ADVANCES IN AGRICULTURAL AND FOOD RESEARCH JOURNAL

Original Research Article

Optimisation of Microwave-Assisted Extraction of Antioxidant Activity from *Pandanus amaryllifolius* Leaf and Evaluation of Blending Properties in Refined Bleached Deodorised Palm Olein Through Response Surface Methodology

Vijehy Balakrishnan[2,3], Fitrien Husin[1], Noorazwani Zainol [1], Nurfatiha Norhisham[1], Rosnani Hasham[2], Harisun Ya'akob[1,2]*

[1]Institute of Bioproduct Development, Universiti Teknologi Malaysia (UTM), Skudai, 81310, Johor Bahru, Johor, fitrienh@gmail.com, azwani@ibd.utm.my, norfatihanorhisham2726@yahoo.com

[2]Department of Bioprocess Engineering, Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, vijehy@gmail.com, r-rosnani@utm.my, harisun@utm.my

[3]Saha Asia Industries, Jalan Enjin 34/3, Alpine Industrial Park, Section 34, 40470 Shah Alam, Selangor, Malaysia, vijehy@gmail.com

*Corresponding author: Harisun Ya'akob, N22, Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia (UTM), Skudai, 81310, Johor Bahru, Johor. harisun@utm.my

Abstract: The demand for natural antioxidants in the food industry has steadily increased due to growing consumer awareness of the health benefits associated with consuming antioxidant-rich foods. Plant-based antioxidants offer a promising alternative to synthetic antioxidants, as they are perceived as safer and more sustainable. Pandan (*Pandanus Amaryllifolius)* leaves, have been recognised for their high antioxidant activity attributed to their rich phenolic content. Microwave-Assisted Extraction (MAE) has emerged as a green and efficient extraction technique for obtaining bioactive compounds from plant materials. This study aims to improve the extraction parameter of MAE for obtaining high antioxidant activity from *P.amaryllifolius* and to determine the optimal blending ratio and frying cycle of *P.amaryllifolius* as a natural antioxidant in Refined, Bleached and Deodorised (RBD) palm olein. The variables examined were the extraction power (350–450 W), extraction temperature (70–88°C) and extraction time (10–20 min), which are crucial factors affecting the extraction efficiency(yield) and antioxidant activity of *P.amaryllifolius*. The antioxidant activity of *P.amaryllifolius* was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and yields were measured. The total yield varied from 12.32 to 21.63% while antioxidant activity varied between 86.7 to 96.6%. Results revealed that the optimal extraction conditions for high yield and antioxidant activity were 450 W, 20 min, and 70°C. Under ideal conditions, the equivalent yield and antioxidant activity values were 20.54% and 92.4%, respectively. Additionally, moisture content and free fatty acid measurements were performed to determine the optimal mixing ratio and frying time of *P.amaryllifolius* in RBD palm olein. RBD palm olein containing 0.4% *P.amaryllifolius* demonstrated the most encouraging result with the highest four cycles of frying. In conclusion, optimising the extraction parameters of *P.amaryllifolius* using MAE provides valuable experimental data

that can guide the formulation of natural antioxidant additives for enhancing the oxidative stability of food products, particularly those containing palm olein as a major ingredient.

Keywords: *Pandanus amaryllifolius;* RSM; antioxidant; MAE, RBD

Received: 11th April 2023

Accepted: 9th July 2024

Available Online: 20th July 2024

Published: 17th October 2024

Citation: Balakrishnan, V., Husin, F., Zainol, N., *et al.* Optimization of microwave-assisted extraction of antioxidant activity from *Pandanus amaryllifolius* leaf and evaluation of blending properties in refined bleached deodorized palm olein through response surface methodology. *Adv Agri Food Res J* 2024; 5(2): a0000498. https://doi.org/10.36877/aafrj.a0000498

1. Introduction

An antioxidant is a substance that is designed to inhibit or slow the oxidation of oxidisable components like lipids and is vital for maintaining the overall quality of the products. This is because lipids are highly vulnerable to oxidation, resulting in colour, flavour, and nutritional value changes in the food products. Oxidation of lipids or fats will also result in the development of undesired chemical molecules such as ketones, aldehydes, and organic acids, reducing the shelf life and nutritional value of lipid-based foods (Saad *et al.*, 2007). There are numerous benefits of incorporating antioxidants in food items, and their use is becoming increasingly important (Krishnawasmy *et al.*, 2013). The complete lack of harmful consequences, on the other hand, is ideal. As a result, extensive research into their effectiveness and side effects is required. Antioxidants prevent these alterations by delaying or slowing the oxidation or rancidity process.

Two main groups of antioxidants can be used in refined bleached deodorised (RBD) palm olein which are synthetic and natural antioxidants. Originally, food companies primarily used synthetic antioxidants to preserve their products, however, as time passed, this tendency has shifted with increasing number of companies opting to employ natural antioxidants to secure their products. Natural antioxidants are defined as naturally occurring additives that attempt to postpone the product's oxidative rancidity and keep its conditions excellent for a longer period. Tocopherols, rosemary extract, and ascorbic acid are some of the most regularly utilised natural antioxidants, which are rapidly being sought after by food producers due to their numerous benefits (Abasolo, 2021).

Pandan, scientifically known as *Pandanus amaryllifolius* Linn. is often used for essential oil production due to its aromatic scent, flavour and colour. Pandan leaves contain many active compounds that contribute to their antioxidant properties such as flavonoids,

alkaloids, tannins, saponins, polyphenols, and others (Djenar *et al.*, 2020). According to Ghasemzadeh and Jaafar (2014), optimum extraction parameters increase the antioxidant activity of Pandan leaves. Extraction of Pandan leaves using microwave-assisted extraction (MAE) showed a high yield of Pandan leaves extract and was found that Pandan leaves extract potentially protects vegetable oils from oxidation in studies conducted by Nor *et al.* (2008) and Ali *et al.* (2015).

Optimisation of the green technology extraction such as the MAE method can significantly influence the quality of the extracted compounds. Green extraction technologies aim to minimise the use of organic solvents and reduce waste generation, leading to lower environmental impact compared to conventional extraction methods. Green extraction technologies like MAE can potentially reduce operational costs associated with extraction processes, making them more economically viable for large-scale industrial applications.

The use of experimental design such as Response Surface Methodology (RSM) in the optimisation process of plant extractions is necessary since this method is the most current method used in various studies. By systematically varying extraction parameters and analysing their effects on extraction efficiency and product quality, researchers can deepen their understanding of the complex interactions between solvents, matrices and target compounds. This knowledge can then be applied to develop more efficient and sustainable extraction processes in various fields such as food, pharmaceuticals and natural product chemistry (Ghasemzadeh & Jaafar, 2014).

RBD palm olein is widely used in the food industry, especially in regions where palm oil is abundant. It is used in a variety of food products such as frying oils, margarine, shortenings and as a cooking oil. The stability of the oil is crucial to ensure the quality and shelf life of these food products. The stability of the oil is essential to prevent oxidation and the formation of harmful compounds, such as free radicals and trans fats, which can occur when oils are exposed to high temperatures for prolonged periods. Instability can lead to rancidity, off-flavours and off-odours (Morales & Przybylski, 2013). Oxidised oils can have detrimental effects on health due to the formation of harmful compounds. Therefore, ensuring the stability of RBD palm olein is important for protecting consumer health.

Previous studies were successfully carried out on natural antioxidants' development from *P. amaryllifolius* (Pandan) leaves but no study was carried out on the optimisation of the extraction parameters especially in the use of green technology extraction such as MAE. Most natural antioxidants fulfil the criteria but there is no evaluation with cooking oil

addition. In addition, the optimisation of extraction from plants such as *P. amaryllifolius* using environmental extraction methods such as the MAE technique material is still unknown and there are no optimisation studies carried out on the best processing method yet. Natural antioxidants should be given more attention as it has fewer adverse effects and have competitive prices compared to synthetically made antioxidants. The information on the potential use of natural antioxidants is also limited and merits more investigation and further research. Therefore, the optimisation of process conditions of obtaining a good quality of natural antioxidants besides the evaluation of blending the plant extract with palm oil needs to be carried out.

2. Materials and Methods

2.1 Sample Preparation

The fresh leaves of *P. amaryllifolius* were obtained from the Institute of Bioproduct Development, Universiti Teknologi Malaysia, Johor, Malaysia. The sample preparation method was conducted based on the method from Zakaria *et al.* (2020). The samples were air-dried and ground into a moderately coarse powder using a Waring blender, then stored in dark vials until further usage. The refined, bleached, deodorised (RBD) palm oil for blending with Pandan leaves extract for its natural antioxidant was obtained from Saha Asia Industries Sdn. Bhd., Shah Alam, Selangor, Malaysia. Fresh potatoes were obtained from the fresh market in Taman Universiti, Skudai, Johor, Malaysia. Fresh potatoes for frying were carefully peeled and sliced to a thickness of 1.5 mm. After soaking for 5 min in a 2.5% salt (NaCl) solution, the sliced potatoes were left to dry before frying.

2.2 Chemicals and Reagents

Analytical grade chemicals such as methanol, ethanol, sodium hydroxide, ether, diethyl ether, and petroleum ether were acquired from Merck Sdn. Bhd. KGaA, Darmstadt, Germany. Standard chemicals such as calcium tetrachloride, potassium iodide, sodium thiosulphate, isooctane, *p*-anisidine, glacial acetic acid, acetic acid, and 2-2-diphenyl-1-picrylhydrazyl (for evaluation of antioxidant activity) were acquired from Merck Sdn. Bhd. KGaA, Darmstadt, Germany. Soy lecithin was obtained from Agrin Chemicals (M) Sdn. Bhd., Malaysia.

2.3 Design of Experiment (DOE)

Using face-centred central composite design (CCD) acquired from Design Expert 7.1.6, the interaction effects between variables (independent and dependent variables) were

examined to determine the optimum extraction conditions. The data collected during the experiments were analysed using analysis of variance (ANOVA), as this determined whether the processing parameters affected the response variables or vice versa. The experimental design in this study is presented in Table 1.

Table 1. Central composite design for extraction of *P.amaryllifolius.*

	Independent Variables		
Experimental Run	X_1 Extraction Power (W)	X_2 Extraction Time (min)	X_3 Extraction Temperatures (°C)
1	-1	0	0
2	-1	1	1
3	0	0	-1
4	-1	1	-1
5	0	0	0
6	1	-1	1
7	0	0	0
8	0	0	0
9	1	-1	-1
10	1	0	0
11	1	1	1
12	0	1	0
13	-1	-1	-1
14	0	0	0
15	-1	-1	1
16	0	0	0
17	0	0	1
18	1	1	-1
19	0	-1	0
20	0	0	0

2.4 Optimisation of P. amaryllifolius Extract Using MAE Method

To extract the natural antioxidant from *P. amaryllifolius* leaves, the extraction was conducted by using the MAS-II Plus Microwave Synthesis Machine Model (Sineo, China) as mentioned by Zakaria *et al.* (2021). The chiller distillatory was linked to the machine to permit the evaporated solvents to condense and maintained in the flask throughout the extraction procedure. The extraction of *P. amaryllifolius* leaves was carried out according to Alara *et al.* (2018) and Zaki *et al.* (2020). An ethanol-water solvent of 75% concentration (Zaki *et al.*, 2020) with a total amount of 1 L was prepared. *P. amaryllifolius* leaves (3 g) were added to the ethanol-water solvent (100 mL) for the extraction of natural antioxidants which were then placed in the set-up microwave oven. By applying the parameters of the extraction process, the MAE process was carried out. The steps were repeated for all

parameters designed. A rotary evaporator (Eppendorf, Germany) under vacuum at 50°C was used to dry the extract under vacuum after each experimental run. Following that, all the extracted samples were analysed for percentage yield and antioxidant activity.

The parameters that were used in the MAE process are extraction power (350-450 W) (Marwah, 2019; Djenar *et al.*, 2020), irradiation time (10-20 min) (Zaki *et al.*, 2020; Djenar *et al.*, 2020), and extraction temperature (70-88°C) (Leong, 2012). Meanwhile, antioxidant activity and percentage of yield were examined as response parameters. Table 1 shows the range of each processing parameter for *P. amaryllifolius* leaves extraction. The study was carried out according to the experimental design created by Design Expert. Each factor was coded according to its level according to the coding scheme. In this study, extraction power (W) was reported as X1, extraction time (min) as X2, and extraction temperature (°C) as X3. The -1 represents as a low level, 0 represents as a medium level, and +1 represents as a high level. Twenty sets of experiments were carried out using CCD.

2.5 Percentage Yield

Following Equation (1), the extraction yield of *P. amaryllifolius* leaf extract was estimated.

$$Extraction\ yield\ (\%) = \frac{Extracted\ material\ mass\ (g)}{Dried\ Pandan\ leaves\ mass\ (g)} \times 100 \quad (1)$$

2.6 Determination of Antioxidant Activity by 2,2-diphenyl-1-picrylhydrazil (DPPH)

In this study, the leaf extract of *P. amaryllifolius* was tested for its ability to scavenge DPPH free radicals using the method outlined by Alara *et al.* (2018). One mM DPPH solution was dissolved in methanol. Then, to make a 0.1 mM fresh working DPPH solution, 10 mL of stock DPPH solution was added to 90 mL of methanol. At optimal conditions, 1.25 mg/mL of the extract was prepared and thoroughly mixed with 2 mL of DPPH solution (0.1 mM). Using a UV-Vis Spectrophotometer (UV-1800, Shimadzu, Japan), the absorbance of the mixture at 517 nm was determined after 30 min of incubation in the dark. The calculation percentage of DPPH scavenging activity is shown in Equation (2).

$$\%\ DPPH\ inhibition = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\% \quad (2)$$

Where $A_{control}$ is the absorbance of a methanol-DPPH solution mixture, while A_{sample} is the absorbance of a sample extract-DPPH solution mixture.

2.7 Blending and Frying Experiment

RBD palm olein was subjected to a frying method through blending with 0.1%, 0.25% and 0.4% of the extracted *P. amaryllifolius* leaves. In order to increase the solubility of *P. amaryllifolius* extract in RBD palm olein, 0.5% of soy lecithin (w/w) was added by thoroughly mixing it by stirring to ensure uniform distribution of the soy lecithin.

In the frying process, fresh potatoes were used. Frying was conducted in batch fryers (Model No TSBQ-12, Zhucheng Tianshun Machinery Co. Ltd., China) according to the method by Jurid *et al.* (2020). To start the frying process, RBD palm olein (2L) was heated for 30 min at 180 ± 5°C in the deep fat fryer. After that, prepared fresh potatoes (300 g) were fried for 8 min in the palm olein. According to Jurid *et al.* (2020), there was a break time of 7 min in between frying cycles. For every successive frying cycle, 100 mL RBD palm olein was collected.

2.8 Moisture Content

The moisture content of the oil was measured using a digital refractometer (Pocket Refractometer) (ATAGO, Tokyo). The method was conducted by dropping the oil sample on the surface glass prism of the refractometer. The measurements were automatically determined. The data were recorded in triplicates and presented as mean ± standard deviation.

2.9 Free Fatty Acid (FFA)

This parameter was determined based on Siew *et al.* (1995) test method. To measure FFA, 5 g of the sample was dissolved in 30 mL of the mixed solvent (ether/ethanol/water, 3:3:2 v/v/v) and then titrated with 0.1 N sodium hydroxide solution (NaOH). FFA were determined in duplicate for each sample, and the average of two measurements was used to further analyse the data. FFA were calculated as a percentage of oleic acid. The calculation to determine the FFA (%) as palmitic acid is shown in Equation (3).

$$\%\ DPPH\ inhibition = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\% \quad (3)$$

where, the formula for determining FFA which is the equivalence factors for palmitic acid, the most abundant fatty acid in palm oil, is 25.6. N stands for NaOH normality, V for the volume of NaOH solution used in mL, and W for the sample weight.

3. Results and Discussion

Table 2 shows the effects of extraction power (350–450 W), extraction time (10–20 min) and extraction temperature (70–88℃) on the extraction yield and antioxidant activity of *P. amaryllifolius* under the experimental test conditions. The total yield varied from 12.32 to 21.63% while antioxidant activity varied between 86.7 to 96.6%. The *F*-test and *p*-value were used to determine the statistical meaning and the significance of each coefficient and results were shown in Tables 3 and 4.

Table 2. CCD and its response variable for extraction of *P.amaryllifolius.*

Experimental Run	Independent Variables			Responses	
	X_1	X_2	X_3	Y_1	Y_2
	Extraction Power (W)	Extraction Time (min)	Extraction Temperatures (°C)	Yield (%)	Antioxidant at 1.25 mg/ml (%)
1	350	10	88	15.56	96.6
2	400	15	79	17.33	92.1
3	400	15	79	17.51	92.8
4	400	15	79	17.84	91.6
5	400	15	88	15.31	93.5
6	350	15	79	19.64	89.1
7	350	10	70	21.63	93.6
8	450	20	70	20.54	92.4
9	350	20	88	12.32	92.3
10	350	20	70	15.62	86.7
11	400	15	79	15.78	93.2
12	400	10	79	15.66	93.0
13	450	10	88	19.22	93.5
14	400	15	79	18.49	93.4
15	400	20	79	18.37	92.3
16	450	10	70	18.26	92.3
17	450	15	79	19.45	90.3
18	450	20	88	19.72	90.5
19	400	15	70	17.64	93.2
20	400	15	79	20.32	91.8

3.1 Modelling and Optimisation of P.amaryllifolius Extraction

In order to optimise the extraction process of *P. amaryllifolius*, 20 experiments with replicates at the centre point designed by CCD were carried out using three-factor variables which were extraction power, extraction time and extraction temperature. According to Eskilsson and Björklund (2000), to extract the analytes from the sample matrix, microwave energy was absorbed to heat the solvent to facilitate the partitioning of the analytes into the solvent. Percentage of yield extract and its antioxidant activity were recorded as their responses as shown in Table 2. The highest percentage of yield extract was observed at

experimental run 7 (21.63%) at conditions of 70℃ of extraction temperatures, 10min of extraction time and 350 W of extraction power. In addition, the highest antioxidant activity when 1.25 mg/mL extract was tested can be seen from experimental run 1 (96.6%) at 88℃ of extraction temperatures, 10 min of extraction time and 350 W of extraction power. The model obtained for the extraction yield (Table 3) and antioxidant activity (Table 4) was significant to reveal the relationship between the independent parameters and responses (p<0.05). Regression analyses were performed at the 95% confidence interval. According to ANOVA, the F-value of both models showed that the derived models were significant.

As shown in Table 3, the variables that had a significant effect on the extraction yield were the linear terms A and C also the interaction between AB (p<0.05), for the antioxidant activity, the variables that had a significant effect on the antioxidant were the linear terms of B and C, and also the interaction between AB and AC as shown in Table 4 (p<0.05). The following quadratic polynomial model for yield response in the form of coded values was given in Equation (4) while the quadratic polynomial model for antioxidant activity was given in Equation (5). In Tables 3 and 4, the significance of each coefficient was found using the F-test and p-values. As the absolute F-value becomes greater, the p-value becomes smaller and the corresponding variables will be more significant. The Lack-of-fit tests were given to check for the quality of both models and the results were found to be not significant, thus indicating that both models could adequately fit the experimental data for all the response variables.

Table 3. ANOVA for yield response

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	70.47	9	7.83	3.03	0.0497
A-power	15.43	1	15.43	5.96	0.0347
B-time	1.41	1	1.41	0.5465	0.4768
C-temperature	13.36	1	13.36	5.17	0.0464
AB	18.09	1	18.09	6.99	0.0246
AC	11.31	1	11.31	4.37	0.0631
BC	0.1225	1	0.1225	0.0474	0.8321
A^2	9.70	1	9.70	3.75	0.0816
B^2	1.17	1	1.17	0.4522	0.5165
C^2	3.91	1	3.91	1.51	0.2471
Residual	25.87	10	2.59		
Lack of Fit	14.69	5	2.94	1.31	0.3856
Pure Error	11.18	5	2.24		
Cor Total	96.34	19		R^2	0.7315

Table 4. ANOVA for antioxidant response

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	63.18	9	7.02	5.44	0.0071
A-power	0.0490	1	0.0490	0.0379	0.8495
B-time	21.90	1	21.90	16.96	0.0021
C-temperature	6.72	1	6.72	5.21	0.0456
AB	8.61	1	8.61	6.67	0.0273
AC	10.81	1	10.81	8.37	0.0160
BC	0.0312	1	0.0312	0.0242	0.8795
A^2	13.75	1	13.75	10.65	0.0085
B^2	1.40	1	1.40	1.08	0.3222
C^2	5.50	1	5.50	4.26	0.0661
Residual	12.91	10	1.29		
Lack of Fit	10.07	5	2.01	3.53	0.0961
Pure Error	2.85	5	0.5697		
Cor Total	76.10	19		R^2	0.8303

$$\text{Yield} = +17.79+1.24A-0.3760B-1.16C+1.50AB+1.19AC+0.1237BC+1.88A^2-0.6523B^2-1.19C^2 \quad (4)$$

$$\text{Antioxidant activity} = +92.26+0.0700A-1.48B+0.8200C+1.04AB-1.16AC-0.0625BC-2.24A^2+0.7136B^2+1.41C^2 \quad (5)$$

3.2. Effects of Operational Factors on Extraction Yield and Antioxidant Activity

To explore the interactions between independent and dependent variables, three-dimensional (3D) response surfaces were plotted to determine the optimum conditions for extraction yield and antioxidant activity. Figure 1 represents the 3D response surface graph for the extraction yield of *P. amaryllifolius*. In order to investigate the effect of extraction power, time and temperature on the yield recovery, experiments were performed by extracting between 10 to 20 min, respectively. The extraction power was set between 350 to 450 W and the temperature between 70 to 88℃. It had been observed from Figure 1(a) that with increasing time and microwave power, the extraction yields increased (p<0.05). The extraction yield improves as the power and extraction time increased, however, high temperatures and long extraction times may cause the thermolabile bioactive chemicals to degrade (Wang & Weller, 2006). Figure 1(b) shows that with the increase in temperature and power, the extraction yield got increased. However, the interaction between the parameters was not significant as (p>0.05). Figure 1(c) represented the decreasing pattern in extraction yield with increasing temperature and time though it was statistically not significant (p>0.05). Hence, it can be concluded that both extraction time and power are pivotal factors. Increasing

microwave power enhances extraction efficiency while prolonging extraction time results in increased extraction yield.

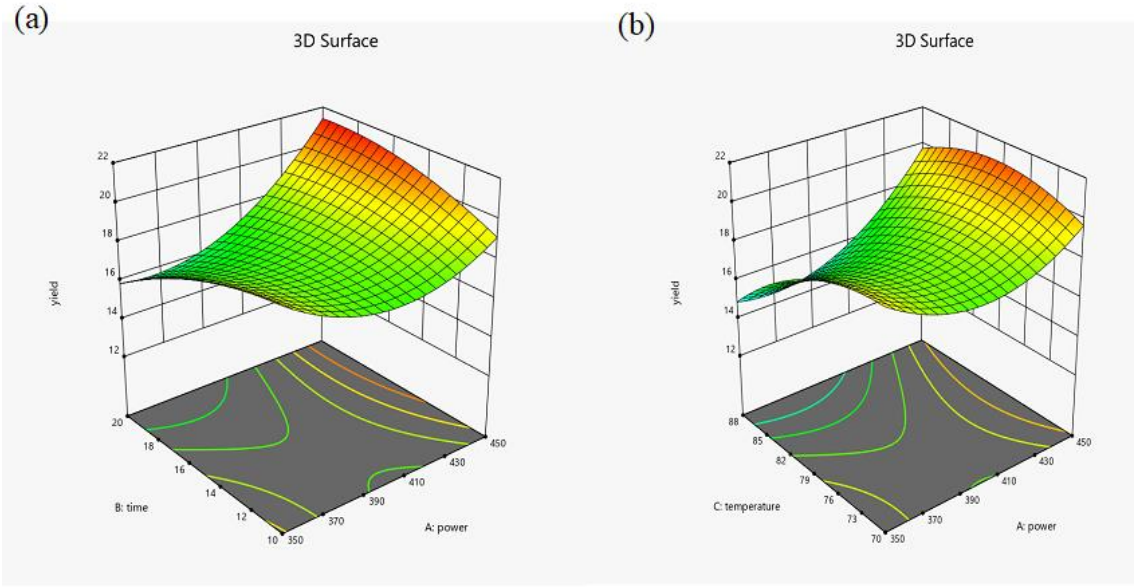
Compared to other approaches, DPPH scavenging ability is a widely utilised method for evaluating antioxidant activity in a relatively short amount of time. Ghasemzadeh and Jaafar (2013), used two types of assays to measure the antioxidant capacity of foods, beverages and supplements that contain polyphenols, the assays that were used are Ferric reducing antioxidant potential (FRAP) assay and mainly 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay. Ascorbic acid was utilised as a reference for determining the antioxidant potential of *P. amaryllifolius*, and the samples from MAE extraction were examined alongside it. Figure 2 shows the effect of the studied parameters on the extraction of antioxidants from the sample matrix. The results indicated that the antioxidant activity deteriorates as the power and extraction time are increased as depicted in Figure 2(a) (p<0.05), similarly, figure 2(b) showed that with the increase in power and temperature, the antioxidant activity got decreased (p<0.05). Increasing microwave power and time generally leads to higher extraction efficiency but can cause thermal degradation of heat-sensitive antioxidants, resulting in reduced overall antioxidant activity. Similarly, higher temperatures can accelerate extraction by enhancing compound solubility and cell disruption but can also promote thermal degradation of the antioxidants leading to reduced overall antioxidant activity. This study was similar to Lovric *et al.* (2017) who found that increasing the extraction time could destroy flavonoids when the microwave was used for an extended period. For the isolation of bioactive compounds from raw plant materials, a suitable temperature for the MAE technique is critical, as over-elevated temperatures could always degrade phenolic and flavonoid compounds as structural decomposition starts at high temperatures (Jin *et al.*, 2017).

As can be seen in Figure 2(c), the antioxidant activity increased with increasing extraction temperature and time (p>0.05). This could be attributed to higher temperatures causing enhanced molecular mobility and flavonoid solubility (Jin *et al.*, 2017). The highest antioxidant activity found in this study was 96.6% in parallel to a study performed by Ali *et al.* (2015), which found that the DPPH radical scavenging activity percentage of their pandan extract ranges from 94% to 96% in BHA, from 61% to 90% in 80% methanol in water, 49% to 82% in methanol and 40% to 74% in ethanol. The difference in the percentage was due to the polarity of the solvent extracts, where the antioxidant activity was higher in a more polar solvent compared to a less polar solvent. In a study conducted by Zaki *et al.* (2020), MAE was used to extract pandan leaves and the results showed that MAE was better at extracting

phenolic compounds from pandan leaves compared to conventional soaking methods. The MAE used 450 W of microwave power at a solvent concentration of 75%. However, the percentage of phenolic compounds obtained was lower than 2% in solvent concentration. In another study conducted by Djenar *et al.* (2020), pandan leaves were also extracted via MAE with various times of extraction and the microwave power, ranging from 10 to 30 min and 180 to 600 W, respectively. In the study, the optimal conditions for extracting phenolic compounds from dried Pandan leaves were found to be 450 W for 20 min. However, even under these ideal conditions, the maximum yield of phenolic compounds was less than 25%.

Nevertheless, the predicted optimum conditions for extraction power, time and temperature in this study were 450 W, 20 min, and 70℃, respectively, which is in close agreement with a study performed by Djenar *et al.* (2020).

The optimal conditions for the selected variables were determined based on the response surface. Under the optimum conditions, the extraction yield was 20.54% and the antioxidant activity was 92.4%, which was very close to the predicted value. The results of experiments and predicted values are presented in Table 5. The high correlation between experimental and anticipated results proved that the regression model was accurate and suitable for *P. amaryllifolius* optimisation. Pandan shows the potential to be used as an alternative antioxidant source in food and edible oil (Ghasemzadeh & Jaafar, 2014). The inclusion of antioxidants as food additives extends the shelf life of many foodstuffs, hence achieving high antioxidants in the produced extracts is a crucial necessity in the food sector. Because lipid oxidation in foodstuffs often results in rancidity and possibly harmful reaction products, attempts are being made to minimise oxidation by increasing antioxidant additions to food. Although synthetic antioxidants have been widely used, antioxidants obtained from plants are more useful in extending the shelf life of food goods and offering health promotion (Kebede & Admassu, 2019).



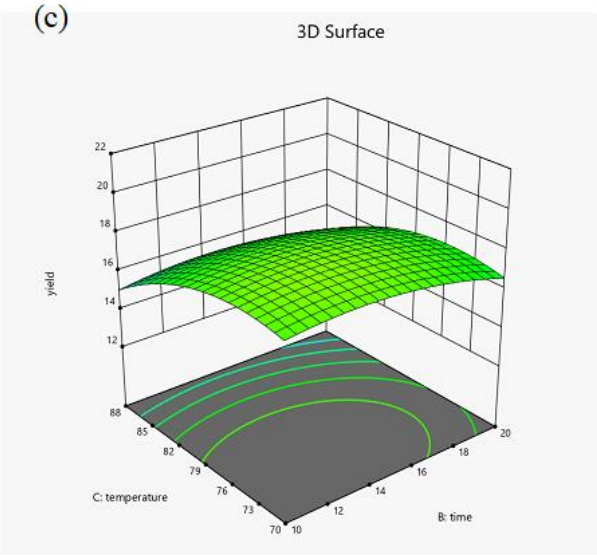


Figure 1. Three-dimensional surface plot for the yield of *P. amaryllifolius* as a function of (a) extraction time and extraction power (b) extraction temperature and extraction power (c) extraction temperature and extraction time.

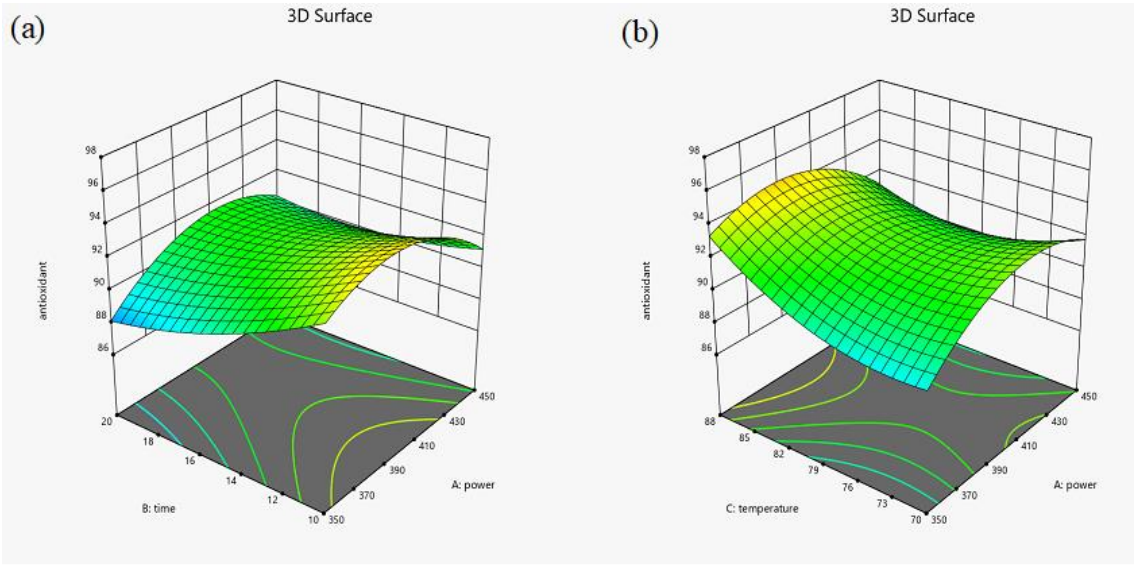


Figure 2. Three-dimensional surface plot for the antioxidant of *P.amaryllifolius* as a function of (a) extraction time and extraction power (b) extraction temperature and extraction power (c) extraction temperature and extraction time.

Table 5. Comparison between predicted and experimental data at the optimum condition of *P.amaryllifolius* extraction

Responses	Predicted value	Experimental value	Difference (%)
Yield of extract (%)	20.04	20.54	0.5
Antioxidant activity (%)	92.18	92.4	0.22

3.3 Blending of RBD Palm Olein with P.amaryllifolius

Moisture content in RBD palm olein blending with extracted *P. amaryllifolius* for this study was illustrated in Figure 3. As part of the frying process, water is lost, oil is absorbed, and heat is transferred. As food and oil contain water, the hydrolysis of the oil is accelerated.

It also prevents the oil from oxidising during frying. Based on the graph in Figure 3, there was no significant difference between all blending treatments and non-blending treatments (p>0.05). This may be due to insufficient exposure to moisture content during the frying process. Therefore, this result may prevent any rancidity and oxidative stress in the oil (Jurid et al., 2020). In continuation to the extraction of *P. amaryllifolius*, RBD palm olein was subjected to the frying method through blending with specific concentration (0.1%, 0.25%, 0.4%) of the extracted *P. amaryllifolius* leaves. The amount of FFA in different frying cycles for different concentrations of *P. amaryllifolius* extract is shown in Figure 4. FFA levels are crucial indicators of oil quality and shelf life. The bar chart showed an increment of FFA for blending and non-blending of RBD palm olein. It may be due to the presence of water in the frying system from potato chips, most likely FFA has increased as a consequence of hydrolysis (Trivedi *et al.*, 2017). All treatments showed a significant difference of p<0.05 respectively to their own treatment.

Elevated FFA levels are often indicative of poor-quality oil. The presence of FFA accelerates the deterioration of oil quality and reduces its shelf life. However, in this study, RBD palm olein blending with 0.4% *P. amaryllifolius* extract resulted in the lowest FFA level and prolonged the quality of the palm olein as long as 4 cycles usage compared with other blending ratios and control. Additionally, the level of FFA even after the 5th cycle of frying was still in the acceptable range, compared to the FFA content of oils in industrial applications which ranges between 0.5 to 0.8% (Orthoefer & List, 2007). Thus, it may be inferred that the addition of *P. amaryllifolius* improved the quality of the oils and lowered the oxidation process led to the development of off-flavours and odours. The finding in this study was similar to the study conducted by Jaswir *et al.* (2000) where it was found that an equal ratio of antioxidant extract derived from rosemary and sage along with citric acid with a 0.15 ratio to a percentage of total refined palm oil used, produced the best antioxidant blend for the oil. The combination of rosemary, sage and citric acid would produce a synergistic effect on retaining the fatty acid content of palm olein during repeated deep-fat frying. In this study, blending of RBD palm olein with 0.4% of *P. amaryllifolius* showed the most promising result up to 4 frying cycles.

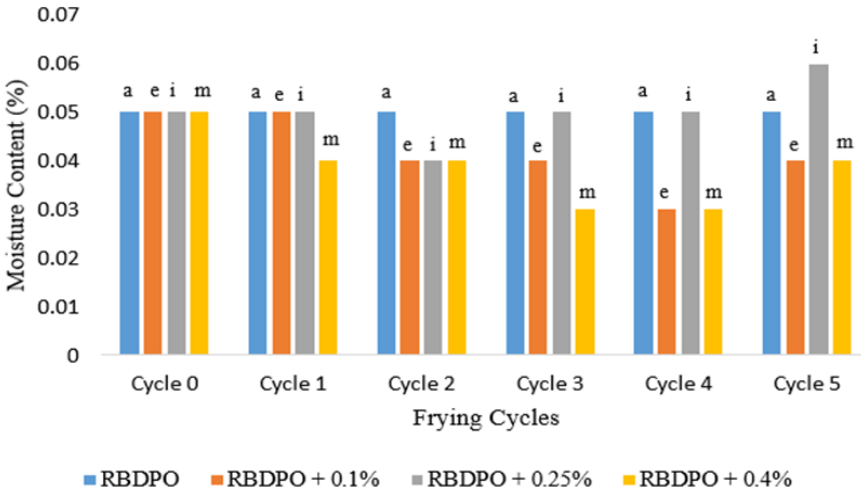


Figure 3. The moisture content of RBD palm olein for different frying cycles. Cycle 0 represented moisture content prior to the experiment

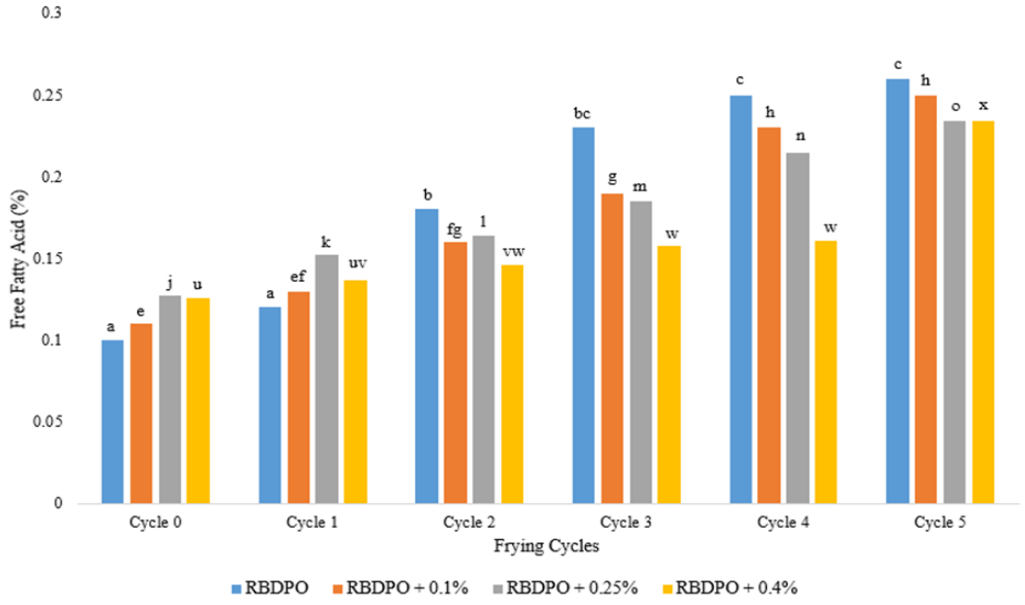


Figure 4. Free fatty acids of RBD palm olein for different frying cycles.

4. Conclusions

A microwave-assisted extraction process has been optimised for effective extraction of *P. amaryllifolius*. The extraction yield and antioxidant activity of *P. amaryllifolius* under the optimal MAE condition (450 W, 20 min, and 70°C) was 20.54% and 92.4%, respectively. The experimental results demonstrated that MAE was a rapid and efficient technique for the extraction of antioxidants from *P. amaryllifolius*. Furthermore, the evaluation of the blending process showed significant improvement in the frying performance of palm olein and this could provide a better solution to food industries. Additionally, *P. amaryllifolius* extract may be served as a potential functional food ingredient. In conclusion, optimising the extraction

parameters of *P. amaryllifolius* using MAE provides valuable experimental data that can guide the formulation of natural antioxidant additives for enhancing the oxidative stability of food products, particularly those containing palm olein as a major ingredient. Furthermore, the utilisation of plant-based antioxidants aligns with the growing consumer preference for clean-label ingredients and sustainable food additives.

Author Contributions: Conceptualisation, methodology, formal analysis, investigation, resources, writing- Vijehy, B., Fitrien, H., Nurfatiha, N. —original draft preparation, writing- Fitrien, H., & Nurfatiha, N. —review and editing- Fitrien, H., Zainol, N., Yaakob, H., & Rosnani, H. All authors have read and agreed to the published version of the manuscript

Funding: This work was funded by the contract research grant R.J130000.7646.4C734

Acknowledgements: In this segment, you may acknowledge any support that is not addressed by the author's contribution or funding sections.

Conflicts of Interest: The authors declare no conflict of interest.

References

Abasolo, C. (2021). *What is a natural antioxidant and why is it useful in food?* BTSA. https://www.btsa.com/en/what-natural-antioxidant/

Alara, O. R., Abdurahman, N. H., Ukaegbu, C. I., *et al*. (2018). Vernonia cinerea leaves as the source of phenolic compounds, antioxidants, and anti-diabetic activity using microwave-assisted extraction technique. *Industrial Crops and Products*, *122*, 533–544.

Ali, M. A., Sabri, R. B. M., LI, K., *et al*. (2015). Impact of pandan leaf extract addition on the oxidative degradation of sunflower oil during microwave heating. *Sains Malaysiana*, *44*(8), 1159–1166.

Djenar, N. S., Mulyono, E. W. S., Saputra, T. R. (2020). The effect of microwave power variations on phytochemical characteristic of pandan leaves (Pandanus Amaryllifolius) using the Microwave-Assisted Extraction (MAE). *Journal of Physics: Conference Series*, *1450*(1), 012007.

Eskilsson, C. S., Björklund, E. (2000). Analytical-scale microwave-assisted extraction. *Journal of chromatography A*, *902*(1), 227–250.

Ghasemzadeh, A., Jaafar, H. Z. (2014). Optimization of reflux conditions for total flavonoid and total phenolic extraction and enhanced antioxidant capacity in pandan (*Pandanus amaryllifolius Roxb*.) using response surface methodology. *The Scientific World Journal*, *2014*.

Ghasemzadeh, A., Jaafar, H. Z. (2013). Profiling of phenolic compounds and their antioxidant and anticancer activities in pandan (Pandanus amaryllifolius Roxb.) extracts from different locations of Malaysia. *BMC complementary and alternative medicine*, *13*(1), 1–9.

Jaswir, I., Man, Y. B. C., Kitts, D. D. (2000). Use of natural antioxidants in refined palm olein during repeated deep-fat frying. *Food research international*, *33*(6), 501–508.

Jin, C., Wei, X., Yang, S., *et al*. (2017). Microwave-assisted Extraction and Antioxidant Activity of Flavonoids from Sedum aizoon *Food Science and Technology Research*, *23*(1), 111–118.

Jurid, L. S., Zubairi, S. I., Kasim, Z. M., *et al*. (2020). The effect of repetitive frying on physicochemical properties of refined, bleached and deodorized Malaysian tenera palm olein during deep-fat frying. *Arabian Journal of Chemistry*, *13*(7), 6149–6160.

Kebede, M., Admassu, S. (2019). Application of antioxidants in food processing industry: Options to improve the extraction yields and market value of natural products. *Advances in Food Technology and Nutritional Sciences*, *5*(2), 38–49.

Krishnaswamy, K., Orsat, V., Gariépy, Y. *et al.* (2013). Optimization of Microwave-Assisted Extraction of Phenolic Antioxidants from Grape Seeds (Vitis vinifera). *Food and Bioprocess Technology*, *6*, 441–45. https://doi.org/10.1007/s11947-012-0800-2

Leong, L. S. (2012). *Potential application derived from the essential oil of pandan leaves (Pandanus amaryllifolis roxb.)* (Doctoral dissertation, Universiti Malaysia Pahang).

Lovrić, V., Putnik, P., Kovačević, D. B., *et al*. (2017). Effect of Microwave-Assisted Extraction on the Phenolic Compounds and Antioxidant Capacity of Blackthorn Flowers. *Food technology and biotechnology*, *55*(2), 243–250

Marwah, A. S. (2019). *Pengaruh Lama Ekstraksi Terhadap Mutu Minyak Atsiri Daun Pandan Wangi (Pandanus Amaryllifolius Roxb.) Menggunakan Metode Microwave Assisted Extraction (MAE)* (Doctoral dissertation).

Morales, M. T., Przybylski, R. (2013). Olive oil oxidation. Handbook of olive oil: analysis and properties, 479–522.

Nor, F. M., Mohamed, S., Idris, N. A., *et al*. (2008). Antioxidative properties of *Pandanus amaryllifolius* leaf extracts in accelerated oxidation and deep-frying studies. *Food chemistry*, *110*(2), 319–327.

Orthoefer, F. T., List, G. R. (2007). Dynamics of frying. In *Deep frying* (pp. 253–275). AOCS Press.

Saad, B., Sing, Y., Nawi, M., *et al*. (2007). Determination of synthetic phenolic antioxidants in food items using reversed-phase HPLC. *Food Chemistry*, *105*(1), 389–394.

Siew, W. L., Tang, T. S., Tan, Y. A., & Malaysia Palm Oil Research Institute of Malaysia. (1995). *PORIM: test methods*. Palm Oil Research Institute of Malaysia.

Trivedi, V., Nivetha, Y., Edwin Chow, P. Y. (2017). Improving the frying performance of RBD palm olein oil using NaturFORT™ TRLG 101 liquid as on-top of TBHQ in deep-fat frying of potato chips. *Journal of food science and technology*, *54*(12), 4035–4041.

Wang, L., Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology*, *17*(6), 300–312.

Zakaria, M. M., Zaidan, U. H., Shamsi, S., *et al*. (2020). Chemical Composition of Essential Oils from Leaf Extract of Pandan, *Pandanus amaryllifolius* ROXB. *Malaysian Journal of Analytical Sciences*, *24*(1), 87–96.

Zakaria, N. A., Rahman, R. A., Zaidel, D. N. A., *et al*. (2021). Microwave-assisted extraction of pectin from pineapple peel. *Malaysian Journal of Fundamental and Applied Sciences*, *17*(1), 33–38.

Zaki, N. A. M., Abd Hashib, S., Ibrahim, U. K., *et al*. (2020). Total phenolic content and antioxidant activity of Pandanus amaryllifolius by soaking and microwave-assisted extraction. In *IOP Conference Series: Materials Science and Engineering*, *778*(1), 012155. doi:10.1088/1757-899X/778/1/012155