

Optimization and Development of Plant Based Textured Protein Supplement

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Abstract

Protein-energy inadequacy remains common among vegetarian populations, driving demand for plant-based protein products that are both nutritionally adequate and sensorially acceptable. This study optimized a plant-based textured protein supplement (TPS) using defatted soy flour, Kabuli chickpea flour, and mushroom paste as protein sources, with carrageenan and guar gum as binders. Using a Central Composite Rotatable Design under Response Surface Methodology, thirteen formulations (TPS1–TPS13) with varying ingredient proportions were developed and texturized by steaming. Proximate analysis identified defatted soy flour as the principal protein contributor ($38.10 \pm 3.96\%$), while chickpea flour added fibre and minerals, and mushroom paste enhanced flavour and moisture. A three-stage screening approach—protein content, Texture Profile Analysis, and sensory evaluation by a trained panel—narrowed selection from 13 to 5 samples. Protein content ranged from 15.7–24.9%, with texture varying significantly across formulations. TPS1, TPS13, and TPS2 emerged as most acceptable, combining high protein, favourable texture (hardness, cohesiveness, springiness, chewiness), and satisfactory sensory scores without added flavourings or colourants, demonstrating a viable vegetarian protein alternative.

Keywords: Soybean, mushroom, chickpea, RSM, texture, sensory

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Introduction

Protein is made from twenty-plus basic building blocks called amino acids. Because we don't store amino acids, our bodies make them in two different ways: either from scratch, or by modifying others. Nine amino acids viz., methionine, phenylalanine, lysine, threonine, tryptophan, histidine, isoleucine, leucine, and valine is known as the essential amino acids, must come from food. And because the body cannot store amino acids, you need to replenish them regularly. Protein exists in various parts of the body, including muscle, bone, skin, hair, and nearly every other tissue. It constitutes the enzymes that drive numerous chemical reactions and the hemoglobin responsible for transporting oxygen in your bloodstream. With at least 10,000 distinct proteins, you are defined and maintained (Wrigley, 2012); (Belitz and Grosch, 2009).

Protein is a key part of any diet. The National Academy of Medicine suggests that adults should consume at least 0.8 grams of protein for each kilogram of body weight daily (National Academies Press, 2005).

Common signs that your body is extremely low on

protein include brittle hair and nails, feeling weak or hungry, muscle weakness (amino acids are essential for building muscle mass). Kwashiorkor is one of the primary forms of severe protein-energy malnutrition. Individuals affected by kwashiorkor experience a significant lack of protein, along with deficiencies in several vital micronutrients. A critical shortage of protein leads to fluid accumulation in the tissues (edema), which sets kwashiorkor apart from other types of malnutrition (Benjamin and Lappin, 2018).

The average Indian diet have a protein deficiency. Because average Indian people do not intake regular food rich in proteins (kidney beans, legumes, mung beans, peanuts, soybeans, daal, paneer, milk in their regular diet).

Textured protein supplements represent a significant leap forward in nutritional science. By focusing on both functionality and sensory experience, they address common challenges associated with conventional protein powders, such as grittiness or limited culinary applications. This innovation makes it easier and more enjoyable for individuals to meet

their daily protein requirements, supporting their fitness and health goals with greater efficiency and satisfaction.

The aim of the present study was to optimize and develop textured protein supplement formulations using plant based protein sources like defatted Soy flour, Kabuli Chickpea flour and Button Mushroom.

Materials and Methods

Raw materials used for the preparation of textured protein supplement were Kabuli Chickpea, white button Mushroom purchased from local market of Jalgaon, Maharashtra India. Defatted Soy cake received from Sanjay Soy Pvt. Ltd. Industry, Avdhan MIDC, Dhule Maharashtra India, to make Defatted Soy flour. Binding agent carrageenan of brand Iampure and guar gum of brand Natures Harvest were used in the formulation.

Proximate Analysis of Raw Protein Sources

Proximate analysis of Defatted Soy cake, Kabuli Chickpea and Button Mushroom were carried out as per AOAC method for moisture, protein, fat, ash, crude fiber and carbohydrate (National Academies of Sciences, Engineering, and Medicine, 2000).

Processing of Protein Sources:

Major raw material Defatted Soy cake, Kabuli Chickpeas were used in the form of flour and Mushroom was used in the form of paste.

➤ Defatted Soy flour

Defatted Soy cake was ground in lab grinder into fine flour to make Defatted Soy flour. (Kellor, 1974).

➤ Kabuli Chickpea flour

Kabuli Chickpea flour process was made by modifying a method given by Ravi and Harte (2009). Around 10% water was added to Kabuli Chickpea seeds and mixed thoroughly for uniform moisture penetration into the cotyledons, followed by an equilibrium period of about 2 hrs. The moistened seeds were then dried at 45–50°C for 10 hrs in lab hot air oven. Splitting of seeds was carried out by milling with household dhal mill and separated by manual winnowing into different fractions such as dhal, partially dehulled whole grains, husk, broken seeds and some flour. The obtained dhal (dehulled grain) is then milled to obtain fine flour.

➤ Mushroom paste

Mushroom paste was made by process mentioned by previous research study. Mishra and Mani (2019); Kumar et. al., (2014); Rosli and Solihah (2014). The white button Mushroom was first rinsed with water and subjected to blanching (in boiling water for 5 min.) and chopped coarsely and ground to paste in lab grinder.

Experimental method

Experimental design Central composite rotatable design (CCRD) (Wan Rosli et.al., 2011). was applied to determine the best formulation of Mushroom paste, chickpea flour and defatted soy flour to optimize the ingredients combination for the development of textured protein supplement using software State–Ease (Design Expert version 13.0.0.5) as shown in Table 1. Mushroom, Kabuli Chickpea flour and Defatted Soy flour were taken as independent variables and cohesiveness, adhesiveness, hardness, gumminess, springiness, and chewiness, protein content, and overall acceptability as responses.

Table 1 shows Experimental design for development of textured Protein supplement formulation made up of plant based protein sources Defatted soy flour, Kabuli Chickpea flour and Mushroom in different percentage.

Table 1: Design of Experiment for Development of Textured Protein Supplement (TPS) Formulation

Run	A: Defatted soy flour		B: Kabuli Chickpea flour		C: Mushroom	
	Parts (g)	%	Parts (g)	%	Parts (g)	%
TPS1	58.5	43.8	37.5	28.1	37.5	28.1
TPS2	50	50.0	25	25.0	25	25.0
TPS 3	37.5	28.1	58.5	43.8	37.5	28.1
TPS 4	37.5	33.3	37.5	33.3	37.5	33.3
TPS 5	50	40.0	25	20.0	50	40.0
TPS 6	25	25.0	25	25.0	50	50.0
TPS7	50	40.0	50	40.0	25	20.0
TPS8	25	20.0	50	40.0	50	40.0

TPS9	25	25.0	50	50.0	25	25.0
TPS10	37.5	28.1	37.5	28.1	58.5	43.8
TPS11	16.5	18.0	37.5	41.0	37.5	41.0
TPS12	37.5	41.0	16.5	18.0	37.5	41.0
TPS13	37.5	41.0	37.5	41.0	16.5	18.0

Table 1 is representing the design of experiment for the development of textured protein supplement and experimental levels of independent variable in terms of actual and coded factors. The code assigned for protein sources were, A: Defatted Soy flour, B: Kabuli Chickpea flour, C: Mushroom paste. The different formulations had been coded from TPS1 to TPS13. The above protein sources composition of formulations (TPS1 to TPS13) were converted into %.

Preparation of the product

All the raw materials were weighed according to formulation obtained by Experimental Design and mix together by adding binding agent carrageen and guar gum (Ulu, 2006); (Demirci, 2014).and some amount of water to make into dough. After dough making, the protein supplement samples were steamed for 20 minutes and cooled (Ahirwar et.al., 2015); (Mishal et.al., 2022); (Kumar et.al., 2012). All the textured protein supplement samples were then subjected for further analysis.

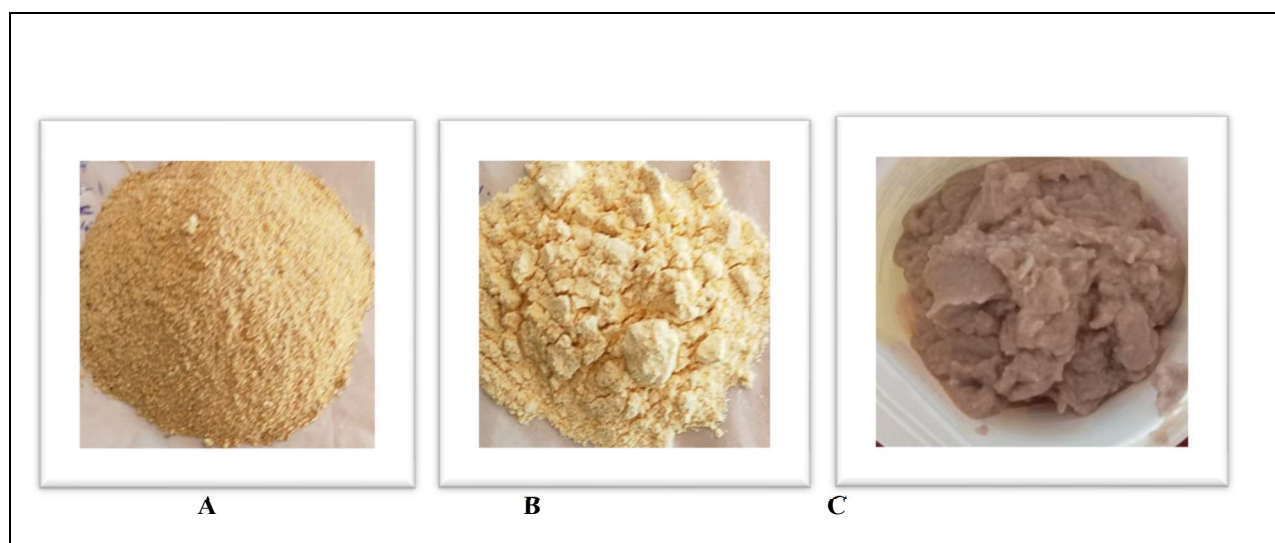
Protein content of protein supplement samples

The AOAC (2002) method was used to determine the proximate composition of 13 selected protein supplement samples for protein analysis. Based on protein content 10 textured protein supplement samples were screened.

Texture profile analysis (TPA)

Results and Discussion

Figure 1 illustrates raw mixture of Defatted Soy flour (A), Kabuli Chickpea flour (B), Mushroom paste (C) and developed Textured Protein Supplement coded as TPS1 –TPS13



The textural characteristics of the 10 textured protein supplement samples in terms of hardness, chewiness, springiness, cohesiveness, adhesiveness and gumminess were established using Texture Analyzer (Texture Pro CT V1.7 Build 28 Brookfield Engineering Labs. Inc.). From these 10 textured protein supplement formulations, 5 textured protein supplement samples were chosen based on the textural parameters.

Sensory Evaluation

Sensory evaluation of preferred 5 textured protein supplement samples were carried out by 10 semi-trained panelists on a 9 point Hedonic scale (9-like extremely to 1- dislike extremely) as per Ranganna, (1999) to establish the overall acceptability of the products. Based on overall acceptability 3 textured protein supplement samples were selected.



TPS1



TPS2



TPS3



TPS4



TPS5



TPS6



TPS7



TPS8



TPS9



TPS10



TPS11



TPS12



TPS13

Figure 1: A:Defatted Soy flour, B:Kabuli Chickpea flour, C: Mushroom paste and Textured Protein Supplement coded as TPS1 –TPS13

Table 2 represents results of proximate analysis results of raw protein sources namely Defatted Soy flour, Kabuli Chickpea flour and Mushroom paste

Table 2: Result of Proximate Analysis of Raw Protein Sources

Parameters	Defatted Soy flour	Kabuli chickpea flour	Mushroom paste
Moisture (%)	5.87± 0.30	7.06 ± 0.46	86.74 ±2.31
Fat (%)	4.95± 0.26	8.57 ± 0.38	1.49 ±0.28
Protein (%)	38.10 ±3.96	18.20 ± 2.14	3.68±0.92
Ash (%)	3.48 ±0.42	8.86 ± 0.92	3.28±0.36
Fibre (%)	3.97 ±0.42	6.47 ±0.66	1.37±0.25
Carbohydrate (%)	43.8 ±3.5	50.84 ± 4.1	3.44±0.68

*Above values are averages of three determinants± standard deviation

Moisture content was lowest in defatted soy flour ($5.87 \pm 0.30\%$) and Kabuli chickpea flour ($7.06 \pm 0.46\%$), consistent with their dry, powdered form, which favors microbial stability and a longer shelf life. Mushroom paste, in contrast, showed a very high moisture content ($86.74 \pm 2.31\%$), typical of fresh mushroom tissue, which is composed largely of water held within its cellular structure. Fat content was highest in Kabuli chickpea flour ($8.57 \pm 0.38\%$), followed by defatted soy flour ($4.95 \pm 0.26\%$), while mushroom paste had the lowest fat content ($1.49 \pm 0.28\%$). The comparatively low fat level in the soy flour is expected, since defatting removes the majority of lipids during processing, which is desirable for improving oxidative stability and extending the shelf life of the final product. The low lipid content of mushrooms is a well-documented characteristic that makes them attractive as a low-fat protein-supplementing ingredient. Protein content was substantially higher in defatted soy flour ($38.10 \pm 3.96\%$) compared to Kabuli chickpea flour ($18.20 \pm 2.14\%$) and mushroom paste ($3.68 \pm 0.92\%$). This confirms soy flour as the primary protein-contributing ingredient in the formulation, consistent with its established role as a high-protein plant-based

ingredient used to fortify textured protein products. Chickpea flour, while lower in protein than soy, still provides a meaningful contribution and additionally brings complementary amino acid and functional properties. The low protein value for mushroom paste is expected given its high moisture content; on a dry-weight basis, mushrooms are known to have a reasonably high-quality protein profile, but on a fresh/wet basis, as analyzed here, the diluting effect of water lowers the measured percentage. Ash content, an indicator of total mineral content, was highest in Kabuli chickpea flour ($8.86 \pm 0.92\%$), followed by defatted soy flour ($3.48 \pm 0.42\%$) and mushroom paste ($3.28 \pm 0.36\%$). This suggests that chickpea flour is a good source of minerals and would enhance the micronutrient density of the final textured protein supplement. Crude fibre followed a similar trend, with Kabuli chickpea flour showing the highest value ($6.47 \pm 0.66\%$), compared to soy flour ($3.97 \pm 0.42\%$) and mushroom paste ($1.37 \pm 0.25\%$). The relatively high fibre content of chickpea flour is consistent with its legume origin and supports its inclusion as a source of dietary fibre in the formulated product, which may contribute functional and health benefits such as improved satiety and

digestive health. Carbohydrate content was highest in Kabuli chickpea flour ($50.84 \pm 4.1\%$), followed by defatted soy flour ($43.8 \pm 3.5\%$), while mushroom paste had a comparatively negligible carbohydrate content ($3.44 \pm 0.68\%$), again attributable to its high moisture and low dry-matter content. Overall, the results indicate that defatted soy flour is the principal protein-dense ingredient, Kabuli chickpea flour

contributes significantly to fibre, mineral (ash), and carbohydrate content while also supplying moderate protein and fat, and mushroom paste despite its low contribution to macronutrients on a fresh-weight basis likely serves the formulation more for its flavor, texture, umami characteristics, and micronutrient/bioactive compound contribution rather than as a major macronutrient source.

Table 3 shows Protein content results of developed textured protein supplement samples Coded as TPS1 to TPS13

Table 3: Result of Protein Content of Textured Protein Supplement Sample

Run	Protein Content (%)
TPS1	22.3 ± 2.94
TPS2	24.9 ± 3.95
TPS3	19.1 ± 2.05
TPS4	19.4 ± 2.95
TPS5	21.2 ± 1.20
TPS6	16.6 ± 1.10
TPS7	23.8 ± 0.95
TPS8	15.7 ± 0.75
TPS9	18.8 ± 1.95
TPS10	17.9 ± 0.95
TPS11	16.4 ± 1.05
TPS12	19.7 ± 1.15
TPS13	24.3 ± 1.85

*Above values are averages of three determinants $\pm$ standard deviation

The protein content of the plant-based textured protein supplement varied considerably across the thirteen experimental runs, ranging from a minimum of 15.7% (TPS8) to a maximum of 24.9% (TPS2), with an overall mean of approximately 20.3%. This range of nearly 9.2 percentage points indicates that the process/formulation variables under investigation had a substantial influence on the protein retention and concentration in the final textured product. Among the runs, TPS2 ($24.9 \pm 3.95\%$), TPS13 ($24.3 \pm 1.85\%$), and TPS7 ($23.8 \pm 0.95\%$) exhibited the highest protein content, suggesting that the combination of parameters used in these formulations was most favorable for protein enrichment. In contrast, TPS8 ($15.7 \pm 0.75\%$), TPS6 ($16.6 \pm 1.10\%$), and TPS11 ($16.4 \pm 1.05\%$) recorded the lowest values, indicating that the conditions employed in these runs may have led to lower protein incorporation, possible dilution by other matrix components, or losses during processing (e.g., due to

extrusion temperature, moisture content, or screw speed, depending on the variables studied). It is also noteworthy that the standard deviations across runs were generally modest (ranging from about $\pm 0.75\%$ to $\pm 2.95\%$), reflecting reasonably good reproducibility of the triplicate determinations and supporting the reliability of the reported means. Runs with comparatively higher variability, such as TPS1 ($\pm 2.94\%$) and TPS2 ($\pm 3.95\%$), may warrant further replication to confirm consistency. Overall, the results demonstrate that protein content is sensitive to the formulation/process parameters tested, and the high-protein runs (TPS2, TPS13, TPS7) can be considered promising candidates for further optimization and selection in subsequent stages of product development, such as sensory evaluation or textural analysis. These findings are consistent with the broader objective of screening formulations to identify conditions that maximize protein content while maintaining acceptable product quality.

Table 4 represents texture profile analysis results of developed textured protein supplement samples TPS1 to TPS13 on the basis of Hardness (as deformation), Adhesiveness, Cohesiveness, Springiness, Gumminess and Chewiness

Table 4: Texture Profile Analysis Result of Textured Protein Supplement Samples

Runs	Hardness as deformation (mm)	Adhesiveness (mJ)	Cohesiveness	Springiness (mm)	Gumminess (g)	Chewiness (mJ)
TPS1	3.6	0.4	0.78	3.97	2.81	11.15
TPS2	5.2	0.5	0.8	4.05	4.16	16.85
TPS3	5.6	0.7	0.51	2.84	2.86	8.11
TPS4	3.8	0.2	0.85	3.23	3.23	10.43
TPS5	3.3	0.6	0.72	3.44	2.38	8.17
TPS7	4.1	0.8	0.97	3.81	3.98	15.15
TPS9	3.1	0.6	1.09	2.1	3.38	7.10
TPS10	3.9	0.5	0.83	1.97	3.24	6.38
TPS12	3.6	0.4	0.64	3.15	2.30	7.26
TPS13	4.8	0.9	1	4.12	4.80	19.78

mm-milimeter, g-gram, mJ-mili joule

During steaming, moist heat drives protein unfolding and denaturation, after which the exposed hydrophobic groups and reactive sites promote protein-protein aggregation; upon cooling, these aggregates set into a three-dimensional gel network that determines the final textural characteristics of the product (Li et.al, 2022). This mechanism explains why samples receiving comparable thermal exposure can still differ substantially in hardness, cohesiveness, and springiness depending on the compactness and uniformity of the network formed during cooling. Hardness, the peak force required to deform the sample, ranged from 3.1 mJ (TPS9) to 5.6 mJ (TPS3). Hardness in heat-set protein gels is understood to increase with the degree of protein unfolding and subsequent aggregate density, since more extensively denatured protein exposes greater numbers of hydrophobic and sulfhydryl groups that drive tighter cross-linking during cooling (Ma and Chen, 2023). The comparatively high hardness of TPS3, however, was accompanied by its lowest cohesiveness value (0.51), a pattern consistent with reports that gels formed from excessively aggregated protein can produce a dense but uneven, poorly bonded network, rather than a uniformly integrated one. Cohesiveness, reflecting the strength of internal bonding and the material's resistance to structural breakdown, was highest in TPS9 (1.09) and TPS7 (0.97). Cohesiveness in heat-induced protein systems is generally regarded as an indicator of how completely the protein network has consolidated during gelation, since better-organized, more regular networks resist deformation more effectively before breaking apart (Yang et al., 2023). The relatively low cohesiveness recorded for TPS3 (0.51) and TPS12 (0.64) suggests these samples may not have achieved a fully continuous gel network during steaming and cooling, potentially due to insufficient protein

unfolding or uneven heat penetration during the steaming step. Springiness and chewiness showed a broadly consistent pattern, with TPS13 recording the highest values for both parameters (4.12 mm and 19.78 mJ) alongside the highest gumminess (4.80 g) and highest adhesiveness (0.9 mJ). Since chewiness is derived as the product of hardness, cohesiveness, and springiness, a sample performing strongly across all three underlying attributes will necessarily register the highest chewiness (Heck et al., 2023). TPS13's consistency across these measures indicates it formed the most elastic and well-consolidated gel structure among the samples tested. TPS10, in contrast, recorded the lowest springiness (1.97 mm) and lowest chewiness (6.38 mJ), which is consistent with a less fully developed protein network, since reduced protein cross-linking and weaker aggregate bonding after cooling generally result in gels that recover poorly after compression (Kuang et al., 2025). Notably, thermal denaturation does not always translate into proportionally higher hardness or springiness; in some heat-treated protein and muscle systems, greater denaturation has been found to correlate negatively with hardness and springiness beyond a certain point, even while increasing shear resistance, due to changes in the balance between hydrophobic interactions and hydrogen bonding within the set gel (Liu et al, 2022). This offers a plausible explanation for why TPS3 achieved high hardness without corresponding gains in cohesiveness or springiness — the steaming conditions applied may have driven the protein toward a stage of aggregation that stiffened the matrix locally without producing a uniformly bonded, elastic network. Taken together, these results indicate that TPS13, and to a lesser extent TPS7 and TPS2, achieved the most favourable balance of gel firmness, internal cohesion, and elasticity following steaming

and cooling, making them the most promising candidates for further sensory and application-based evaluation. TPS3, TPS10, and TPS12, by contrast, would benefit from re-examination of steaming time,

Table 5 shows sensory analysis results of developed textured protein supplement sample on the basis of Appearance, Colour, Flavour, Texture and Overall Acceptability

temperature, or moisture conditions to promote more uniform protein unfolding and a better-consolidated gel network in future optimization trials.

Table 5: Result of Sensory Analysis of Protein Supplement Sample

Run	Appearance	Colour	Flavour	Texture	Overall acceptability
TPS1	7.2±0.79	6.5±0.97	6.5±0.97	6.6±0.52	6.9±0.32
TPS2	7±0.67	6.2±0.79	6.4±0.52	6.4±0.84	6.5 ±0.85
TPS4	6.4±1.07	6.1±0.88	5.8±0.92	6.1±0.99	6.3±0.48
TPS7	5.6±0.52	5.1±0.74	5±0.67	5.3±0.48	5.2±0.42
TPS13	7.1±0.99	6.6±0.84	6.5±1.18	6.6±0.70	6.7±0.82

*Above values are averages of ten determinants± standard deviation

Sensory evaluation of the five selected formulations (TPS1, TPS2, TPS4, TPS7, TPS13) using hedonic scoring across appearance, colour, flavour, texture, and overall acceptability (Fiorentini, 2020) showed TPS1 (6.9 ± 0.32), TPS13 (6.7 ± 0.82), and TPS2 (6.5 ± 0.85) performing best overall, while TPS4 and TPS7 scored lowest, with TPS7 particularly weak in appearance, colour, and flavour. Since no colourant or flavouring was added, the study being focused on optimizing texturization rather than final palatability, appearance and colour scores reflected only the natural surface changes produced by steaming and cooling the plant protein base, consistent with reports that unmodified plant protein matrices generally score lower on visual attributes than formulations with added colourants (Appiani, 2023). Similarly, the lower flavour scores (notably TPS7, 5 ± 0.67) likely reflect the innate "beany"/off-flavour notes typical of unflavoured legume-based proteins, a well-documented challenge in plant-based meat analogue development. Texture scores were highest for TPS1 and TPS13 (6.6 ± 0.52), broadly matching their stronger cohesiveness and springiness in the TPA results (Table 4), supporting texture as a major driver of acceptability in plant protein products (Grasso, 2019). Overall acceptability followed the ranking TPS1 > TPS13 > TPS2, consistent with sensory literature showing overall liking is most strongly tied to taste and texture performance rather than appearance alone. Given the deliberate exclusion of flavour/colour additives at this stage, TPS1, TPS13, and TPS2 are identified as the most promising base formulations, with flavour and colour enhancement recommended in future optimization to further improve consumer acceptability.

Conclusion

The present study concludes that a good-quality, plant-based textured protein supplement can be successfully developed from combinations of defatted soy flour, Kabuli chickpea flour, and mushroom paste, using a Response Surface Methodology-based Central Composite Rotatable Design to optimize ingredient proportions. Defatted soy flour, Kabuli chickpea flour, and mushroom paste together provided a nutritionally and sensorially balanced formulation base, with soy flour supplying the main protein, chickpea flour adding fibre and minerals, and mushroom paste enhancing flavour. Stepwise screening of the thirteen formulations on protein content, texture, and sensory quality successfully identified three optimal samples without compromising nutritional adequacy, structure, or acceptability. Among the formulations evaluated, TPS1 (defatted soy flour 43.8% + Kabuli chickpea flour 28.1% + mushroom paste 28.1%), TPS2 (defatted soy flour 50% + Kabuli chickpea flour 25% + mushroom paste 25%), and TPS13 (defatted soy flour 41% + Kabuli chickpea flour 41% + mushroom paste 18%) emerged as the best-performing samples, exhibiting superior protein content, textural characteristics, and sensory acceptability, with an overall preference ranking of TPS1 > TPS13 > TPS2. These three formulations demonstrate that plant-based ingredients can be successfully texturized via steaming to yield a product capable of meeting vegetarian dietary protein requirements while maintaining acceptable texture and taste. Since this study was focused on optimizing the base formulation and texturization process, flavour and colour additives were deliberately excluded; future work incorporating such enhancements, along with extended shelf-life, nutritional bioavailability, and consumer-scale sensory studies, is recommended to further improve the commercial viability of this textured protein supplement.

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