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Roles of *k*-carrageenan, locust bean gum, and transglutaminase on textural and rheological characteristics of plant-based fishball analogues

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ABSTRACT

Plant protein-based fishball analogues have the potential to contribute to sustainable and alternative protein sources, helping to alleviate the burden of overfishing. However, there is a lack of products that are sensory and nutritionally comparable to commercial fishballs. This study evaluated the impact of mTGase (microbial transglutaminase) and hydrocolloid addition on the texture, microstructure, and sensory properties of mung bean protein-based fishballs. The addition of 1 % mTGase significantly improved texture and water-holding capacity, reducing expressible moisture to 24 % and increasing hardness to 474.69 g. κ-Carrageenan (0.3 %) further enhanced these properties with expressible moisture at 19.55 % and hardness at 794.16 g. The addition of 0.13 % LBG (locust bean gum) with 1 % mTGase increased expressible moisture and reduced gel hardness. Nutritional analysis of mung bean protein-based fishballs formulated with 0.3 % κ-carrageenan, 1 % mTGase, and 0.13 % LBG revealed a higher protein content (12.3 g) and lower carbohydrate content (3 g) compared to conventional surimi products. Sensory evaluation with 61 participants showed high acceptability for mung bean protein-based fishballs, with mean scores of 6.38 for appearance, 6.08 for texture, and 6.02 for overall liking. The results suggest that mung bean protein-based fishballs, optimized with mTGase, LBG, and κ-carrageenan, offer a nutritious and appealing plant-based alternative, meeting consumer preferences for healthy and sustainable food options.

1. Introduction

In 2023, the global meat substitutes market was estimated at USD 18.78 billion and is anticipated to grow at a compound annual growth rate (CAGR) of 42.4 % between 2024 and 2030 (view, 2024). This expansion has led to the proliferation of meat analog products and market saturation. The plant-based seafood market is projected to reach USD 1.3 billion by 2031, growing at a CAGR of 42.3 % during the forecast period from 2021 to 2031 (Research, 2022). Given the unsustainable nature of the current seafood supply chains and the environmental damage caused by overfishing (Halpern et al., 2021; Santo et al., 2020), there is a growing need for innovative and sustainable seafood alternatives. Today, most meat and seafood analogues utilize soy, pea, or wheat proteins (Kyriakopoulou et al., 2021; Santo et al., 2020; Sha and Xiong, 2020). However, these sources present challenges, such as allergenicity and gluten intolerance, limiting their application (Samard and Ryu, 2019). Therefore, it is essential to explore other sources of plant protein. Mung bean protein has emerged as a promising alternative owing to its high nutritional value and functional properties (Dahiya et al., 2015) while low allergenicity in comparison to soy and peanut proteins (Turck et al., 2021).

Mung beans (*Vigna radiata*) are legumes that are widely cultivated in Asia, particularly in India, Myanmar, Thailand, and China. Mung beans are highly nutritious and contain approximately 24 % protein, 61 % carbohydrates, 4.6 % fiber, and 1.2 % fat. Mung bean proteins, primarily storage proteins such as globulin and albumin, are rich in essential amino acids, but lack sulfur-containing amino acids (Dahiya et al., 2015; Yi-Shen et al., 2018).

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The functional properties of mung bean proteins, such as solubility, water-holding capacity (WHC), and gelation, are crucial for their application in food products. Protein solubility influences other properties, such as gelation, foaming, and emulsification, affecting the overall performance of food systems (Vijayan et al., 2024; Yi-Shen et al., 2018). Gelation properties are important in meat and seafood analogues, where protein interactions form viscoelastic gels that trap water within their matrix (Bessada et al., 2019). Mung bean protein has been used in commercial plant-based products, such as the Beyond Burger and JUST Egg. The latter employs mung bean protein isolate and microbial transglutaminase (mTGase) to create an egg-like emulsion via protein cross-linking (Bansal-Mutalik et al., 2019). Protein cross-linking, a method that forms covalent bonds between protein molecules, can impart a meat-like texture to legume proteins, making them suitable for meat analogues (Buchert et al., 2010).

Hydrocolloids, such as kappa-carrageenan (KC) and locust bean gum (LBG), are often incorporated into meat analogue formulations to enhance texture and water-binding properties (Sha and Xiong, 2020). KC, derived from red algae, forms strong gels in the presence of potassium ions and exhibits synergistic effects with LBG, improving the gel strength and elasticity (Brenner et al., 2013). LBG, derived from carob pod seeds, is resistant to pH variations, salts, and heat treatments, making it a valuable thickening and stabilizing agent (Barak and Mudgil, 2014). mTGase is a widely used enzyme in food processing that catalyzes the formation of ε-(γ-glutamyl)lysine bonds between proteins and enhances gel formation and textural properties (Buchert et al., 2010). Mung bean protein is an excellent substrate for mTGase because of its high glutamine and lysine content (Lee and Chin, 2013). The combination of mTGase and hydrocolloids can significantly improve the textural and rheological characteristics of plant-based seafood analogues. To our knowledge, there are no prior studies that have explored the use of mung bean protein isolate, mTGase, *k*-carrageenan, and LBG specifically in the development of plant-based seafood analogs.

This study's objective was to develop a fishball analogue using mung bean protein, hydrocolloids, and TGase to create a product with textural properties similar to those of commercial fishballs. The specific aims of this study include understanding the effect of TGase and hydrocolloids on the textural and rheological properties of the fishball analogue and sensory characteristics of the final product in comparison with that of commercial fishballs. By achieving these objectives, this study seeks to contribute to the development of innovative, sustainable, and nutritious plant-based seafood alternatives.

2. Materials and methods

2.1. Materials

Dehulled mung beans of Indian origin were procured from Sri Murugan Trading Pte. Ltd in Singapore, ground, and sieved through a 60-mesh sieve to produce mung bean flour, which was stored at −18 °C. The mung bean proteins were extracted by following our previously reported method (Vijayan et al., 2024). Commercial surimi products (DoDo Fishball, BoBo Premium Fried Fishball, Peng Wang Lobster Ball, DoDo Crab Flavoured Stick, DoDo Otah Fish Cake) and vegetarian fishballs (Tian Ran Vegetarian Fishball, Friendly Vegetarian Fishball, Makanan Sayuran Vegan Ball, Ahimsa Vegan Atlantic Ball) were obtained from local supermarkets. For protein isolation, food-grade sodium hydroxide (NaOH) (Sigma-Aldrich, St. Louis, MO, USA) and citric acid (RedMan Shop, Singapore) were used for pH adjustment. The preparation of mung bean fishball analogues involved KC, LBG and calcium chloride (CaCl2) from Better 4 U SG, potassium chloride (KCl) from Merck (Darmstadt, Germany), and microbial transglutaminase (Activa TG-SR-MH) with 43 IU/g activity from Ajinomoto (Tokyo, Japan). For microstructural analysis, calcofluor-white (0.2 %) and fluorescein isothiocyanate (FITC; 0.1 % w/w) were obtained from Thermo Fisher Scientific (Waltham, MA, USA). Silicone oil was sourced from Aion Scientific (Singapore) and used to prevent moisture loss. All the ingredients used were of food grade.

2.2. Methods

2.2.1. Mung bean protein-based fishball preparation

All samples (Table 1) were pH adjusted to 7.0 and blended for 3 min. The pH of the samples was adjusted using 2 N sodium hydroxide and 50 % (w/v) citric acid solutions. The samples were placed in 50 mL centrifuge tubes, incubated at 55 °C for 20 min, and cooked at 90 °C for 20 min in a water bath (SW22, Julabo, Seelbach, Germany). The samples were stored at 4 °C overnight, except for those used in the rheological analysis, which were analyzed before heat treatment. The temperature conditions were taken from previous work (Vijayan et al., 2024), and the time conditions were based on preliminary trials for fishball analogue formulation.

2.2.2. Expressible moisture

The expressible moisture content was determined using the method described by Petcharat and Benjakul (2017). Cylindrical gel samples (5 g) were weighed (X), placed between filter papers, and compressed with a 5 kg weight for 2 min. The samples were then reweighed (Y). The expressible moisture was calculated as (X-Y) × 100/X and expressed as a percentage of the initial weight.

2.2.3. Texture profile analysis

Texture analysis was performed using a TA-XT2i texture analyzer (Stable Micro Systems, Surrey, UK) with a 5 kg load cell. The samples were cut into 2 cm cylindrical shapes and compressed twice to 40 % of their height using a 35 mm-diameter flat top cylindrical probe. The test parameters were as follows: pre-test speed 1 mm/s, test speed 1 mm/s, 5 s interval between compressions, 8 mm distance, and 0.05 N trigger force (Sow and Yang, 2015).

2.2.4. Confocal laser scanning microscopy (CLSM)

Microstructural analysis was performed using CLSM with the Zeiss software. The samples were stained with 0.2 % calcofluor white (polysaccharides) and 0.1 % FITC (proteins) and stirred in the dark for 10

Table 1
Key ingredients in the formulations of mung bean fishball analogues.

	Components (%)				
Sample codes	**Water**	**MBPI**	**TGase**	**KC**	**LBG**
P	Upto 100 %				
		45	–	–	–
PT					
0.8		45	0.8	–	–
1		45	1	–	–
1.2		45	1.2	–	–
1.4		45	1.4	–	–
PTK					
0.1		45	1	0.1	–
0.2		45	1	0.2	–
0.3		45	1	0.3	–
0.4		45	1	0.4	–
PTL					
0.13		45	1	–	0.13
0.2		45	1	–	0.2
PTKL					
		45	1	0.3	0.13

1: Key ingredients in the formulation of mung bean protein-based fish balls: MBPI – Mung bean protein isolate, TGase – microbial transglutaminase, KC – κ-carrageenan, LBG – Locust bean gum, P – Control formulation with mung bean protein isolate only, PT – mung bean protein isolate + microbial transglutaminase, PTK – mung bean protein isolate + microbial transglutaminase + κ-carrageenan, PTL – mung bean protein isolate + microbial transglutaminase + locust bean gum, PTKL – Mung bean protein isolate + microbial transglutaminase + κ-carrageenan + locust bean gum.

min. Imaging was performed with a confocal laser scanning microscope (LSM 710) using 405 and 488 nm excitation wavelengths (Ji et al., 2024; Kong et al., 2017).

2.2.5. Rheological analysis

The rheological properties were analyzed using an Anton Paar MCR-102 rheometer with a 25 mm-diameter parallel plate. Samples (0.5 g) were loaded with a 1 mm gap and coated with silicone oil to prevent moisture loss. They were heated from 25 to 55 °C, held for 20 min to simulate the incubation process, and then heated to 90 °C for 10 min. Temperature sweeps were performed at a linear heating rate of 2 °C/min, 0.1 Hz frequency, and 0.5 % strain. The strain value was determined through preliminary stress sweep tests to ensure it remained within the linear viscoelastic region. Frequency sweeps were conducted from 0.01 to 10 Hz at 25 °C (Vijayan et al., 2024).

Creep-recovery tests were conducted using the same rheometer. The samples were subjected to 150 Pa shear stress from 0 to 373.5 s, the stress was removed, and strain variations were recorded from 373.5 to 747 s. Compliance J(t) was recorded over time (Ran et al., 2022).

2.2.6. Nutritional profiling

PTKL refers to the optimized mung bean protein-based fishball formulation incorporating kappa-carrageenan and locust bean gum (Table 1). PTKL samples were analyzed by Setsco Services, Singapore, for nutritional composition (carbohydrates (by difference), fat (AOAC 996.06), protein (AOAC 984.13), cholesterol (AOAC 994.10), saturated (AOAC 996.06) and trans-fatty acids (AOAC 996.06), dietary fiber (AOAC 985.29, sodium (AOAC 937.09), and sugar (AOAC 982.14).

2.2.7. Consumer study

The study was conducted with 61 participants at the Food Innovation and Resource Centre, Singapore Polytechnics. Participants with fish allergies were excluded from this study. Blinded samples of mung bean protein-based, commercial (DoDo fishball), and vegetarian fishballs (Ahimsa Vegan Atlantic ball) were evaluated using the Compusense cloud software (Compusense Inc., Guelph, ON, Canada). For benchmarking purposes, a preliminary sensory trial was conducted to select the most suitable commercial fishball and vegetarian fishball analog for evaluation. The chosen samples were selected based on their superior sensory properties and overall likeability. All samples were freshly prepared on the day of the sensory evaluation. The samples were heated up to 100 °C for 3 min in water to ensure safety and served warm to the participants. Proper food handling practices were followed during preparation and serving to guarantee no microbiological risks. Attributes such as appearance, aroma, taste, flavor, texture, and overall acceptability were rated on a 9-point hedonic scale. Data were analyzed to compare the sensory profiles.

2.2.8. Fourier transform infrared (FTIR) spectroscopy

To prepare for FTIR analysis, the samples were first freeze-dried and finely ground. Spectral data were acquired using a Spectrum Two FTIR spectrometer (PerkinElmer, Massachusetts, USA) equipped with an ATR (attenuated total reflectance) crystal. Scans were collected over the range of 4500 to 400 cm^{-1} at ambient temperature, using a resolution of 2 cm^{-1} and averaging 32 scans per sample. Post-acquisition, spectra were subjected to baseline correction, deconvolution, and curve fitting using Origin software (version 2021b) (Lee et al., 2025).

2.2.9. Statistical evaluation

All measurements are reported as the mean ± standard deviation and were performed in triplicate. Data were analyzed using one-way ANOVA and Tukey's post-hoc test using IBM SPSS Statistics Version 24. Statistical significance was set at $P < 0.05$.

3. Results and discussion

This study examined the effects of mTGase enzyme and hydrocolloid additions on the texture and expressible moisture properties of mung bean protein-based fishball analogues. Initially, the impact of mTGase was analyzed, followed by the addition of hydrocolloids to enhance these properties. The final plant-based fishball mimic was evaluated against commercial fishballs and vegetarian fishballs through consumer research, nutritional and textural comparisons.

3.1. Impact of mTGase addition on texture and microstructure

Table S1 shows the reference range for texture profile parameters for commercial surimi products. The 1 % mTGase addition significantly reduced the expressible moisture content of mung bean protein-based fishballs (PT) to 24.13 % (Fig. 1**a**), indicating a higher water-holding capacity and improved texture and juiciness. However, at 1.4 % mTGase concentration, the expressible moisture increased to 35.35 %, correlating with a poor hardness value of 383.8 g (Fig. 1**b**), indicating reduced gel strength and water-binding capacity. The higher mTGase concentrations likely enhanced protein-protein interactions while reducing protein-water interactions, leading to reduced hardness and increased expressible moisture, as previously reported (Chen and Han, 2011; Ruzengwe et al., 2020).

The hardness of the fishballs increased significantly at 1 % mTGase, reaching 474.69 g (Fig. 1**b**). This value is within the range observed for surimi products (443.60 – 1470.30 g) but lower than commercial vegetarian fishballs (1540.00 – 3075.46 g) (**Table S1**), providing a desirable firmer bite for seafood analogues. The mTGase-induced changes in the protein's secondary structure, specifically the decrease in α-helices and increase in β-sheets, facilitate protein-protein interactions and gel network formation (Zhou et al., 2022). However, at higher mTGase concentrations (1.4 %), hardness decreased, likely because excessive crosslinking inhibited uniform protein network development (Guo et al., 2013). Similar reductions in rheological properties at higher TGase concentrations have been observed in bambara groundnut protein isolates (Ruzengwe et al., 2020).

CLSM images (Fig. 1**c**) revealed a homogeneous protein network in samples treated with 1 % mTGase, indicating enhanced protein cross-linking and gel formation, which improved textural properties, water-binding capacity, and hardness, similar to soy patties treated with mTGase (Lee and Hong, 2020). However, to match the expressible moisture content (3.0–5.5 %) and hardness range (443.60–1470.30 g) of traditional fishballs, the inclusion of hydrocolloids in the formulation was necessary.

3.2. Impact of k-carrageenan & mTGase on texture and microstructure

Numerous studies have demonstrated that polysaccharides serve as effective thickening agents by increasing steric hindrance and chain entanglement density in the protein networks within meat batters. This resulted in a higher apparent viscosity than control samples (Chen et al., 2020; Zhuang et al., 2020). Additionally, Cao et al. (2022) verified that hydrogen bonding and ionic interactions between meat proteins and KC enhanced the viscous behavior of meat batters.

The expressible moisture content of mung bean protein-based fishball analogues (PTK) varied significantly with KC addition (Fig. 2**a**). At 0.1 % KC, the expressible moisture was 26.50 % ± 2.15, while increasing the concentration to 0.2 % reduced the expressible moisture to 20.49 % ± 3.69. Further increasing KC to 0.3 % resulted in a decrease to 19.55 % ± 2.50. However, at 0.4 %, the expressible moisture increased to 28.62 % ± 4.47. This suggested that KC improved the water-holding capacity up to a certain concentration (0.3 %), beyond which the effect diminished, possibly because of the saturation of binding sites or structural changes in the protein network. Previous studies have shown that KC enhanced water retention in protein-based

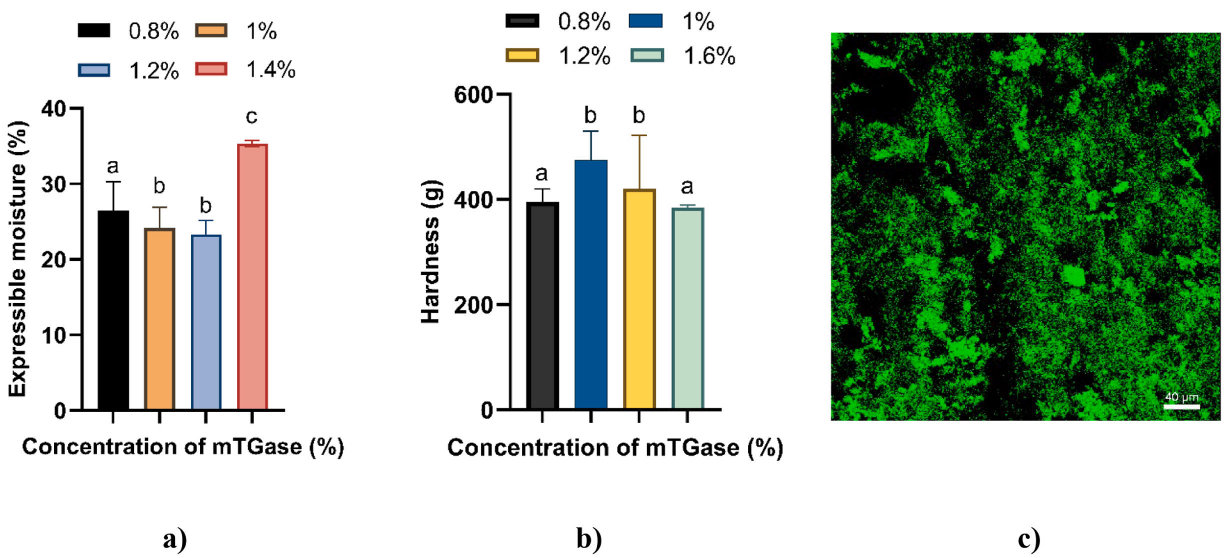

Fig. 1. Impact of mTGase addition on texture and microstructure a) Expressible moisture comparison b) Texture profile analysis comparison – Hardness (g) similar letters indicate $p > 0.05$ (no significant difference). (c): Confocal laser scanning microscopy images using FITC staining – 1 % mTGase sample.

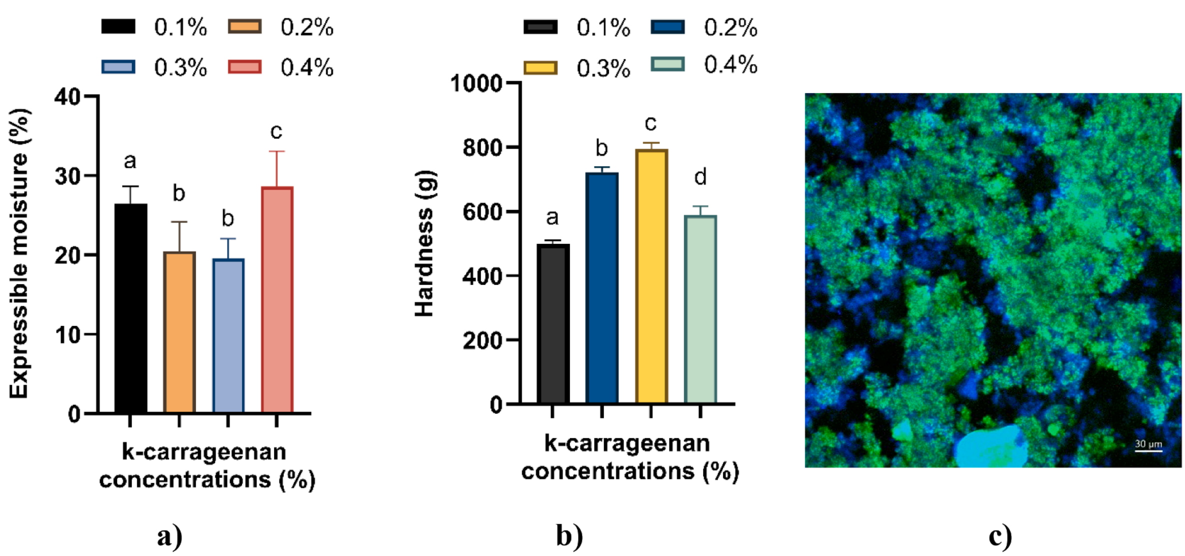

Fig. 2. Impact of KC on a) expressible moisture b) hardness c) microstructure. Similar letters indicate $p > 0.05$ (no significant difference). (c): Confocal laser scanning microscopy images using FITC (protein) and calcofluor staining (KC) – 1 % mTGase and 0.3 % KC sample.

systems by forming a gel network that traps the water molecules (Feng et al., 2024a). The reduction in expressible moisture at lower KC concentrations can be attributed to its ability to form strong hydrogen bonds and ionic interactions with proteins, thereby improving the water-holding capacity (Cao et al., 2022). However, at higher concentrations (0.4 %), the network may become oversaturated, leading to structural changes that reduce water retention, as observed in previous studies where excessive KC concentrations disrupted the gel networks (Wei et al., 2024).

The hardness of mung bean protein-based fishballs responded significantly to the addition of KC (Fig. 2**b**). The mean hardness values increased from 500.653 g at 0.1 % to 794.16 g at 0.3 %, suggesting enhanced gel strength and firmness due to improved protein-KC interactions and network formation. However, at 0.4 %, the hardness decreased to 589.71 g, indicating that excessively high concentrations might disrupt the optimal gel network, resulting in a less firm texture. This increase in hardness can be attributed to KC- protein interactions, forming a more cohesive and denser network, thereby increasing gel strength (Chen et al., 2020; Feng et al., 2024a). The observed decrease in hardness at higher concentrations may be due to KC saturation, which may inhibit further crosslinking and disrupt the network. Additionally, Lu et al. (2022) reported that at higher KC concentrations (0.4 %), there was an increase in KC-KC interactions, reducing protein-hydrocolloid interactions and thus decreasing gel strength and hardness. Zou et al. (2022) indicated that TG-induced crosslinking led to the unfolding, separation, and rearrangement of protein molecules, converting α-helices to β-sheets. When TG and KC were added simultaneously, these changes in protein secondary structure were more pronounced, suggesting that the combination of TG and KC increased hydrogen bond formation, further enhancing the gel network.

CLSM images (Fig. 2**c**) of samples treated with 1 % mTGase and 0.3 % KC revealed a well-organized and dense protein network. The improved microstructure at optimal concentrations (0.3 % KC) can be attributed to a denser and more uniform protein network, which enhances textural properties and water retention. Similar improvements in the microstructure were reported by Feng et al. (2024a), where the KC addition to meat batters resulted in a finer and more cohesive network. Cao et al. (2022) also confirmed that KC enhances protein interactions, contributing to a denser microstructure and improved textural properties. Additionally, Lu et al. (2022) found that at higher concentrations (0.4 %), the microstructure showed signs of over-saturation, with increased hydrocolloid-hydrocolloid interactions leading to less uniform networks. In the current study, at 0.1 % KC, the microstructure (**Fig. S1a**) showed that KC occupied voids within TGase-mediated protein crosslinks. At 0.2 % KC (**Fig. S1b**), leading to a more compact and cohesive protein network. This concentration resulted in improved water-holding capacity and firmness, as evidenced by the decreased expressible moisture and increased hardness. The 0.3 % KC sample (**Fig. S1c**) exhibited the most well-developed microstructure, with a dense and uniformly distributed network. This optimal concentration provided the best balance between low expressible moisture and high hardness, indicating a robust gel formation. The well-developed network is consistent with previous findings that optimal hydrocolloid concentrations improve the gel properties and structural integrity (Cao et al., 2022). However, at 0.4 % KC (**Fig. S1d**), the microstructure began to show signs of over-saturation. The protein network appeared less uniform, with some areas of aggregation and others showing fewer interactions. This disruption likely contributed to the observed increase in expressible moisture and decrease in hardness, suggesting that there is an upper limit to the beneficial effects of KC addition.

3.3. Impact of LBG and mTGase on texture and microstructure

The addition of 0.13 % locust bean gum (LBG) along with 1 % mTGase (PTL) resulted in higher expressible moisture (34.9 %) (Fig. 3**a**), indicating poorer expressible moisture compared to the 1 % mTGase + 0.3 % KC sample. The goal was to incorporate a thickening agent like LBG to improve water-binding capacity, reduce expressible moisture, and enhance the hardness of the sample. However, the increased expressible moisture suggests that LBG incorporation did not achieve the desired effect. The Texture Profile Analysis (TPA) results (Fig. 3**b**) indicate that 0.13 % LBG + 1 % mTGase significantly reduced gel hardness to 537.89 g, making it comparable to the 1 % mTGase sample at 474.69 g. This reduction in hardness is attributed to the hydrogen bonding between LBG and the protein matrix, resulting in a poorer texture profile compared to the 1 % mTGase + 0.3 % KC sample. The latter sample exhibited no visible signs of phase separation and was thus analyzed for TPA. **Fig. S2** illustrates that higher LBG concentrations (0.2 % or more) led to phase separation, causing non-homogeneous distribution of protein and hydrocolloid components after cooking. Consequently, TPA measurements were not performed for gels with >0.13 % LBG + 1 % mTGase. At higher LBG concentrations, hydrogen bonding likely impeded crosslink formation during the mTGase reaction, resulting in poor gel hardness. The CLSM image of the 0.13 % LBG + 1 % mTGase sample (Fig. 3**c**) shows the separation of the hydrocolloid phase and the cross-linked protein phase. This highlights the impact of protein source on interaction magnitude with polysaccharides and the influence of protein/polysaccharide concentration on thermodynamic incompatibility or complex coacervation, depending on the affinity between different biopolymers and the solvent (Ye, 2008). Thermodynamic incompatibility, primarily observed here, occurs when negatively charged proteins interact with neutral or anionic polysaccharides, influenced by pH and ionic strength (Doublier et al., 2000).

The observed viscoelastic properties likely result from three concurrent mechanisms: protein self-association, polysaccharide aggregation, and protein-polysaccharide interactions. The role of each mechanism in rheological properties varies with the biphase volumes of protein and polysaccharide. Mung bean proteins, consisting of globular proteins (Yi-Shen et al., 2018), interact with LBG, a branched polysaccharide with long, linear mannan chains. The exact interaction sites need confirmation through solid/liquid state NMR, a topic for future research (Agarwal et al., 2023).

In this study, the imbalance stems from protein self-association via mTGase cross-linking and polysaccharide aggregation through hydrogen bonding. LBG's low solubility at room temperature, due to its high molecular weight and mannose/galactose ratio (Barak and Mudgil, 2014), affects its dispersion. (Sánchez et al., 1995) noted that the low degree of galactose substitution in LBG leads to high interaction between adjacent mannose chains, forming crystalline regions that hinder dispersion in water. Heating LBG at 80 °C for 30 min disrupts these regions, enhancing solubility. The protein, mTGase, and hydrocolloid mixture in this study was heated at 55 °C for 20 min, followed by heating at 90 °C for 10 min to inactivate the enzyme. During the 55 °C phase, covalent crosslinking of mTGase-mediated Lys-Gln residues likely overpowered LBG-protein interactions. The subsequent heating at 90 °C improved LBG solubility in the aqueous solution, leading to LBG self-aggregation via hydrogen bonding (Fig. 3**c**).

3.4. Impact of mTGase and gums on rheological properties

The rheological behavior of protein solutions or gels is closely linked to their molecular stiffness and interactions, which heat-altered. This

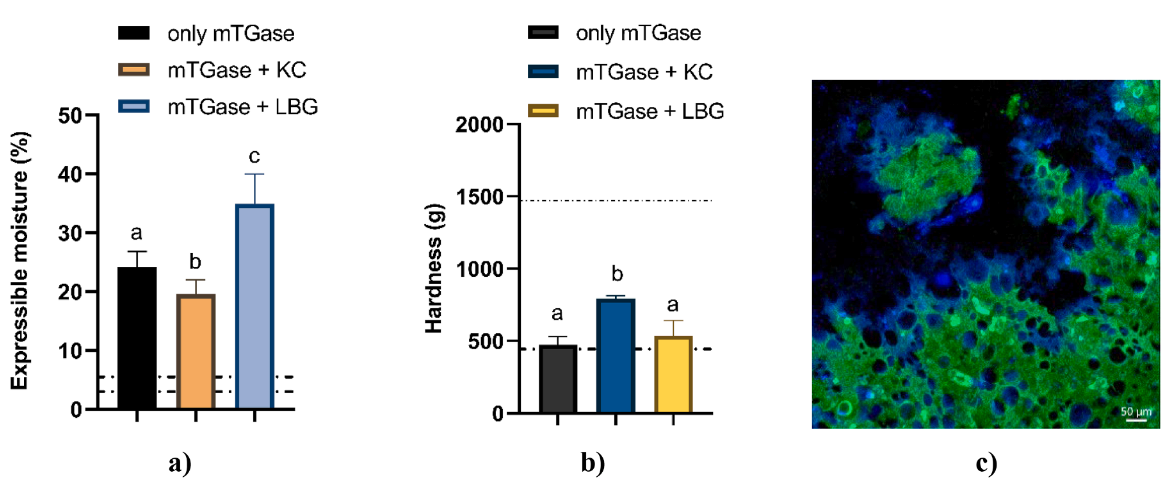

Fig. 3. a) Impact of LBG on a) Expressible moisture comparison of 1 % mTGase, 1 %mTGase and 0.3 % KC, 1 % mTGase and 0.13 % LBG b) Texture profile analysis comparison – Hardness of 1 % mTGase, 1 % mTGase and 0.3 % KC, 1 % mTGase and 0.13 % LBG (c): Confocal laser scanning microscopy images using FITC staining (protein) and calcofluor staining (0.1 % LBG) for) 1 % mTGase and 0.13 % LBG. Similar letters indicate $p > 0.05$ (no significant difference). Dotted lines indicate the reference range for that parameter amongst surimi products.

analysis involved tracking the storage modulus (G′) using a temperature sweep to understand the gelation process. The G′ for P (mung bean protein isolate), PT (1 % mTGase), and PTKL (1 % mTGase + 0.3 % KC + 0.13 % LBG) during thermal gelation are shown in Fig. 4**a**. The changes in G′ in response to the temperature changes were categorized into four distinct phases. Between 25–55 °C, there was a significant rise in G′ for PT and PTKL, suggesting the influence of mTGase concentration on G′ begins before reaching the optimal temperature for mTGase activity at 55 °C, with G′ reaching 637.49 Pa for PT and 2271.93 Pa for PTKL at 55 °C. This aligns with Jia et al. (2016), who demonstrated that crosslinking between glutamine-lysine groups and hydrogen bonding aids in stable three-dimensional network formation in protein gels.

During the holding time at 55 °C for 20 min, the G′ of the control gradually increased, indicating partial protein unfolding and peptide aggregation through hydrophobic interactions, although the aggregation level was too low to form a gel network (Al-Ali et al., 2021). Simultaneously, the G′ value for the PT sample increased from 637.49 Pa to 2417.27 Pa, while the PTKL sample ascended from 2271.93 to 5973.36 Pa, indicating better gel network formation. The significant increase in G′ for PTKL compared to PT and P in both temperature and frequency sweep analyses indicates a superior gelation process facilitated by the combination of mTGase and KC. The enhanced interactions between mTGase, KC, and the protein matrix led to a stronger and more stable gel network. These results align with previous findings where hydrocolloids such as KC combined with enzymes such as mTGase significantly improved gelation and viscoelastic properties (Chen et al., 2020; Feng et al., 2024a). Hydrogen bonding and ionic interactions between proteins and KC enhance the strength and stability of the gel network (Cao et al., 2022).

Between 55–90 °C, the G' values for the PT and PTKL samples initially dropped to a minimum at 81.23 °C, followed by a significant increase during the 20 min heating at 90 °C. This reduction in G′ at the same temperature was not dependent on mTGase concentration or hydrocolloid addition. The initial decrease in G′ could be due to protein denaturation, leading to increased filament 'fluidity (Shand et al., 2008) resulting from reduced system viscosity and protein cluster dissociation (Comfort and Howell, 2002). Moreover, the weakening of hydrogen bonds during heating may trigger a conformational transition, leading to reduced G′ values at 81.23 °C. Following protein unfolding, heat-induced gelation occurs upon heating the sample to 90 °C for 10 min, leading to the incorporation of more unfolded proteins into the gel network (Shand et al., 2008).

During the frequency sweep (Fig. 4**b**), PTKL exhibited the highest G′ values across all frequencies, indicating the strongest and most elastic gel networks. PT showed moderate G′ values, suggesting a decent gel network, but was less robust than PTKL. P had the lowest G′ value, indicating a weak gel formation. The stability of G′ for PTKL with increasing frequency demonstrates its stable and strong gel network.

The interaction between KC and LBG also played a significant role in the rheological properties of the gel. The KC and LBG form networks whose strength depends on the preparation temperature and weight ratio between the components (Pinheiro et al., 2011). The interactions between these polysaccharides, such as hydrogen bonding, contribute to the formation of a stable gel network (Ning et al., 2007). The results suggest physical entanglements and miscibility between these two polysaccharides, potentially leading to changes in the mechanical properties of the gel (Figueiró et al., 2004; Martins et al., 2012).

The addition of galactomannans (LBG) initially increases the water-binding capability of KC but decreases with further increases in galactomannan concentration (Arda et al., 2009). Therefore, the above rheological phenomena may be hydrocolloid concentration dependent. The synergistic interactions between KC and LBG enhance gel strength and water-binding capabilities at optimal ratios, but decrease with higher concentrations, passing through a 'synergistic peak' (Pinheiro et al., 2011).

3.5. Texture, microstructure and nutritional properties of fishball analogues

The effects of mTGase, KC, and LBG addition on the textural properties of mung bean-based fishball analogues are detailed in **Table S1**. The influence of transglutaminase on the texture profile characteristics of plant proteins has been well-documented (Lee and Chin, 2013; Moreno et al., 2020; Shand et al., 2008; Tang et al., 2006). The observed hardness values were consistent with those of previous rheological investigations. Our study's findings for PTKL are consistent with past research, showing that hydrocolloid addition effectively reduces expressible moisture by enhancing water-binding capacity (García-García and Totosaus, 2008). PTKL (mung bean fishball analogues) demonstrates textural properties that are generally comparable to commercial surimi products and vegetarian fishballs but more closely resemble vegetarian fishballs in parameters such as hardness (1312.95 g), chewiness (1099.23 g.mm), and expressible moisture (11.42 %).

The microstructure comparison of fishballs and mung bean protein-based fishball analogues, shown in Fig. 5**a) and b)**, using CLSM revealed distinct differences. The fishballs exhibited a compact protein gel structure with few pores. In contrast, mung bean protein-based fishballs displayed a cross-linked network-like gel structure with larger pores, contributing to the elastic properties of the gel matrix. Ran et al. (2022) suggested that reducing the pore size in plant-based fishballs can be

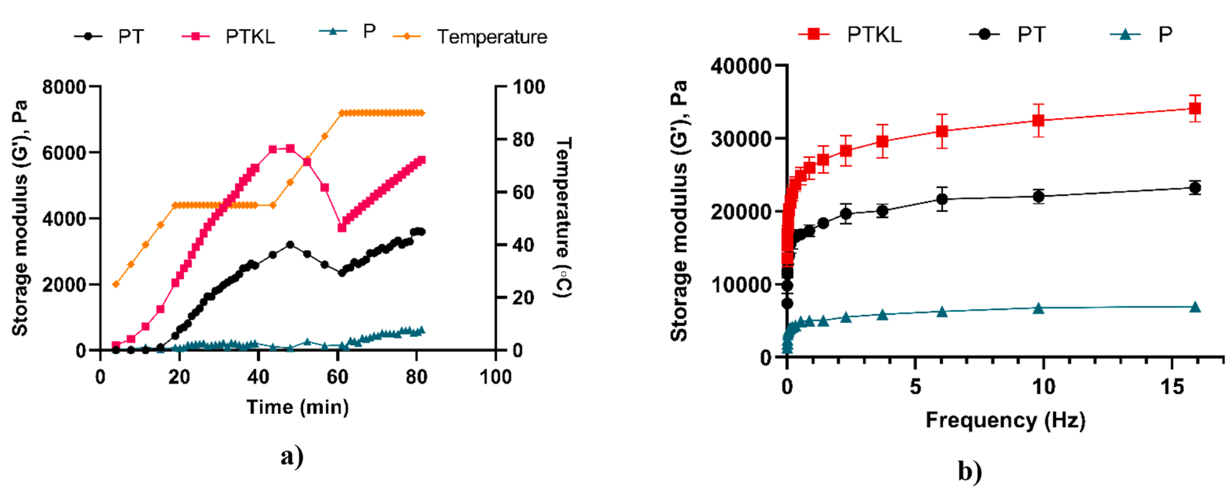

Fig. 4. Impact of mTgase and hydrocolloid addition on rheological properties a) Temperature sweep b) frequency sweep, P- mung bean protein isolate, PT – 1 % mTGase, PTKL – 1 % mTGase + 0.3 % KC + 0.13 % LBG.

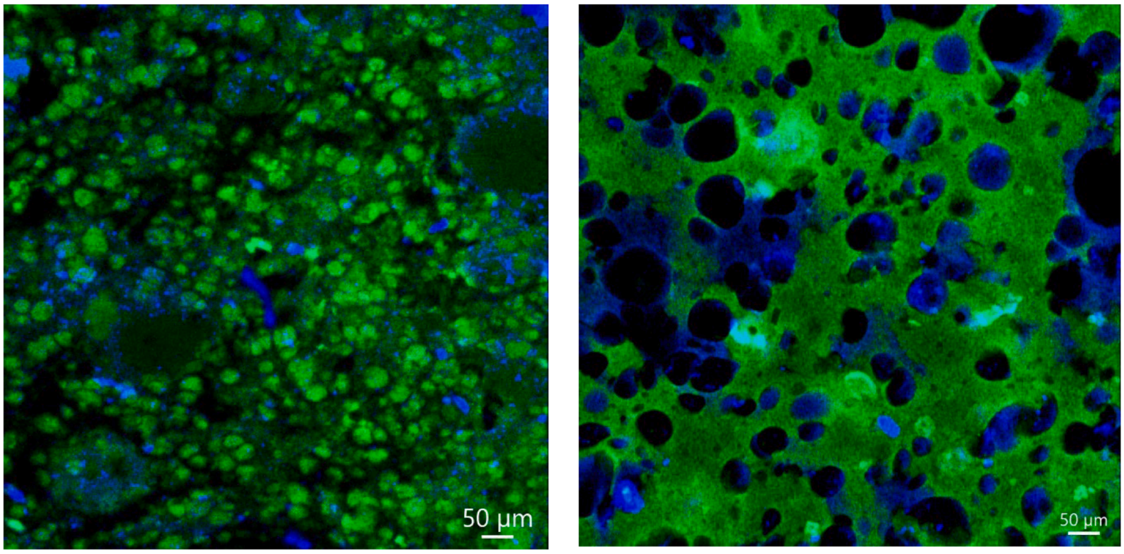

Fig. 5. Confocal laser scanning microscopy images using FITC (for protein) and calcofluor blue (for starch/hydrocolloids) – (a) Dodo fishball sample (b) mung bean protein-based fishball. Scale bar represents 50 µm.

achieved by adjusting the hydrocolloid amount to enhance gum-protein interactions.

Creep recovery tests were conducted to examine the rheological properties of the protein mixtures. In this test, instantaneous stress is applied, causing rapid deformation and an increase in strain over time; upon releasing the stress, the strain decreases (Wu et al., 2010). Recovery refers to the reduction in deformation (strain) over time after stress is removed. Creep compliance (J(t)) measurements were used to compare and determine the structural characteristics of different materials over a longer timescale. A higher creep compliance indicates a more sequential rupture of crosslinks during loading, suggesting that weaker molecular fragments may rupture (Herranz et al., 2013).

Chattong et al. (2015) found that gels with higher elasticity and firmness exhibit lower creep compliance. As shown in Fig. 6, the compliance increased dramatically during the creep phase and then decreased slowly during the recovery phase for all samples. The creep recovery curves of mung bean protein-based fishballs (PTKL) and commercial fishballs are shown in Fig. 6. The lower creep compliance observed in mung bean protein-based fishballs (PTKL) indicated a stronger network and greater resistance to deformation (Pang et al., 2022). This suggested that even if the texture profile properties of the plant-based fishballs match those of commercial fishballs, their resistance to deformation may differ. A nutritional analysis was essential to determine whether mung bean protein-based fishballs have comparable or superior nutritional value compared to conventional fishballs. **Table S2** indicated that mung bean protein-based fishballs offer a higher protein content (12.3 g) than surimi products (7.2 – 9.4 g) and are competitive with commercial vegetarian fishballs (0.9 – 13 g). Additionally, they provide a lower carbohydrate option (3 g) compared to surimi products (6.3 – 13.5 g) and fall within the lower range for commercial vegetarian fishballs (0 – 11 g). This makes mung bean fishballs a nutritionally appealing choice for consumers seeking high-protein, low-carbohydrate, plant-based alternatives.

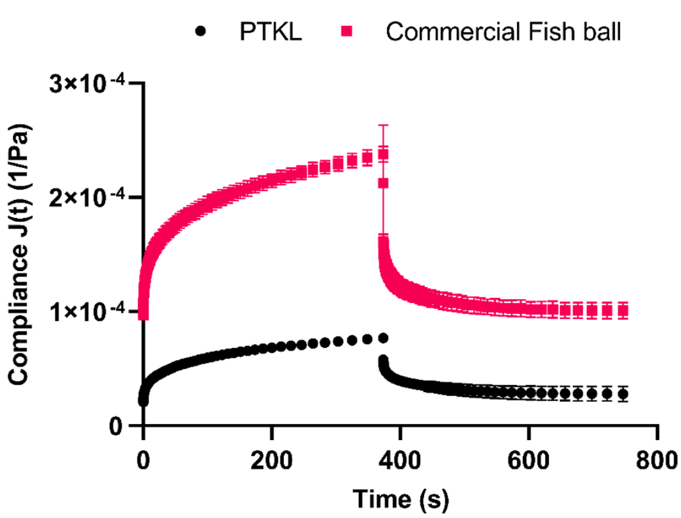

Fig. 6. Creep compliance of PTKL (mung bean protein-based fishball) against commercial fishball sample.

3.6. Secondary structures

The FTIR analysis (Fig. 7) highlights significant secondary structural changes in mung bean protein isolate (P control) and its treated forms (PT, PTK, PTL, PTKL), aligning with the literature. The β-sheet content increased significantly in PT (68.60 % ± 5.77), PTK (55.08 % ± 4.62), PTL (56.19 % ± 1.29), and PTKL (86.25 % ± 4.64) compared to the control (35.49 % ± 0.82). As tightly ordered structures, β-sheets enhance protein-protein interactions and promote gel formation (Feng et al., 2024b; Guo et al., 2024). The synergistic effect of mTGase and hydrocolloids in PTKL maximized β-sheet stabilization, contributing to a denser gel matrix (Li et al., 2019).

The random coil content decreased significantly in PTKL (3.24 % ± 0.20) and PT (3.67 % ± 0.30) relative to the control (15.16 % ± 0.56). Hydrocolloids reduce random coils, promoting structural order and stability (Lin et al., 2017; Sow et al., 2018). The low random coil content in PTKL enhances gel cohesiveness and network strength. The alpha-helix content decreased from the control (33.37 % ± 3.08) to PT (7.29 % ± 0.53), PTK (8.47 % ± 0.60), PTL (4.89 % ± 0.45), and PTKL (7.88 % ± 0.32). mTGase disrupts alpha-helices via cross-linking glutamine and lysine residues, converting them to β-sheets (Li and Xiong, 2015). Hydrocolloids in PTKL partially preserved alpha-helices, contributing to gel elasticity (Li et al., 2020).

The β-turn content was highest in PTK (22.93 % ± 1.90) and PTL (22.83 % ± 1.55) but lowest in PTKL (2.64 % ± 0.17). mTGase-mediated deamidation increases β-turns (Wang et al., 2017), but PTKL's higher β-sheet content optimizes gel stability and strength. These structural changes, particularly the high β-sheet and low random coil

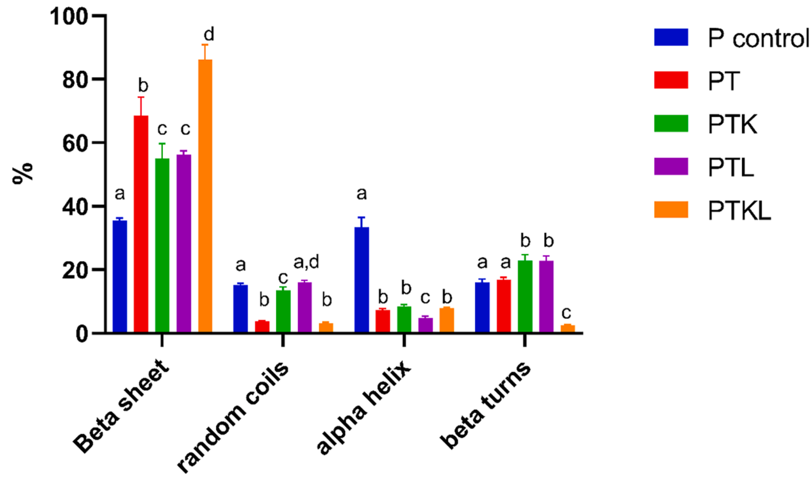

Fig. 7. Secondary structural composition (%) of mung bean protein isolate (P control) and its treated forms (PT: mTGase-treated, PTK: mTGase with κ-carrageenan, PTL: mTGase with locust bean gum, PTKL: mTGase with κ-carrageenan and locust bean gum) analyzed by FTIR. Treatments significantly altered β-sheet, random coil, α-helix, and β-turn content. Different letters indicate significant differences ($p < 0.05$) among samples for each secondary structure.

content in PTKL, correlate with its superior gel strength. β-sheets and reduced disorder enhance protein network stability and cohesiveness, resulting in a robust gel matrix (Zhou et al., 2017).*3.7 Consumer study*

The sensory evaluation of mung bean protein-based fishballs (MBP), commercial fishballs (COM), and vegetarian fishballs (Veg) was conducted with 61 participants in Singapore. The PTKL sample, containing 1 % mTGase, 0.3 % KC, and 0.13 % LBG, was selected as the mung bean protein–based fishball (MBP), as it demonstrated the most comparable textural properties to commercial fishballs (Section 3.5).

The evaluation included parameters such as appearance, aroma, taste, flavor, texture, and overall acceptability. The participants in this study were predominantly female (65.57 %), with a majority aged between 21–30 years (40.98 %), and of Chinese ethnicity (91.80 %). The distribution of family income among participants varied, with the highest proportion earning between 5001 SGD and 10,000 SGD per month (50.82 %). The study revealed that 67.2 % of participants consume fishballs at least once a week or twice a month, with only 18 % consuming them once a month or less frequently.The preferred method of consumption was predominantly boiling in soup (78.69 %). Only 6.56 % of participants reported consuming plant-based seafood, indicating a lower familiarity and potential bias towards traditional fishballs.

As shown in Fig. 8 **(a-e)**, participants preferred commercial fishballs over the other two samples for all sensory attributes. The mung bean protein-based fishballs closely followed, lower in most attributes except aroma. Vegetarian fishballs scored lower across all sensory parameters. In terms of appearance, commercial fishballs were preferred for their traditional look over mung bean protein-based fishballs (**Fig. S3**) or vegetarian fishballs.These differences were statistically significant as indicated by the lettering above the bars ($p < 0.05$).

The sensory evaluation demonstrated that mung bean protein-based

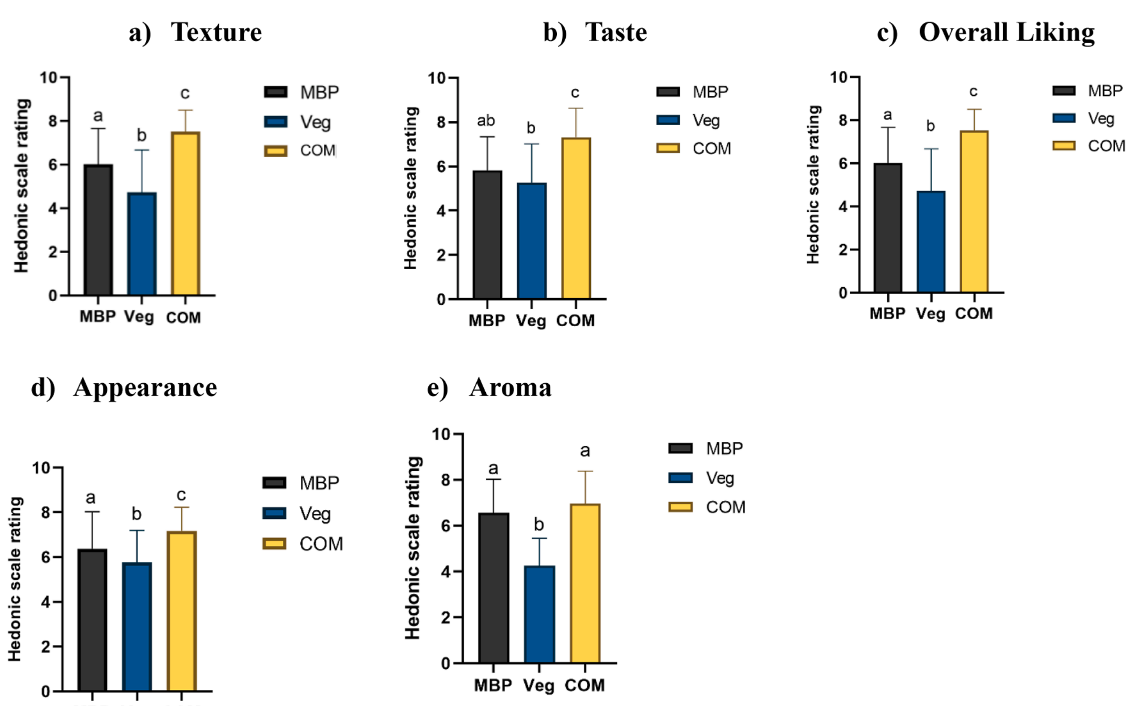

Fig. 8. Sensory evaluation results of MBP – Mung bean protein-based fishball, Veg – Commercial vegetarian fishball, COM- commercial fishball in terms of a) Texture b) Taste c) Overall Liking d) Appearance e) Aroma. Bars with different letters (a, b, c) indicate significant differences ($p < 0.05$).

fishballs have a high potential for consumer acceptance, as reflected in their positive mean scores: 6.38 for appearance, 6.08 for texture, 6.02 for overall liking, and 6.55 for aroma. These scores were significantly higher than those of the vegetarian fishballs ($p < 0.05$), indicating a clear advantage over this alternative, even though they were only comparable to commercial fishballs in aroma.

In recent years, growing health and environmental awareness has significantly influenced consumer expectations toward fish analogues. Plant-based seafood products are increasingly valued as healthier, sustainable, and protein-rich alternatives to conventional seafood. A study by Appiani et al. (2025) found that approximately 27 % of surveyed consumers accepted plant-based canned tuna when it was perceived as healthier or more natural, even if sensory performance was suboptimal.

Although not explicitly measured in the present study, the high protein and low-fat content of the mung bean protein–based fishballs was implicitly perceived as a positive health attribute. This perception aligns with consumer priorities for nutritional quality and realistic sensory characteristics in plant-based seafood. Moreover, broader consumer trends support this shift: Sogari et al. (2024) observed that individuals prioritizing healthy eating were more inclined toward plant-based alternatives.

The texture profile analysis demonstrated that mung bean protein–based fishballs fall within the range of commercial surimi and vegetarian fishball products, yet consumer evaluations revealed noticeable differences in texture, taste, and overall acceptability. While the optimized formulation (PTKL) showed favorable texture profile comparison to commercial surimi products, it did not fully replicate the sensory experience of traditional fishballs. Therefore, further formulation refinement is necessary—particularly to address flavor development, juiciness, and mouthfeel—to bridge the remaining sensory gap.

4. Conclusions

The mTGase and *k*-carrageenan addition significantly improved the textural properties and water binding capacity of mung bean protein-based fishballs, Optimal mTGase (1 %), KC (0.3 %) and LBG (0.13 %) concentrations produced a well-developed, cohesive protein network, enhancing gel strength and texture. In contrast, higher concentrations led to diminished benefits due to excessive crosslinking and network disruption. Locust bean gum, while increasing water retention, negatively impacted gel hardness and uniformity at higher concentrations. Nutritional analysis confirmed that mung bean protein-based fishballs offer a higher protein content and lower carbohydrate levels compared to conventional surimi products, making them a nutritionally appealing choice for health-conscious consumers.

Sensory evaluation revealed that mung bean protein-based fishballs were found acceptable by participants. The study supports the trend towards healthier food options, aligning with consumer preferences for high-protein, low-fat alternatives. The findings indicate that mung bean protein-based fishballs, optimized with mTGase, -carrageenan, have strong potential for market acceptance, providing a nutritious and sensory-pleasing plant-based seafood alternative. This study presented a clean-label formulation using mung bean protein, without synthetic additives or flavor enhancers. The synergistic use of KC and LBG in the presence of mTGase was systematically investigated to mimic the gelation and texture of traditional fishballs, which is a novel approach in the context of plant-based seafood.

Ethical compliance statement

The sensory study protocol was reviewed by the Singapore Polytechnic Institutional Review Board (IRB), which granted an exemption from full IRB review (Protocol No 202,106–02). This exemption was based on the study's minimal risk to participants, as it involved only sensory evaluation of food products without the collection of biological samples or medical interventions. The exemption was confirmed on 22 June 2021.

CRediT authorship contribution statement

Poornima Vijayan: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yige Zhou:** Methodology, Investigation. **Elen Ng Rui Xin:** Formal analysis, Data curation. **Ying Jie Chen:** Supervision, Software, Investigation, Funding acquisition. **Zhixuan Song:** Data curation. **Sixu Wang:** Methodology, Formal analysis, Data curation. **Dingsong Lin:** Formal analysis, Data curation. **Dejian Huang:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2025.100758.

Data availability

Data will be made available on request.

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