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ORIGINAL ARTICLE

Optimization of Process Conditions for Developing Soft Tofu from Kenaf seeds (*Hibiscus cannabinus* L.) using Response Surface Methodology

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ABSTRACT- The growing demand for sustainable protein sources has turned attention to kenaf (Hibiscus cannabinus L.) seeds, which contain 20–25% high-quality protein but remain largely underutilized in food applications. Despite their nutritional potential, kenaf seeds have not been effectively incorporated into mainstream food products due to a lack of optimized processing techniques. Moreover, there is limited research on developing tofu- like products from non-soy legumes which creating a gap in plant-based protein diversification. The objective of this study is to optimize the formulation and processing conditions for producing soft tofu from kenaf seeds using Response Surface Methodology (RSM), thereby addressing critical gaps in plant-based protein diversification. A Box-Behnken Design (BBD) involving 17 experimental runs was employed to evaluate the combined effects of heating temperature (70-90°C), heating time (7-10 minutes) and egg yolk concentration (72-108 grams) on soft tofu textural properties, particularly hardness. The resulting quadratic model demonstrated excelled predictive accuracy (R^2=0.9979), identifying optimal processing conditions at 80°C, 8.5 minutes and 90 gram of egg yolk. Under these optimized conditions, the resulting tofu exhibited a hardness of 425 g, which closely matched the predicted value, indicating minimal deviation between experimental and modeled outcomes. To assess commercial and nutritional viability, the textural properties of the optimized kenaf-based tofu were compared to three commercial soft tofu brands, Brand A (367.70 g), Brand B (442.91 g), and Brand C (461.68 g) as well as soy milk soft tofu (448.26 g) prepared under the same optimized conditions. The kenaf soft tofu demonstrated comparable hardness, being firmer than Brand A, softer than Brand C, and closely aligned with both Brand B and soy milk soft tofu. These findings confirm the potential of kenaf as a viable soy alternative in tofu production. Moreover, the study highlights the effectiveness of RSM in optimizing novel plant-based protein products and underscores the significant influence of processing parameters on texture, offering a scientific foundation for industry application and promoting sustainable food innovation through the valorization of underutilized crops

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INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.) is an industrial crop notable for its rich nutritional profile, particularly in the MH8234 variety. Although kenaf seeds exhibit considerable potential due to their high protein content, their utilization in food applications remains limited. One particularly promising avenue is their

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application in the production of soft tofu, a product traditionally derived from soymilk. However, there is a clear lack of scientific investigation into the use of kenaf seed extract as a base for soft tofu production, representing a critical gap in plant-based protein research and diversification. Addressing this gap, the present study aims to explore the feasibility of producing soft tofu from kenaf seed extract while optimizing the formulation and processing conditions to achieve desirable textural properties, particularly in terms of hardness.

The texture of tofu is influenced by various parameters, and in the case of kenaf-based tofu, optimizing the processing conditions is essential for producing a product with acceptable quality. Key variables include heating time, heating temperature, and the concentration of egg yolk used as an emulsifying and coagulating agent. Prolonged heating durations and elevated temperatures promote protein coagulation; however, excessive heat may result in an undesirably firm tofu texture. Likewise, egg yolk concentration plays a pivotal role in determining tofu hardness, where lower concentrations tend to yield a softer texture and higher concentrations contribute to increased firmness.

To evaluate and fine-tune these interacting variables, this study employs Response Surface Methodology (RSM) as a tool to identify the optimal processing conditions for kenaf-based soft tofu while minimizing the number of experimental runs. Specifically, the Box-Behnken Design (BBD) is adopted to statistically ensure optimal combinations of variables that can yield soft tofu with the desired hardness. The application of RSM in food processing has been well-documented, including its use in tofu production by researchers such as Shih et al. [1] and Ye et al. [2]. Shih et al. [1] optimized processing conditions in soy-based soft tofu by adjusting coagulant concentration, mixing time, and temperature, demonstrating improved smoothness and softness. Similarly, Ye et al. [2] applied RSM in developing a novel kelp-based tofu, highlighting the role of ingredient ratios and coagulation parameters in achieving desirable texture and microstructure. These studies confirm RSM's effectiveness in enhancing the quality of tofu products.

Despite the success of RSM in improving soy- and kelp-based tofu, studies applying this methodology to kenaf-based tofu are still scarce. This underscores the novelty and scientific relevance of the current study, which seeks to establish scientifically validated processing conditions for kenaf seed tofu and evaluate its potential as a plant-based protein alternative to conventional tofu products. Through this approach, the study contributes to advancing sustainable food innovation and the valorization of underutilized crops.

MATERIALS AND METHODOLOGY

Raw Material Preparation

Kenaf seeds of the MH8234 variety were sourced from Zhanpu Zholong Kenaf Seed Co., Ltd., Fujian, China. Pre-treatment followed a modified protocol involving cleaning and soaking to enhance processing efficiency [3]. Eggs locally obtained from Singkwong Market, Sibu Sarawak. The egg yolk was separated from white egg and weight accordingly as in Table 2.

Production of Kenaf Extract

A total of 250 grams of cleaned kenaf seeds were soaked in excess of distilled water in beaker of 200 mL for 12 hours. The soaked seeds were drained and blanched at 80°C for 5 minutes. Following soaking, the mixture was filtered through a plastic strainer and subjected to hot grinding (Waring, USA) at 18,000 rpm with the addition of 1500 mL of hot water. The slurry was filtered using a double-layered muslin cloth and the kenaf extract was collected for further use.

Tofu Preparation from Kenaf Extract

The Box-Behnken Design (BBD) was employed to optimize the processing parameters, including heating time (7-10 min), heating temperature (70-90°C), and egg yolk (72-108 g). Each independent variable was tested at three coded levels: high (+1), medium (0), and low (-1), corresponding to the factorial ranges at Table 1.

Table 1. Independent Variables and Levels in the Box-Behnken Design

Factor	Name	Units	Low		Medium		High	
A	Heating time	Mins	7	(-1)	8.5	(0)	10	(+1)
B	Heating temperature	Degree Celsius	70	(-1)	80	(0)	90	(+1)
C	Egg yolk	Grams	72	(-1)	90	(0)	108	(+1)

Design Expert software (Version 13, State-Ease inc., Minneapolis, USA) generated a total of 16 experimental runs. Kenaf extract was homogenized with egg yolk based on Table 2 to formulate the kenaf soft tofu. The mixture was stirred gently to ensure uniformity, tightly packed and then heated at the designated temperature and duration. The heating time, heating temperature and amount of eggs yolk was optimized via Response Surface Methodology (RSM) with hardness as the key response variables.

Hardness

The hardness of the tofu was measured using a TA-HDi texture analyzer (Stable Microsystem Gloaming, UK) equipped with a 10 kg load cell. The protocol involved a double compression cycle with a 5-second interval using an SMSP/25 probe and a 25 mm diameter cylinder. The test speed was set at 1mm/s, the pre-test speed at 2mm/s and the pasties speed at 5mm/s. The distance used was 2.0cm and trigger force applied was 0.020g as employed by many researchers [5-8].

Experimental Design and Statistical Analysis

The process conditions for soft tofu production were optimized using the Box-Behnken Design (BBD) of response surface methodology (RSM) (Design-Expert, Version 13, Stat- Ease Inc., USA). Each processing parameters such as heating time, heating temperature and the amount of egg yolk were evaluated at three levels (low, median, and high) as shown in Table 1. The design required 17 experimental runs. Analysis of Variance (ANOVA) was conducted to evaluate the significance of the model, R-square values was used to assess model fit and lack of fit (LOF) was checked to ensure the model's reliability. A polynomial equation was generated to elucidate the relationship between the independent variables and the response variable (hardness). Optimization of the process was conducted using the built in-module within the software. The model's validity was assessed by comparing the experimental outcomes with the predicted values [9]. Statistical analysis was performed with a significance level of 0.05 providing a rigorous framework for evaluating the model's accuracy and ensuring it predictive reliability.

RESULTS AND DISCUSSION

The experimental runs including variation in heating temperature, heating time and the amount of egg yolk used during soft tofu preparation are summarized in Table 2.

Table 2. Hardness as a response of heating time, heating temperature and amount of egg yolk

Samples run	Factor 1	Factor 2	Factor 3	Response
	A: Temperature	B: Time	C: Amount of eggs yolk	Hardness
	Celsius	Mins	Grams	Grams
1	80	10	72	260.16
2	70	10	90	323.97
3	80	8.5	90	425.73
4	70	8.5	72	217.13
5	80	7	108	507.96
6	70	8.5	108	513.14
7	80	8.5	90	425.73
8	90	10	90	367.7
9	70	7	90	320.61
10	80	8.5	90	425.73
11	80	7	72	264.13
12	80	8.5	90	425.73
13	80	10	108	532.14
14	80	8.5	90	425.73
15	90	8.5	72	284.95
16	90	7	90	354.13
17	90	8.5	108	541.07

Model Fitting and Response Analysis

An optimal processing condition should enhance the hardness of the soft tofu. Table 2 presents the summary of model fitting results. Fitting of the data to various models (i.e. linear, two factorial interactions (2FI), quadratic and cubic) showed that the reactions between heating temperature, heating time and the amount of egg yolk used in optimizing the hardness of kenaf-based tofu was best described by a quadratic polynomial model. This model provided the most accurate representation of how these factors influenced the tofu's hardness offering a reliable approach for optimizing the process condition.

Table 3. Results Summary of fitting a model in the optimization of hardness in developing kenaf based soft tofu

Source	Sequential P-value	LOF P-value	R-Squared	Adjusted R-Squared	PRESS
Linear	<0.0001	0.0019	0.8988	0.8754	17.93
2FI	0.8154	0.0011	0.9075	0.8519	30.54
Quadratic	<0.0001	0.5265	0.9979	0.9952	1.65
Cubic*	0.5265	-	0.9987	0.9949	-

*Identified as aliased model

The lack of fit (LOF) test examines how well the data conforms to the chosen model. If the model does not align well with the data, the LOF test will return a significant result, suggesting that the model should be discarded. Upon evaluating the model sources, it was found that the LOF test for the quadratic model was not significant, with a P-value of 0.5265. This outcome implies that the quadratic polynomial model is acceptable and could be used to predict the new response. On the other hand, both linear and 2FI models displayed significant LOF results with P-values of 0.0019 and 0.0011, respectively. These small P-values suggest that the models are unsuitable and should be excluded from further consideration. The cubic model was aliased (to the quadratic model) due to an insufficient number of experiments to estimate all terms accurately and thus it was also disregarded in further analysis. The coefficient of determination, R-squared value for the quadratic model was satisfactory high at 0.9979 demonstrating the degree of fit of the model. It may be possible to improve both the R-squared and the adjusted R-squared by applying model reduction to eliminate non-significant terms from the model. Additionally, the quadratic model's predicted residual sum of squares (PRESS) value, which measures how well the model predicts each point in the design, was much lower at 1.65 compared to other models. A lower PRESS value signifies a better fit to the data points selection of the quadratic model for further in-depth study.

Model Reduction

Table 4 presents the result of the ANOVA for the reduce quadratic model. By comparing the initial model with the reduced version particularly in term of probability values, the interaction term AB (the 2FI between heating temperature and heating time) was found to be statistically insignificant. This was supported by the high probability>F value (0.4881) when AB was included in the model. The non-significant terms may be excluded from the model or held constant at a specified level to enhance model precision as documented in previous studies [10-12]. Consequently, the AB term was excluded to enhance the quadratic model. Further analysis through the lack of fit (LOF) test demonstrated a higher probability>F value for the reduced model (0.7536) compared to the initial model (0.5265), suggesting that the reduced quadratic model provides a better fit to the data. The statistical summary of the reduced model shown in Table 4 which includes several key properties. It is recommended that the coefficient of variance (CV) remains below the standardized threshold of 10% to ensure model reliability as indicated in [13,14]. The CV value of the reduced model (0.88112) was well below this threshold. In terms of the PRESS value, its decrease by 0.47 after the removal of AB indicated that excluding this term improved the model.

Table 4. ANOVA Calculated from the reduced Box-Behnken Design model in the optimization of hardness in developing kenaf based soft tofu

Source	Sum of squares	df	Mean square	F value	Prob>F
Model	108.24	7	15.46	521.57	<0.0001
A	2.70	1	2.70	91.16	<0.0001
B	0.3181	1	0.1381	4.66	0.0592
C	94.68	1	94.68	3193.66	<0.0001
AC	0.7478	1	0.7478	25.22	0.0007
BC	0.1787	1	0.1787	6.03	0.0364
A2	5.56	1	5.56	187.41	<0.0001
B2	3.70	1	3.70	124.82	<0.0001
Residual	0.2668	9	0.0296		
Lack of fit	0.1294	5	0.0259	0.7536	0.6252
Pure Error	0.1374	4	0.343		
Cor Total	108.51	16			

*A: Heating temperature, B: Heating time, C: Amount of egg yolk

The statistical summary of the reduced model comprised of several important properties as listed in Table 5. A model is considered as a good fit when the R-squared value at least 0.80 [15,16]. The R-squared for the reduced model suggested that 99.25% of the variation could be explained by the reduced model with its value decreasing if non-contributory terms are included. When AB was part of the model, the adjusted R-squared decreased from 0.99975 to 0.9954 indicating that AB did not significantly affect the response. Adequate precision which measures the signal-to-noise ratio, should exceed 4 to indicate a strong signal. The reduced model's adequate precision value of 71.52 exceeded this benchmark, confirming that the model is suitable for navigating the design space.

Table 5. Summary of statistical obtained from reduced Box-Behnken Design model in the optimization of hardness in developing kenaf based soft tofu

Parameters	Value
Standard deviation	0.1722
Mean	19.54
C.V.	0.8812
PRESS	1.18
R-Squared	0.9975
Adjusted R-Squared	0.9956
Predicted R-Squared	0.9891
Adequate Precision	71.5211

Diagnostic and Optimum Hardness in Developing Kenaf Seeds Soft Tofu

Figure 1 illustrates the relationship between heating time, heating temperature and the amount of egg yolk on the hardness of the kenaf based soft tofu displayed through a three-dimensional plot. The plot encapsulates a saddle surface, Figure 1 (b), with lowest hardness value observed at a heating temperature of 70 °C, heating time of 7 minutes and an amount of egg yolk is 72 grams, respectively.

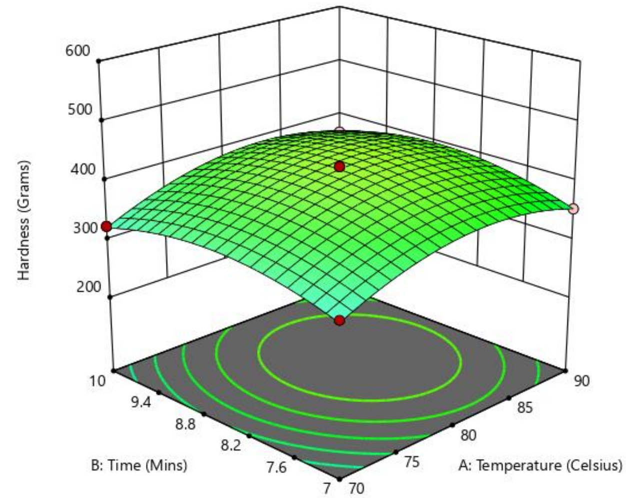

Figure 1 (a)

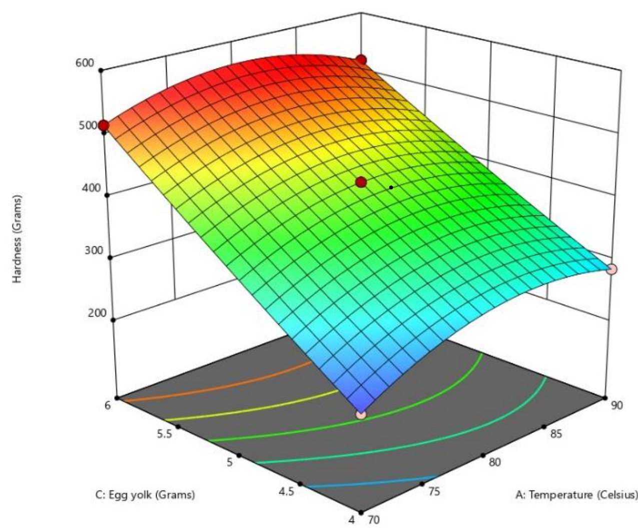

Figure 1 (b)

Figure 1. Three-Dimensional plot shows the relationship between hardness as affected heating temperature, heating time and amount of egg yolk

When seeking the optimal hardness conditions, it is crucial to consider the desired outcomes such as maintaining the kenaf soft tofu within an appropriate hardness range. Given this condition, the target hardness was established within a specific range, ensuring that the heating temperature remained below 90°C and that prolonged heating was avoided to prevent undue hardness. Based on these criteria, the RSM software generated multiple potential solutions for optimal hardness with some examples presented in Table 6.

Table 6. Some examples of optimized hardness conditions generated by the RSM software based on the reduced BBD model

Solution number	Heating temperature (°C)	Heating time (mins)	Amount of egg yolk (g)	Hardness (g)	Desirability
1	70	10	72	321.18	1.00
2	80	8.5	90	421.01	1.00
3	80	10	108	514.46	1.00

Solution number 2 was selected based on the outlined criteria and the minimal variations between the proposed solutions. The optimum conditions for achieving the desired hardness were identified as a heating temperature 80°C, heating time of 8.5 minutes and amount of egg yolk is 90 grams. Under these optimal conditions, the hardness was approximately 421.02 grams. It is worth noting that the desirability values for all solutions were satisfactory with values ranging from zero to one and should be evaluated only concerning the predefined upper and lower limits for the response and variables. In this case, the limits were set according to the study's specified ranges (in range).

Model Verification

Model verification was conducted using three independent trials under the optimized conditions mentioned above and compared against the predicted values from Box-Behnken Design (BBD) model. The results of the validation experiments are shown in Table 7. The predicted hardness was approximately 421.01 gram as indicated by the BBD model while the experimental result yielded values close to this prediction, confirming the adequacy of the validation parameters.

Table 7. Experimental values according to optimized hardness condition

Independent trials	Experimental values for hardness (g)*
Trial 1	425.02±0.075
Trial 2	425.01±0.10
Trial 3	425.02±0.71

* Values expressed as means ± SD using Texture Expert Software (Version 1.22, Stable Micro System Halsemere, Survey UK).

Hardness Variation of Kenaf Soft Tofu across Different Processing Condition

Figure 2 displays the kenaf soft tofu samples, arranged from least to greatest hardness. Visible differences in moisture content, color, and surface texture can be observed among the samples.

(a)

(a) Heating temperature at 70°C for 8.5 minutes with 72 grams of egg yolk

(b)

(b) Heating temperature at 80°C for 8.5 mins with 90 grams of egg yolk

(c)

(c) Heating temperature at 90°C for 8.5 minutes with 108 grams of egg yolk

Figure 2. Visual representation of kenaf soft tofu textural variations across different hardness levels, shown from top and side views

The sample exhibiting the lowest measured hardness of 217.13 grams was produced at processing settings of 70°C for 8.5 minutes using 72 grams of egg yolk. This situation resulted in a soft texture marked by low hardness and a slightly watery texture. The chosen intermediate hardness of 425.73 grams achieved under optimal processing settings of 80°C for 8.5 minutes with 90 grams of egg yolk produced a tofu texture that was firm yet not too hard or soft, exhibiting minimum pore formation. This texture roughly resembles the quality similarly to commercial soft tofu. The maximum hardness measured at 541.07 grams was achieved under processing settings of 90°C for 8.5 minutes using 108 grams of egg yolk. This led to kenaf soft tofu exhibiting an excessively hard texture marked by considerable pore development due to being overcooked. The results demonstrate that alteration in processing conditions directly affect the textural attributes of kenaf soft tofu emphasizing the necessity of optimizing parameters to attain the required product qualities.

Comparative Hardness Analysis with Commercial Brands

The selected kenaf soft tofu sample exhibits a hardness value that is within the range of these commercial products, falling particularly close to Brand B at 442.91 grams. This proximity to the commercial standard validates the selected hardness (425.73g) as both comparable and acceptable.

Table 8. The hardness of the optimum kenaf soft tofu (425.73) was compared against three commercial soft tofu brans (Brand A, B and C).

Type of samples	Hardness (g)
Kenaf soft tofu	425.73
Commercial brand A	367.70
Commercial brand B	442.91
Commercial brand C	461.68

Comparative Analysis of Hardness of Optimize Kenaf Soft Tofu with Soymilk Tofu

Using the optimized conditions, another soft tofu was produced with soymilk as the raw ingredient for comparison purposes. Figure 3 illustrates the visual attributes of soft tofu derived from soymilk, showing both top and side views. The soymilk soft tofu exhibited a hardness value of 448.26 grams, as shown in Table 8. The hardness of the optimized kenaf soft tofu sample is lower than that of the soymilk tofu, which could be attributed to differences in the protein and fat contents of the extract sources [17, 18]. The similarity in hardness between the optimized kenaf soft tofu and the soymilk soft tofu highlights the efficacy of the optimization method. This closeness to the control enhances the acceptance and comparability of kenaf soft tofu as a substitute for soymilk-based soft tofu.

Figure 3. Visual representation of soft tofu from soymilk

Table 9. The hardness of the optimum kenaf soft tofu (425.73) was compared with soymilk soft tofu

Type of samples	Hardness (g)
Kenaf soft tofu	425.73
Soy milk soft tofu	448.26

CONCLUSION

In this study, Response Surface Methodology (RSM) was successfully employed to optimize key processing parameters for the production of soft tofu from kenaf seed extract. The optimal conditions identified—namely a heating temperature of 80 °C, a heating duration of 8.5 minutes, and the incorporation of 90 grams of egg yolk—resulted in a predicted tofu hardness that closely matched the experimental outcome, with a minimal deviation of approximately 0.94%. Notably, the resulting hardness also falls within the typical range observed in commercial tofu products, thereby validating the predictive accuracy and practical applicability of the RSM model in aligning product quality with industry benchmarks. This strong correlation between predicted, experimental, and commercial values underscores the robustness of the model in forecasting and delivering desirable textural attributes. Looking ahead, these findings provide a foundation for the continued development of functional plant-based protein products using underutilized crops such as kenaf. Future research could investigate the nutritional profile and consumer acceptance of kenaf-based soft tofu, as well as explore opportunities for fortification or formulation enhancement. Additionally, scaling up the optimized process for industrial application and evaluating its economic viability would further support the integration of kenaf into sustainable and commercially viable food systems.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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