbing the water to produce larger granules while maintaining their structural properties. On the other hand, when the temperature increased near to boiling points (65°C-95°C), simple starch granules degraded and might change their structure to a starch paste. Tay et.al [2] reported that at the temperature of 50°C, highest extraction yield of 8.24% was obtained using steeping method.

By using ultrasound method, sonication time seems to be important in extracting higher starch yield. Based on a study conducted by González-Lemus et.al [10], the extraction yield of starch increased up to 30% as the sonication time reaches 60 min. However, it was observed that the starch yield decreased when the sonication time increased to 90 min. On the other hand, using steeping method, it is noted that extraction time did not give significant effects to increased starch yield, as the increasing of extraction time has resulted to the lowered starch yield [2]. By using Response Surface Methodology (RSM), starch will be extracted under different variables such as sonication temperature, sonication time and sodium metabisulfite concentration. This approach was applied to determine the optimum conditions (sonication temperature, sonication time and sodium metabisulfite concentration) which require further evaluation by running a laboratory experiment. The optimization work in this study was performed to achieve the best percentage of starch extraction from OPT, using ultrasound-assisted extraction method. Based on the previous literatures, It is believed that the parameters selected for this study are important to achieve higher starch extraction in OPT. The experimental results attained were then compared and employed to verify the predicted result given by Design Expert software.

2.0 METHODOLOGY

2.1 Materials

The oil palm trunks were obtained from Felda Oil Palm Plantation, Mersing, Johor, Malaysia, during replanting activities. The trunks were debarked and the core part of OPT were chopped into chips. Figure 1(a) shows an illustration of core part of OPT used in this study. The OPT chips were initially dried in an oven at 100°C for 2 hours and grinded into powder (Figure 1(b)). The powder was then sieved to mesh size of 40 and oven dried at 105 °C for 24 hours. Prior to the extraction, the sample was kept in a closed container. The solvent used in this study was sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$; 31448, Sigma-Aldrich, ACS Reagent Grade, Dry, 98-100.5%).

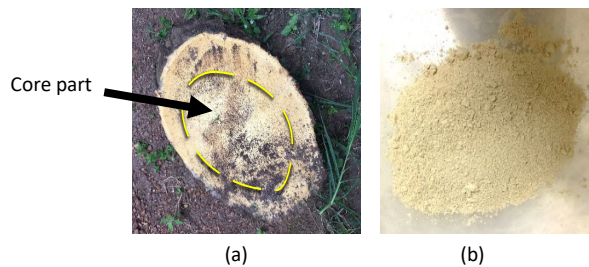


Figure 1. (a) Core part of oil palm trunk (b) Oil palm trunk powder

2.2 Starch Extraction Process

The extraction process used ultrasound-assisted extraction method. A sonication bath machine (Sci-Lab 40kHz, Korea) was used to extract 10 g of OPT powder (immersed into 100 mL of solvent). Table 1 describes all of the extraction conditions. The ratio of the solvent to OPT powder used was 1:10 (w/v). After sonication, the aqueous extract was filtered through 75 μm filter to remove residual bran and impurities. The centrifugation process was done according to Park et.al [11], with slight modification. The slurry was centrifuged (Hettich EBA 21, Germany), at 2,500 rpm for 10 minutes, and the residue was decanted. To remove the precipitates, 40 mL of distilled water was added into the slurry. The slurry was then vortexed for 10 seconds to rapidly mix the solid-liquid. The slurry was then centrifuged for 2 min at speed of 1,500 rpm and freeze dried to obtain the starch extract. Repeated purification and centrifugation may help in reducing the impurity. Then the produced starch was lightly pounded into fine powder in white colour, and stored in 4°C storage until further analysis. The picture of the extracted OPT starch is given in Figure 2.



Figure 2. Oil Palm Trunk Starch

2.3 Determination of Starch Yield

The percentage of starch yield [24] was calculated using the following formula:

$$\text{Yield} = \frac{\text{Weight of dry starch (g)}}{\text{Weight of OPT powder (oven-dried)(g)}} \times 100\% \quad (1)$$

2.4 Process of Starch Identification

Starches are composed of chains of glucose molecules. Starches can be determined through iodine test, using Iodine solution (I_2KI). Iodine solution was made by dissolving 1.3 g iodine (I_2 , Sigma, 7553-56-2, ACS Reagent) and 2.0 g potassium iodide (KI, Sigma, 7681-11-0, ACS Reagent). into 100 ml distilled water. The colour may change into dark blue or black, when a few drops of iodine solutions are added onto the OPT sample, indicating the presence of starch. The core part and outer part of OPT were tested to identified the presence of the starch.

2.5 Optimization Process of Starch Extraction

2.5.1 RSM Design

A central composite design (CCD) was used in this study to estimate the linear and interaction between two variables, investigate the possibility of non-linear effect of the selected variables as well as to determine the optimum operating condition of response. A full 2^3 factorial central composite design with a total of 20 experiments was employed in this study. All of the experiments were run in triplicate, including 6 replicates at center point together with $\pm \alpha$ points. The selected variables were sonication temperature (X_1), sonication time (X_2) and sodium metabisulfite concentration (X_3) which set as independent variables, while the percentage of starch yield (Y_1) was set as the only response for optimization of starch extraction using ultrasound. Table 1 shows the five levels of independent variables, including alpha ($-\alpha$, α) for design experiment. The range for each variable was determined based on the previous literatures. Meanwhile, Table 2 shows the complete design matrix of CCD and experimental result.

2.5.2 Statistical Analysis

The experimental design was carried out using Design expert, version 11.0 (Statease Inc., Minneapolis, USA). This study used Design expert to obtain the regression, graphical and statistical analysis (ANOVA). The second order quadratic polynomial model equation was applied for the analysis as shown in (Equation. 2). The model equation used to determine the effect and performance of independent variables on the response, where Y represents the observed response of the percentage of starch yield, β_0 represents the constant coefficient, and β_i , β_{ii} , and β_{ij} represents the linear, quadratic, and interactive coefficients, respectively. Meanwhile, X_i and X_j represents the independent variables, including sonication temperature, sonication time and sodium metabisulfite concentration. The accuracy of the model and interaction between variables and response was evaluated statistically by ANOVA and F-test (Fisher's test), which then allowed the determination of the significance of each term in polynomial model equation.

obtained was compared with the predicted response value given by the software, to verify the accuracy and suitability of the model. As refer to Table 5, starch yield had 35.83% and 34.21% of the predicted response value and experimental value, resulted in 1.62% percentage error. According to Bezzera [26], the percentage error of less than 10% is commonly acceptable. Hence, following the purpose of the study, it can be concluded that the starch yield model is verified for its acceptability. For further validation of the model, the plot of predicted values versus actual values for starch yield was presented in Figure 4. The plot indicates that the prediction model is adequate as most of the data points located near to the straight line, $y=x$. Therefore, the optimum operating condition for the highest starch extraction using ultrasound-assisted extraction method were 52.84 °C sonication temperature, 30.66 min sonication time and 0.58 wt% sodium metabisulfite concentration, respectively.

Table 5. Comparison of experimental and predicted response at optimum condition

Optimum condition			Response: Starch Yield (g)		
Sonication Temperature (°C)	Sonication Time (min)	Sodium metabisulfite concentration (%)	PV (%)	EV (%)	PE (%)
52.84	30.66	0.58	35.83	34.21	1.62

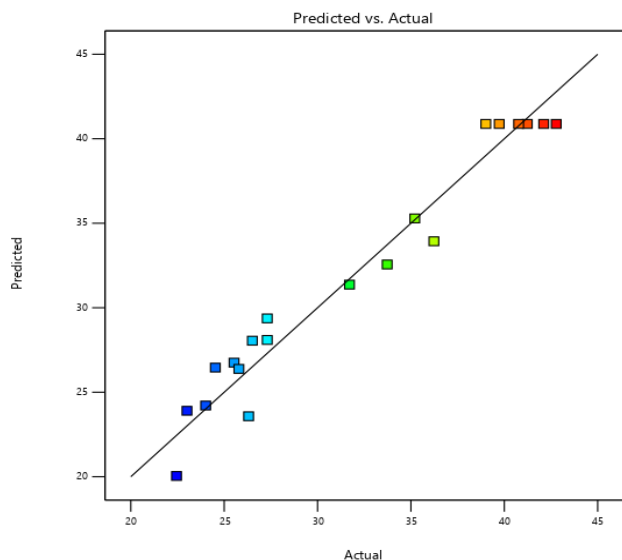


Figure 4. Predicted versus actual plot for starch yield extraction

3.4 Response Surface Analysis of Starch Yield

The 3D surface graph and contour plots were extracted from the model developed to analyses the interaction between the variables and response. All of the data are presented in Figures 5-7. Figure 5 depicts the 3D response surface plot of the sonication temperature (X1) and sonication time (X2) and their interactions on the starch yield at sodium metabisulfite concentration of 1% (center point of design experiment). The sonication temperature is the one of the important parameter affecting the extraction of starch. From the experiment, maximum starch yield of 42.78% was obtained at 45 min and 55 °C. As shown in Figure 5, at the temperature range of 45°C- 55°C, higher extraction of starch was achieved, where the starch yield reached a maximum of 42.78 %. However, as the temperature further increase from 55 °C to 65°C, the starch yield started to decreased. The starch had the lowest extraction yield of 23% when the temperature reached 65°C. Similar result was obtained by Mohamed Noor et.al [27], where the extraction yield did not increase at sonication temperature above 55°C. These findings are in agreement with Jing Ming and Sen Mi [28]. It was observed that the starch granules started to disintegrate at a temperature of 60°C and formed a molecular network. This could be due to the prolonged temperature applied in the experiment, which resulted in the bursting and release of starch from granules into the liquid. This phenomenon is called starch gelatinization. Starch gelatinization occurs when the temperature exceeds a certain degree, causing the disruption of starch molecules and resulted in leaking of starch out of the granules. The starch eventually dissolves into the liquid, causing the reduction of the extraction yield as the starch granules becomes starch paste. Moreover, according to Chemat et.al [22], the increased in temperature near to boiling points (65 °C – 95 °C), led to the decreased of gas solubility and reduced the number of cavitation bubbles, causing to the disintegration of starch granules and hindered the increase of extraction yield. This is related to cavitation phenomena. Cavitation phenomena occurs due to the propagation of ultrasonic waves, causing the formation of cavitation bubbles followed by the collapse event. Due to the intense implosion of cavitation bubbles near the materials, it creates several physical effect such as shockwaves and inter-particles collision, leading to the increased of mass transfer rate between substrate and solvent. At this stage, rapid fragmentation and erosion of the materials could occur, as a resulting to the disruption of the cell wall structure of starch and releasing higher amounts of the starch granules, further leading to the enhance extraction rate and efficiency [22,23] However, higher temperature exceeded with longer extraction time may resulted to the loss of cavitation bubbles and cause a less efficiency of starch extraction

From the figure, it is clearly seen that the extraction yield increased along with the increased of sonication time. Higher extraction yield was obtained when the sonication time increased from 30 min to 45 min. Nevertheless, the starch yield began to decreased after 45 min. Higher temperature and prolonged sonication time could be related to the collapse of cavitation bubble. The penetration and heating of ultrasonic treatment with prolonged sonication time lead to the structural fragmentation of starch granules due to the frequent collapse of cavitation bubbles, resulting in a decrease in starch yield [29-30] Another possible reason for such behaviour may be due to the facts that by 45 min, most of the starch granules embedded in

the parenchyma cells are released. Thus, optimum sonication time is required for the particles to open up the fibrous material structure of OPT and release more starch granules.

Figure 6 shows the effect of varying sonication temperature (X_1) and sodium metabisulfite concentration (X_3) on the starch yield with sonication time fixed at 45 min (center point of design experiment). At minimum sodium metabisulfite concentration and minimum sonication temperature, the extraction yield was less than 30%. Increasing the sonication temperature and sodium metabisulfite concentration, ranging from 0.5 wt% to 1 wt% resulted in better extraction yield. However, further increasing the sodium metabisulfite concentration up to 2 wt% did not significantly increase the starch yield. The similar trend was observed when sonication temperature exceeds the optimum value, the extraction of starch yield dropped. From the Figure 6, it can be seen that the highest starch yield of 42.78% was obtained at sonication temperature of 55°C and sodium metabisulfite concentration of 1 wt%. This is due to the dissolution of sodium metabisulfite in distilled water has resulted to the formation of Na^+ ion and bisulfite ion (HSO_3^-), where it further reacts with H^+ to form sulphur dioxide (SO_2). SO_2 , which act as a reducing agent has the capability to improve the starch yields by breaking down the protein matrix that wraps the starch granules. Moreover, SO_2 capable to create favourable conditions for the growth of lactic acid, which helps to weakened the cell wall and separate the starch, thus allowing the release of higher amount of starch [18].

On the other hand, at maximum sonication temperature and sodium metabisulfite concentration, the starch yield decreased to about 30%. As stated by Pinyo [12], the lower starch yield obtained at higher solvent concentrations could be attributed to the lower amount of delivered energy to break up the protein matrix that wraps the OPT particles. This is due to the increases of viscosity and density of solvent. Greater ultrasonic energy is crucial for disruption process, however, as the viscosity or density of the suspending medium increases, the required delivered energy attenuates and reduces. The observation contradicts the findings by Park et.al [11], as the result showed that lower corn starch was extracted with 0 wt% concentration of sodium metabisulfite. Hence, more studies in this area need to be carried out to determine the factors affecting the sodium metabisulfite concentration on the starch extraction. Therefore, in this study, 0.5% to 1 wt% concentration of sodium metabisulfite is suitable to increase the extraction yield, as excessive concentration might reduce the amount of starch. In the study, based on the optimization work by RSM, 0.58 wt% sodium metabisulfite concentration is sufficient to achieve higher starch extraction.

Figure 7 depicts the 3D response surface plot of the sonication time (X_2) and sodium metabisulfite concentration (X_3) and their interactions on the starch yield while sonication temperature was kept constant at 55°C (center point of design experiment). It was observed that the surface response showed significant changes when sonication time and sodium metabisulfite concentration were varied. Increases in sonication time and sodium metabisulfite concentration could significantly improve extraction yield. At a concentration of 0.5 to 1.0 wt%, higher extraction yield could be obtained with increasing sonication time, ranges from 48 to 54 min. Based on the contour plot, it can be seen that after 54 minutes, the extraction yield started to decrease. It is also noted that the extraction yield increases significantly when the sodium metabisulfite

concentration increased up to 1%, along with the increased of sonication time from 30 min to 45 min. Under a certain condition (sonication time = 45 min and sodium metabisulfite concentration = 1 wt%), a maximum contour with the highest extraction yield of 42.78% was obtained, indicating that further increase in sonication time and sodium metabisulfite concentration would not increase the extraction yield. Starch yield began to decreased when both concentration and time reached the optimal value. The optimal sonication time is necessary to extract a higher percentage of starch from OPT. Again, cavitation involved during the extraction process including acoustic and hydrodynamic are among the factors that contribute to the collapse of microbubble, leading to a decrease in starch extraction when an optimal value is reached [31].

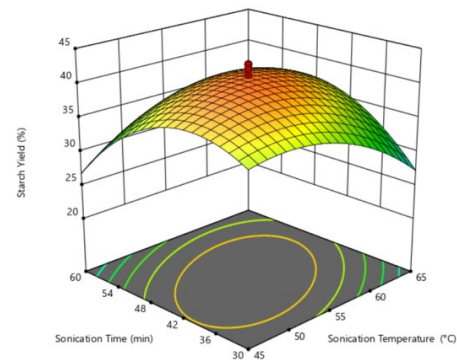


Figure 5. 3D response surface plot representing the relationship between variable (sonication temperature (X_1) and sonication time (X_2)) and starch yield %

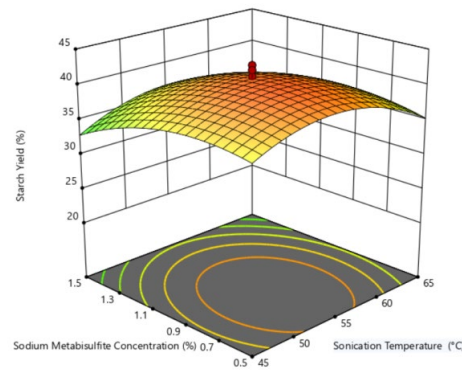


Figure 6. 3D response surface plot representing the relationship between variable (sonication temperature (X_1) and sodium metabisulfite concentration (X_3)) and starch yield %

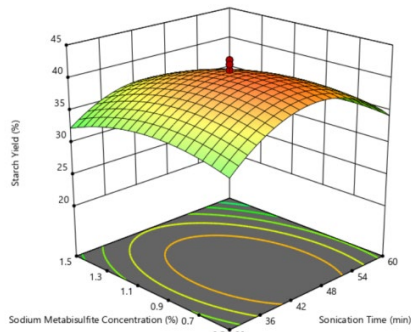


Figure 7. 3D response surface plot representing the relationship between variable (sonication time (X_2) and sodium metabisulfite concentration (X_3) and starch yield %

4.0 CONCLUSION

Ultrasound-assisted extraction was used in the extraction of the starch yield from oil palm trunk (OPT), and was optimized by using the central composite design for the response surface methodology. The CCD was designed with 20 experimental runs to assess the impact of the variables (sodium metabisulfite concentration, sonication temperature, and sonication time) on starch extraction yield. The interaction between the variables in the model are significant ($p < 0.001$), allowing for the incorporation of a quadratic model. The sonication temperature and sodium metabisulfite concentration had a significant effect ($p < 0.05$) on the starch extraction yield from OPT. As per the desirability test, the optimum conditions were found to be, sonication temperature of 52.84 °C, sonication time of 30.66 min and sodium metabisulfite concentration of 0.58 wt%, which resulted in 34.21% yield of starch. Overall, the ultrasound-assisted extraction method has high potential as an effective method to increase the extraction yield of OPT starch, replacing the conventional extraction method.

Acknowledgement

The authors gratefully acknowledge for the sponsorship from UTM Campus Sustainability (UTM-CS) and AUN/SEED-Net. This research is fully supported by Research University Grant of Universiti Teknologi Malaysia (CRG-UTM) under (Grant Project No: 09G81). The authors fully acknowledged Universiti Teknologi Malaysia for the approved fund which makes this important research viable and effective.

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