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ORIGINAL ARTICLE

The Utilisation of Box-Behnken Design as Innovative Approach for Production of Kenaf Seed MH8234 Milk with Maximum Crude Protein Content

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ABSTRACT - The Box-Behnken Design (BBD) was utilised to identify the optimal processing parameters for the production of kenaf seeds milk MH8234 (KSM) with the highest possible crude protein content–CP (%). The independent variables considered in the BBD were soaking time (150, 255, and 360)min, soaking water temperature (30, 60, and 90)°C, grinding water temperature (30, 55, and 80)°C, and grinding time (60, 90, and 120)s. The dependent variable measured was the CP (%). The study's findings indicate that the quadratic model aligns with the experimental data. The study successfully fulfilled its target by using specific processing conditions: soaking time of 150.51 min, soaking water temperature of 30.15°C, grinding water temperature of 36.66°C and grinding time of 120.88 s, and the maximum value of CP (%) KSM at 2.75%. The model's appropriateness was verified by generating the KSM using the optimal values provided by the mathematical model. These findings facilitate the development of a novel variety of plant-derived milk that boasts a substantial amount of crude protein.

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Kenaf seed MH8234, Box – Behnken Design, Plant-based Milk, Processing Conditions, Crude Protein Content.

INTRODUCTION

The Kenaf plant is botanically identified as Hibiscus *cannabinus l*. This plant requires abundant precipitation and consistently high temperatures year-round [1]. Consequently, the majority of kenaf plants exhibit fertility in the northern and eastern coastal regions of peninsular Malaysia, specifically in Kedah, Perlis, Kelantan and Terengganu [2]. The initiative to cultivate kenaf in Malaysia is progressively gaining momentum each year, in response to the Malaysian government's mandate to substitute tobacco crops with kenaf crops. The project was strengthened by the inclusion of the kenaf plant as a significant commodity crop in Malaysia.

Kenaf seeds are a by-product of cultivating kenaf plant. Figure 1 depicted the physical appearance of kenaf seed. According to the NKTB 2020 report, a total of 20 metric tonnes of kenaf seeds were harvested from the cultivation of 200 hectares of kenaf across Malaysia [3]. In addition to its purpose for replanting, most kenaf farmers regard kenaf seeds as waste material due to the limited availability of items that may be derived from this material [4]. This occurs when the primary emphasis of the nation's manufacturing sector is directed towards kenaf-derived products, specifically furniture, textiles, and automobile

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accessories that are exclusively made from kenaf stalks. The relevant government agencies should also give due consideration to this matter as it pertains to the damages suffered by the kenaf farmers, albeit to a small extent.

Figure 1. Kenaf Seed

According to the author's literature assessment spanning from 2010 to 2024, it has been demonstrated that kenaf seeds possess significant potential for the manufacturing of food-based goods. The favourable correlation between the protein and healthy fat level is supported by the research conducted by [4-6]. The writer's enthusiasm for the positive indicator has motivated them to create milk alternatives using kenaf seeds. Moreover, there is still a lack of comprehensive research on the soaking and rotation of kenaf seed milk to enhance its crude protein content. Hence, this investigation was conducted to determine the ideal processing parameters for the production of kenaf seed milk with elevated protein content. To ensure the success of the objective study, the researcher employed the BBD, which is a component of the response surface method. This decision was based on the favourable outcomes observed in prior studies [7-9], which facilitated the researcher's implementation of the study.

MATERIALS AND METHODOLOGY

Source of Materials

25 kg of kenaf seeds MH8234 was imported from Fujian, China, and were transported to University of Technology Sarawak, Malaysia. Upon arrival, the sample was stored under chilled temperature (4 ± 2) ^{o}C.

Table 1. Low, medium and high levels of the factors in Box-Behnken design

Independent Variables	Low		Medium		High	
ST (min)	150	(-1)	255	(0)	360	(1)
SWT (˚C)	30	(-1)	60	(0)	90	(1)
GWT(˚C)	30	(-1)	55	(0)	80	(1)
GT (s)	60	(-1)	90	(0)	120	(1)

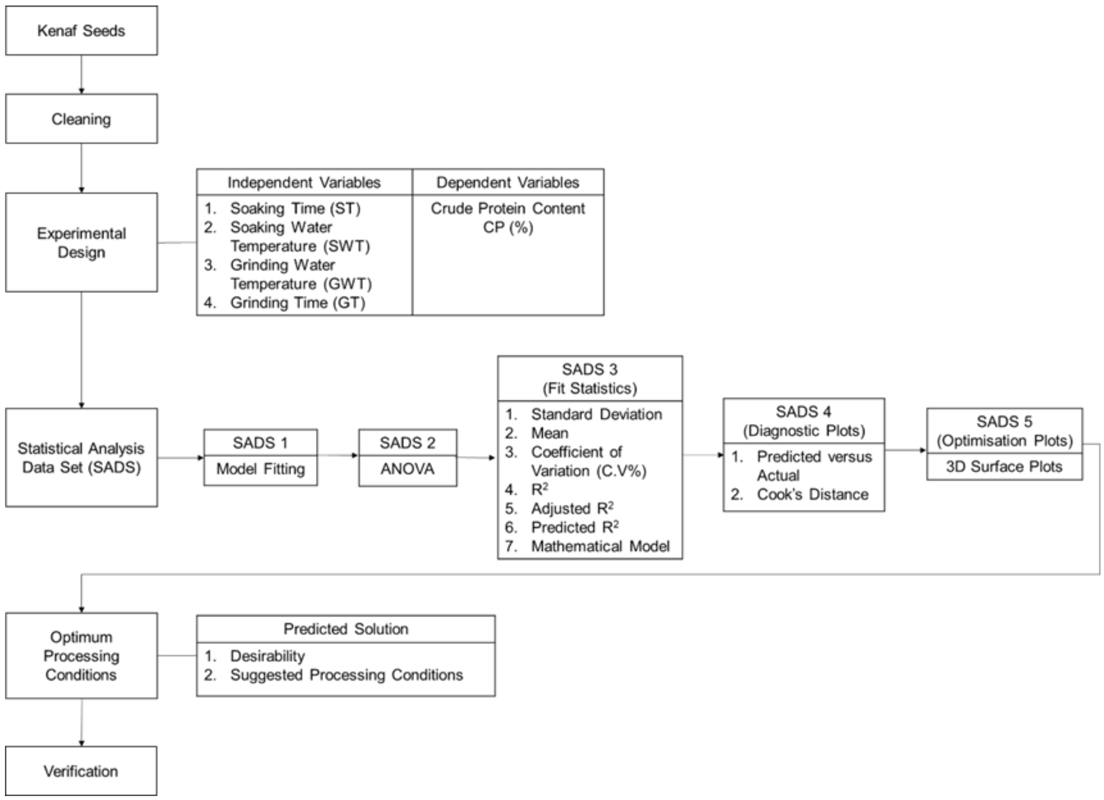

Figure 2. Research summary flow chart.

Experimental Design and Process Flow

The BBD is part of response surface methodology technique, and was utilised to determine the optimal processing conditions for producing KSM with the highest CP(%) value. The experimental design and statistical analysis in this study were conducted using statistical analysis software - Design-Expert 11 software (State-Ease Inc., USA). The study's implementation for the synthesis of KSM with CP(%) incorporates independent variables (ST, SWT, GWT, and GT) and has been executed in accordance with Table 1. According to Table 1, each independent variable will be examined at three distinct levels: low, median, and high. A total of 29 runs have been conducted to satisfy the predetermined experimental design. Meanwhile, the dependent variables pertain to the amount of CP(%) present in each conducted sample. A non-linear regression technique was employed to fit the second-order polynomial equation to the experimental data and determine the significant model terms. To enable the reader's comprehension to understand the concept of the reseach more clearly, Figure 2 has been illustrated to clarify the course of the study that starts from the cleaning process of kenaf seeds till optimisation of verification conditions.

Preparation of KSM

The kenaf seeds were washed meticulously under running water until they were obviously free of dirt. Subsequently, the specimen is immersed in a container filled with 50 ml of filtered water. The immersion conditions were implemented according to the schedule outlined in Table 3. Once the sample has completed the appropriate soaking duration, it is subsequently strained through a plastic sieve and subsequently pulverised with 150 ml of filtered water. The grinding conditions are implemented in accordance with the timetable specified in Table 3. Subsequently, the pulverised sample will be strained through dual layers of muslin fabric. KSM, a yellowish white liquid, is maintained in a reagent bottle for subsequent examination.

Determination of KSM Crude Protein Content – CP (%)

KSM's value for each sample was implemented according to the Kjedahl technique [10].

RESULTS AND DISCUSSION

In this research, multiple regression analyses were performed using response surface methodology, and one established mathematical model resulting from BBD equation [11] to experimental data displayed in Table 1. Upon evaluating the R^2 value between the quadratic and cubic models, it is evident that both models have a similarly high value of 0.99. The experimental system tends to favour the selection of a quadratic model as the mathematical model for the experimental data. The selection of the quadratic model is based on two specific factors: The model has demonstrated a higher degree of complexity with a notable improvement in response elucidation [12]. Specifically, the quadratic model has displayed a larger R^2 value, indicating a stronger relationship between the variables. Furthermore, the adjusted R^2 (0.98) and predicted R^2 (0.94) values have shown a narrow range, suggesting a high level of accuracy in predicting the outcome. However, the cubic model has been dismissed due to being identified as aliased. The presence of an aliased label clearly suggests that there has been a lack of conducted experiments to accurately estimate its terms [13].

The model adequacy and fitness were assessed under the analysis of variance (ANOVA) shown in Table 4. Model reduction has been implemented by removing those terms with p-value that exceed 0.05, this include AB, BC, A^2 and D^2. By deleting insignificant variables, the model reduction has directly increased the significance of other terms by reducing their p-values [14]. Table 5 displays the SADS 2 – Fit Statistic obtained from the experimental data. The values of R^2, adjusted R^2, and predicted R^2 are deemed to be close, with a range between 0.95 and 0.98. The coefficient of variation (C.V%) represents the minimum value and serves as an indicator of the overall reproducibility of the experimental system [15]. The mean model has been able to create a satisfactory signal due to its high precision values. The signal signifies that the acquired data is desirable[16].

Table 2. Summary Result of Fitting a Model to Optimise the KSM's Crude Protein Content

Source	Standard Deviation	R^2	Adjusted R^2	Predicted R^2	PRESS
Linear	0.44	0.62	0.55	0.43	6.98
2FI	0.44	0.71	0.55	0.19	9.98
Quadratic	0.09	0.99	0.98	0.94	0.72
Cubic	0.25	0.99	0.99	0.81	2.29

Table 3. Result of CP(%) for ST, SWT, GWT and GT

Run	A	B	C	D	CP(%)
1	255	60	55	60	1.77
2	255	45	55	90	2.35
3	255	60	55	120	2.09
4	255	30	30	90	2.52
5	360	45	80	90	0.76
6	255	30	55	120	2.65
7	360	45	30	90	2.37
8	360	60	55	90	2.06
9	255	45	80	60	0.95
10	255	45	55	90	2.35
11	255	45	55	90	2.35
12	255	45	30	120	2.65
13	255	45	55	90	2.35
14	255	45	55	90	2.35
15	150	30	55	90	2.45
16	360	30	55	90	2.64
17	255	30	80	90	0.93
18	255	45	80	120	0.59
19	150	45	55	60	1.29
20	255	30	55	60	2.09
21	360	45	55	120	2.04
22	255	45	30	60	1.88
23	360	45	55	60	2.40
24	255	60	30	90	2.08
25	150	45	55	120	2.70
26	150	45	80	90	0.77
27	255	60	80	90	0.85
28	150	60	55	90	2.03
29	150	45	30	90	2.26

Note* Soaking Time: A, Soaking Water Temperature: B, Grinding Water Temperature: C and Grinding Time: D

Figure 3 presents a comparison between the expected response values and the actual response values. Each plot exhibits a comparable linear pattern. Moreover, in the event that the mathematical model is unable to make accurate predictions, the linear plots can be employed to ascertain the value of the response. Figure 4 displays the graphs depicting the Cook's distance in relation to the experimental run number for all the replies. The graphic illustrates that the values at each location are relatively diminutive and in proximity to the lower line. The absence of significant outliers on each plot and the lack of any discernible pattern of errors in capturing data responses are evident in this case [17].

Figure 5 and 6 depict 3D surface plots illustrating the relationship between a significant interaction of AD and CD, as presented in Table 4 (ANOVA). From observations, it is evident that the maximum and minimum CP (%) value is influenced by two factors: the concentration of red and the flare of green and blue; specifically, the highest and lowest points for each plot on the graph. The highest and lowest points for Figure 5 are located on the upper part of the red area at point A: 150 min and D: 120 min, respectively. The lowest point is found on the green area in condition A: 150 min and 60 s. Meanwhile, the highest point on Figure 6 is located in the most intensely red region of the area under the parameters c: 30 ^{o}C and D: 120 s. The lowest point is found in the pale blue region under the conditions c: 70^{o}C and D: 70 s.

The plot clearly demonstrates that a brief immersion period and an extended grinding time have resulted in a significant increase in the CP (%) value. The compact size of the seed is the cause for the little duration of immersion needed for the material to undergo a rapid hydration process [18]. The manufacture of soymilk [19], pistachio milk [20], and tigernut milk [21] differs in terms of the immersion period, which surpasses the optimal period for seed absorption. For this purpose, a brief and ideal duration of soaking has proven to be enough to break down anti-nutritional substances like phytates and tannins, which have the ability to bind with proteins and then become accessible during the filtration procedure [22]. Extended grinding durations have also expedited the filtration process and the creation of KSM with a high protein content [23]. This occurs when the grinding process reduces the physical size of the seeds and increases the surface area by breaking them into tiny fragments. As a result, the protein solubility in the finished product is improved [24]. Put simply, this mechanical action has facilitated the release of proteins from the plant matrix. The study's results align with those provided by [25] about the impact of wet grinding durations on the ultimate yield of soy milk.

Table 4. SADS 1 – Analysis of Variance (ANOVA) Measured from the BBD in the Optimisation of Dependent Variable

Source	Sum of Squares	df	Mean Square	F - Value	p - value	Remarks
Model	12.08	8	1.51	137.24	< 0.0001	Significant
A	0.05	1	0.05	4.85	0.0396	Significant
B	0.47	1	0.47	42.53	< 0.0001	Significant
C	6.62	1	6.62	601.10	< 0.0001	Significant
D	0.46	1	0.46	41.46	< 0.0001	Significant
AD	0.78	1	0.78	71.16	< 0.0001	Significant
CD	0.32	1	0.32	29.00	< 0.0001	Significant
C^2	3.38	1	3.38	306.66	< 0.0001	Significant
D^2	0.13	1	0.13	11.95	0.0025	Significant
Residual	0.22	20	0.01			
Lack of fit	0.22	16	0.21			
Pure Error	0.00	4	0.00			
Cor Total	12.30	28				

Table 5. SADS 2 - Fit Statistics

Components	Value
Standard deviation	0.10
Mean	1.95
C.V%	5.38
R^2	0.98
Adjusted R^2	0.98
Predicted R^2	0.95
Adequate Precision	35.93

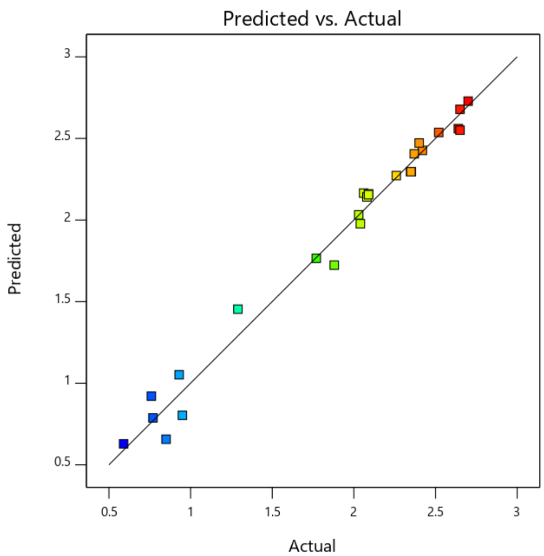

Figure 3. Plot predicted against Actual CP(%) Values for the reduced BBD model in the Optimisation of CP(%)

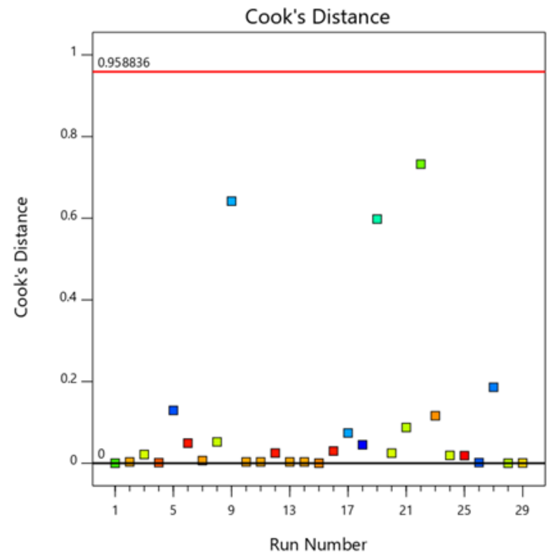

Figure 4. Cook's Distance Plot against run number for the Reduced BBD Model in the Optimisation of CP(%)

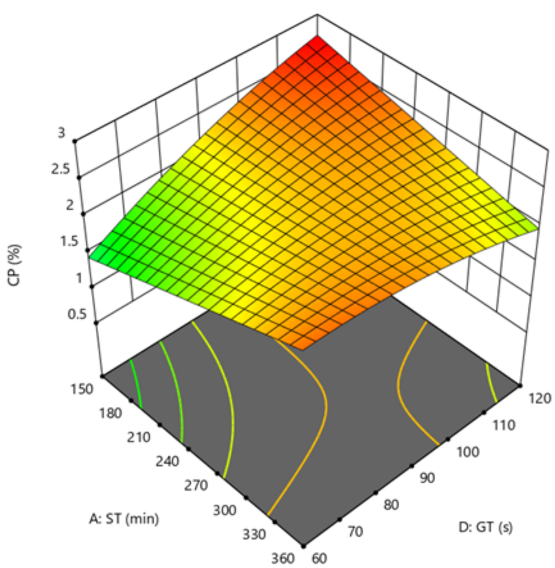

Figure 5. 3D surface Plot Showing the Relationship between CP(%) as affected by A and D

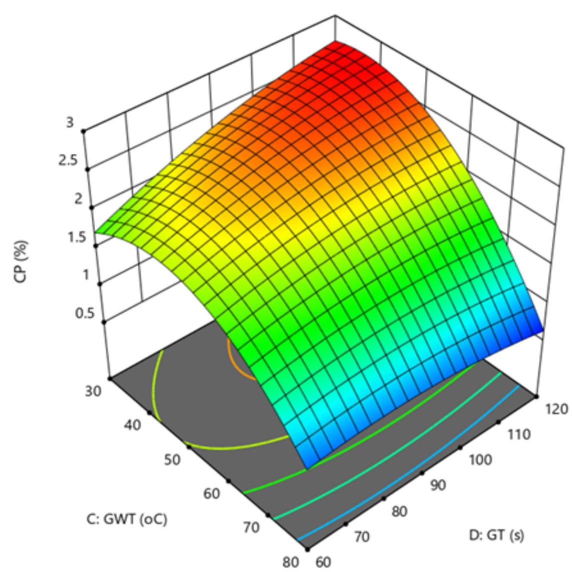

Figure 6. 3D surface Plot Showing the Relationship between CP(%) as affected by C and D

$$CP(\%) : -5.94012 + 0.013278A - 0.013167B + 0.127275C + 0.090646D - 0.00014AD - 0.000377CD - 0.001119C^2 - 0.000153D^2 \quad (1)$$

Where: *A = Soaking Time, B = Soaking Water Temperature, C = Grinding Water Temperature, D = Grinding Time*

Table 6. Optimisation Conditions, Prediction, and Desirability of Model

Solution Number	A	B	C	D	CP(%)	Desirability
1	150.51	30.15	36.66	120.88	2.75	0.99
2	187.09	35.03	38.78	101.69	2.65	0.96
3	200.40	45.36	55.95	91.16	2.46	0.94

Table 7. Experimental Values according to the Optimised A, B, C and D Processing Conditions

Independent trials	CP (%)
1	2.75 ± 0.03[a]
2	2.74 ± 0.05[a]
3	2.73 ± 0.08[a]

Results expressed as means ± standard deviations. All trials in this table were not significantly different ($p > 0.05$).

Table 8. Model Verification of Reduced BBD in Optimising the CP(%) value

Trial	Predicted Mean	Standard Deviation	95% PI low	Data Mean	95% PI high
CP(%)	2.53	0.05	2.42	2.49	2.64

Figure 6 has demonstrated that the water temperature soaking factor at low temperatures has resulted in the production of high-protein KSM. One of the mechanisms contributing to this phenomenon is the enhanced solubility of proteins into the KSM due to the utilisation of low temperatures [26]. Research on the profitability of producing soymilk [19], pistachio milk [20], and tigernut milk [21] has also examined how using low-temperature water affects the overall quality of the end product. Simultaneously, the utilisation of water at elevated temperatures over 60 °C has resulted in a reduction in the levels of protein solubility and hindered the manufacture of CP(%). The negative situation arises from various factors, including the chemical sensitivity of kenaf seeds to high temperatures due to the high albumin content in the protein fraction [5]. Additionally, the geletinization of starch occurs in the carbohydrate composition, leading to the presence of trapped water in the filtered seed debris [27].

The study employed numerical optimisation techniques to determine the optimal processing conditions for producing high-protein KSM's. These conditions included soaking time (A), soaking water temperature (B), grinding water temperature (C), and grinding time (D). The forecast of the optimum processing condition has been produced in Table 6, based on the sequence of mathematical analysis from Table 1 to equation 1. Due to its attractiveness value nearing 1, Solution No. 1 has been designated as a reference. The value has been considered due to its significance in determining the level of certainty regarding the processing circumstances in relation to the study's purpose. Subsequent research have utilised the provided optimal conditions to assess the validity of reserve solution no. 1. The findings of separate trials conducted three times, as indicated in Table 7, revealed that each CP(%) value obtained was about comparable to the value reported in Table 6. Furthermore, the values obtained from these separate experiments exhibited no substantial alteration and were deemed statistically insignificant. The verification model has been successfully implemented and is displayed in Table 8. The results shown indicate that the response data meets the specified requirement, since it falls within the 95% prediction interval.

CONCLUSION

The utilisation of BBD in this study has effectively accomplished the study's objective. Regarding this matter, the most favourable processing conditions at A: 150.51 min, B: 30.15°C, C: 36.66°C, and D: 120.88 s have resulted in the highest value of CP (%) at 2.75%. The further investigation should prioritise the identification of the optimal heating technique and composition for the production of enduring plant-derived milk.

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