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Developing a chickpea protein–flaxseed oil emulsion gel meat analogue using the freeze-alignment technique

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The rising demand for sustainable protein alternatives has driven the development of plant-based meat analogues to address environmental, ethical, and health concerns associated with animal-derived meat. This study aimed to develop a chickpea protein–flaxseed oil emulsion gel meat analogue using the freeze-alignment technique and to optimize its formulation for desirable physicochemical and nutritional properties. A Box–Behnken design was employed to evaluate the effects of freezing temperature (−5 °C to −15 °C), solid-to-liquid (S/L) ratio (5–15% w/w), and chickpea protein isolate-to-flour (CP/CF) ratio (25–75% w/w) on six critical responses: moisture content, water holding capacity (WHC), cutting force, cooking loss, frying loss, and water activity. The optimal processing conditions were identified as a freezing temperature of −10.00 °C, an S/L ratio of 7.00%, and a CP/CF ratio of 75.00%, resulting in a desirability score of 0.8393. Under these conditions, the final product exhibited a moisture content of 65.63%, WHC of 70.38%, cutting force of 18.27 N, cooking loss of 15.02%, frying loss of 24.94%, and water activity of 0.91. The corresponding protein content reached 26.48% on a dry basis, and the fat content was 4.21% (wet basis), reflecting a balanced nutritional profile. Rheological analysis of the emulsion slurry confirmed shear-thinning behavior with low temperature sensitivity (E_a = 10.046 kJ mol^{-1}), indicating stable flow properties. Scanning electron microscopy revealed a porous, fibrous microstructure with uniform ice crystal templating, confirming the effectiveness of the freeze-alignment process. These findings demonstrate the feasibility of producing a nutritionally valuable and structurally stable chickpea-based meat analogue. However, high moisture levels could limit shelf-life, necessitating further investigation into non-freezing preservation methods and sensory evaluation to enhance consumer acceptance and industrial applicability.

Sustainability spotlight

As the global demand for ethical and eco-friendly protein grows, plant-based meat analogues are emerging as a promising solution. This study highlights the development of a chickpea protein–flaxseed oil emulsion gel meat analogue using an innovative freeze-alignment technique. By optimizing key formulation parameters—freezing temperature, solid-to-liquid ratio, and protein isolate-to-flour ratio—researchers achieved a product with high protein content, favourable texture, and desirable moisture retention. The resulting meat analogue showcased a porous, fibrous microstructure and stable rheological behavior, confirming its structural integrity and processing potential. Notably, the use of chickpea and flaxseed oil supports both nutritional balance and sustainability, minimizing reliance on animal-derived ingredients. While elevated moisture levels may affect shelf life, this work sets a strong foundation for further innovation in plant-based meat preservation and sensory enhancement. Overall, this research underscores the viability of chickpea-based systems in advancing sustainable, nutritious meat alternatives.

1. Introduction

Both animal-derived red and white meats are primary sources of essential macro and micronutrients vital for human growth and development. Among these, protein is the most abundant macronutrient, which provides complete amino acids for tissue repair, enzyme production, hormone regulation, muscle growth, and immunity.[1] Consequently, the demand for animal

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protein has surged over the past few decades, paralleling global population growth. According to the OECD/FAO,[2] meat consumption (including beef, pork, poultry, and sheep) is projected to increase by 15% by 2031, necessitating a supply expansion to 377 million metric tons to meet the demand.

However, the meat industry faces significant challenges, including limited natural resources (land and water), rising environmental concerns (water pollution, solid waste disposal, and greenhouse gas emissions),[3] food safety issues (disease outbreaks, antibiotics, pesticides),[4] and ethical considerations.[5] In response, there is a growing demand for alternative protein sources to complement or substitute animal-derived proteins. Plant-based meats have garnered attention for their potential health benefits and lower environmental impact compared to animal-based proteins.[6] Clayton *et al.*[7] defines plant-based meat as structured plant-protein-derived products designed to replace animal meat and marketed as meat substitutes. The plant-based meat market is expected to grow from USD 7.9 billion in 2022 to USD 15.7 billion by 2027, at a CAGR of 14.7%.[8] Consumer demand hinges on the nutritional quality, plant protein source (*e.g.*, grains, legumes, tubers, blends), product type (*e.g.*, plant-based burger patties, sausages, meatballs, strips, nuggets), and the final product's quality (*e.g.*, texture, stability, taste, additives).[9,10]

The textural properties of meat analogs are crucial for consumer acceptance. Dinali *et al.*[11] reported that common animal protein substitutes like tofu, tempeh, Quorn, seitan, and yuba often suffer from poor sensory properties, particularly texture, which fails to mimic animal meat adequately. Therefore, replicating animal meat's fibrous structure is crucial, prompting studies on techniques, conditions, plant proteins, and additives for development. Dekkers *et al.*[12] categorizes structuring techniques into two main strategies: top-down and bottom-up. The bottom-up strategy aims to assemble meat-like structures by mimicking the nanoscale hierarchy of muscle, involving techniques such as cell culturing, wet spinning, and electrospinning. These approaches offer the highest potential to replicate real meat structure due to their ability to control structural elements down to the nanoscale. However, they have significant limitations, including high cost, energy demand, low scalability, and reliance on highly refined or sensitive materials.[12,13] For instance, cultured meat production requires sterile conditions and costly nutrient media like fetal bovine serum,[14] while wet spinning generates substantial wastewater and has limited industrial scalability.[12,15]

In contrast, top-down approaches such as extrusion, shear cell technology, freeze structuring, and hydrocolloid mixing rely on the application of force fields like thermal energy, mechanical shear, or directional freezing to align biopolymer blends and create fibrous textures at the microscale. These techniques are generally more scalable, efficient in resource use, and suitable for industrial applications.[12] Among these techniques, high-moisture extrusion and shear cell processing are the most commonly employed due to their robust processing capabilities. Nevertheless, both methods are associated with high thermal loads, elevated energy requirements, and the risk of structural inconsistency. These challenges can result in excessive protein denaturation, reduced emulsion stability, and limited incorporation of bioactive compounds that are sensitive to heat.[16,17]

Freeze structuring is a novel technique that creates fibrous structures from protein slurries or emulsions through unidirectional heat removal. Ice crystal alignment forms anisotropic, porous textures, with adjustable pore sizes based on freezing rate and temperature. It offers advantages such as minimal protein denaturation, enhanced juiciness, and compatibility with heat-sensitive ingredients, including certain bioactive compounds like vitamins and flavonoids, proteins, and functional polysaccharides that may degrade under high-temperature processing. Furthermore, freeze alignment operates under subzero conditions, enabling lower equipment and energy demands compared to extrusion.[11,12,18] Recent work shows that freezing-based processing can serve as a modular platform for enhancing both texture and functionality in plant-based meats. Directional freezing of soy slurries yields fibrous gels with chicken-like breaking strength,[18] and applying the same principle to cricket-rice blends produces aligned, multilayered networks with improved firmness and adhesion.[19] Freezing-related steps have also been incorporated into multiphase meat-analogue designs: Zhan *et al.*[20] combined freeze-drying of tofu fragments with thermal coagulation to create a biphasic soy matrix containing discrete fibre- and fat-like domains, while Fu *et al.*[21] embedded a soybean-protein/polysaccharide emulsion-gel fat into a pre-formed fibrous SPI scaffold, achieving pork-belly-like hardness and springiness without disturbing fibre alignment. Complementing these textural advances, Dou *et al.*[22] isolated a pectin-rich polysaccharide fraction from freeze-dried raspberry that fortifies gut-barrier integrity and modulates the microbiota, pointing to future opportunities to pair freeze-structured matrices with bioactive polysaccharides. Collectively, these studies highlight the expanding versatility of freeze-related techniques for creating multi-phase, nutritionally enhanced meat analogues.

The selection of raw materials is crucial for the final product quality, alongside the structuring technique. Soy protein and wheat gluten are widely used for developing meat substitutes and analogs due to their availability, rich protein profiles, and processing capabilities.[23,24] However, several studies have reported potential adverse health impacts associated with these raw materials.[25,26] In addition, multiple studies have demonstrated the potential of using fungal biomass, such as molds or mushroom mycelium, to develop meat analogs.[27,28] However, Wang *et al.*[29] highlighted the risk of mycotoxin contamination and the limited availability of effective detection methods in mycelium-based meat analogs. Consequently, there is increasing demand for alternative plant-based proteins, particularly among health-conscious consumers. Chickpea protein has proven to be a good substitute for soy protein. De Camargo *et al.*[30] reported that chickpea provides a healthier alternative to soybean. Furthermore, Webb *et al.*[31] found that extruded chickpea protein could successfully mimic the structure of meat. In addition to being a healthier protein source, using an oil-in-water (O/W) emulsion helps mimic the texture and mouthfeel of animal fat in meat products, providing a juicier and more palatable final product. For instance, Hu *et al.*[32] reported that a soybean oil/water emulsion could successfully replicate the adipose tissue in meat analogs prepared with soy protein.

This study aimed to develop a fibrous meat analogue using chickpea protein and a flaxseed oil/water emulsion, leveraging freeze alignment to achieve a structured texture while enhancing nutritional value through polyunsaturated fatty acids. Unlike previous studies that utilized soy protein with canola oil[18] or alternative protein sources like cricket and rice powder,[19] this research introduces chickpea protein as a novel plant-based alternative. The study's novelty lies in employing response surface methodology with Box–Behnken design to optimize the chickpea protein flour/isolate ratio, solid-to-emulsion ratio, and freezing temperature, ensuring improved product stability. The physicochemical properties of the optimized meat analogue were systematically evaluated.

2. Materials and methods

2.1. Materials

The commercial chickpea flour and chickpea protein isolates were obtained from Anthony's Goods, Los Angeles, California, USA. Food-grade 100% sodium alginate was obtained from Cape Crystal Brands, Summit, New Jersey, USA. Organic cold-pressed flaxseed oil was obtained from Carrington Farms, Closter, New Jersey, USA. The food-grade calcium chloride ($CaCl_2$) was purchased from Pure Original Ingredients, Lindon, Utah, USA. Food-grade glucono-delta-lactone (GDL) was purchased from Soymerica Products, USA, and salt was purchased from a local market. All the chemicals used for physicochemical analysis and TBARS analysis were analytical grade and were purchased from Sigma-Aldrich, St. Louis, Missouri, USA.

2.2. Experimental design

The Response Surface Methodology with Box–Behnken Design (RSM-BBD) was used to optimize and evaluate the individual and synergistic effects of three independent variables: (*A*) the freezing temperature (FT) of the emulsion gel (−5 °C to −15 °C), (*B*) the ratio of total solid to liquid (S/L ratio) of the formula (5–15% w/w), and (*C*) the ratio of chickpea protein isolate to chickpea flour (CP/CF ratio) (25–75% w/w). The dependent variables analyzed were water holding capacity (WHC), moisture content, cooking loss, frying loss, water activity, and texture (cutting force). Fifteen experimental runs (Table 1) were conducted based on the levels of process parameters, with preliminary trials and a study by Chantanuson *et al.*[18] used to determine the levels of independent variables.

2.3. Formulation and production of the meat analog

The formulation and production of the meat analogs were carried out using the methodology explained by Chantanuson *et al.*,[18] with modifications. For a 100 g chickpea–flaxseed oil emulsion slurry, the solid phase (5–15% w/v) consisted of chickpea protein isolate (25–75% w/w), chickpea flour (25–75% w/w), sodium alginate (23% w/w), salt (9% w/w), and glucono-δ-lactone (3% w/w), while the liquid phase (85–95% w/v) consisted of flaxseed oil (91% w/w) and water (9% w/w). Flaxseed oil and water were stirred at 300 rpm for 15 min at room temperature using a magnetic stirrer. The prepared emulsion was mixed with solid ingredients at 300 rpm for 3 min using a mechanical mixer. The resulting chickpea–flaxseed oil emulsion was poured into 4 × 4 × 4 cm silicone molds and frozen at −5 °C to −15 °C in an air blast freezer (air velocity 3.2 ± 1.2 m s^{-1}) for 8 h. After freezing cycle 1, the frozen slurry was removed from molds and soaked in 3% $CaCl_2$ solution for 12 h at 4 °C. The samples were refrozen under the same conditions (freezing cycle 2), thawed at 4 °C. The prepared meat analogs were blotted dry and stored at 4 °C for analysis.

2.4. Product responses

2.4.1. Water holding capacity. The water-holding capacity (WHC) of the meat analog samples was measured using the method suggested by Lakshmanan *et al.*,[33] with minimal modifications. A pre-weighed 50 mL centrifuge tube was filled with 2 ± 1 g of analog sample and a pre-weighed piece of filter paper. The sample-containing tube was then centrifuged using a J2-HS centrifuge model (Beckman Coulter Inc., Fullerton, Calif., USA) at 500 g for 10 min at a temperature of 10 °C. To estimate the weight of the remaining meat analog sample after centrifugation, the wet filter paper was weighed, and the tube was weighed without the paper. Eqn (1) was used to calculate the water-holding capacity (WHC).

$$\mathrm{WHC} = \frac{W_{\mathrm{BC}} - W_{\mathrm{AC}}}{W_{\mathrm{BC}}} \times 100 \tag{1}$$

where W_{BC} and W_{AC} denote the initial weight of the sample (g) before centrifugation and weight (g) after centrifugation, respectively.

2.4.2. Moisture content analysis. According to the AOAC (1995), the moisture content of meat analog samples was assessed by subjecting them to drying in a forced-air oven at 105 °C for 24 h. Subsequently, the percent moisture content of meat analog samples was calculated using eqn (2).

$$\text{Moisture content}(\%) = \frac{W_{\mathrm{i}} - W_{\mathrm{AD}}}{W_{\mathrm{i}}} \times 100 \tag{2}$$

where W_{i} and W_{AD} denote the initial weight (g) of the sample before oven dry and weight (g) after oven drying at 105 °C for 24 h, respectively.

2.4.3. Cooking loss and frying loss. The cooking loss and frying loss were measured using the method explained by Domínguez *et al.*[34] with minor modifications. To measure the cooking loss, 3.0 × 3.0 × 3.0 cm pieces of meat analog samples were cooked in 200 mL of boiling water at 98 °C for 5 min. For the frying loss, samples of the same size were fried in vegetable oil at 170–180 °C for 4 min. The samples were considered cooked when the core temperature reached 70 °C. Following a 5 min drainage period at room temperature (20 °C), the cooking loss and frying loss were calculated using eqn (3) and (4) respectively.

$$W_{iW} - W_{AC}/W_{iW} \times 100 \quad (3)$$

$$W_{iW} - W_{AF}/W_{iW} \times 100 \quad (4)$$

where W_{iW}, W_{AC}, and W_{AF} denote the initial weight (g) of the sample, weight (g) after cooking and weight (g) after frying.

2.4.4. Water activity. The calibrated Rotronic water activity meter (AwQUICK, Rotronic Instrument Corp., Huntington, NY, USA) was used to measure the water activity of the meat analog samples at 25 °C.

2.4.5. Cutting force. Cutting force of meat analog samples was measured using an Instron Universal testing device 196 (Model 5544, Norwood, MA, USA) equipped with a knife blade and a 500 N load cell, according to the method described by ref. 35. The cutting force (N) was determined to be the maximum amount of force required for slicing a 3.0 × 3.0 × 3.0 cm piece of meat analog sample.

2.5. Characterization of the optimized meat analog sample

2.5.1. Flow behavior of the protein slurry. The flow properties of the chickpea–flaxseed oil emulsion slurry with the optimized formula were evaluated using an AR 2000 ex rheometer (TA Instruments, New Castle, DE, USA) with a 20 mm acrylic plate geometry and Universal Analysis Software. A 200 μm gap was used between the rheometer plate and the acrylic plate. The chickpea–flaxseed oil emulsion slurry was allowed to equilibrate to 5, 10, 15, 20, and 25 °C, after which the flow behavior was determined. Shear rates from 0.01 to 100 s^{-1} were used for apparent viscosity (Pa s) measurement, with samples at 10 s^{-1} being reported. The power law model was applied to evaluate the flow behavior of the slurry (eqn (5)).

$$\sigma = K\gamma^n \quad (5)$$

where σ = shear stress (Pa), K = consistency index (Pa s^n), γ = shear rate (s^{-1}), n = flow behavior index. Logarithms of σ and γ were used to construct a scatter plot of log σ *versus* log γ.

A straight line was fitted through the data points on the plot and the magnitude of log K (y intercept) and n (slope) was determined. The Arrhenius relationship (eqn (6)) was used to describe the influence of temperature on apparent viscosity.

$$k = A\,e^{(-E_a/RT)} \quad (6)$$

where k = reaction rate constant, A = frequency factor, E_a = activation energy (J mol^{-1}), R = gas constant (8.314 J mol^{-1} K^{-1}), and T = absolute temperature (K).

The natural logarithm of the apparent viscosity *versus* $1/T$ (absolute temperature in Kelvin) was plotted for chickpea–flaxseed oil emulsion slurry. A trend line was applied, and its slope, intercept, and degree of fit were determined. The slope of the plot was multiplied by the gas constant (8.314 J mol^{-1} K^{-1}) to compute the magnitude of E_a, and A was the exponential of the intercept. The predicted apparent viscosity, obtained using the Arrhenius equation (eqn (6)), was plotted *versus* the experimental viscosity, and the degree of fit of a straight line through the data points was acquired.[36]

2.5.2. Texture profile analysis. The texture profile of the meat analogue was evaluated using an Instron Universal Testing Machine (Model 3342, USA) equipped with a 5 mm cylindrical probe (*P*/5). Each sample was compressed twice to 50% of its original height. Texture parameters including hardness, cohesiveness, springiness, and chewiness were recorded at crosshead speeds of 0.5, 1.0, and 10 mm s^{-1}. All measurements were performed at room temperature (25 ± 1 °C), and results were expressed as the average of three replicates.

2.5.3. Proximate composition analysis. Proximate analyses were conducted on chickpea protein isolate, chickpea flour, prepared meat analogue samples under the optimized conditions, and skinless chicken breast samples were purchased fresh from a local supermarket in Baton Rouge, Louisiana, USA. All samples were analyzed in triplicate. Moisture content was determined using the oven-drying method according to AOAC 930.15, while ash content was measured by incineration in a muffle furnace following AOAC 942.05. Lipid content was assessed using a LECO FA-100 fat analyzer (LECO Corp., USA) based on solvent extraction principles. Nitrogen content was measured using a LECO FP-2000 nitrogen analyzer, and crude protein content was calculated by multiplying the nitrogen percentage by the standard conversion factor of 6.25. All results were expressed on a wet weight basis, and standard deviations were calculated to reflect measurement variability.

2.5.4. Microstructure analysis. Microstructural analysis was performed using scanning electron microscopy (SEM) (Hitachi Tabletop Microscope TM-3000 model). Meat analog samples were securely attached to metal stubs with carbon tape and coated with a thin layer of gold *via* sputtering. They were then positioned in the SEM apparatus for imaging. Images were captured using secondary electrons accelerated at 15 kV and digitally recorded, with magnification at 5000× and 10 000×.

2.6. Statistical analysis and process optimization

RSM-BBD was employed to evaluate the significant differences among variables using a one-way analysis of variance (one-way ANOVA) at a significance level of $p < 0.05$. The Shapiro–Wilk test and Levene's test were used to analyze the normality of the data and the homogeneity of variances, respectively. The Tukey test was employed for pairwise comparisons. The experimental data were fitted using the second-order polynomial equation shown in eqn (7).

$$\begin{aligned} Y_i = {} & \beta_0 \pm \beta_1 A \pm \beta_2 B \pm \beta_3 C \pm \beta_{12} AB \pm \beta_{13} AC \\ & \pm \beta_{23} BC \pm \beta_{11} A^2 \pm \beta_{22} B^2 \pm \beta_{33} C^2 \end{aligned} \quad (7)$$

where Y_i denotes the predicted response, and A, B, and C represent the independent variables. β_0 denotes the intercept, β_1, β_2, β_3 represent the linear effects, β_{12}, β_{13}, β_{23} represent the interaction effects, and β_{11}, β_{23}, β_{33} represent the quadratic effects.

The desirability function method[37] was used to simultaneously optimize the process conditions and product responses for the meat analog by adjusting individual desirability functions for each response variable to attain optimal values.

Table 1 Design matrix and experimental responses for chickpea protein–flaxseed oil meat analogues prepared using response surface methodology[a]

Run	Freezing temperature (°C)	A	S/L ratio (%)	B	CP/CF ratio (%)	C	Moisture content (%)	WHC (%)	Cutting force (N)	Cooking loss (%)	Frying loss (%)	a_w
1	−20	−1	5	−1	50	0	79.3 ± 0.5	73.6 ± 0.3	24.6 ± 2.4	17.7 ± 0.7	27.9 ± 0.9	0.93 ± 0.00
2	−10	1	5	−1	50	0	76.9 ± 0.6	70.3 ± 0.7	23.4 ± 2.4	16.6 ± 1.6	26.9 ± 1.4	0.90 ± 0.00
3	−20	−1	15	1	50	0	82.2 ± 0.5	76.6 ± 0.5	31.2 ± 1.8	21.2 ± 1.1	31.7 ± 1.1	0.96 ± 0.00
4	−10	1	15	1	50	0	79.7 ± 0.5	73.1 ± 0.5	27.4 ± 2.1	19.4 ± 1.8	29.6 ± 1.6	0.92 ± 0.00
5	−20	−1	10	0	25	−1	84.80 ± 0.06	78.2 ± 0.9	31.6 ± 1.3	20.0 ± 1.0	30.3 ± 1.1	0.99 ± 0.00
6	−10	1	10	0	25	−1	83.1 ± 0.5	77.7 ± 0.5	37.2 ± 2.0	20.0 ± 1.3	30.3 ± 1.2	0.97 ± 0.00
7	−20	−1	10	0	75	1	78.8 ± 1.5	77.1 ± 1.9	30.6 ± 0.8	19.6 ± 1.1	29.8 ± 1.1	0.95 ± 0.00
8	−10	1	10	0	75	1	78.0 ± 0.6	72.3 ± 0.7	21.2 ± 2.0	16.6 ± 2.0	26.8 ± 1.8	0.92 ± 0.00
9	−15	0	5	−1	25	−1	83.5 ± 0.5	77.9 ± 0.6	23.6 ± 0.1	20.1 ± 0.9	30.7 ± 0.8	0.97 ± 0.00
10	−15	0	15	1	25	−1	80.7 ± 0.5	75.8 ± 0.4	35.3 ± 0.5	19.7 ± 1.0	29.7 ± 1.0	0.95 ± 0.00
11	−15	0	5	−1	75	1	72.2 ± 0.5	65.6 ± 0.6	20.5 ± 3.2	13.8 ± 1.1	23.8 ± 1.1	0.85 ± 0.00
12	−15	0	15	1	75	1	81.7 ± 0.6	75.8 ± 1.1	27.0 ± 0.8	21.9 ± 1.6	32.1 ± 1.5	0.95 ± 0.01
13	−15	0	10	0	50	0	75.1 ± 0.7	67.9 ± 1.4	31.8 ± 1.0	14.8 ± 1.0	24.9 ± 1.1	0.87 ± 0.01
14	−15	0	10	0	50	0	76.5 ± 0.5	67.3 ± 1.1	33.7 ± 2.2	15.5 ± 1.2	25.5 ± 1.2	0.88 ± 0.03
15	−15	0	10	0	50	0	75.3 ± 0.3	70.0 ± 2.2	30.6 ± 0.6	15.0 ± 1.1	24.9 ± 1.1	0.90 ± 0.02

[a] FT = freezing temperature; S/L ratio = solid-to-liquid ratio; CP/CF ratio = chickpea protein isolate to chickpea flour ratio; WHC = water-holding capacity; a_w = water activity.

3. Results and discussion

3.1. Model characteristics and validation

The Box–Behnken Design (BBD) was used to assess the effects of FT, S/L ratio, and CP/CF ratio on the development of a chickpea protein–flaxseed oil emulsion-based meat analogue. ANOVA results confirmed the quadratic model's significance ($p < 0.001$) for all response variables: moisture content, water holding capacity (WHC), cutting force, cooking loss, frying loss, and water activity (a_w), indicating its suitability for optimization. Among the factors, CP/CF (C) had the most substantial influence, particularly on moisture content ($F = 73.78$), WHC ($F = 63.83$), and cutting force ($F = 44.80$). S/L (B) significantly affected cooking loss ($F = 52.85$) and frying loss ($F = 54.21$), contributing 23.07% and 20.47% to total variation, respectively. The quadratic terms for FT (A^2) and CP/CF (C^2) were highly significant, reflecting nonlinear effects, while interaction terms (AB, BC, AC) showed varying significance across responses. Regression analysis revealed that FT (A) had significant negative effects ($p < 0.001$), while S/L (B) had significant positive effects ($p < 0.001$). CP/CF (C) showed significant negative effects ($p < 0.001$). Quadratic effects were highly significant for A^2 and C^2 and significant for B^2. Model validation (Table 3) showed strong predictive accuracy, with high R^2 values for moisture content (87.69%), WHC (90.19%), cutting force (83.55%), cooking loss (84.72%), frying loss (86.79%), and a_w (94.30%), confirming the model's robustness for optimizing meat analogue processing.

3.2. Product responses

3.2.1. Moisture content, water holding capacity, and water activity. Moisture content, WHC, and a_w are pivotal for the texture, juiciness, stability, and shelf-life of meat analogues, with values ranging from 72.18% to 84.80%, 65.63% to 78.22%, and 0.85 to 0.99, respectively (Table 1). ANOVA (Table 2) confirms that FT (A), S/L (B), and CP/CF (C) significantly influenced all three responses ($p < 0.001$). CP/CF ratio exerted the greatest impact on moisture content, WHC, and a_w, followed by S/L ratio and FT. Regression analysis (Table 3) reveals consistent trends: FT negatively affected moisture content, WHC, and a_w, indicating that lower FT enhances water retention by forming smaller ice crystals, which preserve the integrity of protein networks and reduce water mobility.[38] Similarly, the negative CP/CF ratio coefficients suggest higher protein content enhances water-binding through hydrogen bonding and hydrophobic interactions, lowering free water content and a_w.[39] This trend is consistent with previous findings on soy-protein-based meat analogs where water was more tightly bound at higher protein concentrations.[40] Conversely, S/L ratio positively influenced all responses, attributed to enhanced gel matrix formation through protein–polysaccharide interactions.[18,41] Significant quadratic terms and strong S/L and CP/CF interaction (BC) highlight synergistic stabilization. These relationships are further validated by the response surface plots, where Fig. 1a illustrates how moisture content increases with lower FT and higher CP/CF or S/L ratios, Fig. 1b depicts improved WHC under similar conditions, and Fig. 1f shows reduced water activity, indicating better matrix stability and reduced free water content under optimized conditions. The regression models explain 87.69% (moisture content), 90.19% (WHC), and 94.30% (a_w) of the variation, confirming that lower FT, higher S/L ratios, and balanced CP/CF ratios optimize water-related properties.

3.2.2. Cutting force. Cutting force, a critical quality attribute for meat analogues, was assessed through cutting force, ranging from 20.48 N to 37.16 N (Table 1), reflecting the influence of processing parameters on the analogue's ability to mimic animal meat's fibrous structure. ANOVA (Table 2) indicates that FT (A), S/L (B), and CP/CF (C) significantly affected cutting force. Regression analysis showed that S/L ratio had the

Table 2 Analysis of variance (ANOVA) showing the effects of processing parameters on functional responses of meat analogues[a]

		Moisture content (%)		WHC (%)		Cutting force (N)		Cooking loss (%)		Frying loss (%)		a_w	
		Quadratic		Quadratic		Quadratic		Quadratic		Quadratic		Quadratic	
Source	DF	*F* value	Cont. (%)	*F* value	Cont. (%)	*F* value	Cont. (%)	*F* value	Cont. (%)	*F* value	Cont. (%)	*F* value	Cont. (%)
FT (*A*)	1	4.66*	1.64	26.37***	7.39	9.07**	4.26	9.63**	4.20	10.44**	3.94	49.44***	8.06
S/L ratio (*B*)	1	32.15***	11.31	34.37***	9.63	35.01***	16.45	52.85***	23.07	54.21***	20.47	56.39***	9.19
CP/CF ratio (*C*)	1	73.78***	25.96	63.83***	17.89	44.80***	21.05	17.17***	7.49	20.44***	7.72	7.72***	23.77
$A \times A$	1	36.17***	10.10	65.25***	15.05	2.42	0.36	28.14***	9.16	36.03***	10.22	107.89***	14.38
$B \times B$	1	12.40**	3.30	4.64*	0.61	52.48***	23.58	23.46***	8.54	28.78***	9.07	9.92**	0.84
$C \times C$	1	36.69***	12.91	79.28***	22.22	5.62*	2.64	31.69***	13.83	38.62***	14.58	132.07***	21.52
$A \times B$	1	0.05	0	0.02	0.00	0.83	0.39	0.40	0.17	0.62	0.23	0.03	0.00
$A \times C$	1	1.71	0.38	6.68*	1.87	29.53***	13.88	4.57*	1.99	5.27*	1.99	2.23	0.36
$B \times C$	1	62.77***	22.08	55.44***	15.54	2.00	0.94	37.24***	16.25	49.19***	18.57	99.23***	16.17
Model	9	27.69***	87.69	35.76***	90.19	19.75***	83.55	21.57***	84.72	25.54***	86.79	64.29***	94.30
Lack-of-fit	3	2.09	2.02	2.76	2.02	0.24	0.36	0.10	0.14	0.08	0.10	2.29	1.01

[a] *Significant at $p < 0.05$; **significant at $p < 0.01$; ***significant at $p < 0.001$, FT = freezing temperature; S/L ratio = solid-to-liquid ratio; CP/CF = chickpea protein isolate to chickpea flour ratio; DF = degrees of freedom; cont. = contribution to model variation; WHC = water-holding capacity; a_w = water activity.

Table 3 Regression equations and model fitting for response surface modeling of key functional responses[a]

Response	Regression equation in uncoded units	*R*-sq	*R*-sq (adj.)	*R*-sq (pred.)
Moisture content (%)	$127.84 + 2.977A - 2.075B - 0.7833C + 0.0975A \times A + 0.0571B \times B + 0.003930C \times C - 0.0007A \times B - 0.00325A \times C + 0.02469B \times C$	87.69	84.52	78.60
WHC (%)	$135.48 + 4.344A - 1.671B - 1.0872C + 0.1398A \times A + 0.0373B \times B + 0.006166C \times C - 0.0021A \times B - 0.00860A \times C + 0.02477B \times C$	90.19	87.67	85.43
Cutting force (N)	$13.7 + 0.119A + 4.880B - 0.236C - 0.0459A \times A - 0.2136B \times B - 0.00280C \times C - 0.0257A \times B - 0.03080A \times C - 0.00802B \times C$	83.55	79.32	72.65
Cooking loss (%)	$57.78 + 2.525A - 2.016B - 0.6230C + 0.0765A \times A + 0.0698B \times B + 0.003246C \times C - 0.0087A \times B - 0.00592A \times C + 0.01690B \times C$	84.72	80.80	74.12
Frying loss (%)	$71.47 + 2.738A - 2.219B - 0.6619C + 0.0827A \times A + 0.0739B \times B + 0.003426C \times C - 0.0104A \times B - 0.00608A \times C + 0.01858B \times C$	86.79	83.39	77.77
a_w	$1.5046 + 0.03930A - 0.01755B - 0.010044C + 0.001344A \times A + 0.000408B \times B + 0.000059C \times C - 0.000021A \times B - 0.000037A \times C + 0.000248B \times C$	94.30	92.83	92.13

[a] A = freezing temperature; B = solid-to-liquid ratio; C = chickpea protein isolate to chickpea flour ratio; *R*-sq = coefficient of determination; adj. = adjusted; pred. = predicted; WHC = water-holding capacity; a_w = water activity.

strongest positive effect on cutting, suggesting that higher solid content enhances firmness by increasing the density of the protein–polysaccharide network, likely due to stronger intermolecular interactions such as hydrogen bonding and van der Waals forces between chickpea proteins and sodium alginate.[42] In contrast, CP/CF ratio negatively influenced texture, as higher protein content may lead to excessive gelation, reducing the fibrous alignment by favoring a more amorphous, gel-like structure over anisotropic fiber formation.[18] FT also negatively impacted cutting force, potentially because lower temperatures enhance protein denaturation and aggregation during freezing, strengthening the matrix through increased hydrophobic interactions, whereas higher FT may cause uneven protein unfolding, weakening the structural integrity.[43] The significant quadratic term for S/L ratio indicates a non-linear effect, where excessively high S/L ratios may cause brittleness by overloading the matrix with solids, reducing elasticity, as observed in surface plots (Fig. 1c). Quadratic terms for FT and CP/CF ratio

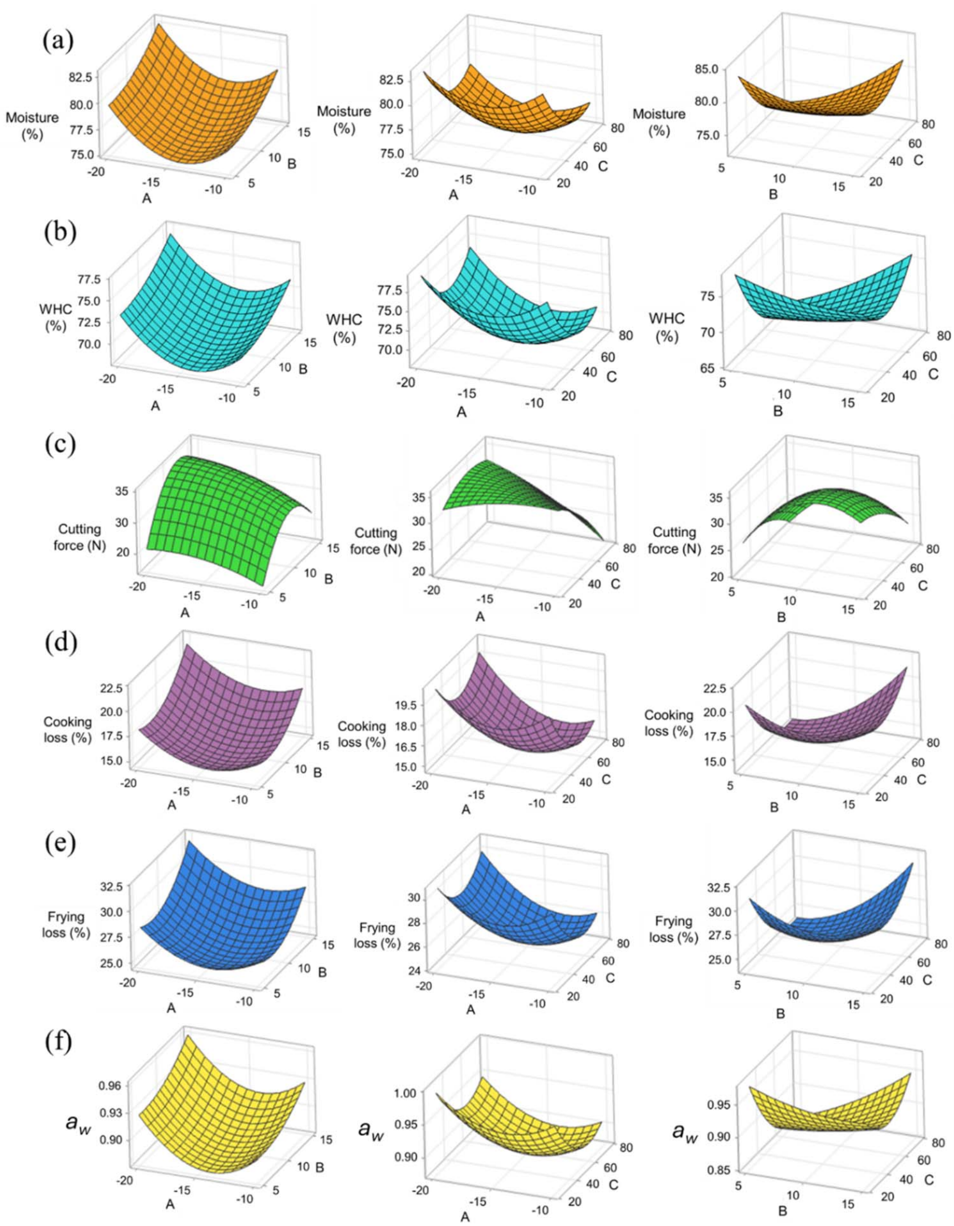

Fig. 1 Response surface plots showing the interactive effects of processing parameters on the physicochemical properties of the chickpea protein–flaxseed oil meat analogue. A = FT (°C); B = S/L ratio (%); C = CP/CF ratio (%). Each row corresponds to a specific response variable: (a) moisture content; (b) WHC; (c) cutting force (N); (d) cooking loss (%); (e) frying loss (%); and (f) water activity (a_w). Within each row, the three 3D surface plots represent the effects of (left to right): $A \times B$ (with C held constant), $A \times C$ (B held constant), and $B \times C$ (A held constant). Hold values for the third variable are: $A = -15$ °C, $B = 10\%$, and $C = 50\%$.

further suggest that extreme conditions can lead to textural inconsistencies, possibly due to phase separation between protein and lipid phases at high CP/CF ratios. The notable interaction between FT and CP/CF ratios highlights their combined effect, where higher protein content may amplify the structural changes induced by FT, affecting fiber alignment. This pattern is supported by Fig. 2c, which demonstrates that cutting force increases at intermediate S/L ratios and declines at extreme CP/CF and FT levels, reflecting the combined influence of these parameters on texture. The regression model (Table 3) explains 83.55% of the variation ($R^2 = 0.8355$), confirming that optimizing FT, S/L, and CP/CF ratios is essential to achieve a firm, fibrous texture, aligning with the sensory expectations for a meat analogue.

3.2.3. Cooking and frying loss. Cooking and frying losses are key quality indicators for meat analogues, impacting texture, taste, and product acceptability, with values ranging from 13.79% to 21.85% for cooking loss and 23.81% to 32.07% for frying loss (Table 1). ANOVA (Table 2) shows that S/L (B), CP/CF (C), and FT (A) significantly influenced both parameters ($p <$

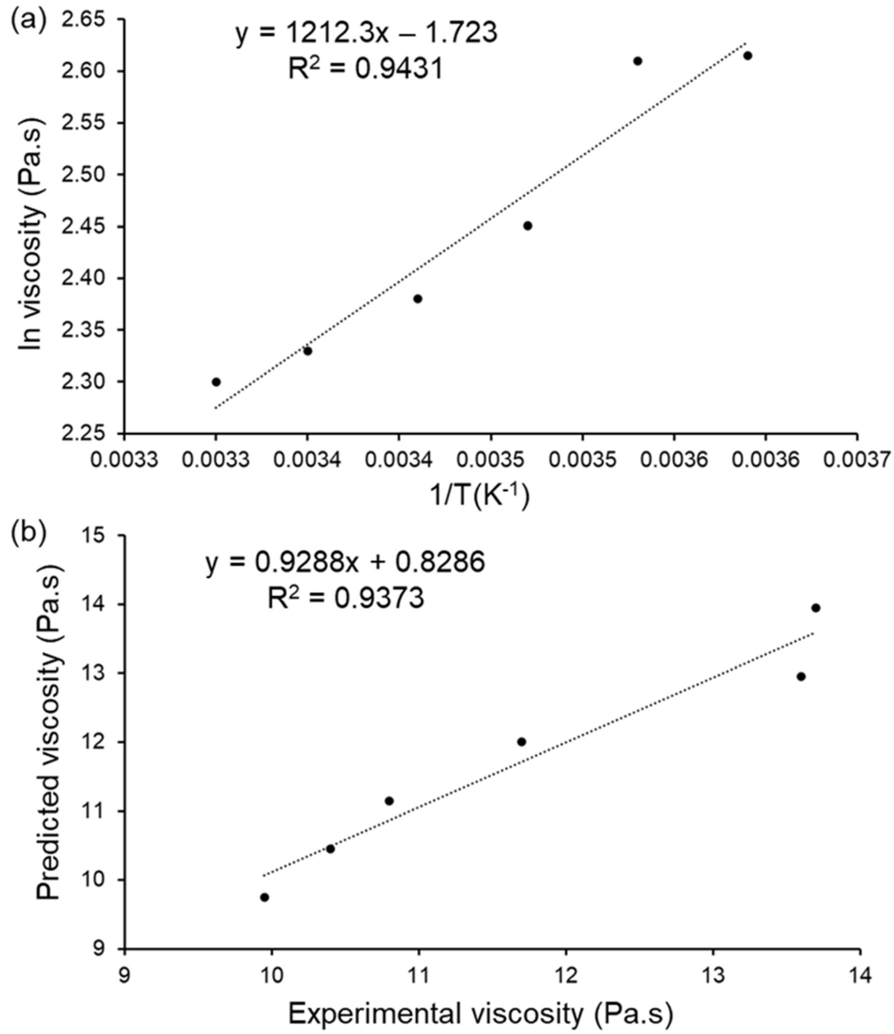

Fig. 2 (a) Arrhenius plot for the apparent viscosity of chickpea protein–flaxseed oil emulsion and (b) comparison of experimental apparent viscosity values (black dots •) with predicted values (dotted line ⋯) derived from the Arrhenius model.

0.001), with S/L ratio having the greatest effect on cooking loss and frying loss, followed by FT and CP/CF ratio. Regression analysis indicated that S/L ratio positively affects both cooking and frying loss, suggesting that higher solid content may weaken the gel matrix's cohesiveness, likely due to reduced emulsion stability, leading to greater expulsion of water and fat during thermal processing.[44] In contrast, CP/CF ratio negatively influences cooking and frying loss, as higher protein content enhances gelation through increased protein–protein interactions, forming a tighter network that retains moisture and fat, consistent with findings in soy protein analogues.[18] FT also negatively affects both responses, indicating that lower FT improves matrix structure due to finer ice crystals that reduce water channel formation and fat migration.[43] Quadratic terms reveal non-linear effects: S/L ratio and CP/CF ratio suggest that extreme ratios disrupt matrix integrity, with high S/L ratios causing brittleness and high CP/CF ratios potentially leading to phase separation, increasing losses.[12] The quadratic term for FT indicates that excessively low or high freezing temperatures may destabilize the gel structure. A significant interaction between S/L and CP/CF ratios underscores their combined role in matrix stability. These outcomes are supported by the response surface plots in Fig. 1d and e, where minimized losses occur at mid-range CP/CF and S/L ratios combined with lower FT, aligning with improved matrix stability and reduced phase separation. The regression models (Table 3) explain 84.72% (cooking loss) and 86.79% (frying loss) of the variation ($R^2 = 0.8472, 0.8679$), confirming that balancing FT, S/L, and CP/CF ratios is essential to minimize losses, thereby improving the thermal stability and sensory quality of the meat analogue.

3.3. Optimization and validation

Optimization of the meat analogue's formulation was conducted using the desirability function, with criteria for each response variable defined based on functional and sensory requirements (Table 4). For moisture content and water-holding capacity (WHC), the goal was to replicate the quality attributes of natural chicken meat. Chicken breast samples analyzed using the same procedures in this study exhibited an average

Table 4 Desirability criteria and optimization targets for functional response variables of meat analogues[a]

Response	Goal	Lower	Target	Upper
Moisture content (%)	Target	71.84	75.00	84.84
WHC (%)	Target	65.10	70.00	78.93
Cutting force (N)	Minimum	18.17	18.17	38.61
Cooking loss (%)	Minimum	12.92	12.92	23.43
Frying loss (%)	Minimum	23.00	23.00	33.54
a_w	Minimum	0.85	0.85	0.99

[a] WHC = water-holding capacity; a_w = water activity.

Table 5 Predicted values for selected responses/functional properties and desirability under optimal conditions: freezing temperature (−10.00 °C); solid/liquid ratio (7.00%); chickpea protein isolate/chickpea flour ratio (75.00%)

	Optimum conditions	
Response	Predicted value	Desirability
Moisture content (%)	74.91	0.9707
WHC (%)	68.89	0.7734
Cutting force (N)	18.83	0.9677
Cooking loss (%)	14.94	0.8076
Frying loss (%)	25.12	0.7987
a_w	0.88	0.7458
Overall desirability		0.8393

moisture content of 76.1 ± 2.0% and a WHC of 69.2 ± 1.3%. Accordingly, the target values were set at 75% for moisture and 70% for WHC to emulate the juiciness and textural functionality of chicken, while the lower and upper limits were defined based on the range of values observed across all experimental trials using different meat analogue formulations. For the remaining responses: cutting force (20.48 N), cooking loss (13.79%), frying loss (23.81%), and water activity (a_w, 0.85), the optimization objective was minimization to enhance tenderness, reduce thermal degradation, and improve microbial safety. Therefore, the lowest observed values from the measured responses (Table 1) were assigned as both the lower and target values, while the upper bounds reflected the highest values obtained across the design space. These selection criteria ensured that the optimization was grounded in experimentally achievable values, while aligning with the functional and safety expectations for high-quality meat analogues.

The optimal conditions identified through the desirability function were a freezing temperature (FT) of −10.20 °C, a solid-to-liquid (S/L) ratio of 7.02%, and a chickpea protein isolate-to-chickpea flour (CP/CF) ratio of 75.00%, which yielded the highest desirability score of 0.8865. For practical implementation, these values were slightly adjusted to more convenient, rounded settings: −10.00 °C for freezing temperature, 7.00% for S/L ratio, and 75.00% for CP/CF ratio. This adjustment resulted in a slightly lower but still acceptable desirability score of 0.8393 (Table 5). A similar approach of adjusting optimal conditions for practicality has been reported in previous optimization studies, such as Tan *et al.*,[45] where small modifications were made to facilitate implementation without significantly compromising overall desirability. Experimental validation confirmed the model's accuracy, with actual values closely matching predicted values as depicted in Table 6. The close agreement between predicted and experimental results validates the robustness of the response surface model and demonstrates the feasibility of these optimized conditions for producing a meat analogue with desirable physicochemical properties, paving the way for scalable production of a sustainable meat alternative.

Table 6 Validation of predicted *versus* experimental values for functional responses under optimized processing conditions[a]

Response	Predicted value	Actual value
Moisture content (%)	74.91	75.51 ± 0.73
WHC (%)	68.89	70.38 ± 1.06
Cutting force (N)	18.83	18.27 ± 0.82
Cooking loss (%)	14.94	15.02 ± 0.89
Frying loss (%)	25.12	24.94 ± 0.93
a_w	0.88	0.91 ± 0.05

[a] WHC = water-holding capacity; a_w = water activity.

To contextualize these findings, the cutting force, water-holding capacity (WHC), and water activity (a_w) of the developed chickpea–flaxseed oil-based meat analogue was benchmarked against a conventional chicken breast and previously reported soy based and other freeze-structured meat analogs. The chickpea–flaxseed oil meat analogue sheared at 18.3 ± 0.8 N, closely matching the ∼20 N breaking strength reported for soy protein gels frozen at −10 °C with 10% solids,[18] but was significantly softer than chicken breast (39.2 ± 1.3 N). The WHC of the chickpea analogue (70.4 ± 1.1%) exceeded that of the soy analogue (52.2 ± 2.6%)[19] and slightly surpassed the value observed in chicken (69.2 ± 1.3%), indicating superior water retention and juiciness, a key attribute for consumer acceptance. The formulation maintained a water activity (a_w) of 0.91 ± 0.05, which is close to values reported for soy-based meat analogues (∼0.88) and favourably lower than fresh poultry (∼0.96), suggesting improved microbial stability and shelf-life potential.[18,46] Overall, this benchmarking validates that the optimized formulation not only meets technical quality metrics but also aligns with consumer expectations and regulatory safety thresholds.

3.4. Characterization of the meat analogue developed under optimized conditions

3.4.1. Flow behavior of the protein–oil emulsion slurry. The rheological properties of the chickpea protein–flaxseed oil emulsion slurry, critical for the freeze-alignment process, were evaluated to understand their impact on phase separation and fiber alignment during structuring (Table 7). The slurry exhibited shear-thinning behavior, with the flow behavior index (n) increasing from 0.799 at 5 °C to 0.848 at 25–30 °C, indicating reduced shear sensitivity as temperature rises. This behavior,

Table 7 Flow characteristics and Arrhenius parameters of the chickpea protein–flaxseed oil emulsion with optimized solid/liquid ratio and chickpea protein isolate/chickpea flour ratio at 5–30 °C[a]

Temperature (°C)	n	K (Pa s^n)	Apparent viscosity (Pa s)
5	0.80 ± 0.02^{c}	34.6 ± 0.8^{a}	13.7 ± 0.6^{a}
10	0.80 ± 0.01^{bc}	34.2 ± 0.2^{a}	13.7 ± 1.0^{a}
15	0.84 ± 0.01^{abc}	24.5 ± 1.3^{b}	11.7 ± 1.1^{ab}
20	0.85 ± 0.01^{a}	21.7 ± 0.6^{c}	10.8 ± 0.1^{b}
25	0.85 ± 0.01^{a}	21.4 ± 0.3^{c}	10.37 ± 0.09^{b}
30	0.843 ± 0.001^{ab}	20.54 ± 0.02^{c}	9.95 ± 0.06^{b}
E_a (kJ mol^{-1})	10.1 ± 1.3		

[a] n = flow behavior index; K = consistency index; E_a = activation energy; different superscript letters (a, b, c) indicate statistically significant differences at $p < 0.05$.

where viscosity decreases with increasing shear rate, enables efficient mold filling and supports directional freezing, essential for fiber formation.[12] The consistency index (K) dropped from 34.563 Pa s to 20.535 Pa s across this temperature range, and apparent viscosity decreased from 13.704 Pa s to 9.949 Pa s, reflecting enhanced flowability at higher temperatures due to weakened hydrogen bonding and hydrophobic interactions within the emulsion matrix.[9] The activation energy (E_a) of 10.046 kJ mol^{-1}, calculated from the Arrhenius model (R^2 = 0.94) (Fig. 2a), suggests low sensitivity to temperature, implying stable rheological properties under varying processing conditions.[36] The high agreement between predicted and experimental viscosities (R^2 = 0.94) validates the model's predictive capacity (Fig. 2b). These rheological traits low temperature sensitivity, shear-thinning behavior, and predictable viscosity are essential for controlling slurry behavior during mold filling and freeze-alignment, ensuring consistent ice crystal templating and fibrous texture in the final product.[18]

3.4.2. Texture profile analysis. The textural attributes of the optimized chickpea–flaxseed oil meat analogue, as evaluated through TPA, revealed a hardness of 4.06 ± 0.06 N, cohesiveness of 25.4 ± 1.2%, springiness of 26.7 ± 0.6%, and chewiness of 0.26 ± 0.04 N. These values fall within the expected range for freeze-structured plant-based matrices. When benchmarked against reference products, the chickpea-based sample exhibited TPA values closely resembling those of a soy-based analogue processed under similar freeze-structuring conditions (hardness: 3.45 N, cohesiveness: 26%, springiness: 29.8%, chewiness: 0.27 N) as reported by Nakagawa *et al.*[19] However, all TPA values remained substantially lower than those of conventional chicken breast (13.82 N, 46%, 65%, 3.85 N for hardness, cohesiveness, springiness, and chewiness, respectively).

This contrast in mechanical properties highlights the softer and less elastic structure of the chickpea-based meat analogue, which is consistent with previous findings on plant-based alternatives. According to Dekkers *et al.*,[12] lower cohesiveness and chewiness in meat analogues stem from the absence of myofibrillar proteins, which are critical for the fibrous network and elasticity in animal muscle. Similarly, Osen *et al.*[47] reported that plant protein extrudates, regardless of formulation, tend to show reduced mastication resistance and structural integrity compared to animal meat. This is often attributed to weaker hydrophobic interactions and less effective protein–protein crosslinking during structuring processes. Although the optimized emulsion matrix mimics the fibrous orientation of meat through freeze-alignment, it lacks the biochemical complexity of muscle fibers, which limits its mechanical robustness. Despite these differences, the lower hardness and chewiness may actually enhance the consumer acceptability of the product, especially among target populations such as older adults or individuals seeking easy-to-chew meat alternatives.[48] Moreover, the springiness and cohesiveness values indicate a resilient gel matrix, supporting structural stability during handling and cooking.

3.4.3. Proximate analysis. The proximate composition of the optimized meat analogue revealed a moisture content of 65.6% ± 1.1%, protein content of 9.10% ± 0.04% (wet basis), and fat content of 4.21% ± 0.02% (wet basis), as shown in Table 8. On a dry basis, the protein concentration reached approximately 26.48%, which, although lower than that of chicken breast (∼32%, dry basis; USDA[49]), is still substantial for a legume-based formulation and comparable to or higher than many commercial plant-based meat analogues.[50] The moderate fat content reflects the addition of flaxseed oil, which is rich in

Table 8 Proximate composition of the prepared meat analogue and selected raw materials

	Chickpea protein isolate	Chickpea flour	Meat analogue
	78.70 ± 0.08	20.6 ± 0.8	9.10 ± 0.04
Total fat	—	7.0 ± 1.2	4.21 ± 0.02
Crude fat (PE)	12.0 ± 1.3	—	—
Crude fiber	<0.50	2.9 ± 0.7	<0.50
Salt	0.8 ± 0.4	0.13 ± 0.09	1.51 ± 0.02
Moisture	8.0 ± 1.2	9.5 ± 1.1	65.6 ± 1.1
Ash	8.4 ± 0.7	3.2 ± 0.4	2.0 ± 0.5

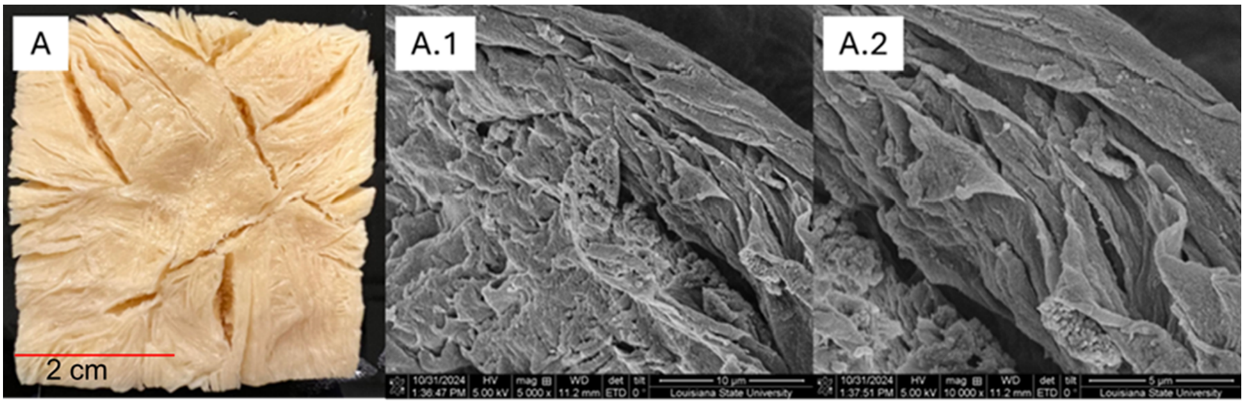

Fig. 3 (A) Photograph and SEM images of the optimized chickpea protein–flaxseed oil meat analogue cross-section at (A.1) 5000× and (A.2) 10 000× magnifications.

α-linolenic acid (ALA), an omega-3 fatty acid known for its cardiovascular and anti-inflammatory benefits.[51] This not only contributes to health-promoting lipid quality but also enhances the mouthfeel and juiciness of the analogue. The low fiber content (<0.50%) is consistent with the high protein purity of the chickpea protein isolate used, while salt (1.51%) and ash (2.00%) levels support ionic interactions and charge shielding necessary for effective protein gelation during freeze-alignment.[52] These compositional attributes contribute to the formation of a coherent protein network, which supports water retention and contributes to textural integrity. Importantly, the moisture level (~65%) is sufficient to replicate the juiciness of real meat products while maintaining a matrix firm enough to withstand handling and cooking. The balanced macro- and micronutrient profile enhances the product's appeal as a sustainable, nutritious alternative for flexitarians and health-conscious consumers.

3.4.4. Microstructure analysis. Fig. 3 illustrates the macrostructure (A) and SEM micrographs (A.1 and A.2) of the optimized chickpea–flaxseed oil meat analogue. The cross-sectional image (A) displays well-defined fibrous alignment and layered separations, visually mimicking the striated appearance of chicken muscle fibers. This directional structuring is a hallmark of freeze-aligned meat analogues and is essential for emulating the fibrous mouthfeel of animal meat. The SEM images confirm these observations at the microscale. At 5000× magnification (A.1), the structure reveals an interconnected, porous matrix with aligned lamellar domains, a clear indication of anisotropic ice crystal templating during the controlled freezing process. The 10 000× magnification (A.2) further highlights the presence of elongated, folded fibrillar structures, suggesting effective phase separation between protein and lipid components, which facilitates fibrous layer formation.

Such structural features are consistent with previously reported freeze-structured meat analogues made from soy proteins.[18] The observed anisotropy enhances mechanical strength and water retention by providing directional channels for moisture entrapment and release during chewing, contributing to improved juiciness and sensory realism. In contrast to the dense and less porous structures often seen in extruded analogues, freeze-structured matrices like the one developed here offer greater microstructural fidelity to natural meat.[12,53] Overall, the uniformity and continuity of the fibrous network observed in both magnifications confirm the robustness of the formulation and the effectiveness of the freeze-alignment strategy in producing a plant-based meat analogue with desirable texture, structural anisotropy, and water-holding potential.

4. Conclusion

This study demonstrated the potential of using freeze alignment to develop a chickpea protein–flaxseed oil emulsion gel-based meat analogue with promising structural and functional attributes. Through Box–Behnken optimization, the formulation achieved a high WHC (70.38%), reduced cooking (15.02%) and frying losses (24.94%), and moderate cutting force (18.27 N), resulting in a balanced combination of moisture retention and textural integrity. With a dry basis protein content of 26.48%, low fat, and favorable water activity, the analogue mimics key nutritional and safety characteristics of conventional meat while promoting sustainability through the use of legume proteins and plant oils. Freeze alignment enabled the formation of a highly porous, anisotropic microstructure that closely resembled the fibrous matrix of muscle tissue. Compared to extrusion and shear cell techniques, which often yield denser, less organized networks, freeze alignment offers a gentler, energy-efficient alternative that better preserves the structural fidelity required for meat-like texture. However, its relatively high moisture content may pose shelf-life challenges, suggesting the need for integrative preservation strategies.

Future research may explore advanced preservation techniques, such as high-pressure processing or pulsed electric field to enhance microbial stability without compromising texture. Additionally, consumer-focused studies evaluating the effects of marination, cooking method, and cultural preferences could inform product positioning across diverse markets. Pairing freeze-aligned matrices with flavor encapsulation, bioactive polysaccharides, or 3D-printed structuring may also enhance nutritional value, mouthfeel, and personalization.

Furthermore, techno-economic and life cycle assessments would be valuable in evaluating scalability and sustainability in comparison to more established structuring technologies. Overall, freeze alignment presents a viable pathway for producing next-generation meat analogues with improved microstructural realism and functional performance, particularly for chickpea-based systems. With further refinement and integration, this method may expand the scope of plant-based meat alternatives to address both consumer expectations and global protein sustainability challenges.

Conflicts of interest

There are no conflicts to declare.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding authors upon reasonable request.

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