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Formulation of plant-based meat alternatives and its optimization by experimental design using response surface methodology†

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The trend of adopting plant-based foods as a substitute for meat is on the rise due to their nutritional benefits. In an effort to develop meat alternatives, response surface methodology (RSM) is used to optimize the formulation. In this study, wheat flour, soy flour, and horse gram were used as the primary ingredients. The process involved an initial screening experiment for the determination of suitable ingredient concentrations followed by a numerical optimization method, RSM-Central Composite Design (CCD). The goal of the optimization was to achieve protein, energy, and carbohydrate efficiencies of 95%, 89%, and 86%, respectively. The final product was tested using specific quantities of ingredients, resulting in maximum amounts of crude protein (20.278 g), carbohydrates (73.488 g), and energy (362.879 kcal). The morphological and textural studies of plant-based meat exhibit comparable characteristics to the available animal meats. This research work highlights the potential advancement of plant-based ingredients in developing nutritionally balanced meat alternatives.

Sustainability spotlight

A research investigation focusing on the ecological implications of plant-based meat substitutes derived from plant sources reveals promising findings regarding the sustainability of plant-based protein sources. Substituting animal-based meat with these components can lead to significant reductions in greenhouse gas emissions, land usage, and water consumption. The plant-based meat not only provides ample protein but also exhibits lower environmental impacts. Furthermore, these ingredients are versatile, making them suitable for a variety of culinary applications, thereby offering a sustainable choice for individuals seeking ethical and environmentally friendly alternatives to traditional animal-derived meats. This study underscores the potential of plant-based alternatives to contribute to a more sustainable and compassionate food system.

1 Introduction

The modern world has witnessed a modification in dietary patterns, marked by reduced meat consumption and a growing demand for flavorful and nutritious meat alternatives. This trend has sparked interest in protein sources like pulses, wheat gluten, and soy protein, which are processed to create meat analogs that mimic the texture, flavor, color, and nutritional composition of different meats. Research has shown that adopting a vegan diet can enhance metabolic activity and contribute to overall physical well-being.[1,2] Meat is valued for its sustainability, high iron content, and perceived quality as a protein source. On the other hand, plant-based meat products are designed to replicate the taste and texture of meat.[3] These alternatives, also known as fake meat, mock meat, or plant proteins, employ various substitutes to mimic flavors, textures, and aromas.[4]

Plant-based meats offer numerous health benefits, and are also rich in protein and fiber, which are essential for weight management. In addition, plant-based meats generally have fewer calories than traditional meats and can help regulate blood sugar levels by slowing down sugar absorption into the bloodstream.[5] Foods high in saturated fats, such as processed beef, lard, full-fat dairy products, and animal fats, are associated with high cholesterol levels and significant health risks. In contrast, plant-based meats do not contain cholesterol. The development of textured vegetable protein in the 1960s played a pivotal role in creating versions of plant-based meat foods like burgers and bacon. The concept of plant-based meat alternatives (PBMA) has continued to evolve, with companies like “Impossible Foods” and “Beyond Meat” introducing a new generation of PBMA that closely resemble animal meat in terms of structure, aroma, and even the appearance of “bleeding” when cooked.[6–8]

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Horse gram is nutrient-dense and contains bioactive compounds with therapeutic and health-promoting effects. It is recognized as a valuable source of proteins, dietary fiber, macro- and micronutrients, and beneficial phytochemicals important for human health. Interest in functional foods and nutraceuticals containing bioactive substances has increased in recent years due to their various health benefits.[9–11] Horse gram is highly nutritious, containing protein (17.9–25.3%), carbohydrates (51.9–60.9%), essential amino acids, low lipid content (0.58–2.06%), minerals, and vitamins,[12] as well as bioactive compounds like phenolic acids, flavonoids, and tannins. Horse gram exhibits high emulsification activity and stability. The functional properties of horse gram enable it to have the stability to hold the proteins during cooking without degradation in meat alternatives.[13] Soy grit and soy flour have gained popularity as meat substitutes, especially in the eastern parts of India. Soy chunks can be consumed in moderation as part of a balanced diet and serve as a valuable protein source for vegetarians and those with protein deficiencies.[14] Soy is rich in omega-3 fatty acids and fiber, while being low in saturated fat. Therefore, the consumption of soy has been associated with a reduction in body weight and fat mass.[15] Besides, soy flour may provide better structural properties to meat alternatives due to its high firmness, compressibility, cohesiveness and springiness.[16]

Cereals, particularly wheat, are integral to global food production and are consumed in various forms. Gluten, a unique protein in wheat, is essential for bread production. Whole wheat, including bran and germ, offers additional health benefits such as vitamin E and has been associated with protection against diseases like heart infection, obesity, and diabetes.[17–19] On the other hand, wheat has a major impact on the physicochemical properties of meat alternatives such as hardness, chewiness and springiness.[20] Based on the above-mentioned factors, the present work reveals the utilization of protein-rich components to create a substitute for meat.

The objective of this study is to formulate plant-based meat alternatives and characterize their nutritional composition (*i.e.* carbohydrates, proteins, and energy). Furthermore, the formulated product is investigated in terms of morphology, texture, functional group and its sensory attributes.

2 Materials & methods

2.1. Experimental design and statistical analysis

2.1.1. Optimization. The response surface methodology utilizing Central Composite Design (Mini Tab 2021 software, V.20.2.1) was chosen for optimizing and formulating the meat substitute. This study applies CCD to establish the relationship between horse gram, soy flour, and wheat flour and their impact on protein, carbohydrate, and energy content.[21] The dependent variables were categorized into three levels: low, medium, and high, along with star points represented by the values −1.68, −1, 0, +1, and +1.68, as shown in Table 1. The analysis of variance provides valuable information about the significance and adequacy of the regression model in explaining the variation in the dependent variables based on the independent variables. It helps assess the overall quality of the model and determines the statistical significance of the model terms.[22]

Table 1 Levels of process variables

Variables	−1.68 Star point	−1 Low level	0 Center point	1 High level	1.68 Star point
Horse gram	0.43	33.66	50.43	75.41	100.43
Soy flour	1.38	21.54	35.00	68.62	91.54
Wheat flour	0.21	16.83	25.22	50.22	67.27

For CCD, the total number of experiments (n) needed is determined by the formula $n = 2k$, where k represents the number of experimental variables and C_0 represents the number of experiments conducted at the center point. In this case, there are three experimental variables ($k = 3$), and six experiments are conducted at the center point ($C_0 = 6$), resulting in a total of 20 experiments. The remaining experimental conditions are kept constant, and the runs are randomized to avoid any potential bias.

In the optimization of shelf-life, three independent variables are investigated *i.e.* horse gram, soy flour, and wheat flour. Each variable is tested at various levels with (−1.68) and (+1.68) representing star points, (1) indicating higher levels, zero (0) representing the center value, and (−1) indicating lower levels. To simplify calculations, the independent variables are assigned coded values, where X_j represents the coded value, X_i represents the actual value, X_0 represents the value at the center point, and Δx represents the shift in the variable X_i given in eqn (1) and (2)

$$X = X_i - X_0;\ i = 1, 2, 3, 4, 5\ (3.4)\ j\ \Delta x \quad (1)$$

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_{12} + \beta_{22} X_{22} + \beta_{33} X_{32} \quad (2)$$

The dependent variable Y can be represented as a function of the independent variables X_1, X_2, and X_3. The Y equation includes various coefficients that account for linear, quadratic, and interactive effects. The constant term is denoted as β_0, while the linear coefficients are represented by β_i. The quadratic coefficients are denoted as β_{ii}, and the interactive coefficients are indicated by β_{ij}.

Specifically, the coefficients β_{11}, β_{22}, and β_{33} correspond to the squares of the levels of X_1, X_2, and X_3, respectively. These coefficients capture the quadratic effects of the independent variables. On the other hand, the coefficients β_{12}, β_{13}, and β_{23} represent the products of the levels of different X variables, accounting for the interactive effects among the variables. In summary, eqn (3) for the dependent variable Y includes a constant term, linear terms for each independent variable, quadratic terms for each independent variable, and interactive terms capturing the interactions between the independent variables.

$$Y = \beta + \sum k\beta X_1 + \sum k\beta X_2 + \sum k \sum k\beta_{XX}\ (3.5); \quad i = 1ii\ i = 1iii\ i = 1j = 2ijij \quad (3)$$

2.2. Preparation of the meat substitute

The horse gram, soy flour, and wheat flour were obtained from a local grocery shop in Coimbatore, Tamil Nadu, India. The meat substitute was prepared according to Sharma *et al.*[23] The horse gram underwent a germination process, involving washing, soaking, and daily rinsing in distilled water. The germinated horse gram was ground and mixed with soy flour, wheat flour, and salt in specific proportions. The mixture was shaped into a round flat form and fried at 195 ± 3 °C for 14–16 minutes formulating the meat substitute.

2.3. Moisture analysis

The moisture content of the samples was determined using a Sartorius MA35 model moisture analyzer, in accordance with the procedure outlined in AOAC (Association of Official Analytical Chemists, 2005). The detailed experimental procedure is given in the ESI.†

2.4. Proximate analysis

2.4.1. Ash. The ash content in the samples was assessed utilizing a methodology specified in AOAC 942.05, 2022.[24] A 2 g of sample was placed in a pre-weighed silica dish and kept in a muffle furnace at 550 °C. The sample was heated and converted into ash and the mineral and organic compounds were identified using eqn (4).

$$\text{Ash}(\%) = \frac{W_1 - W_2}{W_s} \times 100 \quad (4)$$

where W_1 is the weight of the ash + crucible after formation of ash, W_2 is the weight of the empty crucible and W_s is the weight of the sample taken.

2.4.2. Crude fiber. The crude fiber was determined according to AOAC 920.85, 1995.[25] A 2 g of sample was mixed with 1.25% sulfuric acid and subjected to boiling for 30 minutes to break down the complex of carbohydrates and proteins. The solution was filtered to remove the proteins and lipids using 1.25% NaOH solution. The filtered cake was converted into ash using a furnace and the crude fiber content was calculated using eqn (5).

$$\text{Crude fiber}(\%) = \frac{W_1 - W_2}{W_s} \times 100 \quad (5)$$

Here, W_1 refers to the weight of the dry sample, W_2 denotes the weight of the ash sample and W_s is the weight of the sample.

2.4.3. Crude fat. The crude fat of sample was analysed using the method described in AOAC 920.85, 2005.[26] A 2 g of sample was added with 2 mL of ethanol and 10 mL of hydrochloric acid (HCl) solution. The sample was heated until the formation of brown color. Afterwards, 25 mL of diethyl ether and 25 mL of petroleum ether were added to allow for layer separation. The separated compound was kept in a hot air oven set at 105 °C to evaporate the solvent and dry the lipids. The fat content in the samples was calculated using eqn (6).

$$\text{Fat}(\%) = \frac{W_2 - W_1}{S_w} \times 100 \quad (6)$$

where W_2 is the weight of extraction cup after extraction process with fat, W_1 is the weight of the empty extraction cup and S_w is the sample weight.

2.4.4. Carbohydrate. The amount of total carbohydrates found in the sample was estimated using the anthrone method. Following this method, the organic matter's lipid content, crude protein, and ash were removed, and the material that was left over can be classified as carbohydrates.[27]

2.4.5. Protein. The meat sample was weighed approximately along with anhydrous sodium sulphate and copper sulphate. Concentrated sulphuric acid was added to the sample mixtures. The contents were digested until a clear solution appeared. The mixture was dissolved using distilled water, and then transferred to a round bottom flask by adding 60 mL of 50% NaOH. It was then connected to Kjeldahl distillation apparatus. The distillate collector beaker contains weak acid 0.1 N H_2SO_4 and the distillation column was run till the solution volume reaches 150 mL. The collected distillate was titrated against 0.1 N NaOH (AOAC 920.87, 2005).[28] The percentage protein was calculated using the following equations (eqn (7) and (8)).

$$(\%)N = \frac{T - B \times N \times 14}{W_S} \times 100 \quad (7)$$

$$\text{Crude protein } (\%) = \%\ N \times 6.25 \quad (8)$$

Here, T refers to the volume of titration for the sample (mL), B is the volume of titration for the blank (mL), and N refers to the normality of H_2SO_4.

2.5. Energy

The energy value of the meat alternative was considered for the three nutrient groups, crude protein, crude fat and carbohydrates. The energy values of the cookies were calculated according to eqn (9).[29]

$$\text{Food energy (cal)} = [\text{TC } (\%) - \text{CF}] \times 4 + [\text{TF } (\%) \times 9 + \text{CP } (\%) \times 4] \quad (9)$$

Where TC is total carbohydrate, TF denotes the total fat, CP stands for crude protein, CF refers to crude fiber.

2.6. Morphology studies of the meat alternative

A scanning electron microscope (EVO 18, Carl Zeiss, Germany) was used to study the morphology of dried plant-based meat samples. The samples were mounted on the specimen holder and sputter-coated with gold palladium (2 min, 2 mbar) and observed at 15 kV and a vacuum of 9.75×10^{-5} torr.[30]

2.7. Fourier transform infrared spectroscopy

The formulated dried meat alternative sample were analyzed by Fourier transform infrared (FTIR) spectroscopy (Bruker Alpha II, ATR Mode, USA). Similarly, controls such as soy, wheat and horse gram powder were analyzed. The scanning range was kept at 500–4000 cm^{-1}. Pure FTIR-grade KBr was used to prepare all the samples in this experiment.[31]

2.8. Texture characterization

The tensile strength (kPa) of the plant-based meat was evaluated according to the compression test and a TA XT Plus (Stable Micro Systems Ltd, Surrey, UK) was used to obtain hardness (N) by determining the maximum force of the compression. Samples were compressed (test speed 5 mm s^{-1}, load cell 30.0 kg) using a probe P/36 R. The shear force was measured using a force plate (test speed 5 mm s^{-1}, load cell 30.0 kg).

2.9. Sensory evaluation

The formulated plant-based meat alternative was evaluated by a 9-point hedonic scale and the detailed experimental procedure is given in the ESI.† Ten trained healthy subjects (six males, four females, aged between 19 and 30, non-smokers) were recruited for the sensory assessment and they rated each attribute on a scale ranging from 1 (dislike extremely) to 9 (like extremely). Based on multiple sensory attributes and overall acceptability researchers were able to assess the overall quality and potential market acceptance of the product. Written consent was taken from each participant and ethical approval was obtained from Karpagam Academy of Higher Education.

3 Results and discussion

3.1. Optimization of the meat substitute forming parameters by response surface methodology

In this study, RSM is utilized and employs a central composite design comprising both a quadratic model and a linear model. The objective was to investigate the individual and interactive effects of three independent variables, namely horse gram, soy flour and wheat flour on the response variables of energy, carbohydrates, and proteins. The obtained results are presented in Table 2, which showcases the response variable values at different ratios of the independent variables. For the protein content, the minimum value of 12.89 g was attained at a ratio of 33.66 : 21.54 : 16.83 for horse gram, soy flour, and wheat flour, respectively. Conversely, the maximum value of 19.55 g was achieved at a ratio of 50.43 : 35.00 : 25.22 for horse gram, soy flour, and wheat flour, respectively. Regarding carbohydrate content, the minimum value of 42.82 g was obtained at a ratio of 50.43 : 1.38 : 25.22 for horse gram, soy flour, and wheat flour, respectively. In contrast, the maximum value of 70.73 g was attained at a ratio of 75.41 : 68.62 : 50.22 for horse gram, soy flour, and wheat flour, respectively. Regarding energy content, the minimum value of 239.93 kcal was obtained at a ratio of 33.66 : 21.54 : 16.83 for horse gram, soy flour, and wheat flour, respectively. On the other hand, the maximum value of 337.30 kcal was achieved at a ratio of 50.43 : 35.00 : 25.21 for horse gram, soy flour, and wheat flour, respectively. These findings provide valuable insights into the optimal combinations of the independent variables (horse gram, soy flour and wheat flour) that yield desired values of the response variables (energy, carbohydrates, and proteins) in the meat alternative.

3.1.1. Analysis of protein, energy and carbohydrate based on the interactions between the factors. RSM was used to develop a response surface model for the protein, energy, and carbohydrate data. The model selection criteria include a high R-squared value, a small difference between adjusted and predicted R-squared values, a high model F-value, a low model P-value, and a non-significant lack-of-fit P-value as provided in ESI, Table S1,† which led to the suggestion of a quadratic model. The minimal S values obtained for the interactions indicated the significance of the model and its good fit to the data. All coefficients in the model were statistically significant further supporting its validity. The ANOVA results revealed that

Table 2 Central composite design for the meat substitute

Horse gram (g)	Soy flour (g)	Wheat flour (g)	Protein (g per 100 g)	Predicted value of protein (g per 100 g)	Carbohydrate (g per 100 g)	Predicted value of carbohydrate (g per 100 g)	Energy (kcal per 100 g)	Predicted value of energy (kcal per 100 g)
33.660	21.541	16.831	12.89	12.892	43.350	43.355	239.930	239.932
75.412	21.541	16.831	16.903	16.902	42.950	42.950	255.530	255.530
33.660	68.620	16.831	16.580	16.581	42.120	42.120	254.543	254.542
75.412	68.620	16.831	19.140	19.144	51.800	51.809	291.780	291.782
33.660	21.541	50.220	16.850	16.847	43.577	43.571	259.094	259.093
75.412	21.541	50.220	18.310	18.311	50.860	50.863	291.444	291.445
33.660	68.620	50.220	19.203	19.205	53.344	53.348	294.636	294.637
75.412	68.620	50.220	19.222	19.221	70.736	70.735	348.633	348.631
0.430	35.000	25.218	15.986	15.985	44.638	44.637	255.232	255.231
100.43	35.000	25.218	19.373	19.372	58.921	58.916	313.752	313.751
50.430	1.380	25.218	15.589	15.590	42.821	42.821	248.916	248.915
50.430	91.541	25.218	19.460	19.457	58.498	58.492	309.288	309.288
50.430	35.000	0.215	15.642	15.638	42.511	42.504	252.725	252.723
50.430	35.000	67.266	19.027	19.029	58.597	58.599	316.639	316.639
50.430	35.000	25.218	19.555	19.555	69.970	69.970	337.307	337.307
50.430	35.000	25.218	19.555	19.555	69.970	69.970	337.307	337.307
50.430	35.000	25.218	19.555	19.555	69.970	69.970	337.307	337.307
50.430	35.000	25.218	19.555	19.555	69.970	69.970	337.307	337.307
50.430	35.000	25.218	19.555	19.555	69.970	69.970	337.307	337.307
50.43	35.000	25.218	19.555	19.555	69.970	69.970	337.307	337.307

the linear terms of horse gram, soy flour, and wheat flour had a significant effect on the meat alternative, highlighting their contribution to the variation in protein, energy, and carbohydrates. The lack of fit and pure error were both zero, indicating a favourable fit for the energy response.

Further, the RSM analysis provided valuable insights into the relationship between the ingredients (horse gram, soy flour, and wheat flour) and the nutritional composition of the meat alternative. The suggested quadratic models for protein, energy, and carbohydrate responses along with the coefficients, regression equations, and ANOVA results have confirmed the significance and effectiveness of the models in predicting the protein content and energy of the product.

3.1.2. Determination of protein, energy, and carbohydrate interaction between the factors. The identification of minimum and maximum interactions affecting the protein content between horse gram, soy flour, and wheat flour is illustrated in ESI Fig. S1–S3.† The Pareto chart highlights that the highest interaction occurs with variable B, representing soy flour. Specifically focusing on soy flour, interactions with variables C, A, CC, BB, AA, AC, and AB exhibit stronger effects on the protein response. Conversely, the least interaction is observed between soy flour and wheat flour, denoted as BC. The analysis for energy revealed that the highest interaction occurred with variable BB (soy flour). In the case of soy flour, interactions involving variables AA, CC, C, B, A, AB, and BC displayed stronger interaction effects on energy. On the other hand, the lowest interaction was observed between horse gram and wheat flour, specifically in AC. The findings of carbohydrate interactions indicated that the highest level of interaction was observed for CC (wheat flour). Regarding soy flour, interactions BB, AA, C, B, A, BC, and AB demonstrated more favorable effects. Therefore, the least amount of interaction between carbohydrates was observed among horse gram and wheat flour, particularly in the case of AC.

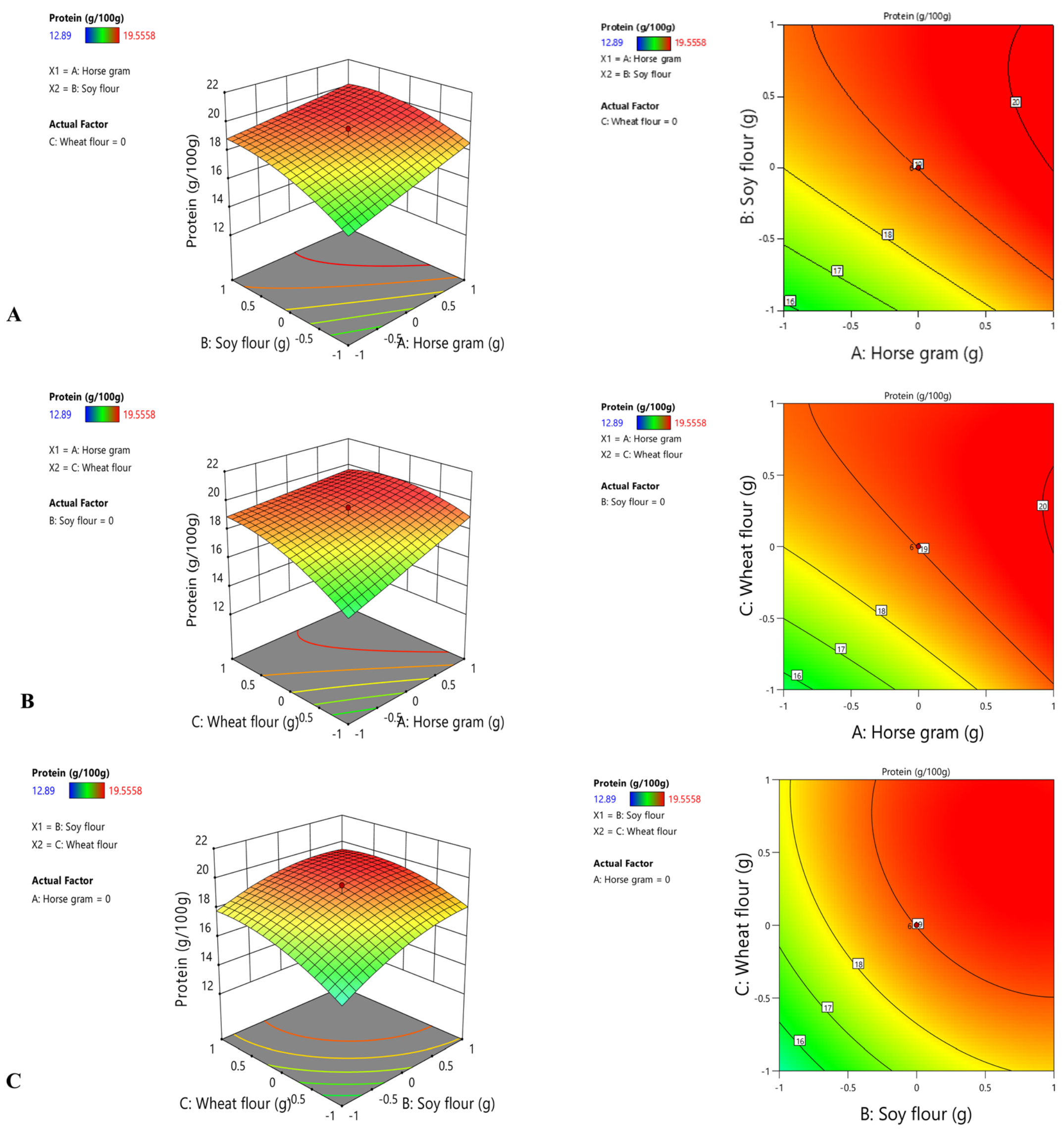

Fig. 1 Response surface plots of protein interactions between (A) soy flour and horse gram, (B) wheat flour and horse gram and (C) soy flour and wheat flour.

3.1.3. Contour and surface plots of protein. The study aimed to analyze the interactions between soy flour and horse gram, wheat flour and horse gram and soy flour and wheat flour in relation to protein values. The results are presented in Fig. 1 which consists of three subplots (A, B, and C) and their interactions. The maximum protein value of 20 was achieved when soy flour and horse gram levels were between 0.5 and 1.0. A protein value of 18 was observed between −1.5 and −1.0, and a value of 16 was obtained at the center (0.0). The minimum protein value of 14 was recorded between −2.0 and −1.5. The surface plot indicated that the maximum interaction occurred at the back surface with medium values at the center and minimum range at the back surface. The surface plots and contour plots provided insights into the behavior and relationships among the variables of horse gram, soy flour, and wheat flour for creating meat alternatives with specific protein contents. The surface plots indicated that the maximum interaction between these variables occurred at the rear surface, suggesting a strong correlation. The center of the plots showed moderate values that represent a balanced combination of the ingredients. Additionally, the narrow range observed at the rear surface indicated consistency within that range, suggesting consistency in the desired characteristics of plant-based meat.

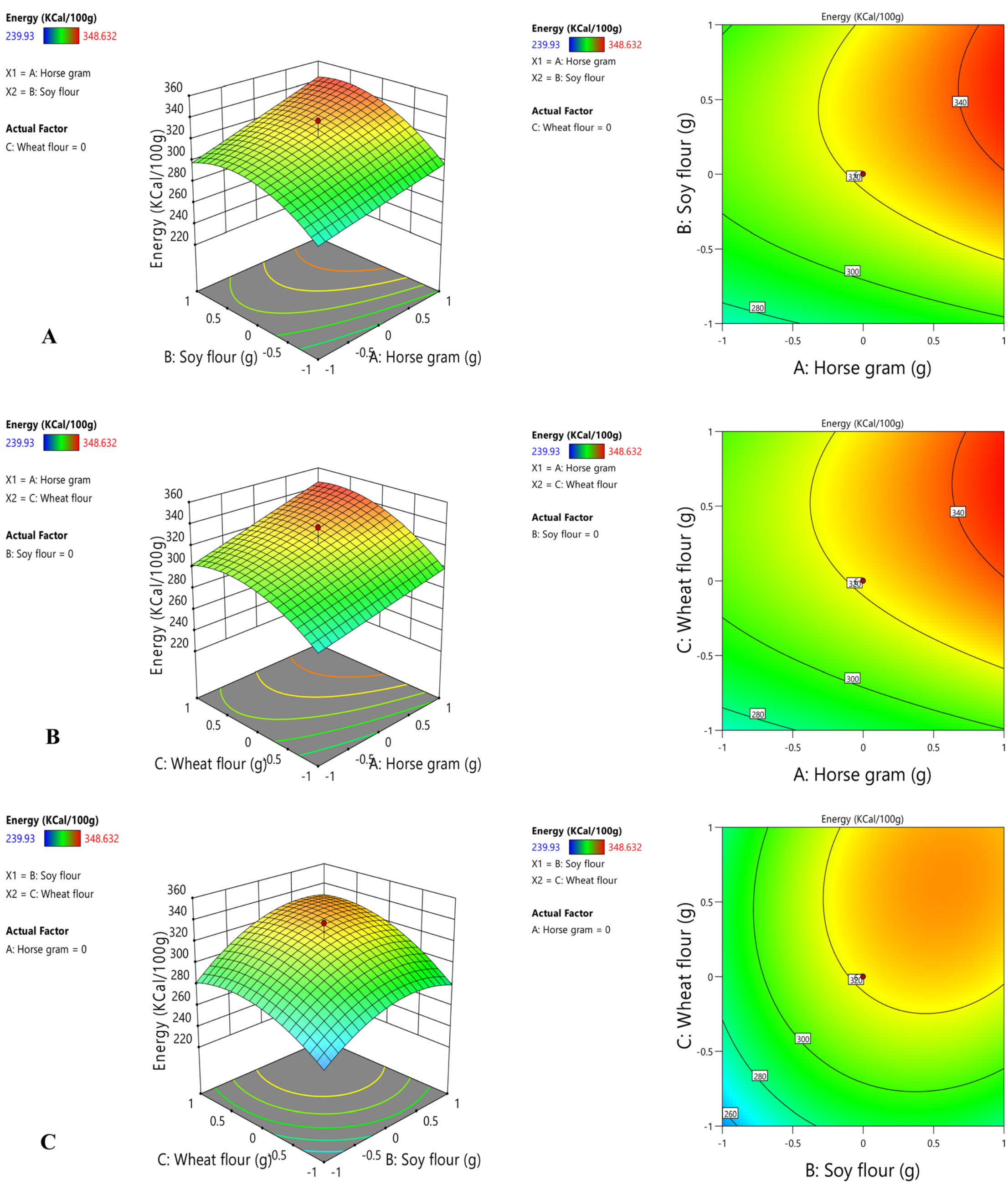

Fig. 2 Response surface plots of energy interactions between (A) soy flour and horse gram, (B) wheat flour and horse gram and (C) soy flour and wheat flour.

These findings align with previous studies,[23,32] with resemblance in the range and surface plot analysis.

Therefore, the graphical representations, including the contour plots and surface plots, provide valuable insights into the behavior and relationships among the variables involved in the meat substitutes. By identifying the optimal combinations of ingredients, these graphical representations contribute to the formulation of plant-based meat products with the desired protein content.

3.1.4. Contour and surface plots of energy. In Fig. 2A–C, the energy range resulting from the interaction between soy flour and horse gram, wheat flour and horse gram, and soy flour and wheat flour is depicted. The highest energy value of 325 was achieved when the levels of horse gram were between 0.0 and 0.5. The surface plot indicated that the maximum interaction occurred at the rear surface with moderate values concentrated at the center. The narrowest range was observed at the rear surface which suggests consistency within that range.

The analysis of surface plots and contour plots provided insights into the behavior and relationships among the variables (horse gram, soy flour, and wheat flour) in achieving specific energy contents in the meat alternative. The strong correlation observed at the rear surface, moderate values at the center, and consistency within a narrow range at the rear surface indicate the influence of these variables on the energy content. The findings in the range and surface plot analysis align with similar results and the significance of the variables in the development of plant-based meat products.[23,32]

3.1.5. Surface and contour plots of carbohydrate. The primary focus of the study was to examine the interactions among soy flour, horse gram, and wheat flour in the production of carbohydrate extracts for meat alternative products. Fig. 3A–C

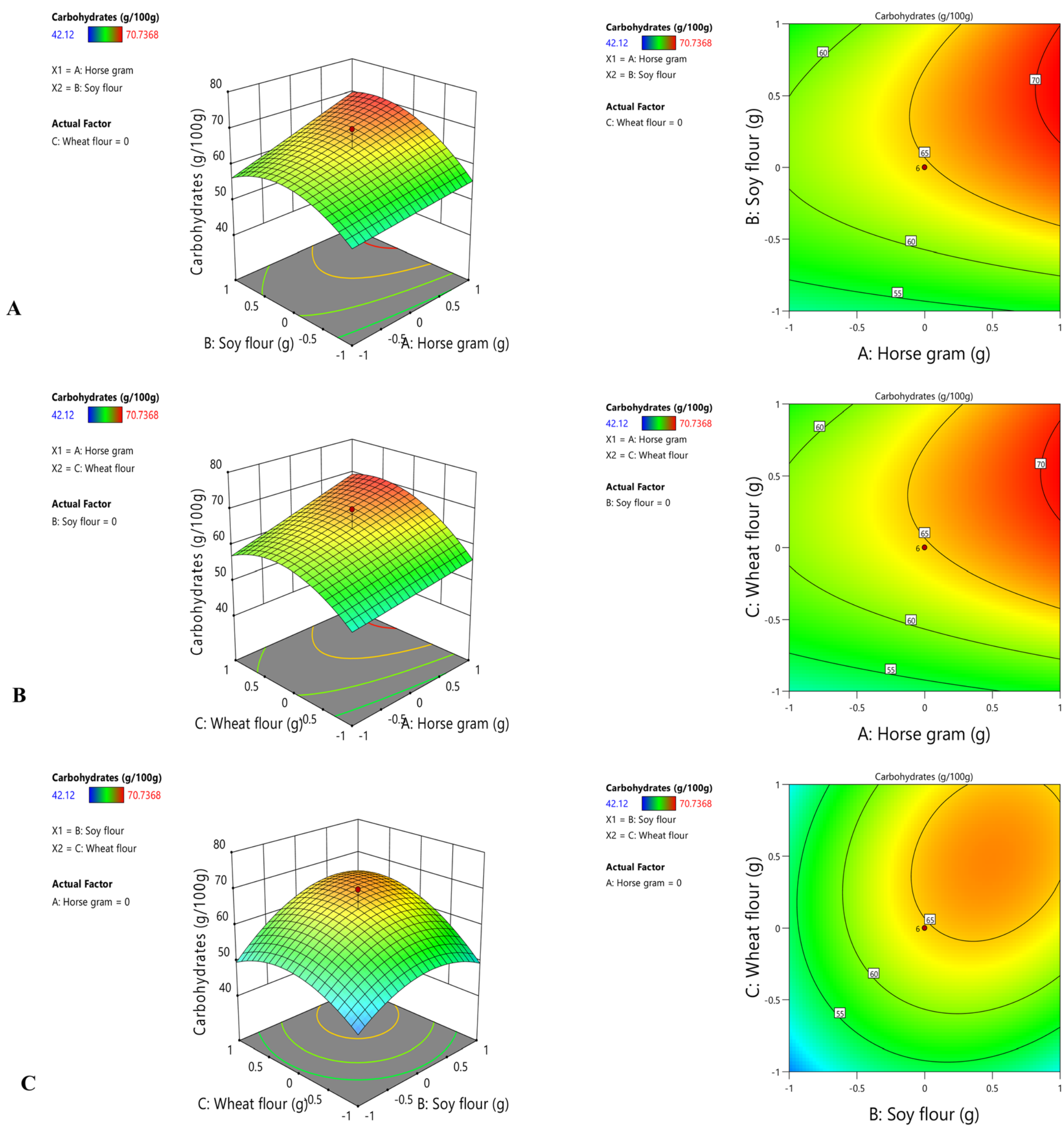

Fig. 3 Response surface plots of carbohydrate interactions between (A) soy flour and horse gram, (B) wheat flour and horse gram and (C) soy flour and wheat flour.

Table 3 Optimized value for the meat substitute

Solution	Horse gram (g)	Soy flour (g)	Wheat flour (g)	Energy (kcal per 100g) (fit)	Carbohydrate (g per 100 g) (fit)	Protein (g per 100 g) (fit)	Composite desirability
1	59.928	51.255	36.467	362.879	73.488	20.278	1

present the carbohydrate range of the products. The results demonstrated that the highest carbohydrate value of 70 was achieved within the range of 0.0 and 0.5 for horse gram, while other ranges yielded values of 40 and 60. Surface plot analysis indicated that the most significant interaction occurred at the rear surface with moderate values concentrated at the center and a narrower range at the rear surface. The range and surface plot analysis resembled the previously reported studies.[23,32]

The surface and contour plots provided valuable insights into the behavior and relationships among the ingredients in the production of plant-based meat products. They indicated maximum interaction at the rear surface, moderate values at the center, and a narrow range at the rear surface, suggesting consistency in achieving desired product characteristics. These graphical representations greatly facilitated the understanding of ingredient behavior and relationships, thereby contributing to the development of the products.

3.1.6. Optimized value for meat substitute forming parameters. The study utilized RSM to identify the optimized concentration ranges for the meat substitute. Table 3 presents the optimized concentration ranges for horse gram, soy flour, and wheat flour. The optimized concentration for horse gram, soy flour, and wheat flour was found to be 59.92 g, 51.25 g, and 36.46 g, respectively. The optimization process involved the use of a desirable function, which yielded a value of 1.0 for the chosen parameters. This value indicates that the selected concentrations of horse gram, soy flour, and wheat flour fall within acceptable limits and are considered optimal based on the experimental data. The desirable function can determine the best combination of ingredients that meets the required amount for the formulation of the meat substitute.[33]

3.2. Formulation of the meat substitute

The meat alternative formulated with horse gram, soy flour, and wheat flour underwent assessment in two distinct forms: raw materials and raw meat substitute. Individual perceptions of springiness, gumminess, and chewiness can be influenced by various factors *i.e.* moisture content, fat content, and food matrix structure. Food manufacturers can consider these factors to precisely adjust the texture of their products in alignment with consumer preferences.[34] Initially, the product was expected to have a soft texture and a high moisture content of 45.68%. However, after deep frying the meat substitute in cooking oil, the texture of the product transformed into a crispy nature. A proximate analysis was conducted on the final product prepared using the optimized values obtained from the RSM. This analysis provided valuable insights into the composition and nutritional profile of the meat substitute. The obtained product results showed 3.91% ash, 20.22% protein, 0.24% fat, 0.32% fiber, and 70.52% carbohydrates. Additionally, the energy value of the product was determined to be 365.12 kcal per 100 g. These findings were provided in ESI Table S2† that represents various components present in the meat substitute and their nutritional profile.

3.3. Morphology of the meat alternative

SEM analysis was carried out to study the morphological characteristics of dried meat alternatives (Fig. 4a–f). In this study, the plant-based meat exhibited irregularly shaped particles at 100× magnification (Fig. 4a), which is comparable to the pea fiber microstructure[35] (Aydogdu *et al.* 2018). Under 500× and 1000× magnification, a compact structure with a porous and irregular network was observed (Fig. 4b and c). Grabowska *et al.* suggested that the formation of porous structures is due to the evaporation of water during the preparation of products.[36] The magnifications of 1000× and 2500× represent the honeycomb based globular structure (Fig. 4d). This is because protein chains significantly form the globular morphology[37] (Muhialdin and Ubbink, 2023).

When magnified to 2500× and 5000×, the meat alternative showed a polymeric network of elongated densely packed morphology with fibrous structures (Fig. 4(e and f)). The morphology of fibrous structures resembles the hydrolyzed wheat protein and soy protein-based meat analogues[38] (Zhang *et al.*, 2023). Likewise, Krintiras *et al.* have prepared structured meat analogues using soy protein isolate and wheat gluten. They exhibited a layered or fibrous structure. Also, it was suggested that the formation of large fibrous structures is made of smaller ones and interconnected with much smaller fibres. Hence, it was reported that these fibres may be gluten. In addition, the fibrous structures are attained when soy protein blends with wheat gluten.[39] Recently, Guo *et al.* investigated high moisture meat analogs and reported that the proteins have transformed from a globular to a fibrous structure.[40] On the other hand, Dekkers *et al.* stated that the ratio of polysaccharides to proteins could be central to the formation of fibrous structures. Therefore, the fibrous structure is of primary interest in plant materials for the development of a meat replacer.[41] The microstructure of meat alternatives serves as a pivotal factor in determining its texture and sensory attributes. The fiber-like structures and protein matrix contribute significantly to the products' chewiness and mouthfeel that successfully mimic the textural qualities of traditional meat. Therefore, the presence of starch globules enhances the overall palatability of the product. A comprehensive grasp of the microstructure of meat alternatives will help in formulating potential guidelines for the design and development of novel products.

3.4. Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopy was performed to investigate the functional group of raw powder (control) and

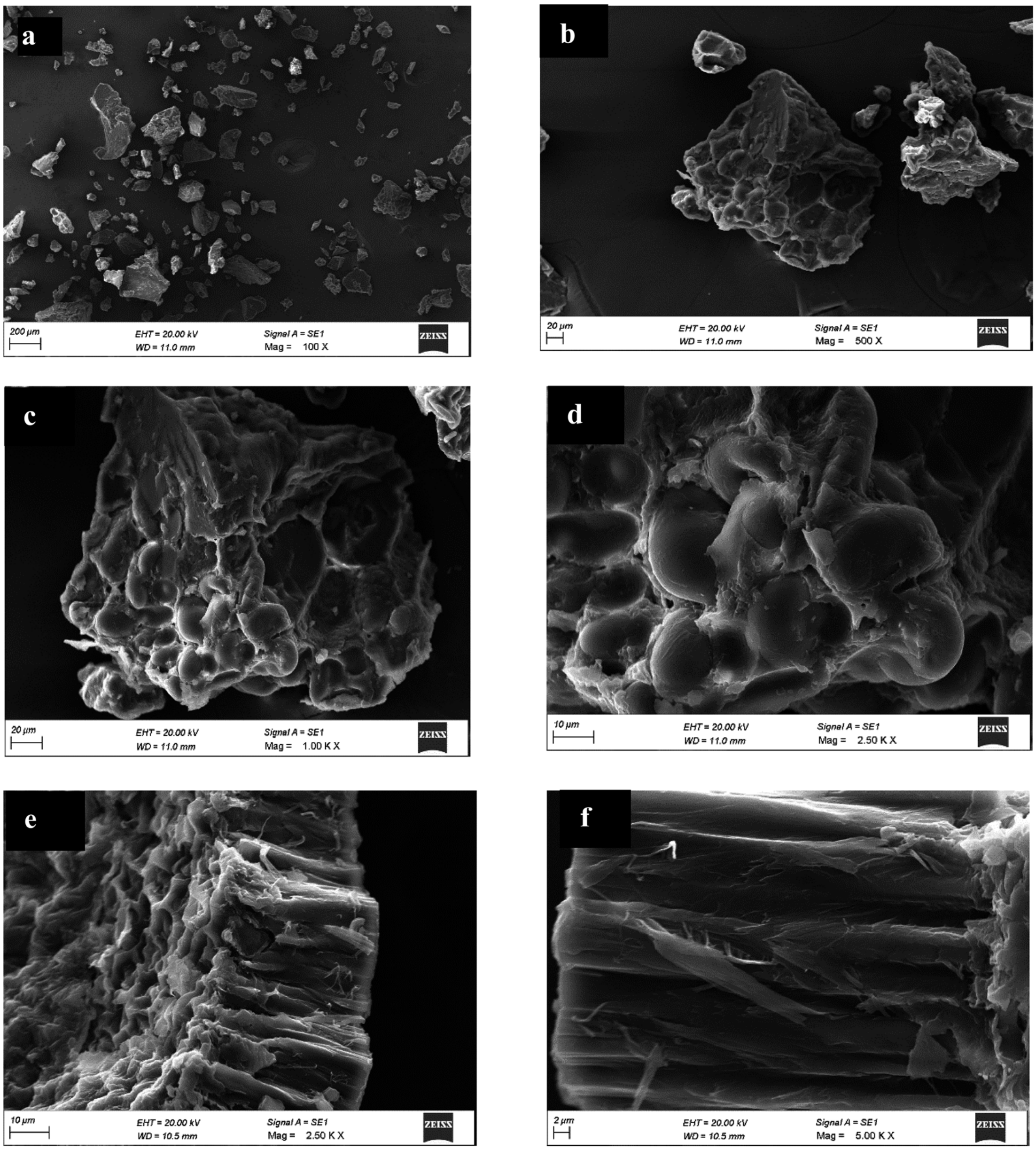

Fig. 4 (a) Morphological characteristics of dried plant-based meat at 100× magnification and (b) dried plant-based meat at 500× magnification. (c) Representation of starch globules in dried plant-based meat at 1000× magnification. (d) Formation of a honeycomb-like structure in plant-based meat. (e and f) Fibrous structure of plant-based meat at 2500× and 5000× magnification.

meat alternatives (Fig. 5). The FTIR spectrum exhibited absorption bands at around 1600 cm^{-1} to 1700 cm^{-1} and 1500 to 1580 cm^{-1} which belong to the proteins for amide I and II bands, respectively. Hence, these peaks represent the specific stretching and bending vibrations of the protein molecule.[42–44] In protein, the amide I band originates from the stretching vibration of the C=O (carbon and oxygen) part of the peptide group. Meanwhile, the amide II band combines NH bending vibration and the effect of C–N stretching.[45] Therefore, the strong spectrum absorbance of NH stretching indicates that the meat alternative has high protein content. The high protein content may originate from wheat, horse gram and/or soy protein. Besides, the spectrum peak obtained at 1520–1650 cm^{-1} is attributed to the C–O and N–H stretching which is influenced by intra and intermolecular H bonding. Likewise, a broad peak was observed in the region of 3100 to 3550 cm^{-1} which signifies the N–H stretching due to flexural vibration of intra and intermolecular H bonding.[43,45] Recently, Banerjee *et al.* extracted protein from under-utilized horse gram seeds and similar results were reported in their study (Banerjee *et al.* 2022).[12] Notable peaks of H and OH bonds were observed at 1640 cm^{-1} to 3300 cm^{-1} which are representative of the wheat flour moisture content.[46] In addition, the absorption region at 2930 cm^{-1} indicates the C–H bonds of fat and carbohydrate content present in the meat alternative.[47] Thus, the observed results highlight the intricate chemical composition within the

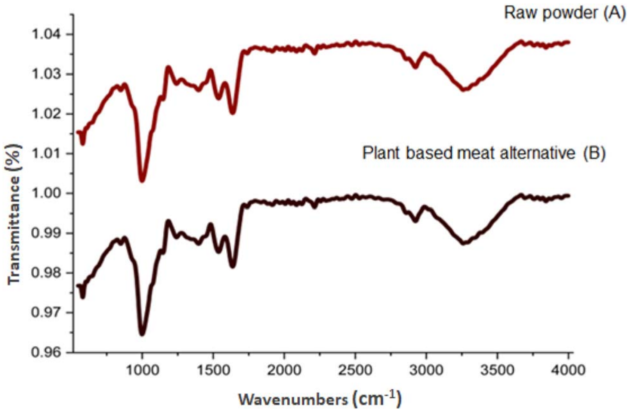

Fig. 5 FTIR spectra of (A) raw meat powder containing horse gram, wheat flour and soy flour and (B) the plant-based meat alternative.

meat alternative sample, comprising a blend of proteins, lipids, and carbohydrates.

3.5. Textural characterization of the product

The results of the texture analysis unveiled significant disparities in textural attributes among the tested plant-based meat products. Hardness values exhibited a range spanning 394.14 ± 2.5 N, signifying differences in the firmness of these products. The cohesiveness of the plant-based meat is 0.33 ± 0.35, indicating varying levels of adhesiveness. Springiness values are in the range 0.94 ± 0.72, denoting differences in the capacity of the product to rebound after compression. Gumminess values displayed a wide span of 61.76 ± 0.72, reflecting variations in overall chewiness. Lastly, chewiness values spanned 35.891 ± 0.83, indicating fluctuations in the effort required for consumption.

The diversity in textural attributes among the tested meat alternative products stands out as an essential factor for detection. This emphasizes that not all plant-based meat alternatives share uniform sensory characteristics. The hardness of plant-based meat products can significantly impact their suitability for various culinary applications. Cohesiveness and gumminess values serve as important indicators of overall mouthfeel and texture perception. Products with higher cohesiveness and gumminess may deliver a more meat-like sensory experience, while those with lower values may be appreciated for their distinctive texture. Springiness and chewiness values reflect the rebound and mastication characteristics of the products, respectively, influencing the overall eating experience. Some consumers may prefer products closely mimicking the behavior of animal-based meat, while others may seek out a distinct texture.[48] Recently, Zahari *et al.* have prepared high-moisture meat analogues (HMMA) using hempseed protein concentrate (HPC) in combination with wheat gluten (WG) and chickpea protein concentrate. It was observed that an HPC : WG ratio of 90 : 10 in HMMA produced improved hardness, resilience and chewiness compared to other samples. Also, it was found that the HMMA with a high amount of HPC showed better acceptability with respect to sensorial attributes, hardness and chewiness. Further, similar springiness was reported in all the samples; however, wheat gluten had better springiness than the other samples.[49] On the other hand, Penchalaraju *et al.* extracted pulse protein concentrate from green gram, horse gram and cowpea by an alkaline/isoelectric precipitation method. Different ratios (20 : 20 : 20, 30 : 15 : 15 and 15 : 20 : 15) of green gram, horse gram and cowpea were used to formulate deep-fried meatballs. It was found that plant-based deep-fried meatballs with a green gram, horse gram and cowpea ratio of 20 : 20 : 20 exhibited similarities to mutton deep-fried meatballs with respect to organoleptic, colour and textural properties, *i.e.* hardness, adhesiveness and cohesiveness.[50] Therefore, the broad spectrum of textural properties observed in this study underscores the pivotal role of texture in the development and marketing of plant-based meat alternatives. Future research endeavors should delve into the intricate relationship between texture and consumer preference in order to further fine-tune plant-based meat products to suit diverse markets and applications.

3.6. Sensory analysis

A sensory evaluation was conducted to assess the quality of the meat alternatives presented in Fig. 6A. The evaluation considered taste, odour, appearance, and overall acceptability as the key parameters. A panel of 10 individuals participated in the assessment, using a 9-point hedonic scale to rate each attribute. The results of this evaluation are provided in ESI Table S3† and Fig. 6 (B) which provide an overview of the sensory evaluation outcomes and the overall acceptability of the plant-based meat substitute. For the deep-fried product, the taste received a score of 5.9, indicating a moderate level of acceptability. The texture was rated at 7.3, indicating a favourable texture. The odour received a score of 7.0, signifying a pleasant aroma. The appearance was rated at 6.8, representing an overall satisfactory visual appeal. To determine the overall acceptability of the meat alternative, the cumulative mean scores from all sensory attributes were calculated. Based on these scores, the overall acceptability of the meat substitute was found to be 6.75. This indicates that the product is moderately favourable for acceptance by the sensory panel. De Angelis *et al.* prepared plant-based meat analogues with mixtures of dry fractionated pea protein, pea protein isolates, soy protein isolates and oat protein through an extrusion method. It was reported that the sensory examination of dry fractionated pea protein and oat protein indicated a strong flavour and taste profile, while the product prepared by extrudates using protein isolates showed better neutral sensory qualities.[51] Similarly, high-moisture meat mimics (HMMA) were prepared using hempseed, wheat gluten and chickpea and their sensory properties investigated. It was reported that high-moisture meat mimics with addition of more hempseed protein concentrate had good acceptability in relation to hardness, chewiness and sensorial attributes.[49] In another study, Sharima-Abdullah *et al.* formulated imitation chicken nuggets (ICNs) with different fractions of chickpea flour and textured vegetable protein (TVP). Five formulations of ICNs were produced and their characteristics examined. It was reported that the ICN prepared with a chickpea flour : TVP ratio

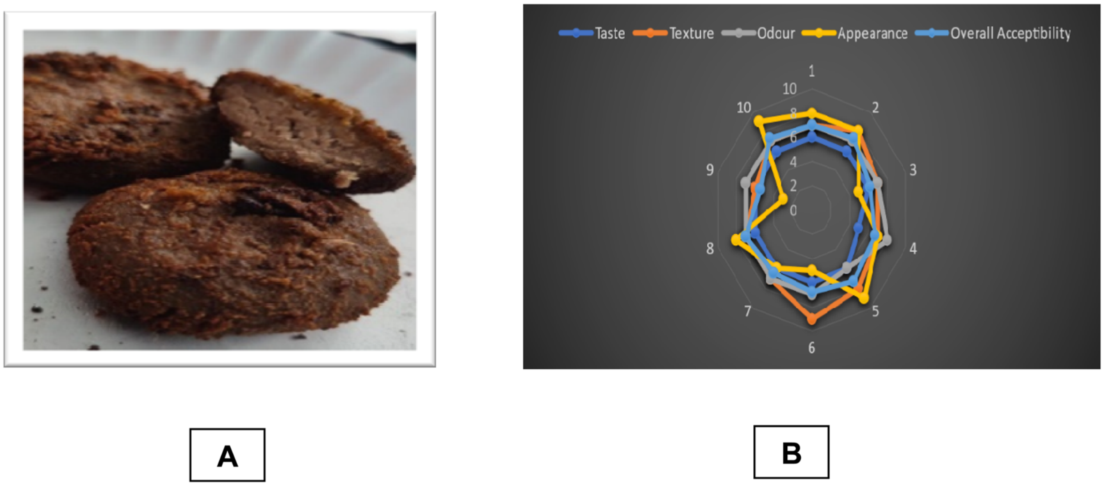

Fig. 6 (A) Formulated plant-based meat alternative and (B) attributes of sensory characteristics for the meat alternative.

of 10 : 30 was the best consumer preferred formulation in relation to taste, texture and overall acceptance in comparison to the other ICN samples. Furthermore, this suggested that the sensory characteristics and the nutritional value of ICNs need to be enhanced in order to make them more like chicken nuggets.[52] Hence, the sensory evaluation provided valuable information regarding the taste, texture, odour, appearance, and overall acceptability of the plant-based meat substitute, helping to assess its quality and consumer satisfaction as suggested.[32]

4 Conclusion

The formulated plant-based meat alternative product could be a sustainable and nutritious replacement for traditional meat. It offers a moderate nutritional profile such as protein, carbohydrates, and energy making it appealing to consumers seeking healthy and environmentally friendly options. The study utilized RSM to determine the optimized concentration ranges for a meat substitute. The optimized concentrations for horse gram, soy flour, and wheat flour were found to be 59.928 g, 51.255 g, and 36.467 g, respectively. The desirability function indicated that these concentrations were within acceptable limits. Sensory analysis indicates acceptability in terms of taste, texture, odor, appearance, and overall satisfaction. The morphology and texture of plant-based meat alternatives revealed comparable structure and properties to those of animal meat. Therefore, this study suggested that this plant-based meat blend is a promising replacement for conventional meat in the future. In addition, it may contribute to ensuring more sustainability for developing a healthier food system for consumers.

Data availability

Data for this article, including quantitative and qualitative data, are available from the corresponding author. Also, the data supporting this article have been included as part of the ESI.†

Author contributions

Archana Devi: conceptualization (lead); investigation (equal); methodology (lead); project administration (lead); writing – original draft (equal). Rahul: data curation (equal); investigation (lead); software (lead); validation (equal). Melvin Joshua: formal analysis (equal); methodology (equal). Naveen: resource (lead); writing – original draft (equal). Pothiyappan Karthik: conceptualization (equal); data curation (equal); supervision (equal); writing – review & editing (lead).

Conflicts of interest

The authors declare no conflicts of interest.

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