Indonesian Food Science and Technology Journal
IFSTJ : Vol 8 No 1, December 2024 (PP: 1-15)
ISSN : 2615-367X

INDONESIAN FOOD SCIENCE AND TECHNOLOGY JOURNAL (IFSTJ)

Journal homepage : online-journal.unja.ac.id/ifstj/issue/archive

Enhancing Instant Fortified Rice Congee (IFRC) for Elderly Nutrition: Collagen and Curcumin Optimisation Using Response Surface Methodology (RSM)

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***Abstract*— Instant rice congee (IRC) fortified with functional ingredients has been specifically formulated to provide enhanced nutrition for the elderly. The optimisation of collagen and curcumin amounts in instant fortified rice congee (IFRC) was determined using Response Surface Methodology (RSM). The study revealed that the optimal conditions for IFRC formulation involved incorporating 7.96 g of collagen and 361 mg of curcumin, resulting in the highest observed values for protein content (32.41%), Total Phenolic Content (TPC) at 24.75 mg GAE/g sample, Ferric Reducing Antioxidant Power (FRAP) at 6.03 mg TE/g sample, adhesiveness at -329.23 g/s and cohesiveness at 0.60. In contrast, the formulation exhibited the lowest values for hardness (581.70 g). These findings, derived from the application of RSM, provide valuable insights into the optimal combination of collagen and curcumin in IFRC, showcasing its potential to enhance key nutritional and textural attributes. The outcomes from this study offer practical guidance for utilising collagen and curcumin as functional ingredients in fortified foods, particularly in the context of creating nutritionally enriched and palatable options for the elderly.**

***Keywords*— Elderly; instant rice congee; porridge; collagen; curcumin**

Manuscript received April 27, 2024; revised May 21, 2024; accepted Nov 22, 2024. Available online December 28, 2024

I. INTRODUCTION

The global aging population is on the rise, and in 2022, the World Health Organization predicts a nearly doubling of the percentage of individuals aged over 60, from 12% to 22%, between 2015 and 2050 [1]. To address the nutritional needs of this expanding demographic, collaboration between food researchers and the food industry is essential to develop convenient and healthy dietary options. Challenges arise from a reduction in meal consumption due to sensory function decline and oral health issues, such as dentures and difficulties in chewing and swallowing, impacting the elderly's eating habits. These issues contribute to a diminished appetite, compromised ingestion, and nutritional deficiencies, including protein deficiency in the elderly, leading to decreased muscle mass and increased prevalence of physical frailty [2]. Additionally, advancing age increases the likelihood of oxidative stress and inflammation, contributing to fatigue, weakness, and impaired mobility in the elderly. Elderly experiencing malnutrition commonly face issues such as muscle weakness, compromised immune function, reduced functionality, and an elevated risk of infections. Specifically, protein-energy malnutrition (PEM), which arises from a decline in both protein and energy intake, has been linked to various health consequences, disease states, and illnesses. Hence, protein-fortified foods (PFF) stands out as a promising alternative to overcome protein-energy malnutrition (PEM) in the elderly [3].

Congee is renowned for its nutritional value, ease of preparation, and digestibility, making it an ideal choice for nourishing individuals who are ill or lack teeth, such as infants and the elderly, who may face challenges with chewing.

Nevertheless, it is important to note that congee, despite its popularity, is a starchy food with relatively low nutritional content [4]. The nutrients in rice congee might not suffice for the elderly, prompting the incorporation of functional ingredients for fortification. Hence, an instant fortified rice congee (IFRC) has been developed to supplement the nutrition of elderly. This product considers characteristics such as a prolonged shelf life, simplicity in preparation, and ease of handling during transportation and distribution [5].

Bioactive compounds, often referred to as functional ingredients, are substances extracted from various food sources, including fruits, grains, vegetables, and by-products of food processing. Importantly, these bioactive ingredients possess the ability to maintain their inherent characteristics even after the extraction process [6]. Curcumin, one of bioactive compounds, serves as a natural component in the creation of functional foods, contributing a unique colour and flavour profile. Beyond its culinary attributes, curcumin also presents potential health advantages, including anti-inflammatory and antioxidant properties, as highlighted by Tripathy et al. [7]. Besides, collagen fibres play a crucial role in maintaining the flexibility of the skeletal system. However, starting from the age of 25, there is a decline in collagen levels in the body. This gradual reduction can lead to decreased flexibility and increased brittleness in ligaments, tendons, bones, and cartilage over time [8]. To address this issue, collagen is extracted from various animal sources, primarily derived from the skin and bones of vertebrate species like bovine and swine. This extracted collagen finds applications in the food, biomedical, cosmetic, and pharmaceutical industries, particularly for combating premature aging, as stated by Hashim et al. [9] and Lupu et al. [8]. Furthermore, the significant interest in the potential utilisation of marine collagen, particularly in the field of food processing, has been ignited by its halal status, making it suitable for adherence to Islamic dietary guidelines among Muslims [9].

Curcumin has been shown to have a significant impact as a functional ingredient, particularly in biscuits, where it enhances the nutritional profile and functionality of composite flour biscuit while boosting antioxidant levels. [10]. Additionally, curcumin-enriched bread developed by Ferguson et al. [11] has shown promise in supporting heart health due to curcumin bioactive properties, including anti-inflammatory, antioxidant, anti-carcinogenic, anti-aggregatory, and mild hypoglycaemic effects. Addition of collagen as a functional ingredient in food products also significantly enhances the rheological properties of surimi, sausages, and frankfurters [9]. Besides, a research conducted by Blinnikova et al. [12] developed fruit jelly sweets enriched with collagen resulted in a 10.13% increase in protein content.

In summary, the results of this study have the potential to significantly contribute to the development of convenience functional foods that are designed to meet the distinct nutritional and textural requirements of the elderly. The optimal combination of collagen and curcumin in IFRC results in the optimal formulation for developing a convenient food tailored for the elderly. This, in turn, can contribute to promoting better health, preventing malnutrition, and enhancing the overall well-being of older individuals.

II. Material and Methods

A. Material

White rice grain (*Oryza Sativa* L.) brand Jasmine Super 5 imported rice were purchased from supermarkets in Selangor, Malaysia. The white rice grain was placed in a tight container and stored in a dark place prior to analysis. Collagen Fortigel B (highly purified bioactive collagen peptides from bovine skin) and curcumin cavacurmin (highly bioavailable curcumin complex with oligosaccharide gamma-cyclodextrin) were sponsored by a local ingredients' supplier (DKSH, Malaysia).

B. Preparation of instant fortified rice congee (IFRC)

The ground rice, approximately 1 mm in size, was cooked using the steaming method with a stainless-steel steamer pot. The ratio of rice to water used to cook rice congee was 1:10 and the heating process was held for 15 minutes at a constant temperature, about 100°C [13]. After the temperature drop to 60°C, 100 g of cooked rice congee were added with 158 to 441 mg of curcumin and 3.9 to 11.0 g of collagen (recommended daily intake for curcumin and collagen, respectively [14–16]). The rice congee was stirred until collagen and curcumin are mixed completely or the texture is smooth without any lumps. The cooked IFRC were spread on the tray for the drying process. Then, the trays were placed in the oven drying at 60°C for 24 hours and until the moisture content of the dried IFRC reaches 6-7% [17, 18]. The dried IFRC were ground and sieved using a 1 mm siever [19]. Then, the dried IFRC were kept in a sealed aluminium bag until further analysis.

C. Experimental Design

The experiment using response surface methodology (RSM) was designed based on two factors which are collagen (X1) and curcumin (X2). The response which are protein content, TPC, FRAP, and texture analysis with the parameters of hardness, cohesiveness and adhesiveness were assumed to be influenced by these two factors. The value for other ingredients in IRC was fixed. Central composite design (CCD), five levels, and their working ranges were chosen in this experiment as shown in **Table 1**. The range of collagen is 3.9 g to 11.0 g [11,12], and curcumin ranged from 158 mg to 441 mg [13] and it was determined based on the recommended daily intake of both functional ingredients for adults. The 13 experimental runs, which resulted in obtained data response variables were shown in **Table 2**.

TABLE 1
CODED AND UNCODED INDEPENDENT VARIABLES USED IN RSM BASED ON CCD DESIGN

Factor	Unit	Notation	Level				
			-α	-1	0	+1	+α
Collagen	g	X_1	3.9	5.0	7.5	10.0	11.0
Curcumin	mg	X_2	158	200	300	400	441

TABLE 2

CODED AND UNCODED FACTORS AND COMPARISON BETWEEN ACTUAL (Y) AND PREDICTED (FITS) RESPONSES

Run No.	Factors		Responses											
	Collagen (g)	Curcumin (mg)	Protein (%)		TPC (mg GAE/g sample)		FRAP (mg TE/g sample)		Hardness (g)		Adhesiveness (g/s)		Cohesiveness	
	X_1	X_2	Y_1	FITS 1	Y_2	FITS 2	Y_3	FITS 3	Y_4	FITS4	Y_5	FITS5	Y_6	FITS6
1	5	200	24.58	24.96	17.28	17.43	5.78	5.84	801.71	802.08	-257.64	-266.21	0.44	0.44
2	10	200	38.98	39.58	26.05	26.09	6.00	5.99	340.80	365.31	-170.43	-177.66	0.67	0.66
3	5	400	22.80	22.82	25.19	25.53	6.21	6.21	786.85	747.12	-258.60	-263.36	0.50	0.51
4	10	400	27.43	27.67	28.97	29.20	6.59	6.52	229.39	213.81	-184.87	-188.30	0.61	0.62
5	3.96	300	23.64	23.49	19.94	19.68	5.49	5.45	1021.74	1046.42	-272.90	-265.95	0.41	0.41
6	11.03	300	37.72	37.26	28.51	28.40	5.73	5.78	369.94	360.47	-155.30	-150.25	0.64	0.64
7	7.5	158.57	32.66	32.10	21.17	21.12	6.39	6.35	454.45	433.71	-245.58	-236.90	0.57	0.58
8	7.5	441.42	22.22	22.17	29.37	29.05	6.95	6.99	251.77	287.72	-245.73	-242.42	0.61	0.60
9	7.5	300	37.84	36.49	21.10	21.24	5.75	5.76	669.37	724.76	-357.39	-364.19	0.57	0.57
10	7.5	300	37.19	36.49	21.10	21.24	5.81	5.76	778.38	724.76	-372.81	-364.19	0.56	0.57
11	7.5	300	36.01	36.49	21.27	21.24	5.73	5.76	772.16	724.76	-364.54	-364.19	0.58	0.57
12	7.5	300	35.64	36.49	21.13	21.24	5.73	5.76	705.45	724.76	-358.34	-364.19	0.56	0.57
13	7.5	300	35.75	36.49	21.57	21.24	5.77	5.76	698.44	724.76	-367.86	-364.19	0.56	0.57

[1]FITS: Fit summary, corrected starting point for the model fitting

D. Analysis of IFRC

Protein determination

Protein content of IFRC was determined using Kjeldahl method by [20]. About 0.6 g of defatted IFRC samples was weighed into the Kjeldahl tube. A catalyst tablet was added and then followed by addition of 25 mL of 98% H_2SO_4. The sample was heated up at 150°C for 30 minutes, then increased to 300°C for 60 minutes and lastly the temperature was increased to 420°C for a 5-hour digestion process. Then, the digestion tube was left to stand for cooling for 2 hours. Following the digestion process, 2% H_3BO_3 and deionised water were added in the tank of the distillation unit. The digestion tube that contains sample was attached to the distillation unit and followed by the addition of 32% NaOH. Then, 0.1 M HCl was prepared in a Schott bottle for the titration process with 2% H_3BO_3. The distillation process was run automatically followed by a titration process. Percentage of protein content was calculated as described below:

$$\text{Kjeldahl nitrogen, \%} = \frac{[(VS-VB)]\times M\times 14.01}{W\times 10} \quad (1)$$

$$\text{Crude protein, \%} = \text{\% Kjeldahl nitrogen N} \times \text{F} \quad (2)$$

where VS = volume (mL) of standardised acid used to titrate a sample; VB = volume (mL) of standardised acid used to titrate reagent blank; M = molarity of standard HCl; 14.01 = atomic weight of N; W = weight (g) of test portion or standard; 10 = factor to convert mg/g to percent; and F = factor to convert N to protein (5.95).

Total phenolic content (TPC)

TPC was determined using Folin-Ciocalteau method by Mayachiew et al. [18] with some modifications. About 200 µL of aliquot from sample extraction was mixed with 1.5 mL of freshly diluted 10-fold Folin-Ciocalteau reagent and the mixture was incubated for 5 minutes. Then, 1.5 mL of 60 g/L Na_2CO_3 solution was added into the incubated solution. The solution was left at room temperature for 90 minutes for another incubation. The absorbance of the solution was measured at 750 nm by using a Genesys 20 spectrophotometer (Thermo Scientific, Waltham, MA, USA). A standard curve ($Y = 0.80X + 0.02$; $R^2 = 0.99$) was prepared by using gallic acid as a standard in a series of concentrations: 0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL and 1.0 mg/mL by using gallic acid as a standard. TPC of the extracted dried rice porridge was expressed as mg gallic acid equivalent (GAE) per g dry sample (mg GAE/g sample).

Ferric reducing antioxidant power (FRAP)

The method stated by Mayachiew et al. [18] was used to determine FRAP of extracted samples with some modifications. FRAP reagent was prepared by mixing 10 mM TPTZ solution in 40 mM HCl, 20 mM $FeCl_3 \cdot 6H_2O$ and 300 mM acetate buffer (pH 3.6) at a ratio of 1:1:10 [v/v/v]. Then, 1.5 mL of prepared FRAP reagent was mixed with 50 µL of the extracted sample in an amber test tube and incubated in a water bath at 37°C for 30 minutes. The absorbance of the ferrous tripyridyltriazine complex solution which is coloured product was measured at 593 nm using the UV-vis spectrophotometer (Thermo Scientific, Waltham, MA, USA). A standard curve ($Y = 1.69X + 0.01$; $R^2 = 0.98$) of Trolox with a series of concentrations: 0.02 mg/mL, 0.04 mg/mL, 0.06 mg/mL, 0.08 mg/mL and 0.10 mg/mL as a standard was prepared. FRAP was expressed as mg Trolox equivalent (TE) per g dry sample (mg TE/g sample).

Texture analysis

About 10 g of dried IFRC samples were rehydrated with 10 mL of boiling water which makes the ratio of rice to water at 1:1. Then, the mixture was stirred until the texture of IFRC is smooth without any lumps. About 10 g of rehydrated IFRC samples with dimension of 23 mm diameter and 35 mm height were placed in a beaker. The rehydrated rice congee was analysed using a texture analyser (Stable Micro Systems, Godalming, UK) fitted with a 50 kg load cell and a cylinder-shaped flat probe of 20 mm diameter. The probe was working at a speed of 1 mm/s and distance of 30 mm into each congee sample. The maximum force value of the first peak, negative area of the graph and the ratio of areas under the two peaks were recorded as hardness (g), adhesiveness (g/s) and cohesiveness values, respectively [19].

E. Statistical Analysis

Minitab Software (ver. 17) was used for the analysis of variance (ANOVA), the multiple regression analysis, and the response surface regression. The one-way ANOVA was carried out to determine the effects of the independent variables X_1 and X_2 to the response variables (Y_1, Y_2, Y_3, Y_4, Y_5, and Y_6) of IFRC. The regression coefficient of determination (R^2) and lack-of-fit value were determined to evaluate the fitness of the polynomial equation to the response variables. The verification of model validity by comparing the experimental values with the predicted values of the optimized model was analysed using t-test method in Statistical Package for the Social Sciences (SPSS) software (version 28). A significant difference was considered at the level of $p<0.05$.

III. RESULT AND DISCUSSION

IFRC production using RSM

Response surface methodology (RSM) was used in this study to determine the optimised amount of functional ingredients fortified in the preparation of IFRC. Data obtained from this study were analysed using MINITAB software version 17 by using five levels CCD, and results on the responses (protein content, TPC, FRAP, hardness, adhesiveness, and cohesiveness) are given in Table 2. Y indicates the experimental value for each response and FITS represents predicted response value. Collagen (g) and curcumin (mg) are represented by X_1 and X_2, respectively.

The experimental and predicted value for each run are listed in Table 2. Under combination of 10 g collagen and 200 mg curcumin, (Run 2), protein (%) shows the highest value in both experimental and predicted value which are 38.98% and

39.58%, respectively. Then, run 3, a combination of 5 g collagen and 400 mg curcumin, exhibits the lowest protein (%) with experimental value of 22.80% and predicted value of 22.82%.

Then, Run 8 (7.5 g collagen, 441.42 mg curcumin) shows the highest experimental value (29.37) for TPC (mg GAE/g sample). However, there is a slight difference in the predicted value (29.20) which shows the highest value under condition Run 4 (10 g collagen, 400 mg curcumin). This result is supported by Siti Roha et al. [22] whereas a similar trend of changes between experimental and predicted value of RSM model were found. This variation might be due to the light exposure of IFRC samples during analysis, which affected the value of TPC. The lowest experimental and predicted value for TPC (mg GAE/g sample) were found at condition of combination factors at 5 g of collagen and 200 mg curcumin, Run 1.

The highest experimental and predicted FRAP (mg TE/g sample) were detected when the factors set at condition 7.5 g collagen and 441 mg curcumin (Run 8). In contrast, Run 5 was detected to have the lowest value of FRAP (mg TE/g sample) for experimental and predicted value, being 5.49 and 5.45, respectively.

The experimental value of hardness (g) ranged from 229.39 to 1021.74 g with different factors combination. The predicted values have a broader range from 213.81 to 1046.42 g. The highest experimental and predicted hardness values were found during Run 5 (3.96 g of collagen and 300 mg curcumin). In addition, the lowest experimental and predicted hardness values were detected when the factors were set at Run 4.

Moreover, Run 10 exhibits the highest experimental and predicted adhesiveness values (g/s), at -372.81 and -364.19, respectively. The lowest experimental (-155.30 g/s) and predicted (-364.19 g/s) adhesiveness values were reported under specific condition of factors at 11.03 g collagen and 300 mg curcumin, Run 6.

The last response, cohesiveness shows the highest value for experimental and predicted at Run 2 with condition of 10 g collagen and 200 mg curcumin. The lowest experimental and predicted cohesiveness were obtained when the factors are set at Run 5 (3.96 g collagen and 300 mg curcumin).

A regression analysis was performed to fit mathematical models using experimental data with the goal of identifying an optimal region for the responses studied. Equations 3, 4, 5, 6, 7, and 8 are quadratic, second-order polynomial equations that can be used to explain the empirical relationship between the input variables and the response variable in terms of uncoded values through the application of multiple regression analysis.

Based on eq. (3), protein (Y_1) is affected by many factors, including linear, quadratic, and interaction terms, which show their effect on the response value. For the linear factors of collagen (X_1) and curcumin (X_2), a positive coefficient suggests that increasing collagen and curcumin leads to an increase in protein of IFRC. Regarding the quadratic factors, collagen (X_1X_1) and curcumin (X_2X_2), the negative coefficients indicate a decrease in protein when these factors interact only within themselves. The interaction factor (X_1X_2) shows a negative coefficient, indicating a decrease in protein as the interaction between these factors decreases.

As shown in eq. (4), TPC (Y_2) presents the negative coefficients for collagen (X_1) and curcumin (X_2) which indicate that TPC of IFRC decreases as both factors decreased. Then, the quadratic factors of collagen (X_1) and curcumin (X_2) present that the positive coefficients increase TPC value when these factors interact only among themselves. The interaction factor (X_1X_2) shows a negative coefficient, indicating a decrease in TPC as the interaction between these factors decreases.

According to eq. (5), FRAP (Y_3) is influenced by the linear factors collagen (X_1) and curcumin (X_2). The positive coefficients indicate that FRAP increases as collagen (X_1) increased. Conversely, negative coefficients imply that FRAP decreases curcumin decreased. Regarding quadratic factors, a negative coefficient for collagen (X_1) indicates that FRAP decreases when this factor interacts only within themselves. In contrast, for curcumin (X_2), the positive coefficient suggests an increase in FRAP when curcumin interacts only with itself. Similarly, as for the interaction factor (X_IX_2), it shows a positive coefficient, indicating an increase in FRAP as the interaction between these factors increased.

In eq. (6), hardness (Y_4) is affected by factors, including linear, quadratic, and interaction terms, which show their effect on the response value. For the linear factors of collagen (X_I) and curcumin (X_2), a negative coefficient suggests that decreasing collagen leads to a decrease in hardness of IFRC. Conversely, positive coefficients indicate that increases in curcumin resulted in increasing hardness of IFRC. Regarding the quadratic factors, collagen (X_1X_1) and curcumin (X_2X_2), the negative coefficients indicate a decrease in hardness when these factors interact only among themselves. The interaction factor (X_1X_2) shows a negative coefficient, indicating a decrease in hardness as the interaction between these factors decreases.

As stated in eq. (7), adhesiveness (Y_5) presents the negative coefficients for the linear factors, collagen (X_1) and curcumin (X_2) which indicate that adhesiveness of IFRC decreases as both factors decreased. Then, the quadratic factors of collagen (X_1) and curcumin (X_2) present that the positive coefficients increase adhesiveness value when these factors interact only within themselves. The interaction factor (X_1X_2) shows a negative coefficient, indicating a decrease in adhesiveness as the interaction between these factors decreases.

Eq. (8) shows that cohesiveness, (Y_6) is influenced by the linear factors collagen (X_1) and curcumin (X_2). The positive coefficients indicate that cohesiveness increases as collagen (X_1) and curcumin (X_2) increased. Regarding quadratic factors, a negative coefficient for collagen (X_1) indicates a decrease in cohesiveness when collagen interact within themselves. In contrast, curcumin (X_2) suggests an increase in cohesiveness when curcumin interact within themselves. For the interaction factor (X_1X_2), it shows a negative coefficient, indicating a decrease in cohesiveness as the interaction between these factors decreased.

$$Y_1 = -59.15+12.213\ X_1+0.3187\ X_2-0.4890\ X_1X_1-0.000468\ X_2X_2-0.00977\ X_1X_2 \quad (3)$$

$$Y_2 = 22.28-0.634\ X_1-0.05000\ X_2+0.2243\ X_1X_1+0.000192\ X_2X_2-0.004990\ X_1X_2 \quad (4)$$

$$Y_3 = 8.547+0.1736\ X_1-0.02637\ X_2-0.01164\ X_1X_1+ 0.000046\ X_2X_2+0.000160\ X_1X_2 \quad (5)$$

$$Y_4 = -344-42.5\ X_1+11.13\ X_2-1.71\ X_1X_1-0.01820\ X_2X_2-0.0965\ X_1X_2 \quad (6)$$

$$Y_5 = 751.4-166.90\ X_1-3.654\ X_2+12.487\ X_1X_1+0.006226\ X_2X_2-0.0135\ X_1X_2 \quad (7)$$

$$Y_6 = -0.0573+0.1187\ X_1+0.000302\ X_2-0.003318\ X_1X_1+0.000001\ X_2X_2-0.000119\ X_1X_2 \quad (8)$$

[1]Y_1 = Protein (%), Y_2 = TPC (mg GAE/g sample), Y_3 = FRAP (mg TE/ g sample), Y_4 = Hardness (g), Y_5 = Adhesiveness (g/s) and Y_6 = Cohesiveness, while X_1 and X_2 are the collagen and curcumin, respectively.

Fitting the model and analysis of experimental design

The regression model's goodness of fit was determined by calculating the coefficient R^2 and adjusted R^2 (multiple correlation coefficient, R), providing a measure of how much variability in the observed response values can be explained by the experimental factors and their interaction [22]. In addition, the R^2 of 1 means that the regression coefficient model is capable in predicting the optimum value with high accuracy [23].

The ANOVA for protein is shown in **Table 3**. The coefficient of determination (R^2) was 99.04%, which indicates that 99.04% of the sample variation in the protein, was attributed to the factors X_1 and X_2. All terms were significant at p-value < 0.05, with the interaction term of X_1X_2 being the least significant term which is 0.001. All the other terms have negative effect on the protein, as indicated by the negative sign of the coefficient value. The squared term which is significant (p-value < 0.05) indicates that the relationship between the factor and the response follows a curved line.

As shown in **Table 3**, the ANOVA data for TPC were tabulated. The coefficient of determination (R^2) is 99.70%, which indicates that 99.70% of the sample variation in the TPC was attributed to the factors X_1 and X_2. All main factors (X_1 and X_2) and their squared terms (X_1X_1 and X_2X_2) are highly significant at p-value of 0.

Table 3 displays the ANOVA value for FRAP. The interaction term (interaction between different factors, X_1X_2) showed a p-value of 0.174, which indicates that there was no interaction between collagen and curcumin. The coefficient of determination (R^2) was 99.08%, which indicates that 99.08% of the sample variation in the FRAP, was attributed to the factors X_1 and X_2. All terms were significant at p-value < 0.050, except for the squared term of X_1X_2 which is not significant. The squared term which is significant (p-value < 0.05) indicates that the relationship between the factor and the response follows a curved line.

The ANOVA results for hardness were presented in **Table 4**. The coefficient of determination (R^2) was 98.09%, which indicates that 98.09% of the sample variation in the hardness, was attributed to the factors X_1 and X_2. All terms were significant at p-value < 0.050, except for the squared term of X_1X_1 and interaction term of X_1X_2 which is not significant. The squared term which is significant (p-value < 0.05) indicates that the relationship between the factor and the response follows a curved line.

Table 4 shows the data of ANOVA for adhesiveness. The coefficient of determination (R^2) was 99.35%, which indicates that 99.35% of the sample variation in the adhesiveness, was attributed to the factors X_1 and X_2. All terms were significant at p-value < 0.050, except for the interaction term of X_1X_2 which is not significant. The squared term which is significant (p-value < 0.05) indicates that the relationship between the factor and the response follows a curved line.

The ANOVA data of cohesiveness were tabulated in **Table 4**. The coefficient of determination (R^2) was 98.62%, which indicates that 98.62% of the sample variation in the cohesiveness, was attributed to the factors X_1 and X_2. All terms were significant at p-value < 0.050. The squared term which is significant (p-value < 0.05) indicates that the relationship between the factor and the response follows a curved line.

The data variation in relation to the fitted model is measured by the lack-of-fit test. It is critical to validate the proposed model's fit using accurate data obtained. The lack-of-fit test is significant ($p<0.05$) if the model does not match the data well [24]. If the results of the lack-of-fit test showed any significance, the model should be rejected [22]. Based on the results, this model has accurately predicted the protein, TPC, FRAP, hardness, adhesiveness, and cohesiveness by showing insignificant lack-of fit ($p>0.05$) with a p-value of 0.700, 0.150, 0.091, 0.600, 0.195, and 0.441, respectively.

TABLE 3
ANOVA FOR THE FITTED QUADRATIC POLYNOMIAL MODEL FOR THE RESPONSE VARIABLE (PROTEIN, TPC, AND FRAP)

Source	DF	Adj SS	Adj MS	F-Value	P-Value	Status
Protein (R^2 = 99.04% R^2 (adj) = 98.35%)						
Regression	5	506.546	101.309	144.21	0.000	Significant
Linear	2	288.223	144.111	205.13	0.000	Significant
X_1	1	189.561	189.561	269.83	0.000	Significant
X_2	1	98.662	98.662	140.44	0.000	Significant
Square (quadratic)	2	194.460	97.230	138.40	0.000	Significant
X_1X_1	1	64.973	64.973	92.49	0.000	Significant
X_2X_2	1	152.112	152.112	216.52	0.000	Significant
Way Interaction	1	23.863	23.863	33.97	0.001	Significant
X_1X_2	1	23.863	23.863	33.97	0.001	Significant
Residual Error	7	4.918	0.703			
Lack-of-Fit	3	1.105	0.368	0.39	0.770	Not significant
Pure Error	4	3.813	0.953			
Total	12	511.464				
TPC (R^2 = 99.70% R^2 (adj) = 99.49%)						
Regression	5	180.299	36.0597	468.52	0.000	Significant

Linear	2	138.944	69.4719	902.65	0.000	Significant
X_1	1	76.075	76.0749	988.44	0.000	Significant
X_2	1	62.869	62.8688	816.85	0.000	Significant
Square (quadratic)	2	35.130	17.5649	228.22	0.000	Significant
X_1X_1	1	13.669	13.6689	177.60	0.000	Significant
X_2X_2	1	25.758	25.7582	334.68	0.000	Significant
Way Interaction	1	6.225	6.2250	80.88	0.000	Significant
X_1X_2	1	6.225	6.2250	80.88	0.000	Significant
Residual Error	7	0.539	0.0770			
Lack-of-Fit	3	0.378	0.1259	3.13	0.150	Not significant
Pure Error	4	0.161	0.0402			
Total	12	180.837				
FRAP (R^2 = 99.08% R^2 (adj) = 98.43%)						
Regression	5	2.10560	0.42112	151.03	0.000	Significant
Linear	2	0.52071	0.26036	93.37	0.000	Significant
X_1	1	0.11031	0.11031	39.56	0.000	Significant
X_2	1	0.41040	0.41040	147.18	0.000	Significant
Square (quadratic)	2	1.57849	0.78925	283.05	0.000	Significant
X_1X_1	1	0.03682	0.03682	13.20	0.008	Significant
X_2X_2	1	1.45445	1.45445	521.61	0.000	Significant
Way Interaction	1	0.00640	0.00640	2.30	0.174	Not significant
X_1X_2	1	0.00640	0.00640	2.30	0.174	Not significant
Residual Error	7	0.01952	0.00279			
Lack-of-Fit	3	0.01504	0.00501	4.48	0.091	Not significant
Pure Error	4	0.00448	0.00112			
Total	12	2.12512				

[1]DF = degree of freedom, Adj SS = adjusted sum of square, Adj MS = adjusted mean square, F = Fischer, P = probability

TABLE 4
ANOVA FOR THE FITTED QUADRATIC POLYNOMIAL MODEL FOR THE RESPONSE VARIABLE (HARDNESS, ADHESIVENESS, AND COHESIVENESS)

Source	DF	Adj SS	Adj MS	F-Value	P-Value	Status
Hardness (R^2 = 98.09% R^2 (adj) = 96.73%)						
Regression	5	725861	145172	72.08	0.000	Significant
Linear	2	491837	245919	122.10	0.000	Significant
X_1	1	470526	470526	233.63	0.000	Significant
X_2	1	21312	21312	10.58	0.014	Significant
Square (quadratic)	2	231694	115847	57.52	0.000	Significant
X_1X_1	1	790	790	0.39	0.551	Not significant
X_2X_2	1	230482	230482	114.44	0.000	Significant
Way Interaction	1	2330	2330	1.16	0.318	Not significant
X_1X_2	1	2330	2330	1.16	0.318	Not significant
Residual Error	7	14098	2014			
Lack-of-Fit	3	4843	1614	0.70	0.600	Not significant
Pure Error	4	9255	2314			
Total	12	739959				
Adhesiveness (R^2 = 99.35% R^2 (adj) = 98.89%)						
Regression	5	75030.9	15006.2	215.05	0.000	Significant
Linear	2	13416.5	6708.2	96.13	0.000	Significant
X_1	1	13386.1	13386.1	191.83	0.000	Significant
X_2	1	30.4	30.4	0.44	0.530	Not significant
Square (quadratic)	2	61569.0	30784.5	441.16	0.000	Significant
X_1X_1	1	42370.2	42370.2	607.19	0.000	Significant
X_2X_2	1	26969.7	26969.7	386.49	0.000	Significant
Way Interaction	1	45.5	45.5	0.65	0.446	Not significant
X_1X_2	1	45.5	45.5	0.65	0.446	Not significant
Residual Error	7	488.5	69.8			
Lack-of-Fit	3	320.1	106.7	2.53	0.195	Not significant
Pure Error	4	168.4	42.1			
Total	12	75519.4				
Cohesiveness (R^2 = 98.62% R^2 (adj) = 97.63%)						
Regression	5	0.063711	0.012742	100.06	0.000	Significant
Linear	2	0.055798	0.027899	219.09	0.000	Significant
X_1	1	0.055214	0.055214	433.60	0.000	Significant
X_2	1	0.000585	0.000585	4.59	0.069	Not significant
Square (quadratic)	2	0.004372	0.002186	17.17	0.002	Significant
X_1X_1	1	0.002992	0.002992	23.49	0.002	Significant
X_2X_2	1	0.000882	0.000882	6.93	0.034	Significant
Way Interaction	1	0.003540	0.003540	27.80	0.001	Significant
X_1X_2	1	0.003540	0.003540	27.80	0.001	Significant
Residual Error	7	0.000891	0.000127			
Lack-of-Fit	3	0.000426	0.000142	1.22	0.411	Not significant
Pure Error	4	0.000465	0.000116			
Total	12	0.064602				

[1]DF = degree of freedom, Adj SS = adjusted sum of square, Adj MS = adjusted mean square, F = Fischer, P = probability

Optimum condition of response surface analysis

The table of comparison values of target, maximum, and minimum with predicted responses for different optimum conditions are shown in **Table 6, Table 7**, and **Table 8**, respectively. The aim of conducting optimisation is to obtain the best combination of collagen and curcumin in producing IFRC with the maximum value of protein, TPC, FRAP, adhesiveness, and cohesiveness while minimum value of hardness. The experiment feasibilities were determined by plotting optimum condition of X_1 and X_2 on the overlaid

contour plot of target, maximum and minimum. The optimum condition is feasible when the plot is on the white area, (target and maximum) while it is not feasible when the plot is on grey area (minimum). Since target and maximum condition show the same values of predicted responses, it can be concluded that target and maximum are equivalent. Target was chosen because it is more optimum compared to maximum. The optimised values of factors were collagen (X_1) = 7.96 g and curcumin (X_2) = 361 mg.

Contour plot and surface plot of responses

The 2D contour plots are the graphical demonstration of the regression equation which is used to illustrate the function of two factors, collagen and curcumin concentration while maintaining other factors at a fixed level. In addition, 3D surface plots are the graphical representatives of the regression equation which is used to demonstrate the function of two factors.

From the analysis, ANOVA and a regression coefficient model were used in analysing the effect of factors on protein. Contour and surface plots, were used for better illustration which showed the effect of collagen and curcumin on the protein on IFRC are shown in **Fig 1**.

As shown in **Fig 1**, contour plot shows a circular pattern. The highest protein is observed at collagen about 10-11 g and curcumin between 200-250 mg. This statement is supported by Hashim et al. [9], whereas drinks added with collagen able to increase the protein content. As illustrated in surface plot, it shows that as collagen and curcumin increase, the protein also increases except for curcumin between 300 - 400 mg, where it shows a decreasing trend. This result is similar to Meshkibaf et al. [25], whereas the addition of curcumin able to increase the protein level but the protein level drop as the addition of curcumin reach a certain amount. This means that protein level depends on the amount of curcumin.

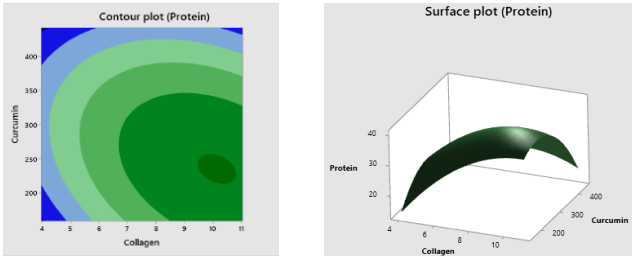

Fig. 1 Contour plot and surface plot of collagen vs curcumin for protein

From the analysis, ANOVA and regression coefficient model were used in analysing the effect of factors on TPC. Contour and surface plots, which showed the effect of collagen and curcumin on the TPC of IFRC, were used for better demonstration are shown in **Fig 2**.

As demonstrated in **Fig 2**, contour plot shows an ellipse pattern. The highest TPC is detected at 11 g of collagen and 400 mg of curcumin. This result agrees with statement by Tripathy et al. [7], which stated that addition of curcumin in crackers improved the phenolic content and resulted in a high antioxidant capacity. The addition of curcumin in waffles also increased the antioxidant capacity [26]. As shown in surface plot, it was observed that TPC increases as collagen and curcumin increased, which could be due to the antioxidant capacity from curcumin and collagen. This result is similar with study by Tóth et al. [27], whereas collagen contributes in antioxidant capacity of egg white-based beverage and collagen also have positive correlation with TPC. Moreover, the concentration of curcumin increases, TPC increased. This result is supported by Adegoke et al. [10], which found that the TPC increased with an increase in the level of substitution of soybean flour and curcumin in the production of functional biscuits. In an earlier study, Lim and Han [28] also discovered that increasing the addition of curcumin up to 10% improved antioxidant properties of yukwa (fried rice snack) due to the high functional phenolic compound in turmeric powder.

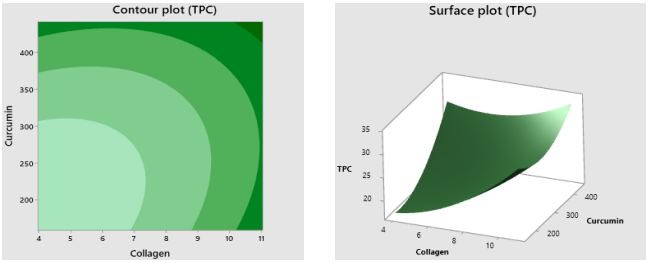

Fig. 2 Contour plot and surface plot of collagen vs curcumin for TPC

Besides, ANOVA and regression coefficient model were used in analysing the effect of factors on FRAP. Contour and surface plots, which showed the effect of collagen and curcumin on the FRAP of IFRC, were used for better demonstration are shown in **Fig 3**.

As illustrated in **Fig 3**, contour plot shows an ellipse pattern. FRAP value is the highest at collagen 10-11 g and curcumin above 400 mg. Curcumin is responsible for the highest value of FRAP because of its high antioxidant content. Collagen also contributes to antioxidant capacity [29]. However, the interaction between collagen and curcumin shows no significant difference ($p>0.05$) in FRAP even though both factors show positive correlation with FRAP. This result is in agreement with Varga-Tóth et al. [27], whereas it is not statistically significant between mixed berries that contain high antioxidant and collagen. As shown in surface plot, as collagen and curcumin increase, the FRAP value also increases. A similar result by Varga-Tóth et al. [27] reported that a higher concentration of mixed berries and collagen gives the highest FRAP value, total antioxidant capacity. Therefore, it can be concluded that both factors (collagen and curcumin) give positive effect on FRAP analysis.

TABLE 5
COMPARISON VALUES OF TARGET AND PREDICTED RESPONSES FOR DIFFERENT OPTIMUM CONDITIONS AND EXPERIMENT FEASIBILITIES

	Goal	Lower	Target	Upper	Optimum Condition		Predicted Response						F/NF
					X_1	X_2	Y_1 (FITS1)	Y_2 (FITS2)	Y_3 (FITS3)	Y_4 (FITS 4)	Y_5 (FITS 5)	Y_6 (FITS6)	
Target	Protein	22.22	38.97	38.98	7.96	361.42	33.08	24.16	6.09	576.22	-331.99	0.59	F
	FITS 1	22.17	39.57	39.58									
	TPC	17.28	29.36	29.37									
	FITS 2	17.43	29.19	29.20									
	FRAP	5.49	6.94	6.95									
	FITS 3	5.45	6.98	6.99									
	Hardness	229.391	1021.743	1021.744									
	FITS 4	213.820	1046.419	1046.420									
	Adhesiveness	-155.308	-372.814	-372.815									
	FITS 5	-150.258	-364.192	-364.193									
	Cohesiveness	0.418	0.672	0.673									
	FITS 6	0.411	0.664	0.665									

[1]X_1 = collagen (g), X_2 = curcumin (mg), Y_1 = Protein (%), Y_2 = Total Phenolic Content (mg GAE/g sample), Y_3 = Ferric Reducing Antioxidant Power (mg TE/ g sample), Y_4 = Hardness (g), Y_5 = Adhesiveness (g/s) and Y_6 = Cohesiveness

TABLE 6
COMPARISON VALUES OF MAXIMUM AND PREDICTED RESPONSES FOR DIFFERENT OPTIMUM CONDITIONS AND EXPERIMENT FEASIBILITIES

	Goal	Lower	Target	Upper	Optimum Condition		Predicted Response						F/NF
					X_1	X_2	Y_1 (FITS1)	Y_2 (FITS2)	Y_3 (FITS3)	Y_4 (FITS4)	Y_5 (FITS5)	Y_6 (FITS6)	
Maximum	Protein	22.22	38.98	38.98	7.96	361.42	33.08	24.16	6.09	576.22	-331.99	0.59	F
	FITS 1	22.17	39.58	39.58									
	TPC	17.28	29.37	29.37									
	FITS 2	17.43	29.20	29.20									
	FRAP	5.49	6.95	6.95									
	FITS 3	5.45	6.99	6.99									
	Hardness	229.391	1021.744	1021.744									
	FITS 4	213.820	1046.420	1046.420									
	Adhesiveness	-155.308	-372.815	-372.815									
	FITS 5	-150.258	-364.193	-364.193									
	Cohesiveness	0.418	0.673	0.673									
	FITS 6	0.411	0.665	0.665									

[1]X_1 = collagen (g), X_2 = curcumin (mg), Y_1 = Protein (%), Y_2 = Total Phenolic Content (mg GAE/g sample), Y_3 = Ferric Reducing Antioxidant Power (mg TE/ g sample), Y_4 = Hardness (g), Y_5 = Adhesiveness (g/s) and Y_6 = Cohesiveness

TABLE 7
COMPARISON VALUES OF MINIMUM AND PREDICTED RESPONSES FOR DIFFERENT OPTIMUM CONDITIONS AND EXPERIMENT FEASIBILITIES

	Goal	Lower	Target	Upper	Optimum Condition		Predicted Response						F/NF
					X_1	X_2	Y_1 (FITS1)	Y_2 (FITS2)	Y_3 (FITS3)	Y_4 (FITS4)	Y_5 (FITS5)	Y_6 (FITS6)	
Minimum	Protein	22.22	22.22	38.98	4.04	158.57	14.70	17.09	6.13	701.99	-150.35	0.37	NF
	FITS 1	22.17	22.17	39.58									
	TPC	17.28	17.28	29.37									
	FITS 2	17.43	17.43	29.20									
	FRAP	5.49	5.49	6.95									
	FITS 3	5.45	5.45	6.99									
	Hardness	229.391	229.391	1021.744									
	FITS 4	213.820	213.820	1046.420									
	Adhesiveness	-155.308	-155.308	-372.815									
	FITS 5	-150.258	-150.258	-364.193									
	Cohesiveness	0.418	0418	0.673									
	FITS 6	0.411	0.411	0.665									

[1]X_1 = collagen (g), X_2 = curcumin (mg), Y_1 = Protein (%), Y_2 = Total Phenolic Content (mg GAE/g sample), Y_3 = Ferric Reducing Antioxidant Power (mg TE/ g sample), Y_4 = Hardness (g), Y_5 = Adhesiveness (g/s) and Y_6 = Cohesiveness

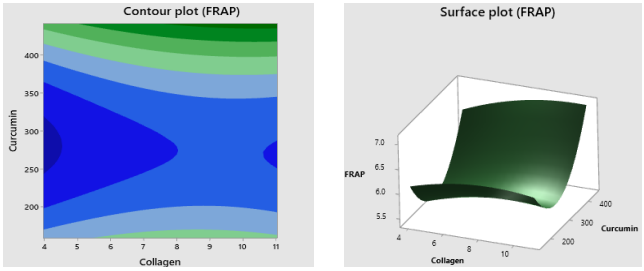

Fig. 3 Contour plot and surface plot of collagen vs curcumin for FRAP

Apart from that, ANOVA and regression coefficient model were used in analysing the effect of factors on hardness. Contour and surface plots, which showed the effect of collagen and curcumin on the hardness of IFRC, were used for better demonstration are shown in **Fig 4**. As demonstrated in **Fig 4**, contour plot shows ellipse pattern. The hardness value is the lowest at collagen above 11 g and curcumin more than 400 mg. Hardness decreases when the amount of functional ingredients increases. This statement supported by Shim and Lim [30], whereas hardness of porridges decreases with the addition of various beans. However, the interaction between collagen and curcumin shows no significant difference ($p>0.05$) in hardness even though both factors show positive correlation with hardness. As shown in surface plot, hardness value is the highest at lowest collagen, and curcumin at 300 mg. Hardness is high because of low concentration of protein from functional ingredients to interrupt starch from swelling and absorb water which resulted in harder texture [21].

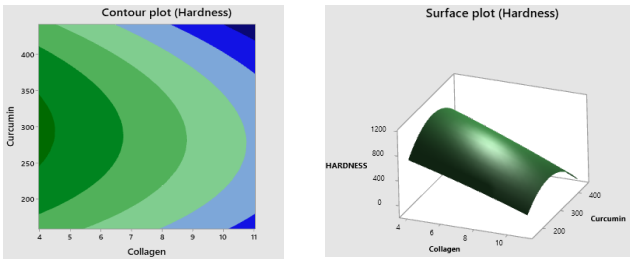

Fig. 4 Contour plot and surface plot of collagen vs curcumin for hardness

Furthermore, ANOVA and regression coefficient model were used in analysing the effect of factors on adhesiveness. Contour and surface plots, which showed the effect of collagen and curcumin on the adhesiveness of IFRC, were used for better demonstration are shown in **Fig 5**.

As shown in **Fig 5**, contour plot shows a circular pattern. Adhesiveness is the highest at 7 g of collagen and 300 mg of curcumin. This means that beyond this point, the addition of collagen and curcumin will decrease the value of adhesiveness. This is supported by Shim and Lim [30], whereas addition of mixed grains into porridges decreased adhesiveness for about 82.8%. However, curcumin shows no significant differences ($p>0.05$) in adhesiveness, which suggests that curcumin does not affect the adhesiveness value in IFRC. This statement is supported by Thuy et al. [26], whereas the addition of turmeric starch (mainly curcumin) is not significantly different ($p>0.05$) in adhesiveness of waffles. A study by Hleap-Zapata et al. [31] also discover that turmeric flour does not give significant difference in adhesiveness. Based on surface plot in **Fig 5**, the centre point of collagen and curcumin gives the highest value of adhesiveness. This shows that the best concentration of collagen and curcumin for adhesiveness value is in the centre point.

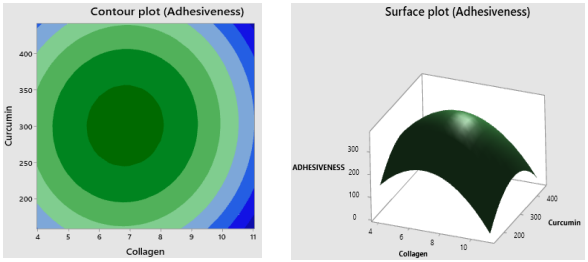

Fig. 5 Contour plot and surface plot of collagen vs curcumin for adhesiveness

Lastly, ANOVA and regression coefficient model were used in analysing the effect of factors on cohesiveness. Contour and surface plots, which showed the effect of collagen and curcumin on the cohesiveness of IFRC, were used for better illustration are shown in **Fig 6**.

As illustrated in **Fig 6**, contour plot shows an ellipse pattern. The highest cohesiveness is observed at 11 g of collagen and less than 200 mg of curcumin. Thus, it meant that at this point, sample is likely to hold strongly its texture after compression. Curcumin has no significant differences ($p>0.05$) towards cohesiveness value. Therefore, cohesiveness value is consistent with the increase addition of curcumin. This result is in agreement with study by Hleap-Zapata et al. [31], whereas there is no significant difference ($p>0.05$) in cohesiveness value of chorizo (raw meat product) with addition of turmeric flour. Based on surface plot in Figure 6, the cohesiveness value increases as the collagen increases while increasing the curcumin content maintain the value of cohesiveness. It is proven that collagen gives significant difference towards cohesiveness. This result is supported by Fan et al. [32] whereas the addition of collagen gives significant differences ($p<0.05$) in cohesiveness value of sausage products. According to Noor Farisya et al. [33] collagen contributes to the rise in cohesiveness and adhesiveness of instant fortified rice congee.

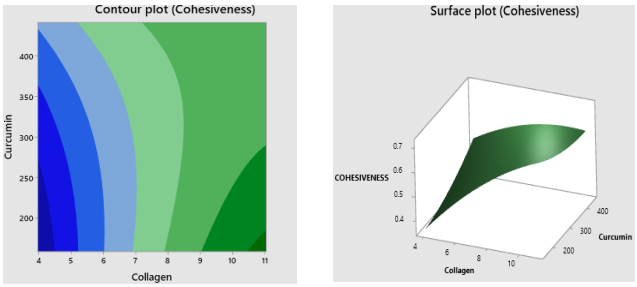

Fig. 6 Contour plot and surface plot of collagen vs curcumin for cohesiveness

Optimisation of IFRC production and experimental validation models

Experimental validation is the final step in the modelling process, and was used to verify the accuracy of the predicted model (regression coefficient model). A validation experiment was carried out under the optimal conditions obtained from the optimisation of target (**Table 8**). This verified the predictability of the model with a comparison of the experimental (actual) values against the predicted figures. The experimental values are presented in **Table 8** with the following values: Y_1, Y_2, Y_3, Y_4, Y_5, and Y_6 are 32.41%, 24.75 mg GAE/g sample, 6.03 mg TE/g sample, 581.702 g, -329.239 g/s, and 0.600, respectively. In comparison, the model predicted values for Y_1, Y_2, Y_3, Y_4, Y_5, and Y_6 were 33.08%, 24.16 mg GAE/g sample, 6.09 mg TE/g sample, 576.225 g, -331.994 g/s, and 0.591, respectively.

TABLE 8
THE COMPARISON VALUES OF EXPERIMENTAL AND PREDICTED VALUES OF RESPONSE VARIABLES UNDER THE OPTIMAL CONDITIONS

Responses (Y)	**Experimental value**	**Predicted value**	**p-value**
Protein, Y_1 (%)	32.41±1.81[a]	33.08[a]	0.586
TPC, Y_2 (mg GAE/g sample)	24.75±3.92[a]	24.16[a]	0.817
FRAP Y_3 (mg TE/g sample)	6.03±0.15[a]	6.09[a]	0.574
Hardness Y_4 (g)	581.70±19.21[a]	576.22[a]	0.669
Adhesiveness Y_5 (g/s)	-329.23±2.12[a]	-331.99[a]	0.158
Cohesiveness Y_6	0.60±0.13[a]	0.59[a]	0.915

Values are expressed in mean±standard deviation (n=3). Means within the same column followed by same superscript lowercase letters indicate no significant differences ($p>0.05$) by Tukey's multiple comparisons test.

Optimisation using actual experimental values was tested using the t-test (SPSS version 28). There are no significant differences ($p>0.05$) detected between experimental and predicted values for any of the response's variables. Hence, this is implying that the RSM-based empirical model can adequately describe the relationship between the independent variables and the target response and, therefore, successfully reveal the optimum process condition in producing IFRC, whereas 7.96 g of collagen and 361 mg of curcumin. IFRC developed using the optimised processing conditions demonstrates potential sensory acceptability among elderly consumers. A sensory evaluation conducted by Noor Farisya et al. [33] revealed that instant rice congee fortified with 12.5 g of collagen and 500 mg of curcumin achieved taste and overall acceptability scores of 5.82±1.73 and 6.56±1.50, respectively, indicating favourable sensory attributes.

IV. CONCLUSION

This research successfully identified the optimisation of protein, TPC, FRAP, hardness, adhesiveness and cohesiveness by adding 7.96 g of collagen and 361 mg of curcumin in IFRC using RSM. It can serve as a food source to fulfil the recommended daily intakes of collagen and curcumin. It was observed that target amount of collagen and curcumin incorporated in IRC showed the best condition of IFRC with the highest results in protein content (32.41%), TPC (24.75 mg GAE/ g sample), FRAP (6.03 mg TE/ g sample), adhesiveness (-329.23 g/s), and cohesiveness (0.60) while the lowest value in hardness (581.70 g). The findings of this research provide practical insights into the effective utilisation of collagen and curcumin as functional ingredients in the formulation of food and beverage products. Additionally, this study has the potential to contribute to the development of functional foods designed to possess desirable texture and nutritional composition, specifically targeting the health needs of the elderly.

ACKNOWLEDGMENT

We would like to thank Universiti Teknologi MARA, Malaysia for financially support of MyRA Research Grant (Grant number: 600-RMC/GPM ST 5/3 (012/2021)) and the Geran Insentif Penyeliaan (grant number: 600-RMC/GIP 5/3 (114/2022)). The authors also acknowledge the sponsorship of materials by DKSH Performance Materials Malaysia Sdn. Bhd. for this study.

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