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ORIGINAL ARTICLE

Optimisation of Total Phenolic Content and Antioxidant Activity of Spray-Dried Ginger-*Kelulut* Powder by D-optimal Mixture Design

*[1,2]Hwong Chia Shing, [1]Chieng Ann Wen Sing, [1,2]Koh Chen Chung and [1,2]Hii Siew Ling

[1]Food Technology Programme, School of Engineering and Technology, University of Technology Sarawak, 96000 Sibu, Sarawak, Malaysia
[2]Centre of Research for Innovation and Sustainable Development (CRISD), University of Technology Sarawak, 96000 Sibu, Sarawak, Malaysia

ABSTRACT - Ginger (*Zingiber officinale*) and *kelulut* honey, which is also known as stingless bee honey, are two natural ingredients that are rich in nutritional values and medicinal properties. The unique flavour of *kelulut* honey mixed with ginger can provide a refreshing taste to consumers when it is taken in the form of a beverage. The objectives of the present work were to optimise the formulation of spray-dried ginger-*kelulut* honey powder and evaluate the effects of the components and their interactions on the total phenolic content (TPC) and antioxidant activity of the formulations. Twenty (20) formulations of ginger-*kelulut* honey mixed with brown sugar and maltodextrin were generated by the D-optimal mixture design of Design-Expert Version 12.0. The findings showed that the TPC of spray-dried ginger-*kelulut* honey powder ranged from 0.96 to 1.44 mg GAE/g powder, and the antioxidant activities were between 51.06% and 75.02%. The TPC was influenced by a higher amount of ginger and a lower amount of brown sugar with *kelulut* honey, whereas an increase in the component of *kelulut* honey and a decrease in the component of ginger and brown sugar resulted in greater antioxidant activity in the spray-dried powder. Maltodextrin was kept at low constraint. Hence, *kelulut* honey was the most influential factor on the antioxidant properties of the spray-dried powder. The optimised formulation of spray-dried powder consisted of 49.81% ginger, 15.68% *kelulut honey*, 8.38% brown sugar, and 26.13% maltodextrin. The experimental values obtained were close to predicted values and thus indicated the adequacy and validity of the model.

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INTRODUCTION

Reactive oxygen species are the by-products generated from cellular redox processes. The accumulation of these free radicals in the body will lead to oxidative stress that can cause damage to the cell structures. Nevertheless, antioxidants have free radical scavenging properties as they can donate electrons to neutralise the free radicals and thus delay or inhibit cellular damage [1]. Phenolic compounds are natural antioxidants found in spices, fruits, and vegetables [2]. It is reported that increased intake of dietary antioxidants may help to reduce oxidative stress in the human body, improve the normal functionalities of physiological systems, and decrease the risk of chronic diseases [3].

Spices such as ginger (*Zingiber officinale Roscoe*) are widely used as traditional remedies to treat various ailments such as colds, nausea, arthritis, migraines, and dyspepsia [4]. Previous studies have shown that ginger essential oils such as gingerol and shogaol possess numerous therapeutic properties,

*Corresponding Author: Hwong Chia Shing. University of Technology Sarawak (UTS),
email: cshwong14@uts.edu.my

including antimicrobial, anti-inflammatory, and antioxidant effects [5]. *Kelulut* honey is a precious bee product of the stingless bee from the genus *Trigona* [6]. The colour of *kelulut* honey is clearer and less viscous than that of common honeybee, and it has a sweet and sour taste with a fermented aroma [7]. *Kelulut* honey contains bioactive compounds such as phenolic acids, flavonoids, and carotenoids that demonstrate excellent antioxidant properties [8].

Fresh ginger undergoes deterioration and loss of nutrients during storage. In the meanwhile, the high viscosity and spontaneous crystallisation of honey have restricted its use in food formulations. Despite the refreshing taste provided by the unique flavour of *kelulut* honey infused with ginger, the traditional method of making a home remedy of ginger honey drink is time-consuming, and people today who live hectic lifestyles are hesitant to produce it themselves. Therefore, ginger and honey in powder form are preferable, and spray drying is considered a good drying approach. According to Phisut (2012) [9], spray drying is a technique used in the food industry to convert a liquid feedstock into dry powder and agglomerates. This drying method can extend the shelf life of products by reducing the water activity and inhibiting microbial growth. It is also convenient for transportation and storage. Spray drying carriers such as modified starch, maltodextrin, and gum Arabic are commonly used as they can increase the glass transition temperature of raw materials and reduce stickiness during spray drying.

The mixture design method can be used to optimise the mixture formulation in order to obtain a product with the desired characteristics. As compared with other designs, the D-optimal mixture design is useful when there are constraints on factor settings in formulation or limited resources for the experiment as it has a smaller number of runs, thus saving time and cost of experimentation. D-optimality also produces a design that best estimates the effects of the factors, making it particularly suited for screening studies [10]. Based on the current literature search, there are very limited studies on the nutritional analysis of spray-dried ginger-*kelulut* honey powder. Therefore, this study aimed to optimise the formulation of spray-dried ginger-*kelulut* honey powder by D-optimal mixture design with a desirable amount of total phenolic content and antioxidant activity as well as to evaluate the effects of components and their interactions on the antioxidant properties of the powder.

MATERIALS AND METHODOLOGY

Materials

Gallic acid monohydrate and DPPH were obtained from Sigma-Aldrich. Folin-Ciocalteu phenol reagent was sourced from Rmstain. Other chemicals used were of analytical grade unless specified otherwise. Ginger (*Zingiber officinale*) was purchased from a local market in Sibu, Sarawak. *Kelulut* honey from the *Heterotrigona itama* species was bought from Need Lee Enterprise, Sibu, Sarawak. Maltodextrin having a dextrose equivalent of 10-15 was used in this study. Brown sugar was purchased from a local bakery shop.

Preparation of Ginger-*Kelulut* Honey Solution for Spray Drying

The ginger was washed thoroughly under tap water and then cut into slices with its skin intact and blended. The ginger pomace and juice were separated by a cloth filter to obtain the juice, and the pomace was discarded. The ginger juice was heated and added with maltodextrin, followed by brown sugar during the cooking process. The mixture was stirred continuously until all of the powdered ingredients were fully dissolved before being allowed to cool to room temperature. Next, *kelulut* honey was added and the solution was thoroughly mixed by continuous stirring for a minute.

After that, the ginger-*kelulut* honey solution was spray-dried using a laboratory scale spray dryer (SD-basic spray dryer, Lab Plant, UK). The operating parameters were inlet temperature 180°C and feed rate 0.21 mL/s [11]. The spray-dried ginger-*kelulut* honey powder was collected in a glass bottle and stored at room temperature until further analysis.

Preliminary Study

Preliminary study was conducted to determine the lowest and highest limits for the amount of each ingredient in order to produce the powder of ginger-*kelulut* honey by spray drying.

Experimental Design

The D-optimal mixture design of Design-Expert version 12.0 software (STAT-EASE Inc., Minneapolis, USA) was employed to evaluate the concentration effects of the blended ginger (A), *kelulut* honey (B), brown sugar (C), and maltodextrin (D) on the response variables of total phenolic content and antioxidant activity of the spray-dried powder formulation. There were lower and upper constraints of components due to the restrictions on the component proportions. Nevertheless, the blend of different proportions of the components was made up to 100% for each formulation. The lower and upper limits of the components were listed in Table 1. According to the design of the software, a total of 20 formulations with five for estimation of lack of fit and five replicates was generated and prepared for spray drying.

Table 1. Constraints of Components

Components	Low (%)	High (%)
Ginger (A)	38.84	58.19
Kelulut honey (B)	14.56	15.68
Brown sugar (C)	0	14.56
Maltodextrin (D)	26.13	32.04

Determination of Total Phenolic Content by Folin-Ciocalteu Assay

The Total Phenolic Content (TPC) of the spray-dried powder was determined using Folin-Ciocalteu assay as described by Kek et al. [12] with slight modifications. One (1) gram of sample was mixed with 20 mL of distilled water and 1 mL of the solution was mixed with 5 mL of 0.2 N Folin-Ciocalteu reagent, followed by 4 mL of 75% w/v sodium carbonate solution. The mixture was then incubated in the dark for 2 hours at room temperature. Next, the absorbance of the sample was measured at 765 nm using a UV-Vis spectrophotometer (Cary 60, Agilent Technologies, USA). A standard calibration curve of gallic acid was constructed within a concentration range of 20 to 100 ppm. The TPC of sample was calculated based on the standard curve and expressed as gallic acid equivalent (mg GAE/g of powder).

Determination of Antioxidant Activities by DPPH Free Radical Scavenging Assay

The antioxidant activity of the spray-dried powder was determined using 2,2-diphenylpicrylhydrazyl (DPPH) assay as described by Suhag and Nanda [13] with minor modifications. Two hundred and fifty (250) milligrams of sample were mixed with 10 mL of 60% acetone and centrifuged at 5000 rpm for 15 minutes. Two (2) millilitres of extract samples were mixed with 2 mL of methanolic solution containing DPPH with a concentration of 6×10^{-5} mol/L. The mixture was homogenised by vortex mixer and then incubated in the dark for 30 minutes. After that, the absorbance of the mixture was measured at 517 nm using a UV-Vis spectrophotometer. The radical scavenging activity was calculated as percentage (%) of inhibition of DPPH by using the following equation:

$$\text{Percentage of inhibition of DPPH} = \frac{Abs_1 - Abs_2}{Abs_1} \times 100\%$$

Where: Abs_1 = *Absorbance of control*, Abs_2 = *Absorbance of sample*

Statistical Analysis

Design Expert Version 12.0 was used for formulations design and statistical analysis of the experimental data. Difference at p value < 0.05 was considered statistically significant. Besides that, the design was expressed by the polynomial regression equation as shown as follows:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{44}X_4^2 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{14}X_1X_4 + \beta_{23}X_2X_3 + \beta_{24}X_2X_4 + \beta_{34}X_3X_4$$

where Y is the predicted response and β_0, β_i, β_{ii} are the linear coefficient, quadratic coefficient, and interaction coefficient, respectively. The actual values of components were converted to L-pseudo-levels before model equations were calculated as pseudo-scaling helps in constructing the design and proper fitting the model. A suitable polynomial equation for the design was chosen according to the fittest model, such as linear, quadratic, special cubic or transformed model.

For a better understanding towards the interaction of the independent variables on the response variables, contour plot and three-dimensional (3D) surface plots of the fit polynomial regression were generated. The plot is in five graduated colour shades: red for the highest value, yellow to orange for medium and high values, green for medium values, light blue for intermediate values between low and medium, and blue for the lowest values [14].

Verification of Model

A series of optimum formulations were generated by the Design Expert software. The formulation with the highest desirability was selected and used for verification. In order to determine the adequacy and validity of the model, the predicted values of TPC and antioxidant activity of the spray-dried powder were compared with the actual experimental values using the following equation:

$$\text{Percentage error (\%)} = \frac{Pred.\ value - Exp.\ value}{Pred.\ value} \times 100\%$$

RESULTS AND DISCUSSION

Screening of the Range of Components

The four components, which were ginger, *kelulut* honey, brown sugar, and maltodextrin, made up the composition of the spray-dried powder. Initially, ginger-*kelulut* honey powder was tasteless after spray drying, and thus brown sugar was added to increase the sweetness. Brown sugar was selected as it contains slightly more minerals than refined white sugar. Due to the properties of high solubility and low viscosity of honey, maltodextrin was used to overcome the problem of stickiness during spray drying, as honey has a low glass transition temperature [15]. Preliminary experiments were carried out to determine the constraints of each ingredient. As shown in Table 1, the recovery of spray-dried ginger-*kelulut* honey powder fell into the accepted ranges of ginger (38.84-58.19%), *kelulut* honey (14.56-15.68%), brown sugar (0-14.56%) and maltodextrin (26.12-32.04%).

Total Phenolic Content and Antioxidant Activity of Spray-Dried Ginger-*Kelulut* Honey Powder

Table 2 shows the responses of TPC and antioxidant activity of 20 formulations. The average TPC was 1.10 ± 0.03 mg GAE/g powder and varied within 0.96 to 1.44 mg GAE/g powder. On the other hand, the mean of antioxidant activity was 61.28 ± 3.68% and varied within 51.06% to 75.02%. TPC of spray-dried ginger-*kelulut* honey powder was higher than TPC of *kelulut* honey of *Heterotrigona itama* (477.30 ± 133.59 mg GAE/kg) as reported by Wong et al. [16] and this might be attributed to the contribution of phenolic content of ginger. Suhag and Nanda [13] reported that TPC and antioxidant activity of spray-dried honey-

Indian gooseberry-basil powder ranged between 0.61 and 0.63 mg GAE/g, and 78.44% to 82.73%, respectively.

Table 2. Ingredients Composition and Responses

Run	Ginger (%)	*Kelulut* Honey (%)	Brown Sugar (%)	Maltodextrin (%)	TPC (mg GAE/g powder)	Antioxidant activity (%)
1	44.65	14.56	11.55	29.23	1.11	61.75
2	52.20	14.56	3.67	29.58	1.26	57.52
3	48.03	15.68	7.83	28.46	1.25	74.54
4	52.28	15.68	0.00	32.04	1.15	57.69
5	56.72	14.95	0.00	28.32	1.25	58.10
6	48.27	14.89	4.90	31.94	1.18	69.12
7	40.78	14.56	14.56	30.10	1.14	63.54
8	38.84	15.68	13.44	32.04	0.96	63.57
9	52.81	14.56	6.50	26.13	1.25	52.32
10	44.54	15.18	14.15	26.13	1.31	71.14
11	50.26	14.56	9.05	26.13	1.27	59.11
12	38.84	15.68	13.44	32.04	0.96	59.06
13	53.40	14.56	0.00	32.04	1.16	56.99
14	58.19	15.68	0.00	26.13	1.23	56.41
15	52.28	15.68	0.00	32.04	1.22	59.74
16	53.40	14.56	0.00	32.04	1.16	51.06
17	47.09	15.68	11.10	26.13	1.39	75.02
18	56.72	14.95	0.00	28.32	1.26	54.76
19	58.19	15.68	0.00	26.13	1.44	61.65
20	43.56	15.11	9.30	32.04	1.20	62.46
Mean					1.10	61.28

Effect of Components on Total Phenolic Content of Spray-Dried Ginger-*Kelulut* Honey Powder

The Analysis of Variance (ANOVA) of the D-optimal mixture design for TPC is shown in Table 3. The mixture model suggested by the Design Expert software was a reduced quadratic model. Transformation was performed to improve the model. The model was significant ($p < 0.05$), and the lack of fit was not significant ($p > 0.05$). This indicated the model was reliable. The R-squared of 0.7307 was acceptable, and this implied that 73.07% of the variation of TPC could be described by mixture models. After square root transformation, the predicted and adjusted R-squared were maximized. As a result, the predicted R-squared of 0.4608 was in reasonable agreement with the adjusted R-squared of 0.6589, where the difference is less than 0.2. Adequate precision measures the signal-to-noise ratio, and a ratio greater than 4 is desirable. In this study, the adequate precision of 9.333 showed an adequate signal, and this model can be used to navigate the design space. The coefficient of variation of 2.86% was small, and this demonstrated the high reliability of the experiments.

Furthermore, only significant independent variables were involved in the model, and non-significant independent variables were eliminated by backward elimination. Hence, the ANOVA results indicated that the linear model terms of ginger (A), *kelulut* honey (B), brown sugar (C), maltodextrin (D), and the combination of AC had a significant effect on the TPC of spray-dried powder. From Table 3, linear and quadratic coefficients were obtained, and an equation as follows was generated that could be used to predict the TPC of spray-dried powder:

$$\text{L\_Pseudo equation: Sqrt (TPC)} = 1.14A + 1.40B + 1.02C + 0.92D + 0.27AC$$

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The positive value of the coefficient indicates the favourable effect, and the negative value of the coefficient indicates the inverse effect between factor and response. From the equation, all the individual ingredients and combination of AC had positive effect on the response of TPC, and with a value of 1.40, *kelulut* honey (B) had the highest influence on the response.

Table 3. Analysis of Variance (ANOVA) for Total Phenolic Content

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value (Prob > F)
Model	0.040	4	0.010	10.18	0.0003
Linear Mixture	0.036	3	0.012	12.05	0.0003
AC	4.502E-003	1	4.502E-003	4.56	0.0497
Residual	0.015	15	9.881E-004		
Lack of Fit	0.011	12	8.906E-004	0.65	0.7470
Pure Error	4.136E-003	3	1.379E-003		
Cor Total	0.055	19			
R^2	0.7307				
Adjusted R^2	0.6589				
Predicted R^2	0.4608				
Adequate Precision	9.333				

CV = 2.86%

$p < 0.05$ indicates significant effect

Figure 1 displays the interaction between ginger (A), *kelulut* honey (B), and brown sugar (C) on the response of TPC, while maltodextrin (D) was kept constant at a proportion of 26.13 %. Maltodextrin was reserved at its low constraint. The purpose was to minimise the addition of a drying agent so that the product was as close to natural ingredients as possible. As illustrated in Figure 1, a higher component of ginger and a lower component of brown sugar with *kelulut* honey resulted in a higher TPC value. Based on the 3-D surface plots, the TPC was positively associated with the amount of ginger and inversely associated with the amount of sugar. According to Stoner [17], gingerols, shogaols, and paradols are the phenolic compounds of ginger that account for the various bioactivities of ginger. On the other hand, in the Da Silva et al. study [18], 14 different phenolic compounds were identified in the methanolic extract of stingless honey. The presence of polyphenolic compounds is commonly found in the nectar of flowers, pollen, and propolis.

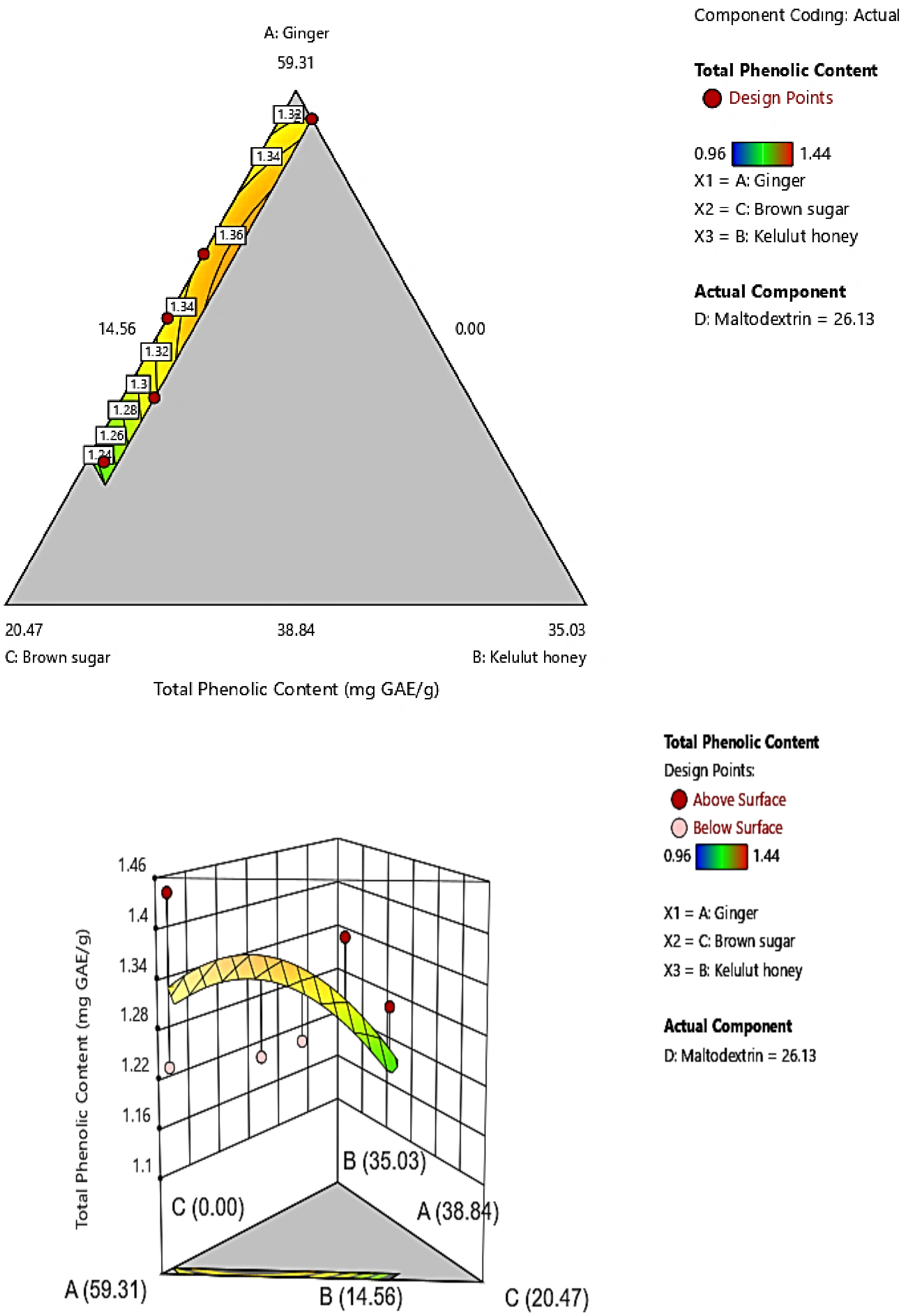


Figure 1. Contour plot and 3-D surface plot for the interaction between three variables: ginger (A), *kelulut* honey (B) and brown sugar (C) on Total Phenolic Content (TPC)

Effect of Components on Antioxidant Activity of Spray-Dried Ginger-*Kelulut* Honey Powder

Table 4 shows the ANOVA results of the D-optimal mixture design for antioxidant activity. The mixture model recommended by the Design Expert software was a reduced quadratic model. The model was significant ($p < 0.05$), and the lack of fit was not significant ($p > 0.05$). This indicated that the model was reliable. The R-squared of 0.7942 was acceptable, and this implied that 79.42% of the variation in antioxidant activity could be described by mixture models. The predicted R-squared of 0.5213 was in reasonable agreement with the adjusted R-squared of 0.6992, where the difference is less than 0.2. The adequate precision of 10.673, which is greater than 4, indicated an adequate signal, and this model can be used to navigate the design space. A coefficient of variation of 6.00%, which is less than 10%, indicated high reliability of the experiments.

The model only involved significant independent variables, as non-significant independent variables were eliminated by backward elimination. Therefore, the ANOVA results showed that the linear model terms of ginger (A), *kelulut* honey (B), brown sugar (C), maltodextrin (D), and a blend of AB, AC, as well as BC had a significant effect on the antioxidant activity of spray-dried powder. The linear and quadratic coefficients were obtained from Table 4, and an equation as follows was generated that could be used to predict the antioxidant activity of spray-dried powder:

$$\text{L\_Pseudo equation: Antioxidant Activity} = 42.26A - 605.77B + 51.72C + 89.38D + 1056.16\,AB + 50.93AC + 991.08BC$$

From the equation, *kelulut* honey (B) with negative coefficient demonstrated an adverse effect on the response but this was overcome by the synergistic effect of the blending of ginger (A) and *kelulut* honey (B), and also the blending of *Kelulut* honey (B) and brown sugar (C).

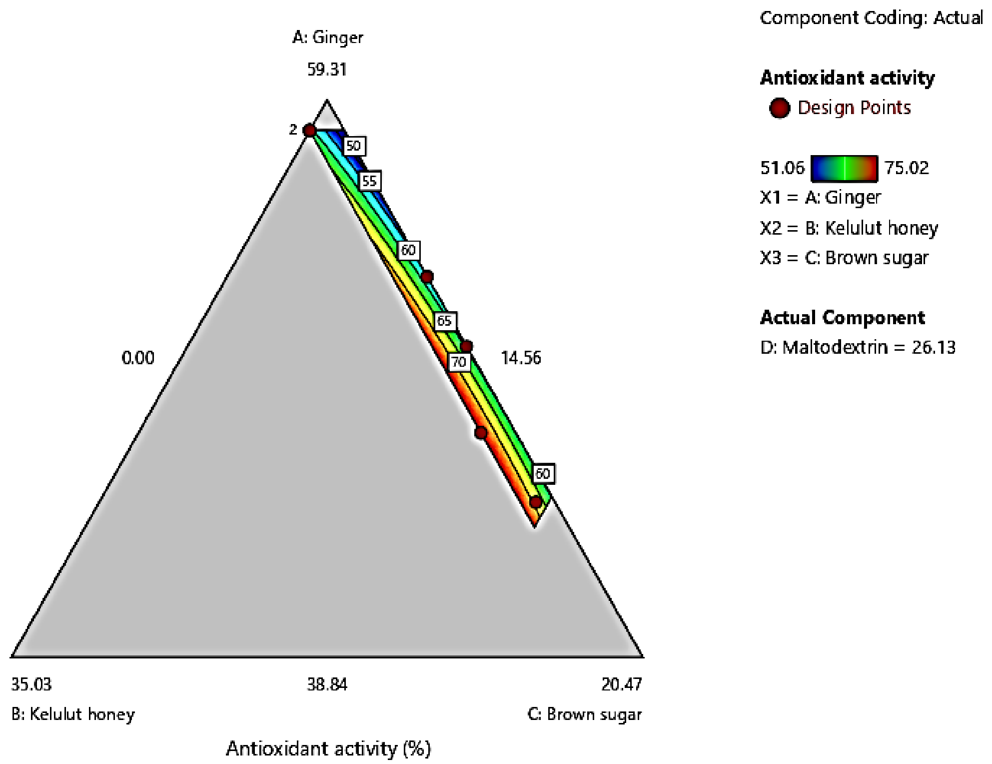
Table 4. Analysis of Variance (ANOVA) for Antioxidant Activity

Source	Sum of Squares	Degree of Freedom	Mean Square	F Value	p-value (Prob > F)
Model	678.19	6	113.03	8.36	0.0007
Linear Mixture	395.90	3	131.97	9.76	0.0012
AB	167.80	1	167.80	12.41	0.0037
AC	158.14	1	158.14	11.70	0.0046
BC	141.31	1	141.31	10.45	0.0065
Residual	175.72	13	13.52		
Lack of Fit	134.24	10	13.42	0.97	0.5796
Pure Error	41.48	3	13.83		
Cor Total	853.91	19			
R^2	0.7942				
Adjusted R^2	0.6992				
Predicted R^2	0.5213				
Adequate Precision	10.673				

CV= 6.00%

$p<0.05$ indicates significant effect.

Figure 2 shows the interaction between ginger (A), *kelulut* honey (B), and brown sugar (C) on the response of antioxidant activity, while maltodextrin (D) was kept constant at a proportion of 26.13 %. As presented in Figure 2, a higher amount of *kelulut* honey with a lower amount of ginger and brown sugar produced higher antioxidant activity in the spray-dried powder. This could be attributed to the polyphenolic compounds in *kelulut* honey. Maringgal et al. [19] stated that polyphenolic compounds could result in a greater antioxidant potential in *kelulut* honey, and the phenolic compounds play a crucial role in the antioxidant activity of honey. Therefore, in this study, *kelulut* honey was the most influential factor on the TPC and antioxidant activity of the spray-dried powder formulation.



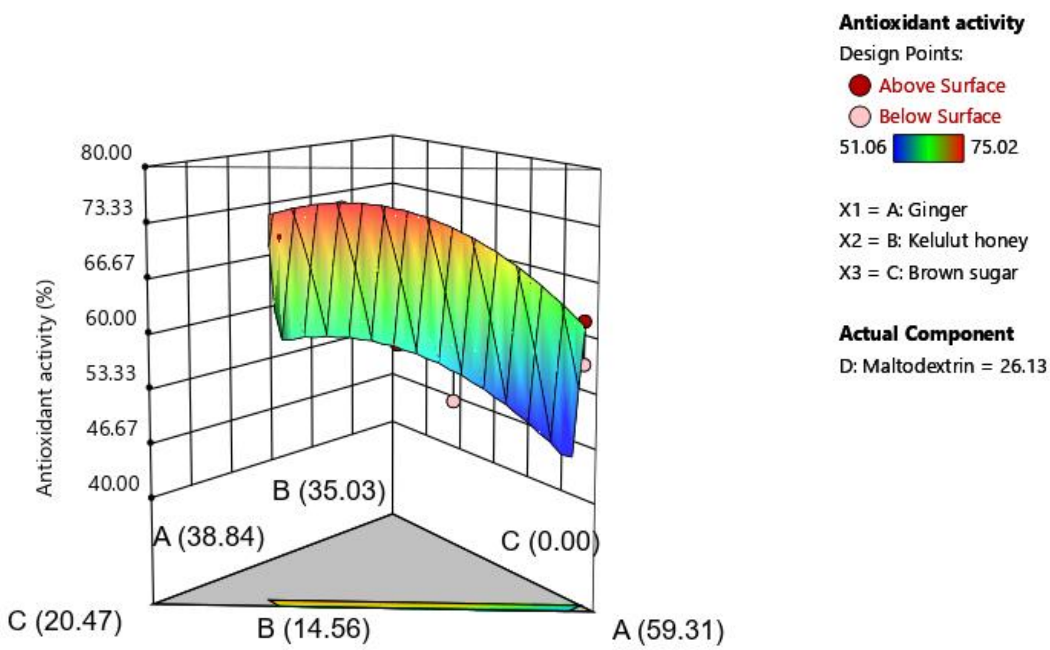


Figure 2. Contour plot and 3-D surface plot for the interaction between three variables: ginger (A), *kelulut* honey (B) and brown sugar (C) on antioxidant activity

Optimisation and Verification of Model

The optimised formulation of the spray-dried ginger-*kelulut* honey powder with the formulation of 49.81% ginger (A), 15.68% *kelulut* honey (B), 8.38% brown sugar (C), and 26.13% maltodextrin (D) was obtained from a series of solutions generated by Design-Expert software (Table 5). With the highest desirability of 0.905, the optimised formulation was selected for the verification process.

Table 5 also tabulates the predicted values, experimental values, the percentage error (PE) of the TPC, and the antioxidant activity of the powder. The experimental value of TPC and antioxidant activity of the optimised formulation were 1.31 mg GAE/g and 73.20%, respectively, and these were comparable with the predicted values of 1.35 mg GAE/g and 75.02% as the percentage error was less than 5%. The result confirmed that the model was satisfactory and accurate for the optimisation of TPC and antioxidant activity of spray-dried ginger-*kelulut* honey powder.

Table 5. Predicted Values, Experimental Values and Percentage Error (%) of Total Phenolic Content and Antioxidant Activity of Optimum Formulation

Optimised formulation				TPC predicted value (mg GAE/g)	Actual value (mg GAE/g)	PE (%)	Antioxidant activity predicted value (%)	Actual value (%)	PE (%)
A (%)	B (%)	C (%)	D (%)						
49.81	15.68	8.38	26.13	1.35	1.31	2.96	75.02	73.20	2.43

Desirability= 0.905

CONCLUSION

The market for functional foods is expanding due to increased health consciousness among consumers nowadays. As ginger and *kelulut* honey are high in nutritional values, they can be used to produce value-added products. The present study using a D-optimal mixture design found that the TPC and antioxidant activity of spray dried ginger-*kelulut* honey powder were within the range of 0.96–1.44 mg GAE/g powder and 51.06%–75.02%, respectively. TPC and antioxidant activity were affected by the different compositions of the formulations, and *kelulut* honey contributed the most to the antioxidant property of the spray-dried powder. This study also revealed that spray-dried ginger-*kelulut* honey powder with optimal TPC and antioxidant activity could be prepared with 49.81% ginger, 15.68% *kelulut* honey, 8.38% brown sugar, and 26.13% maltodextrin.

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