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Research article

Optimization of Modified Starch and Turmeric Powder in Frog Meat Sausage Formulation Using Response Surface Methodology

Nguyen Ngo Phuong Anh[1], Nguyen Thi Dong Giang[1], Le Hoang Khang[1], Cao Thi Tam Nhi[1], Pham Huynh Nhu[1], Eng Keng Seow[2], Nguyen Phung Tien[3], Nguyen Van Muoi[1] and Tran Hong Quan[1]*

[1]Department of Food Technology, Institute of Food and Biotechnology, Can Tho University, Can Tho 94000, Vietnam
[2]Food Science and Technology Program, School of Industrial Technology, Faculty of Applied Sciences, Universiti Teknologi, MARA, Shah Alam 40450, Malaysia
[3]Faculty of Agricultural Technology, Vinh Long University of Technology and Engineering, Vinh Long, 85110, Viet Nam

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Abstract

This study evaluated the effects of modified starch and turmeric powder on the instrumental quality characteristics of frog meat sausage using response surface methodology. A central composite design was applied with modified starch and turmeric powder concentrations ranging from 0.02-0.58% (based on frog meat weight, w/w) and 0.02-4.68% (based on frog meat weight, w/w), respectively. The measured responses included texture profile parameters, water-holding capacity, drip loss, and operationally defined protein interaction-associated fractions. Modified starch increased springiness, cohesiveness, and water-holding capacity while reducing hardness, gumminess, chewiness, and drip loss ($p<0.05$). In contrast, increasing turmeric powder generally reduced springiness, cohesiveness, water retention, and interaction-associated fractions, and increased drip loss ($p<0.05$). The quadratic models adequately described the measured responses and showed insignificant lack of fit ($p>0.05$). Numerical optimization identified 0.50% (based on frog meat weight, w/w) modified starch and 0.93% (based on frog meat weight, w/w) turmeric powder as the optimal formulation. Experimental validation showed close agreement between predicted and observed instrumental responses, with prediction errors below 1%. Fourier-transform infrared spectroscopy (FTIR) spectral differences indicated changes in the molecular environment of protein- and carbohydrate-associated functional groups. Independent sensory evaluation indicated moderate acceptance of the optimized product. These findings demonstrate the technological potential of modified starch and turmeric powder for formulation of frog meat sausage with desirable instrumental texture and moisture-retention properties.

Keywords: frog meat sausage; modified starch; turmeric powder; response surface methodology; texture profile; intermolecular interaction

*Corresponding author: E-mail: thquan@ctu.edu.vn
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1. Introduction

Frog meat has emerged as a promising alternative protein source owing to its high protein (19-22%), desirable amino acid composition, low lipid content (0.5-2.0%), and high digestibility (Indriani et al., 2025). Along with the increasing demand for sustainable animal proteins, frog farming has rapidly expanded in several Southeast Asian countries, particularly Vietnam, Thailand, and Indonesia, due to its relatively short production cycle, efficient feed utilization, and high economic return (Le et al., 2023; Nghia et al., 2023). In Vietnam, Thai frog (*Hoplobatrachus rugulosus*) farming has expanded considerably in the Mekong Delta and several northern provinces, contributing to both domestic consumption and export-oriented production systems (Nguyen, 2014; Le et al., 2023). Frog farming has become an important livelihood model for rural households due to its relatively low investment cost and rapid economic return. Recent surveys in Dong Thap province also reported the increasing scale and intensification of commercial frog farming systems in Vietnam (Le et al., 2023).

Along with the expansion of frog farming, the utilization and commercialization of frog meat products have also diversified. Traditionally, frog meat is mainly marketed in fresh or frozen forms; however, the growing consumer demand for convenient and value-added foods has promoted the development of processed frog-based products, including marinated frog meat, battered and fried products, grilled products, surimi, and sausages (Nguyen, 2014; Musigapan et al., 2022). Sausage-type products are of particular interest because comminution allows the raw material to be transformed into products with more uniform composition, shape, texture, and sensory characteristics. It also facilitates the incorporation of starches, seasonings, and other functional ingredients into the meat matrix (Choe et al., 2013; Jiménez-Colmenero et al., 2001). Despite its nutritional advantages, frog meat exhibits relatively poor technological functionality, including limited water-holding capacity and weak structural stability during processing. Such limitations are associated with the fragile tissue organization and distinctive physicochemical characteristics of amphibian myofibrillar proteins, which may hinder the formation of stable gel matrices in comminuted meat systems (Sun & Holley, 2011; Tohozin et al., 2025).

Hydrocolloids and starches are widely incorporated into comminuted meat products to improve texture, water retention, emulsion stability, and structural integrity during thermal processing (Pietrasik & Janz, 2010; Choe et al., 2013). Among these ingredients, modified starches derived from different botanical sources, such as corn, potato, tapioca, and pea, have been widely used in meat processing because of their improved swelling capacity, thermal stability, and water-binding properties compared with their native counterparts (Bao et al., 2025; Wang et al., 2025b). During heating, starch granules absorb water and gelatinize, thereby altering water distribution and the physical organization of the protein matrix. The incorporation of modified starch into myofibrillar protein systems has consequently been associated with improved water retention and changes in gel texture in comminuted meat and surimi-type products (Sun et al., 2014; Fan et al., 2019).

Besides hydrocolloids, natural spices may also be incorporated into processed meat products to provide characteristic aroma, flavor, appearance, and product identity (Shah et al., 2014). Turmeric powder, derived from the rhizome of *Curcuma longa*, is rich in curcuminoids, volatile compounds, starch, dietary fiber, and other plant-derived constituents (Prasad & Aggarwal, 2011). Its yellow appearance and earthy aroma make it a commonly used culinary ingredient in Southeast Asian foods. In frog meat sausage,

turmeric powder may contribute to flavor development, visual perception, and the masking or modification of undesirable meat odors (Prasad & Aggarwal, 2011; Wang et al., 2025a).

However, excessive incorporation of turmeric powder may adversely affect texture and overall consumer acceptance because insoluble plant particles and dietary fiber components can interfere with the continuity of protein gel matrices and weaken the structural integrity of comminuted meat systems (Bao et al., 2025). In addition, excessive incorporation of spice-derived compounds in meat products may produce overly intense flavor characteristics that impair their acceptance among consumers (Shah et al., 2014). Although previous studies have separately investigated the applications of modified starches and plant-derived additives in processed meat systems (Sun et al., 2014), limited information is available regarding their combined effects on frog meat sausage. Furthermore, the relationships among texture properties, intermolecular interactions, water retention, and sensory quality remain insufficiently understood in frog-based comminuted systems. Therefore, this study aimed to investigate the effects of modified starch and turmeric powder on the texture profile, water-holding capacity, drip loss, and intermolecular interaction-associated protein fractions of frog meat sausage using a central composite design. Numerical optimization was performed using instrumental quality responses, while sensory evaluation was conducted as an independent confirmation of the acceptability of the instrumentally optimized product.

2. Materials and Methods

2.1 Materials

Live Thai frogs (*Hoplobatrachus rugulosus*) were obtained from a local supplier in Can Tho City, Vietnam. Frogs of comparable market size, with an average body weight of approximately 250±20 g, were transported alive to the laboratory and processed on the day of receipt. Food-grade modified starch (INS 1422, acetylated distarch adipate) was purchased from Hoa My Food Additives Co., Ltd. (Ho Chi Minh City, Vietnam). INS 1422 is a cross-linked and acetylated starch produced by esterification with adipic anhydride and acetic anhydride and is widely used as a thickener and stabilizer in processed foods. According to the supplier, the product contained ≥98% dry matter and <12% moisture. Commercial acetylated distarch adipate contains < 2.5% acetyl groups and 0.135% adipate groups.

Turmeric powder was obtained from VIPEP Co., Ltd. (Ho Chi Minh City, Vietnam). The product consisted of 100% *Curcuma longa* L. powder produced in Dak Lak Province, Vietnam, under an ISO 22000-certified manufacturing system. The turmeric powder contained less than 10% moisture and 2-5% (w/w) curcumin. Typical commercial food-grade turmeric powder is supplied as a fine yellow powder with a particle size of approximately 80-100 mesh, volatile oil content of 1-4%, total ash below 9-10%, and acid-insoluble ash below 2.5%.

All other seasonings, including salt and sugar, were of food-grade and purchased from local commercial suppliers. The analytical-grade reagents used throughout the study were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2 Preparation of frog meat sausage

The preparation of frog meat sausage was adapted from the surimi sausage processing method described by Trúc et al. (2016) with modifications to suit frog meat formulation and

the incorporation of modified starch and turmeric powder. The sausage formulation consisted of frog meat (100 g), 2% sugar (based on frog meat weight, w/w), and 1.5% salt (based on frog meat weight, w/w). Modified starch and turmeric powder were incorporated according to the experimental design. A two-factor, five-level Central Composite Design (CCD) comprising 13 experimental runs, including four factorial points, four axial points (α = 1.414), and five center points, was generated using Design-Expert software (Version 13.0, Stat-Ease Inc., Minneapolis, MN, USA) to optimize the levels of modified starch (A) and turmeric powder (B) in the frog meat sausage formulation. The coded and actual factor levels are presented in Table 1.

Live frogs were slaughtered, skinned, deboned, and minced using a meat grinder. The minced frog meat was initially mixed with salt and sugar to facilitate myofibrillar protein extraction, followed by the addition of modified starch, turmeric powder, and crushed ice. The amount of crushed ice was calculated using a moisture balance based on the moisture contents of the frog meat and the other formulation ingredients to obtain a target batter moisture content of approximately 80-85%. The mixture was homogenized using a bowl cutter (84186-38, Hobart, USA) at 1725 rpm for 3 min until a uniform paste was obtained while maintaining the batter temperature of 4±2°C to minimize protein denaturation.

The resulting batter was stuffed into food-grade polyethylene (PE) casings (20-25 mm in diameter), manually tied, and heat-processed in a water bath at 90-95°C for 30 min, until the core temperature reached ≥85°C, as monitored using a digital probe thermometer (TP03, ThermoPro, China). After thermal processing, the sausages were immediately cooled in ice water for 10 min and stored at 4°C for 12 h prior to physicochemical, textural, intermolecular interaction, and sensory analyses. This research was approved by the Ethics Committee of Can Tho University Research Ethics Committee (No. BQ2025-23/CNSH-TP approved date 10 June 2025). Informed consent was received for human studies.

Table 1. Central composite design (CCD) for preparation of frog meat sausage affected by the levels of modified starch (A) and turmeric powder (B)

Std Order	Code Levels		Actual Levels	
	A: Modified Starch (%, w/w)*	B: Turmeric Powder (%, w/w)	A: Modified Starch (%, w/w)	B: Turmeric Powder (%, w/w)
1	-1.00	-1.00	0.10	0.70
2	1.00	-1.00	0.50	0.70
3	-1.00	1.00	0.10	4.00
4	1.00	1.00	0.50	4.00
5	-1.414	0.00	0.02	2.35
6	1.414	0.00	0.58	2.35
7	0.00	-1.414	0.30	0.02
8	0.00	1.414	0.30	4.68
9	0.00	0.00	0.30	2.35
10	0.00	0.00	0.30	2.35
11	0.00	0.00	0.30	2.35
12	0.00	0.00	0.30	2.35
13	0.00	0.00	0.30	2.35

**(%, w/w): based on frog meat weight (w/w)*

2.3 Analysis

2.3.1 Texture profile analysis (TPA)

Texture profile analysis (TPA) of frog meat sausage samples was determined using a texture analyzer (TA.XTplus Texture Analyzer, Stable Micro Systems Ltd., Surrey, UK) equipped with a cylindrical probe. Prior to analysis, sausage samples were equilibrated at 25±2°C for 30 min and cut into cylindrical sections with approximately 20 mm in height and 20 mm in diameter. The samples were subjected to a two-cycle compression test to 50% of their original height at a constant crosshead speed of 1 mm/s with a 5 s interval between compressions. Texture parameters including hardness (N), springiness, cohesiveness, gumminess (N), and chewiness (N·cm) were automatically calculated from the force–time curves generated by the instrument software.

2.3.2 Water-holding capacity (WHC) and drip loss

Water-holding capacity (WHC) was determined following the centrifugal method described by Shand (2000). Approximately 5 g of sausage sample was weighed into a centrifuge tube lined with filter paper and centrifuged at 5000 × g for 10 min at 4°C. WHC was calculated as the percentage of retained water relative to the initial sample weight using the following equation:

$$\text{WHC (\%)} = \frac{W_2}{W_1} \times 100\%$$

where: W_1 = initial weight of the sample before centrifugation (g); W_2 = weight of the sample after centrifugation (g).

Drip loss was determined according to the method described by Honikel (1998). Approximately 10 g of sausage sample was weighed, placed in a sealed container, and stored at 4°C for 24 h. After storage, the released exudate was removed and the sample was reweighed. Drip loss was expressed as the percentage weight loss relative to the initial sample weight.

$$\text{Drip loss (\%)} = \frac{W_1 - W_2}{W_1} \times 100\%$$

where W_1 and W_2 represent the sample weights before and after storage, respectively.

2.3.3 Determination of intermolecular interaction-associated protein fractions

Intermolecular interaction-associated protein fractions including ionic bonds, hydrogen bonds, hydrophobic interactions, and disulfide bonds were determined according to the sequential protein extraction method described by Li et al. (2022). Briefly, 2 g of sausage sample was homogenized sequentially in different extraction solutions to disrupt specific intermolecular interactions.

Ionic bonds were estimated from protein solubility in 0.6 M NaCl solution. Hydrogen bonds were determined using 0.6 M NaCl containing 1.5 M urea. Hydrophobic interactions were evaluated using 0.6 M NaCl containing 8 M urea, while disulfide bonds were estimated after extraction with 0.6 M NaCl containing 8 M urea and 1.5 M β-

mercaptoethanol. After each extraction step, the homogenates were centrifuged at 10000 × g for 15 min at 4°C, and protein concentrations in the supernatants were determined by the Biuret method using bovine serum albumin (BSA) as the protein standard. The contribution of each intermolecular interaction was calculated from the differences in protein solubility between successive extraction systems.

2.3.4 Fourier-transform infrared spectroscopy (FTIR) analysis

Fourier-transform infrared spectroscopy (FTIR) was performed to investigate structural changes and intermolecular interactions in optimized frog meat sausage samples. Freeze-dried samples were finely ground and mixed with potassium bromide (KBr) powder prior to pellet preparation. FTIR spectra were recorded using an FTIR spectrometer (PerkinElmer Spectrum Version 10.5.2, Waltham, MA, USA) equipped with a deuterated triglycine sulfate (DTGS) detector over the wavenumber range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scanning accumulations per measurement.

2.3.5 Sensory evaluation

Sensory evaluation of the cooked frog meat sausage samples was conducted using a nine-point hedonic scale according to the method described by Meilgaard et al. (2016) and Stone et al. (2020). A total of 30 untrained consumers, consisting of students and staff members who were familiar with processed meat products. Participation was voluntary, and all participants were informed about the evaluation procedure and the ingredients used in the samples before assessment. Participants were free to decline or discontinue participation at any time, and no personally identifiable information was collected. The sausage samples were cooked using the processing conditions described in Section 2.2. After cooking, the samples were cooled under controlled conditions and cut into uniformly sized portions of approximately 1 cm thickness (~20 g). Before evaluation, the samples were brought to a serving temperature of approximately 25±2°C. Each sample was placed in an identical odor-free food-grade container and identified using a randomly assigned three-digit code. The serving order was randomized among participants to minimize order and carry-over effects. Drinking water was provided for palate cleansing between samples. Participants were asked to evaluate overall acceptability based on appearance, color, aroma, texture, flavor, and overall preference using a nine-point hedonic scale ranging from 1 (“dislike extremely”) to 9 (“like extremely”). Drinking water was provided between samples to minimize residual flavor effects. Sensory evaluation was conducted independently of the response surface optimization and served only to assess consumer acceptance of the optimized formulation.

2.3.6 Statistical analysis

Experimental data were analyzed using response surface methodology (RSM) with Design-Expert software version 13.0 (Stat-Ease Inc., Minneapolis, MN, USA). A central composite design (CCD) was employed to evaluate the effects of modified starch (A) and turmeric powder (B) on the physicochemical and sensory properties of the frog meat sausage samples.

Experimental responses were fitted to a second-order polynomial model as follows:

$$Y = \beta_0 + \beta_A A + \beta_B B + \beta_{AB} AB + \beta_{AA} A^2 + \beta_{BB} B^2$$

where Y represents the predicted response; β_0 is the intercept; β_A and β_B are linear coefficients; β_{AB} is the interaction coefficient; and β_{AA} and β_{BB} are quadratic coefficients.

Analysis of variance (ANOVA) was performed to evaluate the significance and adequacy of the models. The goodness of fit was assessed using coefficient of determination (R^2), lack-of-fit test, coefficient of variation (CV), and p-values.

2.3.7 Numerical optimization and validation

Numerical optimization was conducted using a desirability-function approach. Instrumental responses, including texture profile parameters, water-holding capacity, drip loss, and intermolecular-interaction-associated protein fractions, were included in the optimization. Hardness, springiness, cohesiveness, gumminess, and chewiness were maintained within the predefined acceptable ranges; water-holding capacity was maximized; drip loss was minimized; and intermolecular-interaction-associated fractions were maintained within ranges associated with a stable gel matrix.

The formulation with the highest overall desirability was prepared in independent batches for model validation. Predicted and observed instrumental values were compared, and prediction error was calculated as:

$$\text{Prediction error (\%)} = (\text{Observed value} - \text{Predicted value}| / \text{Predicted value}) \times 100$$

Following instrumental validation, the optimized product was subjected to an independent sensory evaluation using a nine-point hedonic scale. The sensory result was used to assess product acceptance and was not treated as a response for mathematical optimization.

3. Results and Discussion

3.1 Model fitting and adequacy of regression models

The regression coefficients and statistical parameters of the developed second-order polynomial models are presented in Table 2. The coefficients of determination (R^2) ranged from 0.903 to 0.99, indicating that the developed models adequately explained more than 90% of the variability in all measured responses. Furthermore, the models showed highly significant p-values ($p<0.0001$) and non-significant lack-of-fit values ($p>0.05$), confirming the suitability and reliability of the response surface methodology (RSM) models for predicting the effects of modified starch and turmeric powder on frog meat sausage properties.

The low coefficients of variation (CV = 0.16-6.04%) further demonstrated the good reproducibility, precision, and experimental stability of the developed models, indicating that random variation among experimental runs was minimal. In response surface methodology (RSM), CV values lower than 10% are generally considered indicative of acceptable model reliability and experimental consistency for food system optimization studies. The high coefficients of determination (R^2 = 0.903-0.99), combined with non-significant lack-of-fit values, further confirmed the strong predictive capability and adequacy of the quadratic polynomial models developed in this study (Baş & Boyacı, 2007). These findings suggested that the selected independent variables, namely modified starch and turmeric powder, adequately explained the variability observed in texture properties, water retention, intermolecular interactions, and sensory quality of frog meat sausage.

Table 2. Regression coefficients of second-order polynomial models predicting texture properties (hardness, springiness, cohesiveness, gumminess, and chewiness), water-holding capacity (WHC), drip loss, and intermolecular interaction-associated protein fractions of frog meat sausage as affected by modified starch (A) and turmeric powder (B)

Regression Coefficients (β)	Texture Profile Analysis (TPA)					WHC (%)	Drip loss (%)	Intermolecular Interaction-associated Protein Fractions (mg protein/mL)			
	Hardness (N)	Springiness (cm)	Cohesiveness	Gumminess (N)	Chewiness (N.cm)			Hydrogen	Ionic	Hydrophobic	Disulfide
β_0	49.92	0.92	0.71	36.40	33.62	93.88	1.45	2.22	2.11	2.56	2.64
β_A	-6.79	0.011	0.021	-4.80	-4.12	0.57	-0.24	0.091	0.095	0.14	0.14
β_B	3.06	-0.027	-0.073	2.18	1.88	-0.99	0.45	-0.15	-0.13	-0.22	-0.21
β_{AB}	-4.07	0.001	0.006	-0.38	-0.25	0.15	-0.068	-0.023	-0.005	-0.030	-0.057
β_{AA}	3.14	-0.006	-0.014	2.49	2.34	-0.29	0.11	-0.013	-0.022	-0.027	-0.036
β_{BB}	1.52	-0.011	-0.045	1.06	1.19	-0.49	0.28	-0.070	-0.084	-0.11	-0.12
R^2	0.99	0.98	0.98	0.99	0.99	0.99	0.97	0.99	0.99	0.99	0.903
Lack of fit	0.897	0.063	0.359	0.380	0.064	0.060	0.380	0.825	0.513	0.926	0.879
P - value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	<0.0001	<0.0001	<0.0001
CV (%)	0.37	0.49	1.17	0.62	0.93	0.16	6.04	1.11	1.14	0.77	0.92

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Comparable model performance has been reported in previous optimization studies involving starch–protein composite gels and surimi-based products. Fan et al. (2019) demonstrated that RSM successfully predicted the effects of modified starch properties on the gel behavior and water immobilization capacity of myofibrillar protein systems, with R^2 values exceeding 0.90 and low CV values. Likewise, Zhuang et al. (2019) reported that polynomial models effectively described the influence of insoluble dietary fiber on emulsion gel microstructure and physicochemical properties of meat systems. These studies collectively indicate that RSM is an effective statistical tool for evaluating the complex interactions among hydrocolloids, plant-derived additives, and myofibrillar proteins in comminuted meat matrices. In the present study, the high statistical adequacy of the models also suggested that the combined effects of modified starch and turmeric powder on frog meat sausage quality could be reliably predicted and optimized within the selected experimental ranges.

The negative linear coefficient (β_A) of modified starch for hardness, gumminess, and chewiness suggested that increasing starch concentration reduced rigidity and toughness of the sausage matrix, whereas positive coefficients for springiness, cohesiveness, WHC, and intermolecular interactions indicated improved elasticity and structural stability. In contrast, turmeric powder exhibited positive effects on hardness and drip loss but negative effects on springiness, cohesiveness, WHC, and intermolecular interactions, suggesting that excessive turmeric incorporation adversely affected gel continuity and moisture retention. These findings further support the technological potential of frog meat as a raw material for value-added processed products. Recent studies have highlighted that frog meat possesses favorable nutritional composition and functional characteristics for the development of processed meat products, although formulation optimization remains essential to maximize product quality and consumer acceptance (Indriani et al., 2025; Tohozin et al., 2025).

3.2 Effects of modified starch and turmeric powder on texture profile analysis (TPA)

The effects of modified starch (A) and turmeric powder (B) on the texture profile properties of frog meat sausage, including hardness, springiness, cohesiveness, gumminess, and chewiness, are shown in Figure 1. The second-order polynomial models for all texture parameters showed high coefficients of determination (R^2 = 0.98-0.99), significant model p-values ($p<0.0001$), and non-significant lack-of-fit values, indicating that the developed models were suitable for describing the textural changes of frog meat sausage as affected by the two formulation variables. Texture is one of the most important quality attributes influencing consumer acceptance of emulsified meat products because it determines firmness, elasticity, and overall eating quality (Tornberg, 2005; Toldrá & Reig, 2011). Therefore, optimizing texture characteristics is essential for developing frog meat sausage with properties comparable to those expected for commercial emulsified sausage products.

The regression equations in terms of coded variables were as follows:

$$Hardness\ (N) = 49.92 - 6.79A + 3.06B - 4.07AB + 3.14A^2 + 1.52B^2$$

$$Springiness\ (cm) = 0.92 + 0.011A - 0.027B + 0.001AB - 0.006A^2 - 0.011B^2$$

$$Cohesiveness = 0.71 + 0.021A - 0.073B + 0.006AB - 0.014A^2 - 0.045B^2$$

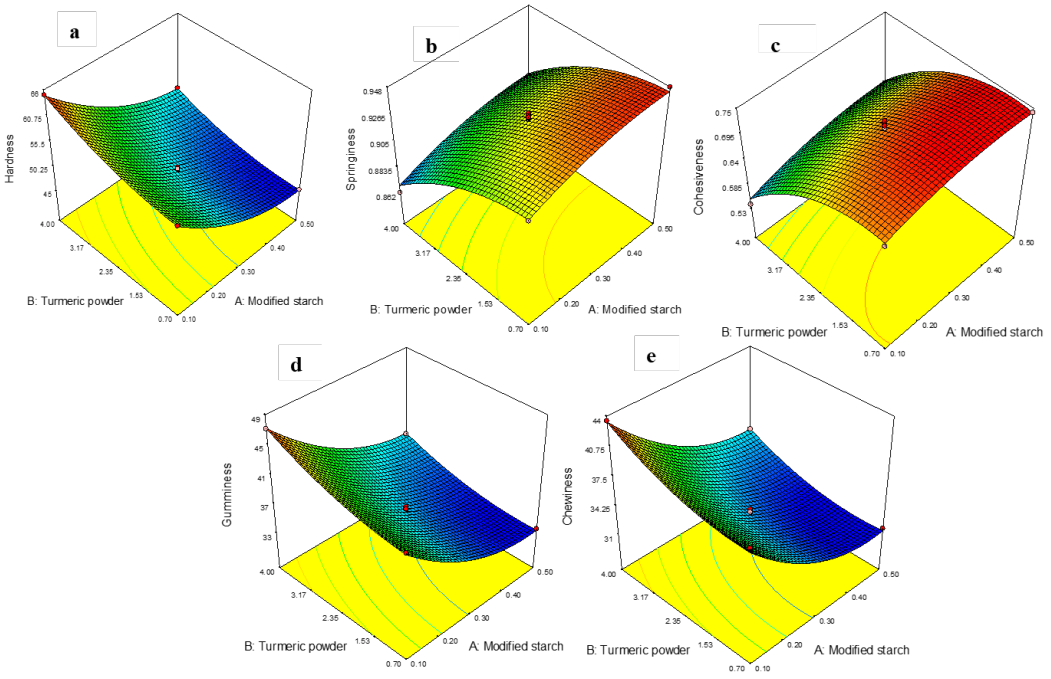


Figure 1. The 3D plots of Hardness (N) (a), Springiness (cm) (b), Cohesiveness (c), Gumminess (N) (d), and Chewiness (N.cm) (e) of frog meat sausage as affected by the levels of modified starch (A) and turmeric powder (B).

$$Gumminess\ (N) = 36.40 - 4.80A + 2.18B - 0.38AB + 2.49A^2 + 1.06B^2$$

$$Chewiness\ (N.cm) = 33.62 - 4.12A + 1.88B - 0.25AB + 2.34A^2 + 1.19B^2$$

The negative linear coefficients of modified starch for hardness, gumminess, and chewiness indicated that increasing modified starch reduced the firmness and toughness of frog meat sausage. This effect may be associated with water absorption, swelling, and gelatinization of starch granules during heating, which could increase water binding within the sausage matrix and reduce its resistance to compression. Similar mechanisms were reported in fish myofibrillar protein systems, where modified starch improved gel properties through water absorption, swelling, and interaction with the protein matrix (Bao et al., 2025).

In contrast, turmeric powder showed positive linear effects on hardness, gumminess, and chewiness, suggesting that higher turmeric increased the resistance of the product to compression. This may be associated with the incorporation of insoluble dietary fiber and cell wall fragments from turmeric powder, which may increase the solid content of the batter and reduce matrix flexibility. At excessive levels, these constituents could interfere with protein–protein associations or dilute the continuous myofibrillar protein phase (Wang et al., 2025b; Bao et al., 2025). Zhuang et al. (2019) documented that insoluble dietary fiber altered the microstructure of myofibrillar protein emulsion gels and affected protein network formation.

For springiness and cohesiveness, modified starch showed positive linear effects, whereas turmeric powder showed negative linear effects. This indicates that modified starch improved the ability of the product to recover after compression and increased its internal binding characteristics. These changes may be related to enhanced starch hydration, water immobilization, and non-covalent associations among starch, water, and myofibrillar proteins during heating. Recent studies on protein–polysaccharide composite gels have likewise demonstrated that hydrated polysaccharides can improve elasticity and cohesiveness by modifying water distribution within the protein matrix (Bao et al., 2025). Similar effects have been reported when turmeric powder was incorporated into processed meat products, where excessive addition altered texture and physicochemical properties because of the presence of insoluble plant constituents.

Gumminess and chewiness followed trends similar to hardness because these parameters are mathematically derived from hardness, cohesiveness, and springiness, reflecting the energy required to disintegrate the product during mastication (Bourne, 2002). The decrease in gumminess and chewiness with increasing modified starch levels indicates that starch incorporation contributed to a softer and more easily chewable product. This effect may be associated with enhanced water immobilization and starch gelatinization, which could reduce the mechanical resistance of the protein-containing matrix during compression and simulated mastication (Bao et al., 2025; Sun et al., 2014). Such textural modification is technologically desirable for frog meat sausage because excessive firmness and chewiness may negatively affect consumer acceptability, particularly in emulsified or surimi-type products.

Similar findings have been reported in previous studies on modified starch incorporation in emulsified meat systems. Pietrasik and Janz (2010) observed that incorporating 4% modified starch into low-fat bologna improved tenderness, binding properties, and overall textural quality. Fan et al. (2019) also demonstrated that modified starch reduced gel rigidity and improved the viscoelastic properties of myofibrillar protein gels by enhancing water retention and stabilizing the protein–starch matrix. More recently, Wang et al. (2025b) reported that the incorporation of 2% resistant starch significantly improved water-holding capacity, texture, and gel characteristics of low-fat meat emulsions through enhanced starch hydration and interactions with myofibrillar proteins. Although the modified starch level optimized in the present study (0.50%, based on frog meat weight) was substantially lower than those reported for conventional meat emulsions, it was sufficient to improve texture and water-holding capacity, likely because frog meat possesses different protein composition and gel-forming characteristics. These findings collectively support the role of modified starch as an effective texture-modifying and water-binding ingredient in comminuted frog meat products.

3.3 Water-holding capacity (WHC) and drip loss

The effects of modified starch (A) and turmeric powder (B) on the water-holding capacity (WHC) and drip loss of frog meat sausage are presented in Figure 2. The developed regression models showed excellent predictive capability, with R^2 values of 0.99 for WHC and 0.97 for drip loss. The regression equations in terms of coded variables were as follows:

$$WHC\ (\%) = 93.88 + 0.57A - 0.99B + 0.15AB - 0.29A^2 - 0.49B^2$$

$$Drip\ loss\ (\%) = 1.45 - 0.24A + 0.45B - 0.068AB + 0.11A^2 + 0.28B^2$$

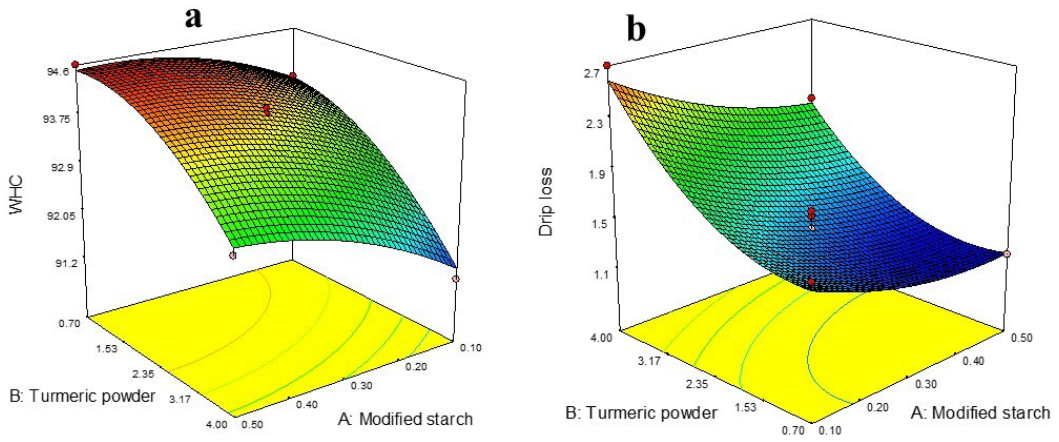


Figure 2. The 3D plots of water holding capacity (WHC) (%) (a) and drip loss (%) (b) of frog meat sausage as affected by the levels of modified starch (A) and turmeric powder (B)

Modified starch increased WHC and reduced drip loss, whereas excessive turmeric powder showed the opposite effect. Similar trends have been reported in emulsified meat systems, where starch gelatinization and swelling enhanced water retention by strengthening the protein–starch gel network (Pietrasik & Janz, 2010; Wang et al., 2025b). Conversely, excessive turmeric powder may introduce insoluble dietary fiber and plant cell wall particles that disrupt network continuity, resulting in lower water retention and greater exudate loss during heating (Zhuang et al., 2019; Wang et al., 2025a).

3.3.1 Water-holding capacity (WHC)

WHC increased significantly with increasing modified starch concentration, and the highest WHC values (>94%) were observed at moderate-to-high modified starch levels combined with low-to-moderate turmeric concentrations. The positive effect of modified starch on WHC may be attributed to the swelling and gelatinization behavior of starch granules during thermal processing, which enhanced water entrapped within the protein–starch matrix (Bao et al., 2025; Wang et al., 2025b). Gelatinized starch can absorb and retain large amounts of water through hydrogen bonding and physical entrapment, thereby reducing water mobility and improving moisture retention in comminuted meat systems (Bao et al., 2025).

In addition, non-covalent associations among starch, water, and myofibrillar proteins may also have contributed to the observed increase in WHC. Such associations, together with starch hydration, could improve water immobilization and reduce syneresis during thermal processing and storage. Fan et al. (2019) demonstrated that modified starch significantly improved gel stability and water immobilization in myofibrillar protein systems due to enhanced starch gelatinization and protein–polysaccharide interactions. Similarly, Sun et al. (2014) reported that modified starch promoted water absorption and improved gel properties in fish myofibrillar protein gels by increasing moisture retention and reducing protein aggregation density.

The 3D plots also indicated that WHC decreased at elevated turmeric levels. Excessive turmeric incorporation may introduce insoluble dietary fiber and plant cell wall components that reduce the continuity of the myofibrillar protein matrix and compete with proteins for available water during thermal gelation. Consequently, less water is retained within the gel network, leading to lower WHC (Shen et al., 2023; Choi et al., 2024). Similar effects have been reported by Zhuang et al. (2019), who showed that insoluble dietary fibers altered the microstructure, water distribution, and functional properties of myofibrillar protein gels. Comparable observations have also been reported in emulsified sausages and low-fat meat emulsions supplemented with wheat fiber, oat fiber, or other insoluble plant-derived ingredients, where dilution of the continuous protein phase reduced gel stability, moisture retention, and water-holding capacity (Shah et al., 2014; Bao et al., 2025). The quadratic effects observed in the regression model further suggested that excessively high starch or turmeric concentrations may negatively affect water retention. Therefore, balanced incorporation of modified starch and turmeric powder is essential for optimizing moisture retention and gel stability in frog meat sausage.

3.3.2 Drip loss

The response surface model indicated opposite effects of modified starch and turmeric powder on drip loss. Increasing modified starch was associated with reduced drip loss, whereas increasing turmeric powder promoted exudate release, consistent with the significant linear effects identified by the regression model. The reduction in drip loss associated with modified starch may be attributed to its water-binding and gel-stabilizing properties. During heating, starch granules absorb water, swell, and gelatinize, forming a hydrated starch phase that increases matrix viscosity and restricts water migration through the protein–starch gel network (Pietrasik & Janz, 2010; Wang et al., 2025b). Furthermore, interactions between gelatinized starch and myofibrillar proteins may enhance matrix continuity, thereby reducing water separation during storage (Shen et al., 2023; Bao et al., 2025).

The negative interaction coefficient β_{AB} in the drip loss model suggested that the combined effects of modified starch and turmeric powder partially compensated for water release at moderate concentrations. However, excessive turmeric incorporation still increased drip loss. This effect may reflect competition for water, dilution of the myofibrillar protein phase, or interference by insoluble turmeric constituents with protein association during heating. Similar mechanisms have been described in meat emulsions supplemented with insoluble dietary fibers (Zhuang et al., 2019; Choi et al., 2024).

Additionally, the phenolic compounds and insoluble fibrous components of turmeric powder may compete with proteins for available water and interfere with protein matrix formation. At elevated levels, these components may create regions with different hydration capacities, promoting water migration and exudate release during storage (Shah et al., 2014; Bao et al., 2025). The results demonstrated that modified starch played an important role in improving moisture retention and minimizing drip loss, whereas excessive turmeric powder adversely affected the water-holding stability of frog meat sausages.

3.4 Intermolecular interactions

The effects of modified starch (A) and turmeric powder (B) on ionic bonds, hydrogen bonds, hydrophobic interactions, and disulfide bonds of frog meat sausage are illustrated in Figure 3. These interaction fractions were operationally estimated from differences in protein

solubility in selective extraction solutions and are expressed as mg protein/mL. The quadratic models showed high coefficients of determination for hydrogen bonds, ionic interactions, and hydrophobic interactions, with R^2 values of 0.990. The disulfide-bond model showed a comparatively lower, although still acceptable, coefficient of determination of 0.903. The lack-of-fit values were non-significant for all four responses ($p>0.05$), indicating that the fitted models adequately represented the experimental data within the investigated formulation range.

The regression equations in terms of coded variables were as follows:

Ionic-interaction-associated fraction = $2.11 + 0.095A - 0.13B - 0.005AB - 0.022A^2 - 0.084B^2$

Hydrogen-bond-associated fraction = $2.22 + 0.091A - 0.15B - 0.023AB - 0.013A^2 - 0.070B^2$

Hydrophobic-interaction-associated fraction = $2.56 + 0.14A - 0.22B - 0.030AB - 0.027A^2 - 0.11B^2$

Disulfide-bond-associated fraction = $2.64 + 0.14A - 0.21B - 0.057AB - 0.036A^2 - 0.12B^2$

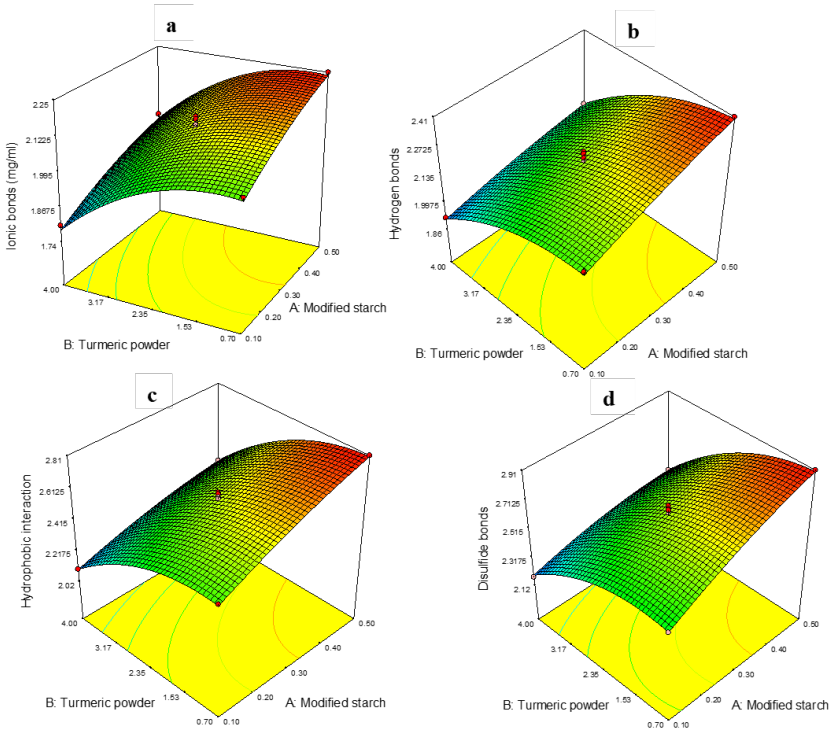


Figure 3. Three-dimensional response surface plots of ionic bonds (a), hydrogen bonds (b), hydrophobic interaction (c), and disulfide bonds (d) of frog meat sausage as affected by the levels of modified starch (A) and turmeric powder (B). The interaction fractions are expressed as mg protein/mL

The positive linear coefficients of modified starch and the negative linear coefficients of turmeric powder indicate that the amounts of protein solubilized in the operationally defined interaction-associated fractions increased with modified starch and decreased with turmeric powder. These changes suggest alterations in the molecular environment and extractability of proteins rather than direct formation or loss of a specific number of molecular bonds (Li et al., 2022). The negative quadratic coefficients further indicate that the responses did not increase indefinitely across the investigated formulation range. Possible explanations include differences in hydration, viscosity, protein accessibility, and interactions among matrix components.

All intermolecular interaction forces increased with increasing modified starch concentration but decreased markedly at elevated turmeric levels. The increase in these operationally defined fractions may reflect changes in protein solubility and molecular associations in the presence of hydrated modified starch. These results were consistent with the observed differences in WHC and texture, but they do not by themselves demonstrate the formation of a more organized, compact, or continuous protein–polysaccharide network. Similar relationships between enhanced intermolecular interactions and improved gel properties have been reported in myofibrillar protein systems supplemented with modified starches and hydrocolloids (Li et al., 2022; Shen et al., 2023).

3.4.1 Ionic and hydrogen bonds

Ionic and hydrogen-bond-associated protein fractions increased significantly with increasing modified starch incorporation, indicating changes in protein extractability under the selective-solvent conditions used in this study. The abundant hydroxyl groups of starch may participate in hydrogen bonding with water and protein functional groups, which could contribute to hydration and water immobilization (Bao et al., 2025; Shen et al., 2023). In addition, starch swelling may also alter the proximity and accessibility of protein molecules.

In contrast, elevated turmeric levels reduced ionic and hydrogen bonding interactions. This response may result from dilution of the extractable protein phase, competition for water, or interactions of turmeric constituents with proteins. Similar reductions in intermolecular interactions and gel stability have been reported in myofibrillar protein systems supplemented with excessive insoluble dietary fibers (Zhuang et al., 2018).

3.4.2 Hydrophobic interactions and disulfide bonds

Hydrophobic and disulfide-associated-protein fractions also increased with modified starch addition. These increases indicate greater differences in protein solubility between the corresponding extraction solutions and may reflect changes in the accessibility or association state of protein groups during heating. Hydrophobic interactions are important contributors to the thermal gelation of myofibrillar proteins, whereas disulfide exchange can contribute to covalent stabilization under suitable processing conditions (Sun & Holley, 2011).

Conversely, excessive turmeric incorporation reduced the hydrophobic- and disulfide-interaction-associated fractions. This response may be related to dilution of the myofibrillar protein phase and physical interference by insoluble dietary fiber and plant cell wall fragments from turmeric powder, which may reduce contact among protein molecules during thermal gelation (Shah et al., 2014). Overall, the interaction-associated fractions showed trends consistent with the TPA, WHC, and drip-loss results. The combined findings suggest that modified starch and turmeric powder altered protein extractability, hydration, and molecular associations within the sausage system.

3.5 FTIR analysis

The FTIR spectra of the control frog meat sausage and the optimized formulation containing 0.50% modified starch and 0.93% turmeric powder (based on frog meat weight, w/w) are presented in Figure 4. The two spectra exhibited similar overall profiles, indicating that the incorporation of modified starch and turmeric powder did not introduce new functional groups but altered the spectral characteristics of several absorption regions. These changes suggest modifications in the molecular environment of proteins and polysaccharides within the sausage matrix.

The broad absorption band observed at approximately 3415–3435 cm^{-1} was assigned to O–H stretching vibrations associated with hydroxyl groups and hydrogen-bonded molecules. Compared with the control, the optimized formulation exhibited a slight shift and increased band intensity in this region, suggesting changes in hydrogen-bonding interactions among water, starch, turmeric constituents, and myofibrillar proteins. Similar variations in the O–H stretching region have been reported in protein–polysaccharide systems where polysaccharide incorporation modifies hydration behavior and water distribution within the gel matrix (Kong & Yu, 2007; Fan et al., 2019). These spectral observations are consistent with the independently measured increase in water-holding capacity and the operationally defined hydrogen-bond-associated protein fraction.

The absorption band at approximately 1642–1648 cm^{-1} corresponds to the amide I region, which mainly arises from C=O stretching vibrations of peptide bonds and is sensitive to changes in the molecular environment of proteins (Kong & Yu, 2007). In the present study, slight differences in band position and intensity were observed following incorporation of modified starch and turmeric powder, indicating alterations in the local environment of peptide carbonyl groups. These spectral changes may reflect protein–water or protein–ingredient associations during thermal processing (Kong & Yu, 2007).

The absorption band around 2928–2929 cm^{-1} was attributed to asymmetric and symmetric C–H stretching vibrations of aliphatic groups. Slight changes in this region may reflect alterations in the molecular packing of protein and polysaccharide components during gel formation. Similar changes have been reported in thermally processed myofibrillar protein systems and have been associated with modifications in the local hydrophobic environment (Sun & Holley, 2011). The FTIR observations are consistent with the increase in the hydrophobic-interaction-associated protein fraction determined using the sequential extraction method.

Additional absorption peaks observed within 1035–1240 cm^{-1} were associated with C–O, C–O–C, and carbohydrate-related stretching vibrations derived from starch molecules and turmeric polysaccharides. The increased intensity in this region was consistent with the presence of the added carbohydrate-containing ingredients in the formulation. Similar carbohydrate-associated FTIR peaks have been reported in hydrocolloid-supplemented surimi and meat gel systems (Fan et al., 2019). The FTIR results indicated that incorporation of modified starch and turmeric powder altered the spectral environment of protein- and carbohydrate-associated functional groups in frog meat sausage.

3.6 Optimization and model validation

Numerical optimization using response surface methodology (RSM) predicted that the optimal formulation of frog meat sausage consisted of 0.50% modified starch and 0.93%

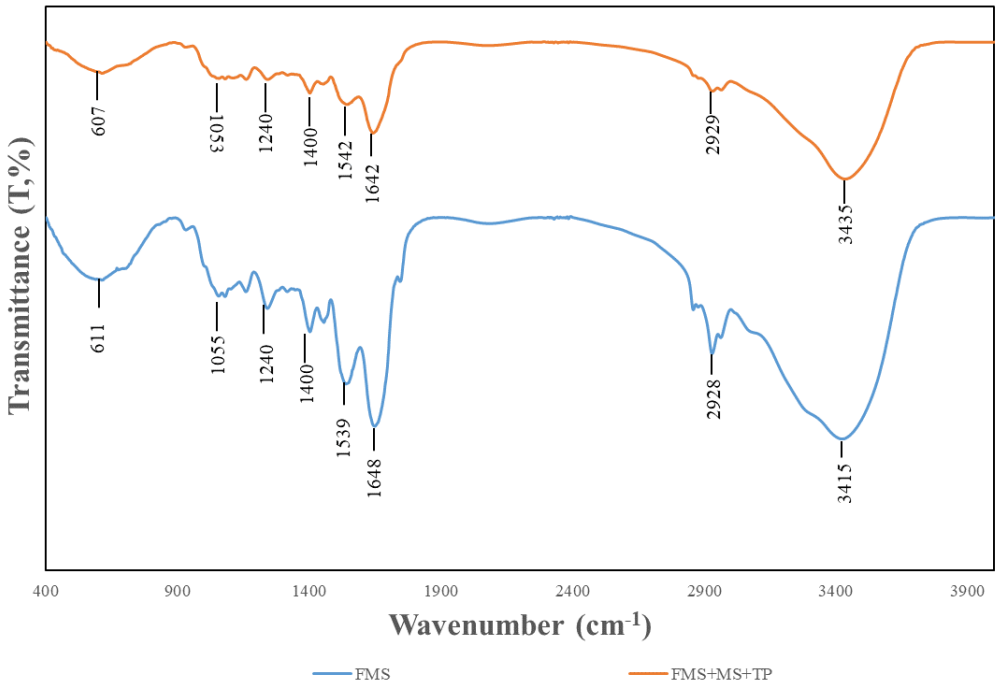


Figure 4. FTIR of frog meat sausage at optimized level of modified starch and turmeric powder. FMS: frog meat sausage; FMS+MS+TP: frog meat sausage added with optimized levels of modified starch and turmeric powder

turmeric powder. Under these optimized conditions, the predicted responses included hardness of 45.37 N, springiness of 0.94 cm, cohesiveness of 0.74, gumminess of 33.64 N, chewiness of 31.71 N.cm, WHC of 94.46%, and drip loss of 1.22% (Table 3). The optimization criteria were established to obtain a sausage formulation with acceptable instrumental texture, high water retention, low drip loss, and interaction-associated protein fractions within the selected experimental ranges.

Experimental validation demonstrated close agreement between predicted and observed values, with all prediction errors below 1%, indicating excellent predictive capability and reliability of the developed quadratic models. The strong agreement between experimental and predicted responses confirmed the adequacy of the RSM approach for describing the interactions between modified starch and turmeric powder in frog meat sausage systems. Similar predictive accuracy and model reliability have been reported in optimization studies involving hydrocolloid–protein interactions and starch-supplemented meat products (Baş & Boyacı, 2007; Fan et al., 2019).

The low prediction errors also indicated that the selected independent variables adequately explained the variability of texture, water retention, and intermolecular interaction responses within the experimental region. Furthermore, the high R^2 values and non-significant lack-of-fit observed in all models confirmed that the developed equations were suitable for process optimization and response prediction in frog meat sausage formulation (Baş & Boyacı, 2007; Khuri & Mukhopadhyay, 2010).

Table 3. Predicted values, observed values and error (%) of the optimized condition of frog meat sausage preparation

Responses	Predicted Values	Observed Values	Error (%)
Hardness (N)	45.37	45.17±6.23	0.44
Springiness (cm)	0.94	0.95±0.03	0.85
Cohesiveness	0.74	0.74±0.06	0.14
Gumminess (N)	33.64	33.47±5.03	0.51
Chewiness (N.cm)	31.71	31.74±6.11	0.09
WHC (%)	94.46	94.58±8.64	0.13
Ionic-interaction-associated fraction (mg protein/mL)	2.26	2.25±0.01	0.44
Hydrogen-bond-associated fraction (mg protein/mL)	2.42	2.41±0.03	0.41
Hydrophobic-interaction-associated fraction (mg protein/mL)	2.83	2.81±0.01	0.71
Disulfide-bond-associated fraction (mg protein/mL)	2.93	2.91±0.05	0.68
Drip loss (%)	1.22	1.21±0.03	0.82

The optimized formulation was subsequently evaluated sensorially as an independent confirmation test. It obtained an overall acceptability score of 5.83±0.04 on the nine-point hedonic scale (data not shown). The sensory score indicated that the product was rated between “neither like nor dislike” and “like slightly,” and close to the “like slightly” category. Thus, although the formulation satisfied the selected instrumental quality criteria, the sensory result also indicated that further refinement of turmeric-related color, aroma, or flavor may be required to achieve stronger consumer liking. The instrumental optimum should therefore be interpreted as a technologically suitable formulation rather than as a fully optimized consumer product.

The present study demonstrates that optimization of modified starch and turmeric powder can substantially improve the technological properties of frog meat sausage. These results complement recent studies that have identified frog meat as a promising alternative protein source for processed food applications because of its favorable nutritional qualities and processing characteristics (Indriani et al., 2025; Tohozin et al., 2025).

4. Conclusions

This study demonstrated that modified starch and turmeric powder significantly affected the physicochemical and structural properties of frog meat sausage. Modified starch improved gel stability, intermolecular interactions, water-holding capacity, and texture quality, while excessive turmeric incorporation weakened gel continuity and reduced moisture retention. The sequential protein extraction and FTIR analyses indicated that modified starch altered the protein interaction-associated fractions and contributed to a more organized protein–polysaccharide matrix during thermal gelation. Numerical optimization based on instrumental responses identified a formulation containing 0.50% modified starch and 0.93% turmeric powder as the optimum within the investigated

formulation range. Experimental validation showed close agreement between the predicted and observed instrumental responses, confirming the adequacy of the developed RSM models for predicting the technological properties of frog meat sausage. Overall, the combined application of modified starch and turmeric powder provides a practical formulation strategy for improving the technological functionality of frog meat sausage, providing useful information for the development of value-added frog meat products.

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6. Authors' Contributions

Ngo Nguyen Phuong Anh performed research and analyzed data;
Nguyen Thi Dong Giang performed research and analyzed data;
Le Hoang Khang performed research and analyzed data;
Cao Thi Tam Nhi performed research and analyzed data;
Pham Huynh Nhu performed research and analyzed data;
Nguyen Van Muoi contributed analytic tools;
Nguyen Phung Tien coordinated research;
Eng Keng Seow coordinated research; and
Tran Hong Quan designed research and wrote the paper

ORCID
Tran Hong Quan https://orcid.org/0000-0002-6807-6867
Eng Keng Seow https://orcid.org/ 0000-0001-8913-0781
Nguyen Van Muoi https://orcid.org/ 0000-0001-6550-4769
Nguyen Phung Tien https://orcid.org/0000-0001-9222-5781

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