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Textural Optimisation Of Fish-Gelatine Stingless Bee Honey Gummies By Response Surface Methodology

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Abstract

Due to concerns over the halal status of gelatine traditionally derived from porcine and bovine sources, the demand for halal-certified alternatives is growing that ensure authenticity and meet dietary requirements, particularly in the gummy industry. Developing this product not only embrace the cultural and religious requirements but also expands the opportunities for the pharmaceutical applications in delivering nutraceutical ingredients. The present work aimed to investigate the effect of starch, fish gelatine and stingless bee honey (SBH) and to optimise their concentration using Response Surface Methodology (RSM) for a formulation that meets target criteria of balanced springiness, cohesiveness, and minimised hardness suitable for halal-certified gummy products. Response Surface Methodology (RSM) with a Central Composite Design (CCD) was employed to optimise. Gummies were prepared using a cold mixing technique then followed by textural analysis, pH value determination, storage stability testing, and microbiological testing. The study factors used were starch (15-25 g), fish gelatine (35-45 g) and (SBH 20-30 g) with 18 experimental runs. The runs included 4 centre points, and the targeted responses were springiness (0.9–1.2 mm, goal 1.04 mm), cohesiveness (0.9–1.14, goal 0.98), and hardness (31–107.7 g, minimised) to satisfy the market standards for gummy texture. For cohesiveness (significant model, $p = 0.0021$ with critical interaction factors AC and quadratic terms A and B significant), hardness (significant model, $p = 0.0333$; fish gelatine significant, $p = 0.007$), the fitted models were quadratic. In contrast, the springiness model (non-significant, $p = 0.2923$). The springiness was considered only as observe texture attribute rather than a primary optimisation target. The optimised formulation (15 starch, 37 fish gelatine, and 25 SBH) was validated, the predicted and actual values closely matched, with only slight variations (hardness deviation within acceptable limits, cohesiveness error ~0.25%, and springiness error ~2.2%). Preliminary storage stability testing over 14 days' period showed the gummies maintained the textural quality. Microbiological evaluation demonstrated no growth of mould and bacterial also maintained the product safety. These findings showed that RSM with CCD can guide the formulation of fish gelatine and SBH-based gummies to achieve desirable cohesiveness and hardness while preserving the safety and texture gummies features.

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Introduction

The incorporation of SBH into gummy formulations presents unique challenges due to the honey's distinct physicochemical properties, which can affect the texture, stability, and shelf- life of the gummies (Esa *et al*., 2022). According to Nordin *et al.* (2018), SBH was observed with higher moisture content than *Apis mellifera* honey. The moisture content of SBH ranging from 13.26 g/100 g to 45.8 g/100 g with a mean of 28.6 g/100 g whereas the International Honey Commission (IHC) threshold for good quality *Apis mellifera* honey is set at 20 g/100 g. Additionally, 97% of 498 honey samples analysed exceeded this threshold, highlighting the consistently higher moisture content in stingless bee honey. The moisture level is an important component in determining honey quality because high moisture content can reduce a honey's shelf life and microbiological stability (Esa *et al*., 2022). According to Marfil *et al.* (2012), gummy jelly is a food made of gel ingredients which contain gelatin. Gummy manufacturing often utilises gelatin because of its thickening, stabilising, and gelling characteristics.

Table 1 : Moisture content comparison between stingless bee honey (SBH) and *Apis mellifera* honey.

Parameter	SBH	*Apis Mellifera* Honey
Moisture Content Range	13.26 g/100 g to 45.8 g/100 g	Standard threshold ≤ 20 g/100 g
Mean Moisture Content	28.6 g/100 g	≤ 20 g/100 g (IHC quality threshold)
Percentage of Samples Exceeding Threshold	97% of 498 samples exceed 20 g/100 g	Threshold for good quality honey
Quality Implication	Higher moisture reduces shelf life & microbiological stability	Lower moisture favours longer shelf life & stability

Since gelatine is traditionally derived from animal tissues such as porcine and bovine, Muslim consumers may be concerned about whether it is halal or not. Porcine gelatine is prohibited in Islam, and bovine gelatine is only halal if it comes from animals that have been slaughtered in accordance with Islamic law (Salama *et al*, 2022). As Muslim consumers increasingly look for assurance about the authenticity and integrity of halal products, there is a growing demand for accurate identification methods and verification of halal-certified goods (Rahma *et al*., 2025). Thus, the necessity for halal alternatives that can meet customer preferences without compromising dietary requirements is essential. Due to this circumstance, there is a need in the market for halal substitutes, especially in the functional foods industry.

The use of fish gelatine can be used as an alternative to bovine or porcine gelatine because it is from permissible sources according to the sharia law. Even though it is not commonly used in the market, fish gelatine exhibits excellent gel forming ability. According to Reza *et al*. (2023), the fish-based gelatine exhibits promising gelling properties in the food and pharmaceutical industry and acts as an alternative to the other gelatines. From the same studies it has been shown that fish-based gelatine possesses similar physicochemical properties to mammalian gelatine, making it suitable for use in a variety of food applications, including gummies. Fish gelatine is still underutilised because of the challenges in production and lack of awareness. It has potential in the halal processed food and pharmaceutical industries highlights a significant innovation for meeting the growing demand for halal products worldwide (Nurilmala et al.*, 2021*).

Response Surface Methodology (RSM) optimises features and qualities by taking into consideration how the components and their interactions influence the responses. This approach is appropriate for food texture optimisation, where several variables simultaneously affect the final product quality, since it effectively models complex relationships and identifies the optimal variable settings (Ghodsi & Nouri, *2024*). This research was examined to optimise the fish gelatine gummies containing SBH by formulating gummies with desired hardness, springiness and cohesiveness using CCD-RSM approach. The formulation of gummies plays critical roles to determine the texture, stability, and mouthfeel of the final product. The chewy texture of the gummy comes from fish gelatine which is rich in amino acids such as glycine, proline, and lysine that provide its thickening and gelling properties (Normah *et al*., 2015). Starch plays a crucial role in controlling firmness and setting properties. Adjusting the starch-to-gelatin ratio allows for the customization of gummy textures, which can range from soft and elastic to firm and brittle. For instance, stringiness and adhesiveness were significantly influenced by gelatin, as stringy and adhesive gels were produced when the concentration of gelatin was reduced (Marfil *et al*., 2012). In contrast, the value of adhesiveness and stringiness will be increased as the starch concentration increases in the gummy (Tarahi *et al*., 2023). Several gelatine sources have been used in attempts to add SBH to gummy compositions which contain SBH and *Nigella sativa* (black seed) oil (A. So'bah *et al.,* 2024). Bovine and porcine gelatines were used in earlier studies, but concerns about halal compliance led to interest in alternatives.

This study aims to develop and optimise fish gelatine-based SBH gummies with desirable springiness, cohesiveness, and hardness, providing a halal alternative with functional benefits.

Materials and methods

Materials

Fish gelatine (Awanti, Sungai Buloh, Malaysia); however, the supplier company did not state any specific fish species from which the gelatine was derived, natural erythritol (Madina, Kuala Lumpur, Malaysia), potato starch (KSFE, Selangor, Malaysia), potassium sorbate (R&M Chemical Company, Selangor, Malaysia), stingless bee honey from Bukit Kuin, Kuantan (Kuin Honey, Kuantan, Malaysia), All other chemicals used were of analytical grade.

Method

Total Phenolic Content (TPC)

TPC content was assessed following the technique according to Agussalim *et al.* (2022) using the spectrophotometric method with Folin-Ciocalteu reagent. Firstly, the sample preparation 2 grams of honey were placed in a 10-mL tube to prepare 20% (w/v) solution. Then the mixture was followed by the addition of 0.5 mL of Folin-Ciocalteu reagent and 7.5 mL of aquabidest. The mixture was allowed to stand for 10 minutes at 18–25 °C, after which 1.5 mL of 20% (w/v) sodium carbonate solution was added. Then, the final volume was adjusted to 10 mL with aquabidest. The solution absorbance was measured at a wavelength of 760 nm and a standard curve was created using 10 mg of gallic acid. The sample was prepared with the same procedure as the sample. The concentrations of gallic acid for the standard curve was prepared using gallic acid (0 - 140 μg/mL, Y = 0.08220x + 0.001566, R^2 = 0.9973). All analyses were performed in triplicate (n=3). Results were expressed as mg of gallic acid equivalents (mg GAE)/100 g of honey.

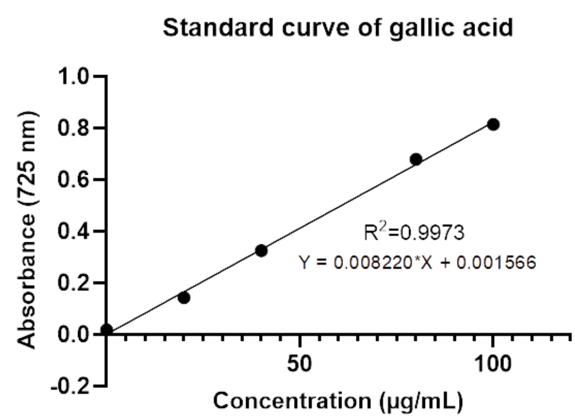


Figure 1 Standard curve of gallic acid with regression Y= 0.008220X + 0.001566 (R^2 = 0.9973).

Total Flavonoid Content (TFC)

TFC was assessed using the spectrophotometric method. According to some researchers, the antioxidant activity of honey increases with its flavonoid content (Moniruzzaman *et al*., 2013; Castiglioni *et al*., 2017). Total flavonoid content was believed to result from plant nectar, pollen and propolis (Albu *et al*., 2022). A 0.10 g sample of honey was dissolved in 10 mL of aquabidest to prepare a 1% (w/v) solution. To this, 0.3 mL of sodium citrate and 0.6 mL of 10% (w/v) aluminium chloride were added. The mixture was stored for five minutes then 2 mL of 1 M sodium hydroxide and aquabidest were added to adjust the final volume to 10 mL. After mixing thoroughly, the mixture absorbance was measured at a wavelength of 510 nm. A standard curve was created with 10 mg of quercetin obtained using the same sample preparation methodology. The concentrations of quercetin for the standard curve were prepared using standard solution of quercetin (0 - 0.742 µg/mL, Y = 0.007376 + 0.004381, R^2 = 0.9961). All analyses were performed in triplicate (n=3). Results were expressed as mg of quercetin equivalents (mg QE)/100g of honey.

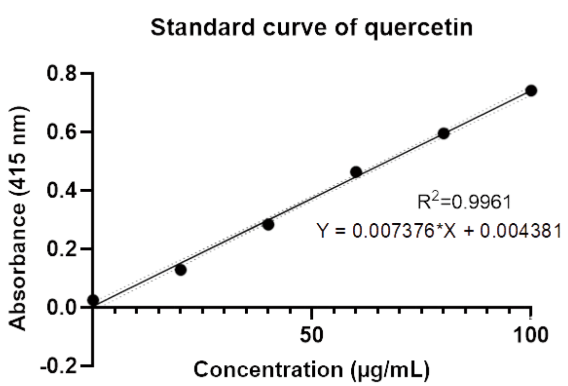


Figure 2: Standard curve of quercetin with regression = 0.007376X + 0.004381 (R^2 = 0.9961)

DPPH Activity

Antioxidant activity was determined by assessing its free radical-scavenging ability of DPPH (2,2-diphenyl-1-picrylhydrazyl) followed by the technique by Gül & Pehlivan, (2018) and Agussalim *et al*., (2022). For the assay, 0.1 mL of honey which was 20% (w/v) was mixed with 0.9 mL of 0.1 mM DPPH methanolic solution. The mixture was shaken. Then, the mixture was then stored in the dark for 30 minutes. After 30 minutes the absorbance of the solution was measured at 517 nm against a methanol blank. The wavelength was chosen because when the free radical DPPH encounters an odd electron, it shows maximum light absorption at a wavelength of 517 nm, giving it a purple color. Antioxidants which act as free-radical scavengers interact with DPPH then turn it to DPPHH. This new form DPPH after H-atom transfer (DPPH-H) absorbs less light than DPPH due to the increased number of hydrogen atoms. The radical form changes to the DPPH-H state when additional electrons are taken up, leading to a loss of color then resulting in a yellow tint (Baliyan *et al*., 2022). The assay was conducted in triplicate (n=3), and results were expressed as % DPPH inhibition.

Preparation of Gummies

According to Mutlu et al. (2018), the gummies were prepared using the cold mixing technique. The cold mixing technique was highly effective because it can preserve over 95% of the diastase enzyme's activity. Gelatine was dissolved in distilled water at various concentrations ranging from 35 to 45 g/100 mL (% w/v). Then, the mixture was heated while being stirred constantly in a water bath (Memmert Water Bath WNE 29, Schwabach, Memmert, Germany) at 80 °C for 10 min. Simultaneously, starch solutions were prepared by dissolving starch in distilled water at different concentrations ranging from 15 to 25 g/100 mL (% w/v) using a magnetic stirrer at 200 rpm. The starch solution was mixed with the gelatine solution and SBH also added at various concentrations ranging from 20 to 30 g/100 mL (% w/v) in 100 mL total volume. Then, 5 g erythritol and 1 g potassium sorbate were added to the mixture. The mixture was stirred using a magnetic stirrer at 220 rpm for one minute. The product was then placed in the silicone mould and kept in the refrigerator. Each batch size was prepared with 6 gummies with a total mass of 18 grams. The silicone mould dimensions are 2 cm width, 1.8 cm length, and 1.3 cm thickness. For the cooling time, 1 hour at 4 °C was set to reach equilibrium texture before mechanical testing or packaging.

Texture Profile Analysis

The textural characteristics of the gummy candies were assessed utilizing a Brookfield Texture Analyser fitted with a cylindrical probe measuring 35 mm in diameter. The evaluation involved compressing the gummy candies at a crosshead speed of 1 mm/s, employing a load cell with a capacity of 5 kg, a trigger force set at 5 g, and a 1.0 mm deformation. Measurements of hardness (g), cohesiveness, and springiness (mm) were taken following the procedure. (Mutlu *et al*, 2018).

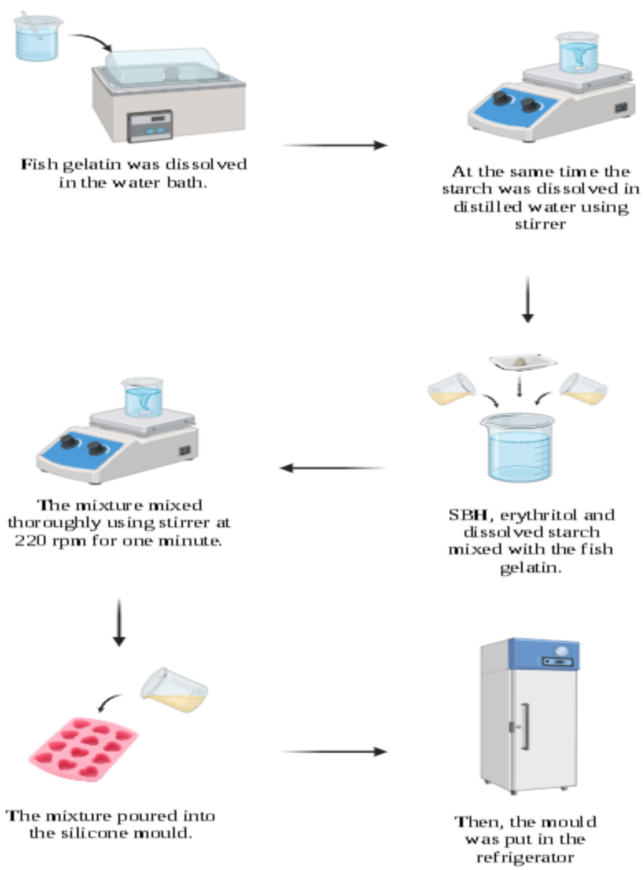


Figure 3: Overview of gummies preparation using Biorender.com

Optimisation of SBH Gummies

To optimize gummy candy formulations, Response Surface Methodology (RSM) utilizing Central Composite Design (CCD) was employed by incorporating three independent variables with four centre points. A total of eighteen experimental trials were conducted to evaluate the impact of these variables which are starch, fish gelatin and stingless bee honey on selected responses, including hardness, cohesiveness, and springiness. Statistical analyses were performed using Design Expert® 11, Stat-Ease Inc., Minneapolis, MN, USA. Numerical optimisation was carried out based on the multiple response desirability function. To validate the predicted optimal concentrations of the mixture and confirm compliance with anticipated responses, experiments were conducted under identical conditions using the determined concentrations of those three variables.

Table 2 Selected variation of proposed model from Design Expert® 11

Starch (%)	15.00
Fish Gelatin (%)	37.00
SBH (%)	25.00
Springiness (mm)	1.045
Cohesiveness	0.98
Hardness (g)	50.48
Desirability	0.872

Determination of pH Value

The pH of the gummy was measured using a pH meter (Mettler Toledo, Zürich, Switzerland) according to the method described by (Delgado & Bañón, 2017) and (Vojvodić Cebin *et al*., 2024). Two gummies (1 g per piece) were cut into pieces and mixed with 8 mL demineralised water to prepare a 20% (w/v) solution. The gummies were then heated in the water bath at 50 °C for 5 minutes until they dissolved with water. The dissolved gummies were carefully transferred to a 10 mL flask. Then the dissolved gummies were left at room temperature for 15 minutes. The pH meter was calibrated prior to measurements using standard buffer solutions at pH 4.00 and 7.00. The pH was measured in triplicate (n=3), and the results are presented as the mean values along with their corresponding standard deviations.

Storage Stability Test

The storage preliminary stability test was conducted according to the previously reported method run by Tireki *et al*., 2021. The prepared gummies were packed into transparent polypropylene zip-lock bags (commercial bags used to store food). The bags were then stored at 30°C for two weeks.

Microbiological Analysis

Total aerobic microbial count (TAMC) and total yeast and mold count (TYMC) enumeration in triplicate plates were used in this analysis to determine the microorganisms present in the gummies. The method was followed by the pour-plate method described by Soheilian *et al.* 2019. In the pour plate method, 1 g of the sample was placed into a sterile petri dish, followed by the addition of 9 mL of culture medium which were Tryptic Soy Agar (TSA) for TAMC and Sabouraud Dextrose Agar (SDA) for TYMC. The contents of the petri dishes were mixed thoroughly, and the culture medium was allowed to solidify at room temperature. The petri dishes were then incubated in an inverted position at 30 to 35 °C up to 3 days for bacterial growth and 20 to 25 °C up to 5 days for yeast growth. The mixtures were plated in triplicates (n=3). The limit of detection is 1 colony-forming unit (cfu) per plate. The criteria for "no growth" was set when absence of any of any bacteria and yeast colonies on plate after incubation and the detection limit was below 10 cfu/g.

Results and discussion

TPC, TPC and DPPH activity Analysis

TPC and TFC and antioxidant activity using the DPPH scavenging assay were used to assess the bioactive properties. According to Table 2, it shows that the Bukit Kuin SBH had a TPC of 57.99 ± 0.381 mg GAE/100g, a TFC of 0.132 ± 0.2562 mg QE/mL, and 66.78 ± 0.4543% DPPH scavenging activity. The observed value of the phenolic content of SBH was align with the previously reported studies about Malaysian SBH that collected from Kelantan, Malaysia and Sabah, Malaysia which range from 33.2 – 60.2 mg GAE/100 g demonstrates its high antioxidant potential due to presence of the phenolic compound. These SBH were analysed not more than nine months and tested within one year duration of storage (Rafiq *et al*., 2021 Ávila *et al*., 2018). Additionally, compared to TPC, the TFC value was very low, demonstrating that flavonoids might not be the main class of phenolic chemicals in this honey. But this value is not aligning with

Table 3: TPC, TPC, and DPPH, of analyzed honey samples (Mean ± SE)

	TPC (mg GAE/100g)	TFC (mg QE/mL)	DPPH scavenging activity (%)
SBH	57.99 ± 0.381	0.132 ± 0.256	66.78 ± 0.454

previous research about stingless bee honey from Kelantan and Sabah, Malaysia which the value ranged from 43.2 – 65.9 mg QE/100 g FW (Rafiq *et al*., 2019). The lower TFC in this study might be attributed because of differences in floral sources or environmental factors influencing the SBH nectar composition (Domínguez-Valhondo *et al*., 2011). The activity of DPPH scavenging observed was 66.78±0.4543%. Strong antioxidant capacity is indicated by 66.78 ± 0.4543%, which shows how effectively the honey neutralizes free radicals. The high scavenging activity is likely due to the synergistic effects of phenolic acids and flavonoids present in the honey. Previously reported research on stingless bee honey stated DPPH inhibition values ranging from 61.43% to 90.28%, which align with this value observed (Agussalim *et al*., 2022). Agus *et al*. (2019) stated that the DPPH antioxidant of SBH had a significant effect on TFC and TPC.

Optimisation of gummies

The numerical optimisation method was used to optimise the multiple responses. The aim of this optimisation was to assess the ideal formulation of gummy that include fish gelatin, starch and SBH. In order to achieve the targeted textural qualities of springiness, cohesiveness, and hardness. The hardness, cohesiveness and springiness of gummies resulted with varying amounts of starch, fish gelatin and SBH. Springiness was ranging from 0.9 mm to 1.2 mm while cohesiveness was ranging from 0.9 to 1.14 and the hardness was ranging from 31 g to 107.7 g. Thus, the fitted models for these parameters were modeled as linear for springiness, quadratic for cohesiveness and linear for hardness with R^2 values between 0.2271 and 0.8059. R^2 values for springiness was low (~0.23) compared to the hardness and cohesiveness indicates a weak model fit. The formulation variables considered do not fully explain the variability in springiness. This limitation implies that other variables or more complex interactions might affect this parameter and suggest for more research.

For the ANOVA results (Table 3) for the responses (springiness, cohesiveness, and hardness) showed the insights into the significance of the model and individual factors. The ANOVA results for springiness suggested that the model is not significant with the Model F-value of 1.37 and p-value of 0.2923 ($p>0.05$). Springiness was excluded from desirability-driven prediction due to non-significant model fit ($p=0.29$). (A-Starch, B-Fish Gelatin, or C-Stingless Bee Honey) showed significant effects on springiness, as all p-values exceeded 0.05. The model's nonexistent significance indicates that additional variables or interactions should be investigated to increase springiness predictability. For cohesiveness, the Model F-value of 7.61 with p-value of 0.0021 ($p<0.05$) demonstrates that the model is significant. There is only a 0.21% chance that an F-value this large could occur due to noise. The interaction between AC and the quadratic terms A^2 and B^2 were the significant terms in this model as their p-values are less than 0.05. Fish gelatin and the quadratic terms A^2 and B^2 highlights their significant role to achieve the optimum cohesiveness. Hardness ANOVA results showed that the Model F-value of 3.86 indicates significance with p-value= 0.0333. Among the factors, only B significantly affects hardness ($p = 0.0070$). A and C are not significant contributors. Fish gelatin is significant in increasing the hardness aligned with the previously reported research that higher the gelatin the higher the hardness of gummy as the hydrogen bonds formed between gelatin of the gummy also increase (Jiamjariyatam, 2018). For the lack of fit test results, all responses fit well with the experimental data. The equations for springiness, cohesiveness, and hardness are expressed as follows:

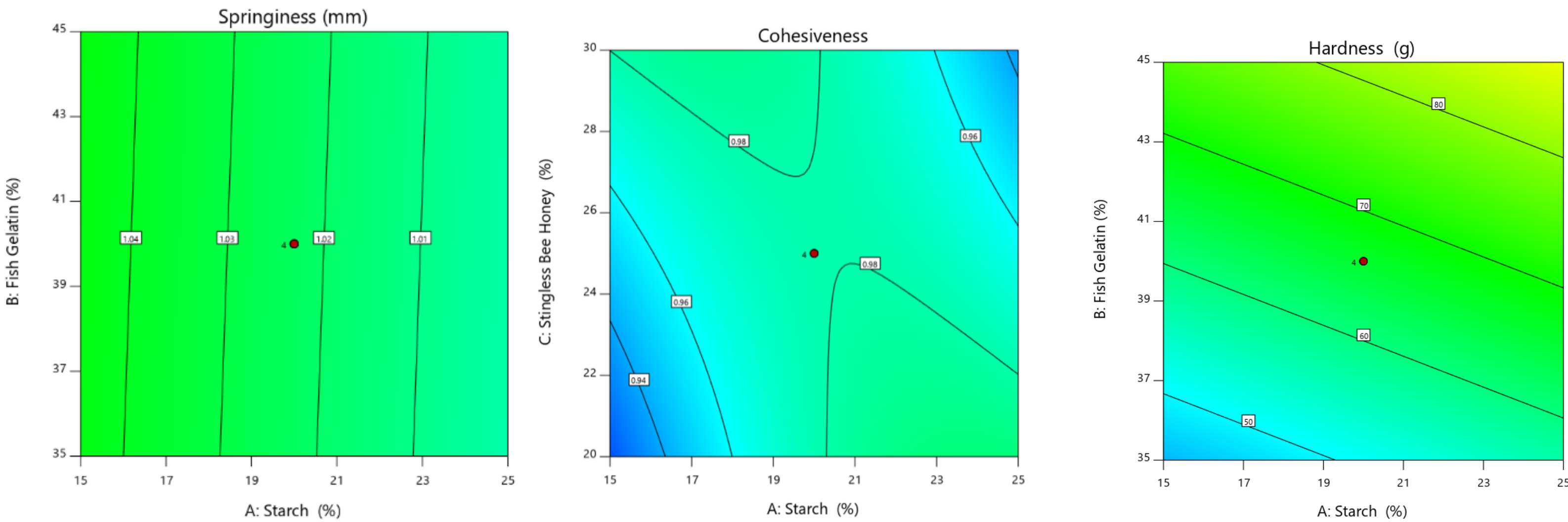
$$\text{Springiness}= 1.02-0.02A+0.00076B-0.050C$$
$$\text{Cohesiveness}=0.979302+0.0069A-0.023B+0.0014C-0.029AC-0.022A^2+0.038B^2$$
$$\text{Hardness}=66.11+5.94A+15.282B-0.769817C$$

(Equation 1)

The results of the springiness response analysis which the values ranged from 0.9 to 1.04 mm. However, statistical analysis showed that the springiness model was not significant, suggesting this response is less robust in the model prediction. The vertical contour lines indicate that starch concentration had the greatest influence on springiness, while fish gelatin had minimal impact. Lower starch percentages (15–17%) produced the best springiness (1.04 mm) demonstrating that a higher starch concentration may affect the gelatin elasticity. The complex relationship between starch and SBH at a 40% fish gelatin concentration is shown by the cohesiveness contour plot. The cohesiveness values showed differences in the formulation's structural integrity with the values ranging from 0.94 to 1.14. A significant curvilinear connection was shown which suggested that the most cohesive texture was formed by intermediate concentration of starch and SBH ingredients. The concentrations of the ingredients had a significant impact on the gummies' hardness.

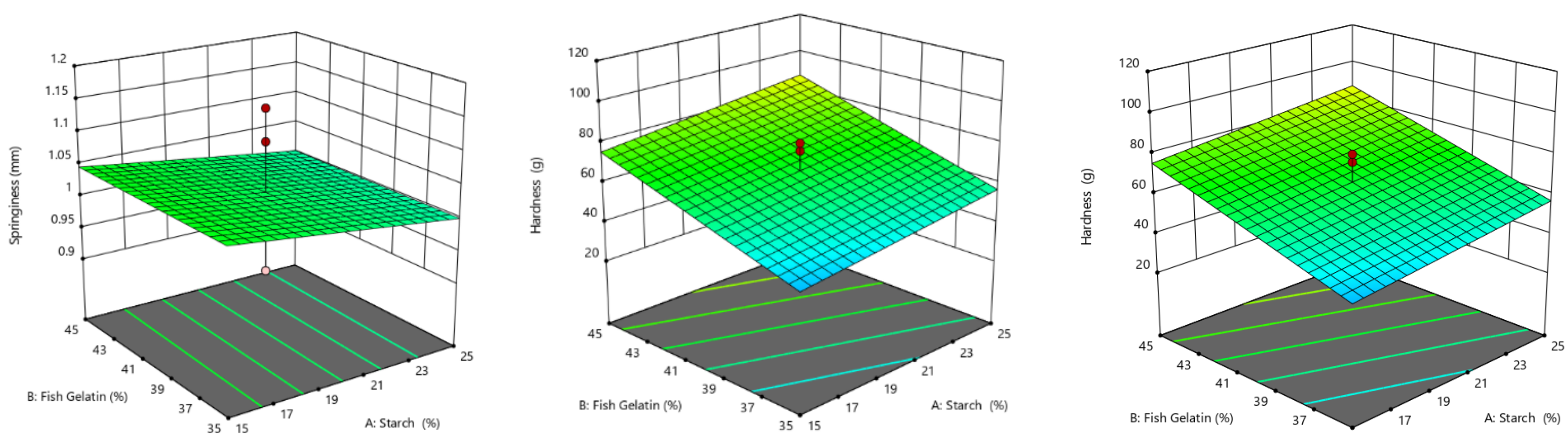
According to the contour plot, the biggest impact on hardness was affected by the concentration of the fish gelatin which varied from 50g to 80g. Increasing the concentration of fish gelatin from 35% to 45% produced increasing hardness of the gummies while the stingless bee honey was maintained at 25%. Fish gelatin levels between 43% and 45%, along with lower starch quantities (15–17%) produced the maximum hardness value (80g).

Figure 4 is a two-dimensional graph of the three responses (springiness, cohesiveness and hardness). This graph examines the relationship between the concentration of SBH, starch and fish gelatin on the responses. Figure 3 presents a three-dimensional representation of two linear models for the springiness and cohesiveness but a quadratic model for the hardness response which is depicted as a curved parabola shape.



(a) (b) (c)

Figure 4 : 2D responses for (a) Springiness (b) Cohesiveness and (c) Hardness



(a) (b) (c)

Figure 5 : 3D responses for (a) Springiness (b) Cohesiveness and (c) Hardness

Table 4: Model summary statistics

Parameters	Source	Sum of square	df	Mean square	F-value	p-value	
Springiness	Model	0.038967	3	0.012989	1.371053	0.292316	not significant
	A-Starch	0.006757	1	0.006757	0.713208	0.41258	
	B-Fish Gelatin	7.62E-06	1	7.62E-06	0.000805	0.977772	
	C-Stingless Bee Honey	0.032203	1	0.032203	3.399146	0.086498	
	Residual	0.132633	14	0.009474			
	Lack of Fit	0.080758	11	0.007342	0.424576	0.872091	not significant
	Pure Error	0.051875	3	0.017292			
Cohesiveness	Model	0.042711	6	0.007119	7.610776	0.00207	significant
	A-Starch	0.000644	1	0.000644	0.688048	0.424464	
	B-Fish Gelatin	0.006952	1	0.006952	7.432184	0.019712	
	C-Stingless Bee Honey	2.47E-05	1	2.47E-05	0.026403	0.873867	
	AC	0.006613	1	0.006613	7.069718	0.022231	
	AA^2	0.007035	1	0.007035	7.52151	0.019144	
	BA^2	0.016608	1	0.016608	17.75597	0.001451	
	Residual	0.010289	11	0.000935			
	Lack of Fit	0.005214	8	0.000652	0.385241	0.875162	not significant
	Pure Error	0.005075	3	0.001692			
Hardness	Model	3558.061	3	1186.02	3.859825	0.033316	significant
	A-Starch	486.2425	1	486.2425	1.582444	0.228986	
	B-Fish Gelatin	3064.043	1	3064.043	9.971727	0.006982	
	C-Stingless Bee Honey	7.775152	1	7.775152	0.025304	0.875885	
	Residual	4301.823	14	307.2731			
	Lack of Fit	3237.636	11	294.3305	0.829733	0.646717	not significant
	Pure Error	1064.188	3	354.7292			

Table 4 outlines the limits for springiness (0.9–1.2 mm), cohesiveness (0.9–1.14), and hardness (31–107.7 g). These goals were derived based on market benchmarks for gummy texture. The goal for the optimisation response goals was set 1.04, 0.98 and minimise for the springiness, cohesiveness and hardness respectively which followed the value of the responses of texturometer from the market gummy (Brand S). A survey about multi-brand gummies reported that commercial gummies brand springiness values were 0.82 to 1.0 and cohesiveness values were 0.62 and 0.92 (Mahat *et al*. 2020). The goals of cohesiveness 0.98 and springiness 1.04 are fair and practical benchmarks to develop gummies with optimal textural qualities and surpassing typical commercial product performance.

Table 5: Optimisation response goal

Dependent Variables	Constraints			
	Lower limit	Higher limit	Goal	Importance
Springiness	0.9	1.2	Target= 1.04	3
Cohesiveness	0.90	1.14	Target= 0.98	3
Hardness	31.0	107.7	Minimise	3

Table 6: Optimised gummies predicted and observed value

Response	Predicted Value	Observed Value
Springiness (mm)	1.0448 ± 0.097	1.02 ± 0.033
Cohesiveness	0.9776 ± 0.031	0.98 ± 0.017
Hardness (g)	51.0049 ± 17.529	66.11 ± 0.882

Validation of the optimised model suggested by RSM-CCD software in which the concentration of starch 15%, fish gelatin 37% and SBH 25% were compared between the predicted value with the observed responses in Table 5. The comparison demonstrates good agreement between those two confirming the reliability of the optimisation model. The model predicted a springiness value of 1.04476 ± 0.097 mm, while the observed value was 1.02 ± 0.033 mm. This minor deviation suggests that the formulation successfully achieved the targeted value (1.04). The predicted cohesiveness value was 0.98 ± 0.017, whereas the expected value was 0.977598 ± 0.0031. The model correctly predicted the gummy's textural behaviour, as evidenced by the small discrepancy between these values (0.002402). The cohesiveness goal of 0.98 was effectively achieved. The observed hardness was 66.11 ± 0.882 g while the predicted value was 51.0049 ± 17.53 g. Minor deviations like this are common complex materials systems which consist of starch-fish gelatin-SBH mixture. The hardness difference, which was about 29% still acceptable within the range. Some limitations may occur during the preparation process or during the physical testing of the gummies. Insufficient experimental data also may lead to this deviation. Experimental replication, model refinement, and expanded data sampling can help reduce such gaps in future optimisations.

Characterisation of optimised model gummies

pH test

The pH value observed for the optimised formulation was 5.52 ± 0.02. The gummies were within the acidic range. A pH of approximately 5.5 helps to maintain the gel structure combined with the preservatives can prevents microbial growth at room-temperature storage. However, if the pH drops too low gummy may fail to form a proper gel (Romo-Zamarrón *et al*., 2019).

Storage Stability Test

For storage stability, no yeast or mould growth was observed on the gummies, throughout the fourteen days' storage period at room temperature as the preliminary testing. The short-term observation did not represent a full-shelf-life study. It is recommended to conduct longer stability study with controlled temperature and relative humidity conditions, alongside regular sensory evaluations, to comprehensively assess product stability and quality over time.

Microbial test

The results demonstrated that there was no microbial growth observed on the test plates for both TAMC and TYMC analyses which use the pour plate method. This indicates that the aerobic bacteria, yeast, and mold within the detection limits were absent from the standard microbiological methods used within the gummies. With the proper handling and proper packaging played a significant role to ensure the microbiological safety of the gummy's final product.

Conclusion

The results showed that starch concentration played a crucial role in determining the springiness value while cohesiveness was decided by an interaction between starch and SBH. Hardness was primarily determined by fish gelatin concentrations. The optimised formulation, which was 15% starch, 37% fish gelatin and 25% SBH were validated experimentally. The result showed minimal deviation between predicted and observed values. These findings highlight the effectiveness of RSM-CCD in developing gummies with desirable textural characteristics.

Authors contributions

Study design, M.M.D.M. and S.A.N.Z. Direction and Coordination, M.S.H. Investigation, M.M.D.N. Resources M.S.H and S.M.R. Writing-Original Draft, M.M.D.N. Writing-Review and Editing M.S.H., S.M.R., M.S.S and S.A.N.Z. Project Administration M.S.H. and M.M.D.N.

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Conflict of interest

The authors declare that there is no conflict of interest in writing this manuscript.

Declaration of generative AI and AI-assisted technologies in the writing process

ChatGPT was used to improve readability and language. The author then review and edit the content as needed. Turnitin was used to check plagiarism for this study.

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