

Statistical optimisation for the formulation of edible bird nest-based instant soup using Response Surface Methodology (RSM)

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Abstract

Scientifically, edible bird nest (EBN) contains protein, carbohydrate, fat, and bioactive compounds that can boost the human immune system, strengthen bones, and improve skin complexion. The inclusion of EBN as the main ingredient in instant soup mix can provide an accomplished nutritional meal with protein, carbohydrates, fat, vitamin etc. The formulation of the main ingredients of EBN-based instant soup is statistically optimised using Response Surface Methodology (RSM) technique, with three main ingredients (i.e., EBN wt%, mushroom powder wt%, and skimmed milk wt%) as input factors meanwhile the antioxidant activity (%) of EBN-instant soup solution as the response. The antioxidant activity (%) is analysed using a standard DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) antioxidant assay. The optimised instant soup mix formulation contains 40 wt% EBN, 20 wt% mushroom powder, and 20 wt% skimmed milk with antioxidant activity of 85.35%. The response fit a linear model with a coefficient of determination (R^2 , 0.9734) and a standard deviation of 0.2. The model is significant with a p-value of < 0.0001 which is below 0.0500. The model has been validated successfully with a maximum error of 5.32%.

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1.0 Introduction

An edible bird nest (EBN) is a general type of bird nest made by edible-nest swiftlets. The main element of EBN is saliva, which is released by the sublingual glands of the swiftlets. The two main species of swiftlets known to produce valuable EBN are *Aerodramus fuciphagus* (white nests) and *Aerodramus maximus* (black nests) (Kang et al., 1991; Nasir et al., 2021). EBN has become one of Malaysia's most important commodities, with most of it exported, particularly to China, due to its alleged health benefits (Noor et al., 2018). Besides China, Hong Kong and Singapore are among countries that import EBN from Malaysia. In addition, Malaysia is the world's third largest exporter after Indonesia and Thailand, accounting for roughly 20 % of the total market for EBN production (Nasir et al., 2021; Noor et al., 2018).

Food is any nourishing substance that is eaten,

drunk, or otherwise ingested into the body to sustain life, provide energy, and promote growth (Shibamoto et al., 2008). Besides, “functional food” is whole, fortified, enriched, or enhanced foods that provide health benefits in addition to essential nutrients (e.g., vitamins and minerals) when consumed at effective levels as part of a varied diet regularly (Hasler, 2002). According to a previous study, there are numerous potential natural resources with functional properties that have yet to be discovered, to produce high-quality functional foods (Shibamoto et al., 2008). EBN is one of the promising sources of high-quality functional foods.

EBN is marketed as a healthy food supplement that can be consumed as a soup, drink, or instant powder. It is believed that eating EBN soup can improve human health by boosting the immune system, respiratory function, and skin complexion (Gan & Hui, 2016; Jamalluddin et al., 2019). Prior to that,

pregnant women from wealthy Chinese families frequently consumed EBN soup to improve the skin complexion of their newborns (Zukefli et al., 2017). Furthermore, consuming EBN strengthen human bones and act as an anti-influenza virus and hemagglutination-inhibitor (Lee et al., 2021). EBN extract also has been added to cosmetic products to rejuvenate new skin cells. The products were advertised as having an anti-ageing effect by maintaining a youthful and radiant complexion (Daud et al., 2021). Hence, the demand for EBN has risen over the last few decades.

Many studies on EBN have been conducted in Malaysia for their biological activities such as antioxidant (Gan et al., 2020; Ghassem et al., 2017; Karamać et al., 2014; Zulkifli et al., 2019), anti-inflammatory effect (Chua et al., 2021; Nur 'Aliah et al., 2016), and chondroprotective agent (Haghani et al., 2017; Seow et al., 2016; Tukiran et al., 2016). Despite of rich in antioxidants, EBN is also rich in protein and carbohydrates. EBN produced in Southeast Asia regions usually will have protein content in the range of 59.8% to 66.9%, while 25.6% to 31.4% the range of carbohydrate content (Darvishmotevalli et al., 2019; Lee et al., 2020; Ling et al., 2020). Besides, it is also rich in vitamins and minerals (Chye et al., 2017). Hence, EBN is very promising in providing high-nutrition functional foods that contain protein, carbohydrates, antioxidants, vitamins, and minerals.

Currently, various researchers are concentrating on the production of nutraceuticals instant food by employing a variety of plants (Jabeen et al., 2021; Wadikar & Premavalli, 2013; Yadav et al., 2022), therefore EBN is the best alternative to plant-based nutritious instant food. In this study, EBN and mushroom powder will be formulated as the main ingredient in nutritious instant soups normally consisting of vegetables and meat, and skimmed milk is used as nutrient addition (Chen et al., 2021). Response surface methodology (RSM) as a statistical optimisation tool will be employed in this study. Generally, the main composition (i.e. vegetables and meat), auxiliary composition (i.e. seasoning and starch), and nutrition composition of the instant soup mix is used as the independent variables and variables such as carotenoids (Wadikar & Premavalli, 2013), acidity (Wadikar & Premavalli, 2013), antioxidant (Demircan, 2022), and total phenolic content (Demircan, 2022) as responses. The main advantage

of this technique is the ability to change input factors simultaneously to understand the interaction effect between input factors. Moreover, the use of RSM aids in unbiased product development and improved ingredient optimisation for the intended responses (Wadikar & Premavalli, 2013) faster than one factor at a time (OFAT) technique. The optimised main ingredients formulation of the instant soup will be proposed, and model validation will be performed.

2.0 Methodology

2.1 Material

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) was purchased from Sigma Aldrich. Methanol, ACS analysis grade, 99%, J.T. Baker brand was purchased from Fisher Scientific. Ultrapure water with a conductivity of 18.2 mΩ was used throughout the experiment. Raw EBN sample was purchased from a local supplier in Permatang Pauh, Penang, Malaysia. Meanwhile, mushroom powder, skimmed milk, parsley, oregano, salt, and sugar were purchased from a local supplier in Permatang Pauh, Penang, Malaysia.

2.2 Methods

The raw EBN was cleaned by immersing it in ultrapure water for one hour. Then, the attached feathers were removed using a tweezer and rinsed with ultrapure water. The cleaned raw EBN was then dried in an oven at 50 °C for 12 hours until the moisture content was reduced to 15%. The dried EBN was ground with mortar and pestle and sieved through a 0.4 mm mesh filter.

The EBN was extracted using a modified heat extraction method by Marfo & Oke (1989). First, 0.5 g of the EBN powder was dissolved in 50 mL ultrapure water and mixed at 150 rpm at 30, 40, 60, 80, and 100 °C for 3 hours. Then, the solution was centrifuged at 4000 rpm for 15 minutes. The EBN hydrolysate was then dried at 50 °C for 12 hours and further used in the antioxidant assay.

The DPPH antioxidant assay was performed using the previous method by Ho et al., 2020. First, 0.1 mM of DPPH solution was prepared. Next, a sample solution was prepared by dissolving 2.5 g of various weight percentages of EBN: mushroom powder: skimmed milk: and others (i.e., parsley, oregano, salt, and sugar) in 10 mL ultrapure water. The solution was then filtered using a 90 mm Whatman filter. Then, 1 mL of each sample solution was mixed with 2.9 mL

DPPH solution and incubated for 30 minutes at room temperature. For control, 1 mL of ultrapure water was added to 2.9 mL DPPH solution. The absorbance of the control and samples were measured at 517 nm using a UV-vis spectrophotometer. The percentage of the DPPH scavenging was calculated using Eq. (1):

$$\text{DPPH scavenging (\%)} = \frac{A_0 - A_1}{A_0} \times 100 \quad (1)$$

where: A_0 is the absorbance of DPPH in the absence of an EBN-based sample solution, and A_1 is the absorbance of DPPH in the presence of an EBN-based sample solution. Methanol was used as the blank in this experiment.

Randomised Central Composite Design (CCD) in Design Expert version 13 software, was used to optimise the formulation of the EBN-based instant soup mix. The main input factors used were EBN (wt%), mushroom powder (wt%), and skimmed milk (wt%). Table 1 tabulated the design parameters used in RSM. The CCD number of experiments was formulated based on a quadratic polynomial equation in Eq. (2) (Hasanzadeh et al., 2021).

$$Z = \rho_0 + \sum_{i=1}^n \rho_i K_i + \sum_{i=1}^n \rho_{ii} K_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^n \rho_{ij} K_i K_j + \kappa \quad (2)$$

where: Z is response variable; ρ_0 and ρ_i are a constant and linear coefficient. ρ_{ii} and ρ_{ij} are quadratic and interaction coefficients. K is input variable and κ is statistical error. Since there are 3 input variables (i.e., edible bird nest (wt%), mushroom powder (wt%), and skimmed milk (wt%), $K = 3$) with a total of 14 non-centre points run. Meanwhile centre point runs are equivalent to 6 and the total number of runs is 20 as tabulated in Table 2.

3.0 Results and discussion

3.1 Prediction of antioxidant activity (%) in EBN-based instant soup using RSM

In this study, randomised CCD is used to predict the antioxidant activity (%) by manipulating the EBN in the range of 30 to 40 wt%, mushroom powder and skimmed milk in the range from 15 to 20 wt%. Meanwhile, the experimental value of the antioxidant activity (%) is used as the response. Table 2 shows the analysis of the experimental and predicted value of antioxidant activity from RSM. It was observed there is only a slight deviation between the experimental value and predicted value (± 0.2 to ± 1.8) as shown in Table 2 proving the accuracy of the prediction model.

The model predicted that the EBN : mushroom powder : skimmed milk weight composition of 26.59 : 17.5 : 17.5 wt% would yield the lowest antioxidant activity (66.94%). Moreover, the maximum antioxidant activity of 90.02% was obtained at the composition of EBN : mushroom powder : skimmed milk weight composition is 43.41 : 17.5 : 17.5 wt%. Since the composition of the mushroom powder is the same as skimmed milk, it can be suggested that the higher wt% of EBN the greater the antioxidant activity (%). This is supported by the interaction between individual parameter and antioxidant activity (%) as shown in Fig. 1, the higher the wt% of EBN, the higher the antioxidant activity (%) whereas the antioxidant activity (%) remain constant when mushroom powder and skimmed milk wt% were increased. This attributes to the main source of antioxidants in the instant soup mix coming from EBN. The previous study identified that EBN is rich in bioactive components including antioxidant glycopeptides (Ariff et al., 2019; Ismail et al., 2021; Wang et al., 2022). Moreover, in terms of instant soup formulation, EBN is also found to have more antioxidant activity compared to chicken and fish soups (Daud et al., 2021).

3.2 Model analysis

The relationship between the manipulated variables of EBN (wt%), mushroom powder (wt%) and skimmed milk (wt%) and antioxidant activity (%) were well fit with the linear model in RSM. Eq. (3) shows the linear regression model for antioxidant activity (%).

$$AA(\%) = 6.86A + 0.0043B + 0.0043C + 78.48 \quad (3)$$

where: AA is the antioxidant activity, A is the EBN (wt%), B is the mushroom powder (wt%), and C is the skimmed milk (wt%).

Table 3 summarised the analysis of the variance of the linear model. The coefficient of determination (R^2) of the model is 0.9734, which is close to 1.0 attributable to the accuracy of the model. The closer the R^2 value to 1.0, the better the empirical model fits the experimental data (Domingues et al., 2013). The model is also significant with a p-value (< 0.0001) less than 0.05 since a p-value greater than 0.1000 indicate that the model term is not significant (Demirel & Kayan, 2012; Salar et al., 2016). The lack of fit obtained is not significant with p-value of 0.9621. The non-significance lack of fit clarified that the linear model is valid when the p-value is greater,

Table 1: Design parameters for RSM

No.	Factors	Units	- α -1.682	Low -1	Mean 0	High +1	+ α +1.682
1	Edible Bird Nest	wt%	26.59	30.00	35.00	40.00	43.41
2	Mushroom Powder	wt%	13.30	15.00	17.50	20.00	21.71
3	Skimmed Milk	wt%	13.30	15.00	17.50	20.00	21.71

Table 2: The experimental value of antioxidant activity (%) and predicted value of antioxidant activity (%) from RSM corresponding to the wt% of the main ingredients

Run	Edible Bird Nest (wt%)	Mushroom powder (wt%)	Skimmed Milk (wt%)	Experimental AA (%)	RSM predicted AA (%)	±SD
1	26.59	17.5	17.5	66.56	66.94	0.2
2	30	20	20	71.25	71.63	0.2
3	30	15	15	71.23	71.61	0.2
4	30	20	15	71.24	71.62	0.2
5	30	15	20	71.24	71.62	0.2
6	35	17.5	17.5	78.10	78.48	0.2
7	35	17.5	13.3	78.10	78.48	0.2
8	35	17.5	17.5	79.00	78.48	0.3
9	35	13.3	17.5	78.10	78.48	0.2
10	35	17.5	17.5	82.00	78.48	1.8
11	35	17.5	17.5	80.00	78.48	0.8
12	35	21.71	17.5	78.11	78.49	0.2
13	35	17.5	21.71	78.11	78.49	0.2
14	35	17.5	17.5	79.00	78.48	0.3
15	35	17.5	17.5	78.10	78.48	0.2
16	40	15	20	84.96	85.34	0.2
17	40	20	20	84.97	85.35	0.2
18	40	15	15	84.95	85.33	0.2
19	40	20	15	84.96	85.34	0.2
20	43.41	17.5	17.5	89.64	90.02	0.2

Table 3: Analysis of variance (ANOVA) for the quadratic model

Source	Sum of square	DF	Mean square	F-value	P-value	
Model	642.56	3	214.19	195.06	<0.0001	significant ^a
A-EBN	642.56	1	642.56	585.19	< 0.0001	
B-mushroom powder	0.0002	1	0.0002	0.0002	0.9882	
C-skimmed milk	0.0002	1	0.0002	0.0002	0.9882	
Residual	17.57	16	1.10			
Lack of Fit	6.75	11	0.6135	0.2835	0.9621	not significant ^b
Pure Error	10.82	5	2.16			
Cor Total	660.13	19				

R² = 0.9734, predicted R² = 0.9682

^a significant under 95% confidence level

^b not significant relative to the pure error due to noises

Table 4: Model validation between the optimum predicted value and experimental value of AA %

EBN (wt%)	Mushroom powder (wt%)	Skimmed milk (wt%)	AA (%) predicted	AA (%) Actual	Error (%)
40.00	20.00	20.00	85.35	80.85	5.32
40.00	20.00	20.00	85.35	81.33	4.75
40.00	20.00	20.00	85.35	82.56	3.31
40.00	20.00	20.00	85.35	82.88	2.94
40.00	20.00	20.00	85.35	84.97	0.19

than 0.05. Moreover, from the model validation the maximum error between experimental and predicted data is 5.32 % as tabulated in Table 4, which is acceptable since it is less than 10% (Esfe et al., 2022). Furthermore, the R^2 and p-value of the model also proved that the model fits the experimental data. Moreover, the significant factors towards the antioxidant activity (%) are EBN (A and A^2) with a p-value of 0.0001 and 0.0045 and the interaction between mushroom powder and skimmed milk (BC) with a p-value of 0.0411.

3.3 Interaction between EBN (wt%), mushroom powder (wt%), skimmed milk (wt%) and antioxidant activity (%).

The 3D surfaces in Fig. 2 show the relationship between the input factors (i.e., EBN (wt%), mushroom powder (wt%), and skimmed milk (wt%)) and antioxidant activity (%). Fig. 2(a) shows the relationship between EBN (wt%) and mushroom powder (wt%) towards AA (%), Fig. 2(b) shows the relationship between EBN (wt%) and skimmed milk (wt%) towards AA (%) and Fig. 2(c) shows the relationship between mushroom powder (wt%) and skimmed milk (wt%) towards AA (%).

From Fig. 2(a), at high EBN (40 wt%), there is a small effect of mushroom powder (wt%) on AA (%), as there is a reduction of 0.01% AA when mushroom powder (wt%) reduced from 20 to 15 wt% at 40 wt% EBN. Whereas at low EBN (30 wt%), there is a slight increase in AA (%) of approximately 0.02% when mushroom powder (wt%) is reduced from 20 to 15 wt%. Similarly, the pattern is also the same for the interaction between EBN (wt%) and skimmed milk (wt%) as shown in Fig. 2(b) on AA (%). It can be inferred that there is no direct correlation between EBN and mushroom powder, or between EBN (wt%) and skimmed milk on AA (%). The slight changes of AA (%) when there is an increment of mushroom powder (wt%) and skimmed milk (wt%) are considered insignificant. The interaction between mushroom powder (wt%) and skimmed milk (wt%) on AA (%) is also analysed and shown in Fig. 2(c). It can be observed that when skimmed milk (wt%) increased from 15 to 20 wt% at constant mushroom powder (i.e., 20 wt%), the AA (%) increased by 2%. Meanwhile, when mushroom powder (wt%) increased from 15 to 20 wt% at constant 20 wt% skimmed milk, AA (%) increased by 5%. In comparison to skimmed milk, the effect of mushroom powder on AA (%) is more substantial. Many species of mushrooms have

been discovered to contain antioxidant properties that allow them to neutralise free radicals (Kozarski et al., 2015; Sánchez, 2017).

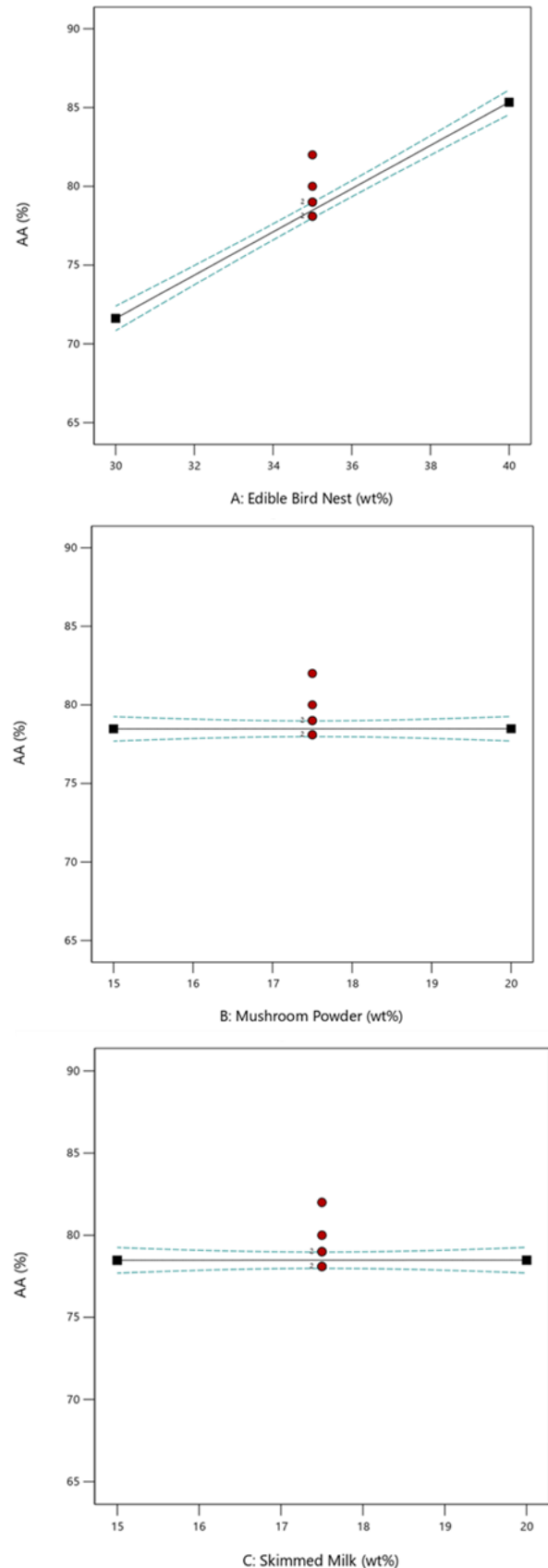


Fig. 1: Interaction of individual parameters on antioxidant activity (%) at factor 35 wt% EBN, 17.5 wt% mushroom powder, and 17.5 wt% skimmed milk

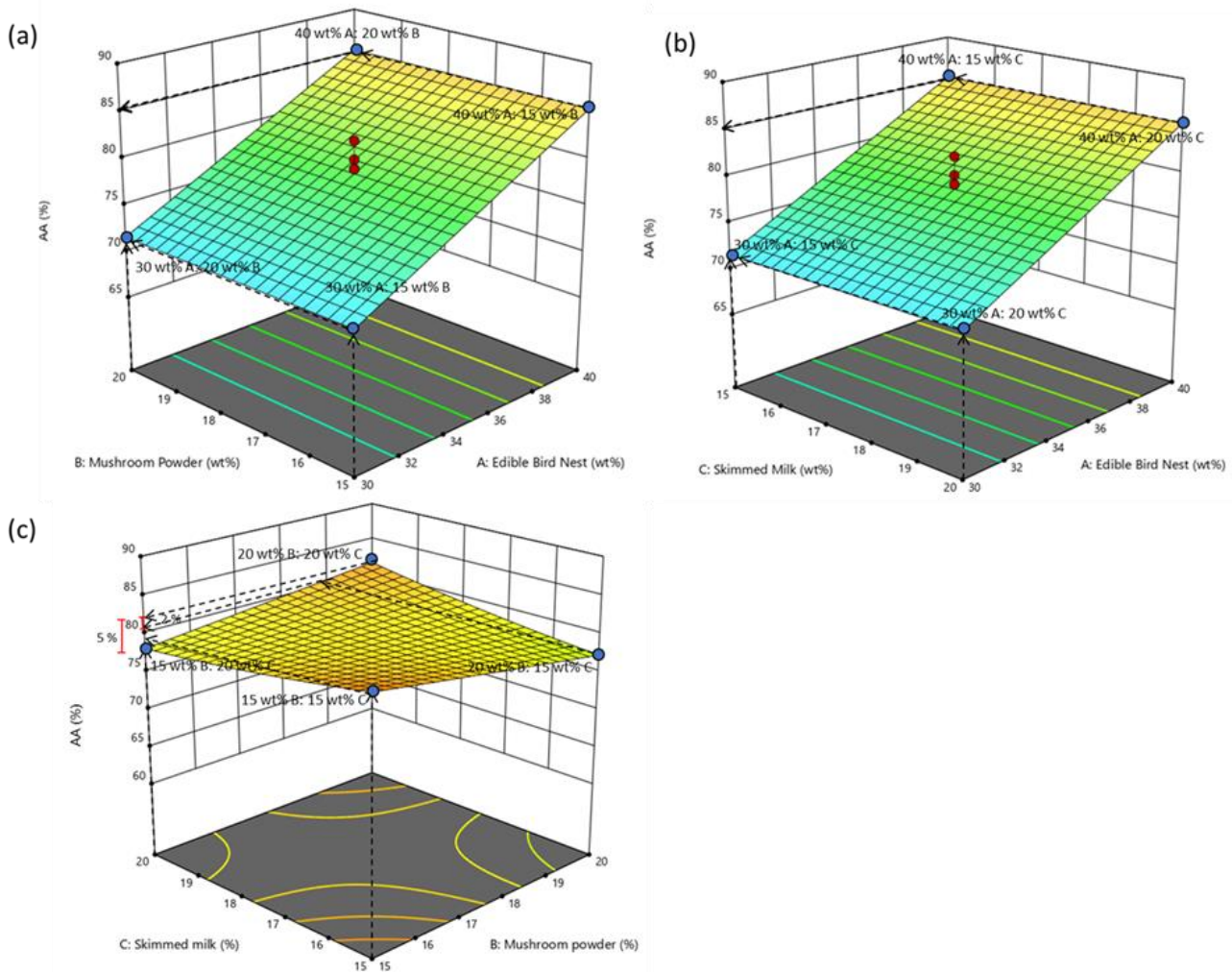


Fig. 2: Interaction of parameters on antioxidant activity (%) at factor 35 wt% EBN, 17.5 wt% mushroom powder, and 17.5 wt% skimmed milk

3.4 The optimised formulation of EBN-based instant soup and model validation

RSM provided 100 solutions, and the optimum formulation for EBN-based instant soup was chosen with the highest AA (%). The constrain parameter of AA (%) has a low range of 66.56% and a high range of 89.64%. Based on the maximised desirability (1.000), the optimal condition of AA is 85.35% at 40 wt% of EBN, 20 wt% of mushroom powder, and 20 wt% of skimmed milk. To validate the model, the optimum AA % is compared to the experimental value of AA % at 40 wt% EBN : 20 wt% mushroom powder : 20 wt% skimmed milk. The standard error was calculated between the optimum value and experimental value and tabulated in Table 4.

4.0 Conclusions

The optimised formulation of EBN-based instant soup was obtained from RSM using a randomised centre composite design. The prediction and

optimisation of the AA (%) based on different weight compositions of EBN, mushroom powder, and skimmed milk fitted the linear model with R^2 of 0.9734 and a p-value of < 0.0001 . The obtained R^2 portrays the accuracy between the predicted and experimental value of the AA (%), meanwhile p-value less than 0.05 proved that the model is significant. Moreover, EBN is found to be the main ingredient influencing AA (%). Since there is no substantial variation in AA (%) when mushroom powder and skimmed milk (wt%) are reduced. Meanwhile, a 5% increment of AA was found when mushroom powder (wt%) increased from 15% and 20 wt% at constant skimmed milk (wt%). It can be concluded that EBN (wt%) is the main contributor to AA (%) with a p-value of < 0.0001 and the interaction between mushroom powder and skimmed milk also slightly affects the AA (%) with a p-value of < 0.0001 . Furthermore, the optimum formulation of the EBN-based instant soup is obtained at 40 wt% of EBN, 20 wt% of mushroom powder and 20 wt% of skimmed milk with antioxidant activity of 85.35%. The

percentage error obtained from the comparison between the predicted value and actual experimental value of AA is 5.32% maximum from 5 repeated runs. This demonstrates that the model is validated for use in the optimal formulation of the EBN-based instant soup mix.

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