

OPTIMIZATION OF LEGUME BASED MEAT ALTERNATIVE USING FREEZE STRUCTURING AND EVALUATION OF ITS QUALITY INDICES

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Abstract

The continuous development and increasing demand of the fast-growing market for meat alternatives will offer abundant opportunities for food scientists and entrepreneurs to innovate new technologies. Product development and applied research primarily focus on the sensory qualities related to the texture of meat analogues. The preference for sustainable plant-based meat over animal meat worldwide is primarily driven by the growing concerns regarding the environment and health. In this study, various plant-based composites were investigated to create plant-based meat alternatives with distinctive texture profiles using an innovative freeze-structuring technique. Statistical ANOVA was utilized to optimize the composite consisting of pea proteins isolate (PPI) and soy protein isolate (ISP). The physicochemical, textural, and sensory properties of the analogs were thoroughly examined. The findings indicated that the analog with a ratio of 40:60 (PPI:ISP) was the most favored compared to other formulations. The incorporation of soy protein seemed to have an impact on the textural properties. The optimized composition level (40:60) exhibited a hardness of 842.87g, chewiness of 721.35 N/cm, and the highest sensory properties on an 8.3 hedonic scale. Additionally, the water activity was measured to be 0.553, classifying the product as an intermediate moisture food. The nutritional analysis shows that optimized meat substitutes have 16 % increased protein levels when compared to conventional chicken meat. This study improved the comprehension of the impacts of composites on the structure and physicochemical characteristics of plant-based meat substitutes. It highlighted the potential of utilizing plant protein composites in the development of satisfactory meat analogues.

Keywords: Freeze structuring; Legume composites; Meat analogues; Nutritional analysis; Protein isolates; Textural attributes

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Introduction

Meat consumption has changed over time. Consumer attitudes toward meat continue to be favorable, and the worldwide production of meat is expected to rise over the next decade. However, a growing group of consumers is actively seeking ways to decrease the amount of animal protein in their diet, which is increasing the need for alternative meat products. Overall, consumer attitudes, concerns about health, and price sensitivity all influence meat demand, and the increasing popularity of alternatives to meat products will likely have a major part in the future (Biscarra *et al.*, 2023, Frank *et al.*, 2022). The inefficient utilization and conversion of plant proteins into animal proteins in the food cycle results in a loss of value. The meat industry significantly contributes to the rising CO₂ levels than other food sectors (Raney *et al.*, 2009). Also, the resources such as water, feed, and electricity were needed for raising animals, and meat production was very high. Other than environmental impact, animal meat is not a sustainable food meeting the demands of the rising global population. In contrast to the past, where consumers primarily considered intrinsic quality attributes such as sensory experience, safety, shelf-life, and packaging when purchasing meat, modern consumers are now more concerned with extrinsic qualities like organic production, animal welfare, fair trade practices, and environmental impact due to the saturation and abundance of food choices available on market shelves. The meat industry has been marred by negative attention due to the exposure of unethical, unsafe, unhygienic, and non-standard practices in some animal slaughterhouses, brought to light by social media and animal rights movements. Multiple consumer studies, which have focused on various demographics, have indicated a growing inclination towards plant-based, green foods. The aforementioned reasons have been cited as the driving force behind this shift in the perception of quality (Asgar *et al.*, 2010).

Plant-based meat alternatives have been recognized as favorable sources of protein compared to animal-derived meat, presenting advantages in terms of societal, ecological, and well-being aspects. These alternatives have the potential to contribute towards reducing meat consumption and promoting sustainability. (Profeta *et al.*, 2021). The potential outcome is that the implementation of substitutes for meat, derived from combinations of diverse plant-based proteins, could potentially be regarded as a sustainable and efficacious approach. Plant-based meat analogs

encompass products that possess the appearance, flavor, and fibrous consistency akin to animal meat. (Pietsch *et al.*, 2017). Green foods, which are predominantly derived from plant sources like soybean, pea, and other legumes, have come to define a notable shift in consumer preference towards plant-based foods over recent years. This trend can be attributed to a heightened awareness of health and environmental consciousness among consumers. Notably, the growth of plant-based meat analogs or texturized vegetable proteins has been remarkable. (Devanampriyan Rajan *et al.*, 2023). By effecting chemical, mechanical, and thermal modifications on the functionalities of globular proteins in plants, a texture and mouth feel similar to animal proteins can be delivered. Furthermore, legume-based meat, derived from proteinaceous legumes that can grow under extreme biotic and abiotic stress conditions, is a more affordable source of protein than animal meat. Different shapes, such as sheets, patties, and strips, can be produced using extrusion-based technologies to create meat analogs (Ahmad *et al.*, 2022). Several methods have been proposed for achieving these desired structural attributes most of them striving for texture simulation. These traditional and innovative processes are aimed at producing fibrils that are similar in structure to individual muscle fibers and can provide desired organoleptic properties such as Hardness and chewiness similar to meat. Traditional structuring processes like extrusion by using thermomechanical methods and Printing technologies have had some success (Dekkers *et al.*, 2018; Sha *et al.*, 2020). The aggregates formed by highly denatured plant proteins, on the other hand, do not have the three-dimensional anisotropic structure of fibers from muscles. Consumers frequently claim about the dryness (lack of juiciness) of cooked samples.

The research utilized the freeze-structuring technique, which involved freezing a protein emulsion to produce a distinct fibrous arrangement. Upon elimination of ice crystals, a permeable and fibrous microstructure would emerge, akin to that found in animal meat muscles, composed of numerous interconnected sheet-like proteins. The fibrous arrangements of meat replacements produced through the freeze-structuring technique are also highly dependent on the plant-based protein used. This is attributable to the fact that diverse protein sources may exhibit dissimilar structure-function associations in terms of water retention and solubility, gelation process, and texture formation, all of which exert an influence on the ultimate

composition of meat substitutes (Consolacion *et al.*, 1986; Geerts *et al.*, 2018).

The utilization of legume protein as an ingredient in processed foods has seen a significant increase in recent years, driven by motives such as nutritive value, mouthfeel, functional properties, and economic considerations (Riascos *et al.*, 2010). Pea-based structures, for example, tend to be softer compared to soy-based products. To address this, researchers are exploring ways to enhance gel strength by modifying protein bonding. This can involve adding salts with chaotropic ions or optimizing conditions such as pH, temperature, and protein content Sun and Arntfield (2012). In addition, studies on Chickpea, lentils, and lupine have revealed their impressive emulsion and foam stabilization capacities. Moreover, by thermally modifying the proteins to enhance hydrophobic interaction, these legumes can also act as effective gelling agents (Bader *et al.*, 2011).

Pea protein has grown in acceptance among high-moisture extrusion for meat substitute manufacturers because it serves as an excellent complement to other protein sources with distinct processing characteristics. Due to the global availability, low price, and processing capabilities, soy proteins are the most widely used constituents for meat-alternative products. Soy flour and soy protein isolates are two of the most commonly used soy products. Soy protein isolate is made by alkaline extraction preceded by precipitation in acidic conditions, yielding a product with 90% protein content. In addition to its high protein purity, the benefit of soy protein isolate is its light color and bland flavor when compared to other soy ingredients (Cheftel *et al.*, 1992; Geerts *et al.*, 2018). Wheat gluten is another commonly used plant protein. It is insoluble in water. It contains Glutenin and gliadin proteins of wheat gluten, which are responsible for elasticity and extensibility; therefore, the use of gluten as a key ingredient in the formation of fibrous and viscoelastic structures (Don *et al.*, 2003). Composites of various plant proteins with varying nutritional compositions, as well as other ingredients that include hydrocolloids such as carrageenan and alginates, are frequently used in plant-based alternatives to improve texture consistency, bind water, and reduce syneresis. These ingredients might enhance the nutritional and textural properties of meat analogs. A small study has been conducted to date on the use of the freeze-structuring technique for creating plant-based analogs using a composite of different plant-based proteins.

As a result, this proposed study aimed to create an optimal blend of plant proteins such as pea protein isolate and soy protein isolate were used as

legume source protein, and also wheat protein (known as vital wheat gluten) was used as major protein source and other ingredients to create a plant-based meat analog similar to chicken meat with acceptable meat-like properties using a freeze structuring technique. The physicochemical properties of the developed meat analogs, such as textural, emulsion stability, sensory attributes, and nutritional analysis, were done for fried chicken meat, optimized meat substitute samples, and compared with the commercial meat substitute available in the market which have been investigated. In this study, freeze-structuring techniques were used to create a unique plant-based protein structure. The findings of this study could pave the way for potential implementation, particularly in small-scale domestic industries where investing in alternative manufacturing techniques such as extrusion is both impossible and expensive. The result findings of this study could pave the way for the use of legume proteins as substitutes in animal-derived products in the future.

Materials and Methods

Raw materials

Pea protein isolate PPI (Nutrova) was obtained from Axys Nutrition Products Pvt Ltd, which is composed of 80 g protein, 6.48% moisture, 4.08% w/w ash content, and 1.15% w/w of total fat content (Amazon, 2023a). Soy protein isolates ISP was obtained from M/S Profoods, India, as per manufacturer instruction composition contains 90 grams of protein per 100 grams (Amazon, 2023c). Vital wheat gluten VWG (CCDS) were obtained from E-commerce, amazon portal (Amazon, 2023b). which consists of 23% of crude protein, 4% moisture, 0.8% w/w ash and 0.86% w/w crude fat. Kappa Carrageenan was obtained from the urban platter in Mumbai. Wheat starch was obtained from Angel Starch and Food Pvt Ltd, Erode. Other ingredients like salt (NaCl), refined sunflower oil, and Texturized vegetable protein (TVP) were bought from a local bakery ingredients shop. Eviscerated chicken meat (internal parts removed) was purchased from a local market (Erode, India) and was repeatedly washed with chlorinated water (0.01 ppm). Following this, an antimicrobial treatment was applied using a 2% turmeric solution at a temperature of 37°C. After a soaking period of 20 minutes, the water was completely drained off and the meat was then covered with ice cubes., chicken meats were transported to the laboratory at once and then stored in refrigerated condition (6°C) until use and it was then prepared for analysis. The commercial meat substitutes used for comparison

were produced in Good Dot Enterprises which procured from E-commerce (Herbivo, 2023).

Flour Blend Optimization

Statistical analysis with Minitab 20 Statistical software (Trial version) was performed in an optimized condition to analyze the significant difference in the mean values. For the Protein blends such as PPI and ISP were considered for the study. Analysis of variance was performed final data were found to be significant at an amount of $p < 0.05$ (95% confidence amount) and using Duncan's test significant differences one-way analysis of variance by (ANOVA) technique. Comparison of the mean values, Standard deviation were estimated as per the study by (Subramani *et al.*, 2022; Sangeetha *et al.*, 2023). The identification of relationships between the dependent and independent variables, pertaining to liking. Parameters such as textural attributes and sensory attributes were measured after frying. Instrumental data were averaged for each of the attributes being investigated.

Preparation of freeze-structured meat substitute

The analog was prepared according to the protein blends with a total of 6 different ratios of flour composites. Initially, protein emulsion was prepared by mixing (1:4:5) ratio from the (part of protein isolates; oil: water) to form a past type emulsion. Oil and water were blended at the ratio levels of 1:10 to produce the liquid phase emulsion. For the 100 g of protein blend (Flour basis). Other minor ingredients such as ice water, 30% water of (-4°C), 3% vegetable oil, 1.5% salt, 2% wheat starch, 0.4 % Kappa carrageenan, Texturized vegetable protein of 5%, and 1% vital wheat gluten were formulated in order to make each composite protein analog. The slurry was subjected to homogenized mixing, and the samples were then placed in cubic molds (3.8×3.0×2.0 cm). The top and bottom area of the molds were covered with a lid, the samples were initially subjected to and frozen at -10°C for 8 h for Stage one freezing step. After 8 h of freezing, the frozen samples were removed from the molds and stabilized by soaking in a 3 wt.% CaCl₂ solution at 4°C overnight, and then the second stage freezing step was applied to obtain a stable gel between the protein isolates in the same manner at -10°C for 8 h (2nd freezing step) as the methodology followed by author (Chantanuson *et al.*, 2022). All samples were stored at 4°C until testing. A seasoning blend mix of 5% (w/w) was prepared as per the research work done for the prawn chips for seasoning formulated by (Devanampriyan *et al.*, 2023) and was added during frying preparations for frozen meat substitute which contains a blend of dried chili, cumin, Asafoetida,

thyme, black pepper and bay leaf powder which can improve aroma and can naturally masking the floury-note from protein blends.

The frozen analog was applied seasoning on both sides and fried in a deep fat fryer containing pre-heated (190°C) refined sunflower oil. The freeze-structured analog sample was then subjected to deep frying for 1 minute 30 seconds on each side piece (Yuliarti *et al.*, 2021). The final thickness of the meat analog, found to be (2.5×2.5×1.0 cm) was considered for the analysis. The uncooked meat substitutes (Before frying) were analyzed for the purpose of quantifying their color attributes. While the deep-fried meat substitutes were assessed for textural, water activity, and sensory parameters. Figure 1 illustrates an overall schematic process flow of the freeze-structured meat substitute.

Texture analysis

Texture attributes such as Hardness, Adhesiveness, Gumminess, Cohesiveness, springiness, and chewiness were determined for the fried analog sample were determined using a Texture Analyser model (TA.HD. Plus, Stable Micro Systems, England). Compression probes were used (P 75 mm compression platen). Two complete cycles of compression and decompression of meat analogs, which were studied in the analog sample, were in the dimension of (2.5×2.5×1.0 cm). The test system with a back extrusion rig with a cylindrical probe of a 35 mm diameter was used to test the subject. The compression disk is attached to an extension bar by using a 50 kg load cell for analysis. The probe travelled at 2mm/s speed which was measured at ambient temperature (25°C). Readings for textural attributes of the meat

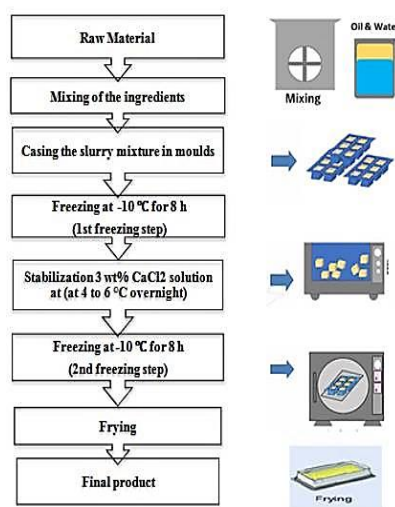


Figure 1. illustrates the process methodology for freeze-structured meat substitutes

substitutes were noted for each trial response (Das *et al.*, 2015). The analysis was done in triplicates to verify the reliability and validity of the findings.

Water activity measurement

Water activity is the amount of water available for the growth of microorganisms. The fried sample of meat substitute was tested using a water activity meter with a sensor Chilled-mirror dew point; infrared temperature type from Aqua lab, pre bench top water activity meter has ± 0.01 aw accuracy.

Colour analysis

The uncooked samples before deep frying were considered for the color analysis study. The determination of color was carried out using a Minolta Chromameter (Model CR-400, Minolta Co., Osaka, Japan) by employing the Hunter L*, a*, and b* values. The a* value was utilized to measure redness, which is positive, and greenness, when negative, while the b* value was employed to determine yellowness is positive and blueness is negative. Further, the L* value was utilized to measure the level of lightness on a scale of 0-100 from black to white. The readings were accurately recorded on a sample handling plate filled with raw dough of freeze-structured meat analogs and placed on the analyzing port. Samples were measured on the surface of the analog samples before frying. The measurements were taken at three different spot points on the surface of each raw sample.

Sensory analysis

Sensory analysis employs techniques for the fried sample of meat substitutes that were subjected to this study. The discipline demands the employment of panels of human assessors who evaluate samples while also documenting their responses. The randomly coded samples were compared with the standard meat (Fried chicken) and rated for their attributes such as Fibrousness, Firmness, Juiciness, Elasticity, Tenderness, Taste, and flavor. Based on a 9-point hedonic scale, the highest value of 9 indicates a soft scale, with the greatest score being extreme Like), and the lowest score of 1 indicating (Hard) (Extreme Dislike). the panel of semi-trained judges (n=10) age group between 25 to 35 age rated for various sensory qualities for the fried meat substitute. Scores of overall acceptability were considered for the final values. (McClements *et al.*, 2021)

Emulsion viscosity measurements

The rheological properties of the protein emulsion in a smooth paste consistency were determined using a rheometer (DV-III™ Ultra Rheometer, AMETEK Brookfield, USA) with a

spindle of CPE-40. The data were measured at shear rates from 0.075 to 0.375 s⁻¹. For the viscosity measurement, the protein emulsion sample was poured directly into a cylinder of capacity 25 µ. The measurements were conducted until reproducibility was obtained (Giridharaprasad *et al.*, 2021).

Emulsion stability measurement

The emulsion is added in order to improve the stability, texture, and cohesiveness of the product. It can lead to a better mouth feel and juiciness. The protein emulsion stability was measured during the preparation of raw dough. About 25 g of raw meat substitute dough was weighed and then placed in a centrifuge tube with a known weight and centrifuged for 1 min at 2700 rpm. The centrifuge tube was subjected to heat treatment at 70°C water bath for 30 min, and again, it was centrifuged for 3 min at 2700 rpm. The supernatant was collected into a crucible, and the centrifuge tube (including the pellet) was reweighed. The crucible was heated for 3 hrs and then re-weighed Total expressible fluid (TEF) and Fat (%) were calculated. Emulsion stability studies for sheep tail fat in order to determine TEF were done by Aktas and Genccelep (2006).

$$TEF = \frac{\text{weight of (Centrifuge tube and sample)} - \text{(centrifuge tube and pellet)}}{(1)}$$

$$TEF (\%) = \left(\frac{TEF}{\text{Sample weight}} \right) \times 100 \quad (2)$$

$$\text{Fat} (\%) = \frac{((\text{weight of crucible} + \text{dried supernatant}) - \text{(weight of empty crucible)})/TEF}{(3)} \times 100$$

Chemical composition analysis

The samples (TC, T1, T2, T3, T4, and T5) were subjected to chemical composition analysis as per the method of testing for meat substitutes AOAC methods referred from (McClements *et al.*, 2021), Analysis such as moisture content, ash content, protein content, fat content, and dietary fiber content were analyzed which was tabulated in Table 4.

Comparative nutritional analysis

For the comparative nutritional analysis, three samples were considered. Firstly, the chicken meat (Fried), Optimized fried meat substitute (Fried), and commercial meat substitute sample (Fried). These three samples were applied seasoning mix and then deep fat fried for 1 min 30 seconds at (190°C). Parameters such as moisture by Hot air over method

(AOAC 950.46), ash content by the muffle furnace method (AOAC 920.153), Protein content by the Kjeldahl method (AOAC 981.10), Fat content analyzed by the Soxhlet extraction method (AOAC 960.39) and Dietary fiber were analyzed by Acid hydrolysis method and available Carbohydrate content was estimated by percentage difference from non-carbohydrate compounds. Energy (Kcal) was calculated by theoretical formula method (AOAC 2000) and mineral content of the samples was analyzed using a flame photometer (AOAC 969.23-1971) and cholesterol determination was done by Spectrophotometric method done by (Kolaric *et al.*, 2020), Iron content were determined by the Spectrophotometric method analyzed by (Siong *et al.*, 1989). All parameters were analyzed and compared in Table 8. This nutritional analysis was done (based on the nutritional attributes of commercial meat substitutes used as the reference) from (Herbivo, 2023). All these testing methods were listed in the method of analysis for plant-based meat substitutes by (McClements *et al.*, 2021). Values are based on a triplicate analysis of the sample.

Results and discussion

Preliminary studies

Preliminary studies were carried out to fix the acceptable levels. A trial was done based on the addition of pea protein isolate and Soy protein isolates. It was observed that increasing pea protein levels would affect the sensory profile of meat substitutes and create an off taste to the product. So, pea protein levels in the formulation should be in decreasing trend. This was determined by using trial and error method. Levels of pea protein isolate from

preliminary trials varied from 10 to 60 %, and Soy protein levels were substituted from 20 to 80 %. Out of these variations, based on sensory properties and the Hardness profile of the final fried product, the best combinations were finalized. At the conclusion of the preliminary trials, the best blend was considered for the levels of ANOVA optimization in the statistical mathematical tool. These preliminary compositions were taken on the basis of initial trials (data not reported). Pea protein isolates and soy protein isolates were blended in the ratio as shown in Table 1. Sample TC served as a control. T1-T6 codes were assigned to the samples respectively. These preliminary trials from the ANOVA can be used for future work for optimization using response surface methodology (RSM) for plant-based meat substitutes using freeze structuring (Chitradevi *et al.*, 2023).

Effect of textural attributes and water activity on fried meat substitute

Textural properties of the fried meat substitute were tabulated in Table 2 containing various amounts of pea isolates and soy protein blends. The results show that the hardness of the meat substitute increased significantly ($p < 0.05$) when soy protein concentration was increased. The control sample of fried chicken meat has a lower hardness which only contains pea protein. A similar study was done for plant-based meat using methylcellulose, which has lowered hardness values (Bakhsh *et al.*, 2021). The chewiness of the meat substitute was negatively influenced by the inclusion of pea isolate, which is due to the reduced amount of gluten content which significantly ($p < 0.05$) with the improved amount of pea protein isolates. The addition of pea protein in the formulation had a significant effect on texture properties ($p \leq 0.05$). Hardness values of the fried meat substitutes were increased from 527.33 N to 1911.88 N, which indicates that the maximum force has been exerted to compress the meat substitute sample. While the chewiness values were increased from 459.31 to 1435.22 N/cm. Due to the formation of a stable and elastic network structure, pea isolates promote the formation of a stronger gel network, resulting in a firmer texture. The solubilization of

Table 1. shows the formulation of protein blends using ANOVA

S.no	Sample code	Pea isolate (PPI)%	Soy isolate (ISP) %
1	TC	100	0
2	T1	80	20
3	T2	60	40
4	T3	40	60
5	T4	30	70
6	T5	20	80

Table 2. shows the effect of textural attributes on various compositions of fried meat substitutes

*Note: ANOVA blend optimization for selected varieties.
* Protein composite of 100% protein blend on a flour basis.

Parameters	Sample code					
	Tc	T1	T2	T3	T4	T5
Hardness(g)	527.33±0.26 ^a	545.67±0.12 ^a	719.21±0.62 ^a	842.87±0.02 ^a	1700.34±0.02 ^a	1911.88±0.55 ^a
Adhesiveness (NS)	-29.68±0.15 ^f	-23.17±0.71 ^f	-17.97±0.14 ^f	-11.07±0.31 ^g	-5.49±0.25 ^g	-3.24±0.11 ^g
Springiness (g)	0.38±0.45 ^d	0.44±0.25 ^d	0.503±0.15 ^d	0.58±0.45 ^d	0.67±0.45 ^d	0.79±0.15 ^d
Cohesiveness (ratio)	0.45±0.45 ^c	0.44±0.25 ^d	0.41±0.75 ^e	0.39±0.50 ^e	0.373±0.50 ^e	0.35±0.45 ^e
Gumminess(N/cm ²)	105.65±0.21 ^b	110.74±0.11 ^b	279.120±0.26 ^b	347.53±0.26 ^b	421.40±0.15 ^b	517.38±0.36 ^b
Chewiness (N/cm)	459.31±0.36 ^c	466.56±0.11 ^c	671.44±0.58 ^c	721.35±0.66 ^c	1410.33±0.01 ^c	1435.22±0.60 ^c
Water Activity	0.564±0.01	0.537±0.01	0.542±0.01	0.553±0.01	0.534±0.01	0.596±0.01

*Different superscript letters in the same column indicate statistical significant difference of mean value ($p < 0.05$)
Each value is a mean of triplicate observations (n=3)

proteins, which tends to reduce the availability to form a network, has been studied by (Flores *et al.*, 2023). Adhesiveness and cohesiveness ranges were decreased from -29.68 to -3.24 Ns and 0.45 to 0.35, respectively. Springiness values have slight changes, which decreased from 0.38 to 0.79g, which shows that the samples have a higher ability to recover from the original form after the deforming force was removed. Moreover, Changes in texture can be attributed to the chemical linkages and protein interactions formed by Soy protein isolates, such as disulfide bonds, hydrophobic interactions, and hydrogen bonds. The compact network formed by soy protein leads to varying springiness (Muhialdin *et al.*, 2023)

Gumminess values were decreased from 105.65 to 517.38 N/cm². Similar Trends were done for texturized protein by the authors Kitcharoenthawornchai and Harnsilawat (2015), showing that the addition of texturized vegetable protein supports mastication. Pea protein isolates have been utilized as some prime raw materials for the development of fibrous meat alternatives. The results showed that the addition of Texturized vegetable protein maximized the force necessary to disintegrate the sample, which was needed for swallowing; therefore, TVP was added at lower levels for easier mastication during sensory analysis. Trial T3 has moderate levels of hardness and chewiness, which contain (a 40:60) ratio of pea and soy isolates. It can be good palatable during the mastication. The incorporation of soy isolates has reduced the level of force required to cut the sample and also reduced the compression needed through the sample. Pea protein isolates have been shown to be valuable raw materials for the development of fibrous whole-muscle meat alternatives. Moreover, the addition of kappa carrageenan to pea protein isolate and wheat gluten in the formulations has been shown to improve the quality attributes of plant-based meat alternatives, including the formation of visible fibers (Muhialdin *et al.*, 2023). The inclusion of ISP along with PPI for the meat analog constituent resulted in a tender consistency in the meat substitutes, which can form a fibrous structure. This result was further substantiated by the visual portrayal of the specimen. Figure 2(a) illustrates the cross-sectional image, which contains 40% PPI levels. Figure 2(a) indicates the cross-sectional raw sample and 2(b) indicates the deep-fried meat substitute sample.

Water activity levels in fried meat substitutes should be controlled since higher levels of water activity can result in the formation of mutagenic substances and prone to microbial contamination. The addition of fat or oil in the process of frying can both boost the levels of water activity. Deep-frying

of meat substitutes at appropriate times and temperature combinations can reduce the water activity levels. Water activity values were increased from 0.564 to 0.596, which has slightly increased significantly ($p < 0.05$) when compared to the control sample of fried chicken meat. Increased levels of soy isolates, which lead to improper mass transfer during frying, also due to decreasing pea isolates, which have negatively correlated with the water activity. More wheat gluten leads to more water uptake and traps the water inside the gluten-fibrous structure. Improper mass transfer during frying leads to improved water activity. Trial T5 sample has less water activity values since it has higher levels of textural values and can be neglected, and Trial T3 has a moderate water activity level of 0.553 since the levels can be classified as intermediate moisture foods (Intermediate moisture foods, $0.60 < a_w < 0.85$). Furthermore, appropriate water activity levels are critical for meat product preservation because they can alter the color, aroma, and shelf-life of the meat substitutes. The deviation in the values may vary due to the levels of protein isolates in the formulation, which influences the frying time and can make the product moist, which results in a deviation of water activity levels.

Effect of sensory attributes on fried meat substitute

Sensory attributes of meat substitutes, such as Fibrousness, Juiciness, Elasticity, Tenderness, Taste, and overall acceptability, of meat substitutes are shown in Table 2. The developed meat substitute was compared with the control sample of fried chicken. Improving the amounts of soy protein isolates significantly lowers the scores of the sensory properties ($p < 0.05$). But the fried sample developed with moderate levels of PPI was liked by all the panellists. The fried samples were examined for pea protein content, and it was discovered that the sample with 40% pea isolate protein had the highest scores. Additionally, it was established that this sample scores were significantly ($p < 0.05$) similar to the control sample (Meat). Furthermore, it was determined that the sample was significantly ($p < 0.05$) similar to the control sample. Figure 3 was



Figure 2. illustrates the process methodology for freeze-structured meat substitutes

found to have higher sensory scores of 8.3, including all the sensory attributes such as Fibrousnesses, juiciness, Tenderness etc. A study by (Hamid *et al.*, 2020) shows that the incorporation of jackfruit peel and wheat gluten on higher levels decreases overall acceptability. Increasing levels of soy protein lead to the bitterness in the fried sample this can be due to increasing the concentration of the tri hydroxylated genistein in most of the soy-based foods which exceed the limits, whereas those of other soy isoflavones are around or below their respective threshold value, indicating that genistein might be one of the main contributors to the bitterness of soy products. In addition to genistein, other isoflavonoids such as glycitin, genistin, acetyl genistin, and malonyl genistin also activate bitterness caused by soy (Zhang *et al.*, 2023). Bitter fraction of soy contains small peptides with molecular weight less than 1000 Daltons, which are hydrophobic amino acids. The overall acceptability score of meat substitutes with 40% pea isolate and 60% soy isolate is 8.3, which was the highest score recorded by the panelists shown in Table 3.

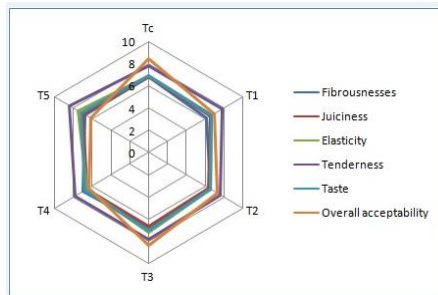


Figure 3. shows the sensory scores of the fried sample

Effect on Chemical composition characteristics of fried meat substitutes

The physiochemical measurements of fried meat substitutes were tabulated in Table 4. The inclusion of protein composites was found to increase the protein content significantly ($p < 0.05$) when compared to the control sample. Increasing the concentration of soy protein has also significantly ($p < 0.05$) increased the fat content of fried meat substitutes due to the increase in oil uptake content product appears to be soggy after frying. This soggy effect after frying mainly due to the texture influenced by the protein isolates, such as water absorption capacity (WAC). High WAC of pea protein isolate (PPI) and soy protein isolate (ISP) can result in greater moisture retention and a softer texture which absorbs more oil during frying and leads to soggy texture (De Angelis *et al.*, 2020). Additionally, the freeze-structuring process used to produce the meat substitutes can also affect the texture. Pea protein isolate (PPI) has been found to create a more porous structure in the final product, potentially contributing to a softer texture, which results in the freeze-structuring process influencing the soggy texture of the final fried meat substitutes (Webb *et al.*, 2023). It is compatible with the study proposed by soy protein, which has a higher fat content it is because of its ability to absorb and hold oil, and it increases the fat content of meat substitutes during frying. When mixed with water, ISP has a high water absorption capacity and creates a gel-like structure, allowing it to bind to and hold onto oil during frying. As a result, the ISP absorbs greater amounts of oil during the process of frying (Vernaza *et al.*, 2020), increasing the fat content. Furthermore, the structures of ISP, which develop by heat denaturation and polypeptide cross-linking,

Table 3. shows the effect of sensory attributes on various compositions of fried meat substitutes

Parameters	Sample code					
	Tc	T1	T2	T3	T4	T5
Fibrousnesses	6.80±0.84 ^b	6.20±0.51 ^b	6.30±0.84 ^{ab}	6.70±0.78 ^b	6.00±1.15 ^{ab}	6.80±0.78 ^b
Juiciness	6.80±0.91 ^b	6.60±0.51 ^b	6.40±1.07 ^b	6.60±0.51 ^b	6.60±1.07 ^b	7.00±0.66 ^b
Elasticity	6.90±0.73 ^{ab}	6.70±0.94 ^b	6.60±1.07 ^{ab}	7.20±0.78 ^{ab}	6.60±0.84 ^b	7.50±0.47 ^b
Tenderness	7.80±0.63 ^a	7.80±0.63 ^a	7.60±0.84 ^a	7.80±0.78 ^a	7.80±0.78 ^a	8.40±0.51 ^a
Taste	6.90±0.31 ^{ab}	6.70±0.48 ^b	6.60±0.84 ^{ab}	7.00±0.66 ^{ab}	6.80±0.78 ^{ab}	7.20±0.42 ^b
Overall acceptability	8.40±0.63 ^b	7.00±0.66 ^{ab}	7.30±0.51 ^b	8.30±0.51 ^b	6.35±0.51 ^b	6.16±0.42 ^b

*Different superscript letters in the same column indicate statistical significance differences ($p < 0.05$)
Each value is a mean of ten observations (n=10)

Table 4. shows the chemical analysis on various compositions of fried meat substitutes

Samples code	Moisture (%)	Ash content (%)	Protein (%)	Fat (%)	Dietary fibre (%)
Tc	23.68±0.18 ^a	0.87±0.05 ^b	14.20±0.54 ^e	6.10±0.01 ^f	4.41±0.35 ^g
T1	16.62±0.31 ^a	0.89±0.01 ^b	15.04±0.02 ^d	4.30±0.01 ^e	4.62±0.25 ^f
T2	17.80±0.08 ^b	0.91±0.001 ^a	16.32±0.09 ^{bc}	4.52±0.02 ^c	4.71±0.14 ^e
T3	18.22±0.08 ^c	0.92±0.001 ^b	19.66±0.02 ^{ab}	4.34±0.03 ^c	4.76±0.16 ^c
T4	19.83±0.11 ^c	0.94±0.002 ^b	19.78±0.02 ^c	4.45±0.01 ^d	4.85±0.15 ^d
T5	20.60±0.10 ^c	0.96±0.001 ^b	20.29±0.01 ^{ab}	4.71±0.01 ^b	4.92±0.31 ^b

*Different superscript letters in the same column indicate statistical significance differences ($p < 0.05$)
Each value is a mean of triplicate observations (n=3)

develop a more permeable and open structure, which allows for higher oil penetration (Rareunrom *et al.*, 2008).

The amount of protein, fat, and dietary fiber was found to be the greatest at the amount of 40% pea isolate supplementation. Moisture levels of the samples vary from 16-20% due to the protein properties and freeze-structuring process conditions influencing moisture content. Because of the freeze-structuring process and high moisture content, the product has a more layered and fibrous structure that can absorb more water. Similarly, when soy protein isolate, and pea protein isolate are combined to create meat analogs, the resulting products have higher moisture content than chicken meat. The combination of these protein isolates changes the quality characteristics of the meat analogs, making them more tender (Lee *et al.*, 2023).

Dietary fiber levels were increased from 4.4 to 4.9 % due to the addition of pea protein isolate, and wheat gluten, resulting in the formation of fibrous materials with higher fiber content. Soy protein isolate has excellent gelation properties and has the ability to form fibrous structures, which contributes to the increased fiber content. Interactions between

pea protein isolate and amylopectin, a component of starch, aided in the formation of protein anisotropic structures and increased the fibrous degree of the extrudes, resulting in increased fiber content (Diaz *et al.*, 2022).

Effect of color properties on raw meat substitute

Color parameters such as L*, a*, and b* were studied based on the effects of pea protein isolate, which significantly affect the lightness value of the pea protein isolate substituted with higher composition. Incorporating pea isolate flour into a meat substitute resulted in a notable reduction of color values, particularly in lightness (L), redness (a), and yellowness (b) values, with a significance level of $p < 0.05$. The reduced color values observed in raw uncooked samples containing pea isolates could be attributed to its inherent lightweight property. This effect may be due to the lower carotenoid content in protein isolates. In this study, the uncooked (raw samples obtained from freeze structuring) were used for measuring the color properties. The results are shown in Table 5. The increase in the level of pea isolates percentage results in a significant increase in the lightness of the sample. Moreover, the addition of soy protein isolate decreased the redness and yellowness (positive b* value) of the sample. This can possibly be due to the color of soy isolates (whitish color). ISO bright values were decreased from 7.96 to 3.13 as the pea isolate addition decreased which implied higher intensity of the product colour. A study by the authors (Kitcharoenthawornchai and Harnsilawat, 2015) shows that the incorporation of texturized protein resulted in reduced lightness values.

Table 5. shows the effect of color properties of freeze-structured raw meat substitute

Sample code	L*	a*	b*	ISO Bright
TC	45.93	8.22	21.43	7.96
T1	40.23	5.53	17.96	4.61
T2	35.34	5.47	17.82	4.63
T3	40.65	6.39	19.77	6.81
T4	31.36	4.67	14.33	3.98
T5	29.63	8.53	15.75	3.13

Table 6. shows the viscosity measurements of the protein emulsion

RPM levels	Viscosity (cps)	Shear stress (D/cm ²)	Shear rate (1/sec)	Torque (%)
00.01	06099	4.55	0.075	19.8
00.02	55083	6.83	0.150	29.4
0.03	44097	9.31	0.225	39.9
00.04	33977	12.1	0.300	51.4
00.05	3 3848	16.2	0.375	68.3

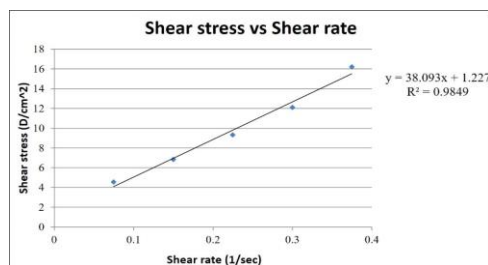


Figure 4. shows the Shear stress vs. shear rate relationship on the viscosity of protein emulsion

Effect on emulsion viscosity measurements

Emulsion viscosity controls its flow characteristics and stability, and both are important for its consistency as well as the quality of substitutes for meat. It directly influences the effect of different components as well as processing parameters on the stability and texture of an emulsion by measuring its viscosity. Protein emulsion was prepared pea isolates: oil: water at (1:4:5) ratio using pre-shearing action until the reproducibility was achieved different levels were measured and tabulated in Table 6 and the shear stress vs. shear rate relationship graph illustrated in Figure 4 shown as a behavior of Newtonian fluid using Ostwald de-wale equation fluid behave like oil viscosity which cannot affect the fluid behavior during mixing of the raw batter of meat substitute during freeze structuring which as shown in Table 4. The emulsion mixture exhibits Newtonian fluid behavior of $n=1$, represents the relationship

Table 7. shows the emulsion stability measurement

S.no	Parameters	values
1	TEF (%)	2.76
2	Fat released (%)	1.12
3	Water released (%)	2.37

behavior of shear stress and shear rate relationship. A study by (Alvarez *et al.*, 2011) shows that emulsion stability was done for oil-based products such as added canola oil. Olive oil has a lower binding and is used as salad dressings. Overall, emulsion viscosity analysis provides helpful information into the formulation and processing of meat replacements, assisting in the optimization of texture and quality.

Effect on emulsion stability on meat substitutes

Emulsion stability testing is critical for meat replacements since it helps define the product's physicochemical and sensory qualities. Drip loss, liquid holding capacity, microstructure, hardness, and overall acceptability can be affected by emulsion stability. Emulsion stability is critical in meat-emulsified model systems for generating a coherent protein network, excellent distribution of fat, and emulsion stability. Table 7 provides an illustration of the assessment of the emulsion's stability, which serves as an indication of its ability to retain moisture and fat during the processing stage. The results of this test can be analyzed by observing the hydration and binding properties of the emulsion. It is observed that an increase in the percentage of added hydrocolloid kappa carrageenan 0.4% leads to a reduction in the total expressible fluid. This phenomenon can be attributed to the maximum absorption capabilities of modified starches, including wheat starch, also added to the formulation, which exhibits the pasting property. The dry blend of flour is when it gets exposed to the water introduced during formulation, which has increased due to the gelatinization of the starch. The heating of starch-water systems to higher temperatures results in the release of a lesser amount of water, which implies that a greater number of water molecules can be absorbed into the polymer matrix after being subjected to higher temperatures, such as frying meat substitutes. Typically, increasing emulsion viscosity leads to increased emulsion stability. These relationships between emulsion viscosity and emulsion stability were considered to be important quality parameters in meat processing, especially for emulsified meat products. As a result, a high-viscosity emulsion is difficult to break and may produce a more uniform and viscoelastic end product (Aktas and Genccelep, 2006). A study by (Fenton *et al.*, 2021) found that, in contrast to other electrolytes, potassium,

rubidium, and cesium ions preferentially bond to the helix structure of kappa carrageenan, hence promoting helix formation and gelation at significantly lower concentrations of sodium salts. Consequently, compared to, for instance, sodium chloride, kappa carrageenan gel is substantially stronger when potassium chloride is present. When compared to kappa, iota generates weaker, more elastic gels; however, this ion selectivity is not observed. This is most likely because the degree of helix self-association is diminished by the greater concentration on the iota carrageenan chains. A study by the addition of kappa carrageenan has been found to have a positive impact on lipid metabolism, promoting energy expenditure and suppressing the bioavailability of ingested lipids. (Taghian *et al.*, 2023) It has been shown to upregulate the expression of Sirtuin1, which is involved in fatty acid oxidation and lipid metabolism.

Effect of Comparative Nutritional Analysis

The nutritional values of the three samples were compared and tabulated in Table 8 as follows. The method of preparation employed for the three samples was frying. However, in commercial samples, the recommended method of preparation was sautéing. Nevertheless, in this particular study, the ultimate end application of chicken meat and the optimized sample called for frying. Consequently, the process for commercial samples was modified to frying instead of sautéing in order to align with the process and to prevent deviation in the values. The selection of the commercial sample was based on ingredient compatibility. The commercial samples were chosen based on a market analysis from the website (Mintel, 2023). Based on the primary ingredients used in this study were pea and soy isolate and vital wheat gluten, which closely matched the commercial ingredient label of the meatless mince kit from Good dot (Herbivo, 2023). Furthermore, the final optimized fried product is minced, making the results even more comparable to the commercial sample, which would be a major consideration for this study. Since commercial meat substitutes are currently produced mainly by MSME, the chosen nutritional parameters can be better compared when raw material and standard operating procedures (based on the nutritional attributes of commercial meat substitutes used as the reference). The nutritional compositions of meat substitutes were analyzed, and market-sold meat substitutes are determined and compared in Table 8.

The optimized sample has a considerably higher moisture content ($p < 0.05$) compared to the fried chicken. This is due to increased dietary fiber content, which has a tendency to retain water and is

Table 8. shows the comparative chemical analysis on fried chicken meat and optimized meat substitute sample composition T3 (40:60) (PPI: ISP) and fried commercial sample

Parameters	Chicken meat (Fried)	Optimized composition T3 (40 % PPI:60 % ISP) (Fried)	Fried Commercial sample (Good dot meatless mince kit)
Moisture content (%)	19.68±0.18 ^a	18.22±0.08 ^a	12.65±0.20 ^a
Ash content (%)	3.14±0.04 ^b	3.92±0.03 ^b	0.98±0.07 ^a
Protein content (%)	13.19±0.31 ^{ab}	29.66±0.22 ^{ab}	50.76±3.62 ^{ab}
Carbohydrate content (%)	52.97±2.31 ^{ab}	74.64±3.31 ^{ab}	26.3±2.84 ^{bc}
Dietary fibre (%)	6.2±0.26 ^c	5.86±0.16 ^c	5.71±2.24 ^c
Fat content (%)	6.82± 0.53 ^e	4.34±0.03 ^e	3.94±0.03 ^e
Energy value (Kcal)	342.82	435.3	397.5
Cholesterol (mg)	0.0	0.0	0.0
Sodium (mg/100g)	137.61± 2.53 ^e	414.2± 3.01 ^{ab}	1498.32±4.01 ^{ab}
Potassium (mg/100g)	330.4 ± 1.33 ^{bc}	64.22 ± 2.38 ^{ac}	90.89 ± 1.08 ^{ac}
Lithium (mg/100g)	0.05± 0.08 ^{ab}	0.03± 0.02 ^{ac}	0.02± 0.05 ^{ac}
Calcium (mg/100g)	46.33± 0.58 ^{ac}	42.88± 0.88 ^{ab}	40.23± 1.38 ^{ab}
Iron (mg/100g)	9.23±1.24 ^c	7.56± 3.78 ^{ac}	13.23±2.94 ^c

*Different superscript letters in the same column indicate statistical significance differences ($p < 0.05$). This nutritional analysis was done based on the commercial sample nutritional label (Herbivo.in Good dot meatless mince kit). Each value is a mean of triplicate observations ($n=3$).

directly correlated with moisture levels. Furthermore, the fat content of fried chicken meat was higher than the optimized sample. Also, it has lower calories due to an increase in carbohydrate content, mainly this occurs due to fried meat does not contain any additional components. Protein levels increased in the optimized sample as a result of the addition of legume protein isolates and wheat gluten, which contains about 90% protein in the raw material form. Optimized meat has both minor and major ingredients used in the formulation, which leads to an increase in the carbohydrate content compared to fried chicken meat. Calcium and iron levels were increased as compared to the optimized sample, which may be related to the fact that frying can absorb oil while frying., which may contain more calcium and iron, which can lead to a significant increase in micronutrients (Artz *et al.*, 2005).

Compared to the commercial meat substitute, the optimized sample has significantly lower moisture, fat, and harnessed energy (in Kcal) ($p < 0.05$) due to different processing conditions on the industrial scale. Protein levels were higher in the commercial sample (Herbivo, 2023) because it comprised hydrolyzed groundnut protein and other blended flours such as soy flour, wheat flour, and barley flour which would serve as a good source of proteins in the raw form. So the protein levels were higher in the commercial sample (Herbivo, 2023). The findings of the nutritional analysis evidently show that the optimized meat substitute contains approximately three times less sodium than commercially available meat substitutes. This would be a major benefit for making healthier options for consumers without compromising the taste and the quality. Low sodium levels help reduce health problems associated with high sodium intake, such as heart disease, high blood pressure, and

kidney disease (Galina *et al.*, 2020). Also, the optimized meat substitutes are more nutritious as their ash (minerals) contents are significantly higher than those of the commercial product. However, the dietary fiber content of the optimized product and the commercially available product were not significantly different. Although the carbohydrate content of the optimized sample is higher than that of the commercial sample, the overall energy (in Kcal) obtained from the optimized sample is lower due to the lesser contribution from protein than a commercial sample.

Conclusion

The findings of the proposed study evidently show that the freeze-structuring technique was capable of forming a fibrous structure using plant-based protein, which can resemble chicken meat. However, these freeze-structuring techniques were also highly dependent on the protein sources used to resemble meat. The addition of 40% pea isolate and soy isolate at 60 % shows that ideal hardness levels of 842.87 g and springiness of 0.58 g can provide a similar texture to the meat. Sensory attributes show the improved fibrousnesses and juiciness of the meat substitute, which were considered to be an essential parameter for meat ratio of (40:60) of PPI and ISP formulations were more preferred by the panelists, which had more excellent overall acceptability scores of 8.3 on the scale during consumption. Nutritional analysis shows that meat substitutes have 16% increased protein when compared to conventional chicken meat and a less significant difference in dietary fiber levels. Even essential nutrients from legume sources such as pea and soy isolate would serve as a potential source that can create a fibrous structure similar to meat.

The color values show a significant decrease in the supplementation of soy isolate, which has reduced values of lightness (L), redness (a), and yellowness (b) values of uncooked meat substitutes. The strength of the gel network within protein molecules was found to be a significant factor in the texture of meat analogues. Plant protein isolate supplementation was also noted to be a significant contributor to developing a fibrous texture that closely resembles the meat, which can be an essential component of meat analog composites. Utilization of various composites of plant proteins by freeze structuring technique can produce meat analogues.

The growing importance of meat substitutes can be attributed to shifting customer choices, health issues, and sustainability concerns. Sources such as edible insects, cultured meat, and plant-based meat substitutes are some of these substitutes, which provide nutritional benefits and can be consumed by vegetarians and also those who follow halal diets. However, laboratory-grown cultured meat that is made from animal cells is quite similar to conventional meat, which can be used in place of real meat as a source of protein in the future.

Moreover, considering cost and technological difficulties can arise in mass production of meat substitutes. This field lacks research on technology advancements, cost containment, commercialization, and outpacing studies concerning the regulatory aspects of meat substitutes. The most recent developments in biotechnology, like precision fermentation, which creates microbial cells, and cultured animal cells, have the potential to produce a new generation of meat substitutes. In conclusion, more exploration is required to understand the customers' perspectives on meat alternatives because they are unfamiliar with the product. Technologies need potential implementation, particularly in small-scale domestic industries, where investing in alternative manufacturing techniques such as extrusion is impossible and expensive. However, this proposed study can be more feasible to adapt this freeze-structuring technology to small-scale domestic industries, which can be alternative manufacturing to create plant-based meat substitutes that can replace conventional meat, which has the ability to satisfy consumer demands and manage environmental issues while offering sustainable solutions.

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