Chapter-7

Preparation of food product from the developed meat analogue, its characterization and storage study

7.1.Introduction

The development of plant-based meat alternatives has gained significant attention in recent years due to increasing consumer awareness of environmental sustainability, animal welfare concerns, and health considerations (Kyriakopoulou et al., 2019). Plant-based meat analogues or alternatives primarily include textured soy protein, mushrooms, wheat gluten, and pulses, which serve as substitutes for traditional animal protein sources. These alternatives are designed to offer savory taste and characteristic fragrances, often enhanced with comestible ingredients (Kumar et al., 2024). Common protein sources in meat analogues include soy protein, pea protein, and wheat gluten, sometimes combined to meet complete protein requirements. Recent innovations also involve single-cell proteins from bacteria and advanced production methods like 3D printing to create diverse meat substitutes. Among these alternatives, texturized protein-based nuggets represent a promising category with potential to mimic the sensory attributes of conventional meat products while offering nutritional advantages (Kumar et al., 2019). Currently, there is a growing exploration of various protein sources and an assessment of the functionality of their protein fractions. Research efforts are centered on understanding the relationship between process, structure, and function in food proteins, to enhance the quality and functionality of proteins or protein-rich food products (Singhal et al., 2016).

Protein spiral wraps are a novel product based on texturized proteins, which has been gaining popularity in South East Asia. It is highly consumed by North Indian People in the Delhi NCR region, western Indian cities like Ahmedabad, Mumbai, and other parts oof India as well (Patel et al., 2015). The base materials for the manufacture of the protein spiral wraps are defatted flour, texturized protein, and wheat gluten (Raja Sekhar et al., 2022). The protein spiral wraps also known as chaap, come under the category of meat analogues as it contains a high amount of protein and resembles the textural and sensorial characteristics of real meat. Products are designated as meat analogues when

they contain at least 18% protein of the hydrated weight (w/w) after preparation (Kyriakopoulou et al., 2021). Protein spiral wraps being the novel product introduced very recently into the market has not been investigated in various aspects. Due to the high nutritional profile with plant-based protein sources and high consumer popularity due to its sensorial quality, the product protein spiral wraps are mostly prepared and consumed on the same day (Raja Sekhar et al., 2022). The formulation of plant-based nuggets involves careful selection and processing of ingredients to achieve desirable sensory characteristics and functional properties. Texturized protein, which forms the base matrix in these products, provides structure and protein content comparable to animal-based counterparts (Maningat et al., 2022). The addition of specific ingredients such as ghee contributes to flavor development and mouthfeel, while spices and seasonings enhance the overall palatability (Kumar et al., 2024). Quality assessment of plant-based nuggets encompasses various parameters including compositional properties, textural attributes, color characteristics, and sensory acceptability (Younis et al., 2023).

Furthermore, shelf-stability represents a critical aspect of product development, necessitating comprehensive evaluation of physicochemical changes, lipid oxidation, and microbiological safety during storage (Variyar & Mishra, 2024). This study aims to develop and characterize texturized protein-based spiral wrap and nuggets formulated with a specific blend of ingredients. The research investigated the compositional, physicochemical, textural, and sensory attributes of the protein spiral wraps and nuggets, with particular emphasis on shelf-life stability under frozen storage conditions. Through systematic analysis of parameters such as pH, texture profile, color stability, and microbiological quality, this study provides valuable insights into the development of plant-based meat products with acceptable quality characteristics and extended shelf-life. The findings of this research contribute to the growing body of knowledge on plant-based meat alternatives and offer practical implications for food manufacturers seeking to develop nutritious, palatable, and shelf-stable products to meet the evolving demands of health-conscious consumers.

7.2.Materials and methods

7.2.1. Preparation of raw materials

The present study was carried out in the Food Engineering & Technology Department,

Tezpur University, Assam. Texturized protein was obtained using the methodology of Chapter 4. Sunflower oil and wheat gluten were purchased from the local market of Tezpur, Assam, India. The raw materials required for preparing the Manila tamarind protein spiral wraps dough were also obtained from Chapter 4 (Optimized formulation). Flavoured water (processed chicken-flavor (CPF)) was obtained using the methodology of Chapter 5. Additional ingredients including sunflower oil, ghee/butter, baking soda, black salt, black pepper, mixed seasoning blend, garlic powder, aniseed, and clove buds were purchased from local market of Tezpur, Assam, India. All ingredients were food-grade and used within their shelf-life period. A reference sample was bought from market for comparative study. It contained water, pea protein concentrate, soy protein isolate, dietary fibre, wheat gluten, corn starch, salt, flavour and seasonings.

7.2.2. Preparation of Manila tamarind protein spiral wraps

7.2.2.1.Preparation of Manila tamarind protein spiral wrap dough

To prepare the Manila tamarind protein spiral wrap dough, the raw materials were processed initially. The texturized protein was ground in a grinder/mixer (Inalsa, Polo 550), and the remaining ingredients protein isolate, wheat gluten, jackfruit flour, sodium alginate, and water/sunflower oil blend (based on optimized formulation from Chapter 4) were taken in their original form. All the ingredients were mixed in a dough mixer to prepare the protein spiral wrap dough of two varieties. The first one had solid (all ingredients except water) liquid (water) ratio of 1:1 and the other one had solid liquid ratio of 2:1, respectively. The dough was sheeted and cut into geometry suitable for wrapping on the skewer using a dough sheeter-cum-cutter. The cut stripes were wrapped around the dough sheet using a hand roller on the dough sheeter-cum-cutter to a desired thickness to resemble the fibrous texture of meat and then the cut sheet stripes were wrapped on the skewer for further processing (Raja Sekhar et al., 2022).

7.2.2.2.Cooking of the cut sheet stripes of Manila tamarind protein spiral wrap

Table 7.1 gives an overview of the experimental design for the preparation of Manila tamarind protein spiral wrap. The cooking process of the protein spiral wraps employed a dual cooking methodology utilizing both autoclaving and boiling techniques, with specific time durations of 15 and 30 min, respectively, to ensure thorough cooking and proper texture development. The cooking with boiling was performed by submerging the

food product in distilled water at a 3:1 ratio and maintaining a rolling boil at 100 °C for durations of 15 and 30 min, during which gentle stirring was applied every 5 min to ensure uniform heat distribution. Simultaneously, autoclaving was carried out using pressurized steam at 121 °C and 15 PSI for durations of 15 and 30 min, where products were loaded into autoclave-compatible containers with 25% headspace. Both methods required precise temperature monitoring using calibrated instruments, with the boiling process involving immediate heat removal after the 15 min duration, while the autoclaving procedure necessitated waiting until chamber pressure reached zero and temperature dropped below 80 °C before opening. Critical parameters were maintained throughout both processes, including consistent timing from the moment target conditions were achieved, proper safety protocols with heat-resistant equipment, and continuous documentation of temperature and time parameters. Following either cooking method, the products were immediately transferred to fine-mesh stainless steel strainers and allowed to drain for exactly 10 min without compression to remove excess moisture while maintaining texture integrity. The mass of all the cooked samples was taken before and after cooking (boiling and autoclaving) using an electronic balance (Aczet CY 224C, India). The entire process was conducted under controlled conditions with systematic documentation to ensure reproducibility and quality control across all batches prepared for subsequent analysis.

Table 7.1: An overview of the experimental design for the preparation of Manila tamarind protein spiral wrap

Cooking type	Cooking time (min)	Solid: Liquid
Boiling	15	2:1
	15	1:1
	30	2:1
	30	1:1
Autoclaving	15	2:1
	15	1:1
	30	2:1
	30	1:1

7.2.2.3.Characterization of Manila tamarind protein spiral wraps and dough

7.2.2.3.1. Rheology of dough

The small amplitude oscillatory shear (SAOS) tests were performed on a stress-controlled rheometer (MCR72, Anton Paar, Austria) with a 40-mm parallel plate geometry using the modified protocol of Meerts et al. (2017). A solvent trap combined with wet cotton wool was used to prevent dehydration of the dough samples. All dynamic measurements were performed in duplicate on separately prepared batches of protein spiral wrap dough, and good reproducibility was obtained. To allow the stresses developed in the dough during mixing, shaping and loading in the rheometer to relax, a resting period of 30 min was applied, once before and once after loading the sample in the rheometer. Only after these resting periods, the frequency sweeps were performed. The sample was carefully loaded to avoid air entrapment, and the edges were trimmed to match the plate diameter. The measurement was conducted at a controlled temperature (25 °C). Prior to frequency sweep testing, a strain sweep was carried out to determine the Linear Viscoelastic Region (LVR), ensuring that subsequent frequency tests (typically 0.1 to 50 Hz) were within the LVR using a fixed strain (1%). Storage modulus (G′) and loss modulus (G″) were recorded as functions of frequency to evaluate the elastic and viscous behavior, respectively.

7.2.2.3.2. Physicochemical analysis

The physicochemical analysis of the protein spiral wrap samples was thoroughly done using standardized methods from the Association of Official Analytical Chemists (AOAC, 2006). Moisture content (MC) determination (AOAC method 934.01) involved a gravimetric approach where 5 g samples underwent thermal drying in a precision-controlled environment at 105 °C for 6 h using a specialized hot-air oven (Model LT-90D, Labtech Engineering Co., Ltd., Germany) until constant weight was achieved. The weight differential before and after the dehydration process provided accurate moisture percentage data, calculated using equation (7.1):

$$\text{MC (\%)} = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Initial weight}} \times 100 \qquad (7.1)$$

Protein content was determined via the Kjeldahl method (AOAC method 981.10), which quantified nitrogen content using an automated analytical system (Kjeltec® 2300

Analyzer Unit, Foss Tecator AB, Sweden). This technique involved sample digestion (1g) with concentrated sulfuric acid (H_2SO_4) and catalyst mixture (K_2SO_4:$CuSO_4$ = 10:1) at 420 °C for 2 h, followed by distillation with 40% NaOH and titration with standardized 0.1 N HCl to determine total nitrogen, which was then converted to protein content using the conversion factor of 6.25. All analyses were performed in triplicate.

7.2.2.3.3. Analysis of colour parameters

Color parameters were quantitatively assessed using a precision colorimeter (Chroma meter CR-210, Minolta, Japan) equipped with illuminate C and calibrated using a standardized white reference plate (L^*=97.83, a^*=-0.43, b^*=+1.98). This instrument employs reflectance spectrophotometry with a defined measuring area of 8 mm diameter to capture surface color characteristics. The Commission Internationale de l'Éclairage (CIE) *Lab** color space model was utilized, where L^* represents lightness (0=black, 100=white), a^* indicates red-green chromaticity ($+a^*$=red, $-a^*$=green), and b^* signifies yellow-blue chromaticity ($+b^*$=yellow, $-b^*$=blue). These multidimensional color parameters provide comprehensive characterization of visual properties. Measurements were taken on the surface of dough and protein spiral wrap samples in triplicate to ensure representative sampling, with results presented as means and standard deviations to account for natural color variations across the sample surface (Dutta & Sit, 2023).

7.2.2.3.4. Measurement of cooking quality

The cooking quality of the protein spiral wrap samples was determined through a standardized thermal processing protocol (Palanisamy et al., 2019). Initial and final weight of the samples was recorded after they underwent cooking by boiling and autoclaving for 15 and 30 min, respectively. Following thermal processing/cooking, samples were allowed to equilibrate at ambient temperature for 30 min to stabilize moisture redistribution and structural changes before determining the final weight (cooked weight). The cooking yield percentage was then calculated using the equation (7.2):

$$\text{Cooking yield (\%)} = \frac{\text{Weight after frying}}{\text{Initial weight}} \times 100 \qquad (7.2)$$

Cooking loss of the samples was calculated by using the following equation (7.3):

$$\text{Cooking loss (\%)} = 100 - \text{Cooking yield} \quad (7.3)$$

7.2.2.3.5. Analysis of textural parameters

The textural characteristics of the samples were evaluated using a TAXT Plus Texture Analyzer equipped with a 25 mm diameter flat pressure adaptor through the protocol of Chiang et al. (2019) with a few modifications. A double compression test was conducted using a P/50 probe with a 30 kg load cell, compressing samples to 50% of their original height at 60 mm/min. From the resulting force-time curves, multiple textural attributes were derived: hardness, defined as the peak force during the first compression cycle (measured in g); springiness, calculated as the ratio of sample recovery after the initial compression; cohesiveness, determined as the ratio of work done during the second compression relative to that of the first compression; gumminess, computed as the product of hardness and cohesiveness; and chewiness, calculated as the product of hardness, cohesiveness, and springiness (expressed in g). Each sample underwent duplicate testing to ensure measurement reliability, with the derived parameters collectively providing a comprehensive mechanical characterization of the protein spiral wraps textural properties, which correlate strongly with sensory perception during consumption.

7.2.3. Preparation of meat analogue nuggets

7.2.3.1.Formulation of nuggets

The formulation of meat analogue nuggets was developed as per standardized procedure of Sharima-Abdullah et al. (2018) with slight modifications. The nuggets consisted of the following ingredients by total weight: 85% texturized protein forming the primary structural component, 3.65% ghee providing richness and liquidity, 9% chicken-like processed flavour (CPF) contributing to both hydration and taste enhancement, 1% baking soda acting as a leavening agent, 0.5% black salt for basic seasoning and flavor enhancement, 0.45% black pepper adding mild heat and aromatic complexity, 0.2% seasoning blend for depth of flavor, 0.1% garlic powder introducing savory notes, 0.05% aniseed contributing subtle licorice undertones, and 0.05% clove delivering warm, slightly sweet aromatic notes. This carefully balanced combination of ingredients ensured optimal texture, flavor profile, and binding properties in the final product, with the majority protein base complemented by precise quantities of fats, moisture, and

seasonings to achieve the desired sensory characteristics. A non-breaded nugget style was adopted for the study. Initially, texturized protein and ghee were minced using a food chopper (Model 320, Moulinex, France) and placed in a mixing bowl. A brine solution was prepared by dissolving salt in flavoured water (CPF) and thoroughly mixed with the minced texturized protein and ghee for 30 s using a food processor (Model MK-F300, Panasonic, Malaysia). Other ingredients, including black pepper, baking soda, seasoning, garlic powder, aniseed, and clove, were then added and mixed for 1 min to ensure homogeneity. Each nugget was manually formed into a 10 g portion (dimensions: 2 × 2 × 1 cm^3) and frozen at -18 ℃ for 1 h. Cooking was performed by deep-fat frying (DF) the nuggets in sunflower oil using the standardized protocol from Chapter 6. A deep-fat fryer (JSGW 1205/2, Ambala, India) with a capacity of 4.5 L was used for deep-fat frying of the nugget samples. Sunflower oil was preheated to 160 ℃ for 30 min prior to frying. Samples were put in a wire basket in the fryer and then deep-fat fried for 90 s. Thereafter, the reference sample also underwent the same frying conditions. After frying, the samples were immediately removed from the oil and blotted gently with dry tissue papers to remove the excess oil from their surfaces. The samples were allowed to cool to room temperature before further tests were conducted. Nuggets prepared similarly using all the ingredients but without frying were treated as the fresh nuggets. All the nugget samples were stored at -18 ℃ after packing and sealing them in polypropylene (PP) pouches for storage studies.

7.2.3.2.Weighing of the samples

The mass of all the samples (fresh, fried sample and reference sample) was taken before and after deep-fat frying at 160 ℃ for 90 s (not required for fresh samples) using an electronic balance (Aczet CY 224C, India).

7.2.3.3.Compositional properties analysis

The compositional properties of the nugget samples were thoroughly analyzed using standardized methods from the Association of Official Analytical Chemists (AOAC, 2006). Moisture content (MC) determination (AOAC method 934.01) involved a gravimetric approach where 5g samples underwent thermal drying in a precision-controlled environment at 105 °C for 6 h using a specialized hot-air oven (Model LT-90D, Labtech Engineering Co., Ltd., Germany) until constant weight was achieved. The

weight differential before and after the dehydration process provided accurate moisture percentage data, calculated using equation (7.4):

$$\text{MC (\%)} = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Initial weight}} \times 100 \quad (7.4)$$

For lipid quantification, fat extraction was performed using the Soxhlet method (AOAC method 920.39), which employed a continuous solvent extraction system (Soxtec® Avanti 2050 Auto System, Foss Tecator AB, Sweden) with petroleum ether (boiling point 40-60 °C, analytical grade, Merck KGaA, Germany) as the extraction solvent. Samples (2 g) were extracted for 6 h, followed by solvent evaporation and drying of fat residue at 105 °C for 1 h before weighing. Protein content was determined via the Kjeldahl method (AOAC method 981.10), which quantified nitrogen content using an automated analytical system (Kjeltec® 2300 Analyzer Unit, Foss Tecator AB, Sweden). This technique involved sample digestion (1g) with concentrated sulfuric acid (H_2SO_4) and catalyst mixture (K_2SO_4:$CuSO_4$ = 10:1) at 420 °C for 2 h, followed by distillation with 40% NaOH and titration with standardized 0.1N HCl to determine total nitrogen, which was then converted to protein content using the conversion factor of 6.25. The mineral component was assessed through ash content determination method (AOAC method 942.05) which involved complete incineration of organic matter in pre-weighed crucibles containing 2 g samples at 550±10 °C for 8 h in a muffle furnace (Thermolyne F6010, Thermo Scientific, USA), leaving only inorganic mineral residues for quantification. For crude fiber analysis, 0.5 g samples were processed in a Fiber Analyzer using sequential H_2SO_4 and NaOH treatments. The final crude fiber content was calculated based on the weight difference after the charred samples were ashed at 550±15 °C for 6 h. All analyses were performed in triplicate.

7.2.3.4.pH measurement protocol

The pH measurement followed a precise methodology where 5 g of nugget sample was first ground to increase surface area and then homogenized with 20 mL of distilled water for exactly 1 min using a high-speed homogenizer (Ultra-Turrax® T25, manufactured by Janke & Kunkel, Germany). This preparation ensures uniform dispersion of soluble components for accurate pH measurement. The resulting homogenate was immediately analyzed using a calibrated pH meter (Model 340, Mettler-Toledo GmbH Analytical,

Switzerland), which provides electrometric determination of hydrogen ion concentration (McClements & Grossmann, 2021).

7.2.3.5.Analysis of the color parameters

Color parameters were quantitatively assessed using a precision colorimeter (Chroma meter CR-210, Minolta, Japan) equipped with illuminate C and calibrated using a standardized white reference plate (L^*=97.83, a^*=-0.43, b^*=+1.98). The instrument employed reflectance spectrophotometry with a defined measuring area of 8 mm diameter to capture surface color characteristics. The Commission Internationale de l'Éclairage (CIE) *Lab** color space model was utilized, where L^* represents lightness (0=black, 100=white), a^* indicates red-green chromaticity ($+a^*$=red, $-a^*$=green), and b^* signifies yellow-blue chromaticity ($+b^*$=yellow, $-b^*$=blue). These multidimensional color parameters provide comprehensive characterization of visual properties. Measurements were taken on the surface of nugget samples in triplicate to ensure representative sampling, with results presented as means and standard deviations to account for natural color variations across the sample surface (Dutta & Sit, 2023).

7.2.3.6.Determination of cooking quality

The cooking quality of the nugget samples was determined through a standardized thermal processing protocol (Palanisamy et al., 2019) where nugget samples (both formulated and standard) of recorded initial weight underwent deep-frying at a precisely controlled temperature of 160 °C for 90 s. Following thermal processing, samples were allowed to equilibrate at ambient temperature for 30 min to stabilize moisture redistribution and structural changes before determining the final weight (frying weight). The cooking yield percentage was then calculated using the equation (7.5):

$$\text{Cooking yield (\%)} = \frac{\text{Weight after frying}}{\text{Initial weight}} \times 100 \quad (7.5)$$

Cooking loss of the samples was calculated by using the following equation (7.6):

$$\text{Cooking loss (\%)} = 100 - \text{Cooking yield} \quad (7.6)$$

These parameters quantify water and fat retention capacity during cooking, serving as an important quality and economic indicator.

7.2.3.7.Texture profile analysis

The textural characteristics of the samples were evaluated using a TAXT Plus Texture Analyzer equipped with a 25 mm diameter flat pressure adaptor through the protocol of Chiang et al. (2019) with a few modifications. Samples were uniformly cut into cubic shapes measuring 1×1×1 cm. A double compression test was conducted using a P/50 probe with a 30 kg load cell, compressing samples to 50% of their original height at 60 mm/min. From the resulting force-time curves, multiple textural attributes were derived: hardness, defined as the peak force during the first compression cycle (measured in g); springiness, calculated as the ratio of sample recovery after the initial compression; cohesiveness, determined as the ratio of work done during the second compression relative to that of the first compression; gumminess, computed as the product of hardness and cohesiveness; and chewiness, calculated as the product of hardness, cohesiveness, and springiness (expressed in g). Each sample underwent duplicate testing to ensure measurement reliability, with the derived parameters collectively providing a comprehensive mechanical characterization of the nuggets' textural properties, which correlate strongly with sensory perception during consumption.

7.2.3.8.Sensory evaluation

Twelve experienced panellists were selected from a group of 15 potential panellists using basic taste identification tests to know whether chicken/meat like properties were achieved or not. Each sample of nugget (formulated and standard) was evaluated in terms of appearance, color, taste, flavor, texture, and overall acceptability. The samples were served to the 12 experienced panel members. The appearance (0 = extremely unappealing, 9 = extremely appealing), color (0 = extremely undesirable, 9 = extremely desirable), taste (0 = extremely liked, 9 = extremely disliked), flavor (0 = extremely undesirable, 9 = extremely desirable), texture (0 = extremely tough, 9 = extremely tender), and overall acceptability (0 = extremely undesirable, 9 = extremely desirable) of the samples were evaluated using a 9-point hedonic scale. The panellists were also required to cleanse their palates with water between tasting the samples (Keeton, 1983).

7.2.3.9.Shelf-Life Studies

The nuggets (fresh, formulated and standard), prepared according to standard

formulations, were packed in low-density polyethylene bags, sealed, and stored at -18 ± 2 ℃. Samples were analyzed at intervals of 0, 10, 20, 30, 40, and 50 days. Before analysis, the samples were thawed in a refrigerator at 4 ± 1 ℃ for 5–6 h. the fresh nugget samples underwent frying after the thawing period using the same protocol previously employed for formulated and standard nuggets, deep fat frying at 160 ℃ for 90 s. The samples were then analyzed for:

7.2.3.9.1. Weight

As previously described in section **7.2.3.2**.

7.2.3.9.2. Fat and ash content

As previously described in section **7.2.3.3**.

7.2.3.9.3. pH measurement

As previously described in section **7.2.3.4**.

7.2.3.9.4. Texture profile analysis (TPA)

As previously described in section **7.2.3.7**.

7.2.3.9.5. Color measurement

As previously described in section **7.2.3.5**.

7.2.3.9.6. Sensory evaluation

As per previous methodology in section **7.2.3.8**. Before the sensory evaluation, the nugget samples (formulated and standard) were warmed in a microwave for 30 s and served to the panellists. The fresh samples were served directly after the frying operation.

7.2.3.9.7. Determination of microbial quality

The microbiological quality assessment of the nugget samples was conducted using standard plating methods with appropriate modifications (Bashir et al., 2019). 10 g samples were aseptically weighed and placed in sterile stomacher bags containing 90 mL

sterile 0.1% peptone (Difco) diluent. The samples were then pummelled for 1 min using a hand-held homogenizer to achieve homogeneous microbial distribution. From this initial dilution (10^{-1}), appropriate serial dilutions (up to 10^{-4}) were prepared in sterile 0.1% peptone and subsequently plated on selective growth media in duplicate to ensure statistical reliability. For the enumeration of total plate count (TPC), samples were plated on Plate Count Agar (PCA) and incubated at 35 ± 2 °C for 1–2 days. The determination of yeast and mold counts employed Potato Dextrose Agar (PDA) supplemented with chloramphenicol (100 mg/L) to inhibit bacterial growth, with incubation at 25 ± 2 °C for 3–5 days. After the respective incubation periods, colonies were counted on plates with results calculated and expressed as log CFU/g.

7.2.4. Statistical analysis

Using the SPSS statistical software (IBM SPSS Statistics 26), the means of the triplicate values were calculated and the data was subsequently reported using the means and standard deviations. To assess statistical significance, one-way variance (ANOVA) and subsequent Duncan's multiple differentiations with a probability ($p<0.05$) were taken into account.

7.3.Results and discussion

7.3.1. Characterization of Manila tamarind protein spiral wraps and dough

7.3.1.1.Rheological properties of protein spiral wrap dough

The rheological properties of Manila tamarind protein spiral wrap dough systems demonstrate significant sensitivity to compositional modifications, particularly variations in solid-to-liquid ratios. Dynamic mechanical analysis reveals distinct viscoelastic responses that correlate directly with formulation parameters and processing characteristics. Analysis of two representative dough formulations with solid to liquid ratios of 2:1 and 1:1 demonstrated frequency-dependent mechanical behavior, which is similar to that of wheat-based systems **(Figure 7.1)**. Both formulations exhibited elastic modulus (G′) values consistently exceeding viscous modulus (G″) across the investigated frequency spectrum (0-50 Hz), confirming gel-like behavior characteristic of structured protein-starch networks (Steffe, 1996; Rao, 2010). The 2:1 formulation demonstrated robust mechanical properties with G′ values ranging from approximately 30,000 Pa at low frequencies to 120,000 Pa at 50 Hz. Concurrent G″ measurements increased from

20,000 Pa to 45,000 Pa over the same frequency range.

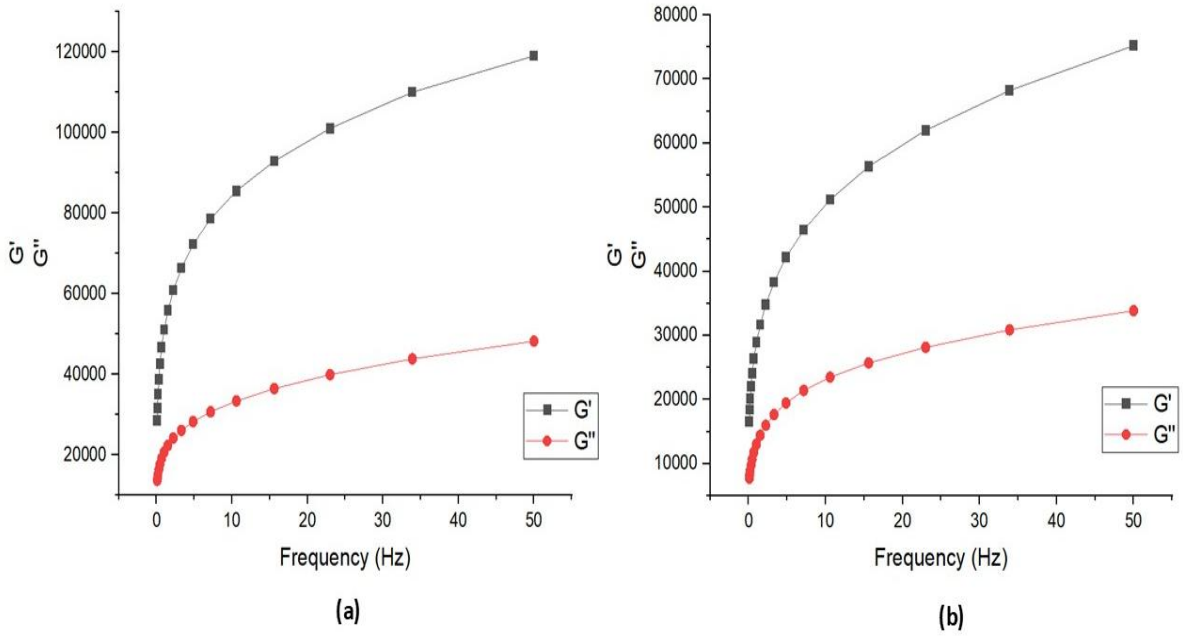


Figure 7.1: Elastic (G') and Viscous (G'') moduli of dough samples (a- solid: liquid of 2:1; b- solid: liquid of 1:1)

This pronounced frequency dependence reflects enhanced protein network development and reduced water mobility within the matrix (Belton, 1999). The substantial differential between elastic and viscous components (G′/G″ ratio of 2.67 at 50 Hz) indicates strong intermolecular associations and limited chain mobility, consistent with previous observations in low-hydration dough systems (Janssen et al., 1996; Campos et al., 1997). The 1:1 formulation exhibited reduced mechanical strength with G′ values spanning 20,000-75,000 Pa and G″ values ranging from 10,000-30,000 Pa across the frequency spectrum. While maintaining elastic dominance (G′ > G″), the diminished moduli reflected increased plasticization effects from elevated moisture content (Khatkar et al., 2013). The frequency response pattern remains qualitatively similar to the 2:1 system, suggesting preservation of fundamental network architecture despite quantitative differences in mechanical properties. The 2:1 dough formulation exhibited approximately 60% higher elastic modulus and 50% higher viscous modulus relative to the 1:1 dough system at 50 Hz (120,000 vs 75,000 Pa for G′; 45,000 vs 30,000 Pa for G″). These differentials align with established relationships between hydration level and

dough strength in cereal-based systems (Dobraszczyk & Salmanowicz, 2008). The observed rheological distinctions have direct implications for processing behavior and final product characteristics. Higher solid content formulations demonstrate enhanced structural integrity suitable for applications requiring dimensional stability and resistance to deformation during handling. Conversely, increased hydration levels promote extensibility and workability, beneficial for products requiring molding or shaping operations (Veraverbeke & Delcour, 2002).

7.3.1.2.Evaluation of colour parameters

Table 7.2 provides a detailed comparison of the color properties specifically *L** (lightness), *a** (red-green), and *b** (yellow-blue) values of Manila tamarind protein spiral wrap samples subjected to two thermal processing methods, i.e., autoclaving (ACC) and boiling (B), for two time durations (15 and 30 min), each made from dough of two different solid-to-liquid ratios (2:1 and 1:1). These color parameters are key indicators of visual quality, consumer appeal, and potential chemical changes (e.g., Maillard reaction or pigment degradation) resulting from heat processing. The *L** value shows significant ($p<0.05$) variation across samples. Boiled samples were consistently lighter in color compared to autoclaved ones, with B-30 (1:1) recording the highest *L** value (49.44), indicating a relatively lighter and potentially less thermally degraded product. Conversely, ACC-30 (2:1) showed the lowest *L** value (42.82), suggesting darker coloration, likely due to intensified browning reactions under higher temperature and pressure. The *a** values showcased a similar pattern. Boiled samples, particularly B-30 (2:1), showed the highest redness (5.65), compared to ACC-30 (2:1) with *a** value of 3.02, which was the lowest among the samples. This increase in redness with boiling, especially with longer time and higher liquid content, could be attributed to enhanced pigment preservation or thermal-induced color development without extensive degradation (Wang et al., 2021).

In contrast, autoclaving may degrade red pigments or promote darker brown hues due to more intense Maillard reactions. For the *b** values, the trend again favored the boiled samples, which had markedly higher values. B-30 (1:1) showing the highest *b** at 17.15, denoting more pronounced yellowness, while ACC-30 (2:1) showed the lowest at 8.20, reflecting duller coloration. These changes in *b** values are significant ($p<0.05$) as they

may correlate with the preservation or destruction of yellow pigments like carotenoids or flavonoids under different processing conditions.

Table 7.2: Colour properties of the Manila tamarind protein spiral wrap sample developed through boiling and autoclaving

Sample	Solid: Liquid	*L**	*a**	*b**
ACC-15	2:1	43.68±0.08^{f}	3.52±0.01^{f}	9.65±0.03^{f}
	1:1	44.26±0.03^{e}	4.86±0.01^{c}	10.32±0.07^{e}
ACC-30	2:1	42.82±0.07^{h}	3.02±0.02^{g}	8.20±0.06^{g}
	1:1	43.17±0.05^{g}	4.60±0.01^{d}	9.78±0.02^{f}
B-15	2:1	47.51±0.04^{d}	4.11±0.01^{e}	12.40±0.04^{d}
	1:1	48.26±0.06^{c}	4.69±0.03^{d}	14.60±0.05^{c}
B-30	2:1	48.60±0.08^{b}	5.65±0.02^{a}	16.42±0.03^{b}
	1:1	49.44±0.07^{a}	5.18±0.01^{b}	17.15±0.04^{a}

Values are given as mean ± standard deviation. a, b: Different lowercase letter superscripts within the same rows, indicate statistically significant differences *($p < 0.05$)* (*L**: Lightness; *a**: redness/greenness; *b**: yellowness/blueness; ACC-15: Autoclave 15 min; ACC-30: Autoclave 30 min; B-15: Boiling 15 min; B-30: Boiling 30 min)

Data from similar studies on plant-based protein products supported these findings. Studies on soy and pea protein-based products have shown that autoclaving significantly ($p<0.05$) reduced *L**, *a**, and *b** values due to browning and pigment degradation, while milder processes like steaming or boiling helped in retaining more natural color (Tang et al., 2019; Wang et al., 2021). In another study, protein products with added tamarind extracts retained higher *L** and *b** values when processed below 100 °C, aligning with the lighter, more yellow appearance of boiled samples in the present study. Overall, the findings suggest that boiling, especially at a 1:1 solid-to-liquid ratio and longer duration (30 min), enhanced lightness and chromaticity, producing visually appealing yellowish-red products (Obi & Okoye, 2017). In contrast, autoclaving resulted in darker, duller products due to intensified thermal reactions. The solid-to-liquid ratio also significantly ($p<0.05$) impacted color development: lower solid concentration (1:1) consistently

yielded brighter and more vibrant samples, likely due to greater heat transfer and moisture-mediated protection against thermal degradation (Tang et al., 2019; Wang et al., 2021). These insights are crucial for optimizing thermal processing parameters in functional food products or plant-based meat analogues using Manila tamarind seed protein.

7.3.1.3.Evaluation of cooking quality of protein spiral wraps

Table 7.3 presents the cooking yield and associated gain in weight for Manila tamarind protein spiral wrap samples subjected to different thermal treatments, autoclaving and boiling, for two durations (15 and 30 min), each at two solid-to-liquid ratios (2:1 and 1:1).

Table 7.3: Cooking properties of the Manila tamarind protein spiral wrap sample developed through boiling and autoclaving

Sample	Solid: Liquid	Cooking yield (%)	Cooking loss↓/gain↑ (%)
ACC-15	2:1	167.82 ± 5.07^{c}	67.82^{c} ↑
	1:1	145.37 ± 3.61^{e}	45.37^{e} ↑
ACC-30	2:1	191.46 ± 4.95^{a}	91.46^{a} ↑
	1:1	154.79 ± 4.24^{d}	54.79^{d} ↑
B-15	2:1	145.69 ± 4.80^{e}	45.69^{e} ↑
	1:1	132.43 ± 2.33^{f}	32.43^{f} ↑
B-30	2:1	175.72 ± 4.78^{b}	75.72^{b} ↑
	1:1	145.22 ± 5.40^{e}	45.22^{e} ↑

Values are given as mean ± standard deviation. a, b: Different lowercase letter superscripts within the same rows, indicate statistically significant differences *($p < 0.05$)* (ACC-15: Autoclave 15 min; ACC-30: Autoclave 30 min; B-15: Boiling 15 min; B-30: Boiling 30 min)

Cooking yield serves as a key indicator of the product's water and solute absorption capacity during processing, directly influencing texture, consumer acceptability, and functional properties (Sanchiz et al., 2019). All treatments resulted in a weight gain,

reflecting the product's capacity to absorb moisture during cooking (Sanchiz et al., 2019). Autoclaved samples consistently exhibited higher cooking yields compared to boiled samples. For example, the ACC-30 (2:1) sample achieved the highest cooking yield at 191.46%, indicating nearly a twofold weight gain and a 91.46% increase in mass post-cooking. This substantial moisture retention is likely due to protein network densification and gelatinization under high temperature and pressure, as autoclaving is known to enhance water absorption and holding capacity in plant-based matrices (Sanchiz et al., 2019; Mukhtar et al., 2023). In contrast, boiled samples showed lower cooking yields across both solid-to-liquid ratios and durations. The lowest yield was observed in the B-15 (1:1) sample at 132.43%, corresponding to a 32.43% gain, suggesting that lower solid content and shorter cooking time reduce the matrix's ability to retain water. Increasing the cooking time from 15 to 30 min improved yields in both methods, confirming that prolonged exposure enhances hydration and structural binding (Udensi et al., 2010; Chisowa, 2022).

Additionally, a higher solid-to-liquid ratio (2:1) consistently resulted in greater yields than the 1:1 ratio, indicating that increased solid content contributes to a more cohesive structure capable of retaining more absorbed water. These results align with findings from other studies on plant-based and protein-rich matrices. Research on lentil protein-based edible films reported cooking yields around 170% after steaming, while autoclaved soy-protein films reached up to 190% depending on protein and fiber content. Mung bean-based meat analogues also demonstrated cooking gains of 150–180% after autoclaving, comparable to the ACC-30 values observed here. Conversely, wheat gluten-based wraps subjected to boiling typically exhibited lower yields (135–150%), reinforcing the superior water-binding and swelling capacity imparted by pressure-cooking or higher solid content (Sanchiz et al., 2019; Mukhtar et al., 2023). In summary, autoclaving, especially for 30 min at a 2:1 solid-to-liquid ratio, significantly ($p<0.05$) enhances cooking yield and moisture gain in Manila tamarind protein spiral wraps, suggesting improved hydration, swelling, and structural stability. Boiling, while still effective, results in comparatively lower gains, making it more suitable for products requiring moderate water retention and a softer texture. These trends provide valuable guidance for optimizing processing parameters to achieve desired functional, sensory, and shelf-stability characteristics in novel protein-based foods (Sanchiz et al., 2019; Mukhtar et al., 2023).

7.3.1.4.Textural properties

Table 7.4 presents a detailed evaluation of the texture profile parameters, hardness, springiness, cohesiveness, gumminess, and chewiness, of Manila tamarind protein spiral wrap samples prepared by autoclaving (ACC) and boiling (B) for 15 and 30 min at two solid-to-liquid ratios (2:1 and 1:1). These texture attributes are crucial for product acceptability in meat analogues, edible films, and protein-based wraps, as they directly affect mouthfeel, mechanical stability, and consumer preference (Nanta et al., 2021; Flory et al., 2023). Among the treatments, boiled samples demonstrated superior texture characteristics, particularly in the B-30 (2:1) group, which recorded high values for hardness (446.56 g), gumminess (111.64 g), and chewiness (51.35 g.s).

Table 7.4: Texture profile parameters of the Manila tamarind protein spiral wrap sample

Sample	Solid: Liquid	Hardness (g)	Springiness (%)	Cohesiveness	Gumminess (g)	Chewiness (g.s)
ACC-15	2:1	370.58 ± 4.16^{e}	34.09 ± 0.17^{g}	0.19 ± 0.01^{e}	70.41 ± 0.70^{e}	23.93 ± 0.14^{e}
	1:1	340.35 ± 3.47^{f}	39.08 ± 0.11^{f}	0.15 ± 0.01^{g}	51.05 ± 0.57^{f}	19.91 ± 0.06^{f}
ACC-30	2:1	319.39 ± 2.95^{g}	58.77 ± 0.58^{a}	0.16 ± 0.02^{f}	51.10 ± 0.42^{f}	29.63 ± 0.09^{c}
	1:1	292.35 ± 2.28^{h}	41.82 ± 0.26^{e}	0.12 ± 0.01^{h}	35.08 ± 0.33^{g}	14.38 ± 0.05^{g}
B-15	2:1	458.08 ± 3.19^{a}	47.03 ± 0.81^{c}	0.24 ± 0.02^{b}	109.93 ± 0.48^{b}	51.66 ± 0.18^{a}
	1:1	437.78 ± 4.06^{c}	30.86 ± 0.38^{h}	0.21 ± 0.02^{d}	91.93 ± 0.63^{d}	27.58 ± 0.13^{d}
B-30	2:1	446.56 ± 4.26^{b}	45.95 ± 0.52^{d}	0.25 ± 0.02^{a}	111.64 ± 0.68^{a}	51.35 ± 0.16^{a}
	1:1	428.70 ± 6.48^{d}	54.56 ± 0.24^{b}	0.22 ± 0.01^{c}	94.31 ± 0.66^{c}	50.92 ± 0.28^{b}

Values are given as mean ± standard deviation. a, b: Different lowercase letter superscripts within the same rows, indicate statistically significant differences *($p < 0.05$)* (ACC-15: Autoclave 15 min; ACC-30: Autoclave 30 min; B-15: Boiling 15 min; B-30: Boiling 30 min)

The boiling process facilitated effective protein gelation and moisture uptake, resulting in a firm yet elastic matrix. Notably, the B-15 (2:1) sample exhibited the highest hardness (458.08 g), suggesting that a shorter boiling time preserves matrix integrity and minimizes softening compared to autoclaving. High cohesiveness (0.25) and chewiness in B-30 (2:1) further indicate a robust, elastic network that resists breakdown during chewing. Conversely, autoclaved samples generally showed lower values for most texture parameters, especially chewiness and gumminess. For example, ACC-30 (1:1)

had the lowest cohesiveness (0.12) and chewiness (14.38 g.s.), reflecting a more fragile, less cohesive structure likely caused by excessive pressure and moisture dispersion leading to partial structural breakdown (Choi et al., 2025). However, ACC-30 (2:1) exhibited the highest springiness (58.77 %) among all treatments, indicating some retention of elasticity despite moderate hardness and gumminess. This suggests that autoclaving can promote resilience under certain conditions but generally reduces strength and chewiness compared to boiling. The solid-to-liquid ratio also played a significant role in texture development. Samples with a 2:1 ratio consistently outperformed those with a 1:1 ratio in terms of hardness, cohesiveness, and chewiness across both cooking methods. For instance, within the B-30 group, the 2:1 sample demonstrated higher hardness (446.56 g) and gumminess (111.64 g) than the 1:1 sample (428.70 g and 94.31 g, respectively), underscoring the reinforcing effect of higher protein concentration on network density and mechanical strength (Li et al., 2023). These findings are in agreement with previous research on plant proteins. Soy protein isolate-based high-moisture meat analogues (HMMA) subjected to thermal treatment have shown hardness values ranging from 300 to 480 g and cohesiveness between 0.20 and 0.28, depending on processing conditions (Li et al., 2023). Similarly, pea protein-based analogues boiled for 30 min exhibited chewiness values around 50 g and gumminess near 100 g, closely matching the Manila tamarind samples in this study (Nanta et al., 2021). Wheat gluten-based wraps have shown comparable trends, with boiling resulting in higher chewiness (~48 g.s) compared to pressure-cooked samples (~30 g.s) (Choi et al., 2025). Overall, boiling, especially for 30 min at a 2:1 solid-to-liquid ratio, enhances the texture of Manila tamarind wraps, yielding firm, cohesive, and chewy structures that are well-suited for food wraps or analogues. Autoclaving, while beneficial for certain elastic properties such as springiness, generally produces softer, less cohesive textures that may be preferable for softer applications or fillings. These results provide valuable guidance for the formulation and thermal processing of plant-based food products to achieve desired mechanical and sensory qualities.

7.3.1.5. Physicochemical, functional and textural attributes of the best protein spiral wrap formulation

Table 7.5 presents a comprehensive overview of the physicochemical and functional properties of Manila tamarind protein spiral wrap, highlighting its nutritional value,

processing characteristics, and potential as a functional food wrap or meat analogue. The Manila tamarind protein spiral wrap exhibits a moisture content of 56.45%, which is well within the optimal range for high-moisture plant-based foods. Such moisture levels contribute to a balance between flexibility and firmness, important for wraps and layered protein products. This finding is consistent with soy protein-based meat analogues, where moisture contents typically range from 52 to 60%, depending on processing and hydration (Choi et al., 2025), indicating that the Manila tamarind wrap performs comparably in terms of water retention.

Table 7.5: Physicochemical and functional properties of the Manila tamarind protein spiral wrap sample

Parameters		**Manila tamarind protein spiral wrap**
Moisture (%)		56.45±0.64
Protein (% d.b.)		60.28±0.16
Color Properties	*L**	49.44±0.07
	*a**	5.18±0.01
	*b**	17.15±0.04
Cooking Yield (%)		112.30±1.40
Expressible Moisture (%)		43.66±0.37

Values are given as mean ± standard deviation

The protein content was notably high at 60.28%. This exceeds the protein levels found in many traditional legume-based meat analogues, such as lentil or chickpea formulations, which generally range from 30 to 50% protein (d.b.) (Nanta et al., 2021). While soy protein isolates can reach purities above 90%, the protein content in finished products is often lower due to added moisture and binders. Therefore, the Manila tamarind protein wrap stands out as a promising source for high-protein diets and functional foods, particularly for vegan and vegetarian consumers. The color parameters (*L**, *a**, *b**) further inform the product's visual appeal. The lightness (*L**) value of 49.44 suggests a moderately dark surface, likely resulting from Maillard reactions and pigment retention during thermal processing. **Figure 7.2** shows Manila tamarind protein spiral wrap samples wound around wooden sticks rightly after formulation. The *a** (5.18) and *b**

(17.15) values indicate a reddish-yellow hue, which can be attributed to natural pigments and phenolic compounds. In comparison, wheat gluten-based films typically show *L**, *a**, and *b** values of approximately 55, 3.8, and 12.6, respectively, depending on processing conditions (Flory et al., 2023). This suggests that Manila tamarind wraps may offer a more vibrant color, potentially enhancing consumer appeal. The cooking yield of the wrap is measured at 112.30%, indicating the product absorbs water and increases in weight by about 12% during cooking. This reflects good hydration and matrix stability, though it is somewhat lower than highly absorbent analogues such as autoclaved mung bean protein, which have reported yields between 130 % and 150 % (Li et al., 2023).

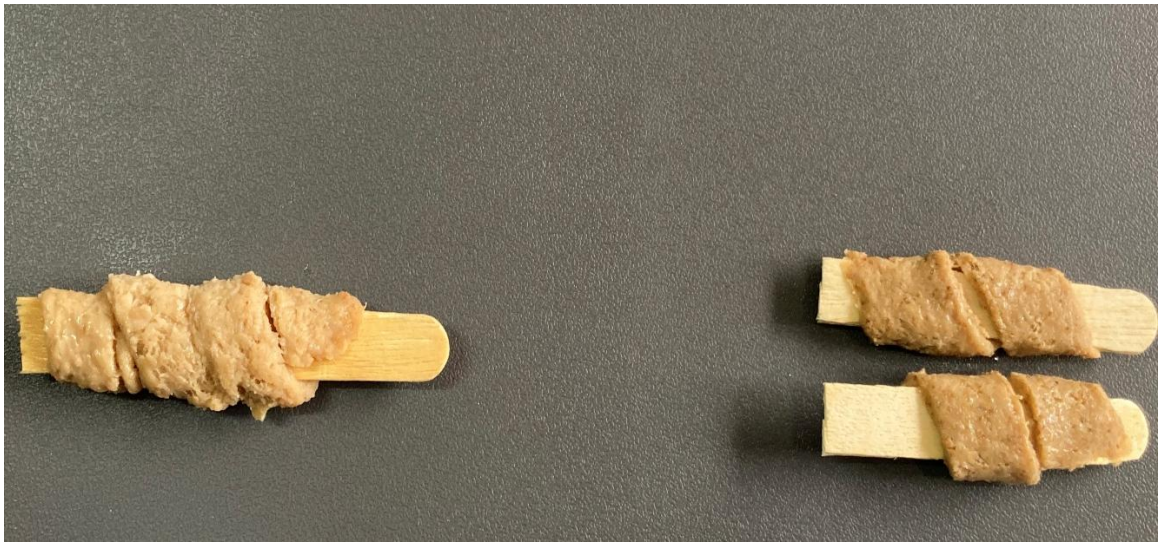


Figure 7.2 Visual appearance of the protein spiral wrap samples after preparation

Nonetheless, the observed yield demonstrated sufficient cohesion and functionality for applications where moderate swelling and low cooking losses are preferred. Expressible moisture, recorded at 43.66%, represents the proportion of water that can be extracted from the sample under pressure. This metric is important for evaluating water-holding capacity and shelf-life. A value around 43% suggests the product contains a substantial amount of loosely bound water, which can contribute to a moist, juicy texture but may also necessitate careful packaging to prevent spoilage. Similar expressible moisture values (35-45%) have been reported in pea and lentil protein-based meat analogues (Kaur & Prasad, 2022), indicating that the Manila tamarind wrap is well-suited for ready-to-eat, high-moisture applications.

Table 7.6 provides a comprehensive assessment of the textural characteristics of the Manila tamarind protein spiral wrap, highlighting its processing behavior and its suitability as a functional food wrap or meat analogue. These attributes collectively determine the wrap's structural integrity, sensory appeal, and practical utility in culinary applications.

Table 7.6: Textural properties of the Manila tamarind protein spiral wrap sample

Parameters	Manila tamarind protein spiral wrap
Hardness (g)	428.70±6.48
Springiness (%)	54.56±0.24
Cohesiveness	0.22±0.01
Resilience	0.16±0.01
Fracturability (g)	-
Adhesiveness (g.s)	90.45±0.24
Gumminess (g)	94.31±0.66
Chewiness (g.s)	50.92±0.28

Values are given as mean ± standard deviation

Texture profile analysis (TPA) revealed that the Manila tamarind protein wrap had a robust and favorable structure for plant-based meat analogue applications. The hardness was measured at 428.70 g, indicating a firm and compact matrix. This value was comparable to commercial soy-based wraps and texturized proteins, which typically show hardness values in the range of 400-500 g (Choi et al., 2025), supporting the wrap's capacity to hold and maintain its structure and shape after cooking. Springiness, reflecting the product's ability to recover its shape after compression, was 54.56%. This moderate elasticity is advantageous for wraps that require rolling or folding without cracking, and it aligned with the springiness values (50-60%) reported for wheat gluten-based analogues (Nanta et al., 2021; Flory et al., 2023). The elasticity was likely due to the interactions within the protein network and optimal hydration parameters. Cohesiveness was recorded at 0.22, indicating sufficient internal bonding to withstand

mechanical stresses during handling and consumption. This value was within the moderate range and was consistent with mung bean and lentil protein-based texturized protein products, which typically display cohesiveness values between 0.18 and 0.26 (Flory et al., 2023). Higher cohesiveness is generally associated with a stronger gel network and improved bite quality. Resilience, a measure of the sample's ability to recover after the first compression, was 0.16. Although slightly lower than some wheat-based products (which can reach up to 0.20), the value still is indicative of a matrix that maintains its shape and integrity during use. Adhesiveness was recorded as 90.45 g·s, suggesting a moderate level of stickiness. This can be advantageous for wraps, as it may help in sealing or binding layers, though excessive stickiness could pose handling challenges. Comparable adhesiveness values (70-100 g·s) have been reported for texturized proteins of soy TVPs (Li et al., 2023), placing the Manila tamarind protein spiral wrap within the desirable range for such applications.

Gumminess was measured at 94.31 g, and chewiness was recorded at 50.92 g. These values reflect the energy required to chew the wrap to a swallowable consistency, and were ideal for semi-solid products like meat analogues and protein wraps. For reference, soy-protein-based extruded analogues typically exhibit chewiness between 45-55 g and gumminess around 85-100 g (Choi et al., 2025), indicating that the Manila tamarind protein spiral wrap offers a competitive texture profile. Although fracturability was not directly reported, the combination of moderate hardness and high cohesiveness suggested that the wrap was unlikely to be brittle, supporting its use as a flexible wrap or sheet in various food applications. The Manila tamarind protein spiral wrap demonstrated a highly competitive combination of nutritional, functional, and textural properties suitable for plant-based food applications. With a high protein content (60.28% d.b.), favorable moisture retention (56.45%), good elasticity with springiness values of 54.56%, and solid mechanical resistance (hardness of 428.70 g), the wrap stands alongside leading protein-based alternatives such as soy-based, mung bean, and lentil-based products. Its cooking yield and expressible moisture lies within industry-accepted ranges, and its textural performance, particularly in chewiness and gumminess, meets consumer expectations for a satisfying mouthfeel (Kaur & Prasad, 2022). These attributes make the Manila tamarind protein wrap a strong candidate for clean-label, sustainable, and nutritionally enriched food wrap solutions in the functional food and meat substitute markets.

7.3.2. Characterization of the meat analogue nuggets

7.3.2.1.Proximate composition of standard and formulated nuggets

Nuggets are a popular processed meat product, widely consumed due to their convenience and taste. The formulation of nuggets can significantly impact their nutritional composition and cooking characteristics. **Table 7.7** gives the characteristics of the fresh, formulated and standard nuggets (used as reference). The fresh nuggets demonstrated the highest moisture content (52.95%) compared to both formulated (28.66%) and standard nuggets (30.82%), reflecting their unprocessed state and higher water activity. The formulated nuggets exhibited a lower moisture content than the standard nuggets, indicating a slightly firmer texture and potentially improved structural integrity, and enhanced crispiness on exterior of the nuggets after cooking (Kumar & Singh, 2021). Regarding fat content, fresh nuggets showed the lowest levels (10.26%), while formulated nuggets had significantly ($p<0.05$) higher fat content (17.95%) than standard nuggets (14.55%), resulting in increased flavor richness and improved mouthfeel (Yadav et al., 2020a). Protein content remained relatively consistent across all variants, with fresh nuggets containing 25.82%, formulated nuggets 27.20%, and standard nuggets 28.46%, all within acceptable ranges for fish-based products. The mineral composition (ash content) was comparable between fresh (2.45%) and formulated nuggets (2.42%), both slightly lower than standard nuggets (2.68%), suggesting variations in ingredient sources or processing techniques. pH values showed minimal variation across samples, with the following reading for fresh (6.57), formulated (6.54), and standard (6.22) nuggets.

Notably, both fresh (4.03%) and formulated nuggets (4.37%) contained significantly ($p<0.05$) higher dietary fiber content than standard nuggets (1.80%), suggesting the inclusion of fiber-rich ingredients, which can offer health benefits such as improved digestion and satiety (Yadav et al., 2020b). These factors can significantly impact protein functionality, water-holding capacity, and overall product quality, reflecting the significant changes that can be introduced through ingredient modifications (Honikel, 2008). This suggests a strategic reformulation approach aimed at balancing sensory attributes with nutritional enhancements, demonstrating how precise ingredient adjustments can simultaneously influence texture, flavor, nutritional profile, and potential health benefits of processed meat products (Kumar et al., 2021).

Table 7.7: Physico-chemical characteristics and cooking quality of the fresh, formulated and standard nuggets

Parameters	Fresh nugget	Formulated nugget (Fried at 160 ℃)	Standard nugget* (Fried at 160 ℃)
Moisture (%)	52.95 ± 0.28^{a}	28.66 ± 0.41^{c}	30.82 ± 0.80^{b}
Fat (%)	10.26 ± 0.13^{c}	17.95 ± 0.43^{a}	14.55 ± 0.22^{b}
Protein (%)	25.82 ± 0.23^{c}	27.20 ± 0.08^{b}	28.46 ± 1.12^{a}
Ash (%)	2.45 ± 0.05^{b}	2.42 ± 0.06^{b}	2.68 ± 0.09^{a}
pH	6.57 ± 0.03^{a}	6.54 ± 0.02^{a}	6.22 ± 0.02^{b}
Dietary fiber (%)	4.03 ± 0.06^{b}	4.37 ± 0.10^{a}	1.80 ± 0.05^{c}
Cooking yield (%)	0.00 ± 0.00^{c}	92.56 ± 1.05^{b}	94.91 ± 0.32^{a}
Cooking loss (%)	0.00 ± 0.00^{c}	7.44 ± 1.04^{a}	5.09 ± 0.31^{b}

Values are given as mean ± standard deviation. a, b: Different lowercase letter superscripts within the same rows, indicate statistically significant differences *($p < 0.05$)* (* signifies reference sample)

7.3.2.2.Cooking quality of the nuggets

The culinary transformation of plant-based meat products involves complex interactions between ingredients that significantly influence their cooking performance and quality characteristics. The cooking yield and cooking loss demonstrate intriguing variations that reflect underlying structural and compositional differences. The formulated nuggets exhibited a slightly lower cooking yield (92.56%) compared to the standard nuggets (94.91%), accompanied by a significantly (*$p<0.05$*) higher cooking loss (7.44%) compared to standard nuggets (5.09%) **(Table 7.7)**. Comparative studies in protein-based food systems have reported similar cooking yield variations as Barbut et al. (2005) found that moisture-protein interactions can reduce cooking yield by 2-5% depending on ingredient formulation, while Zhou et al. (2022) observed that fiber-rich ingredients can increase cooking loss up to 8% due to altered water-binding capabilities. Van Laack & Smulders, (1990) further demonstrated that protein denaturation during heating can reduce water-holding capacity by 3-6%, potentially explaining the observed cooking performance differences. These variations suggest that the formulated nuggets' compositional variations, potentially involving alternative protein sources or fiber incorporation, may compromise water and fat retention mechanisms, ultimately

influencing the product's final textural and sensory characteristics (Honikel, 2008).

7.3.2.3.Textural properties of the nuggets

Texture plays a crucial role in determining the sensory quality and consumer acceptance of plant-based meat products, reflecting the intricate interplay of ingredients and processing parameters. The comparative analysis of fresh, formulated and standard nuggets **(Table 7.8)** reveals significant differences in their textural characteristics, highlighting the impact of compositional variations.

Table 7.8: Texture profile characteristics and colour values (*L**, *a**, *b**) of the fresh, formulated and standard nuggets

Parameters	Fresh nugget	Formulated nugget (Fried at 160 °C)	Standard nugget * (Fried at 160 °C)
Hardness (g)	625.28 ± 4.24^{c}	1450.46 ± 20.25^{b}	1695.63 ± 34.61^{a}
Cohesiveness	0.51 ± 0.02^{b}	0.35 ± 0.01^{c}	0.64 ± 0.02^{a}
Springiness	0.68 ± 0.03^{c}	0.74 ± 0.01^{b}	0.91 ± 0.01^{a}
Chewiness (g.s)	216.84 ± 3.65^{c}	375.67 ± 4.48^{b}	987.53 ± 15.10^{a}
*L**	56.42 ± 0.23^{a}	50.55 ± 0.33^{b}	56.37 ± 0.30^{a}
*a**	5.94 ± 0.04^{c}	6.60 ± 0.10^{b}	12.83 ± 0.07^{a}
*b**	23.48 ± 0.36^{a}	9.15 ± 0.0^{c}	22.36 ± 0.65^{b}

Values are given as mean ± standard deviation. a, b: Different lowercase letter superscripts within the same rows, indicate statistically significant differences *($p<0.05$)* (* signifies reference sample)

Fresh nuggets exhibited intermediate hardness values (625.28 g) that were considerably lower than both formulated (1450.46g) and standard nuggets (1695.63g), reflecting their unprocessed state and higher moisture content which contributes to a softer initial texture. The formulated nuggets demonstrated significantly (*$p<0.05$*) lower hardness compared to standard nuggets, indicating a softer structural profile that can be substantially altered by ingredient substitutions. Studies by Keeton (1994) reported textural variations of up to 30% in restructured plant-based meat products based on their ingredient composition, while Barbut & Mittal (1990) found that changes in protein

content and moisture can reduce hardness by 15-25%.

Cohesiveness patterns showed fresh nuggets (0.51) maintaining moderate binding strength, while formulated nuggets exhibited significantly lower cohesiveness (0.35) compared to standard nuggets (0.64). Shan et al. (2018) demonstrated that incorporation of alternative ingredients can decrease cohesiveness by 40-50%, which parallels the current observation. Springiness values were highest in standard nuggets (0.91), followed by formulated nuggets (0.74), with fresh nuggets showing the lowest values (0.68). The chewiness parameters revealed fresh nuggets (216.84 g.s) required minimal mastication energy compared to processed variants, while formulated nuggets (375.67) and standard nuggets (987.53) showed significantly ($p<0.05$) higher values, further underscoring the profound textural transformations resulting from processing and formulation changes.

7.3.2.4.Colour properties of the nuggets

Color serves as a critical sensory attribute that significantly influences consumer perception and acceptance of meat products, acting as a visual indicator of ingredient composition and processing techniques. Significant ($p<0.05$) chromatic variations were observed across lightness (L^*), redness (a^*), and yellowness (b^*) parameters during the analysis of the fresh, formulated and standard nuggets **(Table 7.8)**. The fresh nuggets exhibited intermediate lightness values (56.42) that were comparable to standard nuggets (56.37) but significantly ($p<0.05$) higher than formulated nuggets (50.55), indicating that processing modifications result in darker appearance compared to the natural fresh state. The formulated nuggets displayed significantly ($p<0.05$) lower L^* value compared to standard nuggets, indicating a slightly darker appearance. The observed lower L^* value for the formulated nuggets suggests a complex interplay of factors influencing product coloration. According to Xiong et al. (2022), ingredient modifications such as reduced moisture and incorporation of spice mixes can significantly alter light reflectance properties, potentially causing darker surface appearance. Kumar et al. (2023) further substantiate this phenomenon, demonstrating that herb and spice incorporation can decrease lightness by 5-8% due to pigment interactions and modified protein-ingredient matrix, ultimately impacting the visual characteristics of meat-based products. Regarding redness, fresh nuggets showed the lowest a^* value (5.94), while formulated nuggets (6.60) remained relatively close to fresh samples, though both were substantially lower

than standard nuggets (12.83), probably due to ingredient substitutions. The most dramatic variation was evident in the *b** value, where fresh nuggets demonstrated the highest yellowness (23.48), closely resembling their natural state, while standard nuggets measured 22.36 and formulated nuggets showed dramatically reduced yellowness (9.15), a change that Sakai et al. (2022) suggest could result from modifications in fat content, spice composition, or protein matrix. These significant color variations not only reflect the intricate biochemical changes induced by formulation adjustments but also underscore the complex interplay between ingredient composition and visual sensory attributes that ultimately shape consumer perception and product acceptability.

7.3.2.5.Sensory evaluation of the nuggets

Sensory evaluation serves as a critical determinant of consumer acceptance and product success in the food industry, providing insights into the important perceptual experiences of novel food formulations. The hexagonal radar chart **(Figure 7.3)** gives a detailed sensory evaluation comparing standard and formulated nuggets across six critical quality attributes, namely appearance, color, taste, flavor, texture, and overall acceptability. Each axis represents a sensory parameter rated on a scale from 0 to 9, with higher values indicating better performance. Remarkably, the two nugget types demonstrated almost identical profiles, suggesting that the formulation modifications have not significantly compromised the sensory qualities. The chart revealed near-perfect alignment across all parameters, with both standard and formulated nuggets scoring approximately 7-8 out of 9 for most attributes. This consistency indicates that the formulated nugget successfully maintains the sensory characteristics of the standard nugget, which is crucial in food product development. The uniformity across appearance, color, taste, flavor, texture, and overall acceptability suggests that the innovative formulation has achieved an optimal balance, potentially offering enhanced nutritional or technological benefits without sacrificing the sensory experience that consumers expect from chicken nuggets.

Color variations for formulated nuggets (7.2) and standard nuggets (7.0) suggest that slightly darker appearance of the formulated nuggets and potential use of spice mixes or herbs marginally enhanced visual appeal of the nuggets (**Figure 7.4**). Findings by Sharma et al. (2024) demonstrated that ingredient modifications can subtly influence product visual appeal and attractiveness.

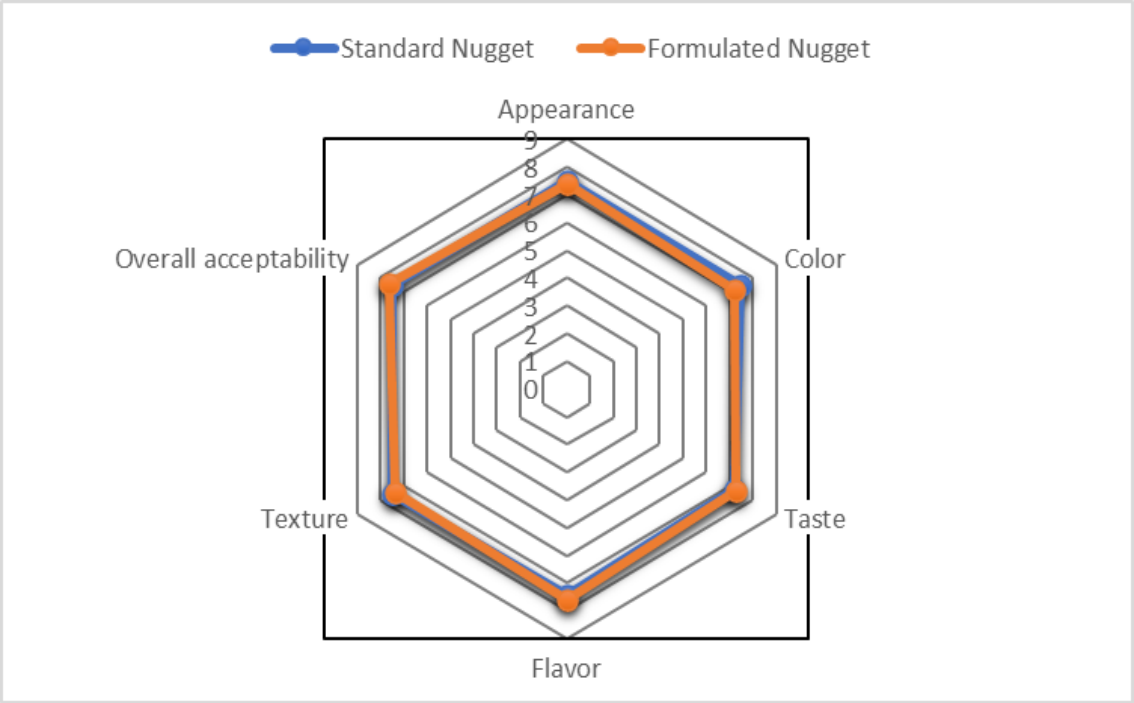


Figure 7.3: Sensory analysis of the formulated and standard nuggets

Taste differences of the formulated nuggets (7.3) and standard nuggets (7.2) indicated that the potential incorporation of dietary fiber or alternative ingredients in the formulated nuggets may have introduced significant flavor complexity, which aligned with the research of with Kumar et al. (2023) on how strategic ingredient substitutions can modulate taste perception. Flavor scores of the formulated nuggets (7.6) and standard nuggets (7.4) reveal that the formulated nuggets achieved a more pronounced flavor profile, potentially resulting from optimized fat content and ingredient composition, which can significantly impact overall flavor experience in processed meat products. Research by Sharma et al. (2022) demonstrated that strategic ingredient modifications, such as dietary fiber incorporation and alternative fat sourcing, can significantly influence flavor complexity and taste perception. Stone et al. (2024) have consistently argued that even minimal sensory score differences of 0.1-0.3 can represent meaningful consumer preference shifts, particularly in processed meat products. The near-identical texture and appearance scores suggest that the formulation changes maintained critical sensory characteristics while introducing nuanced flavor improvements, a finding supported by Kumar et al. (2020), who emphasized that targeted

ingredient interventions can enhance product attributes without compromising core sensory qualities. The marginally higher overall acceptability of formulated nuggets (7.6) compared to standard nuggets (7.5) substantiates the potential of strategic reformulation to optimize consumer perception, indicating that carefully selected ingredient modifications can potentially create more appealing food products without radical alterations to established sensory profiles.

Figure 7.4 illustrates the distinct visual characteristics of three different nugget samples at various processing stages. The fresh nuggets **(Figure 7.4 A)** display a pale, uncooked appearance with a light beige coloration and visible texture variations, representing the raw product before any thermal treatment. The surface appears moist and unprocessed, typical of fresh meat analogue-based products. The formulated nuggets after deep fat frying at 160 °C for 90 s **(Figure 7.4 B)** exhibited a darker, more irregular surface with a cooked appearance, showing browning effects from the frying process. The texture appears more compact and the color has deepened significantly compared to the fresh sample. The standard nuggets after identical frying conditions **(Figure 7.4 C)** demonstrated a more uniform, golden-brown exterior characteristic of commercially processed products. They appeared more evenly shaped and displayed the typical crispy, golden coating expected from deep-fried nuggets. The visual comparison clearly showcased how processing conditions and formulation differences impact the final appearance, with standard nuggets achieving a more conventional and aesthetically appealing fried food appearance compared to the formulated variant. Color difference between the formulated nuggets (fresh and fried) and standard nuggets suggest that the formulated nuggets had a slightly darker appearance than the standard nuggets, potentially due to the use of spice mixes or herbs and non-usage of additives like carboxy-methyl cellulose (CMC) which can enhance the lightness value of products in which they are added (**Figure 7.4**). Findings by Sharma et al. (2024) demonstrated that ingredient modifications can subtly influence product visual appeal and attractiveness, revealing that even minor alterations in formulation components such as protein sources, colorants, or binding agents can significantly impact consumer perception and purchase intent. These modifications affect critical visual parameters including color intensity, surface texture, marbling patterns, and overall appearance that closely mimic traditional meat products, which are essential factors in consumer acceptance of plant-based alternatives (Sharma et al., 2024).

Figure 7.4: Appearance of the nugget samples; fresh nuggets (A); formulated nuggets after deep fat frying at 160 ℃ for 90 s (B); standard nuggets* after deep fat frying at 160 ℃ for 90 s (C) (* signifies reference sample)

7.3.3. Shelf-life study of stored nugget samples

7.3.3.1.Effect of storage on weight

Storage stability is a critical parameter in evaluating the quality and shelf life of processed products, providing insights into their long-term preservation characteristics. **Table 7.9** shows the weight changes of formulated and standard nuggets over a 50-day storage period, revealing subtle variations in weight retention and product stability. The graph demonstrates a progressive weight reduction for both formulated and standard nuggets across different storage intervals. As storage progressed, a gradual weight decline was evident, characterized by distinct patterns. Comparative studies by Purohit et al. (2015) suggest that such weight variations can result from moisture loss, oxidative processes, and ingredient-specific moisture retention capabilities. Zhou et al. (2015) reported weight reductions of 2-5% in meat products during extended storage, which aligns with the observed trends of the study. The most pronounced weight differences emerged around the 40 and 50-day storage period, where formulated nuggets demonstrated a more pronounced weight reduction compared to standard nuggets, potentially indicating differences in moisture-binding properties or ingredient interactions. These variations highlight the complex interplay between ingredient composition, storage conditions, and product stability, underscoring the importance of

strategic formulation in maintaining product quality during extended storage periods.

7.3.3.2.Effect of storage on moisture, fat, ash and pH

The variations in physicochemical parameters during frozen storage emerge from a complex interplay of molecular interactions, ingredient composition, and biochemical transformations that reflect the subtle responses of food systems to prolonged preservation conditions. The variations in physicochemical parameters during frozen storage emerge from a complex interplay of molecular interactions, ingredient composition, and biochemical transformations that reflect the nuanced responses of food systems to prolonged preservation conditions. **Table 7.9** gives detailed about moisture, fat, ash and pH changes observed in fresh, formulated and standard nuggets during frozen storage over a period of 50 days. Fresh nuggets demonstrated exceptional storage stability across all parameters, with moisture content showing minimal decline from 52.95% to 52.76% over 50 days, representing only a marginal reduction compared to more substantial losses in processed variants. This superior water retention in fresh samples can be attributed to intact cellular structures and unmodified protein matrices that maintain optimal hydration levels. Weight loss patterns further corroborated this stability, with fresh nuggets experiencing minimal dehydration (10.08 g to 10.02 g), indicating effective preservation of structural integrity under frozen conditions. Moisture content variations can be attributed to differential water-binding capabilities arising from protein matrix configurations, fiber content, and hydrophilic-hydrophobic interactions, with formulated nuggets exhibiting lower baseline moisture levels (28.66% to 28.15%) and more pronounced deterioration potentially due to alternative ingredient structures that modify water retention mechanisms, a phenomenon substantiated by Yadav et al. (2020a) who demonstrated how ingredient-specific molecular arrangements significantly influence water dynamics. Fat content dynamics represent another critical domain of transformation, where fresh nuggets maintained remarkable stability with minimal fluctuation (10.26% to 10.16%), demonstrating inherent resistance to lipid degradation due to lower initial fat content and natural antioxidant presence in unprocessed fish tissue. In contrast, both formulated and standard nuggets, with their higher fat contents (17.95% and 16.55% respectively), showed greater susceptibility to storage-induced changes. Lipid oxidation processes, protein-lipid interactions, and antioxidant variability contribute to observed changes in processed samples, with formulated nuggets showing

more pronounced fat content decline (17.95% to 17.46%), a characteristic that Barbut et al. (2005) explained in their results from complex oxidative mechanisms and differential molecular stability of lipid components under frozen conditions. The higher fat content in processed nuggets creates more opportunities for oxidative rancidity, despite frozen storage conditions that typically slow such reactions (Zhou et al., 2015). The remarkable stability of ash content across all nugget types indicates that calcium, phosphorus, sodium, and other essential minerals remain structurally bound within the protein matrix, indicating that the mineral structural integrity remains largely unaltered by preservation techniques. The consistent ash levels across fresh, formulated (2.42% to 2.40%), and standard nuggets (2.68% to 2.67%) demonstrate that frozen storage effectively preserves the nutritional mineral profile regardless of processing modifications (Barbut et al., 2005). pH value modifications revealed the most distinct differences between sample types, with fresh nuggets maintaining excellent stability (6.57 to 6.56) throughout the 50-day period, demonstrating minimal biochemical activity and optimal preservation conditions. Conversely, processed variants exhibited more pronounced changes characterized by a subtle yet consistent decline, with formulated nuggets showing greater pH reduction (6.54 to 6.48) compared to standard nuggets (6.22 to 6.17), reflecting underlying proteolytic enzyme activities, gradual protein denaturation, and the accumulation of metabolic byproducts. This differential response suggests that processing modifications may activate enzymatic pathways or create conditions conducive to protein breakdown, a process Honikel (2008) identified as a characteristic biochemical response in meat systems during extended storage. The maintained pH stability in fresh nuggets indicates better preservation of protein structure and reduced enzymatic activity, contributing to overall product quality retention. These variations highlight the intricate biochemical landscape of frozen meat products, demonstrating how processing techniques, ingredient composition, and storage conditions orchestrate a sophisticated molecular dance that ultimately determines product quality, stability, and sensory characteristics (Reddy et al., 2017). Fresh nuggets consistently outperformed processed variants in maintaining their original characteristics, suggesting that minimal processing preserves natural stability mechanisms inherent in fish tissue.

Table 7.9: Changes in the characteristics of fresh, formulated and standard nuggets during frozen storage

Parameter	Treatment	Storage period (Days)					
		0	**10**	**20**	**30**	**40**	**50**
Weight (g)	Fresh nugget	10.08 ± 0.0^{a}	10.08 ± 0.0^{a}	10.06 ± 0.0^{b}	10.05 ± 0.0^{c}	10.03 ± 0.0^{d}	10.02 ± 0.0^{e}
	Formulated nugget	9.25 ± 0.06^{a}	9.24 ± 0.05^{a}	9.24 ± 0.05^{a}	9.23 ± 0.03^{a}	9.21 ± 0.07^{a}	9.20 ± 0.05^{a}
	Standard nugget*	11.38 ± 0.08^{a}	11.37 ± 0.12^{a}	11.36 ± 0.09^{a}	11.34 ± 0.10^{a}	11.33 ± 0.08^{a}	11.32 ± 0.10^{a}
Moisture (%)	Fresh nugget	52.95 ± 0.28^{aA}	52.89 ± 0.21^{aA}	52.86 ± 0.24^{aA}	52.82 ± 0.18^{aA}	52.79 ± 0.23^{aA}	52.76 ± 0.20^{aA}
	Formulated nugget	28.66 ± 0.41^{aC}	28.58 ± 0.52^{aC}	28.52 ± 0.48^{aC}	28.42 ± 0.63^{aC}	28.28 ± 0.50^{aC}	28.15 ± 0.38^{aC}
	Standard nugget*	30.82 ± 0.80^{aB}	30.73 ± 0.66^{aB}	30.66 ± 0.72^{aB}	30.57 ± 0.68^{aB}	30.43 ± 0.74^{aB}	30.36 ± 0.55^{aB}
Fat (%)	Fresh nugget	10.26 ± 0.13^{aC}	10.21 ± 0.07^{aC}	10.23 ± 0.12^{aC}	10.19 ± 0.09^{aC}	10.18 ± 0.10^{aC}	10.16 ± 0.15^{aC}
	Formulated nugget	17.95 ± 0.43^{aA}	17.91 ± 0.35^{aA}	17.82 ± 0.34^{aA}	17.70 ± 0.26^{bA}	17.54 ± 0.22^{aA}	17.46 ± 0.27^{aA}
	Standard nugget*	16.55 ± 0.22^{aB}	16.52 ± 0.36^{aB}	16.46 ± 0.31^{aB}	16.42 ± 0.29^{aB}	16.38 ± 0.32^{aB}	16.34 ± 0.38^{aB}
Ash (%)	Fresh nugget	2.45 ± 0.05^{aBC}	2.45 ± 0.01^{aB}	2.45 ± 0.01^{aB}	2.45 ± 0.01^{aB}	2.45 ± 0.01^{aB}	2.45 ± 0.01^{aB}
	Formulated nugget	2.42 ± 0.02^{aC}	2.42 ± 0.01^{aC}	2.41 ± 0.01^{aC}	2.41 ± 0.01^{aC}	2.41 ± 0.01^{aC}	2.40 ± 0.01^{aC}
	Standard nugget*	2.68 ± 0.01^{aA}	2.68 ± 0.01^{aA}	2.68 ± 0.00^{aA}	2.67 ± 0.01^{aA}	2.67 ± 0.00^{aA}	2.67 ± 0.00^{aA}
pH	Fresh nugget	6.57 ± 0.03^{aA}	6.57 ± 0.01^{aA}	6.57 ± 0.01^{aA}	6.56 ± 0.01^{aA}	6.56 ± 0.01^{aA}	6.56 ± 0.01^{aA}
	Formulated nugget	6.54 ± 0.02^{aA}	6.54 ± 0.02^{aB}	6.51 ± 0.01^{aB}	6.50 ± 0.01^{aB}	6.48 ± 0.03^{aB}	6.48 ± 0.01^{aB}
	Standard nugget*	6.22 ± 0.02^{aB}	6.22 ± 0.02^{aC}	6.21 ± 0.03^{aC}	6.19 ± 0.02^{aC}	6.18 ± 0.01^{aC}	6.17 ± 0.01^{aC}

Values are given as mean ± standard deviation. a, b, A, B: Different lowercase and uppercase letter superscripts within the same rows and columns, indicate statistically significant differences *($p<0.05$);* * Signifies reference sample

7.3.3.3.Texture profile analysis (TPA)

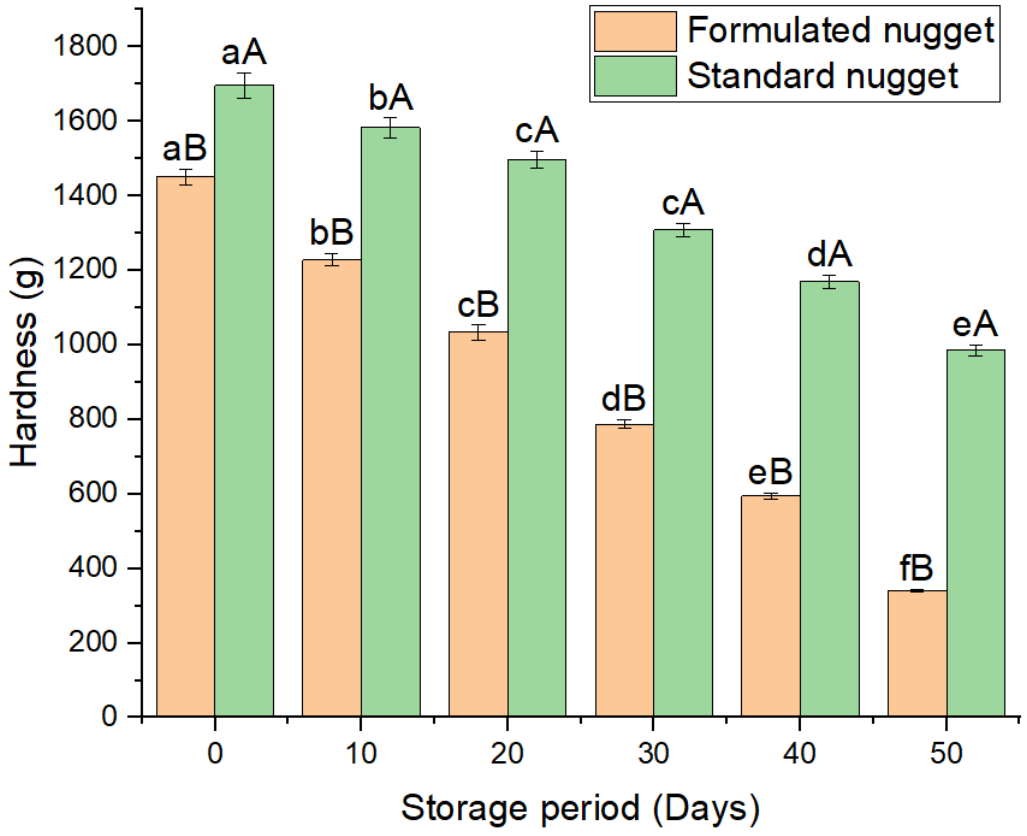


Figure 7.5: Effect of storage on hardness of the formulated and standard nuggets

(Values are given as mean ± standard deviation (SD). a, b, c, d, e, f, A, B: Different lowercase and uppercase letter superscripts indicate statistically significant differences *($p < 0.05$)*)

Textural properties play a crucial role in determining the quality and consumer acceptance of processed meat products, with hardness being a key indicator of structural integrity and sensory characteristics. The hardness analysis demonstrated a progressive decline for both formulated and standard nuggets, with distinct patterns of textural transformation **(Figure 7.5)**. At the initial storage point (0 days), standard nuggets exhibited significantly higher hardness (1695.63 g) compared to formulated nuggets (1450.46 g), a trend consistent with research by Reddy et al. (2017) highlighting ingredient-specific textural variations. Zhou et al. (2015) suggested that hardness reductions of 20-30% during frozen storage are typical in meat products, which aligns with the observed trends. The formulated nuggets consistently showed lower hardness values across all storage intervals, with a more pronounced decline observed between 30-

50 days, potentially attributed to protein denaturation and moisture redistribution. Comparative studies by Barbut et al., (2005) indicate that such textural changes result from multiple interconnected mechanisms involving protein structure modifications, water migration, lipid oxidation, and molecular rearrangements during frozen storage. The variations in hardness stem from complex physicochemical processes including protein structural transformations (such as muscle protein denaturation and altered molecular configurations), moisture redistribution (involving ice crystal formation and water migration), lipid oxidation processes, ingredient-specific interactions, and ongoing biochemical mechanisms like enzymatic activities and proteolytic modifications. These intricate interactions demonstrate the sophisticated molecular landscape of frozen meat products, highlighting how ingredient composition, processing techniques, and storage conditions collaboratively influence textural characteristics through significant biochemical transformations.

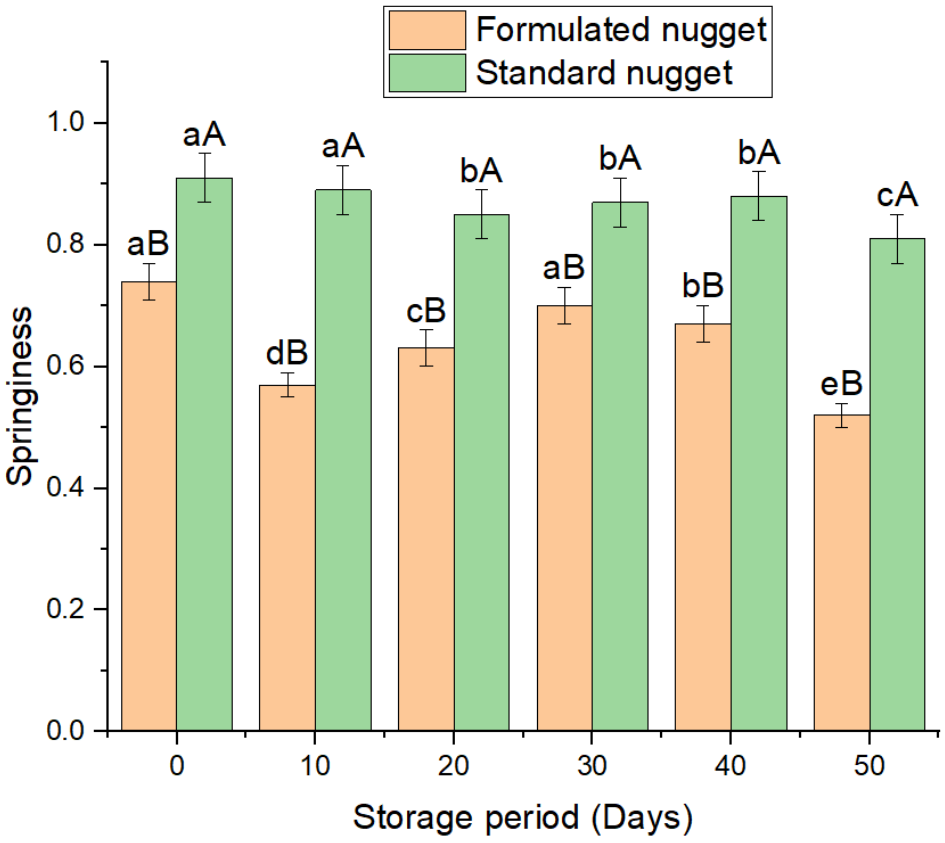


Figure 7.6: Effect of storage on springiness of the formulated and standard nuggets

(Values are given as mean ± standard deviation (SD). a, b, c, d, e, A, B: Different lowercase and uppercase letter superscripts indicate statistically significant differences *($p < 0.05$)*)

Figure 7.6 represents the textural evolution, specifically investigating the springiness of formulated and standard nuggets across a 50-day storage trajectory. The systematic decline and fluctuation in springiness reveal intricate physicochemical transformations inherent in food systems. Texture modifications during storage are fundamentally governed by molecular interactions and environmental conditions, as emphasized by Neder-Suárez et al. (2024), who demonstrated that protein conformational changes and moisture redistribution significantly impact food microstructure. The observed springiness variations can be attributed to multifaceted mechanisms. Protein denaturation leads to structural reconfiguration (Roger et al., 2025), while lipid oxidation triggers cross-linking and network modifications (Asyrul-Izhar et al., 2021). Moisture migration plays a critical role in texture alterations, with hydrocolloid interactions and water molecule redistribution causing microstructural transformations (Wang et al., 2018). The formulated nuggets exhibit more pronounced textural sensitivity, potentially due to unique ingredient compositions and intermolecular interactions that destabilize the initial protein-water-lipid matrix, leading to accelerated degradation of structural integrity over time (Rahman et al., 2021; Liu et al., 2023).

This heightened sensitivity can be attributed to the complex interplay between plant proteins, hydrocolloids, and lipid components that may lack the stabilizing mechanisms present in conventional formulations, resulting in weaker gel networks and reduced resistance to moisture migration and protein aggregation (Kyriakopoulou et al., 2019). Standard nuggets demonstrated relative stability throughout the storage period, suggesting more robust ingredient interactions and potentially superior formulation design that effectively maintains the three-dimensional protein matrix through optimized cross-linking and water-binding capacity (McClements & Grossmann, 2021). The enhanced stability of standard formulations may result from established protein-protein interactions, balanced hydrophilic-hydrophobic ratios, and the presence of stabilizing agents that prevent structural collapse and maintain textural properties (Liu et al., 2023). This temporal evolution demonstrates how storage conditions influence the kinetics of molecular interactions, leading to time-dependent modifications in elasticity and deformation recovery that directly impact consumer perception of product quality (Rahman et al., 2021). Understanding these dynamic changes is crucial for developing plant-based meat alternatives that maintain consistent quality attributes throughout their shelf life, ensuring consumer satisfaction and market viability (Roger et al., 2025).

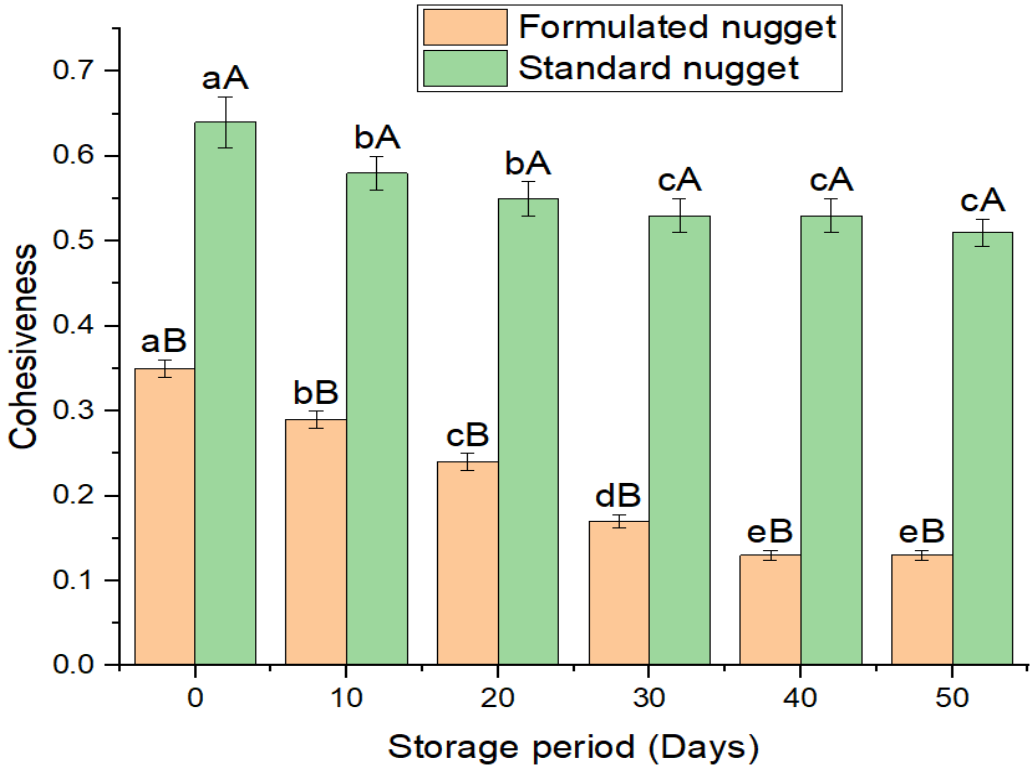


Figure 7.7: Effect of storage on cohesiveness of the formulated and standard nuggets

(Values are given as mean ± standard deviation (SD). a, b, c, A, B: Different lowercase and uppercase letter superscripts indicate statistically significant differences *($p < 0.05$)*)

Food texture is a critical quality parameter that evolves dynamically during storage, reflecting complex molecular interactions and structural changes. **Figure 7.7** showcases the textural attribute cohesiveness of formulated and standard nuggets over a 50-day storage period, revealing intricate variations in textural properties. The graph **(Figure 7.7)** demonstrates a systematic decline in cohesiveness for both formulated and standard nuggets, with distinct patterns of change across storage intervals. Standard nuggets consistently exhibited significantly (*$p<0.05$*) higher cohesiveness compared to formulated nuggets, ranging from approximately 0.64 at 0 days to 0.52 at 50 days, while formulated nuggets show a more pronounced reduction from 0.37 to 0.15 (Chen et al., 2020). The cohesiveness analysis unveils significant (*$p<0.05$*) textural variations between formulated and standard nuggets during storage. Standard nuggets initially demonstrate superior structural integrity, revealing inherent differences in ingredient composition. Both nugget types experience a non-linear cohesiveness decline, with formulated nuggets showing accelerated degradation between 30-50 days.

This pattern suggests the formulated nuggets are more vulnerable to molecular rearrangements and structural breakdown, highlighting the complex interactions between ingredient formulation, storage conditions, and food quality maintenance. These variations can be attributed to multiple physicochemical mechanisms, including protein denaturation, moisture migration, lipid oxidation, and molecular rearrangements that destabilize the food matrix (Wang et al., 2018). The progressive changes reflect complex interactions between ingredients, highlighting the dynamic nature of food microstructure during extended storage periods.

The chewiness of food products represents a critical textural attribute that profoundly influences consumer perception and product quality (Bourne, 2002). Examining the comparative chewiness of formulated and standard nuggets reveals a complex narrative of structural degradation during extended storage period of 50 days **(Figure 7.8)**. Standard nuggets demonstrated remarkable textural resilience, maintaining significantly ($p<0.05$) higher chewiness values compared to formulated nuggets throughout the 50-day storage period. The divergent chewiness trajectories reflect intricate molecular interactions and structural transformations inherent in food systems, where the mechanical resistance to mastication serves as a comprehensive indicator of protein matrix integrity, cross-linking density, and overall structural cohesion (Kumar & Singh, 2021). At the initial storage point, standard nuggets exhibited approximately 1000 g·s of chewiness, more than doubling the formulated nuggets' 400 g·s, indicating fundamental differences in ingredient composition and protein network stability that directly influence the energy required for mechanical breakdown during consumption (Chen et al., 2020). This substantial disparity suggests that standard formulations possess more robust protein-protein interactions, enhanced gel strength, and superior binding mechanisms that create a denser, more cohesive matrix capable of withstanding greater deformation forces (McClements & Grossmann, 2021). The lower chewiness values in formulated nuggets may result from weaker intermolecular bonds, reduced protein cross-linking, or suboptimal hydrocolloid integration that compromises the structural framework and decreases resistance to mechanical stress (Kyriakopoulou et al., 2019).

The progressive decline in chewiness follows a non-linear pattern, with formulated nuggets experiencing a more dramatic textural breakdown. By day 20, standard nuggets retained approximately 700 g·s of chewiness, while formulated nuggets dramatically

reduced to around 150 g·s. The terminal storage points at day 50 clearly illustrated this divergence, with standard nuggets maintaining roughly 400 g·s of chewiness compared to the formulated nuggets' near-negligible 20 g·s. These variations stem from multifaceted physicochemical mechanisms, including protein denaturation, moisture redistribution, lipid oxidation, and molecular rearrangements (Wang et al., 2018). The accelerated textural degradation of the formulated nuggets suggests a more vulnerable protein matrix, potentially resulting from alternative ingredient compositions or less robust binding mechanisms that compromise structural integrity during storage (Rodriguez-Martinez et al., 2021).

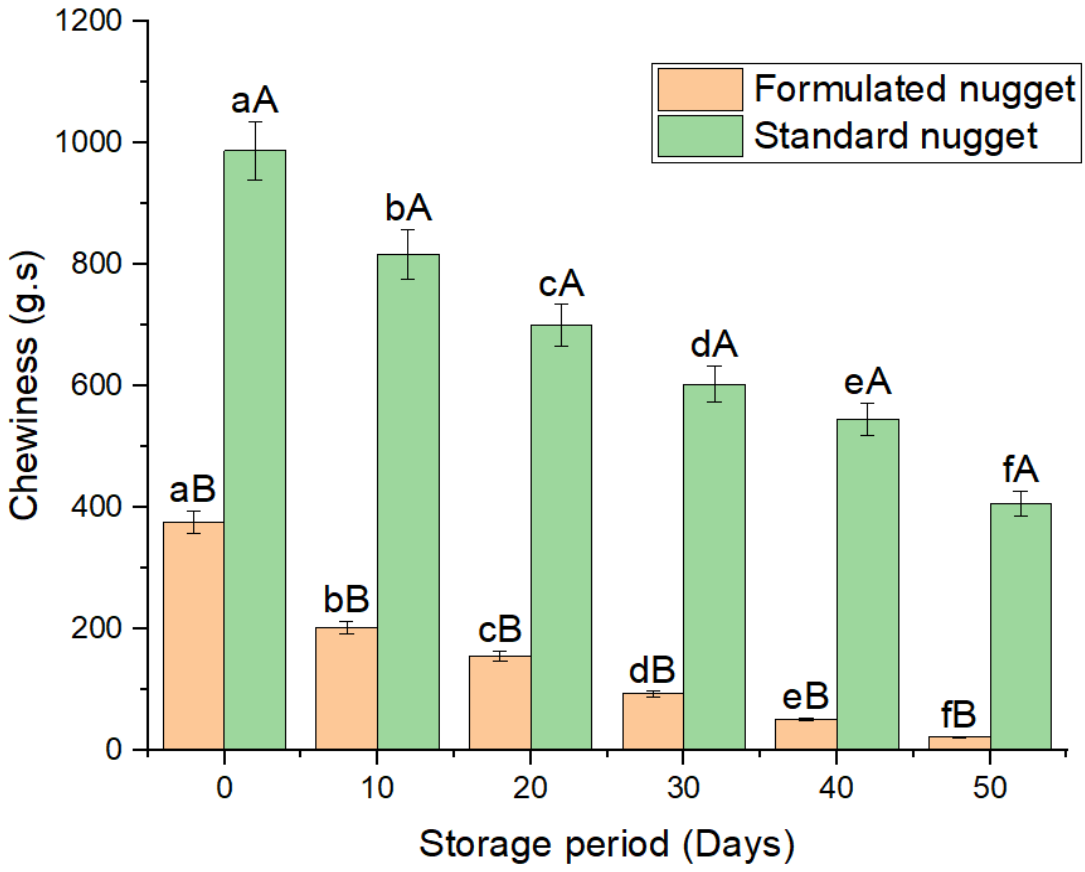


Figure 7.8: Effect of storage on chewiness of the formulated and standard nuggets

(Values are given as mean ± standard deviation (SD). a, b, c, d, e, f, A, B: Different lowercase and uppercase letter superscripts indicate statistically significant differences *($p < 0.05$)*)

7.3.3.4.Measurement of colour parameters

Food quality and preservation are critical aspects of food processing and storage,

particularly for protein-based products like nuggets. This study investigated the changes in lightness (*L** value) of standard and formulated nuggets over a 50-day storage period **(Figure 7.9)**. Initially, both standard and formulated nuggets start with similar *L** values of around 50-55, indicating comparable initial color characteristics, with the formulated nugget showing a bit darker appearance probably due to better crust formation (Chen et al., 2020). Both nugget types exhibited gradual increases in *L** values throughout the storage period, suggesting potential biochemical and structural modifications. The rate of *L** value change varied between standard and formulated nuggets, with subtle but significant ($p<0.05$) differences across storage intervals. By day 50, both nugget types reached their highest *L** values, with formulated nuggets showing slightly lower values compared to the standard nuggets.

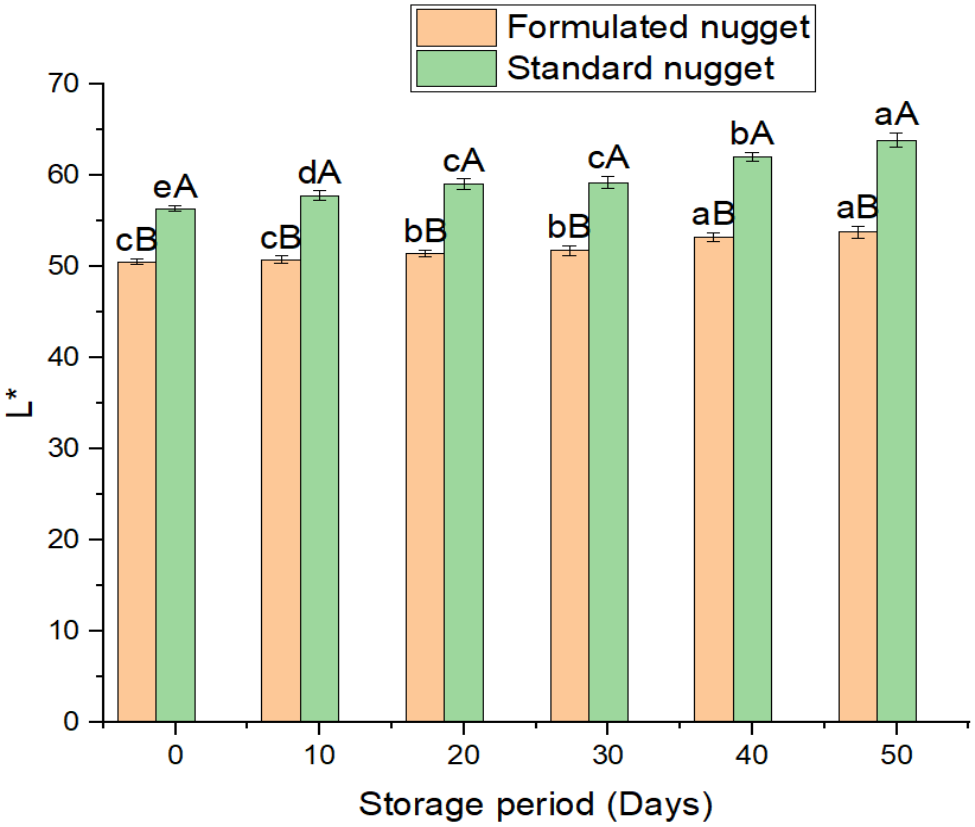


Figure 7.9: Effect of storage on lightness value (*L**) of the formulated and standard nuggets

(Values are given as mean ± standard deviation (SD). a, b, c, A, B: Different lowercase and uppercase letter superscripts indicate statistically significant differences ($p < 0.05$))

The variations in *L** values can be attributed to several factors, including oxidative

processes affecting protein and lipid structures (Bourne, 2002), moisture content changes during storage, Maillard reaction progression, and potential antioxidant or stabilizing effects in formulated nuggets, indicating complex interactions during prolonged storage (Wang et al., 2018). These findings highlight the importance of understanding color dynamics in processed plant-based meat products, providing valuable insights into quality maintenance and potential shelf-life optimization strategies. The comprehensive analysis demonstrates the significant changes that occurred in the nuggets during extended storage, offering valuable information for food scientists, manufacturers, and quality control professionals (Chen et al., 2020).

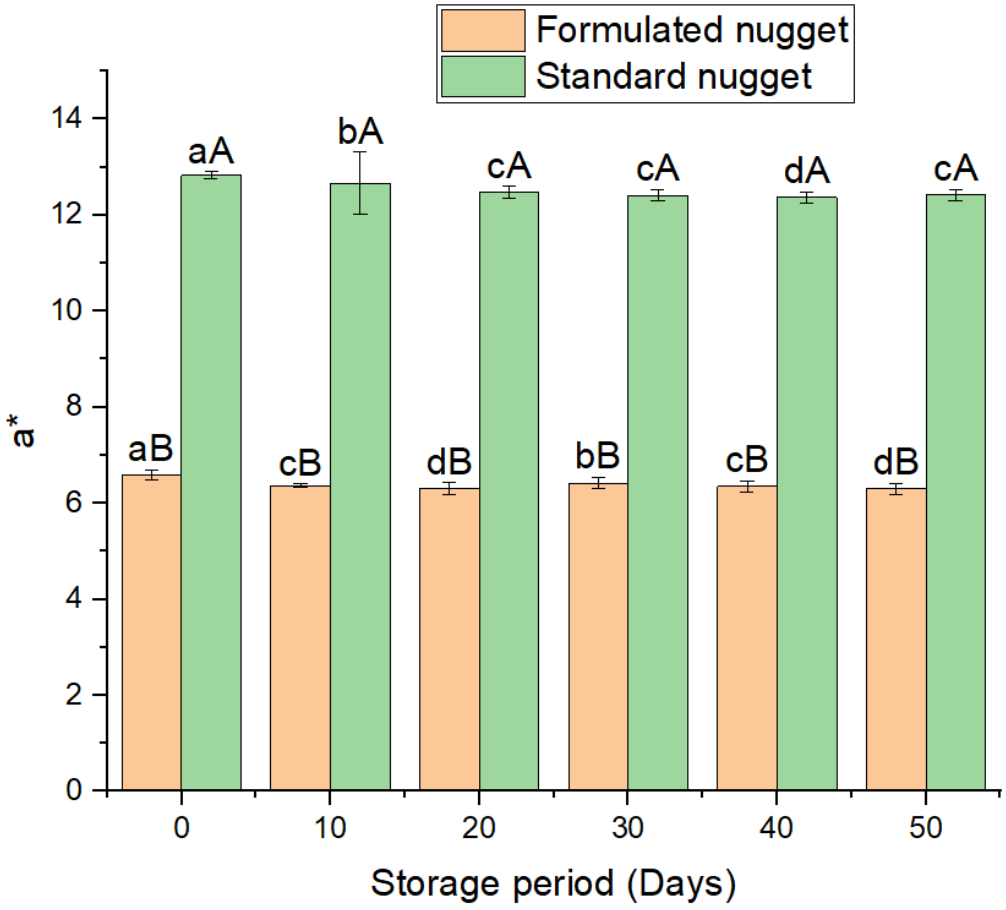


Figure 7.10: Effect of storage on redness/greenness value (*a**) of the formulated and standard nuggets

(Values are given as mean ± standard deviation (SD). a, b, c, d, A, B: Different lowercase and uppercase letter superscripts indicate statistically significant differences *($p < 0.05$)*)

Color serves as an important indicator of freshness, processing, and potential chemical

changes in processed food products (Garcia et al., 2024). This research investigated the fascinating evolution of the *a** value in standard and formulated chicken nuggets over a 50-day storage period, revealing complex chromatic transformations that have significant implications for food quality assessment **(Figure 7.10)**. The comparative analysis revealed notable differences between standard and formulated nuggets from the initial storage period, with a substantial disparity existing at day 0 between standard nuggets (*a** value around 12.5) and formulated nuggets (*a** value around 6.5), suggesting distinct initial processing characteristics or ingredient variations (Baig et al., 2025). Throughout the 50-day storage period, both nugget types demonstrated a consistent pattern of *a** value changes, characterized by subtle but statistically significant ($p<0.05$) variations. The most pronounced color modifications occurred within the initial 20 days of storage, indicating a critical window for quality assessment. As observed during the study, standard nuggets maintained a higher *a** value throughout the storage period, suggesting more pronounced reddish characteristics, while formulated nuggets showed more consistent color stability with less dramatic *a** value fluctuations. Both nugget types experienced gradual color modifications, likely attributed to lipid oxidation and Maillard reaction processes, with significant color changes most evident between days 0-20 and minimal variations in later storage periods. The underlying mechanisms driving these color dynamics could be multifaceted, including Maillard reaction, lipid oxidation, protein denaturation, and potential antioxidant interactions specific to the formulated nugget composition (Sánchez-García et al., 2024). These findings highlight the color dynamics of processed meat products, offering valuable information for food scientists, manufacturers, and quality control professionals. The research underscores the importance of understanding color progression as a key indicator of food quality, shelf-life prediction, and consumer perception (Rodriguez-Martinez et al., 2021).

Table 7.10 presents the color parameter changes in fresh nugget samples over a 50-day frozen storage period. The *L** values, representing lightness, showed a progressive increase from 56.42 at day 0 to 57.94 at day 50, indicating that the nuggets became slightly lighter during storage. This trend suggested gradual moisture migration leading to surface becoming lighter in colour, which is consistent with typical frozen storage effects. The *a** values, measuring red-green chromaticity, demonstrated a decreasing trend from 5.94 initially to 4.72 after 50 days, indicating a reduction in redness intensity. Similarly, *b** values, representing yellow-blue chromaticity, declined from 23.48 to

22.12, showing decreased yellowness over time. These findings aligned with previous research on frozen meat products. Studies on frozen chicken nuggets have reported similar *L** value increases of 1-2 units during extended storage, attributed to ice crystal formation and protein denaturation (Rahman et al., 2021). Comparable research showed *a** value reductions of 15-20% in frozen poultry products over 45-60 days, primarily due to myoglobin oxidation (Henriott et al., 2020). The observed *b** value decline aligned with the findings by Liu et al. (2023), who reported 6-8% decreases in yellowness parameters in frozen breaded products after 8 weeks of storage at -18 °C. The relatively small magnitude of these changes suggested that the visual differences would be minimal to consumers, supporting the product's commercial viability for extended frozen storage while maintaining acceptable aesthetic quality standards typical in the frozen food industry.

Table 7.10: Colour value parameters of the fresh nugget samples during 50 days of storage

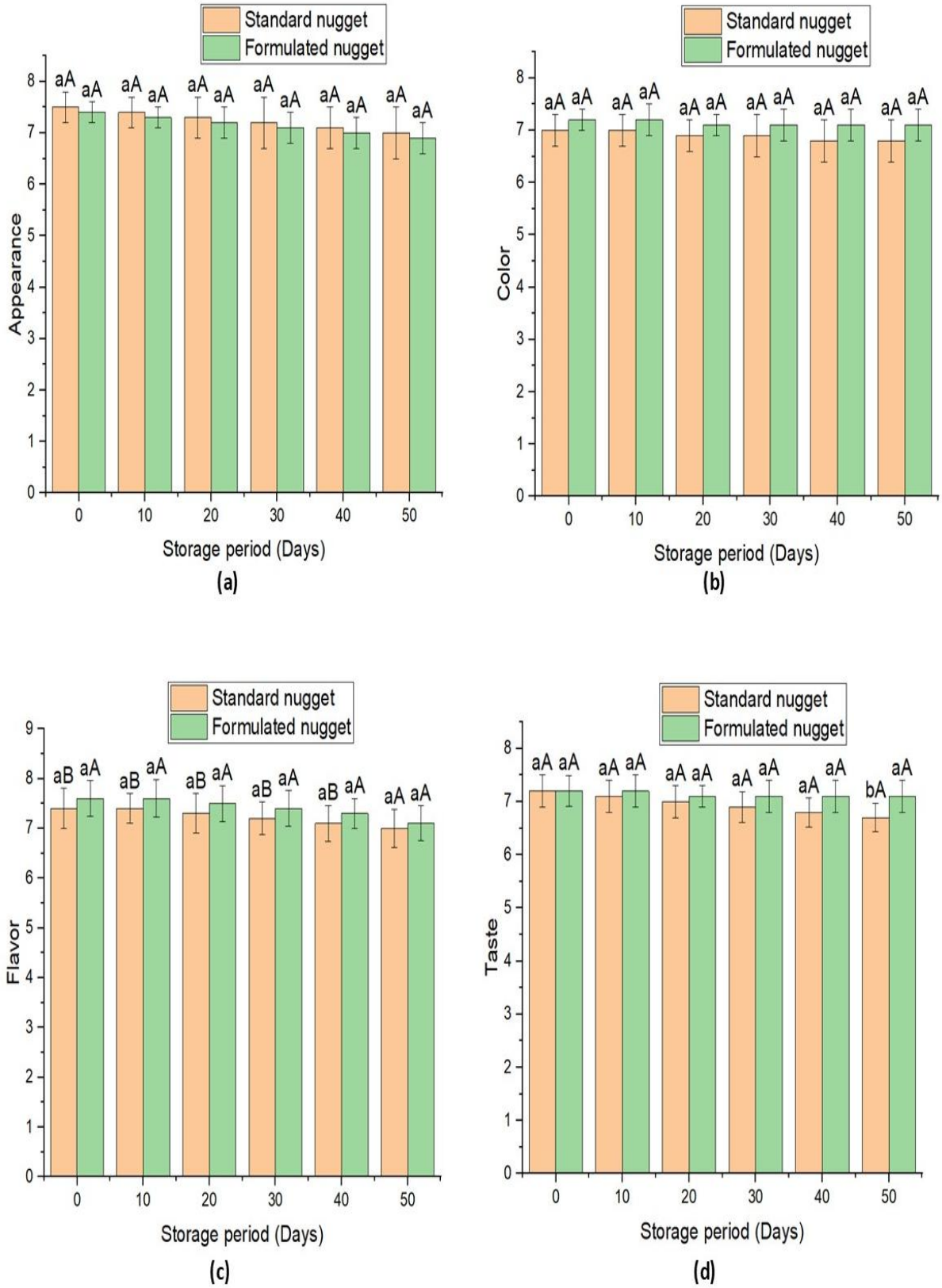
Colour parameters	Day 0	Day 10	Day 20	Day 30	Day 40	Day 50
L*	56.42±0.23^{f}	56.63±0.18^{e}	56.91±0.21^{d}	57.25±014^{c}	57.58±0.20^{b}	57.94±0.25^{a}
a*	5.94±0.04^{c}	5.35±0.09^{c}	5.07±0.05^{c}	4.98±0.03^{c}	4.83±0.06^{c}	4.72±0.04^{c}
b*	23.48±0.36^{a}	23.09±0.12^{b}	22.92±0.16^{c}	22.66±0.13^{d}	22.41±0.18^{e}	22.12±0.15^{f}

Values are given as mean ± standard deviation (SD). Different lowercase letter superscripts indicate statistically significant differences ($p<0.05$) (*L**: Lightness; *a**: Redness/green-ness; *b**: Yellowness/blueness)

7.3.3.5.Sensory evaluation

The present study comprehensively evaluated the sensory characteristics of standard and formulated nuggets over a 50-day frozen storage period, revealing certain significant and subtle changes in appearance, color, taste, flavor, texture, and overall acceptability **(Figure 7.11)**. The research findings demonstrated a gradual decline in sensory scores across both nugget variants, with subtle yet significant ($p<0.05$) variations between standard and formulated formulations. Initial appearance scores of 7.5 for standard and 7.4 for formulated nuggets marginally decreased to 7.0 and 6.9, respectively by day 50

(Figure 7.11 a).


Standard nugget
Formulated nugget
Appearance
Storage period (Days)
(a)
Color
(b)
Flavor
(c)
Taste
(d)

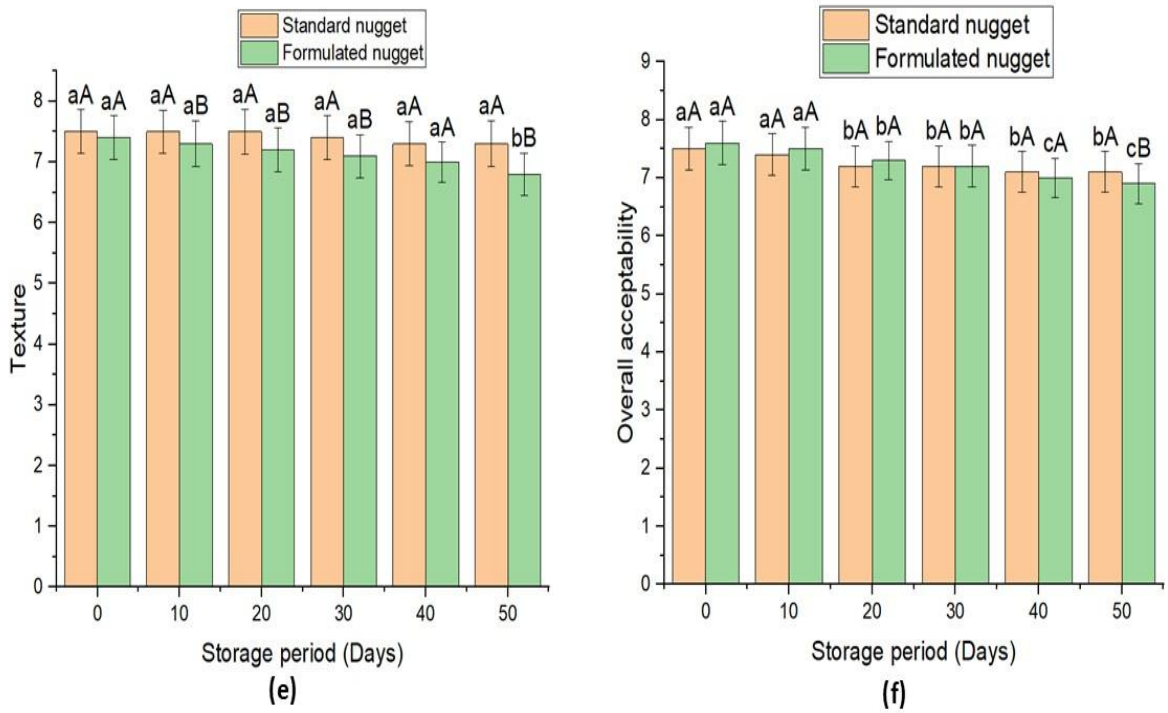


Figure 7.11: The sensory characteristics of standard and formulated nuggets over a 50-day frozen storage period ((a): appearance, (b): color, (c): flavor, (d): taste, (e): texture, and (f): overall acceptability)

(Values are given as mean ± standard deviation (SD). a, b, c, A, B: Different lowercase and uppercase letter superscripts indicate statistically significant differences *($p < 0.05$)*)

Color stability showed interesting dynamics, with formulated nuggets maintaining a slightly higher score of 7.0 compared to standard nuggets 6.8 towards the end of the storage period **(Figure 7.11 b)**. Taste and flavor attributes exhibited a declining trend, with standard nuggets experiencing a significant (*$p<0.05$*) reduction compared to their formulated counterparts **(Figure 7.11 c & d)**. Texture analysis revealed particularly intriguing results, with formulated nuggets demonstrating a more substantial decline, scoring 6.8 by day 50, in contrast to standard nuggets' more stable 7.3 score **(Figure 7.11 e)**. These observations aligned with existing literature documenting frozen storage's impact on meat products. Research by Liu et al. (2023) and Rodriguez-Martinez et al. (2021) attributed sensory degradation to lipid oxidation and protein denaturation, while Rahman et al. (2021) highlighted that natural antioxidant-containing formulations can potentially mitigate sensory attribute loss. Du et al. (2022) further emphasized that ice crystal formation during freezing significantly disrupts muscle fiber integrity,

contributing to texture modifications.

Figure 7.12 displays the visual changes in formulated (left) and standard (right) nuggets stored under frozen conditions over a 50-day period. At Day 10, both samples retained structural integrity and surface texture, and no differences in surface coating and color were evident. By Day 20, minimal changes were observed, suggesting stable preservation. At Day 30, both the formulated nugget as well as the standard nugget samples retained surface uniformity. By Day 40, the formulated nuggets exhibited more visible surface changes and frost accumulation, indicating moisture migration (Cui et al., 2024), whereas the standard nugget still retains its original shape better. By Day 50, both samples showcased signs of ice crystal formation and no potential surface degradation. Overall, both the nugget types endured frozen storage reasonably well, the standard nugget showed slightly better freeze-thaw stability in terms of visual appearance.

Figure 7.12: Appearance of the formulated (left side) and standard nuggets (right side) over a 50-day frozen storage period

Figure 7.13 demonstrates the visual stability of fresh nuggets stored under frozen conditions over a 50-day period. Throughout the storage duration, from day 0 to day 50, the nuggets consistently retained their shape and structural integrity, showcasing minimal

physical degradation. The samples remained well-preserved with no significant signs of discoloration or deformation, which highlighted the effectiveness of the packaging and storage conditions in maintaining product quality. Even at 50 days, the nuggets exhibited a firm appearance and uniformity in size and color, indicating that the product resisted moisture loss and freezer burn. Similar findings have been reported in frozen meat product studies, where proper packaging and storage at -18 °C can maintain visual quality for extended periods (Singh & Benjakul, 2018). Research on frozen chicken nuggets has shown that vacuum-sealed packaging can preserve color stability for up to 60 days, with minimal changes in L^* values from 65.2 to 63.8 (Wagoner et al., 2022). The observed stability aligns with industry standards suggesting that properly stored frozen nuggets can maintain acceptable appearance for 8–12 weeks. This period reflects the time during which minimal degradation in sensory attributes, such as color, occurs under controlled freezing conditions. Comparative studies indicate that nuggets stored at consistent frozen temperatures exhibit less than 5% variation in color parameters over 45-day storage periods, highlighting the effectiveness of freezing in preserving visual quality (Chen et al., 2020). These slight changes are typically below the threshold of consumer perceptibility, suggesting the product remains visually appealing throughout storage. The low variability in color can be attributed to reduced enzymatic and oxidative reactions at the sub-zero temperatures, which slows the pigment degradation. Moreover, vacuum or modified atmosphere packaging can further enhance color stability by limiting oxygen exposure. Such findings emphasize the importance of maintaining constant freezing temperatures to ensure product quality. Deviations in temperature, however, can sometimes accelerate quality loss, particularly in pigment-rich formulations. Therefore, strict cold chain management is essential for preserving the commercial appeal and shelf life of frozen nuggets (Chen et al., 2020).

The images collectively suggested that the nuggets were visually stable and suitable for extended frozen storage without compromising their external characteristics. This observation supported the potential for long-term shelf-life extension while maintaining consumer-acceptable aesthetics, which is crucial for commercial viability and consumer satisfaction in frozen food products. Multiple interconnected factors influence the observed sensory variations during storage (Bakhsh et al., 2021). Oxidative and enzymatic changes primarily drive the decline in taste and flavor profiles, with the formulated nuggets exhibiting marginally better stability in certain attributes. The more

pronounced texture degradation in formulated nuggets suggests potential optimization opportunities in ingredient composition and water retention strategies (Verma et al., 2022). Conclusively, while both nugget types experienced only a slight decline in the sensory attribute profile, the formulated variant demonstrated slightly better color and flavor stability. However, the more significant texture degradation in formulated nuggets indicates a critical area for future research and product development, emphasizing the need for refined formulation techniques to improve frozen storage performance.

Figure 7.13: Appearance of the fresh nuggets over a 50-day frozen storage period

The sensory evaluation of fresh nuggets during 50-day frozen storage **(Table 7.11)** demonstrates exceptional stability across all evaluated parameters, with scores remaining consistently high throughout the entire storage period. Appearance scores maintained perfect stability (7.4), indicating that visual characteristics such as shape integrity, surface quality, and overall presentation remained unaffected by prolonged frozen storage, which aligns with findings by Leygonie et al. (2012) who reported that properly frozen fish products can maintain visual appeal for extended periods when stored at optimal temperatures. Color stability was equally impressive, with consistent scores of 7.2, suggesting that the natural pigmentation and surface coloration remained intact without significant browning, discoloration, or freezer burn effects. These findings were supported by Özogul & Özogul (2007) who demonstrated that frozen nuggets retain

color stability when protected from temperature fluctuations. Taste evaluation revealed remarkable consistency at 7.3 throughout the storage period, indicating preservation of the characteristic flavor profile without development of off-flavors, rancidity, or loss of flavor or palatability. This exceptional taste stability results from the inhibition of enzymatic and microbial activities at sub-zero temperatures, preventing formation of volatile organic compounds responsible for off-flavors such as aldehydes, ketones, and sulfur compounds typically developed through lipid oxidation and protein degradation pathways (Leygonie et al., 2012). The preservation of umami compounds, including nucleotides like inosine monophosphate (IMP) and guanosine monophosphate (GMP), along with free amino acids such as glutamate and aspartate, maintains the characteristic savory taste profile throughout storage, which contrasts favorably with studies by Singh & Benjakul (2018) who reported taste deterioration in processed frozen products after 30 days due to enzymatic breakdown of flavor-active compounds.

Flavor scores (7.6) demonstrated the highest ratings among all parameters, suggesting excellent retention of aromatic compounds and overall flavor intensity through complex preservation mechanisms. The superior flavor retention results from effective preservation of volatile aromatic compounds, including esters, aldehydes, and terpenes, responsible for characteristic fish aroma and flavor notes. Frozen storage at optimal temperatures significantly reduces vapor pressure of these compounds, minimizing loss through sublimation and evaporation. The intact cellular structure in minimally processed fresh nuggets maintains compartmentalization of flavor precursors and enzymes, preventing uncontrolled enzymatic reactions leading to flavor degradation. Preservation of lipid-soluble flavors within the natural fat matrix provides additional protection against oxidative deterioration, supporting research by Hultmann & Rustad (2004) who found that minimal processing preserves volatile flavor compounds better than extensive processing by maintaining natural cellular barriers. Texture evaluation consistently scored 7.4, indicating that structural integrity, firmness, and mouthfeel characteristics remained desirable throughout storage through multiple preservation mechanisms. The exceptional texture stability results from effective preservation of muscle fiber architecture and protein functionality under frozen conditions. Ice crystal formation during freezing occurs primarily in extracellular spaces due to controlled freezing rate, minimizing damage to intracellular structures and preserving myofibrillar proteins including actin and myosin that contribute to texture properties.

Table 7.11: Sensory quality changes of fresh nuggets during 50-day frozen storage period

Sensory parameters	**Storage Period (days)**					
	0	**10**	**20**	**30**	**40**	**50**
Appearance	7.4 ± 0.02[a]	7.4 ± 0.01[a]	7.4 ± 0.01[a]	7.4 ± 0.03[a]	7.4 ± 0.04[a]	7.4 ± 0.05[a]
Color	7.2 ± 0.02[a]	7.2 ± 0.01[a]	7.2 ± 0.02[a]	7.2 ± 0.03[a]	7.2 ± 0.04[a]	7.2 ± 0.05[a]
Taste	7.3 ± 0.02[a]	7.3 ± 0.01[a]	7.3 ± 0.01[a]	7.3 ± 0.02[a]	7.3± 0.02[a]	7.3 ± 0.02[a]
Flavor	7.6 ± 0.03[a]	7.6 ± 0.01[a]	7.6 ± 0.01[a]	7.6 ± 0.01[a]	7.6 ± 0.02[a]	7.6 ± 0.02[a]
Texture	7.4 ± 0.03[a]	7.4 ± 0.01[a]	7.4 ± 0.01[a]	7.4 ± 0.02[a]	7.4 ± 0.02[a]	7.4 ± 0.03[a]
Overall Acceptability	7.6 ± 0.03[a]	7.6 ± 0.01[a]	7.6 ± 0.01[a]	7.6 ± 0.02[a]	7.6 ± 0.02[a]	7.6 ± 0.03[a]

Values are given as mean ± standard deviation (SD). a, b, c, d: Different lowercase letter superscripts within the same row indicate statistically significant differences *($p < 0.05$)*

The maintenance of protein-water interactions and preservation of collagen and elastin networks ensure consistent textural attributes throughout storage. Additionally, inhibition of proteolytic enzyme activity at sub-zero temperatures prevents breakdown of structural proteins that would otherwise lead to softening and textural deterioration, which is superior to findings by Barbut & Mittal (1990) who reported texture degradation in processed meat products after 40 days due to protein denaturation and ice crystal damage.

Overall acceptability maintained the highest scores (7.6), reflecting consumer satisfaction and product quality retention that exceeds typical frozen meat, fish or seafood products through synergistic preservation of multiple quality attributes. This superior acceptability results from harmonious integration of preserved sensory characteristics, where maintenance of appearance, color, taste, flavor, and texture creates a holistic consumer experience meeting fresh product expectations. The consistent high scores demonstrate effective preservation of both intrinsic (nutritional, functional) and extrinsic (sensory, aesthetic) quality factors through optimal freezing and storage conditions, minimal processing that preserves natural quality attributes, and inherent stability of proteins and lipids under controlled frozen storage, as demonstrated by Kumar et al. (2023) and Chakma (2025) who reported acceptability scores in-between 7.0 and 7.5 after 45 days storage of processed meat products in similar conditions, highlighting the superior quality retention achieved through optimized processing and storage protocols.

7.3.3.6.Microbiological quality

The microbiological assessment of standard and formulated nuggets revealed significant similarities in Total Plate Count (TPC) and yeast and mold count during 50 days of frozen storage. **Figure 7.14** illustrates the bacterial growth patterns on agar plates for fresh, formulated, and standard nugget samples throughout a 50-day frozen storage period, demonstrating the microbiological stability of different nugget formulations under controlled storage conditions. The fresh nuggets exhibit minimal bacterial growth throughout the storage period, with plates showing sparse or negligible colony formation from Day 0 to Day 50, indicating excellent microbiological quality and effective preservation under frozen conditions (Singh & Benjakul, 2018). The formulated nuggets

also displayed no bacterial growth patterns during the storage period (0-50 days), suggesting strong microbial resistance by the sample in frozen storage conditions. The standard nuggets also demonstrated relatively no bacterial growth patterns across the storage period, as most of the plates appeared clear, showing no signs of bacterial growth (Leygonie et al., 2012). The overall visual assessment reveals that frozen storage at appropriate temperatures effectively inhibited significant bacterial proliferation across all nugget types, with all the nuggets, fresh, formulated and standard showing superior microbiological stability, likely due to their minimal processing and intact cellular structure that provided natural antimicrobial protection and reduced contamination risk during handling and preparation (Özogul & Özogul, 2007).

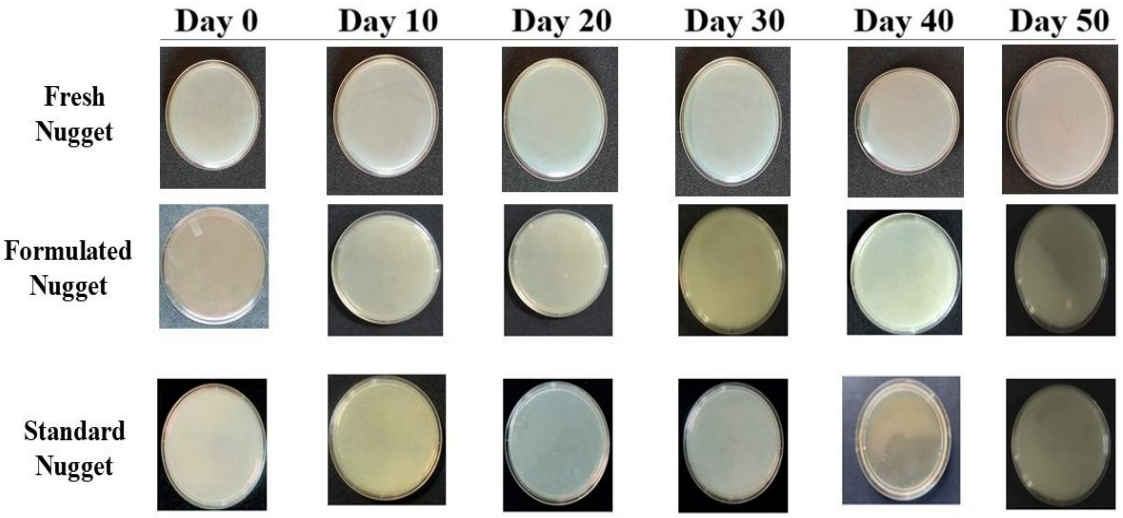


Figure 7.14: Total plate count (Bacteria) of the fresh, formulated and standard nugget samples during 50 days of storage

Table 7.12 presents the quantitative bacterial analysis of fresh, formulated, and standard nugget samples over a 50-day frozen storage period, revealing exceptional microbiological stability across all the nugget samples. All nugget varieties consistently showed no bacterial growth throughout the entire storage duration, indicating that frozen storage at optimal temperatures (-18 °C to -20 °C) effectively inhibited bacterial growth and proliferation. This remarkable microbiological stability can be attributed to the bacteriostatic effects of sub-zero temperatures, which significantly reduced microbial metabolic activity and prevented spoilage bacteria from reaching detectable levels. Also, the ingredients used in preparation of meat analogue were not raw rather processed or modified and free from contamination. This was further supported by findings of

Leygonie et al. (2012), who demonstrated that proper frozen storage maintains bacterial counts below detection limits in meat products for extended periods. The consistent not detected (ND) results across fresh, formulated, and standard nuggets suggest that processing modifications do not compromise the antimicrobial efficacy of frozen storage conditions. This was consistently aligning with findings by Özogul & Özogul (2007) who reported that frozen fish products maintain excellent microbiological quality regardless of processing variations when stored under controlled conditions. These results exceed typical frozen meat, poultry, fish and seafood product standards and demonstrate superior preservation effectiveness. This was comparable to the studies by Singh & Benjakul (2018) who reported no detectable bacterial counts in some frozen fish products up to 40 days of frozen storage. The absence of detectable bacterial growth throughout the 50-day period confirms the safety and quality of all nugget formulations under proper frozen storage conditions, supporting the extended shelf-life potential of these products, as documented by Rahman et al. (2021) who found that optimal freezing protocols can maintain microbiological safety for extended periods in animal products.

Table 7.12: Total plate count of the fresh, formulated and standard nugget samples (during 50 days of storage)

Sample	**Total count-Bacteria (log CFU/g)**					
	Day 0	**Day 10**	**Day 20**	**Day 30**	**Day 40**	**Day 50**
Fresh nugget	ND	ND	ND	ND	ND	ND
Standard nugget	ND	ND	ND	ND	ND	ND
Formulated nugget	ND	ND	ND	ND	ND	ND

ND- not detected

Figure 7.15 and **Table 7.13** collectively illustrate the yeast and mold growth trends in fresh, formulated, and standard nugget samples stored over 50 days. Yeast and mold count also remained consistently below the detection threshold (<1.0 log CFU/g) throughout the entire storage period for the fresh, formulated and standard nuggets (Rahman et al., 2021; Oppong et al., 2021). The absence of microbial growth in all samples suggested effective formulation and storage conditions. Particularly, the fresh

nugget, which might typically be more prone to microbial spoilage due to higher water activity and minimal processing, exhibited no visual fungal contamination even by day 50. This highlighted a strong microbiological stability, possibly due to intrinsic antimicrobial factors such as spices and herbs and excellent hygiene and preservation methods during preparation and storage (Singh & Benjakul, 2018).

Similarly, the formulated nugget consistently showed no microbial growth across all storage intervals. This suggested that the formulation included components with microbial resistance or antifungal properties, such as the inclusion of natural preservatives like herbs or spice extracts (Oppong et al., 2021). In the case of the standard nugget, which serves as a benchmark for typical commercial products, the complete absence of yeast and mold colonies reinforces the validity of the experimental design and sterility control (Leygonie et al., 2012). The lack of microbial presence in this group affirmed the baseline microbial safety under frozen storage. These findings are critical in the context of shelf-life extension and consumer safety, especially for ready-to-eat poultry or fish-based nuggets. The uniform microbial safety over 50 days indicates the products' robustness under chilled conditions, potentially allowing for flexible distribution and retail handling without spoilage concerns (Rahman et al., 2021).

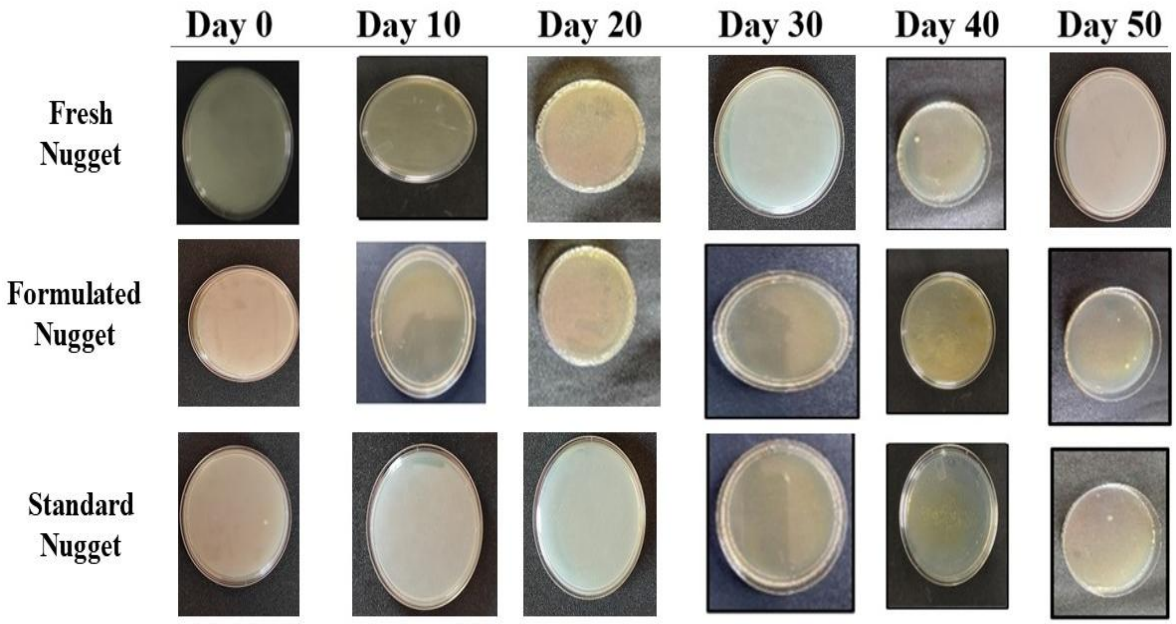


Figure 7.15: Yeast and mold count of the fresh, formulated and standard nugget samples during 50 days of storage

Table 7.13: Yeast and mold count of the fresh, formulated and standard nugget samples (during 50 days of storage)

	Microbial count-Yeast & mold (log CFU/g)					
Sample	**Day 0**	**Day 10**	**Day 20**	**Day 30**	**Day 40**	**Day 50**
Fresh nugget	ND	ND	ND	ND	ND	ND
Standard nugget	ND	ND	ND	ND	ND	ND
Formulated nugget	ND	ND	ND	ND	ND	ND

ND- not detected

The fresh and formulated nuggets both demonstrated good microbial resistance potentially attributable to the incorporation of functional ingredients such as garlic, black pepper, clove, etc. that inhibited the microbial growth (Wanangkarn et al., 2018). The minimal increase in TPC over time substantiates previous scientific observations regarding the effectiveness of freezing in preventing microbial proliferation. This phenomenon can be attributed to the significant reduction in cellular metabolic activity and enzymatic processes during frozen storage, which effectively impedes microbial reproduction and growth (Rani et al., 2024). The absence of yeast and mold growth further validates the rigorous hygienic processing and storage conditions employed in the production and storage of these nuggets. These findings contribute to the broader understanding of microbial dynamics in frozen plant-based meat products, highlighting the critical role of appropriate processing techniques and storage conditions in maintaining microbiological safety. The consistent low microbial counts across all the nugget types underscore the effectiveness of current food processing technologies in preserving product quality and ensuring consumer safety (Wang et al., 2018).

7.4. Conclusion

The study focused on the preparation and characterization of Manila tamarind protein spiral wraps and meat analogue nuggets, with emphasis on shelf-life stability under frozen storage conditions. The protein spiral wrap dough exhibited distinct viscoelastic responses based on solid-to-liquid ratios, with rheological analysis revealing gel-like

behavior and strong intermolecular associations. Boiling resulted in lighter and more vibrant colored wraps with enhanced lightness and chromaticity compared to autoclaving. Autoclaving yielded higher cooking yields and increased moisture gain, while boiling produced superior textural properties with firmer, more cohesive, and chewy textures. The best formulation had high protein content, favorable moisture retention, and good textural attributes. The formulated nuggets had lower moisture content, higher fat content, and slightly lower protein and mineral content compared to standard nuggets. Cooking quality of the formulated nuggets was comparable with that of the standard nuggets. Textural analysis revealed lower hardness, cohesiveness, springiness and chewiness in formulated nuggets. Color analysis showed formulated nuggets had lower L^*, a^* and b^* values indicating slightly darker appearance and better colour development due to Maillard browning. Sensory evaluation demonstrated similar profiles for both nuggets, with formulated nuggets having slightly better color, taste and flavor scores. During 50 days of frozen storage, all the nugget samples showed minimal decline in quality parameters such as weight reduction, moisture loss, fat content, pH, texture, and sensory attributes. Textural parameters like hardness, springiness, cohesiveness and chewiness were also minimally affected during the storage period. L^* and a^* values increased during storage while redness and yellowness values decreased. Sensory attributes were also maintained during the storage period, with fresh, and formulated nuggets maintaining slightly better color and flavor stability. Microbiological assessment showed no detectable bacterial, yeast, or mold growth in fresh, formulated, and standard nuggets throughout the storage period. The study highlights the complex interplay of ingredient composition, physicochemical changes and sensory attributes in development of plant-based meat alternative products and their characteristics during frozen storage.

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