

The Influence of Sodium Carbonate, Tween 80 and Soy Lecithin Concentrations on Pasteurised Kenaf Seed MH8234 Emulsion Physical Stability Under Ambient Temperature Storage_A Study Using Box-Behnken Design Approach

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Abstract - The Box-Behnken design was utilised to determine the optimum conditions for formulated pasteurised kenaf seed emulsion (PKSE) physical stability towards the ambient temperature. The Box-Behnken design including independent variables concentration such as sodium carbonate (0.1, 0.3 and 0.5), Tween 80 (0.2, 0.4 and 0.6) and soy lecithin (0.1, 0.2 and 0.3) was used. The responses (dependent variables) that evaluate the physical stability were sedimentation height, creaming height and emulsion stability index. The second-order model obtained for responses coefficient determination was between 0.995 and 0.998. Sedimentation height and creaming height were primarily affected by the sodium carbonate concentration. The optimum goal set was to lower sedimentation height, lower creaming height, and higher emulsion stability index. These goals were achieved at sodium carbonate 0.278, tween 80 0.500 and soy lecithin 0.260. These conditions produced PKSE at the sedimentation height of 8.41 mm, creaming height of 11.43 mm and emulsion stability index of 80.18%. The model adequacy was confirmed by producing formulated pasteurised ks emulsion under optimum values given by the model. These results help produce formulated PKSE to be stored at ambient temperature with stable conditions.

Index Terms - Kenaf seeds MH8234, pasteurisation, Box-Behnken design, ambient temperature, physical stability

INTRODUCTION

Kenaf is also known as *Hibiscus Cannabinus* l. This plant is commonly planted using seeds. Kenaf seed (KS) is triangular with acute angles, with a small dimension (6 mm long x 4 mm wide) and dark brown. Mariod et al., 2010 have conducted a study related to kenaf seed (ks) V36 and QP3 variety and found that both samples contain high crude fat amounts. Cheong et al., 2018 have reported that kenaf seed has poor water solubility with a high amount of monounsaturated fatty acids. Despite these hurdles, research related to ks has prevailed that this underutilised and so-called agricultural waste has potential for future food and pharmaceutical products [1]–[5].

The author's literature search revealed that most KS emulsion was developed with KS oil. Moreover, the KS oil extraction process is either harmful or expensive, as it involves the utilisation of hexane as a chemical extraction solvent and supercritical fluid extraction equipment. Ibrahim et al., 2020 has produced ks KB6 tofu and applied heating treatment of 90°C for 5 min towards ks KB6 extract before adding coagulant. It was observed that the ks KB6 extract remained stable without any separation. However, the author believed that ks extract under different varieties react differently towards applied heating treatment. This statement is due to several comments from authors [2]–[4], stating that KS is low in emulsifying properties. Hypothetically, the low emulsifying properties are due to the abundance of crude fat content over crude protein content.

Pasteurisation implements a high-temperature short time (HTST) or low-temperature low time heating treatment (LTST) technique that functions to eliminate microbial growth [7]–[9]. As emulsion is thermodynamically unstable, the pasteurised emulsion will tend to separate. The separation may be triggered by the creaming and sedimentation phenomenon. These processes are linked with coalescence and flocculation conditions [10], [11]. Without proper formulation, the KS extract undergoing pasteurisation is expected to separate. Thus, applying emulsifiers with amphiphilic properties and stabiliser is necessary for stabilising PKSE.

According to [12], the formulated surfactant emulsified emulsion, which undergone heat treatment, required thorough care. Heat treatment with above 62°C may cause the emulsified emulsion to destabilise due to phase inversion temperature. However, this condition is subjected to applying "Tween 80" alone. McClements, 2011 also suggested that the surfactant emulsified emulsion which undergone heat treatment (> 62°C) required immediate chilling storage at (4°C) to restore the function of Tween 80. Studies have demonstrated a consistent link between the application of tween 80 and soy lecithin, which can produce stable canola

and soy emulsion [13], [14]. In the meantime, the combination of two emulsifiers in emulsion formulation has been suggested and proven to prolong and improve the physical stability and emulsion quality [10], [14].

Response surface methodology optimised technique can be described as mathematical modelling that evaluates the interaction between independent and dependent variables with the selected criteria and searches the significance between both variables (Gunst et al.; Mirhosseini et al., 2008). The response surface methodology technique has become the incumbent method for practical explanations to reduce the number of experimental trials needed to evaluate multiple parameters and their interactions. This research paper aims to identify the optimum concentration of surfactants (Tween 80 and soy lecithin) and stabiliser (sodium carbonate) towards the optimum physical stability of PKSE under ambient temperature ($27^{\circ}\text{C} \pm 2^{\circ}\text{C}$) for seven days.

MATERIALS AND METHODS

I. Source of Materials

Kenaf seeds Ming Hong MH8234 were bought from Zhanpu Zhonglong Kenaf Seeds Co., Ltd, Fujian China and transported to University College of Technology Sarawak. The material was further stored at chilled temperature ($4 \pm 2^{\circ}\text{C}$). Sodium carbonate, Tween 80 and soy lecithin were purchased from a local supplier. All the ingredients are either analytical or food grade.

II. Experimental Design

Response surface methodology was selected to identify the optimum concentration (surfactants and stabiliser) in formulated pasteurised kse towards its physical stability parameters (creaming height, sedimentation height and emulsion stability index). Design Expert 11 (Stat-Ease Inc., USA) was employed to perform statistical analysis and experimental design. Moreover, Box – Behnken design with three levels and three factors was selected to evaluate the three independent variables' mixed effects: the concentration of Tween 80, soy lecithin, and sodium carbonate. Each of the independent variables was examined at three levels: lowest (-1), median (0) and highest (1). The whole independent variables tested conditions are displayed at Table 1. Contrary, the dependent variables are sedimentation height, creaming height and emulsion stability index. There were 17 with three replicates in the center region and a non – linear regression method was used to fit the second-order polynomial (Eq. (1)) to the experimental data to resolve the significant model terms.

$$Y = \beta_0 + \sum_{j=1}^k \beta_j x_j + \sum_{j=1}^k \beta_{jj} x_j^2 + \sum_i \sum_{<j=2}^k \beta_{ij} x_i x_j + e_i \quad (\text{Eq. 1})$$

Where Y is the response; x_i and x_j (i and j range from 1 to k). β_0 is the model coefficient; β_j , $\beta_{(jj)}$, β_{ij} are interaction coefficients of linear, quadratic and second-order terms, respectively; k is the number of independent parameters (k = 3 in this study); e_i is the error. The analysis of variance (ANOVA) was performed and the experimental data was assessed using several descriptive statistical analyses such as standard deviation, mean, determination coefficient (R^2), predicted coefficient (R^2), percentage of coefficient variation (C.V%), lack of fit and p-value. These data reflected the statistical significance of the developed quadratic mathematical model. Upon fitting the data to the models, the generated data were applied to plot response surfaces.

III. Preparation of PKSE

25 g of KS were soaked in 75 ml of filtered water at ambient temperature for 6 hours. The soaked KS were further filtered using a plastic strainer and ground with 150 ml filtered water in a laboratory blender (Waring, USA) at 18,000 rpm. The ground slurry was then filtered using cheesecloth, and the remaining was discarded. The yielded KS extract was then mixed with the ingredients mentioned in Table 1. and homogenised using a digital homogeniser (OMNITECH, United Kingdom) under 15,000 rpm for 180 s with 10 mm probe. The homogenised mixture was transferred to the pasteuriser (ARMPFIELD PT 1275, United Kingdom). The pasteuriser pumping speed was set at 8.5 ml/s, and the heating condition was fixed at 72°C for 15 s and directly chilled at 4°C . The pasteurised output was kept in a 250 ml measuring cylinders and stored at ambient temperature $27 \pm 2^{\circ}\text{C}$.

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

Factors	Low	Medium	High
Sodium carbonate % (w/v)	0.1 (-1)	0.3 (0)	0.5 (1)
Tween 80 % (w/v)	0.2 (-1)	0.4 (0)	0.6 (1)
Soy lecithin % (w/v)	0.1 (-1)	0.2 (0)	0.3 (1)

IV. Measurement of sedimentation height, creaming height and emulsion stability index

Table 3. Anova table showing the variables as linear, quadratic and interaction terms on each response for kse

	Sedimentation (mm)		Creaming (mm)		Emulsion Stability Index (%)	
	Coefficient	p value	Coefficient	p value	Coefficient	p value
Constant	8.51		13.48		78.01	
A	3.19	< 0.001	-12.74	< 0.0001	9.55	< 0.0001
B	0.13	0.042	-0.07	0.951 (ns)	-0.06	0.961 (ns)
C	-0.05	0.374 (ns)	-0.99	0.404 (ns)	1.04	0.374 (ns)
AB	0.29	0.006	-0.50	0.761 (ns)	0.22	0.893 (ns)
AC	-0.07	0.384 (ns)	4.13	0.035 (ns)	-4.06	0.035
BC	0.09	0.255 (ns)	-1.46	0.388 (ns)	1.37	0.408 (ns)
A ²	0.92	< 0.001	11.32	0.000	-12.23	< 0.0001 (ns)
B ²	-0.08	0.351 (ns)	-1.14	0.485 (ns)	1.21	0.451 (ns)
C ²	0.15	0.069 (ns)	0.49	0.757 (ns)	-0.65	0.681 (ns)
Model Fitting						
R ²	0.998		0.964		0.955	
Adjusted R ²	0.996		0.919		0.898	
Predicted R ²	0.981		0.754		0.692	
CV%	1.620		17.14		4.29	
Adequacy Precision	62.361		13.873		12.769	
p value		< 0.001		0.0003		0.0006
Lack of Fit		0.198 (ns)		0.553 (ns)		0.5628 (ns)

A: Sodium Carbonate %(w/v), B: Tween 80 %(w/v), C: Soy Lecithin %(w/v)
(ns) = Not Significant (p value > 0.05)

The sedimentation and creaming measurements were measured using a calibrated Vernier scale (Hanaki, Japan) with a 0.01 mm range. The creaming was examined by measuring the opaque emulsion layer (cream) height, while sedimentation was determined by the sediment's height on the container's bottom. The emulsion stability index was calculated according to [19].

RESULT & DISCUSSION

Table 2. Experimental and Predicted data based on the design in Table 1.

No. of Runs	Independent Variables			Dependent Variables					
	A	B	C	Sedimentation height (mm)		Creaming height (mm)		Emulsion Stability Index (%)	
				(E _o)	(E _i)	(E _o)	(E _i)	(E _o)	(E _i)
1.	0.3	0.4	0.2	8.52	8.51	12.82	13.48	78.66	78.01
2.	0.3	0.2	0.3	8.47	8.33	11.39	13.37	80.14	78.30
3.	0.5	0.6	0.2	13.01	12.96	9.63	10.3	77.36	76.70
4.	0.3	0.4	0.2	8.64	8.51	8.45	13.48	82.91	78.01
5.	0.5	0.2	0.2	12.01	12.14	11.26	11.49	76.73	76.37
6.	0.3	0.4	0.2	8.39	8.51	16.24	13.48	75.37	78.01
7.	0.3	0.6	0.1	8.54	8.68	17.20	15.22	74.26	76.10
8.	0.3	0.2	0.1	8.64	8.61	13.94	12.44	77.42	78.95
9.	0.1	0.6	0.2	6.14	6.01	37.05	36.82	56.81	57.17
10.	0.3	0.4	0.2	8.41	8.51	13.16	13.48	78.43	78.01
11.	0.1	0.4	0.1	6.39	6.37	40.94	43.15	52.67	50.47
12.	0.1	0.4	0.3	6.32	6.41	34.17	32.90	59.51	60.69
13.	0.5	0.4	0.1	12.98	12.89	8.15	9.42	78.87	77.69
14.	0.5	0.4	0.3	12.64	12.65	17.90	15.69	69.46	71.66
15.	0.3	0.6	0.3	8.73	8.76	8.81	10.31	82.46	80.93
16.	0.1	0.2	0.2	6.28	6.33	36.67	35.96	57.05	57.71
17.	0.3	0.4	0.2	8.61	8.51	16.71	13.48	74.68	78.01

A: Sodium Carbonate %(w/v), B: Tween 80 %(w/v), C: Soy Lecithin %(w/v)
(E_o): Experimental Value, (E_i): Predicted Value

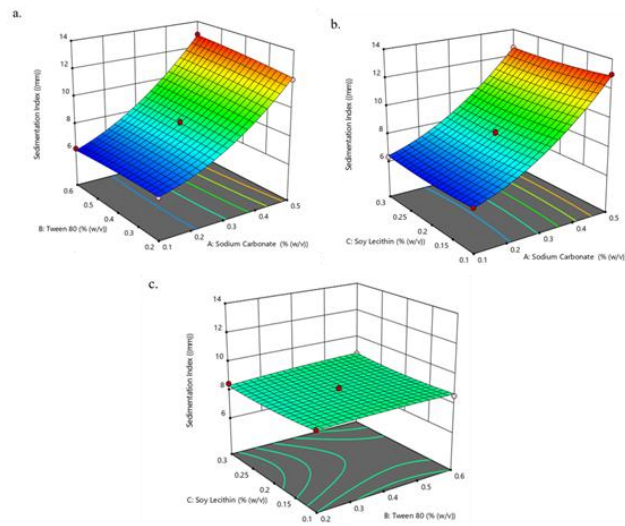


FIGURE 1. RESPONSE SURFACE PLOTS (3D) SHOWING THE INFLUENCE OF SODIUM CARBONATE, TWEEN 80 AND SOY LECITHIN CONCENTRATION ON PKSE SEDIMENTATION'S HEIGHT (A – C)

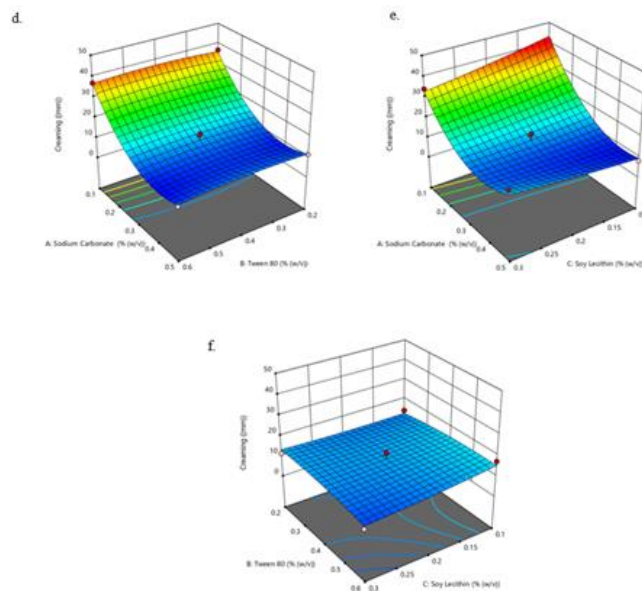


FIGURE 2. RESPONSE SURFACE PLOTS (3D) SHOWING THE INFLUENCE OF SODIUM CARBONATE, TWEEN 80 AND SOY LECITHIN CONCENTRATION ON PKSE CREAMING'S HEIGHT (D – F)

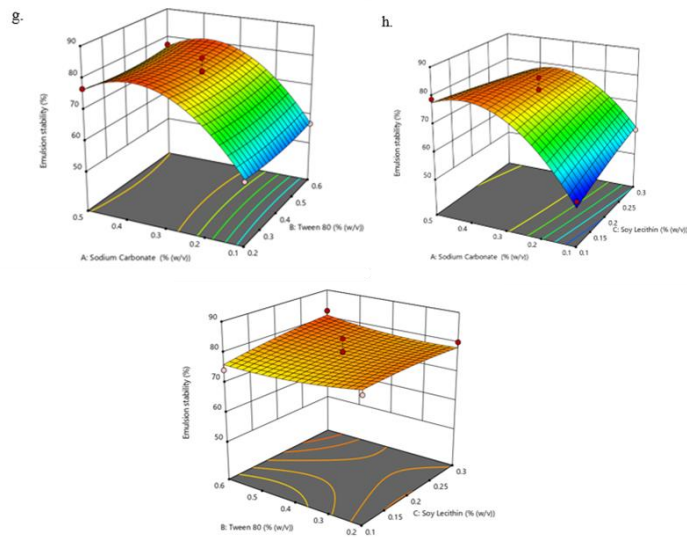


FIGURE 3. RESPONSE SURFACE PLOTS (3D) SHOWING THE INFLUENCE OF SODIUM CARBONATE, TWEEN 80 AND SOY LECITHIN CONCENTRATION ON PKSE EMULSION STABILITY INDEX (G – I)

Table 4. Final Regression Models Significant Response Variables

Response Variables	Final Regression Model
Sedimentation height	$6.97256 + 0.01750A - 0.98500B - 7.34500C + 7.12500AB - 3.37500AC + 4.50000BC + 22.91875A^2 - 1.76875B^2 + 15.71500C^2$
Creaming height	$62.65181 - 269.6925A + 40.7975B - 62.63000C - 12.56250AB + 206.50000AC - 73.00000BC + 282.89375A^2 - 28.48125B^2 + 49.82500C^2$
Emulsion stability index	$30.37562 + 269.67500A - 39.81250B + 69.97500C + 5.43750AB - 203.12500AC + 68.50000BC - 305.81250A^2 + 30.25000B^2 - 65.00000C^2$

Table 5. Experimental values according to the optimised PKSE processing condition

Independent trials	Sedimentation (mm)	Creaming (mm)	Emulsion Stability Index (%)
1	8.41 ± 0.03	11.43 ± 0.06	80.18 ± 0.04
2	8.40 ± 0.05	11.41 ± 0.03	80.17 ± 0.09
3	8.42 ± 0.04	11.42 ± 0.07	80.15 ± 0.05

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

I. Model Fitting

In this research, multiple regression analyses were performed using response surface methodology, and there were three established models resulting from Equation 1 to experimental data displayed in Table 1. The model adequacy and fitness were assessed under the analysis of variance (ANOVA) in Table 3. The statistical configuration shows that all the responses (sedimentation height, creaming height and emulsion stability index) were suggested to form under the quadratic model. The model adequacy was monitored with ten attributes displayed in Table 3: model significance, standard deviation, mean, R², adjusted R², predicted R², C.V%, adequate precision, lack of fit and p – value. Table 3 presents that all responses are significant ($p < 0.05$). The attained correlation coefficient value (R²) is between 0.995 and 0.998. Thus, these amounts are considered excellent for the model.

The model adequacy evaluation compares the R² and adjusted R² value [20]. Table 3 shows that the adjusted R² value is between 0.898 and 0.996. These amounts show no significance, revealing no difference in each model that the model might not have applied a factor to the irrelevant term. The C.V% expresses minimum mean value variation, and the low C.V% indicates the system with good reproducibility [20], [21]. The C.V% values for the emulsion stability index are lower than 10%, while the sedimentation is higher than 10%. Each response's adequate precision value is higher than what explains the mean model produces a passable signal, and the collected data were desirable. The response variations have accurately predicted the models. Moreover, the lack of fit value for all responses are not significant ($p > 0.05$).

II. Sedimentation Height

Figure 1. presents the response surface plots on formulated kenaf seed extract sedimentation with the effect of varied concentration of sodium carbonate, tween 80 and soy lecithin. Based on this experiment, this response is monitored after the sample being left for three days at ambient temperature ($27\text{oC} \pm 2$). By referring to Table 3, shows that the factors which significantly affect sedimentation height ($p < 0.05$) are: linear factor of concentration of sodium carbonate (A), the concentration of tween 80 (B); the interaction between the concentration of sodium carbonate and tween 80 (AB) and the quadratic factor of sodium carbonate (A²). Figure 1. recorded that the highest sedimentation value is at 13.01 mm, while the lowest sedimentation height at 6.28 mm. Sodium carbonate with a higher concentration produces greater sedimentation height than the higher concentration of other independent variables (soy lecithin and tween 80).

This study selected sodium carbonate as part of the emulsion stabilising agent. The added sodium carbonate concentration is between 0.1% and 0.5%, which is coherent with the Malaysian Food Act 1985 regulation. Stabilising agents modify the emulsion's pH and escalate the repulsive force, thus emulsifying properties. This statement agrees with (Hosseini-Parvar et al., 2016; Liang et al., 2016), stating that the emulsion under alkalise condition produces smaller initial droplet sizes and stable against coalescence. Figure 1 shows that the increased concentration of sodium carbonate leads to higher sedimentation. In comparison to others variables (tween 80 and soy lecithin) which had less effect on sedimentation height. Generally, sedimentation happens when molecules with a higher density than the surrounding liquid phase move downwards due to gravitational separation. Furthermore, the occurred sedimentation contributed by the high amount of sodium carbonate is due to ion binding and electron shielding [10], [22]–[24].

III. Creaming Height

Creaming is defined as a process of droplets moving upwards due to its density lower than the surrounding emulsion phase [10], [25], [26]. The creaming process is part of the gravitational separation process, and its motion speeds depend on the droplet's size, density and aqueous phase viscosity. This research determines creaming height by measuring the formed (creaming) height. A calibrated vernier caliper is used to achieve precise reading. By referring to Figure 2d. shows that both graphs pattern interaction between A & B (sodium carbonate and tween 80) and B & C (sodium carbonate and soy lecithin) are in declined form. However, the interaction graph between B & C (Tween 80 and soy lecithin) is displayed in a flat pattern. This can be foreseen that the addition of sodium carbonate at low concentration has stimulated the creaming phenomena. The prediction is supported by Table 3, showing that sodium carbonate (A) linear and quadratic factors (A) are significant.

In this experiment, the PKSE were left at ambient temperature for seven days. The observation shows that there is no coagulation formed on the first day. However, the creaming started to form on the second day till the last day of experiments. Figure (d-f) shows that a low level of sodium carbonate contributing a greater amount of creaming height. The PKSE solubility is much affected by its pH. The low sodium carbonate concentration has caused the emulsion to be less alkalise and resulted in low

electrostatic repulsion. This condition caused the pasteurised emulsion droplets to aggregate and flocculate and form creaming. Compared to the mixed surfactants (Tween 80 and soy lecithin), these components had less effect on creaming. This condition is due to the effective synergistic effect between both components towards emulsifying the emulsion (Dickinson, 2010; McClements, 2016).

IV. *Emulsion Stability Index*

Emulsion stability index is signified as the capability of an emulsion to remain stable considering gravitational separation to occur. The gravitational separation occurred due to the creaming and sedimentation process mentioned previously. Emulsion with high emulsion stability index is reflected towards stable emulsion. Conversely, low emulsion stability is linked with insoluble emulsion. Principally, a high value of emulsion stability index is achieved through proper formulation and suitable storage conditions. Hence, this has decelerated the effect of gravitational separation derived from thermodynamic and kinetic stability [10]. By referring to Figure 3(g and h), the surface plots show that the interaction of A&B (sodium carbonate and tween 80) and A&C (sodium carbonate and soy lecithin) are in declined form, as compare to the interaction of B&C (tween 80 and soy lecithin) which show a flat pattern. Figure 3(g) displays that the lowest emulsion stability index is at 57.05% and achieved through the lowest concentration of sodium carbonate 0.1% and tween 80 0.2%. This can be predicted that both factors at low concentration efficiently maintain the emulsion stability index. On the contrary, the highest emulsion stability index is at 78.43% is attained through 0.3% sodium carbonate and 0.4% tween 80. The addition of sodium carbonate at a higher concentration has fully alkalisied the whole emulsion. The pH modification is needed to amplify the electrostatic interaction and reduce the creaming process that adheres to Stoke's law.

Both figures (g & h) presented a similar pattern. The decreased concentration of sodium carbonate with other (tween 80 or soy lecithin) yielded the lowest stability index. Figure h. demonstrated that maximum emulsion stability is achieved at 0.3% sodium carbonate and 0.2% soy lecithin. The minimum emulsion stability results from 0.1% sodium carbonate and 0.1% soy lecithin. Thus, it can be justified that the lower amount of both components (sodium carbonate and soy lecithin) cannot fully stabilize the emulsion, thus causing the emulsion to be prone to emulsion instability. Figure i. display a flat pattern of the surface plot. The topmost value of the emulsion stability index is at 82.46% at 0.3% of soy lecithin and 0.6% of Tween 80, while the bottommost result, 77.42% is achieved at 0.1% of soy lecithin and 0.2% of Tween 80. As no declination occurred, this revealed that the addition of Tween 80 and soy lecithin has efficaciously stabilised the emulsion. In this study, the applied emulsifiers (Tween 80 and soy lecithin) shared similar properties - amphiphilic. These emulsifiers tend to attract and bind the components of oil and water. The application of two emulsifiers in emulsion formulation has directly improved its emulsion stability index as both factors complement each other [10], [30].

V. *Optimisation of PKSE Formulation*

This study employs numerical optimization to establish a formulation that adheres to function desirability. Table 6. shows the characteristic of optimum formulation conditions. This research aims to produce a PKSE formulation with a high emulsion stability index and less amount on the factors (creaming and sedimentation) that affected the emulsion's physical stability. This objective can be achieved by minimising both independent variables: creaming and sedimentation and maximising the emulsion stability index. By referring to the optimiser, these goals can be achieved by applying the formulation condition suggested by the optimiser to be at (A: 0.278, B:0.500 and C:0.260) with selected desirability function at 0.819. Furthermore, the optimiser's proposed condition is followed, and the experimented and theoretical data was further compared and projected by crossed design. In the meantime, both set values were evaluated using the t-test method for validity justification. The generated data between experimented and theoretical deduced non-significant ($p > 0.05$). Table 5. signified that the experimental value for sedimentation height, creaming height, and emulsion stability index was 11.43, 8.41 and 80.18%. The experimented data is considered satisfactory as the gathered data is non-significant as compared with the theoretical value. Thus, it can be regarded as that the suggested formulation has met the goal set and can be utilised for further application.

CONCLUSION

This work was devoted to identifying the optimum concentration of stabiliser and emulsifiers: sodium carbonate, Tween 80 and soy lecithin with the Box – Behnken design application on PKSE physical stability under ambient temperature. The optimum concentration obtained was sodium carbonate 0.278, Tween 80 0.500 and soy lecithin 0.260, which yielded an emulsion at desired criteria. The emulsion produced under optimum concentration had a sedimentation height of 8.41 mm, creaming height of 11.43 mm and emulsion stability index of 80.18%. These experimental values were in agreement with theoretical value, thus indicating the adequacy of the fitted model and response surface methodology found to be a valuable tool. Further studies shall be focusing on the impact of different storage conditions towards optimised formulated pasteurised ks emulsion physicochemical properties.

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REFERENCES

- [1] A. A. Mariod, S. F. Fathy, and M. Ismail, "Preparation and characterisation of protein concentrates from defatted kenaf seed," *Food Chem.*, vol. 123, no. 3, pp. 747–752, 2010.
- [2] A. M. Cheong, C. P. Tan, and K. L. Nyam, "Emulsifying conditions and processing parameters optimisation of kenaf seed oil-in-water nanoemulsions stabilised by ternary emulsifier mixtures," *Food Sci. Technol. Int.*, no. 1, 2018.
- [3] A. M. Cheong, K. W. Tan, C. P. Tan, and K. L. Nyam, "Kenaf (*Hibiscus cannabinus* L.) seed oil-in-water Pickering nanoemulsions stabilised by mixture of sodium caseinate, Tween 20 and β -cyclodextrin," *Food Hydrocoll.*, vol. 52, pp. 934–941, 2016.
- [4] A. M. Cheong, K. W. Tan, C. P. Tan, and K. L. Nyam, "Improvement of physical stability properties of kenaf (*Hibiscus cannabinus* L.) seed oil-in-water nanoemulsions," *Ind. Crops Prod.*, vol. 80, pp. 77–85, 2016.
- [5] M. H. A. Basri, A. Arifin, J. Nasima, A. H. Hazandy, and A. Khalil, "Journey of kenaf in Malaysia: A Review," *Sci. Res. Essays*, vol. 9, no. 11, pp. 458–470, 2014.
- [6] I. G. Shafa'atu, N. A. Mat Noh, W. Z. Wan Ibadullah, N. Saari, and R. Karim, "Water soaking temperature of kenaf (*Hibiscus cannabinus* L.) seed, coagulant types, and their concentrations affected the production of kenaf-based tofu," *J. Food Process. Preserv.*, no. April, pp. 1–13, 2020.
- [7] S. Jeske, J. Bez, E. K. Arendt, and E. Zannini, "Formation, stability, and sensory characteristics of a lentil-based milk substitute as affected by homogenisation and pasteurisation," *Eur. Food Res. Technol.*, vol. 245, no. 7, pp. 1519–1531, 2019.
- [8] M. Igual, C. Contreras, M. M. Camacho, and N. Martínez-Navarrete, "Effect of Thermal Treatment and Storage Conditions on the Physical and Sensory Properties of Grapefruit Juice," *Food Bioprocess Technol.*, vol. 7, no. 1, pp. 191–203, 2014.
- [9] D. M. Horsfall, C. A. Simeon, and P. Michael, "Stabilised cocosoy beverage: physicochemical and sensory properties," *J. Sci. Food Agric.*, pp. 1839–1846, 2006.
- [10] D. J. McClements, *Food Emulsions, Principles, Practices, And Techniques*. 2016.
- [11] Z. Ahmadian-Kouchaksaraei, M. Varidi, M. Javad Varidi, and H. Pourazarang, "Study of stability characteristics of sesame milk: Effect of pasteurization temperature, additives, and homogenisation pressure," *Qual. Assur. Saf. Crop. Foods*, vol. 7, no. 5, pp. 677–686, 2015.
- [12] D. J. McClements, "Edible nanoemulsions: Fabrication, properties, and functional performance," *Soft Matter*, vol. 7, no. 6, pp. 2297–2316, 2011.
- [13] L. C. B. Züge, C. W. I. Haminiuk, G. M. Maciel, J. L. M. Silveira, and A. D. P. Scheer, "Catastrophic inversion and rheological behavior in soy lecithin and Tween 80 based food emulsions," *J. Food Eng.*, vol. 116, no. 1, pp. 72–77, 2013.
- [14] T. Mehmood and A. Ahmed, "Tween 80 and Soya-Lecithin-Based Food-Grade Nanoemulsions for the Effective Delivery of Vitamin D," *Langmuir*, vol. 36, no. 11, pp. 2886–2892, 2020.
- [15] R. H. Myers and D. C. Montgomery, "Response Surface Methodology: Process and Product Optimization Using Designed Experiments," *Technometrics*, vol. 38, no. 3, p. 285, 1995.
- [16] H. Mirhosseini, C. P. Tan, A. Aghlari, N. S. A. Hamid, S. Yusof, and B. H. Chern, "Influence of pectin and CMC on physical stability, turbidity loss rate, cloudiness and flavor release of orange beverage emulsion during storage," *Carbohydr. Polym.*, vol. 73, no. 1, pp. 83–91, 2008.
- [17] J. P. Maran and S. Manikandan, "Dyes and Pigments Response surface modeling and optimization of process parameters for aqueous extraction of pigments from prickly pear (*Opuntia ficus-indica*) fruit," *Dye. Pigment.*, vol. 95, no. 3, pp. 465–472, 2012.
- [18] G. Fan, Y. Han, Z. Gu, and D. Chen, "Optimizing conditions for anthocyanins extraction from purple sweet potato using response surface methodology (RSM)," *LWT - Food Sci. Technol.*, vol. 41, no. 1, pp. 155–160, 2008.
- [19] M. N. Nasrabadi, S. Amir, and H. Goli, "Stability assessment of conjugated linoleic acid (CLA) oil-in-water beverage emulsion formulated with acacia and xanthan gums," *FOOD Chem.*, vol. 199, pp. 258–264, 2016.
- [20] O. Gul, I. Atalar, F. T. Saricaoglu, and F. Yazici, "Effect of multi-pass high pressure homogenization on physicochemical properties of hazelnut milk from hazelnut cake: An investigation by response surface methodology," *J. Food Process. Preserv.*, vol. 42, no. 5, pp. 1–11, 2018.
- [21] Ž. Šumić, A. Vakula, A. Tepić, J. Čakarević, J. Vitas, and B. Pavlić, "Modeling and optimization of red currants vacuum drying process by response surface methodology (RSM)," *Food Chem.*, vol. 203, pp. 465–475, 2016.

- [22] E. Keowmaneechai and D. J. McClements, "Influence of EDTA and citrate on physicochemical properties of whey protein-stabilized oil-in-water emulsions containing CaCL₂," *J. Agric. Food Chem.*, vol. 50, no. 24, pp. 7145–7153, 2002.
- [23] A. Schwenzfeier, A. Helbig, P. A. Wierenga, and H. Gruppen, "Emulsion properties of algae soluble protein isolate from *Tetraselmis* sp.," *Food Hydrocoll.*, vol. 30, no. 1, pp. 258–263, 2013.
- [24] J. L. Li, D. W. Sun, and J. H. Cheng, "Recent Advances in Nondestructive Analytical Techniques for Determining the Total Soluble Solids in Fruits: A Review," *Compr. Rev. Food Sci. Food Saf.*, vol. 15, no. 5, pp. 897–911, 2016.
- [25] Q. Liu, H. Huang, H. Chen, J. Lin, and Q. Wang, "Food-grade nanoemulsions: Preparation, stability and application in encapsulation of bioactive compounds," *Molecules*, vol. 24, no. 23, pp. 1–37, 2019.
- [26] N. H. C. Marzuki, R. A. Wahab, and M. A. Hamid, "An overview of nanoemulsion: Concepts of development and cosmeceutical applications," *Biotechnol. Biotechnol. Equip.*, vol. 33, no. 1, pp. 779–797, 2019.
- [27] A. M. Cheong, J. X. Jessica Koh, N. O. Patrick, C. P. Tan, and K. L. Nyam, "Hypocholesterolemic Effects of Kenaf Seed Oil, Macroemulsion, and Nanoemulsion in High-Cholesterol Diet Induced Rats," *J. Food Sci.*, vol. 83, no. 3, pp. 854–863, 2018.
- [28] S. C. Chew, C. P. Tan, and K. L. Nyam, "In-vitro digestion of refined kenaf seed oil microencapsulated in β -cyclodextrin/gum arabic/sodium caseinate by spray drying," *J. Food Eng.*, vol. 225, pp. 34–41, 2018.
- [29] E. Dickinson, "Flocculation of protein-stabilized oil-in-water emulsions," *Colloids Surfaces B Biointerfaces*, vol. 81, no. 1, pp. 130–140, 2010.
- [30] T. Mehmood, A. Ahmed, Z. Ahmed, and M. S. Ahmad, "Optimization of soya lecithin and Tween 80 based novel vitamin D nanoemulsions prepared by ultrasonication using response surface methodology," *Food Chem.*, vol. 289, no. March, pp. 664–670, 2019.

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