A study on physicochemical properties of protein gels and patties made from faba bean protein isolate and New Zealand *Perna canaliculus* concentrate

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Abstract

This study examines the physicochemical, textural, and microstructural characteristics of protein gels and patties made from faba bean protein isolate (FBPI) and New Zealand *Perna canaliculus* in the form of defatted mussel powder (DMP).

The initial investigation explored the influence of different proportions of FBPI and DMP proteins (100:0, 75:25, 50:50, 25:75, and 0:100 by weight) at a total protein concentration of 12.5% on the gelation process, water-holding capacity (WHC), texture, color, and microstructure of protein gels. Various methods such as rheology, confocal laser scanning microscopy (CLSM), particle size distribution analysis, and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were employed. The gelation temperature ranged from 20 °C to 90 °C, with the strength of the gel significantly affected by the FBPI to DMP ratio. The 50:50 protein mixture of FBPI and DMP proteins exhibited an increase in gel strength compared to gels made from either protein individually. The water-holding capacity (WHC) of the gels decreased as the amount of DMP increased, with the 50:50 protein ratio showing a slight improvement in WHC, suggesting better water retention. Textural analysis showed that the hardness of the gels decreased with higher DMP content, indicating a reduction in structural rigidity. Particle size distribution analysis indicated that smaller particles were associated with a denser gel network and higher storage modulus (G') values. Color analysis showed that the lightness of the gels decreased after heating, and the addition of DMP increased redness and yellowness. CLSM images revealed that FBPI formed a more continuous and denser gel structure than DMP, with the latter forming a weaker gel network. SDS-PAGE analysis offered insights into the protein composition and the effects of heat treatment on protein solubility and aggregation, showing that the solubility of proteins in the mixtures was influenced by intermolecular interactions, with different levels of solubility observed across various extraction solvents and protein ratios.

When not mixed together, FBPI and DMP exhibited distinct differences in their gelation properties. FBPI gels (100:0) demonstrated a higher gel strength and better water-holding capacity compared to DMP gels (0:100). FBPI gels also showed a denser and more continuous network structure, as observed through CLSM, while DMP gels formed a weaker and less

cohesive network. Additionally, FBPI gels had higher hardness values, indicating greater structural rigidity, whereas DMP gels were softer and more pliable.

The second study examined the development of plant and seafood blended patties by investigating the effects of varying FBPI and DMP protein ratios (100:0, 75:25, 50:50, and 0:100 w/w%) on texture, color, and physicochemical properties. The previous study found that a 50:50 protein blend of FBPI and DMP proteins was the best for maximizing gel strength and water-holding capacity. To further improve the processing attributes of the patties, the total protein content was increased to 15% by weight, and polysaccharides were added. The study focused on blended protein mixtures (75:25 and 50:50 w/w%) combined with polysaccharides to enhance the textural attributes and stability of the analogue patties. The research aims to assess how varying protein proportions and the inclusion of polysaccharides affect the texture, color, and physicochemical attributes of the patties. Adding polysaccharides, such as methylcellulose (MC) at concentration 2 ,3, 4 w/w% and konjac glucomannan (KGM) 3, 4, 5 w/w%, significantly influenced the texture, color, and physicochemical characteristics of the analogue patties. The study utilized various analytical methods, including texture profile analysis, color measurement, cooking loss analysis, and microstructure analysis, to assess the effects of different protein ratios and polysaccharide additions. The findings indicated that the inclusion of polysaccharides can improve the patties texture, reduced cooking loss, and enhanced shape retention and structural integrity after cooking. Additionally, the color analysis revealed changes in color attributes before and after cooking, with higher DMP content leading to increased redness and yellowness. The microstructure analysis demonstrated that polysaccharides can create a denser and more uniform matrix within the patties, enhancing their overall quality. The research offered significant findings for the food sector, guiding the development of sustainable and nutritionally rich protein substitutes that align with consumer preferences for nutritional content, texture, and appearance. It highlighted the promise of integrating FBPI and DMP, along with polysaccharides, to produce blended patties with favorable characteristics. Notably, the 50:50 protein blend performed exceptionally well in the patties, demonstrating improved gel strength, water-holding capacity, and overall textural quality, making it a promising candidate for further development and commercialization.

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FBPI	Faba bean protein isolate
DMP	Defatted green-lipped mussel powder
CLSM	Confocal Laser Scanning Microscopy
DLS	Dynamic light scattering
G'	Storage modulus
G''	Loss modulus
LVR	Linear viscoelastic region
Fig.	Figure
Min	Minute
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
WHC	Water holding capacity
TPA	Texture profile analysis
SEM	Scanning Electron Microscopy
DTT	Dithiothreitol
KGM	Konjac Glucomannan
MC	Methylcellulose

1.0. Chapter 1 : Introduction

1.1. Overview of the research

Nowadays, consumers are increasingly prioritizing sustainability and seeking ways to minimize their environmental impact. One significant way many are choosing to do this is by transitioning toward a more plant-based dietary approach. For example, in Denmark, the percentage of the population identifying as vegetarians increased from 1.8% in 2017 to 3% in 2022, with a higher rate of 7.4% among the 18 to 34 age group (Genet et al., 2023). This shift in dietary habits is supported by the increasing accessibility of plant-derived alternatives, such as non-dairy yogurt, dairy-free milk, and meat substitutes (Mehany et al., 2024; Montemurro et al., 2021; Zioga et al., 2022). Furthermore, plant-derived alternatives like egg, ice cream, and fish alternatives are currently in the initial phases of development (McClements & Grossmann, 2021).

More people are embracing flexitarian diets, choosing to eat less meat and exploring plant-based alternatives for concerns regarding animal welfare, environmental sustainability, and health advantages. This approach, which emphasizes plant-based foods while allowing for moderate meat consumption, offers a pragmatic middle ground for those who find the transition to a fully vegetarian or vegan lifestyle challenging (Lang, 2020). At present, the limited adoption of sustainable plant-derived meat alternatives is mainly attributed to difficulties in product formulation, which impact key sensory qualities including texture and taste (Michel et al., 2021). While taste plays a major role in food choices, meat consumption is also driven by emotional connections and social factors, as it is often perceived as the best source of protein and an essential part of traditional family meals (Salgaonkar & Nolden, 2024). A promising approach to enhance the acceptance of plant-based meat alternatives involves the development of hybrid meat or seafood products that combine plant-derived ingredients with animal meat in various proportions to improve both texture and flavor (Rajagopal & Burnkrant, 2009; Spencer & Guinard, 2018). This approach narrows the gap by matching the sensory qualities of plant-based alternatives with those of conventional meat, making the shift to plant-based options more enjoyable and attractive for consumers (Neville et al., 2017).

In this evolving landscape, hybrid protein options have become an innovative option, meeting the needs of consumers who want to savor the texture and flavor of meat while embracing the benefits of plant-derived ingredients (Spencer et al., 2018). Plant-based ingredients, especially soy and wheat, have been used by the meat industry for years, not just to reduce costs but also for their functional benefits, including fat emulsification, water retention, and gelling properties (Asgar et al., 2010; Singh et al., 2008). These products feature a range of plant-derived ingredients, such as fruits, pulses, grains, and vegetables, are designed to offer the best of both worlds. By blending the well-known texture and flavor of meat with the health benefits of plant-based ingredients, hybrid products can make health and sustainability more accessible to a wider audience (Grasso & Jaworska, 2020). The market has already seen the launch of several hybrid meat products across different countries, including the UK, Denmark, Austria, and the US. These products span a range of categories, from sausages and burgers to meatballs and mince, and have shown promise in terms of consumer acceptance. Research suggests that hybrid products might attract more consumers than purely plant-based options, potentially due to their ability to mimic the sensory qualities of meat more closely (Grasso et al., 2022).

Moreover, the ecological advantages of hybrid meat products are significant. Studies indicate that by replacing a portion of meat with plant proteins, it is possible to achieve substantial reductions in emissions of greenhouse gases (Baune et al., 2021). This reflects the growing consumer awareness and concern about the pandemic's lasting impact on food sustainability and the broader food industry. Hybrid meat products stand out as a promising advancement in the evolving discussions about food sustainability, potentially having a significant impact on the shift towards more sustainable and health-conscious eating practices By addressing both the sensory and ethical dimensions of meat consumption, these products offer a tangible pathway towards a more balanced and environmentally friendly future (Boyacı-Gündüz et al., 2021).

Wheat gluten and soy protein are frequently used in plant-based meat alternatives due to their well-known gel-forming properties, as different proteins exhibit varying gelation characteristics (Tan et al., 2023). The quality and yield of meat products are determined by the gelation properties of myofibrillar proteins (MPs). Changes to MP functionality, brought about by either natural or artificial covalent modifications that affect protein structure and aggregate formation, can lead to varied effects on the final product's quality (Zhao et al., 2021). In

addition, the gelation ability of proteins is vital for the production of various food items, including hybrid meat analogues. This process entails proteins unfolding and then aggregate together to form a three-dimensional network that holds water and other components, resulting in a gel-like texture. This property is particularly important for mimicking the consistency and sensory experience of meat, which is often characterized by its ability to retain moisture and exhibit a certain degree of chewiness (Lu et al., 2024). Several factors affect a protein's ability to form gels, including the protein type, its concentration, the inclusion of other ingredients, pH levels, and processing conditions like temperature and time (Akharume et al., 2021). In the context of hybrid meat products, the gelation property of proteins is exploited to create a matrix that can bind both plant-based and animal-derived proteins together. This allows for the creation of products that have a texture similar to that of meat, which is crucial for consumer approval (Arora et al., 2023). By manipulating the gelation process, can adjust the texture and composition of these products to fulfill specific criteria, thereby enhancing their sensory characteristics and overall quality.

In this study, the focus was on faba bean and green-lipped mussel, exploring their unique gelation properties and how these can be leveraged to create innovative blended products. Legumes are regarded as the most traditional, nutrient-rich, and accessible plant-based protein sources (Neacsu et al., 2017). Among legumes, the faba bean (*Vicia faba L.*), commonly referred to as the broad bean, horse bean, or fava bean is notable as one of the oldest crops cultivated worldwide (Mínguez & Rubiales, 2021). It offers a high protein content, between 20% and 41%, and is rich in essential amino acids like lysine, arginine, and leucine (Millar et al., 2019; Walter et al., 2022). In addition to their amino acid profile, faba beans contain bioactive compounds that are significant for both nutrition and medicine, especially in preventing and managing chronic noncommunicable diseases (Abu-Reidah et al., 2017). Faba beans are an important component in a diverse range of food products due to their substantial protein content, essential amino acids, bioactive compounds, and dietary fiber. They offer numerous health advantages and serve as an economical source of nutrients for both animal and human food industries (Barbosa et al., 2024). Faba beans are increasingly valued by the food industry for their nutritional benefits and sustainability as a vegetable protein source. They are being used to create protein concentrates and isolates for food supplements and incorporated as partial or complete protein substitutes in various products. This includes applications in pasta, biscuits, mayonnaise, sausages, and other items, demonstrating their versatility and nutritional advantages in modern food production. Additionally, faba beans are

being developed into products designed for nutritional enrichment and convenient, everyday use (Dangi et al., 2022; Vioque et al., 2012).

Seafood is commonly recommended for its high nutrient content and beneficial effects on health, including cardiovascular improvements, reduced inflammation, and enhanced immunity (Willett et al., 2019). In the last 50 years, there has been an increase in global seafood consumption, driven by rising population numbers and the growth of aquaculture (Marwaha et al., 2020). Among the diverse seafood options, the New Zealand green-lipped mussel (*Perna canaliculus*), also known as kutai or kuku, stands out as a culturally significant bivalve mollusk from the Mytilidae family. Recognizable by its green edge and typically reaching about 200 mm in size (Murphy et al., 2002), this species is harvested for both recreational and commercial purposes and serves as a model indicator species in New Zealand (Chandurvelan et al., 2016). Valued for its rich content of omega-3 fatty acids, protein, iodine, and carbohydrates, the green-lipped mussel is also praised for its peptides, amino acids and bioactive proteins, which offer anti-inflammatory, antibacterial, antiarthritic, and anticancer benefits (Grienke et al., 2014).

The research began with a detailed examination of the gelation properties of faba bean protein isolate (FBPI) and defatted green-lipped mussel powder (DMP), which was essential for understanding how to combine these proteins to replicate meat's texture and functional qualities. Building on this foundation, the study then focused on developing blended seafood patties that incorporate FBPI and DMP, aiming to create a sustainable and nutritious alternative to traditional seafood patties. To ensure these new products meet quality and market standards, comprehensive analyses, including Texture Profile Analysis (TPA) and color evaluation, were conducted. These assessments not only evaluate the sensory characteristics of the patties but also compare them to commercially available plant-based patties, checking their texture and visual appeal. However, there are no existing studies that explore the gelation properties of FBPI and DMP in the context of blended patties. This gap in research underscores the novelty and potential impact of the current study, which aims to pioneer the exploration of these proteins' gelation capabilities and their application in the creation of sustainable blended meat products.

1.2. Research objectives

The objectives of this research were:

- To investigate the impact of varying ratios of faba bean protein isolate (FBPI) and defatted green-lipped mussel powder (DMP) on the microstructure and rheological properties of protein gels.

- To develop plant and seafood blended patties by optimizing the ratios of faba bean protein isolate (FBPI), defatted green-lipped mussel powder (DMP) and polysaccharides to achieve texture and color properties comparable to commercial patties.

1.3. Thesis structure

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2.0. Chapter 2 : Literature Review

2.1. Faba bean *(Vicia faba)*

Chickpeas, lentils, and beans are widely recognized for their nutritional, economic, and ecological advantages. Among them, the faba bean (*Vicia faba L.*) is an exceptional yet underutilized source of sustainable, premium protein, due to its rich protein content and various agronomic advantages (Erbersdobler et al., 2017). This versatile legume has been cultivated for centuries, primarily in the Mediterranean and Middle Eastern regions. Its significance as a food and feed legume is underscored by its ability to thrive in cool weather conditions and its classification into three main subtypes based on seed size: minor, equina, and major. The lack of identified wild forms of faba bean suggests that its genetic diversity is more influenced by geographical isolation than by botanical traits (Etemadi et al., 2018). The faba bean, known for its high nutritional value, is non-genetically modified (non-GMO) and is not classified as a major allergen. These qualities give it an edge over soy, which is often genetically modified and a common allergen (Calabrò et al., 2014).

The faba bean seed consists of the seed coat, which represents 15% of the total seed weight, the two cotyledons, making up 84%, and the embryo, comprising 1% (Karataş et al., 2017). The cotyledons are the main source of protein (25–32%) and carbohydrates (40–50%) (Mattila et al., 2018). In contrast, the seed coat is thick and fibrous, housing the majority of the seed's phenolic compounds, including tannins and minerals (Karataş et al., 2017). The weight of 100 seeds varies by cultivar, ranging from 21 g to 196 g, and their dimensions span from 10 × 13 mm to 20 × 30 mm (Santos et al., 2022). Additionally, faba bean seeds can be found in various colors, including white, green, and brown (Santos et al., 2018).

In modern agriculture, faba bean has found a new role as a cover crop, valued for its exceptional ability to fix nitrogen, which surpasses that of other pulses. This trait, combined with its high protein content and benefits in crop rotation, has driven a significant increase in its global production over the past two decades. The Food and Agriculture Organization (FAO) has acknowledged this growing trend, highlighting the increasing relevance of faba bean in global agriculture (Khazaei et al., 2019). With their high protein and fiber content and low fat levels, faba beans are an excellent ingredient for plant-based food products. Their primary

storage proteins are globulins, which constitute 69.5% to 78.1% of the total protein content, similar to other pulses (Stone et al., 2024). In faba bean seeds, globulins account for over 80% of the total protein content. These globulins are categorized into two types based on their sedimentation properties: legumin-type (11S) and vicilin-type (7S) (Warsame et al., 2018). With the rising demand for plant-based proteins, faba bean presents a sustainable and abundant source of these essential nutrients. Its utilization in the food industry not only contributes to dietary diversity but also supports environmental sustainability through its nitrogen-fixing capabilities and efficient use as a crop rotation option.

2.1.1. Faba bean protein

Rahate et al. (2021) found that faba beans are notable for their high protein content, which is roughly twice that of cereal grains. The protein composition in faba beans includes several fractions, with globulins being the most prevalent at 60%, followed by albumins at 20%, glutelins at 15%, and prolamins at 8%. Faba beans contain between 20% and 41% total protein, reflecting significant variability. This variation can be attributed to several factors, including varietal differences, the type of source (flour, fraction, or isolate), and agricultural practices such as fertilization technique, planting site and growing season (Multari et al., 2015; Yang et al., 2018).

In cereals, prolamins are key storage proteins, whereas in legumes, globulins serve as the primary storage proteins. These proteins are essential in defining the rheological and textural characteristics of legumes. In faba beans, the storage proteins are located in the seed cotyledons and remain enzymatically inactive, supplying vital nutrients for both seed germination and seedling development (Warsame et al., 2018). The storage protein in faba beans is composed of two primary subunits: globulins and albumins. In faba beans, globulins are characterized by their 7-11 S fractions. Globulins in faba beans are low in amino acids with sulphur lack, especially cysteine and methionine. Moreover, albumins are another type of proteins present in faba beans, are richer in sulphur-containing amino acids, such as methionine and cysteine. This enhances their value component of the protein fraction in faba beans, helping to mitigate the deficiency of these amino acids found in globulins. Albumins in faba beans contain trypsin inhibitors and phytolectins. Trypsin inhibitors are proteins that prevent trypsin, an enzyme important for digesting proteins, from functioning effectively (Rahate et al., 2021).

Incorporating plant protein concentrates or isolates into product formulations offers significant advantages, both nutritionally and functionally. These proteins are made up of a diverse range of polypeptide units, with molecular weights spanning from 10 to 150 kDa (kilodalton). In faba beans, the 7S globulin has a molecular weight between 48 and 75 kDa, while the 11S globulin ranges from 20 to 30 kDa. Both types of globulins are composed of acidic and basic subunits, which contribute to their structural and functional diversity (Sivasankari et al., 2019). Although lower in sulphur-rich amino acids, faba bean proteins still supply essential amino acids and offer functional benefits like emulsification, gelation, and water-holding capacity, making them versatile for various food applications (Vojtíšková et al., 2010).

Plant proteins, in comparison to animal proteins, typically lack sulphur-containing amino acids like methionine and methionine, which reduced their overall protein quality. Faba beans, however, provide essential amino acids such as leucine, isoleucine, methionine, lysine, phenylalanine, tyrosine, histidine, valine, threonine and tryptophan, along with non-essential ones like glutamic acid, aspartic acid, arginine, alanine, proline, serine and glycine. The amino acid composition of faba beans can vary depending on the specific bean variety (Nosworthy, 2020).

2.1.2. Nutritional properties

Faba beans are used worldwide for consumption, livestock feed, fodder, and therapeutic applications. They are a rich plant-based protein source, providing around 30% protein, particularly high in lysine. Beyond protein, faba beans offer essential nutrients, dietary fiber, and bioactive compounds like γ-aminobutyric acid (GABA), antioxidants and phenols, all of which enhance their health advantages and nutritional benefits (Khazaei et al., 2019).

Faba beans are a nutrient-rich legume with carbohydrate content ranging from 51% to 68%. However, starch is the predominant form of carbohydrate in faba beans, accounting for 41–58% of the total carbohydrate content (Dhull et al., 2022) . Faba beans are abundant in dietary fiber, which aids digestive health by facilitating regular bowel movements, reducing disease risk, and aiding in satiety. However, they also contain considerable amounts of soluble sugars, such as oligosaccharides like stachyose, verbascose, and raffinose which are

indigestible and may cause flatulence. These oligosaccharides can limit faba bean consumption for individuals with sensitivities or digestive concerns (Labba et al., 2021). Alternatively, soaking or cooking faba beans can help reduce the levels of these oligosaccharides and make them more digestible (Njoumi et al., 2019).

Faba beans are rich in starch, which makes up between 22% and 45% of their composition. This starch primarily consists of two key components: amylopectin and amylose (Punia et al., 2019). Faba bean starch granules exhibit a variety of shapes, featuring round, oval, elongated, and uneven shapes, with surface cavities. These structural features have been identified through the use of scanning electron microscopy (Sofi et al., 2013). The solubility and swelling power of faba bean starch are relatively low, at 9.92 g/100 g and 12.67 g/g, respectively. This is attributed to the strong binding forces that integrate the starch granules, making them less soluble and less prone to swelling when exposed to water (Zhang et al., 2019). Faba bean starch is notable for its resistance to enzymatic hydrolysis. This resistance is reflected in its high content of resistant starch (RS), which constitutes 46.7% of the total starch. In contrast, the starch contains only 15.3% rapidly digestible starch and 34.5% slowly digestible starch. The high RS content indicates that a significant portion of the starch in faba beans is not easily broken down by digestive enzymes, contributing to its slow digestion and absorption in the human body (Bello-Pérez et al., 2007).

According to Singh et al. (2013), faba beans are a rich source of both soluble and insoluble dietary fiber. In a comparison with flours from other beans such as pinto, red kidney beans, and lima, faba bean flour (FBF) was found to have the highest fiber content (Gu et al., 2020). Whole faba beans provide dietary fiber ranging from 15% to 30%, primarily composed of hemicellulose, with cellulose and lignin also present. The seed coat of faba beans is particularly notable for its high dietary fiber content, reaching up to 82.3%, making it a valuable source of phenolic compounds, minerals and fiber. To fully benefit from these nutrients, it is advisable to consume faba beans with their seed coat intact (Karataş et al., 2017).

2.1.3. Methods for the production of protein isolates

Within food sector, producing protein isolates is essential for concentrating proteins from diverse sources, which improves their functional characteristics and nutritional benefits. Protein isolates are usually produced through two main methods, which are dry extraction and wet extraction techniques (Stone et al., 2015).

2.1.3.1. Dry extraction

Dry fractionation is a process where legume seeds rich in starch are ground and then separated using air classification, yielding different products such as flours, enriched flours (with 40-50% protein), and protein concentrates (with 60-80% protein). This air classification sorts particles by size, shape, and density within an airstream and can be optimized through multiple rounds to improve separation accuracy. Prior removal of the seed coat can enhance the purity of the protein and reduce fiber content.According to Coda et al. (2015), applying pin milling and air classification to dehulled faba bean seeds produced two separate fractions, with the lighter fraction containing 51.49% protein, 23.38% starch, and substantial amounts of ash, fat, and dietary fiber. The heavy, coarse fraction contained 16.73% protein, 65.82% starch and had a low-fat content. Dry fractionation has the advantage of less impact on the original protein structure and requires less energy and water, but it may result in lower protein purity due to contamination between fractions (Boye et al., 2010).

2.1.3.2. Wet extraction

Wet extraction of plant proteins involves dissolving the proteins in solvents like alkali, acid, or water, followed by separation through centrifugation and precipitation. The specific extraction process is influenced by factors such as ionic strength, pH, temperature, and solvent type. This method is often used in laboratories to produce protein concentrates with over 60% protein content and isolates with more than 90% protein content (Stone et al., 2015).

a) Alkaline extraction-isoelectric precipitation (AE-IP)

AE-IP exploits the impact of pH on protein solubility. At the isoelectric point (pI), proteins have minimal electrostatic repulsion and the lowest solubility (Klupšaitė & Juodeikienė, 2015). For most plant globulins, the pI is within the pH range of 4-5 (Boye et al., 2010). Proteins are dispersed in alkaline solutions (pH 8-11) to increase solubility, followed by centrifugation to remove insoluble components. The supernatant's pH is then adjusted to the pI or close to it, causing proteins to precipitate and become neutralized. According to Karaca et al. (2011), protein isolates obtained through alkaline extraction and isoelectric precipitation (AE-IP) exhibited a higher overall protein content (85.6%) compared to those made with salt extraction (78.4%) across various legumes such as lentil, faba bean, chickpea, pea, and soybean. This observation aligns with Stone et al. (2015) on pea protein isolates (PPI), which also showed higher protein content using AE-IP. The alkaline extraction step helps to solubilize the proteins by breaking the interactions that hold them within the plant matrix, while the isoelectric precipitation step allows for the selective precipitation of proteins at their isoelectric point, where they are least soluble.

b) Salt extraction

In protein extraction, salts leverage their ability to dissolve legume and pulse proteins, with different ionic strengths affecting the process (Muranyi et al., 2016). Commonly used salts include ammonium sulfate, sodium chloride, and calcium chloride. The extraction process involves two main steps: initially, the salting-in process uses salts with low ionic strength (less than 0.15 M) to enhance protein solubility without denaturing them. This is followed by the salting-out process, where a high ionic concentration salt (around 5 M) is used to aggregate and precipitate the dissolved proteins (Jiang et al., 2021). The resulting protein extracts are then separated from carbohydrates and insoluble fibers, diluted further with cold water, and subjected to additional purification steps (Lam et al., 2018). Karaca et al. (2011) found that salt extraction yielded lower protein content for FPI (81.98%) compared to AE-I (84.14%) SE has been shown to cause less denaturation than AE-IP and to yield PPI with superior functional properties.

c) Ultrafiltration

Ultrafiltration (UF) is a membrane filtration technique that relies on molecular size to separate proteins without the need for heat, preserving their functional integrity (Klupšaitė & Juodeikienė, 2015). It has gained popularity as a viable alternative to isoelectric precipitation, particularly when used after alkaline extraction to further refine and purify proteins. UF, when combined with diafiltration (DF), can significantly enhance both the recovery and purity of protein isolates, resulting in a higher-quality end product (Singhal et al., 2016). The method's efficiency in producing protein-rich concentrates was demonstrated by Boye et al. (2010), who reported that UF-extracted protein concentrates from legumes such as peas, chickpeas, and lentils contained higher overall protein levels compared to other extraction methods. Furthermore, Fuhrmeister and Meuser (2003) observed that concentrates derived from wrinkled peas using UF achieved a protein content of 70-80% and a reduced fat content of 2.3%. In comparison, isolates obtained through isoelectric precipitation had a slightly lower protein content of 68% and a higher fat content of 3.8%. This demonstrates the superiority of UF in not only maximizing protein yield but also in producing a lower-fat, more nutritionally balanced product. Additionally, the ability of UF to avoid the use of extreme pH adjustments or high temperatures, common in other extraction techniques, helps maintain the functional properties of proteins, making them more suitable for various food applications. Thus, UF has become an increasingly important method within food sector for producing high-quality, protein-rich concentrates and isolates.

In summary, selecting the most appropriate extraction method for producing protein isolates involves considering several factors, including the functional properties desired in the final product, its nutritional composition, and the extent to which anti-nutritional compounds must be reduced. Each extraction technique offers distinct benefits and drawbacks, such as differences in efficiency, purity, and impact on protein functionality. Therefore, the decision on which method to use typically depends on the specific goals of the formulation, the application of the end product, and the inherent characteristics of the protein source being processed. For instance, wet extraction methods may be favored for higher protein purity, while dry fractionation could be chosen for cost-effectiveness and scalability. Ultimately, the balance between nutritional value, functionality, and processing efficiency will guide the selection process, ensuring that the chosen method aligns with the targeted product outcomes.

2.1.4. Technofunctional properties of legume proteins in food applications

Legume proteins are essential for determining the performance of various food products, as they affect sensory attributes, physicochemical properties, and structural integrity, all of which contribute to consumer acceptance and market success. These proteins' functional characteristics depend on numerous variables, including the choice of raw ingredients, the techniques used for their extraction, and the production conditions they undergo (Multari et al., 2015). Here are some key aspects of legume protein in food applications:

a) Protein solubility

Protein solubility is a fundamental factor influencing the functional properties of proteins in food systems, as it directly affects their capacity to form emulsions, gels, and other structural components. Several factors, including the hydrophilic-lipophilic balance, isoelectric point, ionic strength, temperature and pH, significantly affect protein solubility. Fernández-Quintela et al. (1997) reported that faba bean protein isolate exhibited optimal solubility at a pH of 9, which is comparable to soybean protein isolate and higher than that of pea protein isolate. This enhanced solubility renders faba bean proteins particularly suitable for applications requiring efficient water dispersion. Setia et al. (2019) also reported that faba bean flours demonstrated a protein solubility of 78.1%, a characteristic that enhances their functionality in food products. Additionally, processes such as soaking and germination further increased solubility, attributed to the activity of proteolytic enzymes that break down proteins into smaller, more soluble peptides. This increased solubility improves the versatility and functional potential of faba bean flours in a variety of food formulations.

b) Water-binding capacity (WBC) and oil-binding capacity (OBC)

The WBC and OBC of faba bean proteins play a crucial role in preserving the consistency, flavor, and sensory experience of food products. WBC refers to the ability of proteins to absorb and retain water, with retained water being essential for maintaining the integrity of the protein structure. OBC, on the other hand, is associated with the interaction between fats and the hydrophobic, non-polar amino acids found on the surface of the protein molecules (Kiosseoglou & Paraskevopoulou, 2021). Fernández-Quintela et al. (1997) found that faba bean protein isolate had a higher WBC compared to pea and soybean protein isolates. This superior water-binding ability is attributed to the structural properties of faba bean proteins, which allow them to form strong interactions with water molecules. The WBC is

further enhanced by processes such as soaking and germination, as shown by (Setia et al., 2019). These processes weaken the association between starch and protein/fiber, exposing more hydrophilic groups that can interact with water. This physical change not only improves the WBC but also enhances the overall functional properties of faba bean flours, making them more versatile in food formulations.

The oil-binding capacity (OBC) of faba bean proteins is another key functional attribute that has been widely examined. Research by Eckert et al. (2019) reported a notably high OBC of 6.12 g/g for faba bean protein, highlighting its strong capacity to absorb and retain oil within a food matrix. This property is particularly beneficial in emulsion-based products, where stabilizing oil droplets is crucial for maintaining texture and consistency. The high OBC of faba bean protein isolates, as demonstrated by Vioque et al. (2012), is attributed to the partial denaturation of proteins during the isolation process. This denaturation exposes hydrophobic residues, which favor oil binding by interacting with the non-polar components of oil. Similarly, Setia et al. (2019) studied the OBC of faba bean flour, finding a value of 1.5 g/g. While lower than that of isolates, it still demonstrates significant oil-binding capacity. They concluded that soaking and germination did not notably impact OBC, indicating that the isolation process itself plays a more crucial role in determining this functional property.

c) Foaming

Foaming abilities are vital for applications in products such as sweet treats, confections, whipped creams, frozen desserts, and leavened baked items (Eckert et al., 2019). Foams are formed when proteins unfold and absorb at the boundary between air and water, forming a film around the air bubbles. The effectiveness of a protein in creating and stabilizing foams is influenced by its physical properties, with solubility being a major determinant. For a protein to efficiently form a film and establish strong bonds—such as hydrogen and hydrophobic interactions—it needs to have high solubility. These bonds are essential for preserving the foam's structure. (Klupšaitė & Juodeikienė, 2015). Eckert et al. (2019) found that poor foaming capacity (FC) values for faba bean protein, with 31.2% at pH 5 and 66.7% at pH 7. These values were lower compared to pea protein (167.4% at pH 5 and 243.7% at pH 7) and lentil protein (403% at pH 5 and 425% at pH 7). The low FC of faba bean protein can be linked to its lower solubility, which hinders the rapid formation of a protein film around air bubbles. However, they found good foam stability (FS) for faba bean protein, with 83.8% at pH 5 and 76.7% at pH 7 of the foam remaining after 30 minutes of storage at room temperature. Despite

the lower FC, the foam formed by faba bean protein was resistant to stress and could maintain its volume. Setia et al. (2019) also found a stronger FC for raw yellow pea flour compared to faba bean flour, attributing the difference to the poor solubility of faba bean protein. They demonstrated that soaking did not affect the FC, but germination elevated it. This improvement can be attributed to the alteration in protein conformation during germination, which enhances the protein's ability to form films around air bubbles. However, they observed no notable difference in foaming stability (FS) between raw yellow pea flour and faba bean flour. They proved that soaking did not affect the FS, but germination elevated it, similar to the FC. The relationship between the emulsifying activity index (EAI) and emulsifying stability index (ESI) with foaming capacity (FC) and foaming stability (FS) indicates that alterations in protein structure and physicochemical characteristics affect both emulsifying and foaming abilities.

d) Emulsifying

Proteins are essential in the formation and stabilization of emulsions, as they create electrostatic repulsion on the oil surface. This capability is influenced by the balance between their hydrophilic and hydrophobic properties, which is determined by their amino acid composition (Klupšaitė & Juodeikienė, 2015). The emulsifying activity index (EAI) assesses the stabilized surface area per unit weight of protein by measuring the turbidity of a diluted emulsion, while the emulsifying stability index (ESI) evaluates the emulsion's stability over time under specific conditions. Both indices are used to determine a protein's effectiveness in forming and maintaining emulsions. Eckert et al. (2019) found that faba bean protein has a relatively low emulsifying activity index (EAI) of 36.4 m^2/g at pH 7, which is lower than the EAI values of proteins from pea, lentil, and chickpea, as previously reported by Karaca et al. (2011). This lower EAI in faba bean protein is likely due to its limited solubility and compact structure, which make it less effective at adsorbing onto the oil-water interface and forming stable emulsions (Eckert et al., 2019). In contrast, Setia et al. (2019) discovered that while raw yellow pea flour had lower EAI and emulsifying stability index (ESI) values compared to raw faba bean flour, soaking did not significantly alter these properties. However, germination improved both EAI and ESI, likely because the germination process causes partial unfolding and dissociation of proteins, enhancing their ability to interact with and stabilize emulsions.

2.2. The green-lipped mussel, *Perna canaliculus*

Mussel meat is a highly sustainable and environmentally friendly protein source, offering a nutritious and affordable option for human consumption (Chen et al., 2023). In 2020, mussels, a significant type of shellfish, were produced at a volume of 2 million tons, highlighting their potential as a sustainable option compared to meat, poultry, and fish. Mussel farming is linked to low greenhouse gas emissions and is considered non-polluting, with little adverse effect on the environment and biodiversity (Rebouças et al., 2023; Venugopal & Gopakumar, 2017). This type of farming does not necessitate the use of antibiotics or feed, and it generates only 0.6 kg of CO2 per kilogram of edible product. The environmental advantages of mussels are spurring increased interest in their meat as a viable and eco-friendly protein source (Suplicy, 2020).

The New Zealand green-lipped mussel, scientifically referred to as *Perna canaliculus* and trademarked as Greenshell™ mussels, is a unique bivalve species that has garnered attention for its distinctive features and potential health benefits. This mussel is larger than the commonly known blue mussel (*Mytilus edulis*) and boasts a green-lipped shell, making it easily recognizable. Its commercial farming and wild harvesting contribute significantly to New Zealand's economy, with exports reaching a substantial value in 2021 (Miller et al., 2023).

Renowned for their richness in essential nutrients, including vitamins, minerals, iron, lipids, and proteins, green-lipped mussels are especially valued for their high content of Omega 3 polyunsaturated fatty acids, which are linked to potential anti-inflammatory benefits. Lipid extracts from these mussels, such as those used in products like Lyprinol, are widely marketed for their anti-inflammatory properties (Webb, 2017).

Although mussel powder offers well-documented health benefits, its use in food products has been constrained mainly due to its intense and often unpleasant flavor, primarily caused by lipid oxidation. However, incorporating mussel powder into food products, such as biscuits, has shown promising results in terms of digestibility (Klunklin & Savage, 2018). Fortified biscuits have demonstrated a lower Glycemic Index (GI), a phenomenon attributed to the interaction between the mussel proteins and the starches in the biscuits. This interaction forms a protective matrix that slows down the digestion process, reducing the rate of digestibility (Klunklin & Savage, 2018).

The New Zealand green-lipped mussel is a valuable species that offers both economic and health benefits. Its unique characteristics and nutritional profile position it as a significant player in the seafood industry, with potential applications in food and nutraceutical products. While challenges such as off-flavor and lipid oxidation exist, innovative approaches to incorporating mussel powder into food products could unlock its full potential, offering consumers health-promoting foods with improved digestibility.

2.2.1. Proteins

Mussels, particularly the species *Mytilus edulis*, are recognized for their rich protein content, with 8 to 13 grams of protein per 100 grams of raw, edible meat. Over 30 distinct proteins have been identified in this species. (Qiao et al., 2018). According to Naik and Hayes (2019) the proteins found in mussel meat are typically divided into three major groups: sarcoplasmic proteins, myofibrillar proteins, and stroma proteins. Sarcoplasmic proteins, easily soluble in water and weak salt solutions, mainly include enzymes involved in glycolysis, along with parvalbumin, myoglobin, and creatine kinase. Myofibrillar proteins, requiring high-concentration salt solutions to dissolve, include myosin, actin, tropomyosin, troponin, and paramyosin, all of which play a key role in preserving the quality of raw mussel meat and its products. On the other hand, stroma proteins, which are insoluble in both water and high-salt solutions, consist of extracellular matrix proteins like collagen and elastin. The composition of these protein fractions is influenced by the origin of the raw materials (Ochiai & Ozawa, 2020).

Konieczny et al. (2021) reported that protein bands for actin (43-46 kDa) and paramyosin (98-107 kDa) made up a significant portion of the total protein at 63%, while myosin accounted for only 4.55%. They also noted the presence of protein bands over 250 kDa, which could indicate the presence of protein aggregates and heavy myosin.

Amino acids, the fundamental building blocks of proteins, are vital for the health and well-being of both humans and animals. They participate in numerous biological functions, such as energy metabolism, nervous system communication, hormone and enzyme production, and cellular repair. The green-lipped mussel is an excellent source of protein due to its diverse amino acid profile, which includes both essential and non-essential amino acids. Glutamic acid, present in higher concentrations of 51.8–58.8 mg/g, is accompanied by aspartic acid, which

ranges between 42.8 and 44.0 mg/g. Moderate amounts of serine (18.9–19.5 mg/g) and histidine (7.8–8.5 mg/g) are also found, alongside significant levels of glycine (40.9–43.8 mg/g) and threonine (18.9–21.2 mg/g). Additionally, arginine (27.0–35.9 mg/g), alanine (18.3–24.5 mg/g), valine (14.6–16.3 mg/g), and methionine (8.5–9.5 mg/g) contribute to the profile. Other important amino acids include phenylalanine (14.8–16.2 mg/g), isoleucine (16.0–17.7 mg/g), lysine (29.2–51.3 mg/g), leucine (17.9–23.6 mg/g), proline (14.5–19.7 mg/g), L-cysteine (6.1–6.6 mg/g), tyrosine (13.9–15.4 mg/g), and tryptophan (4.9–5.2 mg/g), which highlight the green-lipped mussel's rich amino acid profile, emphasizing its nutritional value.(Coulson et al., 2015).

2.2.2. Nutritional properties

The approximate composition of mussels can change with the seasons and depends on their habitat and water temperature. Fresh whole green-lipped mussel meat typically contains 76–82% moisture, 1.6–2.2% fat, 2–3% ash, 3–6% carbohydrates, and 12–14% protein. In contrast, dried whole mussel meat has less than 5% moisture, 6–12% fat, 2–25% ash, 10–25% carbohydrates, and 36–67% protein (Miller et al., 2023). Based on dry matter, green-lipped mussels (*Perna canaliculus*) consist of 49.5% crude protein, 9.05% lipid, 16.74% ash, 4.33% moisture, and 20.38% carbohydrates (Vijaykrishnaraj & Prabhasankar, 2015).

Green-lipped mussels are highly nutritious, and packed with micronutrients, vitamins, protein, and polyunsaturated fatty acids. They are notably high in omega-3 fatty acids, which are widely recognized for their advantages in promoting cardiovascular well-being and enhancing cognitive function. The local Maori often consume green-lipped mussels to help alleviate symptoms of osteoarthritis, allergies, and asthma (Miller et al., 2023)

2.2.3. Extraction method

The limited functional capabilities of myosin and myofibrillar proteins in low ionic aqueous conditions pose challenges for using mussel meat protein in functional foods or beverages. To address this challenge, food scientists are dedicated to boosting both the extraction yield and the overall functionality of these proteins. They are implementing a range of cutting-edge processing technologies, such as pH-shifting, which alters protein solubility and functionality; high-pressure homogenization, which improves protein dispersion and texture; pulsed electric fields, which enhance protein extraction and stability; and ultrasound, which increases protein solubility and bioactivity. These innovative approaches are being employed to achieve more efficient protein extraction and to optimize the performance and applications of protein isolates in various food products (Chen et al., 2020; Naik & Hayes, 2019; Surasani et al., 2018).

2.2.3.1. pH-shifting

The solubility of a protein plays a pivotal role in its extractability and the development of new food products. One effective method for protein extraction is the pH shift technique, which utilizes highly acidic solutions (with pH values ranging from 2.0 to 3.5) or alkaline solutions (with pH values between 10.0 and 13.0) to solubilize and extract proteins from mussel meat. This approach leverages the fact that proteins possess numerous ionizable groups, which can generate strong electrostatic repulsion when the pH deviates significantly from the protein's isoelectric point, thereby facilitating their solubilization (Chen et al., 2023; Surasani et al., 2018; Vareltzis & Undeland, 2012). The process begins by solubilizing the proteins in either an acidic or alkaline environment, followed by centrifugation to remove insoluble components from the homogenized mussel meat. The resulting protein-rich supernatant is then precipitated at the protein's isoelectric point, typically found between pH 4.0 and 6.0. The pH shift method is widely favored for its simplicity, speed, cost-effectiveness, and economic viability, making it a popular choice for isolating proteins from a diverse array of sources, including meat, fish, shellfish, legumes, grains, and nuts (Kamal et al., 2021; Kumar et al., 2021).

2.2.3.2. High-pressure homogenization

High-pressure homogenization is a sustainable and widely adopted technology for modifying protein structures. This method leverages a combination of mechanical forces and brief heating effects to alter protein conformation (Chen et al., 2020). The modification of proteins' structural and functional characteristics through this method depends on various factors, including temperature, pressure, the number of processing cycles, valve configuration, and the flow rate applied during the process. The high-pressure homogenization process operates by applying intense shear forces, generating cavitation, creating turbulent flow, and introducing brief heating. These combined effects work to disrupt and unfold the tightly packed, high molecular weight proteins and protein aggregates. As a result, more internal hydrophobic and charged groups are exposed, and the particle size of the proteins is reduced (Yu et al., 2018; Zou, Zhao, Shi, et al., 2020). This technology enhances the functionality of proteins by modifying their structural characteristics, making them more suitable for various applications.

Yu et al. (2018) investigated the effects of high-pressure homogenization on the structural and functional properties of myofibrillar proteins from mussels (*Mytilus edulis*). Applying pressures ranging from 0 to 100 MPa over three cycles resulted in notable structural modifications. Specifically, the content of α-helix and β-turn structures in the proteins decreased up to 80 MPa and continued to decline at 100 MPa. Conversely, the β-sheet content initially increased up to 80 MPa but then diminished at 100 MPa. As pressure increased, there was a rise in both free sulfhydryl content and surface hydrophobicity. Between 40 and 80 MPa, the mean particle size and degree of aggregation decreased, while the absolute ζ-potential value increased. However, at 100 MPa, the ζ-potential value saw a reduction. Surface hydrophobicity increased steadily from 0.1 to 80 MPa but showed a slight decrease at 120 MPa. Functionally, the solubility of the proteins improved by 7.4%, and their oil holding capacity saw a dramatic increase of 1300%, although water holding capacity declined. Additionally, the proteins exhibited enhanced emulsifying activity and stability, improved foaming ability, and better foam stability. These findings underscore the effectiveness of high-pressure homogenization in augmenting the functional properties of mussel myofibrillar proteins, thereby broadening their potential applications in various food products.

2.2.3.3. Pulse electric field

Recent studies have highlighted the effectiveness of Pulse Electric Field (PEF) technology for extracting active ingredients from natural products. This innovative method significantly enhances the extraction rates and yields of a wide range of active compounds while maintaining the quality of the extracted materials. PEF offers several advantages, including its non-thermal nature, which prevents heat-induced degradation of sensitive components, its speed and efficiency in processing, as well as its energy efficiency and minimal environmental impact. These benefits make PEF an increasingly popular choice for non-thermal pasteurization of foods and for the extraction of valuable compounds from various natural sources (Ye et al., 2012).

In the specific context of mussel protein extraction, PEF has demonstrated substantial improvements over traditional methods. The application of PEF technology in this area involves using a combination of high-intensity electric fields and controlled pulse sequences to facilitate protein release from mussel tissues. Response Surface Methodology (RSM) has been employed to systematically explore the effects of different processing times and PEF parameters on the efficiency of protein extraction. The results have shown that PEF can achieve faster extraction and higher yields compared to conventional methods such as salting out or the pH shift technique. For example, under optimal conditions specifically, an electric field intensity of 20 kV/cm, eight pulses, and a 2-hour enzymolysis process at a maximum protein yield of 77.08% was attained (Zhou et al., 2017). This substantial improvement underscores the potential of PEF technology to enhance the efficiency and effectiveness of protein extraction from mussels, making it a promising method for various applications in the food industry and beyond.

2.2.3.4. Ultrasonication

Ultrasound involves the use of sound waves with frequencies exceeding 20 kHz, and it plays a significant role in modifying the physical, structural, and functional characteristics of food proteins. Typically, to achieve these modifications, low-frequency ultrasound ranging from 20 kHz to 100 kHz is utilized alongside high intensities between 10 to 1000 W/cm² (Ampofo & Ngadi, 2022). The effects of ultrasound on protein structure are primarily driven by several mechanisms, including cavitation, dynamic agitation, hydrodynamic shear forces, localized heating, and turbulence (Kentish & Feng, 2014).

Cavitation specifically is a crucial process in which reactive free radicals such as hydroxyl radicals (•OH) and perhydroxyl radicals (•OOH) are generated through the breakdown of water molecules. This process can cause substantial changes in the secondary and tertiary structures of proteins, facilitating crosslinking and aggregation, including the formation of disulfide (SS) bonds, while potentially lowering allergenicity (Rahman et al., 2020; Xu et al., 2017).

The extent of structural and functional changes induced by ultrasound treatment is highly dependent on a range of operating conditions. These include factors such as sonic energy density, treatment duration, the type of solvent used, the degree of temperature increase, the design of the sonoreactor, the ratio of substrate to slurry, and the specific type of protein being treated (O'sullivan et al., 2016; Rahman & Lamsal, 2021). Extensive research has demonstrated that ultrasound treatment can significantly improve the hydrosolubility, functionality, and digestibility of various proteins. This includes proteins derived from milk, plant sources, and meats, highlighting the versatility and effectiveness of ultrasound as a tool for enhancing protein properties across different food types (Kang et al., 2021; O'sullivan et al., 2016; Shokri et al., 2022) .

The solubility of proteins in water is a fundamental aspect of their functionality, significantly impacting their performance in various applications. Recent research has shown that ultrasound treatment can markedly enhance the solubility of mussel myofibrillar proteins, increasing it from 11% to 33% as the ultrasound power is raised to 600 W (Yu et al., 2022). This improvement in solubility can be attributed to several key factors.

First, ultrasound treatment facilitates the exposure of hydrophilic groups within the protein structure. Additionally, it leads to a reduction in particle size and promotes the dissociation of protein subunits (Kang et al., 2021). As the particle size decreases, the surface area of the protein molecules increases, which enhances their interaction with water molecules. This increase in molecular surface area allows for better hydration and solubilization of the proteins.

Furthermore, the ultrasound process induces partial denaturation of the proteins, which exposes more internal hydrophobic groups. This exposure enhances the ability of mussel proteins to quickly adsorb and spread at interfaces, such as the air-water and oil-water interfaces (Daba & Morris, 2022; Kang et al., 2021). The result of these changes is an enhanced capacity of the proteins to accumulate at these interfaces, where they form a thick protein layer. This layer significantly improves the emulsifying and foaming properties of the mussel proteins (Daba & Morris, 2022; Kang et al., 2021; Yu et al., 2022). In summary, the application of ultrasound not only increases the solubility of mussel proteins but also optimizes their functionality by improving their interaction with water and their ability to form stable emulsions and foams.

2.3. Developing hybrid protein systems

Recently, the trend of incorporating plant proteins as partial replacements for animal proteins in food formulations has emerged as a promising strategy to minimize the environmental impact of food production. This approach seeks to maintain or even improve the nutritional content and characteristics of food products while reducing ecological impact. By integrating plant proteins, which offer advantages such as lower costs, reduced allergenicity, and distinct nutritional benefits, this strategy addresses some of their inherent limitations, including unique taste profiles and limited water solubility. The combination of plant proteins with animal proteins helps to balance these drawbacks and leverage the complementary strengths of both sources, thus improving overall product performance and sustainability (Alves & Tavares, 2019).

The creation of mixed protein systems offers various benefits. One key advantage is the ability to manipulate the size of protein aggregates, which can be tailored to influence the texture and stability of food items. Additionally, these systems can enhance the overall protein content of gels without major changes to their flow properties, potentially increasing their nutritional value. Furthermore, integrating plant proteins can lower production costs, enhancing the economic feasibility of food products. Also, new market opportunities, the creation of innovative products with mixed protein systems can open up new markets, particularly for plant-based dairy substitutes that cater to specific consumer preferences, such as those in Southeast and East Asia. Lastly, cultural acceptance, when using proteins that are common in certain cultures, such as mung bean protein in Asia, can facilitate the acceptance of new products by consumers who are familiar with the flavors and health benefits associated with those proteins (Grasso & Jaworska, 2020).

Proteins are intricate molecules distinguished by their specific three-dimensional shapes, which result from various molecular forces such as electrostatic interactions, ion pairing, van der Waals forces, hydrogen bonds, the hydrophobic effect, and conformational entropy. Notably, the hydrophobic effect is essential in promoting protein folding, leading to the compact forms typical of globular proteins. The internal arrangement of a protein includes secondary structures like alpha helices, beta sheets, and beta turns, all of which are primarily influenced by steric constraints and hydrogen bond formation. Differences in polypeptide sequences and the unique conditions of their native environments give rise to variations in the

structural configurations of proteins from plant and animal sources. These structural differences affect secondary and tertiary structures, which in turn influence the functional properties of proteins, including solubility, gelation, emulsification, and foaming abilities. Moreover, variations in structure also impact the nutritional aspects of proteins, affecting digestibility and the availability of essential amino acids (Day et al., 2022).

Despite the increasing interest in developing hybrid protein formulations, there remains a notable lack of research specifically dedicated to seafood-plant protein hybrids. This gap in the current body of research represents an opportunity for exploration that could significantly advance our understanding of how to enhance both the sustainability and nutritional profile of seafood products. Addressing this research void could lead to innovative approaches that combine the benefits of seafood and plant proteins, potentially resulting in products that are not only more environmentally friendly but also richer in nutritional value. This unexplored area offers promising potential for improving the quality and sustainability of seafood alternatives and blends.

Table 1 presents an overview of recent research findings focused on hybrid proteins.

Table 1 Recent studies conducted on preparation and characterization of animal-plant hybrid protein systems

Type of hybrid protein		Main Findings	Applications	References
Animal protein	**Plant protein**			
Chicken	Yellow pea, chickpea, lentil (0, 25, 50, and 75%)	• The hybrid products are generally softer than control patties, which are typically made from 100% meat. This softening effect is attributed to the addition of plant-based ingredients that alter the overall texture. • The addition of plant-based components results in reduced cohesiveness and springiness. • When the proportion of plant-derived ingredients reaches 50% or higher, there's a noticeable decrease in various texture parameters. This suggests that the more plant-based ingredients are added, the further the texture deviates from that of a traditional meat product.	Burger	Chandler and McSweeney (2022)
Pork	Lentil, chickpea, pea, bean (10–44%)	• Hybrid meat products tend to have higher cooking yields compared to traditional meat products. This means they retain more of their weight during the cooking process, which can be advantageous for both manufacturers and consumers. • During cooking, meat products often shrink in size. Hybrid products, however, tend to experience lower diameter reductions. This is beneficial as it means the product maintains its shape and size better during cooking, potentially leading to a more appealing final product.	Burger	Argel et al. (2020)

Chicken	Hempseed meal (10, 20, 30, 40%)	• The addition of plant-based ingredients can significantly increase the overall phenolic concentration and improve antioxidant effectiveness. These phenolic substances are recognized for their antioxidant capabilities, which help guard against lipid degradation and prolong the product's shelf life. • Tend to have decreased cooking loss, which is beneficial for both yield and texture. Less moisture loss can result in a juicier product, while reduced fat drippings can lead to less shrinkage and a more stable cooking process.	Sausage	Sun et al. (2022)
Beef	Soy-based textured vegetable protein (10–40%)	• Better at retaining moisture, which can contribute to a juicier and more appealing texture. Also, higher water holding capacity that helps in maintaining the weight and volume of the product during cooking, which is beneficial for both yield and consumer satisfaction. • The use of an Electronic Tongue System to evaluate the products has shown that it tends to exhibit higher levels of sourness, astringency, umami, and saltiness. • Lower cooking loss, consequently improved WHC.	Patties	Bakhsh, Lee, et al. (2021a)
Chicken	Pea protein isolate (12–30%)	• The best fibre structure at 20% indicates that at this concentration, the plant-based ingredients effectively contribute to a desirable fibrous texture.	3D-Nuggets	Wang et al. (2023)

2.4. Gelation of plant protein

Gelation of proteins is essential in the food industry as it facilitates the transformation of proteins from a liquid form into a gel-like consistency, providing a variety of appealing qualities to food products. This change occurs when polypeptide chains undergo cross-linking to establish a three-dimensional network. Various molecular interactions, such as hydrogen bonds, ionic bonds, disulfide bonds, and hydrophobic interactions, contribute to the formation of this network. The nature of these interactions is influenced by the protein's structural attributes and the isolation methods employed. Notably, storage proteins commonly used in food formulations can participate in different chemical reactions and interactions, including intermolecular disulfide bonds, due to the presence of amino acids like cysteine and glutamine. This versatility enables the production of gels with diverse structural compositions, which is vital for creating food products with specific textural and functional characteristics (Yada & Dee, 2024).

Proteins are widely utilized as gelling agents in a diverse range of food products, such as yogurt, cheese, eggs, and meats, where they play a crucial role in achieving the desired texture and sensory attributes. The classification of protein gels can be approached from several perspectives. For instance, based on thermal stability, gels can be categorized into thermo-reversible gels, which can return to a liquid state upon reheating, and thermo-irreversible gels, which maintain their gelled state even after heating. Additionally, the nature of the protein aggregates within the gel can distinguish between opaque gels, which scatter light and appear cloudy, and transparent gels, which allow light to pass through, giving them a clear appearance. Another classification criterion is based on the method used for gel formation, leading to heat-induced gels that form through heating processes and cold-induced gels that form under cooling conditions. The performance and characteristics of protein gels are influenced by a variety of factors, including the specific molecular structure of the protein, which affects how the proteins interact and form cross-links during gelation, and the conditions of the gelation process itself. Each of these factors contributes to a unique mechanism of gel formation, which can significantly impact the final properties of the gel, including its texture, stability, and sensory characteristics (Zheng et al., 2022).

2.4.1. Heat-induced gelation process in plant protein

The process of heat-induced gelation is a widely used technique for gelling plant globular proteins and involves several key stages, including denaturation, aggregation, and the formation of a three-dimensional network. At the outset, heat disrupts the natural structure of protein aggregates, breaking them down into smaller particles that subsequently reorganize into nearly spherical shapes. As the heating progresses, the protein molecules unfold, leading to the exposure of previously hidden hydrophobic regions, disulfide bonds (S–S), and sulfhydryl groups (–SH). These newly exposed groups allow the proteins to form covalent disulfide bonds, hydrogen bonds, and hydrophobic interactions with adjacent proteins. The resulting protein particles then begin to aggregate and cluster together, eventually forming a cohesive three-dimensional network that defines the gel structure. This complex process not only alters the molecular conformation of the proteins but also impacts the texture and structural integrity of the final product, making it an essential step in the creation of various food products (Tang et al., 2024).

The properties of heat-induced gels derived from plant globular proteins are influenced by multiple factors, including the protein source, its chemical compositions, molecular configuration, and external conditions such as pH, ionic strength, and temperature. As the pH deviates from the protein's isoelectric point, typically around pH 4 to 5, the proteins develop a stronger charge, enhancing their solubility in water. This increased solubility causes a notable change in gel characteristics, transitioning from disordered, opaque structures, known as particulate type structures, to more organized, translucent formations referred to as strand type structures. This transformation occurs due to the shifting balance of molecular interactions, including electrostatic repulsion, hydrogen bonding, and hydrophobic interactions, among protein molecules during gelation. The specifics of this transformation are significantly affected by the protein's origin and the conditions applied during heat treatment, resulting in considerable variations in the final gel structure and its properties (Tang et al., 2024).

2.5. Gelation of animal protein

The gelation process of animal proteins is a highly complex phenomenon in which protein molecules transition from a liquid, or sol, state to a solid, gel-like form. This transformation is critical in many food applications, such as producing gelatine and creating meat analogues, where the right texture and consistency are key. The specific mechanisms that drive gelation vary considerably depending on the type of animal protein being used. For example, globular proteins like globulins behave differently from non-globular proteins, such as collagen and muscle proteins, in the way they bond and interact during gel formation. Globular proteins generally unfold and aggregate through heat-induced denaturation, while collagen and muscle proteins form more fibrous gel networks due to their distinctive structural makeup. These differences in gelation are affected by factors such as temperature, pH, and salt levels, all of which influence the final texture, firmness, and stability of the gel (Floret et al., 2023).

Globular proteins, such as globulins, are normally soluble in their native state but undergo significant conformational changes when exposed to elevated temperatures or pressures. These environmental conditions disrupt the internal molecular bonds, causing the protein's structure to alter and expose reactive amino acids that were once hidden. As these proteins unfold, new intermolecular bonds form between them, leading to their aggregation and the establishment of a network structure that results in gel formation. This gelation process is affected by several factors, including pH and ionic strength. Changes in pH can interfere with internal electrostatic bonds or cause localized electrostatic repulsion, which enhances the unfolding of the proteins. Nevertheless, to fully achieve gelation and complete the protein aggregation process, additional factors and conditions are often required beyond mere pH and ionic strength adjustments. Non-globular proteins, such as collagen and muscle proteins, differ significantly from globular proteins in both structure and solubility. Unlike globular proteins, which are typically soluble and compact in their native form, non-globular proteins have an extended-chain structure and are inherently insoluble and amorphous when in their fibrous state. To induce gelation in these non-globular proteins, distinct conditions are required compared to those used for globular proteins. Specifically, collagen and muscle proteins often need to be solubilized through hydrolysis, which is achieved under strong acidic or alkaline conditions. This process effectively disrupts the electrostatic interactions that hold together the bundles of fibrils and fibers within these proteins. By breaking down these interactions, the

proteins are converted into a form that can subsequently undergo gelation, leading to the formation of a gel structure. The unique properties and requirements of non-globular proteins for gelation highlight the complexity of their behavior compared to their globular counterparts. Muscle proteins undergo a transition from a sol state to a gel state when subjected to thermal or pressure treatments. This transformation is primarily driven by hydrophobic interactions, which are further reinforced by hydrogen bonds, calcium bridges, and disulfide linkages. These interactions and bonds help to stabilize the protein network as it forms a gel. Additionally, the process of gelation can be further facilitated at lower temperatures and pressures through the application of transglutaminases. These enzymes play a crucial role in forming cross-links between glutamine and lysine amino acids, thereby promoting and enhancing the gel formation. This combined effect of heat, pressure, and enzymatic activity ensures a robust gel structure, which is essential for various food applications where texture and consistency are important (Zhao and Zhou, 2021).

Recently, research have found that the gelation of animal proteins, such as those from mussels, involves a complex process that is significantly influenced by both the composition and the structure of the proteins. According to Naik and Hayes (2019), mussel protein comprises approximately 11.39% matrix proteins, 17.19% myofibrillar proteins, and 48.4% sarcoplasmic proteins. Sarcoplasmic proteins, characterized by their water solubility and globular structure, play a crucial role in the gelation process. These proteins, which dissolve easily in aqueous environments, contribute significantly to the formation and stability of gels by interacting with other protein types and participating in the network formation essential for gel development. When the temperature rises and exceeds the deformation temperature of these proteins, their structure unfolds, exposing hydrophobic groups and active sites. This unfolding process causes the proteins to aggregate through hydrophobic interactions, electrostatic forces, and hydrogen bonds. With further temperature rise, these protein aggregates undergo stronger interactions, including the formation of disulfide bonds, which leads to the development of a stable three-dimensional network structure, ultimately resulting in gel formation (Park, 2005).

Most studies on seafood protein gelation have focused on the gelation mechanism of myofibrillar proteins (Li et al., 2023). Unlike globular proteins, myofibrillar proteins are fibrous and soluble in salt, with a heat-induced gelation mechanism that is distinctly different. Myosin, a key protein in myofibrillar gelation, is essential for the gelation of meat products since actin alone cannot form gels (Sun & Holley, 2011). As the temperature increases, the

myosin light chain begins to de-helixes, and the head (S1 region) of the myosin molecules attract each other through hydrophobic and electrostatic interactions, creating initial aggregates. These aggregates form spherical clusters at the head, while the tail of the myosin extends outward. With further heating, the myosin tail (S2 and light chain regions) folds and crosslinks with other tails. The denatured myosin molecules then form a stable network by forming disulfide bonds, hydrophobic interactions and hydrogen bonds (Xie et al., 2024).

2.6. Food polysaccharide

Food polysaccharides, due to their diverse functional properties, play a significant role in shaping the interactions among components within food systems. These interactions directly impact the texture of food products, which is a crucial factor in determining food quality and consumer satisfaction. Recent research in food science has increasingly focused on how polysaccharides can be manipulated to enhance or create unique textures. This is particularly important for tailoring food products to meet the needs of individuals with dietary restrictions or challenges in consuming regular foods. The growing body of work in this area underscores the vital role of polysaccharides in improving or innovating food textures to accommodate a wide range of dietary needs and preferences.

Polysaccharides are increasingly valued in the food industry for their safety, accessibility, sustainability, and health benefits (Bernaerts et al., 2019). Food polysaccharides are known for their outstanding rheological properties, including their ability to thicken, stabilize, gel, and emulsify. Even when present in small quantities, these polysaccharides can significantly modify the texture of food products. Their ability to alter texture has been well understood for many years, with research recognizing their important role in food science (Guimarães et al., 2020). These polysaccharides are also valued for extending the shelf life of food items by influencing factors such as water crystallization, creaming, freeze-thaw stability, and the retrogradation of starch. Due to these properties, polysaccharides are essential in the formulation of a wide array of food products. Their influence can be seen in the creation of dairy products, frozen foods, confectioneries, beverages, fruit juices, and baked goods such as bread and pastries. The use of food polysaccharides not only improves the overall quality and texture but also enhances the convenience and longevity of many food items, making them critical components in modern food production.

Polysaccharides can have linear or branched structures, be charged or neutral, and some even exhibit both hydrophobic and hydrophilic characteristics, classifying them as amphiphilic polysaccharides (Yang et al., 2020). The abundance of hydroxyl (-OH) and carboxyl (-COOH) groups in the molecular chains of natural polysaccharides enables the attachment of various functional groups through a range of chemical modifications. These modifications include processes like methylation, sulfation, carboxymethylation, selenylation, etherification and acetylation (Xu et al., 2019). **Figure 1** provides a classification diagram for different types of polysaccharides. Konjac glucomannan and methylcellulose are two polysaccharides that have found extensive use in food applications due to their unique functional properties. Both polysaccharides contribute significantly to improving texture, mouthfeel, and stability in a variety of food formulations.

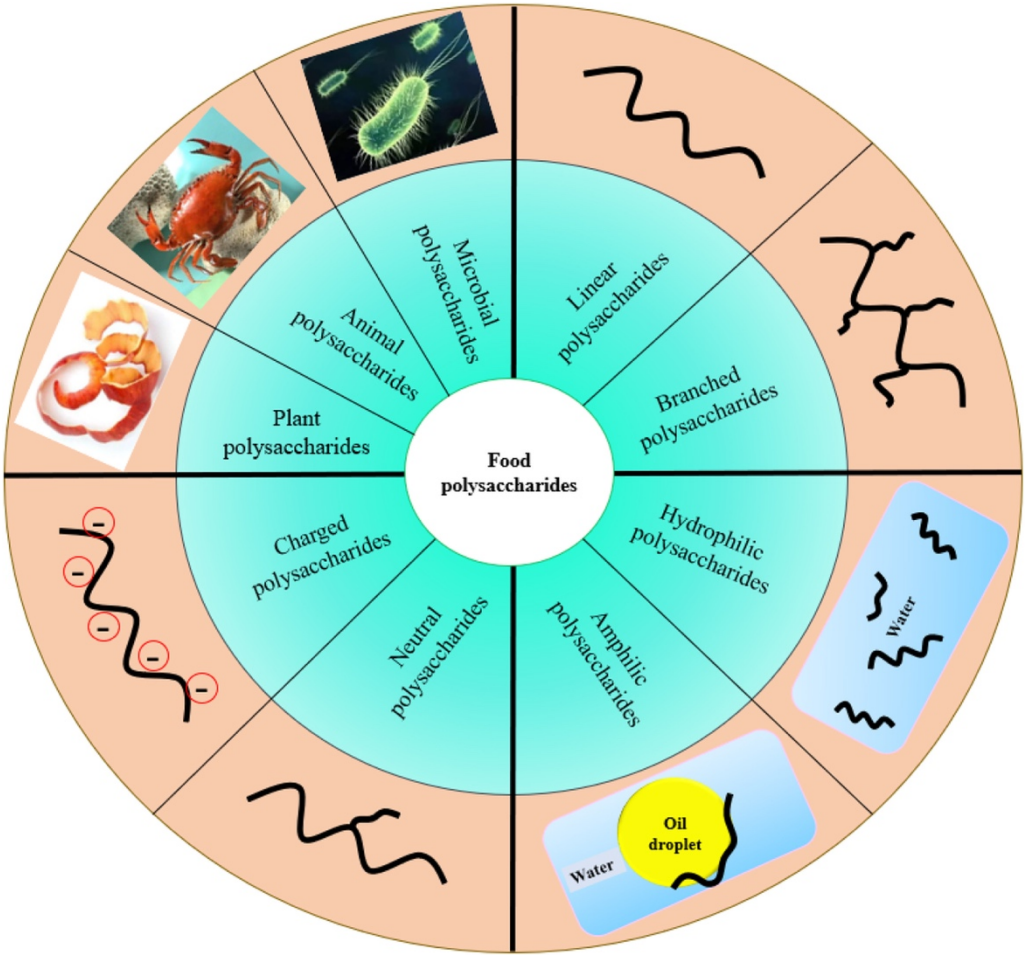


Figure 1. Classification of food polysaccharides (Yang et al., 2020).

2.6.1. Konjac glucomannan

Konjac glucomannan (KGM) is a polysaccharide that dissolves in water and consists of α-mannose and α-glucose units linked by β-1,4 bonds, arranged randomly along its chain. Its structure also features a low level of acetylation and numerous hydroxyl groups. These characteristics greatly enhance its ability to form gels, which makes KGM highly useful as a thickening, stabilizing, and gelling agent in a wide range of food products. Its excellent gel-forming capacity is particularly appreciated for its adaptability in both traditional and contemporary food applications (Mu et al., 2017). Konjac (*Amorphophallus konjac*), a perennial species from the Araceae family, is predominantly cultivated in subtropical and tropical regions of Asia and is recognized for its tubers, which serve as a rich source of konjac glucomannan (KGM). Approximately 40% of the tuber mass consists of KGM, a water-soluble polysaccharide with a partially branched structure, featuring about 8% β-1,3 glycosidic bonds. The molecular weight of KGM varies between 500 and 2000 g/mol, depending on factors such as the raw material and the extraction methods utilized. Typically, KGM exhibits a normal distribution of molecular weights and a polydispersity index of 1.21, comparable to other common polysaccharides (Zhang & Yang, 2014).

The molecular structure of KGM, illustrated in **Figure 2**, features hydroxyl and carbonyl groups that facilitate interactions with water molecules via hydrogen bonding and Van der Waals forces. This ability enables KGM to form robust gels that effectively retain moisture, which is advantageous in various food applications where maintaining a consistently moist environment is crucial (Zhang & Yang, 2014). Additionally, the hydroxyl groups in KGM play a role in hindering the formation of disulfide, hydrogen, and hydrophobic bonds in proteins, thereby mitigating protein aggregation and preventing denaturation. This property makes KGM useful for protecting protein components in food products (Wang et al., 2017).

KGM, a hydrocolloid derived from processed konjac tuber flour, is noted for its distinctive physiological, functional, and technological properties. Its exceptional rheological and gelling characteristics make it highly effective as a stabilizer and emulsifier in a variety of processed foods, beverages, and cosmetic products (Singh et al., 2018). The United States Food and Drug Administration (FDA) has recognized KGM as Generally Recognized as Safe (GRAS) since 1994, confirming its safety for use in food products under specified conditions.

In 1996, konjac glucomannan (KGM) received approval from the United States Department of Agriculture (USDA) to be used as a binder in meat and poultry products, emphasizing its effectiveness in enhancing texture and stability in various food items. The Food Chemical Codex (FCC) establishes standards for food ingredients and recognizes KGM as a versatile thickening agent, gelling agent, emulsifying agent and film former in the U.S., illustrating its important role in improving product attributes (Wang et al., 2017). Furthermore, KGM has been shown to enhance gel strength in surimi at a concentration of 2.0% (Buda et al., 2021), and its gel-forming capabilities are currently being explored for potential use in edible food-packaging films, which could offer significant benefits (Pruksarojanakul et al., 2020).

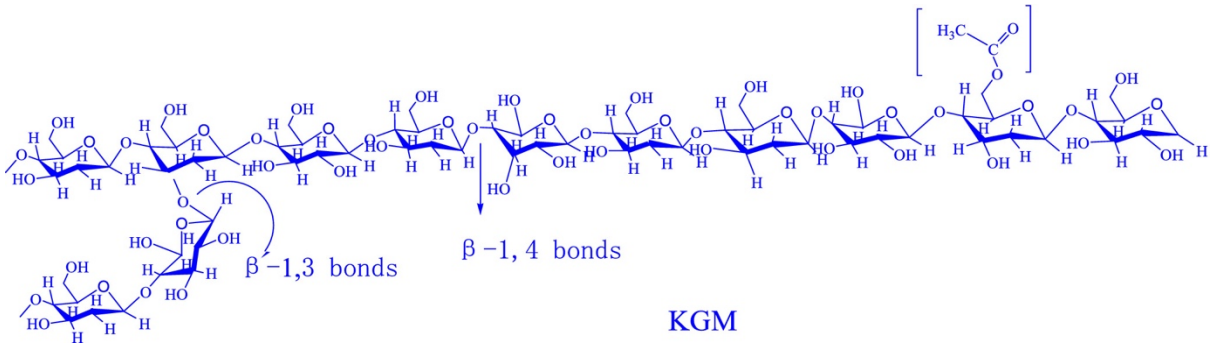


Figure 2. Molecular structure of konjac glucomannan (Gong et al., 2019)

2.6.2. Methylcellulose

Methylcellulose (MC), a recognized derivative of cellulose ether, is classified as safe for consumption by the U.S. Food and Drug Administration, and is produced by partially substituting cellulose hydroxyl groups with methoxy groups via a reaction with methyl chloride in a basic solution (Health & Services, 2015). MC is a vital ingredient in plant-based burger patties, where its gelling and emulsifying abilities are used to bind textured vegetable protein (TVP) effectively. This polymer is produced by modifying cellulose through etherification with dimethyl sulphate in a caustic alkaline solution. MC plays a crucial role in products from leading plant-based meat brands such as Beyond Meat, Impossible Meat and Harvest Gourmet Nestle. However, due to its thermo-reversible properties, it often requires the addition of stabilizers and hydrocolloids to maintain the stability of the gelled structure in the final product. Research indicated that a concentration of 3% MC is optimal for producing meat analogues, achieving a texture that closely resembles beef (Bakhsh, Lee, et al., 2021b). MC has been found

to surpass transglutaminase in enhancing texture, reducing cooking loss, and preserving flavor in plant-based burger patties (Chen et al., 2023). Moreover, patties that incorporate MC exhibit enhanced attributes such as increased hardness, improved springiness, greater cohesiveness, and superior chewiness when compared to patties formulated with κ-carrageenan and xanthan gum (Tunnarut et al., 2022). Although MC is highly effective, the search for alternative binders that offer a cleaner label while maintaining similar functionality is ongoing, reflecting a continuous effort to improve plant-based meat products.

2.7. Protein-polysaccharide interactions

The use of emulsifiers, solubilizers, and dispersing agents is crucial in achieving the desired consistency and stability in food items. The combination of polysaccharides and proteins stands out for its exceptional capacity to alter the texture and rheological characteristics of food colloids. This interaction plays a critical role in extending the shelf life of food products, underscoring its significance in both the sensory qualities and stability of different formulations (Gentile, 2020). These combinations can create amphiphilic compounds that adhere tightly to the interface between oil and water through the hydrophobic regions of proteins, forming a viscoelastic film. The non-adsorbing polysaccharide component enhances steric stabilization, potentially leading to gel-like properties.

Protein-polysaccharide interactions can occur either through covalent or noncovalent bonding. Covalent bonds typically form via Maillard-type reactions, which enhance the heat stability of protein-polysaccharide conjugates. These reactions are sensitive to specific conditions, such as temperature and pH, that must be carefully regulated for optimal results. Given the large molecular size of polysaccharides, their attachment to proteins is limited in number, with more compact, folded proteins like ovalbumin and lysozyme usually having fewer bonding sites than more extended, unfolded proteins like casein. This reaction is largely influenced by the presence of lysine, which reacts with the reducing sugar of polysaccharides (Li et al., 2022).

In an ideal scenario, where the interactions between polysaccharides and proteins are perfectly balanced, both components would distribute uniformly in the solvent and act independently, as if the other were not present. However, this situation is uncommon in practice. Polysaccharides and proteins typically dissolve together only under certain conditions, such as in dilute solutions or when their intermolecular complexes remain soluble. This ideal situation, where they create a macroscopic single-phase system, is uncommon. In most cases, their compatibility is affected by several factors, leading to incompatibility and phase separation instead of forming a uniform solution (McClements, 2006). This incompatibility is largely due to the low mixing entropy between these molecules, which plays a central role in driving their thermodynamic instability. Consequently, creating stable mixtures where proteins and polysaccharides dissolve together can be difficult without precisely adjusting factors like concentration, pH, and temperature (Turgeon et al., 2007).

The relationship between polysaccharides and proteins are influenced by various intermolecular forces, including hydrogen bonds, hydrophobic interactions, Van der Waals forces, and electrostatic attractions. Among these, electrostatic forces are particularly essential for forming complexes and coacervates, playing a crucial role in binding the macromolecules (McClements & Grossmann, 2021). The formation of polysaccharide-protein complexes generally starts with the neutralization of charges, driven by the electrostatic attraction between molecules carrying opposite charges. Once the neutralized complexes form, they can aggregate further, resulting in a three-dimensional network structure. This process gives rise to coacervates, which are composed of highly hydrated systems featuring liquid droplets rich in biopolymers. These coacervates establish a dispersed phase, concentrating the biopolymer droplets and leading to unique structural characteristics (Turgeon et al., 2007).

The formation of polysaccharide-protein complexes and coacervates is influenced by thermodynamics, specifically the total Gibbs free energy change (ΔG). A negative ΔG indicates that the complexation can happen spontaneously. This change in Gibbs free energy depends on the relationship between enthalpy (ΔH) and entropy ($-T\Delta S$) (Schmitt et al., 2009). Entropic effects arise from the release of counterions and water molecules, as the polymers compact and increase the system's disorder. The enthalpy change reflects the energy either released or absorbed, considering contributions from dilution, mixing, solvent rearrangement, and the creation of noncovalent bonds including, hydrophobic forces, electrostatic interactions and

hydrogen bonding at the interfaces of the complex. These factors work together to determine the likelihood and stability of the complex's formation under certain conditions (Turgeon et al., 2007).

The phase behavior of mixtures containing polysaccharides and proteins is governed by a complex interplay of intermolecular interactions, thermodynamic principles, and external conditions such as pH, salt concentration, mixing ratios, and the total polymer concentration. Mastery of these factors is essential for accurately controlling the formation of complexes and coacervates, which has significant implications for applications in both biological systems and industrial processes (Yang et al., 2020). The impact of pH is significant because it affects both the ionization level of proteins and the formation of complexes. Proteins possess an isoelectric point (pI) at which they carry no overall charge. When the pH is above this point, proteins are negatively charged, whereas they are positively charged when the pH is below the pI. Specific pH values, known as critical pH values (pHc and pHØ), are important as they define the conditions under which complexation and macroscopic phase separation take place, respectively (Turgeon et al., 2007). The electrical equivalence pH (EEP) represents the pH level at which the charges on two interacting polymers are balanced and maximized, resulting in the highest degree of complex formation and the largest volume of coacervate. The addition of salt influences this process by screening the charges on the polymers, thereby reducing their ability to interact. Increased ionic strength can further inhibit complexation by diminishing the release of counterions. To achieve optimal complex formation, it is important to determine the ideal ratio of polysaccharide to protein that ensures full charge neutralization. This optimal ratio can be established through methods such as electrophoretic mobility measurements or by assessing turbidity and scattered light intensity. As the total polymer concentration increases, counterions become more concentrated, potentially suppressing complexation and coacervation. There is a critical concentration above which the entropic advantage of forming complexes is suppressed, leading to reduced coacervation (Yang et al., 2021).

2.8. Analysis techniques

2.8.1. Rheology

Rheology is the field of study that examines how materials respond and change their shape when subjected to applied forces. It focuses on understanding the flow and deformation behavior of various substances under different conditions (Ghanbari et al., 2020). Liquids and dispersions, which are made up of molecules or particles, exhibit movement as these components slide past one another. The measurement of resistance to flow in fluids, resulting from internal friction, is a key aspect of rheology (Ramaswamy, 2022). Rheology plays a vital role across numerous industries, including food processing, pharmaceuticals, personal care products, paints and coatings, and oil and gas. In these sectors, comprehending the flow and deformation behavior of materials is essential for optimizing product development, refining manufacturing processes, and ensuring quality control. Rheological measurements are commonly conducted using specialized instruments such as rheometers and viscometers. These devices apply controlled stresses or strains to a material and then measure the resulting deformation or flow behavior, providing detailed insights into the material's rheological properties.

2.8.1.1. Oscillatory rheology

Oscillatory rheology is a technique used to analyse the viscoelastic properties of materials by applying a sinusoidal oscillatory stress or strain and observing the material's corresponding response. By measuring the resulting strain or stress, this method provides valuable information on how the material behaves in both its elastic (solid-like) and viscous (liquid-like) states (Sharma et al., 2023). When a material is exposed to oscillatory stress or strain, it undergoes deformation, with its response depending on the material's inherent properties. In a perfectly elastic solid, this deformation would be instantaneous and fully recoverable upon removal of the stress. In a purely viscous liquid, the deformation would be time-dependent and not recoverable; the liquid would simply flow (Gupta, 2022).Viscoelastic materials, however, exhibit a combination of these behaviors. When an oscillatory stress is applied to a material, some of the energy is stored within its structure, resulting in a reversible, elastic deformation, while the remaining energy is lost as heat, causing an irreversible flow that represents the viscous behavior of the material (Gutierrez-Lemini, 2014).

The storage modulus (G') represents the elastic component of a material's behavior, indicating how much energy is stored and recovered during each deformation cycle. It reflects the material's stiffness or resistance to deformation. In contrast, the loss modulus (G'') measures the viscous aspect, showing how much energy is dissipated as heat with each cycle, which relates to the material's flow characteristics or its capacity to dampen applied forces (Gupta, 2022).

The phase angle (δ) measures the delay between the applied stress and the resulting strain in a material, indicating the contributions of its elastic and viscous properties. A fully elastic material corresponds to a phase angle of 0 degrees, while a phase angle of 90 degrees signifies a purely viscous fluid. The tangent of the phase angle (tan δ) is a dimensionless parameter calculated as the ratio of the loss modulus (G'') to the storage modulus (G'), providing insight into the material's viscoelastic behavior and illustrating the balance between elasticity and viscosity (Micaelo et al., 2022).

Oscillatory rheology can be conducted over a range of frequencies, allowing researchers to observe how the material's properties change with the rate of deformation. This is particularly useful for understanding how materials will behave under different dynamic conditions, such as those encountered in processing or in-service use. The technique is widely employed in the study of polymers, foods, cosmetics, and other complex materials where understanding the interplay between elasticity and viscosity is essential for the effective creation of products and for upholding rigorous quality control standards.

2.8.1.2. Techniques to measure the oscillatory rheology

Oscillatory rheology is a technique employed to evaluate the viscoelastic properties of substances, including gels, by applying a fluctuating strain and recording the corresponding stress response. This approach offers valuable information about the material's storage modulus (G'), representing its elastic or solid-like properties, and its loss modulus (G''), reflecting its viscous or liquid-like attributes. The key techniques involved in oscillatory rheology include:

2.8.1.2.1. Strain sweep (Amplitude sweep*)*

This technique involves applying oscillatory strains of increasing magnitude to the sample while keeping the frequency constant. The objective is to establish the linear viscoelastic region (LVE), where the stress response is linearly related to the strain, in accordance with Hooke's Law. When the material is tested outside this region, it may display nonlinear characteristics, which can offer insights into its structural attributes (Stojkov et al., 2021).

2.8.1.2.2. Frequency sweep

In a frequency sweep, the material is subjected to oscillatory stress or strain at various frequencies, with the strain amplitude kept within the linear viscoelastic region (LVE) to avoid nonlinear effects. This test provides information about the material's response to different rates of deformation and is useful for understanding its behavior under dynamic conditions. During frequency sweeps, both the temperature and oscillation amplitude are kept constant. When the material demonstrates characteristics of solid-like behavior, the storage modulus (G') will surpass the loss modulus (G'') throughout a wide frequency range (Fasolin et al., 2023).

2.7.1.2.3. Time sweep

A time sweep test applies constant stress to the material over time (creep) and then removes the stress to observe the recovery behavior. This test can reveal details about the material's stability over time and its viscoelastic properties (Stojkov et al., 2021).

2.7.1.2.4. Dynamic moduli (G' and G'')

During oscillatory testing, both the storage modulus (G') and the loss modulus (G") are assessed. The storage modulus represents the material's elastic behavior, while the loss modulus indicates its viscous characteristics. By calculating the ratio of the loss modulus to the storage modulus (tan δ), one can gain insights into the material's overall viscoelastic balance (Stojkov et al., 2021).

2.7.1.2.5. Phase angle (δ)

The phase angle measures the delay between the applied stress and the resulting strain during an oscillatory test. It reflects the material's viscoelastic properties, where a phase angle of 0 degrees signifies a material that is perfectly elastic, and a phase angle of 90 degrees indicates a material that behaves as a purely viscous fluid (Stojkov et al., 2021).

2.7.2. Color measurement

The visual appearance of food, especially its color, is often the first attribute that consumers notice. It can influence their perception of the food's flavor, freshness, and overall quality, thereby affecting their decision to purchase the product (Plasek et al., 2020). Color is often used as an indirect indicator of food quality because it is easily perceived and can be linked to other important factors, such as flavor, texture, aroma, and even nutritional content. It is a simple and fast method that can provide immediate feedback on the condition of the food. These involve human observers evaluating the color of food. This method is subjective and can be influenced by personal biases, lighting conditions, and other factors (White et al., 2020). This method uses instruments like colorimeters or spectrophotometers to measure color in a more objective and reproducible manner. These devices can quantify color by providing numerical values that correspond to specific color coordinates. The Lab* color space, introduced by the Commission Internationale de l'Eclairage (CIE) in 1976, is intended to mimic human vision. It offers a standardized color scale, where L* measures the lightness of a color, and a* and b* indicate the color's position along the red to green and yellow to blue spectrums.This system is widely used because it aligns well with human perception of color differences (Rampáčková et al., 2021).

2.7.3. Texture profile analysis

The International Organization for Standardization (ISO) defines food texture as a diverse set of qualities that can be detected through multiple senses, such as touch, vision, and hearing. Texture involves not just the physical structure of the food, but as well as its reaction to applied mechanical forces, is closely linked to its rheological properties (Yang et al., 2020).

TPA is a widely used technique for assessing the textural attributes of food products. The method involves applying two compressions to the food sample using a texture analyser with a probe, simulating the act of biting or chewing. These compressions are designed to replicate the initial two bites a consumer takes, enabling the measurement of key textural properties such as hardness, cohesiveness, springiness, gumminess, and chewiness. TPA results offer valuable insights into the sensory characteristics of food, which are essential for product development, quality assurance, and consumer satisfaction (Rahman et al., 2021).

This study concentrated specifically on the hardness characteristic measured through TPA of the samples. Hardness, a key textural parameter, represents the amount of force needed to compress the sample, mimicking the pressure of the initial bite during consumption (Shin & Choi, 2021). By concentrating on hardness, the study aimed to understand its impact on the sensory perception of samples, which is a key factor in consumer acceptance and overall product quality. Hardness is a key sensory attribute that greatly affects how consumers perceive and accept food products. It can also be an indicator of quality, as certain levels of hardness may be associated with freshness, doneness, or the integrity of the food structure. To meet consumer expectations and ensure product consistency, understanding and controlling the hardness of food items, such as patties, is essential in product development and quality control.

2.7.4. SDS-PAGE

Polyacrylamide gel electrophoresis (PAGE) is a crucial technique in both molecular biology and biochemistry, utilized to separate proteins and other molecules based on their size and charge. SDS-PAGE, a specific type of PAGE, is particularly valued for its straightforwardness and effectiveness in resolving proteins according to their molecular weights (EE Farg et al., 2024).

In SDS-PAGE, the anionic detergent sodium dodecyl sulfate (SDS) denatures proteins and attaches to them, providing a uniform negative charge-to-mass ratio. This treatment masks the proteins' natural charges and shapes, allowing for their separation primarily according to molecular weight. The separation is further improved by employing a discontinuous polyacrylamide gel system, which includes both a stacking gel and a resolving gel. The stacking gel first concentrates the proteins prior to their entry into the resolving gel. Within the resolving gel, the proteins are then separated according to their size as they migrate through the gel under the application of an electric field. The composition of the stacking and resolving gels, as well as the electrophoresis buffer, is critical for achieving fine resolution of proteins. The consistent binding of SDS to proteins guarantees that separation occurs based on molecular weight, with smaller proteins migrating through the gel more rapidly than larger ones. Although SDS-PAGE is an effective technique for protein separation, quantifying proteins can be difficult because of the protein bands' shape and resolution. However, advancements in analysis software and the use of densitometers have greatly improved the accuracy and reliability of quantifying proteins from SDS-PAGE gels (Jin et al., 2024). SDS-PAGE is used for a range of applications, including analyzing the relative abundance of proteins in a sample, estimating the relative molecular weight of proteins and protein hydrolysates, and identifying and quantifying molecules and impurities within samples (Pulikkottil Rajan, 2024).

2.7.5. Scanning Electron Microscopy (SEM)

SEM serves as a highly advanced method for examining specimen surfaces, delivering high-resolution images that detail intricate surface topographies. An SEM system generally consists of an electron gun, which includes an electron source and an accelerating anode, electromagnetic lenses for focusing the electron beam, a vacuum chamber that accommodates the specimen stage, and a variety of detectors for collecting emitted signals. The electron gun accelerates electrons across a voltage range of 1 to 30 kV, with routine imaging typically conducted at 15 to 30 keV. For enhanced resolution of secondary electrons and reduced electron penetration, a low-voltage SEM (LVSEM) operates at accelerating voltages between 1 and 5 keV (Morishita et al., 2020).

In SEM, the specimen stage is a critical component that allows for the accommodation of specimens ranging in diameter from 3 to 20 cm. The design of this stage typically includes motorization and sometimes computer control, providing up to 3 to 5 degrees of freedom. This includes the capability for linear translation in the x, y, and z axes, as well as tilt and rotation. Such functionality is vital for precisely positioning the specimen in relation to the electron beam, which enhances the imaging of different specimen areas and optimizes signal collection. For mounting the specimen, various holders are used, with flat metal disks, known as specimen stubs, being commonly employed. Proper mounting is essential to ensure that the specimen is securely fixed and maintains good electrical contact with both the holder and the stage. This prevents the accumulation of electrostatic charges, which could affect imaging quality. Additionally, vertical positioning of the specimen is necessary to ensure that it is within the optimal focal range of the electron beam. The correct setup of these parameters is crucial for achieving high-quality SEM images and accurate analysis (Suri, 2020).

SEM is a versatile tool for surface analysis, offering high-resolution imaging and a wide range of operational modes to suit different specimen types and analysis requirements. Its ability to reveal detailed surface features at the nanometer scale makes it invaluable in materials science, biology, and various other fields of research.

2.7.6. Confocal Laser Scanning Microscopy (CLSM)

CLSM represents an advanced imaging method that provides distinct advantages over traditional widefield microscopy, especially in specialized areas such as food science and nanotechnology. CLSM captures images from a single focal plane, reducing out-of-focus light and providing clearer, more detailed images of specific layers within a sample. The laser scanning aspect means that only the area illuminated by the laser is imaged, which can lead to higher-quality images with better resolution than full sample illumination in wide field microscopy (Fried et al., 2021). Using various laser wavelengths, CLSM can excite different food constituents including proteins, polysaccharides, and lipids enabling precise three-dimensional mapping of these components throughout the material (Metilli et al., 2020). CLSM enables detailed visualization of complex structures and interactions by utilizing multicolor fluorescence staining and labelling, which allows for the differentiation of nano-encapsulated food ingredients.

CLSM is capable of imaging dynamic processes such as aggregation, phase separation, and coagulation without disturbing the sample, offering insights into real-time changes in food systems (Gallegos-Cerda, Hernández-Varela, Chanona-Pérez, et al., 2023). Compared to methods such as Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM), the preparation of samples for Confocal Laser Scanning Microscopy (CLSM) is relatively simple. Typically, the sample preparation for Confocal Laser Scanning Microscopy (CLSM) involves adding a dye to the sample, mixing it thoroughly, and subsequently placing it onto a glass slide. CLSM further facilitates electronic adjustment of magnification and offers the capability to visualize samples in both vertical (x-z and y-z) and horizontal (x-y) planes, thereby providing a detailed and comprehensive view of the sample's structure. (Falsafi et al., 2020).

However, there are some limitations. The availability of suitable lasers and efficient fluorophores can be a limiting factor in CLSM. High-intensity laser illumination can potentially damage the sample. CLSM also has a limited resolution compared to TEM and SEM, with a maximum resolution of around 100 μm, whereas TEM can achieve resolutions below 50 picometers, and SEM can achieve resolutions between 0.5 to 4 μm (Gallegos-Cerda, Hernández-Varela, Arredondo-Tamayo, et al., 2023).

This literature review includes an introduction to the faba bean and New Zealand green-lipped mussels, focusing on their nutritional properties and proteins. It also introduces the gelation processes for both plant and animal proteins, including hybrids derived from both sources. Recent studies have explored the properties of hybrid proteins from plant and animal sources, including the interactions between polysaccharides and proteins. Nevertheless, there is a scarcity of research exploring how these hybrid proteins, specifically those derived from green-lipped mussels and faba beans, influence the gelation properties. The existing literature does not extensively cover how the integration of proteins from these sources affects the gelling behavior and structural characteristics of gels, leaving a gap in our understanding of their potential impacts on gelation. This review also highlights the various analytical techniques employed to study these properties, such as rheological analysis and microscopy, which are crucial for understanding the gelation mechanisms and optimizing the formulation of hybrid protein-based products.

3.0. Chapter 3: The effect of different ratios of faba bean protein isolate (FBPI) and defatted green-lipped mussel powder (DMP) on the microstructure and rheological properties of protein gels

Keywords: Faba bean protein isolates; defatted mussel powder; gelation; microstructures; water holding capacity; rheological property; protein solubility

3.1. Introduction

Recently, combining animal and plant proteins has been acknowledged as a strategy to improve proteins' technological and nutritional features in the formulation of innovative food products (Hinderink et al., 2020). The demand from the global society seeks a new approach to resources that are healthy and sustainable for nature. Green-lipped mussels, known scientifically as *Perna canaliculus*, are indeed a significant species in New Zealand, both ecologically and culturally. The green-lipped mussel industry is a significant part of New Zealand's economy. The projection by the New Zealand government for annual aquaculture revenues to reach NZ$3 billion by 2030 indicates a strong commitment to expanding the industry. This growth is likely to be driven by increasing global demand for seafood, the health benefits associated with mussel consumption, and the industry's focus on sustainable practices (Stenton-Dozey et al., 2021). These mussels are harvested for human consumption and are known for their rich flavor and high nutritional value. Additionally, the mussels are used in the production of supplements, particularly those aimed at supporting joint health, as they are believed to have anti-inflammatory properties. The nutritional profile of green-lipped mussels, rich in protein, carbohydrates, lipids, vitamins, and omega-3 fatty acids, positions them as a valuable dietary supplement and food source. This nutritional richness has made them not only a staple in the diet of the local Māori people but also a sought-after product in international markets (Abshirini et al., 2022). Green-lipped mussels are a rich source of various proteins, including sarcoplasmic proteins, stromal proteins, and myofibrillar proteins. Myofibrillar proteins, the primary structural proteins in mussel muscle tissue, contribute to the mouthfeel and texture of the mussel meat. Sarcoplasmic proteins, found in the sarcoplasm, play roles in energy metabolism and protein synthesis, providing essential amino acids and other nutrients. Stromal proteins, part of the mussel's connective tissue, support the mussel's structure and function, influencing its texture and processing characteristics. These diverse proteins make green-lipped mussels a valuable ingredient in enhancing the nutritional profile of foods, improving texture, and developing innovative products in the seafood and plant-based food sectors (Zou et al., 2021).

Legumes, including faba beans, have become increasingly popular as a source of high-quality protein in various food products, catering to the growing demand for vegetarian and plant-based options. These products not only offer a sustainable alternative to animal proteins but also contribute to dietary diversity and nutritional balance. Faba beans, in particular, are noteworthy for their protein content, which is around 27% on a dry weight basis. This makes them a valuable ingredient for formulating protein-rich foods. Compared to soybeans, which are the leading source of vegetable protein worldwide, faba beans have a lower lipid content (1% compared to 18% in soybeans) and contain less of the flatulence-causing factors raffinose and stachyose than cowpeas. These attributes make faba beans an attractive option for use in a wide range of food products. The major proteins found in faba beans are globulins, which include legumin (11S) and vicilin (7S) (Langton et al., 2020). These proteins are similar in structure and function to those found in soybeans, suggesting that faba beans could serve as a viable alternative or supplement to soy in various applications. The functional properties of these proteins, such as their ability to form gels and emulsions, make them suitable for use in products like tofu, tempeh, and plant-based milk alternatives. The versatility of faba beans in food processing opens up opportunities for innovation in plant-based foods. As consumers become more health-conscious and environmentally aware, the demand for sustainable, high-quality plant proteins is likely to continue growing. Faba beans, with their nutritional profile and functional properties, are well-positioned to play a significant role in meeting this demand (Augustin & Cole, 2022).

Consequently, the combination of plant proteins and animal proteins has been shown to be advantageous for maintaining a healthy body. Combining both plant and animal proteins ensures a comprehensive amino acid profile, crucial for vital functions such as muscle repair, immune system support, and overall health, as animal proteins provide the essential amino acids lacking in some plant sources (Nichele et al., 2022). Therefore, there is a growing focus on analyzing and understanding the aggregation, gelation, and emulsifying properties of mixing plant and animal proteins due to high market demand. When protein concentrations exceed the critical level necessary for gelation, the proteins initiate a phase of aggregation, forming initial clusters. The gelation mechanisms of proteins are fundamental in determining their functional properties, playing a pivotal role in the development of protein-based products with specific textures and stability profiles. This understanding is crucial for optimizing protein behavior across diverse applications. Thermal stability, a critical characteristic influenced by a protein's complex tertiary and quaternary structures, significantly affects its functionality under

varying temperature conditions. Proteins are sensitive to temperature changes, which can lead to unfolding or denaturation. The thermal stability of a protein is measured by the energy needed to disrupt its stable conformation, a process that involves breaking non-covalent interactions such as hydrogen bonds, ionic bonds, and hydrophobic interactions. The gelation process of proteins, a complex series of physicochemical transformations, is crucial for the development of protein-based products with specific textures and stability profiles. This process typically unfolds in three sequential stages: co-fusion, nucleation, and growth. During heating, proteins undergo denaturation, unfolding their native structures and exposing reactive and hydrophobic sites. These denatured proteins then coalesce into initial small aggregates, which serve as nuclei for further aggregation. Nucleation, a key step, can occur homogeneously throughout the solution or heterogeneously, initiated by impurities or surface irregularities. Following nucleation, the aggregates grow by incorporating more protein molecules, eventually interconnecting to form a three-dimensional network that converts the fluid into a gel, trapping water, air, and solutes within its structure. The gelation process is influenced by various factors, including protein concentration, temperature, pH, ionic strength, and the composition of the protein solution, whether it's a single protein or a blend, each affecting the final properties of the gel (Ji et al., 2024). Moreover, heat-induced gelation is a specific type of gelation mechanism where heat is the primary factor causing proteins to unfold, aggregate, and eventually form a gel. This process is widely used in the food industry for creating products like surimi (a fish paste), protein-based meat analogues, and certain dairy products. The temperature at which gelation occurs varies depending on the protein source and its concentration. The existing scientific literature primarily examines the gelation properties of protein composites sourced from animal or dairy origins, leaving a noticeable gap in research concerning hybrid compositions that blend animal and plant proteins. The combination of plant and animal proteins has emerged as a promising approach to overcome the functional and nutritional limitations inherent in each (Khalesi & FitzGerald, 2021). For instance, mixing soy milk with bovine and/or buffalo milk, this strategy not only enhances the sensory characteristics of products like yogurt but also offers the potential for probiotic delivery (Ismail et al., 2017). By blending proteins from different sources, it's possible to achieve a more balanced amino acid profile at a lower overall protein content, thereby reducing calorie intake and the risk of intestinal discomfort (Khalesi & FitzGerald, 2021). However, there is no available information regarding the combination of FBPI and DMP proteins in hybrid gelation systems. This represents a significant gap in the research and development of innovative food products that could leverage the unique properties of both plant and marine-based protein sources. This study

aims to investigate the gelation behavior and microstructural characteristics of these protein mixtures at various ratios, while maintaining a fixed protein concentration of 12.5 w/w%. The anticipated outcome of this study is to provide valuable insights for the development of blended gel-based food products that incorporate both animal and plant proteins.

3.2. Materials and Methods

3.2.1. Materials

Faba bean protein isolate (FBPI), containing 85% protein, was purchased from NZProtein in New Zealand and stored under dry conditions to preserve its quality. The composition of FBPI, as indicated on the packaging label, is presented in **Table 2**. Defatted green-lipped mussel powder (DMP), provided by Sanford Ltd., New Zealand, the composition of DMP is described in **Table 3**. Additionally, analyses of the mineral content of DMP (**Table 4**) and the amino acid composition of both FBPI and DMP (**Table 5**) were conducted at the Nutrition Laboratory of Massey University in Palmerston North, New Zealand.

The chemicals and reagents used included dithiothreitol (DTT), sodium dodecyl sulphate (SDS), isopropanol, and urea solution, all sourced from Sigma-Aldrich in New Zealand. Additional reagents such as sodium chloride (NaCl), sodium azide, sodium hydroxide (NaOH), hydrochloric acid (HCl), and glacial acetic acid were obtained from Ajax Fine Chem, New Zealand. Other materials, including β-mercaptoethanol, Coomassie brilliant blue, Bradford reagent, bovine serum albumin (BSA), 4x Laemmli sample buffer, molecular weight marker (Precision Plus Protein™ Dual Xtra Prestained Protein Standards), and fast green, were supplied by Fisher BioReagents in New Zealand. All chemicals and reagents used for characterization were of analytical grade.

Table 2. Composition of faba bean protein isolate (FBPI) (as per packaging label)

Nutritional information		
Composition	**Average value per serving (31.25g)**	**Average value per (100g)**
Energy	522 kJ	1670 kJ
Calories	125	399
Protein	26.5 g	85 g
Fat -total	1.7 g	5.4 g
-saturated	0.5 g	1.2 g
Carbohydrate	1.2 g	3.8 g
-sugar	<0.5 g	<1.0 g
-fibre	<0.5 g	<0.5 g
Sodium	91 mg	290 mg

Table 3. Composition of DMP (as provided by Sanford Ltd, New Zealand)

Nutritional information	
Composition	**Percentage (%)**
Moisture	4.0
Ash	29.1
Crude Protein	48.6
Fat	4.0
Carbohydrate	14.3

Table 4. Mineral content of DMP (as per laboratory analysis)

Mineral information		
Composition	Value	Units
Calcium	8100	mg/kg
Magnesium	4300	mg/kg
Potassium	9000	mg/kg
Sodium	30000	mg/kg
Phosphorus	6700	mg/kg
Iron	770	mg/kg
Arsenic	11.7	mg/kg
Cadmium	0.33	mg/kg
Chromium	4.4	mg/kg
Copper	5.2	mg/kg
Iodine	18.2	mg/kg
Lead	0.42	mg/kg
Manganese	25	mg/kg
Mercury	0.058	mg/kg
Selenium	1.94	mg/kg
Zinc	49	mg/kg
Chloride	5.2	g/100g

Table 5. Amino acid composition of FBPI and DMP (as per laboratory analysis)

AMINO ACIDS mg/100mg protein	**FBPI**	**DMP**
Aspartic Acid	11.87	8.96
Threonine	3.87	3.65
Serine	5.16	3.31
Glutamic Acid	16.81	8.35
Proline	4.62	2.79
Glycine	4.48	7.33
Alanine	4.34	3.17
Valine	5.14	3.63
Methionine**	0.95	-
Isoleucine	4.33	3.23
Leucine	7.99	4.35
Tyrosine	3.98	2.81
Phenylalanine	4.56	3.44
Histidine	2.42	1.29
Lysine	6.88	6.23
Arginine	9.53	5.46
Taurine	ND	5.60
Cysteine		1.15
Methionine***		2.29

Methionine** refers to the amino acid profile includes cysteine and methionine, with the methionine result coming from performic acid oxidation.

Methionine*** refers to the amino acid profile using HCl hydrolysis, however, there may be about a 10% loss due to the hydrolysis process.

3.2.2. Methods

3.2.2.1. Preliminary study

In the initial phase of this study, the minimum gelation concentration (MGC) of proteins from both plant (FBPI) and animal (DMP) sources were determined. The MGC represents the lowest concentration at which protein can form a stable gel under specific conditions. It was observed that solutions containing 12.5% of total protein concentration from both FBPI and DMP sources were capable of forming a gel when subjected to heating at 90°C for 30 min and adjusted to pH 7.

The study assessed the protein content of the powders, which included FBPI (85%) and DMP (48.6%). Thus, the mixtures with 12.5% total protein at various ratios (100:0, 75:25, 50:50, 25:75 and 0:100) are presented in **Table 6**.

Table 6. Protein ratio compositions and Total weights for FBPI and DMP mixtures at 12.5% Total Protein Concentration.

Conc. (w/w)	**FBPI : DMP (%)**				
	100 : 0	**75:25**	**50:50**	**25:75**	**0: 100**
FBPI	2.94	2.21	1.47	0.74	0.00
DMP	0.00	1.30	2.60	3.91	5.21
Distilled Water + 0.02 w/w% sodium azide	17.06	16.49	15.93	15.36	14.79
Total weight (g)	20	20	20	20	20

3.2.3. Sample preparations

The steps outlined below detail the preparation process for all methods of characterization.

A 12.5 weight percent protein solution was prepared in five different ratios (% w/w): 100:0, 75:25, 50:50, 25:75, and 0:100. This was achieved by dispersing the FBPI powder and DMP in distilled water containing 0.02 weight percent sodium azide to inhibit microbial growth. The mixture was gently stirred magnetically for 24 hours at room temperature to ensure complete hydration. Following hydration, the pH of the solution was measured and adjusted to 7 using 1 M hydrochloric acid or 1 M sodium hydroxide, while stirring continuously.

3.2.4. Visual appearance and Color analysis

The color profiles of the samples were analysed both before and after heating in an oven at 90 °C for 30 min using a Minolta Chroma Meter CR 300. This analysis utilized the CIELAB color space, measuring parameters such as L* (lightness, with a range from 0 to 100), a* (where positive values indicate redness and negative values indicate greenness), and b* (where positive values denote yellowness and negative values denote blueness). Calibration of the equipment was performed using a white tile with reference values Y = 86.6, x = 0.3162, and y = 0.3232. The reported results represent the average of measurements taken in triplicate, with five measurements per sample.

3.2.5. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

The SDS-PAGE method was employed to assess the protein profiles of the solutions according to their molecular weights (He, 2011; Liu et al., 2009). The analysis was conducted under both reducing and non-reducing conditions. For sample preparation, 2.0 g of each solution (prior to heat treatment) and gel (post-heat treatment) were diluted with 2.0 ml of 2 wt% SDS solution in Eppendorf tubes. This mixture was rotated continuously at room temperature overnight. Under reducing conditions, 25 µl of the 12.5% mixture solution were combined with 1.25 µl of a reducing agent (β-mercaptoethanol). In contrast, for non-reducing conditions, 25 µl of the 12.5% mixture solution were mixed with 1.25 µl of deionized water, then boiled for 10 min. 10 µl of boiled samples were loaded into the wells of a Mini-PROTEIN TGX Gel (Bio-Rad Laboratories, USA). Electrophoresis was carried out at 130 V in running

buffer for approximately 45 min. Following this, the gel was stained in staining solution for 45 min under constant shaking. The gel was then destained 3–4 times, with the solution in the container being checked regularly. The destaining process was stopped once the solution appeared clear and no longer blue. To determine the protein bands, a protein molecular weight marker (Precision Plus Protein Dual Xtra Standards, Catalog #161-0377, Bio-Rad, USA) between the range of 10 and 250 kDa was run alongside the samples on the same gel. ChemiDoc touch imaging system (Bio-Rad, USA) was used to scan the gel.

3.2.6. Microstructure observation

The solutions, prepared at a concentration of 12.5 w/w %, were made using the same method as previously described. A confocal laser scanning microscope (CLSM) was employed to examine the microstructures of the mixtures before and after heat treatment, comparing the solutions and gels formed post-treatment. Approximately 50 μL of the sample was mixed with 10 μL of fast green dye (Sigma Aldrich, USA) in an Eppendorf tube, gently shaken, and allowed to sit for 2 min to ensure thorough staining. The stained samples were then transferred onto a microscopy slide with a single cavity and covered with a glass coverslip. The slides were incubated in an oven at 90 °C for 30 min to form gel samples. To prevent evaporation, nail polish was applied to seal all the samples. The microstructural characteristics of the solutions and gels were observed using a confocal laser scanning microscope equipped with a 100× oil immersion objective lens, and the resulting images were analyzed using Image J software (USA) (Wang et al., 2019).

3.2.7. Particle size analysis

The Malvern Mastersizer 3000 (Malvern instruments Ltd. Worcestershire, UK) was used to analyse the angle-dependent light scattering volume-based size particles of FBPI/DMP in native state (before heat treatment). Samples of 450 - 500 μl were analyzed at 2000 rpm using reverse osmosis (RO) water as the dispersion medium. Refractive indices of 1.47 and 1.33 were applied to samples and dispersant, respectively, to estimate the average refractive index of solutions (Kayeye, 2023).

3.2.8. Intermolecular forces determination

Approximately 2 grams of both solution and gel samples, before and after heat treatment, were dissolved in 10 mL of each of the following solutions: A (0.05 M NaCl), B (0.6 M NaCl, pH 7.0 adjusted with 0.1 M NaOH or 0.1 M HCl), C (0.6 M NaCl, 1.5 M urea), D (0.6 M NaCl, 8 M urea), and E (0.6 M NaCl, 8 M urea, 0.5 M DTT). The mixtures were stirred for approximately 1 hour and then subjected to centrifugation at 10,000 rpm for 30 min at 4°C.

To assess the intermolecular forces of the solutions with varying ratios of FBPI and DMP, a method with minor modifications was used (Li et al., 2022) . Following centrifugation, the supernatant was carefully pipetted, and the soluble protein concentration in the supernatants was quantified using a Bradford Protein Assay Kit (Bio-Rad, USA) with bovine serum albumin (BSA) as the standard. The protein concentration was determined by measuring the absorbance at 595 nm using a UV-Vis spectrophotometer (1900, Shimadzu, Japan).

$$(\%)\ \text{Protein Solubility} = \frac{\text{Protein content in the supernatant}}{\text{Total protein content}} \times 100 \qquad \textbf{(Equation 1)}$$

3.2.9. Gel characteristic analysis

3.2.9.1. Rheological measurements

The gelation was formed using a heat treatment method at 90 °C for 30 min. Rheological measurements were conducted using a stress-controlled rheometer (DHR-3, TA Instruments, New Castle, DE, USA) equipped with a parallel plate geometry, with a 40 mm diameter and a 1 mm gap., following the methodology of Yang et al. (2022). Approximately 1.5 mL of mixture was carefully placed on the rheometer plate using a dropper, with adjustments made to ensure the sample fit the geometry. To prevent evaporation during the measurements, soybean oil was applied around the sample.

The rheological analysis was performed as follows: Initially, the samples was heated from 20°C to 90°C at a rate of 2°C/min, held at 90°C for 30 min, and then cooled from 90°C to 20°C at the same rate, followed by a 15 min holding period at 20°C. Throughout this process, strain and frequency were maintained at 1% and 1 Hz, respectively. Next, a frequency sweep was

performed with a constant strain of 1%, varying the frequency between 0.01 and 10 Hz. Finally, a strain sweep was carried out at a constant frequency of 1 Hz, with strain ranging from 0.01% to 1000%.

3.2.9.2. Gel hardness

Texture profile analysis was performed with modifications based on Liang et al. (2021). Approximately 10 grams of each 12.5 w/w% solution were transferred into containers, covered with lids, and then heated in an oven at 90°C for 30 min to form gels. After heating, the samples were allowed to cool to room temperature for 30 min (**Figure 3**).The texture analysis was carried out using a textural analyzer (TA.XT.plus, Stable Micro Systems, UK) equipped with a 5 kg load cell. Each sample was placed on a heavy-duty platform and analyzed using a 35 mm cylindrical probe with double compression. The test parameters were set to 50% deformation, a trigger force of 5.0 g, and pre-test, test, and post-test speeds were all set to 1.0 mm/s. This analysis aimed to assess the hardness of the samples, with each measurement being repeated in duplicate to ensure accuracy.

Hardness (N) = Maximum force of the first compression **(Equation 2)**

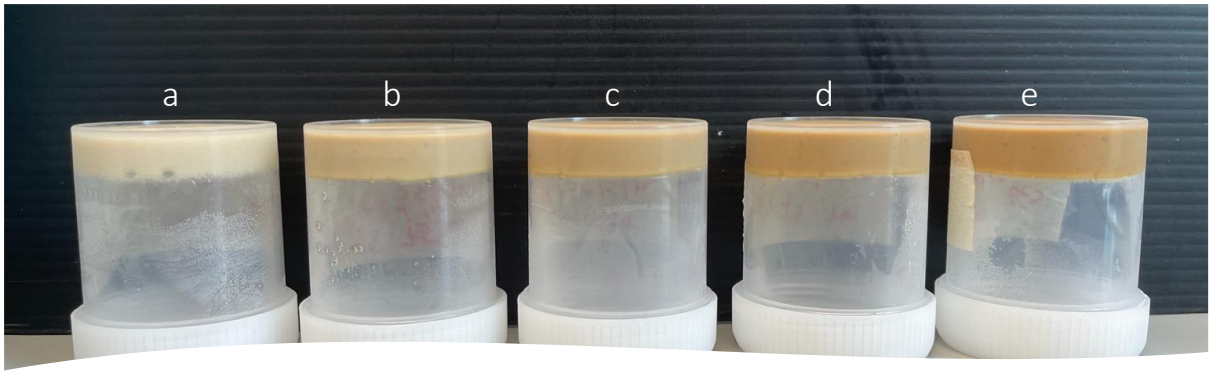


Figure 3. Protein gel samples with varying ratios of FBPI to DMP: (a) 100:0, (b) 75:25, (c) 50:50, (d) 25:75, and (e) 0:100, respectively.

3.2.9.3. Water holding capacity (WHC)

The WHC was assessed following slight modifications of the method outlined by He et al. (2021). Approximately 2 g of each 12.5% solution were placed in Eppendorf tubes, heated in an oven at 90 °C for 30 min to form gels, and then cooled at room temperature for 30 min. After cooling, the samples were centrifuged at 6000 x g for 10 min at 20°C using a Heraeus Pico 17 centrifuge (Thermo Scientific, USA). Excess water on the gel surfaces and within the centrifuge tubes was carefully removed with filter paper. The WHC (%) was then calculated according to the formula in Equation 3.

Water holding capacity (%) = ($(W_2 - W_0)$) / ($(W_1 - W_0)$) x 100 **(Equation 3)**

3.2.10. Statistical data analysis

All experiments were conducted in duplicate or triplicate, and results were reported as the mean ± standard deviation from at least two experiments. A one-way analysis of variance (ANOVA) was performed using SPSS version 21.0 (Chicago, II, USA) to evaluate significant differences between samples. Tukey's test was applied for post hoc comparisons, with a significance level set at $P < 0.05$. Figures were generated using Origin Pro Software (Origin Pro 2024b, USA), and Image J was utilized for image processing.

3.3. Results and Discussions

3.3.1. Visual appearance and color analysis

Figure 4 showed the mixtures of FBPI to DMP with different protein ratios before and after heat treatment for 30 min at 90°C. The lightness value tends to decrease after heating for all FBPI ratios. For instance, the 100:0 (FBPI) has a lightness value before heating and decreases slightly after heating. Then, 75:25 50:50, 25:75, and 0:100 ratios all follow the same pattern, with decreasing lightness values post-heating, indicating a darkening effect due to heating. As shown in **Figure 5 (A)**. the lightness decreases as the proportion of the mussel powder increases. This suggests that FBPI was lighter in color that the mussel protein. Krause et al. (2023) found that the solubility and structural properties of FBPI were prone to alterations upon exposure to heat, leading to changes in color attributed to protein denaturation and aggregation. Investigations on faba bean protein isolates reveal that heating can induce a decrease in lightness (L* value), causing the protein to appear darker.

From **Figure 5 (B)**, it can be observed that heating generally increased the a* values across all ratios, shifting the color towards redness. Higher proportions of DMP powder led to higher initial a* values, indicating that mussel powder is inherently more red compared to FBPI. This effect was more pronounced in samples with higher DMP content, suggesting that mussel powder significantly contributed to the color change upon heating. The natural pigments present in mussels, including carotenoids, were responsible for these color alterations (Naik & Hayes, 2019). Heating caused the oxidation of some compounds, intensifying the red hue (Konieczny et al., 2021). Additionally, the high protein content and bioactive compounds, such as omega-3 fatty acids and peptides, in mussel powder influenced color stability and response to heat.

Figure 5 (C) illustrated that yellowness (b* value) increased as the proportions of DMP increased. Klunklin and Savage (2018) found that green-lipped mussel powder, with its significant protein and mineral content, was associated with color transformations occurring upon exposure to heat. The heating process induced Maillard reactions, leading to enhanced brown pigmentation as well as increased red and yellow hues.

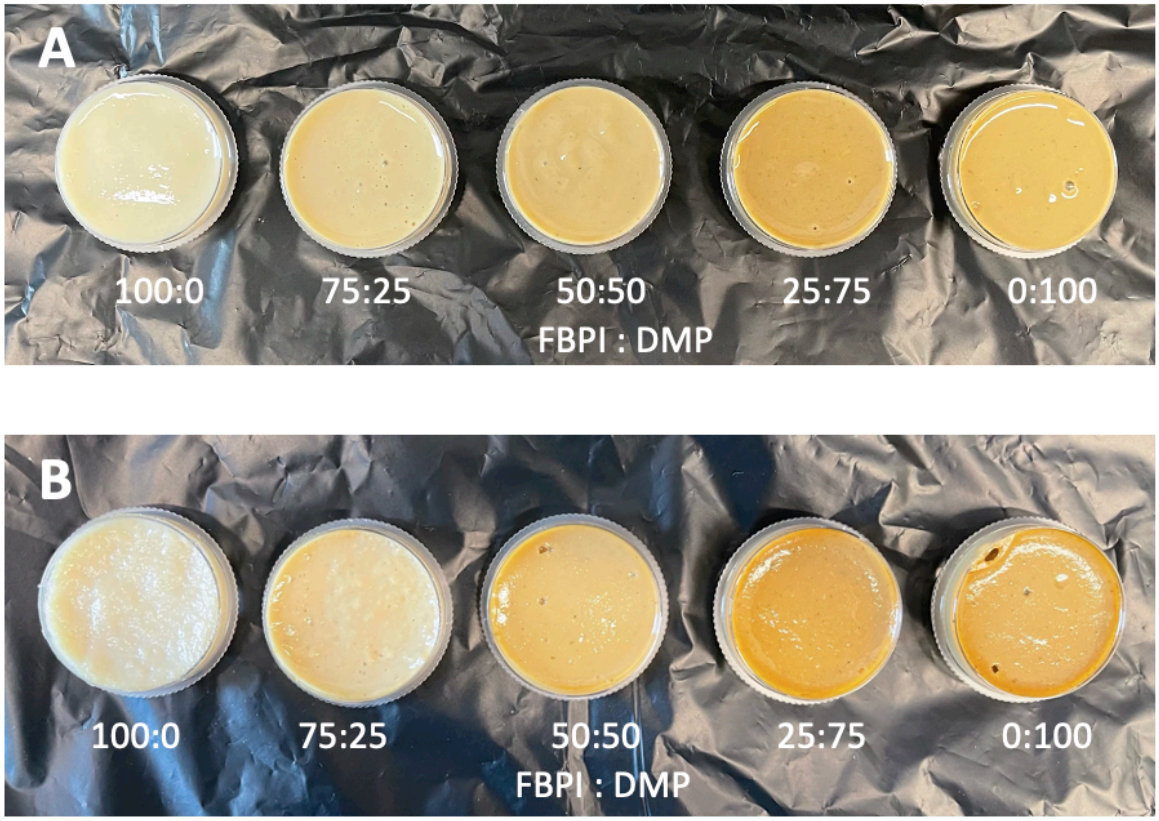


Figure 4. Color analysis of the mixture before heat treatment (A) and after heat treatment (B) with five different protein ratios of FBPI to DMP.

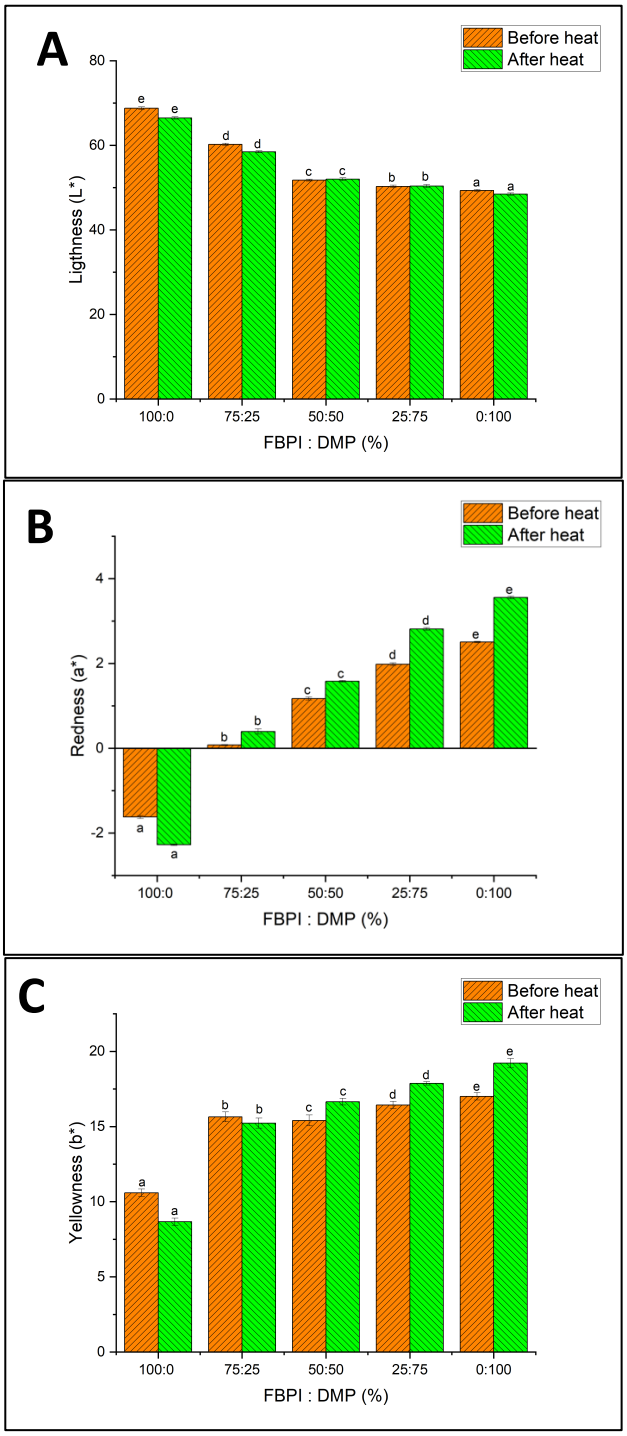


Figure 5. The lightness (L*), redness (a*), and yellowness (b*) of the mixtures were measured before and after heat treatment in an oven at 90°C for 30 min. The figure indicated that the particle size distributions of the samples were significantly different, with different letters above the bars representing statistically significant differences ($p < 0.05$). In the figure, 100:0 represented 100% FBPI with 12.5% total protein in the mixture. The other ratios were as follows: 75:25 for 75% FBPI and 25% DMP; 50:50 for 50% FBPI and 50% DMP; 25:75 for 25% FBPI and 75% DMP; and 0:100 for 100% DMP.

3.3.2. SDS-PAGE

The SDS-PAGE analysis served as an initial tool to visualize the protein composition of the mixtures and to identify any changes in the protein banding patterns that could suggest interactions between the different protein sources. The presence of new bands or shifts in molecular weight may indicate complex formation or conformational changes. SDS-Page was performed under reducing (**Fig. 6 A**) and non-reducing (**Fig. 6 B**) conditions to analyze the protein-protein interaction of the mixtures. The non-reducing SDS-PAGE method maintains the denaturing properties that alter the protein structure and confer a negative charge on it. This negative charge facilitates the separation of proteins solely based on their size, while the protein remains undivided and intact as a single unit without disassembling into individual monomers.

Conversely, the reducing SDS-PAGE technique imparts a negative charge to the protein and disrupts the disulfide (S-S) bonds, transforming them into -SH bonds. As a result, the protein undergoes separation into its constituent monomers. Under reducing conditions, the breakdown of disulfide bonds can lead to the dissociation of protein complexes into their individual subunits or cause a change in the protein's conformation that makes it more compact (Alrosan et al., 2022). This can result in a thicker band on a gel because the reduced protein may be more uniform in size and shape, migrating more cohesively through the gel.

It was observed that a fraction of the proteins failed to enter into the gel, as evidenced by the presence of sizable or insoluble material in the diagram. This finding indicates that the commercial plant protein isolates and animal protein under investigation contain some constituents that exhibit resistance to dissolution, even in the presence of both SDS and a reducing agent. The primary constituents of FBPI were legumin-like 11S proteins, with α- and β-polypeptides being the prominent components. Faint bands were detected, indicating the presence of vicilin and convicilin (Nivala et al., 2021).

The SDS-PAGE analysis of the FBPI/DMP mixture showed bands corresponding to legumin (~50-60 kDa), convicilin (~40-50 kDa) and vicilin (~20-25 kDa), with varying intensities depending on the ratio of the FBPI protein sources in the mixture.In the SDS gels with only green lipped mussel (F4), there were hardly any bands present both before and after heat treatment, indicating a minimal amount of soluble protein in non-reducing condition. Nevertheless, lighter bands were slightly visible in reducing condition. Furthermore, in formulations F2, F3, and F4, which contained DMP, **Figure 6 B** shows visible protein bands at 100 kDa, 43 kDa, and 15 kDa. Based on previous study on mussels, these bands could correspond to paramyosin (98-107 kDa), actin (43-46 kDa) and light chain myosin (15-20 kDa), respectively (Konieczny et al., 2021; Liu et al., 2009). Heat treatment leads to significant aggregation and loss of soluble proteins, particularly under non-reducing conditions, while reducing conditions help maintain some solubility. After heat treatment in reducing condition (B) it can been seen that the bands (F4) appear lighter compared to before heat treatment. This was because when heat applied, these reduced proteins can form large aggregates that are not effectively solubilized in the SDS sample buffer, resulting in their absence or lower intensity on the gel.

In non-reducing conditions (**Fig. 6 A**), the protein structure may not be fully disrupted because disulfide bonds were intact. These bonds can hold protein aggregates together, and without the presence of a reducing agent like β-mercaptoethanol, these aggregates may not enter the separation gel or may not separate well, resulting in larger aggregates situated at the top of the gel. In contrast, when reducing conditions are used, β-mercaptoethanol acts as a strong reducing agent that can cleave disulfide bonds between cysteine residues. This action helps to disrupt the quaternary structure of proteins, which was the assembly of multiple protein subunits into a functional protein complex. By breaking these disulfide bonds, the protein complexes can be dissociated into their individual subunits, which will then migrate through the gel more effectively (Zhu et al., 2018). The use of a reducing agent like β-mercaptoethanol typically results in clearer and more distinct bands on the gel because it helps to break down aggregated proteins into their constituent parts. This can improve the resolution of the gel, making it easier to identify and analyze individual protein bands.

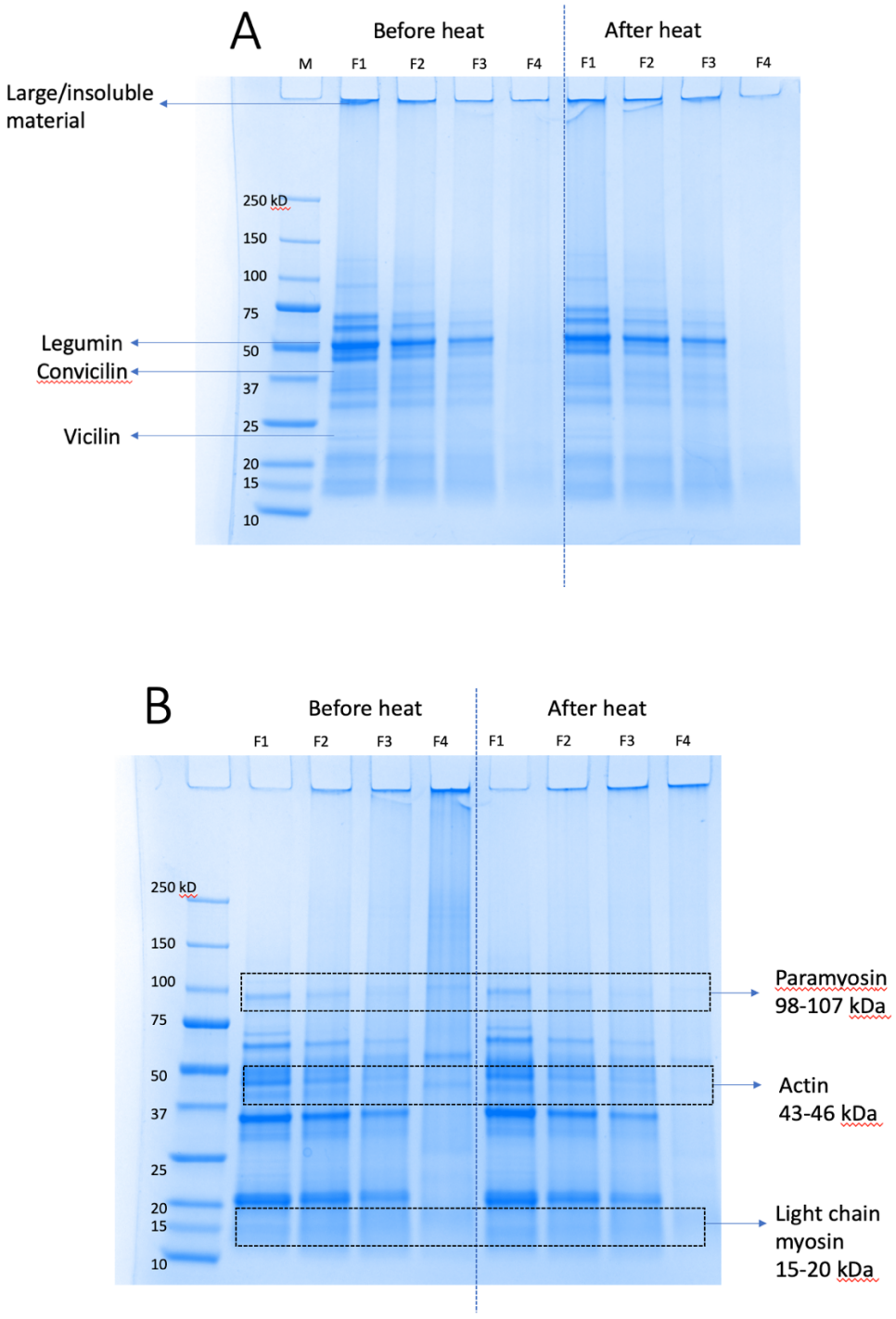


Figure 6. SDS-PAGE analysis was conducted under non-reducing (A) and reducing conditions (B) for the solution created by dissolving the FBPI and DMP mixture both before and after heat treatment. The samples, labeled F1, F2, F3, and F4, corresponded to different protein ratios of FBPI to DMP at 100:0, 75:25, 50:50, and 0:100, respectively.

3.3.3. Microstructure observations using CLSM

Figure 7 illustrated the microstructure of the mixture as examined by CLSM, highlighting the changes before and after heat treatment. The proteins, stained with Fast Green, appeared green, while the spaces or pores between the protein particles were shown in black. The CLSM images labeled (A) show the microstructure of the protein mixtures before they are subjected to heat treatment at 90°C for 30 min. At this stage, the proteins were expected to be in a soluble form, possibly showing a dispersed or slightly aggregated state depending on the protein concentration and the ratio of FBPI to DMP. Then, the CLSM images labeled (B) illustrated the microstructure of the protein mixtures after heat treatment. This process is expected to induce significant changes in the protein structure, leading to aggregation and, at sufficient protein concentrations, the formation of a gel network. The images showed larger protein aggregates and a continuous gel network, indicating that the heat treatment has caused the proteins to unfold and interact with each other, forming a three-dimensional matrix that traps water and other components, thereby transforming the mixture into a gel (Du et al., 2021).

From observations, the transitions from a solution to a gel state in pure FBPI (100:0) involve a significant change in microstructure. Initially, the solution exhibits a relatively uniform structure with small, dispersed particles. Upon gelation, the microstructure becomes more continuous and denser. A previous study observed the microstructure of faba bean protein gels at pH 7 was found to be dense and homogeneous (Langton et al., 2020). The presence of only FBPI seems to have the ability to adjust the formation structure, partly because it can interact with the protein at the interface and form a gel network in the continuous networking forming upon gelation. These features arise from the strong gelation properties due to their high content of hydrophobic and sulfur which contains amino acids which can form strong protein-protein interactions (Shi, 2022). However, mussel powder disrupts the gel network formation of FBPI leading to a weaker and heterogenous gel structure. It can be observed that pure mussel powder (0 : 100) does not form a strong gel network consistent with its lower storage modulus (*G'*) in rheological measurements. This could be due to their amino acid composition which lacks sufficient hydrophobicity and covalent bonding capacity resulting in weaker gels (Zhao et al., 2021). Also, the particle size and purity of the proteins may also influence gel formation. Larger particles or impurities can hinder the formation of a uniform gel network. As the quantity of DMP increased, the gel microstructure became progressively

more heterogeneous, irregular and coarser resulting in the development of larger structures which showed a reduction in gel strength and other physical properties (Zhang et al., 2024).

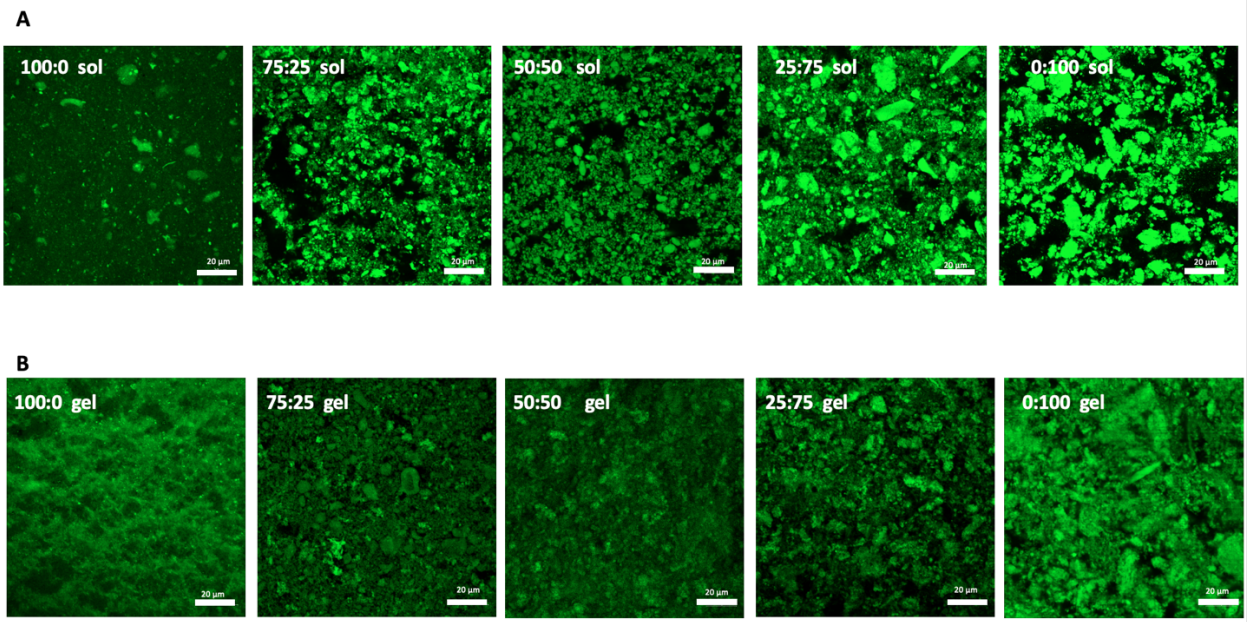


Figure 7. CLSM images of protein mixtures with varying ratios of FBPI and DMP before (A) and after (B) heat treatment at 90°C for 30 min.

3.3.4. Particle size analysis

The distribution of particles within the aggregations of the mixture was analysed using Dynamic Light Scattering (DLS). **Figure 8** displayed the particle size distribution of the mixture aggregate. The distribution showed a peak around 30 μm, indicating that the particles in pure FBPI were relatively larger compared to the mixtures containing DMP. As the substitution ratio of DMP increased, the particle size of the hybrid proteins declined. This trend was evident from the leftward shift of the distribution peaks in the figure. The reason behind this could have been the inherently smaller particle size of DMP compared to FBPI. When FBPI was partially or completely replaced by DMP, the overall particle size distribution of the mixture decreased.

The particle size of the mixtures played a crucial role in determining the physicochemical characteristics of the solutions, which influenced the stability, appearance, and sensory attributes. Larger particle size was frequently associated with an undesirable gritty mouthfeel. It was observed that the particle size of 100% FBPI (100:0) was considerably smaller in comparison to the other mixture. This finding highlights the superiority of faba bean protein isolates over all other components. By supporting the discussion regarding the determination of *G'* value in **section 3.4.6** and the particle size of only FBPI showed the smaller particle size compared the others, this will lead to the formation of much denser and compact gel network. The particle size (D[3,2] values) influences the storage modulus (*G′*) of the protein mixtures. According to **Figure 9 (A)**, smaller particles, as seen in the 100:0 and 75:25 protein ratios, tend to have a larger surface area available for interactions, which can enhance the network structure and gel strength, resulting in higher *G′* values. Conversely, larger particles in the 25:75 and 0:100 protein ratios may have fewer interactions, leading to weaker gel structures and lower *G′* values. Thus, the observed *G′* values align with the particle size distribution, indicating a relationship between particle size and gel strength.

Figure 9 (B) indicated that the particle size (D[4,3]) increased as the ratio shifts from 100:0 to 0:100. As the proportion of mussel powder increased in the mixture (moved from 100:0 to 0:100), the average particle size (D[4,3]) increases. The smallest particles were observed with pure faba bean protein isolate (100:0), while the largest particles were seen with pure mussel powder (0:100). Particle size can affect the texture, solubility, and overall mouthfeel of food products. Smaller particle sizes (e.g., in the 100:0 ratio) might result in smoother textures, while larger particle sizes (e.g., in the 0:100 ratio) could lead to coarser textures. Moreover, larger aggregates can sometimes disrupt the uniformity and connectivity of the network protein. When protein aggregates become too large, they may not interconnect effectively, leading to a weaker overall gel structure. At higher concentrations of mussel protein (0:100) the phase separation might occur, where the proteins separate into distinct regions rather than forming a homogeneous network. This can decrease the gel's ability to store elastic energy, leading to a lower G' (**Section 3.4.6**). Larger aggregates might also alter the water-binding properties of the gel. If the water was not evenly distributed or trapped in large aggregates, it can reduce the network's cohesiveness and elasticity. Supawong et al. (2021) also observed that the mixtures of rice protein isolate and fish protein hydrolysate showed improved solubility and gelation properties. The particle size distribution influenced the texture and stability of the food products, making the blends suitable for diverse applications. Moreover, larger particles (as seen in higher FBPI ratios) tend to create stronger gels, likely due to increased interactions and entanglements between the particles while, smaller particles (as seen in higher DMP ratios) may result in weaker gels because they do not interact as extensively, leading to a less robust network as can be seen in CLSM. This particle size variation significantly affects the rheological properties, with higher FBPI ratios, larger particles forming stronger gels and higher DMP ratios, smaller particles forming weaker gels..

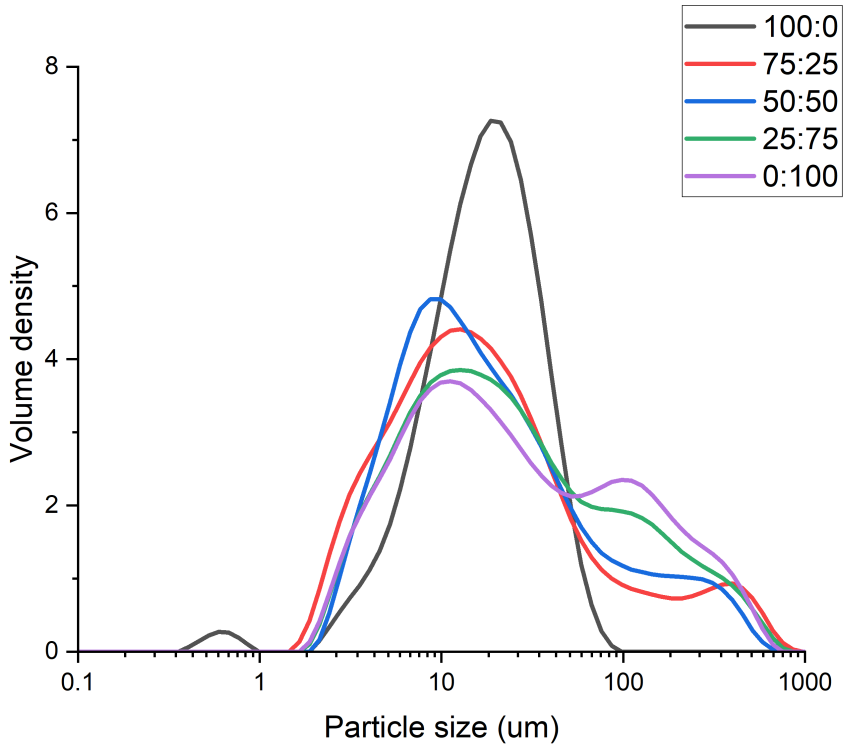


Figure 8. The particle size distribution of mixtures before heat treatment was analyzed for different ratios of FBPI to DMP. In the figure, 100:0 represented 100% of 12.5% total protein of FBPI in the mixture, followed by 75:25, which represented 75% of FBPI and 25% of DMP; 50:50 represented 50% of FBPI and 50% of DMP; 25:75 represented 25% of FBPI and 75% of DMP; and 0:100 represented 100% of DMP.

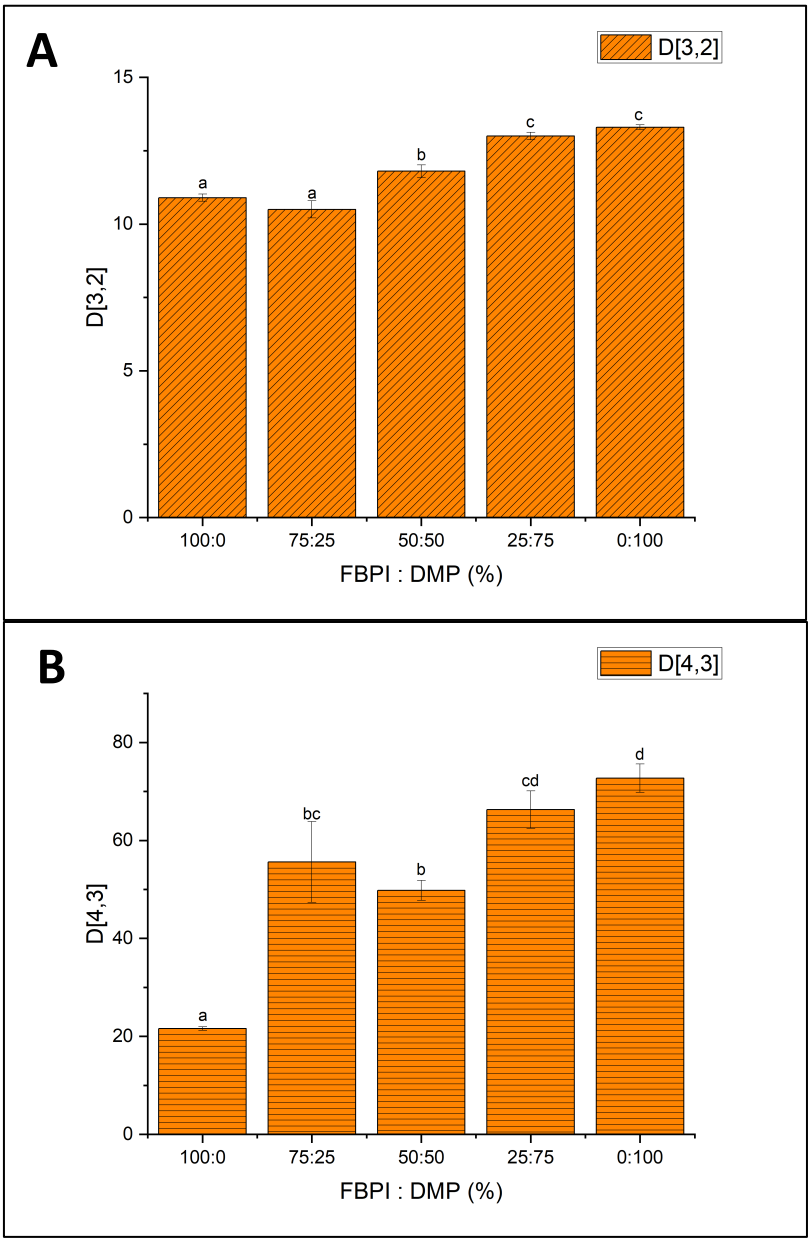


Figure 9. Particle size distribution (D[3,2]) (A) and (D[4,3]) (B) of mixtures before heat treatment were measured for different ratios of FBPI to DMP. Each bar showed the mean ± SD (n=3). Different letters above the bars indicated that the particle size distributions of the samples were significantly different ($p < 0.05$). In the figure, 100:0 represented 100% of 12.5% total protein of FBPI in the mixture, followed by 75:25, which represented 75% of FBPI and 25% of DMP; 50:50 represented 50% of FBPI and 50% of DMP; 25:75 represented 25% of FBPI and 75% of DMP; and 0:100 represented 100% of DMP

3.3.5. Intermolecular forces determination

Figure 10 illustrated the dominant interactions responsible for the gelation of hybrid mixtures. Disulfide bonds were the most significant, while hydrophobic interaction play a secondary role in the gel structure. Each column in the figure represents the protein solubility achieved using a different extraction solvent. The structural arrangement of protein was mainly upheld by a mix of forces, such as nonspecific binding (A), ionic bonding (B), hydrogen bonding (C), hydrophobic interactions (D) and disulfide bond (E) (Li et al., 2024). According to the figure, protein shows the lowest solubility in solvents A compared to the other treatment solvents. FBPI (100:0) generally shows the higher solubility across all solvents, while only DMP (0:100) show lower solubility in most solvents.

Additionally, the hydrophobic interaction plays a crucial role in protein gel formation. Enhancing surface hydrophobicity leads to the creation of a denser gel network that effectively traps additional water (Wang et al., 2020). Research has shown that urea aids in the denaturation of proteins, either by directly engaging with the protein to promote the solvation of its polypeptide chain by water and urea, or indirectly by changing the structure of water molecules, which in turn alters the solvent's behavior and diminishes the hydrophobic effect (Acharya & Chaudhuri, 2021). Moreover, the study by Din et al. (2021) illustrated that the addition of reducing agents like DTT (Dithiothreitol) can enhance protein solubility by breaking disulfide bonds, which aligns with the observed high solubility in solvent E (0.6 mol NaCl + 6 mol Urea + 0.5 mol DTT). Among the group of proteins studied, FBPI/ DMP at 100:0 faba was found to have the highest concentration of disulfide bonds compared to others proteins. According to a recent analysis, that soy proteins with a higher 11S ratio led to a higher storage modulus suggest that the 11S fraction plays a significant role in the gel-forming ability of soy proteins (Wang et al., 2020). Proteins with higher surface hydrophobicity tend to interact more strongly with each other through hydrophobic interactions. These interactions can lead to a stronger and more cohesive gel network, contributing to a higher storage modulus. Therefore, the results indicate that hydrophobic interactions are crucial for the organization of proteins within the hybrid gels.

Additionally, the presence of disulfide bonds in the mixture gels appears to be significantly associated with their mechanical properties. Additionally, the increased solubility of the 25:75 protein ratios combination of FBPI and DMP, in contrast to the 50:50 protein ratios combination, can be ascribed to the differential effects of molecular interactions within these protein mixtures. The presence of salts and other ions in the solvent milieu likely influences ionic bonding, hydrophobic interactions, disulfide bonds and hydrogen bonding, all of which impact the solubility of proteins (Bharmoria et al., 2024). The 25:75 protein ratio may have established an ionic environment that favored salting-in, thereby boosting protein solubility by weakening ionic bonds and disrupting the hydrogen bond network (Zhang et al., 2022). Furthermore, this ratio might have optimized hydrophobic interactions and the stability of disulfide bonds, contributing to an overall rise in solubility. These interaction effects underscore the importance of considering the ionic environment and protein composition when formulating protein blends for optimal solubility and functional properties.

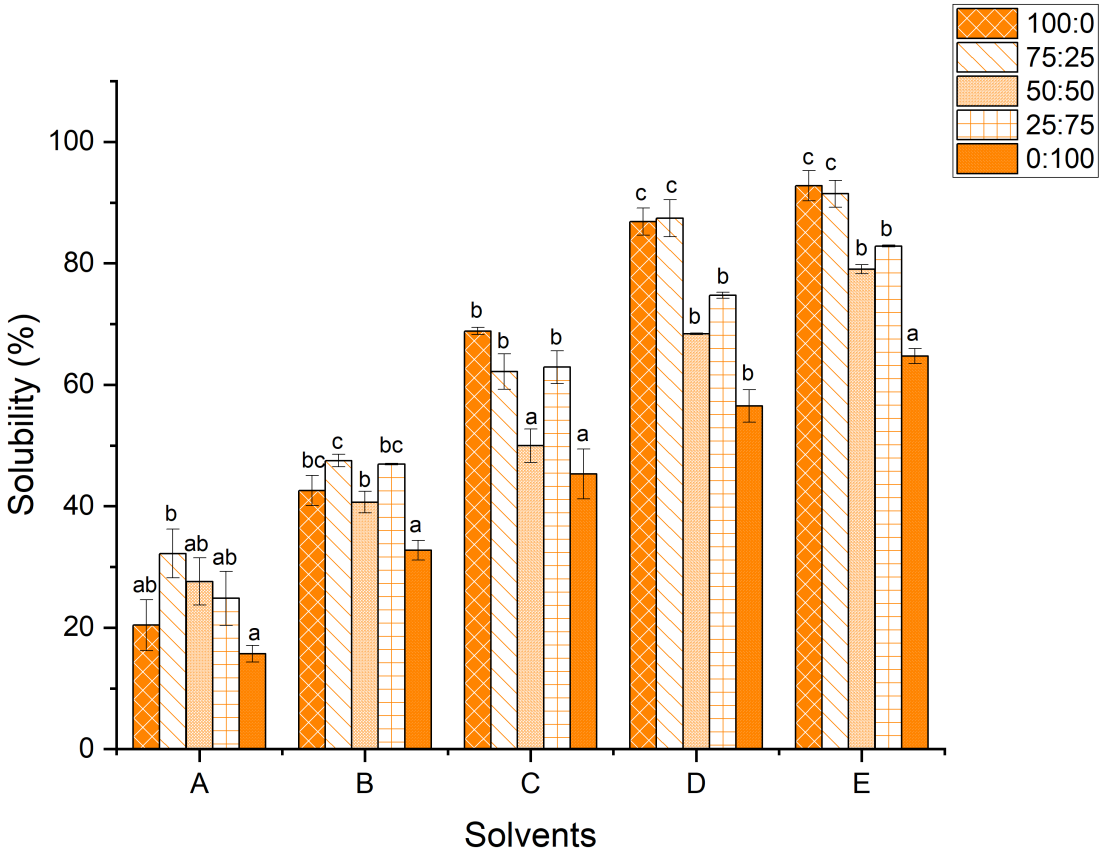


Figure 10. Protein solubility percentages in five mixtures of different protein ratios (100:0, 75:25, 50:50, 25:75, and 0:100) were measured in five different extraction solvents. Each bar showed the mean ± SD (n=3). Different letters above each extraction treatment indicated that the results were significantly different ($p < 0.05$). In the figure, 100:0 represented 100% of 12.5% total protein of FBPI in the mixture, followed by 75:25, which represented 75% of FBPI and 25% of DMP; 50:50 represented 50% of FBPI and 50% of DMP; 25:75 represented 25% of FBPI and 75% of DMP; and 0:100 represented 100% of DMP. For the solvents, A represented 0.05 mol NaCl; B represented 0.6 mol NaCl at pH 7; C represented 0.6 mol NaCl + 1.5 mol Urea; D represented 0.6 mol NaCl + 6 mol Urea; and E represented 0.6 mol NaCl + 6 mol Urea + 0.5 mol DTT.

3.3.6. The effect of the mixture on the rheological properties during the gelation process

The phenomenon of the protein gelation involves in conversion of protein from a liquid state to a solid, gel-like state. The gels strength was determined by analysing the storage modulus (*G′*) through oscillatory rheology analysis. In **Figure 11**, the storage modulus *G'*, loss modulus *G"* and temperature were measured over time. There was a clear trend of increasing *G'* and *G"* for all samples throughout the temperature ramp, temperature holding and temperature decreased. The data clearly demonstrates that when the mixture consists of 100% FBPI, there was a noticeable increase in *G′* and *G"* as the heating progressed, resulting in a higher final *G′* value. In contrast, when the mixture consisted of 100% DMP, the rate of increased in *G′* and *G"* was slower. Then, the *G'* and *G"* continuous to increase at the stage of temperature ramp and holding temperature of 90°C. This indicated that the protein molecules undergo denaturation process which causing the hydrophobic group that were initially hidden within the protein to become more exposed (Zhang et al., 2021). As a results, the protein aggregates and a network structured were formed.

To better present and examine, the bar chart in **Figure 12** illustrated the changes in *G'* (storage modulus) for different ratios of FBPI to DMP at various temperatures (20 °C, 60 °C, 90 °C and cooled back to 20 °C). It can be observed that the *G'* increases progressively from 20 °C to 90 °C. This can be attributed to the development of a thermo-irreversible hybrid protein network through the process of aggregation and crosslinking (Ding et al., 2022). Pure FBPI (100 : 0) shows the largest increase in *G'* upon heating, indicated significant changed towards the hardness in its structure during heating. The lower *G'* observed for pure DMP (0 : 100) can be attributed to its distinct protein composition and the weaker gelation compared to FBPI. There is a clear trend of decreasing of the *G'* with an increase of DMP ratios. As mentioned, β-conglycinin and other plant proteins can inhibit the self-aggregation of myosin heavy chains, which is a critical step in muscle protein gelation. Without proper aggregation, the gel network will be compromised (Zhao et al., 2024).

While, the 50:50 protein ratio composition shows a higher *G'* value compared to the 75:25 and 25:75 compositions. The combination of FBPI and DMP at 50:50 ratio may result in stronger intermolecular reaction between proteins compared to other compositions. This enhances interaction can lead to a denser protein network, resulting in higher *G'* values. This indicates the mixing between the two proteins in equal amounts of protein results in a material with better strength.

This synergistic effect can be due to the interactions between FBPI and DMP that create a network. The combination of diverse protein sources has a positive impact on the overall amino acid profile and nutritional value (Dimina et al., 2022). Specifically, when animal and plant proteins were mixed, they create a more balanced composition of amino acids and exhibit improved functional properties compared to using proteins in isolation. It was clear that the FBPI and DMP mixture combined the strengths of each protein to create a more balanced amino acid profile (Appendix B). The mixing of the two protein sources, FBPI and DMP, in various ratios resulted in a synergistic effect, where the amino acid profile of the mixture was enhanced by the inclusion of amino acids that were absent or present in lower concentrations in one of the sources. For instance, when mixed in a 50:50 ratio, the mixture benefited from the contribution of taurine and cysteine from DMP, which were not found in FBPI when it stood alone. This approach to combining complementary protein sources allowed for the creation of a product with a more comprehensive array of essential amino acids, thereby potentially increasing its nutritional value and suitability for a broader spectrum of dietary requirements.

Animal protein typically considered as complete protein since they contain all nine essential amino acids that humans cannot synthesize and must obtain from their diet. These essential amino acids include leucine, isoleucine, methionine, lysine, phenylalanine, tyrosine, histidine, valine, threonine and tryptophan. Plant protein, on the other hand, may be limited in one or more of these essential amino acids (Lopez & Mohiuddin, 2024). For example, the amino acid profile of faba beans typically includes higher levels of other essential amino acids such as lysine, which is often found in lower amounts in cereal grains (Dhull et al., 2022). This synergistic effect was believed to be the underlying reason for the better performance observed in their study with the 50:50 protein ratios mixtures. To enhance better understanding of the amino acid profile in protein mixtures and to guide future work, it is necessary to employ analytical methods such as high-performance liquid chromatography (HPLC) or mass spectrometry (MS). These techniques allow for the precise separation, identification, and

quantification of individual amino acids, providing valuable insights into the nutritional composition and functional properties of the protein blend. Although this study did not include such analyses, these methods are recommended for future research to fully characterize the amino acid profile of the protein mixtures under investigation.

During the cooling phase, the *G'* keeps continuously increasing and eventually reached a plateau at approximately 20 °C after 5000 sec from the initiation of the heating-cooling cycle, which can be attributed to the consolidation of intermolecular forces such as hydrogen bonding and van der Waals attraction (He et al., 2021). Additionally, this increases may be further explained by the formation of disulfide bonds.

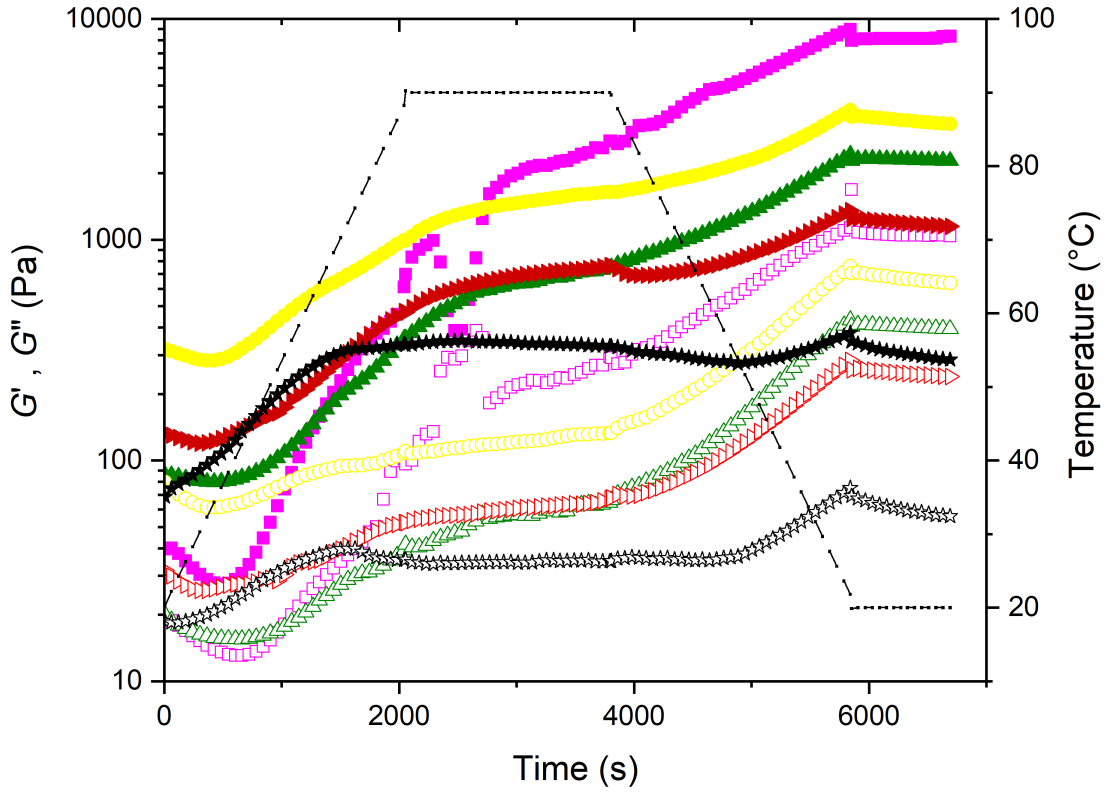


Figure 11. The dependence of *G'* (solid symbol) and *G"* (open symbol) for mixtures with different sample compositions at a frequency of 0.1 Hz. Represented as 100:0 (■), 75:25(▲), 50:50 (●), 25:75 (▶), 0:100 (★). All samples were set at pH 7.

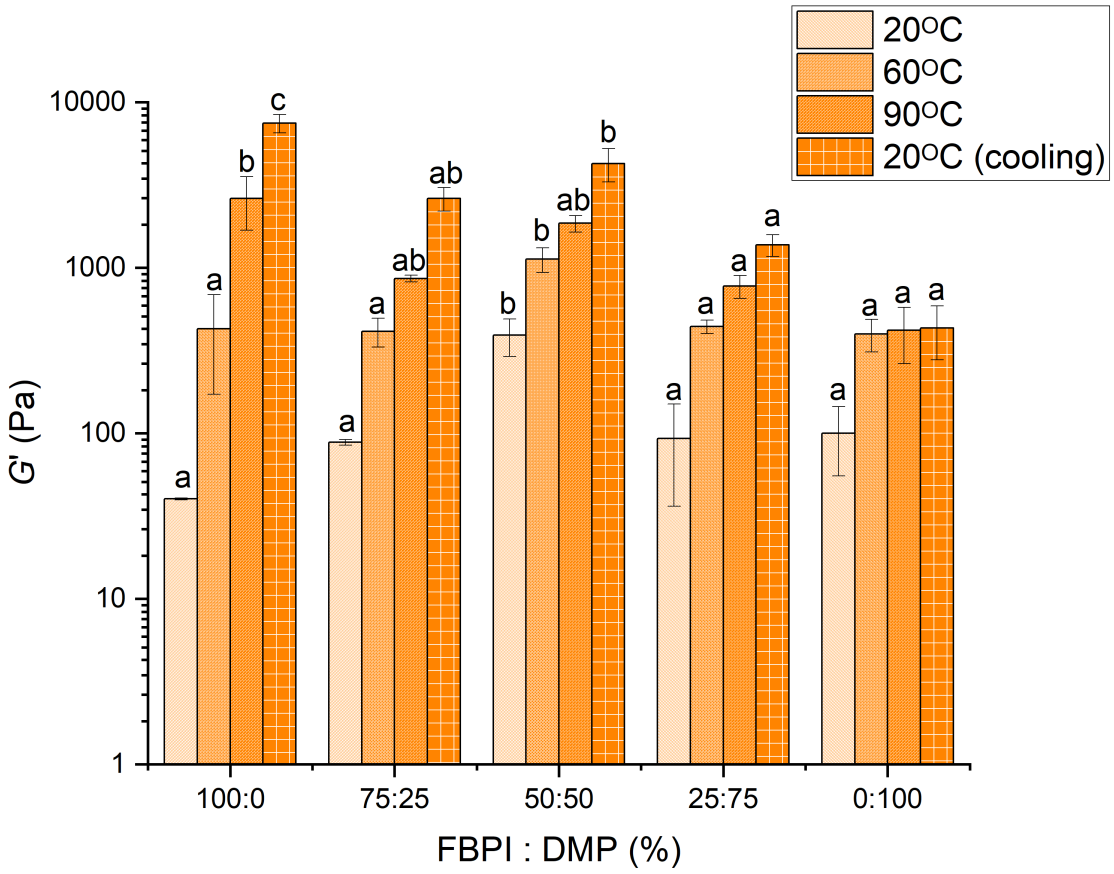


Figure 12. The changes in *G'* (storage modulus) for different protein ratios of FBPI to DMP at various temperatures (20 °C, 60 °C, 90 °C and cooled back to 20 °C). A significant difference ($P<0.05$) in storage modulus *G'* is shown by different letters above the columns.

3.3.7. The effect of the mixture on the rheological properties after the gelation process

After cooling the samples were given 15 min to equilibrium before carrying out frequency and strain sweeps. The G' and G'' show a consistent parallel relationship across all samples, with slight increases in frequency as shown in **Figure 13**. For all ratio concentrations, both G' and G'' tend to increase with increasing frequency. For each ratio, the filled symbols for G' were consistently greater than the empty symbols for G'' across the frequency spectrum. This finding suggested that the solid-like elastic behavior dominated the liquid-like viscous behavior under these concentrations and conditions. To enhance the comparison of gel strength across various samples, the bar graph illustrated G' values at 1 Hz, as depicted in **Figure 15**. By combining both *G'* and *G"*, the complex modulus gives a more complete picture of the material's mechanical properties, especially its ability to resist deformation under oscillatory stress at 1 Hz. The letters above the bars (a, b, ab, c) indicate statistical groupings. The 100:0 FBPI to DMP protein ratio showed the highest gel strength, significantly differing from all other samples, with bars sharing the same letter indicating no significant differences. This strong gel in the 100% FBPI mixture may be due to protein aggregates, as observed in CLSM analysis.

Figure 14 presented the strain sweep of the mixture gels, illustrating G' and G'' plotted against the applied strain. At low strain levels, both G' and G'' remained constant, indicating linear viscoelastic behavior that was unaffected by the strain amplitude. The findings confirmed that a 1% strain for temperature and frequency sweeps was within the linear viscoelastic region. As strain increased, both G' and G'' decreased, revealing strain-thinning behavior typical of colloidal gels, like those from soybean and hemp proteins (Dapčević-Hadnađev et al., 2020; Zhao et al., 2020). The breaking stress outcome **Figure 16** corresponded with the G' measurement at 1 Hz. As the proportion of DMP in the mixture increased, the breaking stress decreased. Mixtures that were 100% FBPI had the maximum breaking stress, suggesting they were capable of resisting higher stress levels compared to other samples without incurring damage. As the applied strain was further increased, G'' exceeded G', indicating a major breakdown in the gel structure. The rheological behavior observed under large deformation conditions was consistent with that seen under small deformation conditions.

In summary, the rheological behavior aligns closely with the microstructural features identified through CLSM analysis. These features include the size of protein aggregates and microstructural separation. This connection showed that the rheological properties were significantly affected by the material's microstructure, as highlighted by the CLSM analysis.

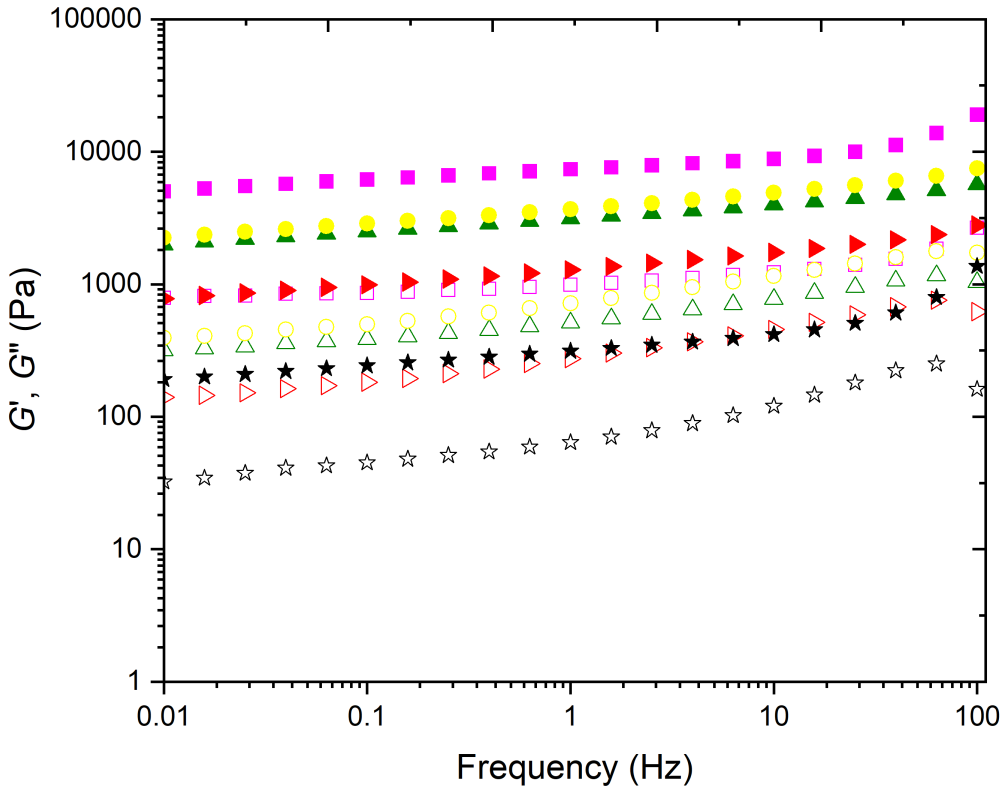


Figure 13. The *G'* (solid symbol) and *G"* (open symbol) as a function of frequency at 12.5 wt% with different protein ratio concentration measured at 20 °C. Represented as 100:0 (■) , 75:25 (▲), 50:50 (●), 25:75 (▶), 0:100 (★).

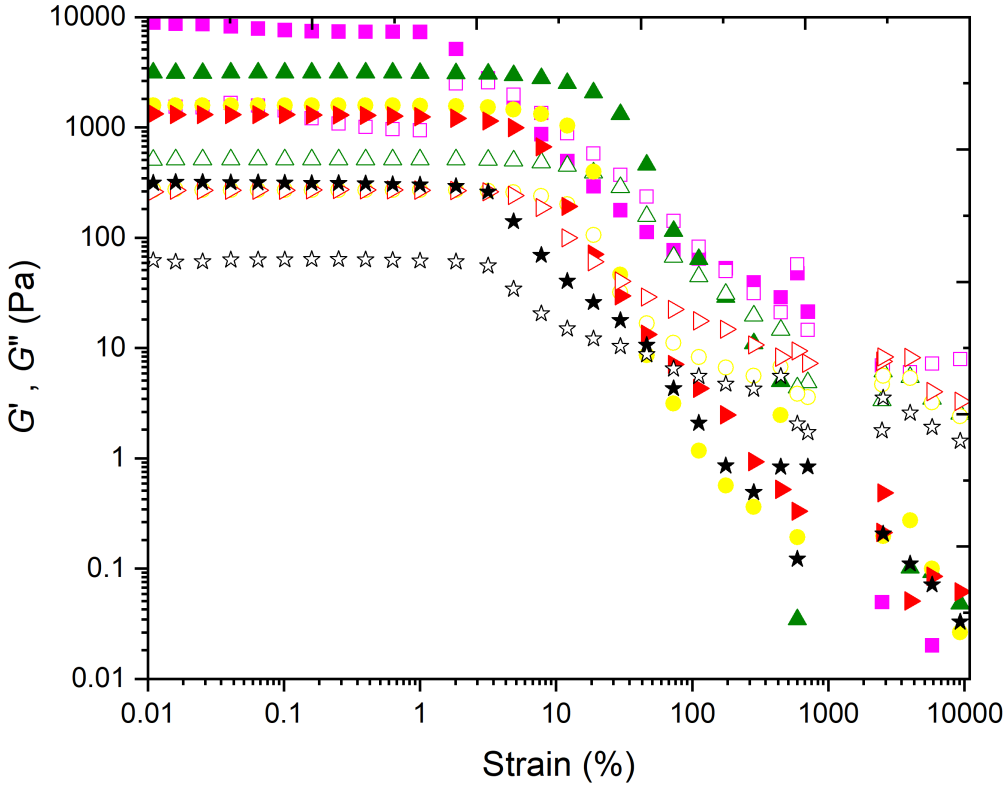


Figure 14. The *G'* (solid symbols) and *G"* (open symbols) as a function of strain at 12.5 wt% with different ratio concentrations. Represented as 100:0(■) , 75:25 (▲), 50:50 (●), 25:75.(▶), 0:100 (★).

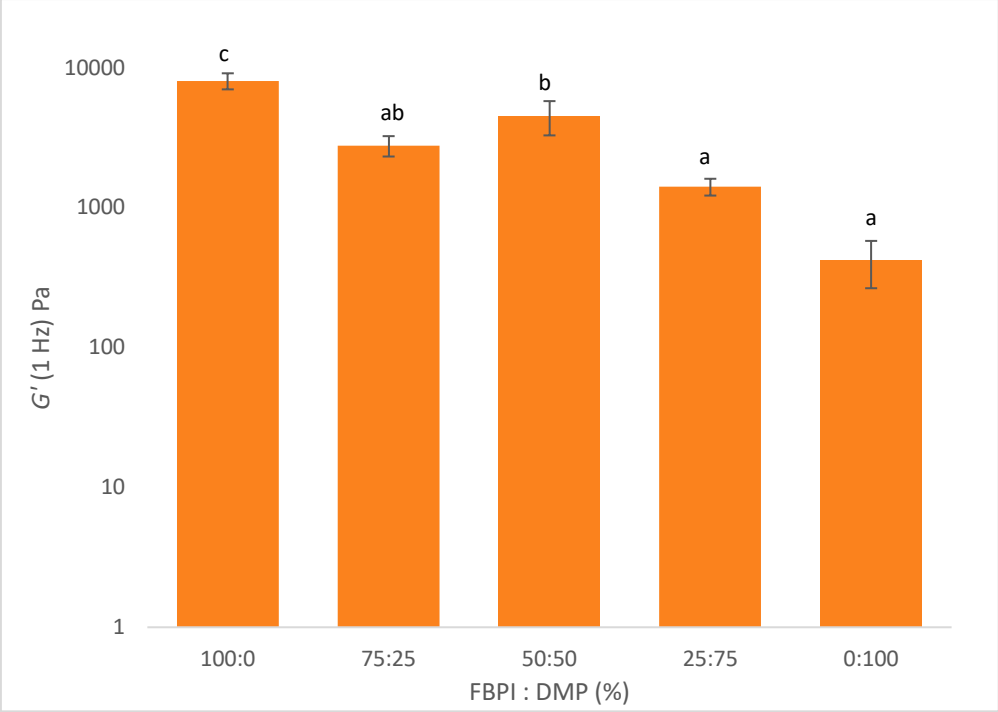


Figure 15. The storage modulus *G'* at 1 Hz of the gels as a function of different protein ratio concentrations. Each bar indicates significant difference ($p < 0.05$).

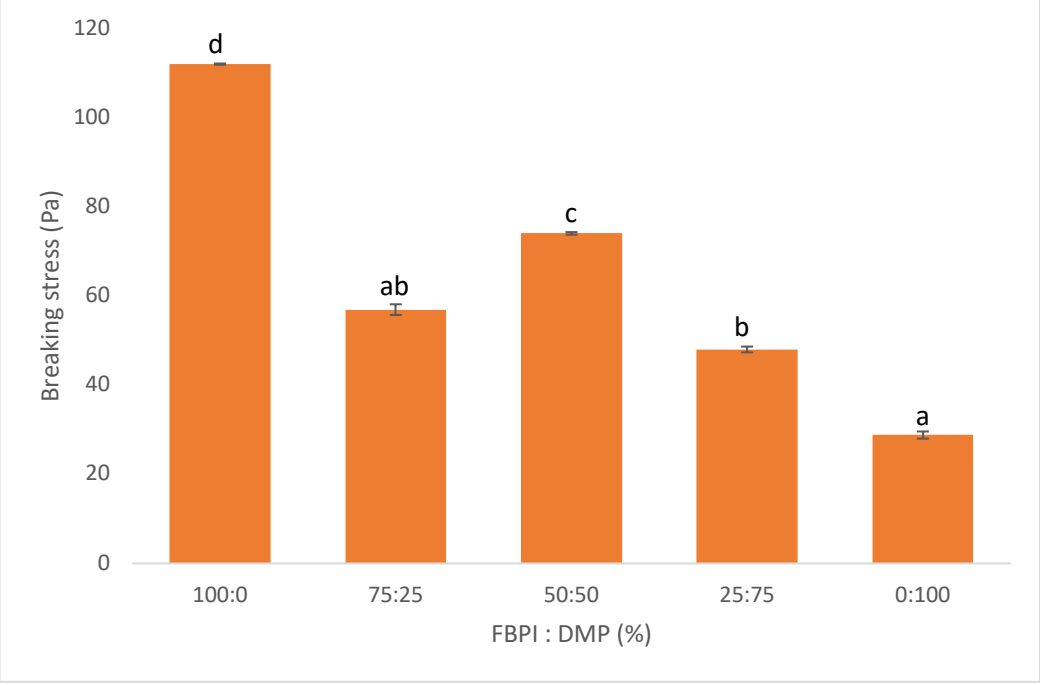


Figure 16. The breaking stress of the gels as a function of different protein ratio concentrations. Each bar indicates a significant difference ($p < 0.05$).

3.3.8. Textural determination

As shown in **Figure 17**, the hardness of the mixtures decreased as the proportion of mussel powder increased. This finding aligned with Klunklin and Savage (2018), who reported a reduction in biscuit hardness with higher concentrations of mussel powder. The findings showed that as the DMP content increased, the hardness decreased, highlighting DMP's impact on the textural characteristics of the mixtures. The presence of higher concentrations of DMP may have contributed to a softer texture, likely due to its functional properties impacting the structural integrity of the gels. The observed softer texture with higher DMP concentrations may have resulted from its high moisture retention capacity, which enhanced water retention and increased the moisture content within the gels, ultimately softening their texture and reducing hardness (Fuentes et al., 2009).

This study focuses on hardness and its correlation with rheology data because these parameters provide a comprehensive understanding of the textural and structural properties of the protein mixtures. Hardness serves as a key indicator of the overall texture and consumer acceptability of food products, while rheological measurements, such as the storage modulus (*G'*), offer insights into the mechanical properties and structural integrity of the gels. By examining these parameters together, we can better understand how different protein ratios influence the textural and structural characteristics of the mixtures, thereby providing a more complete picture of their performance.

To conclude, by increasing the mussel powder content reduces the structural rigidity as reflected in both textural hardness and the *G'* values (**Figure 15**). In addition, FBPI's effective water-binding properties support the structural stability of the mixtures, resulting in a more solid texture (Nilsson et al., 2023).

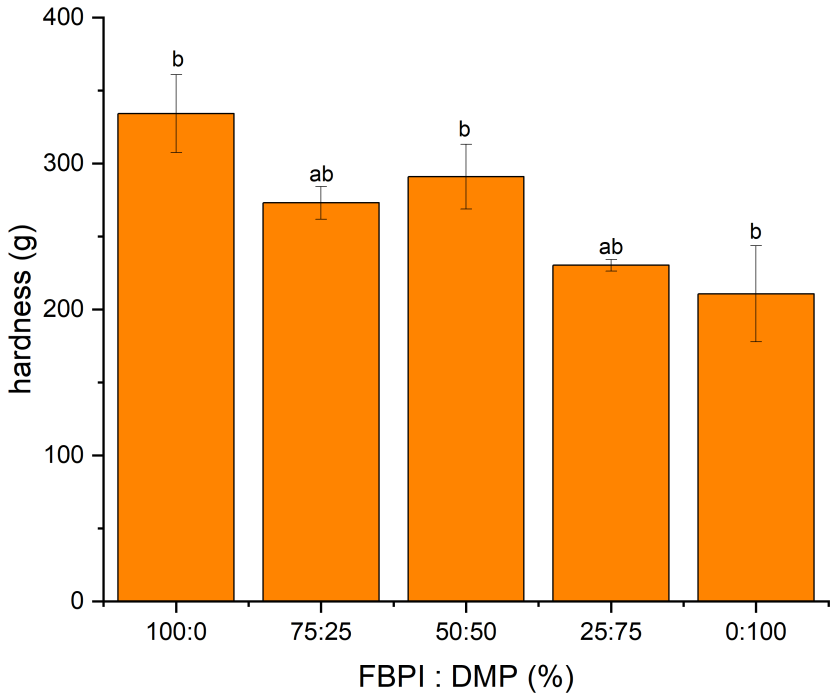


Figure 17. Hardness of the mixtures after heat treatment in an oven at 90°C for 30 min. Each bar represents the mean ± standard deviation (n=2). Different letters above the bars indicate significant differences in sample hardness ($p < 0.05$). In the figure, 100:0 represented 100% of 12.5% total protein of FBPI in the mixture, followed by 75:25, which represented 75% of FBPI and 25% of DMP; 50:50 represented 50% of FBPI and 50% DMP; 25:75 represented 25% of FBPI and 75% of DMP; and 0:100 represented 100% of DMP.

3.3.9. Impact of protein mixtures on water holding capacity (WHC)

Figure 18 illustrated the relationship between the maximum value of *G'* observed in 100% FBPI and its gel strength, which was directly associated with the highest WHC. This capability to retain water eventually reinforced the gel's structure (Qin et al., 2021). The correlation coefficient observed in the *G'* measurements across all samples aligns with that of the WHC, indicating a consistent connection between gel strength and WHC. When FBPI on its own can often exhibit high viscosity, which poses challenges during mixing. By adding DMP can dilute the concentration of FBPI in the mixture, which may help to reduce the overall viscosity.

The trend shows a decrease in both *G'* value and WHC with increasing mussel powder, indicating that faba bean protein contributes significantly to both properties. The WHC decreases from 100:0 to 0:100, with a notable drop at the 75:25 protein ratio and slight increase at 50:50 indicating that the combinations from both proteins enhanced the ability to retain water. According to Lima et al. (2023) have shown that the blending of milk and plant proteins can enhance the functional attributes of food systems, including gel formation and water retention. These results imply that the combination of plant and animal proteins can optimize texture and moisture retention, thereby enhancing the overall quality of food items. The data obtained from these studies further reinforces these findings. Specifically, when a 50:50 protein ratio of FBPI and DMP was used, it was observed that the resulting *G'* and WHC values were superior to those obtained from other protein combinations. This indicates a beneficial interaction between the plant and animal proteins, further supporting the idea that their combination can enhance the overall standard of food products. The WHC significantly affected the quality of the gel. This characteristic demonstrates how effectively proteins interact with water interaction within the food system, thereby highlighting its importance. It was commonly observed that during the cooling phase, the increase in moduli was more pronounced, probably because of the participation of ionic interactions and the creation of covalent bonds (Tanger et al., 2022).

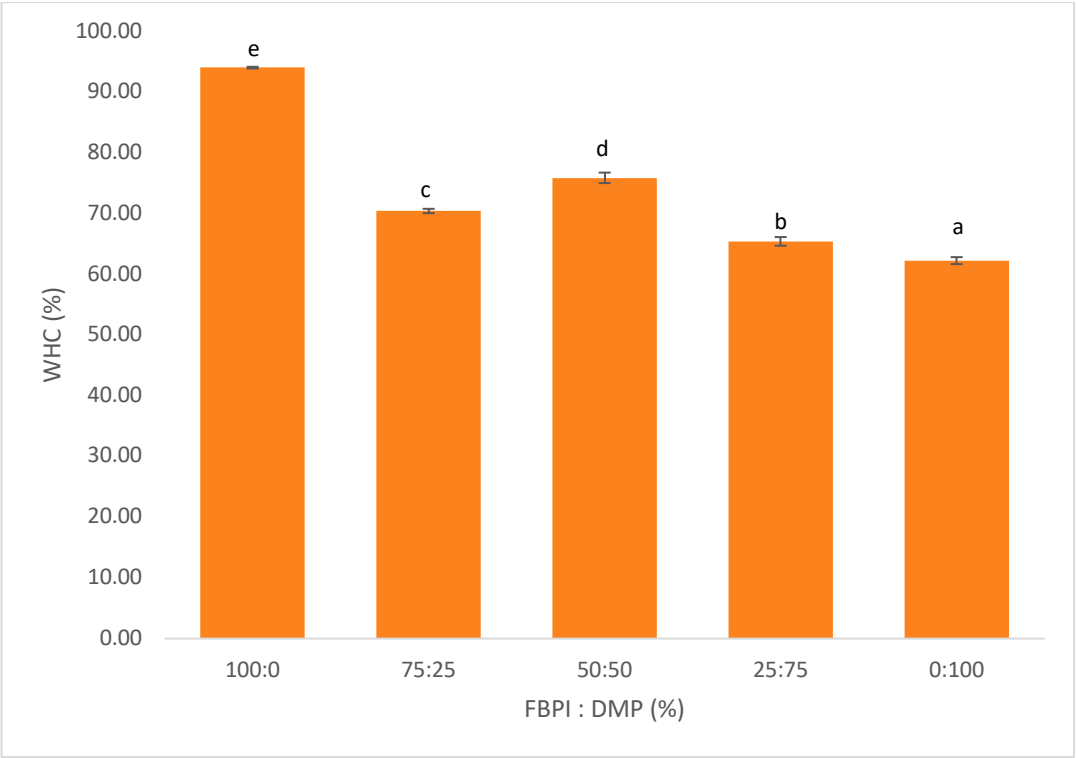


Figure 18. The WHC of gels was assessed based on different protein ratio concentrations, with each bar representing significant differences ($p < 0.05$). The 100:0 represented 100% of 12.5% total protein of FBPI in the mixture, followed by 75:25, which represented 75% of FBPI and 25% of DMP; 50:50 represented 50% of FBPI and 50% DMP; 25:75 represented 25% of FBPI and 75% of DMP; and 0:100 represented 100% of DMP

3.4. Conclusion

In conclusion, the study concludes that a 50:50 protein ratio mixture of FBPI and DMP offers the most promising results regarding gel strength and WHC, suggesting that this combination could be beneficial for developing sustainable and nutritious food products with enhanced functional properties. The research contributes to the understanding of how plant and animal protein mixtures can be utilized to create innovative food products that meet the demands for healthier and more environmentally friendly food options. Furthermore, mussel powder contributes a unique seafood flavor that is difficult to replicate with plant-based proteins alone. Thus, a product made with 100% FBPI might lack the depth of flavor that mussel powder can provide. Moreover, the study highlights the importance of particle size in determining the gel's strength, with smaller particles correlating to a stronger gel network. The type of protein used was also important in determining the gel's properties. The color analysis indicates that the addition of DMP influences the color of the gel, making it redder and yellower, which could be a consideration for product development. The microstructural observations using CLSM confirm that FBPI forms a denser gel structure, while the presence of mussel powder can disrupt the network formation. This understanding of the microstructure can guide the formulation of protein gels with specific textural properties. Despite these promising findings, the study noted several limitations that should be considered in future research. It does not delve into the effects of minerals and amino acids present in the mixtures of FBPI and DMP on the functional properties of the gel, so future work should focus on understanding how these components influence gelation, texture, and nutritional value. Additionally, the long-term stability and shelf-life of the protein gels were not evaluated; thus, further research should investigate how the gel properties change over time under various storage conditions. By addressing these limitations and expanding the scope of the research, a deeper insight into the functional properties and possible uses of mixtures of FBPI and DMP can lead to the development of innovative and sustainable food products.

4.0. Chapter 4: Optimizing protein-polysaccharide mixtures for a plant and seafood blended - patty concept development

Keywords: Faba bean protein isolate; Defatted green-lipped mussel; Polysaccharides; Konjac Glucomannan; Methylcellulose; Blended patty; Microstructures

4.1. Introduction

In the wake of the global shift towards sustainable and healthy dietary choices, the demand for plant-based protein products has surged. The development of meat or seafood analogues that mimic the sensory qualities of traditional meat or seafood while offering nutritional benefits and environmental sustainability was a pressing challenge for the food industry. The role of consumer preferences was critical in the commercialization of meat or seafood analogues. Historically, the development of these analogues faced significant challenges, primarily due to the lower nutritional quality of plant proteins and the lack of meaty sensations. An innovative approach to address these challenges involved incorporating high-quality animal protein offcuts or by-products into the formulation of hybrid meat or seafood analogues. This strategy aimed to enhance both the nutritional value and sensory characteristics of the products, thereby offering a potential solution to bridge the gap in nutritional quality and improve consumer acceptance (Kyriakopoulou et al., 2019).

The faba bean, a pulse known for its high protein (25–40%), carbohydrate (47–68%), and fibre content (11–30%), has emerged as a crop of interest due to its potential environmental benefits. Its cultivation contributes positively to the environment by fixing nitrogen, enhancing soil fertility for subsequent crops, and seamlessly fitting into crop rotation practices. Recognized by the EU Smart Protein Project as a promising alternative to animal protein, the faba bean has been explored in various food applications, including bread, pasta, and meat and dairy analogues (Augustin & Cole, 2022). Despite its nutritional, functional, and environmental advantages, the faba bean remains underutilized in the food industry, presenting an opportunity for greater exploitation as a sustainable protein source (Nawaz et al., 2021).

Moreover, green-lipped mussels, a type of farmed shellfish native to New Zealand, were celebrated for their high nutritional value, offering a rich source of micronutrients, vitamins, protein, and polyunsaturated fatty acids. These mussels were cultivated through aquaculture since the 1960s and New Zealand became a significant supplier of this seafood to the world (Miller et al., 2023). Beyond their consumption as fresh or frozen food, green-lipped mussels were also utilized in the form of dietary supplements for humans, such as powder and oil extracts, and as ingredients in pet food for dogs and cats (Konieczny et al., 2021). The mussels contained a variety of proteins, with myofibrillar proteins being a key component, consisting mainly of myosin, paramyosin, and actin (Zou, Zhao, Sun, et al., 2020). However,

research has been scarce on the detailed properties of proteins derived from New Zealand green-lipped mussels, highlighting a gap in our understanding of their potential applications (Jayaprakash & Perera, 2020). Therefore, FBPI and DMP were selected as the key ingredients to develop plant and seafood hybrid patties.

One of the most common methods for forming gels was through heat-induced gelation. This approach involved heating a concentrated protein solution above its denaturation temperature, resulting in the unfolding of proteins and the exposure of hydrophobic and sulfhydryl groups (Tanger et al., 2022). This unfolding initiated hydrophobic and covalent disulfide bonds among the protein molecules, leading to the formation of a three-dimensional network that endowed the gel with elastic properties (Akharume et al., 2021). The degree of protein aggregation and the nature of the resulting network, whether filamentous or particulate, depended on the electrostatic interactions among proteins, which were affected by the pH and ionic strength of the solution (Tanger et al., 2022). Therefore, by adjusting the heating conditions and solution properties, protein gels with varied characteristics could be created.

A common problem that was faced with these blended gels, particularly those combining seafood and plant proteins, was their texture, which was overly soft and fragile. As a result, the gels were prone to breaking apart with just a small amount of stress, unlike the robust and pliable texture of an analogue patty. To increase the gel strength of seafood analogue patties, various methods can be employed, including the addition of enzymes and polysaccharides (Xie et al., 2024). Among these approaches, the use of additional binding agents has been a popular strategy to improve gelation, texture, and stability.

The investigation focused on the effects of two specific polysaccharides, konjac glucomannan (KGM) and methylcellulose, selected for their distinct properties and proven efficacy as binding agents in food products. Derived from the tubers of the konjac plant, KGM is a water-soluble polysaccharide that has been extensively applied in the food sector as a multifunctional component and a nutritional additive. Its increasing popularity stems from its multiple potential health benefits, including aiding in the management of diabetes, obesity, and elevated blood sugar levels. Moreover, its exceptional water-holding capacity and gel-forming abilities make it highly effective in improving texture and moisture retention in plant-based and hybrid patties (Jiang et al., 2019). This compound was composed of a random linear copolymer structure, featuring a specific linkage pattern between glucose and mannose

molecules, and it also includes a side chain adorned with acetyl groups, which make up 5–10% of its composition (Jiang et al., 2019). Furthermore, KGM was designated as Generally Recognized as Safe (GRAS) and has seen widespread use across multiple European nations (Jimenez-Colmenero et al., 2013). KGM served as a fat alternative in the creation of products like low-fat mayonnaise, skimmed yoghurt, and cheese, showcasing its effectiveness in enhancing texture, flow properties, and shelf life (da Silva et al., 2016; Xu et al., 2020). In addition, KGM was applied in making low-fat frankfurters, restructured gilthead sea bream, restructured pork nuggets, and low-fat sausages, where it significantly boosted water retention, chewiness, cohesiveness, and gel strength (Jiménez-Colmenero et al., 2012). Furthermore, Ran et al. (2022) observed that incorporating KGM into soy protein-based fishball analogues reinforced protein crosslinks, leading to improved texture and structural integrity of the analogues. They found that by incorporating 5.0% KGM into Soy Protein Isolate (SPI), they could produce a Plant-Based Fishball (PFB) with a gel-like and chewy texture, closely resembling that of traditional fishballs. However, there was limited accessible research regarding the use of KGM in the development of hybrid plant and seafood products and its impact on the textural and rheological properties of these alternatives.

Methylcellulose (MC) was chosen for its binding and thickening abilities, which are widely recognized in the food industry for creating a reversible heat-set gel (Coughlin et al., 2021). At lower temperatures, water effectively hydrates the methoxy groups present in MC. As the temperature rises, the hydrogen bonds between these groups and water molecules weaken, altering the gel's structure. This change enhances the hydrophobic interactions between the polymer chains, leading to the formation of a heat-set hydrogel. Conversely, when the temperature drops, the hydrogen bonding increases, allowing the methoxy groups to rehydrate and breaking the hydrophobic interactions, which returns the gel to a sol state (Coughlin et al., 2021). Bakhsh, Lee, Lee, Sabikun, et al. (2021) found that optimal results were achieved in plant-based meat analogue (PBMA) patties made with commercial texture vegetable protein (C-TVP) and enhanced with 3% methylcellulose (MC), closely replicating the texture and quality of the control beef patties.

A primary objective was to assess how varying protein ratios (w/w%) of FBPI to DMP, specifically 100:0, 50:50, 25:75, and 0:100, and different polysaccharide concentrations, specifically konjac glucomannan (KGM) at 3, 4, and 5% (w/w%) and methylcellulose (MC) at 2, 3, and 4% (w/w%), influenced the texture, color, and physicochemical properties of the blended patties. By identifying the optimal protein ratio and polysaccharide combination, this research aimed to contribute to the formulation of sustainable and nutritious meat alternatives that met consumer expectations for texture, nutritional value and visual appeal. The findings were of significant interest to the food industry, providing guidance to formulate plant and seafood blended patty products with improved textures and structures.

4.2. Materials and Methods

4.2.1. Materials

Faba bean protein isolate (FBPI) was purchased from NZProtein, New Zealand and was stored under dry conditions which provided the composition as indicated on the packaging label (**Table 2, Chapter 3**). Defatted green-lipped mussel powder (DMP) was kindly provided by Sanford Ltd, New Zealand. It consisted of 48.6% crude protein, 4% fat, 14.3% carbohydrate, 4% moisture, and 29.1% ash (w/w). Polysaccharides including konjac glucomannan was kindly provided by Kapiti Health Food, New Zealand and Methylcellulose was kindly provided by Sherratt Ingredients, New Zealand. Garlic, ginger, cinnamon, coriander, sugar and black pepper were purchased from a local supermarket (Pak N Save, Albany, Auckland NZ).

4.2.2. Preparation of plant and seafood hybrid patties

The sample preparation involves initially mixing faba bean protein isolates (FBPI) with defatted green-lipped mussel powder (DMP) in different protein ratios (w/w%) (100:0, 75:25, 50:50, and 0:100) at a total 15% protein concentration using an overhead IKA stirrer (RW 20 Digital, IKA 3593000, NZ) until fully dissolved. This ensures a uniform distribution of the mussel powder within the FBPI solution. Methylcellulose, konjac glucomannan, and other ingredients were then gradually incorporated into the mixture, ensuring complete dissolution. Continue stirring slowly for 5 min to ensure these ingredients were fully dissolved and evenly distributed in the mixture. The slow incorporation helps prevent clumping and ensures a smooth mixture. The spices (garlic, ginger, cinnamon, coriander, sugar, black pepper) were incorporated at the last step. Additionally, since DMP has a higher sodium content as reported

by Mititelu et al. (2022), no additional salt was included in the recipe. The mixture undergoes heat treatment in a water bath at 90°C for 30 min to enhance the solubility of components in the mixture and can induce gelation, leading to a solidified or gel-like structure as proteins and other molecules denature and aggregate, followed by refrigeration at 4°C overnight to allow for proper hydration and stabilization. Then, the mixture is divided into 40-gram portions, each shaped into a ball and then flattened into patties with a 7 cm diameter and 1 cm thickness. The patties were placed on the baking paper and covered with polyethylene film and stored at -20°C overnight which helps in hold their texture and quality for future analysis.

The control sample, which did not include any added polysaccharides, consisted of a total protein content of 15% (w/w). The formulations comprised FBPI and DMP in the following protein ratios: 100:0, 75:25, 50:50, and 0:100 (w/w%), as presented in **Table 7**.

Table 7. Formulation of the control mixtures

Ingredients (w/w)	FBPI: DMP			
	100 : 0	75:25	50:50	0 : 100
FPI (g)	17.65	13.24	8.82	0.00
Mussel powder (g)	0.00	7.81	15.63	31.25
Garlic (g)	1.00	1.00	1.00	1.00
Ginger (g)	1.00	1.00	1.00	1.00
Cinnamon (g)	0.20	0.20	0.20	0.20
Coriander (g)	0.20	0.20	0.20	0.20
Sugar (g)	0.40	0.40	0.40	0.40
Black pepper (g)	0.20	0.20	0.20	0.20
Water (g)	79.35	75.95	72.55	65.75
Total weight (g)	100	100	100	100

The study then examined blended protein formulations containing a total protein content of 15% (w/w) specifically targeting protein ratios of 75:25 and 50:50, which were identified as optimal for the intended purposes of the protein mixture. These formulations, which included the integration of polysaccharides, were detailed in **Table 8**.

Two commercial plant-based patties were also tested in the study. This comparative study aimed to assess the effectiveness of the experimental protein mixtures against established plant-based alternatives. The results from these tests provided valuable insights into the potential improvements and innovations that could be achieved with the novel protein formulations.

Table 8. Formulation of patties with different protein ratios of FBPI to DMP with different concentrations of Konjac Glucomannan (KGM) and Methylcellulose (MC).

Ingredients (w/w)	Control (75:25)	MC-2%	MC-3%	MC-4%	KGM-3%	KGM-4%	KGM-5%	Control (50:50)	MC -2%	MC-3%	MC-4%	KGM-3%	KGM-4%	KGM-5%
FBPI	13.24	13.24	13.24	13.24	13.24	13.24	13.24	8.82	8.82	8.82	8.82	8.82	8.82	8.82
Mussel powder	7.81	7.81	7.81	7.81	7.81	7.81	7.81	15.63	15.63	15.63	15.63	15.63	15.63	15.63
Methylcellulose	0.00	2.00	3.00	4.00	0.00	0.00	0.00	0.00	2.00	3.00	4.00	0.00	0.00	0.00
KGM	0.00	0.00	0.00	0.00	3.00	4.00	5.00	0.00	0.00	0.00	0.00	3.00	4.00	5.00
Garlic	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ginger	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cinnamon	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Coriander	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Sugar	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Black pepper	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Water	75.95	73.95	72.95	71.95	72.95	71.95	70.95	72.55	70.55	69.55	68.55	69.55	68.55	67.55
Total weight	100	100	100	100	100	100	100	100	100	100	100	100	100	100

4.2.3. Cooking

The cooking procedure for the patties followed the method described by Chandler and McSweeney (2022), with minor modifications. The first step involved preheating a non-stick pan to a specific temperature of 120°C before placing the patties on it. Each patty was then cooked for 1.5 min on both sides, with a total cooking time of 12 min to achieve an internal temperature of 75°C, which was verified using a probe thermometer (Thermapen MK4 Thermocouple). Following the cooking process, the patties were left to cool down naturally to room temperature for a period of 30 min before undergoing analysis.

4.2.4. Texture Profile Analysis (TPA)

TPA was carried out using a TA-XT Plus Texture Analyzer (Stable Microsystems, Godalming, UK), based on the method by Flory et al. (2023) with slight adjustments. Samples were cut into cubes measuring 4 cm by 4 cm by 1 cm. A 5 kg load cell was used, equipped with a 35-mm diameter aluminium cylindrical probe, and samples were compressed to 50% of their original height. The duration between the two compression cycles was set at 5 seconds. Only hardness was analysed for the patties. The decision to focus on hardness was made because it is a critical parameter that significantly influences the sensory perception and consumer acceptance of plant and seafood hybrid patties. Hardness provides essential information about the structural integrity and mouthfeel of the product, making it a key attribute for quality evaluation.

4.2.5. Color Measurement

Color measurements of the patties, both before and after cooking, were conducted using a Minolta Chroma Meter CR 300. The parameters analysed included L* (which indicates lightness on a scale from 0 to 100), a* (where a positive value represents redness and a negative value indicates greenness), and b* (with positive values indicating yellowness and negative values indicating blueness). These measurements were obtained using the CIELAB color space. The device was calibrated with a white tile (Y = 86.6, x = 0.3162, y = 0.3232) as a reference (El-Anany et al., 2020). The given result is the average of measurements taken in triplicate (five measurement for each sample).

4.2.6. Cooking loss (CL)

The CL was assessed by weighing the patties before and after they were cooked. The excess liquid released during cooking was removed using paper towels. Each measurement was performed twice. The calculation for CL was carried out as follows (El-Anany et al., 2020):

$$\text{Cooking loss (\%)} = \frac{raw\ patty - cook\ patty}{raw\ patty} \times 100 \quad \textbf{(Equation 4)}$$

4.2.7. Microstructure

The microstructure of the cooked patties was analysed with a Scanning Electron Microscope (SEM, TM3030 Plus). Initially, the samples were frozen at -20°C overnight. They were then freeze-dried using a Labconco freeze dryer (model 7753034, USA) at -20°C for about four days. The samples were attached to aluminium stubs using double-sided carbon tape. After mounting, a thin layer of platinum, approximately 4 nm thick, was applied via sputter coating using a Leica EM SCD050 sputter coater from Leica Microsystems, Germany. The images were captured at a magnification of 100x (Rahmawati et al., 2020).

4.2.8. Statistical analysis

All experiments were performed in duplicate, and the results were presented as the mean ± standard deviation. A one-way analysis of variance (ANOVA) was conducted using SPSS version 21.0 (Chicago, IL, USA). Significant differences among samples were determined using Tukey's test, with a significance level set at $P < 0.05$.

4.3. Results and Discussion

4.3.1. Visual appearance

Figure 19 illustrated the external look of the patties for the control group, showing their condition before (A) and after (B) cooking, without the addition of any polysaccharides.he appearance before cooking showed the samples were uniform in color and texture with a smooth surface. However, after cooking the patties were unable to retain their shape, especially in the protein ratios of 100: 0 and 0: 100 protein ratios of FBPI: DMP (w/w%).

Given these observations, the study focused on exploring the protein ratios (w/w%) of 50:50 and 75:25, this time incorporating polysaccharides. This adjustment was made in light of the study goal, which was to develop a blended protein formulation that could offer improved structural integrity and stability. As shown in **Figure 20**, it can be seen that higher concentrations from both KGM and MC contribute to better shape retention and structural integrity after cooking. This was observed in both formulation bases 75:25 (w/w%) and 50:50 (w/w%). The 50:50 (w/w%) generally shows better structural integrity and shape retention compared to the 75:25 (w/w%), especially at higher concentrations of KGM and MC. For comparison, images of commercial plant-based patties (A and B) were included, revealing that the shape and color of the experimental patties were closely similar to those of the commercial products.

Previous research extensively described the function of MC as a powerful binding agent, emphasizing its ability to preserve the structure and consistency of a range of food products, such as Impossible Burgers and Beyond Burgers (Bohrer, 2019). Similarly, it was noted that the outer and inner textures of textured vegetable protein (TVP) patties improved in homogeneity and cohesiveness as the concentration of MC increased. This suggests that adding MC could help reduce the grainy texture often associated with TVP in patty formulations (Bakhsh, Lee, Lee, Sabikun, et al., 2021). Furthermore, methylcellulose act as an effective binder, particularly in meat or seafood analogues due to its unique properties that do not require pre-heating for gel formation. This characteristic can be attributed to its distinct thermal gelling capabilities, which, combined with its emulsifying properties, provide significant advantages during the processing of meat or seafood analogue products (Sanz et al., 2005). The results of this study aligned with previous findings, especially concerning the protein ratios of 50:50 and 75:25 (w/w). These results support the documented advantages of methylcellulose, affirming

its beneficial impact on the structural integrity of hybrid patties. Furthermore, previous studies have indicated that increasing the concentration of KGM in gels made from peanut protein isolate (PNPI), pea protein isolate (PPI), or soy protein isolate (SPI) resulted in significant improvements in the hardness and chewiness of these gels. This observation is further supported by visual inspections, which suggest that KGM enhances the textural qualities of protein-based gel products (Yao et al., 2023).

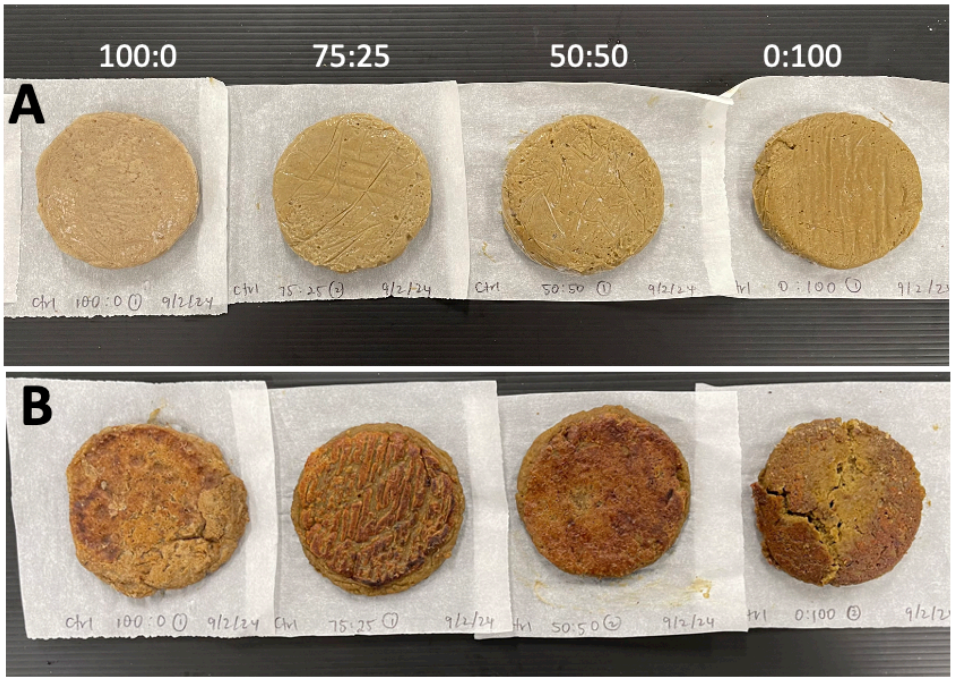


Figure 19. The external appearance of the control patty without adding polysaccharide before cooking (A) and after cooking (B) with different formulations of FBPI: DMP (w/w%).

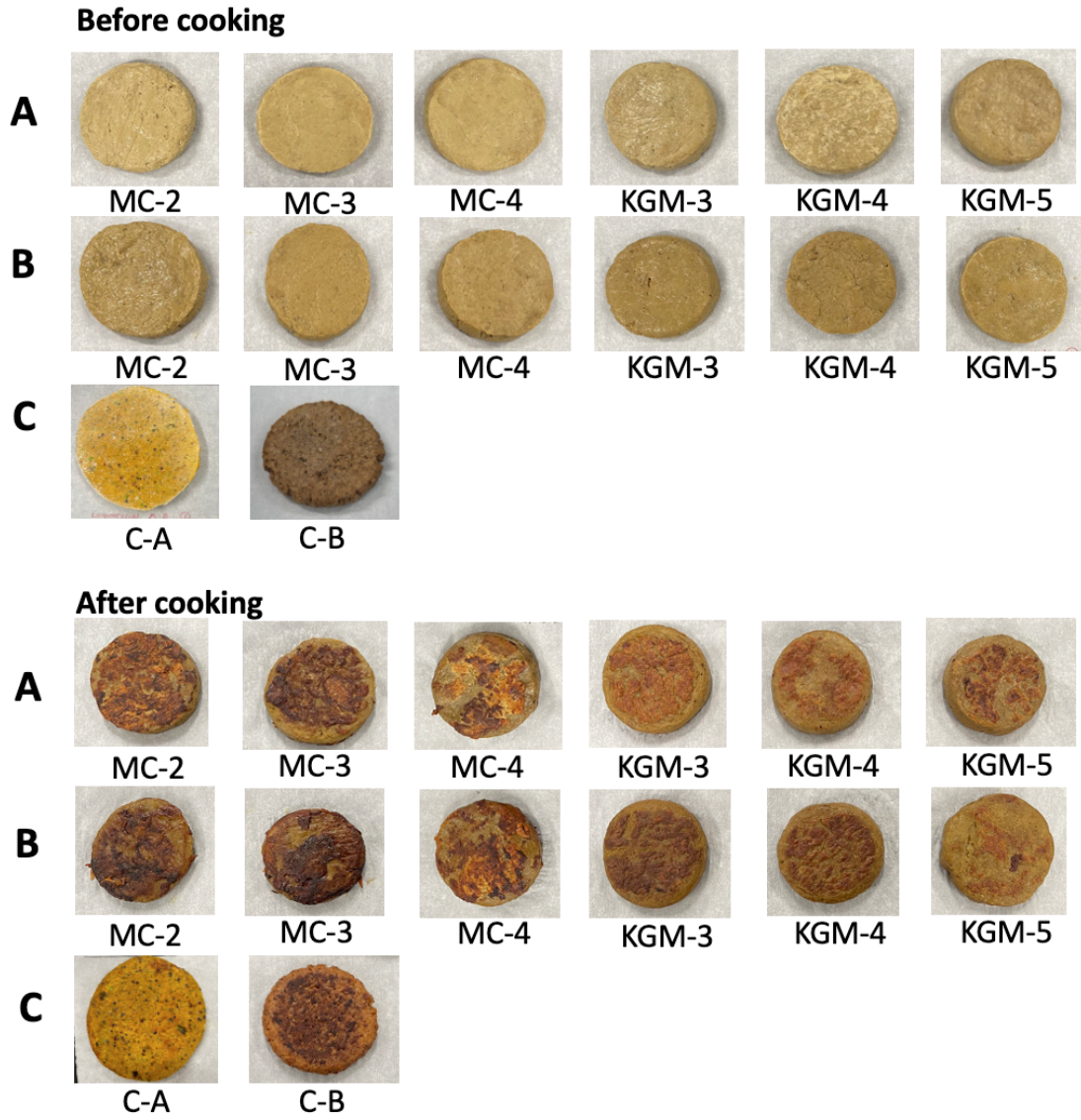


Figure 20. Visual appearances of seafood analogue patty with different protein ratios (w/w%) and concentrations of polysaccharides. A: patty with 75:25 (w/w%); B: patty with 50:50 (w/w%); C: commercial plant-based patty; MC-2: patty with 2% methylcellulose added; MC-3 patty with 3% methylcellulose added; MC-4: patty with 4% methylcellulose added; KGM-3: patty with 3% konjac glucomannan added; KGM-4: patty with 4% konjac glucomannan added; KGM-5: patty with 5% konjac glucomannan added; C-A: commercial plant-based patty A; C-B: commercial plant-based patty B.

4.3.2. Textural determination

The partial replacement of meat or seafood with plant proteins had the most significant impact on hardness and resilience (Santos et al., 2022). 100:0 protein ratios of FBPI to DMP, demonstrated the most pronounced values for these parameters, which can be attributed to heat denaturation. Denaturation in plant proteins involves the loss of their native structure, causing them to unravel into a more linear form. This can occur at different temperatures but is especially when cooking up to 80 °C (Santos et al., 2022).The findings correlate with earlier studies that have measured gel hardness and storage modulus at specific ratios. For example, the current study's finding that a 50:50 protein ratio of FBPI to DMP yields a higher hardness compared to a 75:25 protein ratio aligns with an earlier study that reported increased storage modulus and gel strength at similar ratios of protein. This suggests that the balanced interaction between FBPI and DMP at a 50:50 protein ratio (w/w%) optimizes the gel network, enhancing textural properties (**Chapter 3, Figure 15**). In this study, the control mixture's hardness was likely to be influenced by the protein ratios and the absence of polysaccharides. The differences in hardness among the various groups, marked by the letters (a and b), indicate that the protein ratios and the inclusion of polysaccharides significantly influence the textural attributes of the hybrid patties. This finding was consistent with earlier research that suggested the protein ratio was essential in determining both the hardness and the textural properties of the hybrid patties, based on measurements of gel hardness and storage modulus at specific protein ratios (**Chapter 3, Figure 15 and Figure 17**). The findings shown in **Figure 21** provide essential insights into the effects of protein ratios on the textural characteristics of the patties, improving the understanding of the ideal protein mixture for creating high-quality hybrid patties. Similarly, other studies have highlighted the significant influence of protein ratios on the textural properties of food products. Nie et al. (2024) found that combining muscle protein with pea protein in a hybrid protein system can produce a strong hybrid gel, even with lower salt concentrations. The hardness of the hybrid gel demonstrated a significant increase, reaching up to 24 times for the muscle gel, and 11-fold and 4-fold increases for hybrid gels with 25% and 50% pea protein substitution, respectively.

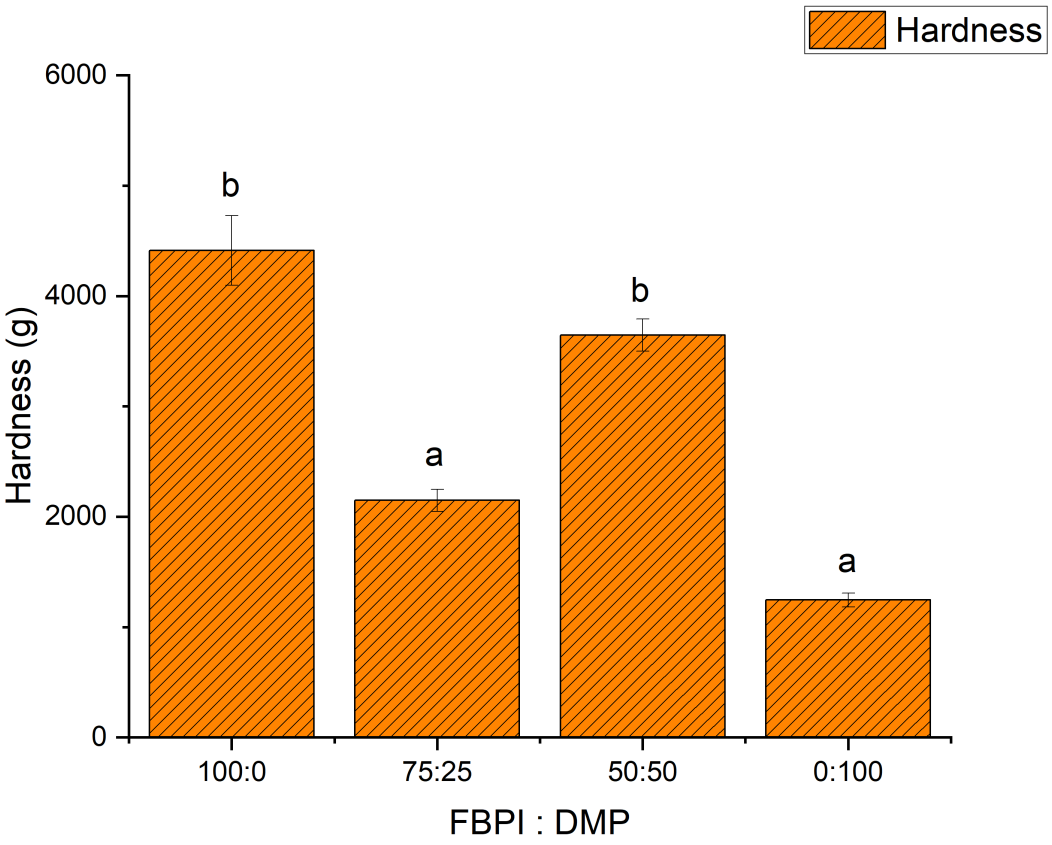


Figure 21. Hardness of seafood analogue patty with different protein ratios (w/w%) of FBPI and DMP without adding polysaccharides.

After conducting the control formulation, the study proceeded to focus on the 75:25 and 50:50 protein ratios of FBPI and DMP in combination with the incorporation of polysaccharides. **Figure 22** presents the hardness of the mixture with polysaccharides. The figure illustrated how the inclusion of polysaccharides altered the textural characteristics of the hybrid patties. The inclusion of different levels of polysaccharides, specifically methylcellulose (MC) and konjac glucomannan (KGM) allows for the evaluation of how these additives affect the hardness of the patties. The varying levels of polysaccharides, as indicated in **Table 8**, enable a comparative analysis of their influence on the textural attributes of the patties. Methyl cellulose appears to be more effective than KGM in increasing hardness. As both MC and KGM addition increased, the hardness significantly increased. Bakhsh et al. (2021) found that increase of methylcellulose concentration in their plant-based meat patty increases the hardness of all their patties. Moreover, increase in methylcellulose (MC) concentration from 2% to 4% was found to enhance the hardness of all patties. This observation was consistent with the findings that increasing the concentration of binding agents, such as carrageenan, soy protein concentrate, casein, and xanthan gum, led to a proportional rise in properties like hardness, chewiness, gumminess, and compression values in mushroom-based sausage analogues (Arora et al., 2017).

Methylcellulose (MC) was recognized for its ability to form strong gels upon heating, which significantly enhances the hardness and structural integrity of food products. This property likely explains why the patties with added MC exhibited a firmer texture compared to those with Konjac Glucomannan (KGM). Notably, the formulations containing MC at 4% concentrations demonstrated a hardness closely resembling that of commercial A patties. This similarity in texture suggests that MC is highly effective in replicating the desirable firmness found in commercially available products. A study by Yuliarti et al. (2023) found that MC enhances the hardness of meat analogs by forming a robust gel matrix that provides structural integrity. Additionally, as reported by Tunnarut et al. (2022), plant-based patties containing MC exhibited significantly greater hardness, springiness, cohesiveness, and chewiness than those made with κ-carrageenan and xanthan gum.

Moreover, previous research revealed that KGM functioned as a structuring agent, enhancing the textural and physicochemical qualities of fishball analogues made from Soy Protein Isolate (SPI). KGM was chosen for its exceptional gelling properties and ability to retain water, which were essential for replicating the texture and structural integrity of traditional fishballs (Ran et al., 2022). By comparing the hardness values of the patties with polysaccharides to the commercial standard, one can assess the extent to which the polysaccharide additions have influenced the textural properties. If the hardness values of the patties with polysaccharides closely align with the commercial standard, it suggests that the polysaccharide combinations have effectively enhanced the textural attributes, potentially achieving a texture comparable to commercial products.

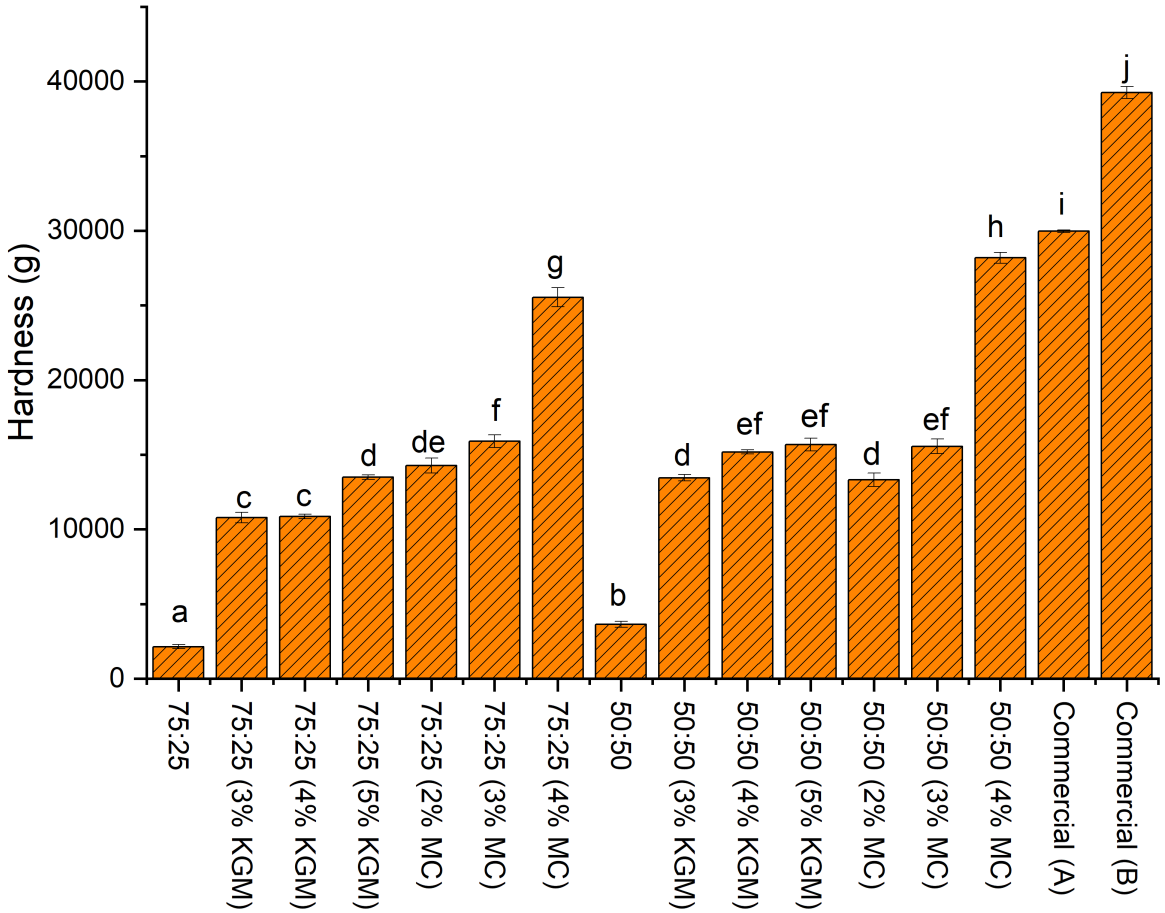


Figure 22. Hardness of different formulations involving FBPI and DMP with various concentrations of konjac glucomannan (KGM) and methyl cellulose (MC), compared to two commercial samples (A and B).

4.3.3. Color analysis

The study examined the color parameters of blended patties made from plant and seafood, using different ratios (w/w%) of FBPI to DMP. These patties were evaluated at a constant protein concentration of 15%, with and without the addition of polysaccharides. The objective was to assess how the proportions of different ingredients influenced the color attributes of the patties both before and after cooking. Color was a critical factor in consumer preference and served as a key indicator of quality that directly impacted consumer choice (Luo et al., 2017). The color characteristics were evaluated using L* to measure lightness, a* to represent the red-green axis, and b* for the yellow-blue axis, both prior to and following cooking. These measurements helped to determine the visual quality of the patties, which played a crucial role in consumer acceptance.
4o mini

Color was measured before cooking and after cooking for control formulations without polysaccharides (**Figure 23**). Generally, cooking reduces lightness and increases redness and yellowness, with the effects more pronounced in samples with higher DMP content. This was likely due to Maillard browning reactions and the natural pigments in DMP powder, which intensify upon cooking. The brownish color seen in the patties after cooking can be explained by the development of new pigments, specifically those produced by Maillard reactions occurring between proteins and carbohydrates on the surface of the patties (Zhou et al., 2022). These brown pigments decrease the light scattered from the patties, leading to a decrease in their overall lightness (L*).

Cooking increased redness (a*) in the patties, particularly in samples with a higher DMP content (0:100). This effect was attributed to the natural pigments in mussel powder, which intensified during cooking. Additionally, an increase in yellowness (b*) was observed in all samples after cooking, with the most pronounced effect seen in the 0:100 ratio. The intensity of the green color also increased as the DMP ratio in the formula rose, a finding consistent with Klunklin and Savage (2018) research, which demonstrated that incorporating green-lipped mussel enhances the greenness of bread samples.

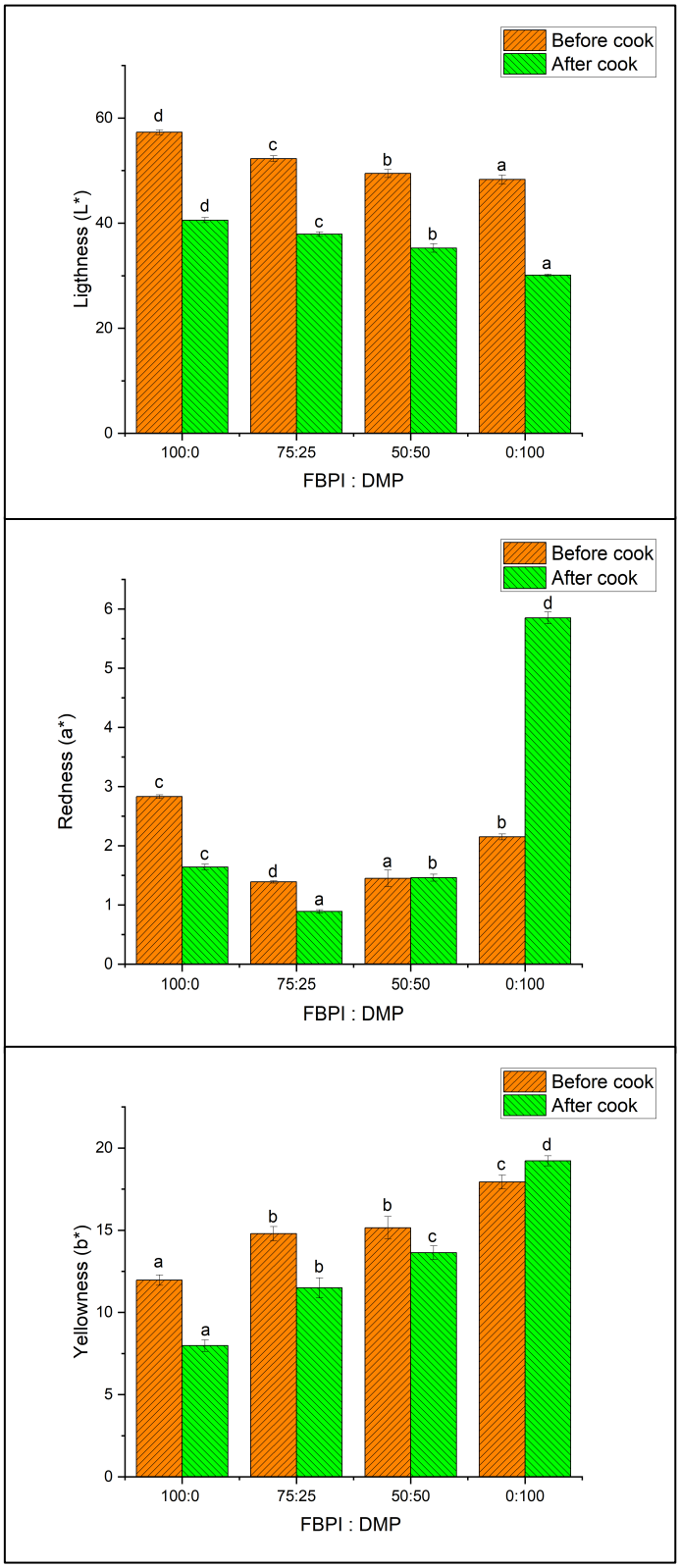


Figure 23. The color parameters (lightness, redness, and yellowness) of patties before and after cooking with varying protein ratios of FBPI to DMP without polysaccharides. A significant difference ($P < 0.05$) in varying protein ratios is shown by different letters above the column, respectively.

To elaborate further, **Table 9** displayed the color characteristics of plant and seafood blended patties formulated with different concentrations of polysaccharides. The polysaccharides used were KGM (konjac glucomannan) and MC (methylcellulose), which were added at different weight percentages (w/w%) to the patties. The concentrations ranged from 2% to 5% for KGM and from 3% to 4% for MC. The control patties (Ctrl) did not contain any polysaccharides. Additionally, the table included data for two commercial plant-based patties (A and B) for comparison. The table indicated that the color values varied significantly ($P \leq 0.05$) across different formulations, as shown by the superscript letters following the average values for each color parameter.

The data indicated that KGM was added to the patties at concentrations of 3%, 4%, and 5%, affecting their color characteristics compared to the control patties without polysaccharides. Before cooking, the KGM-containing patties had lower L* values, meaning they appeared darker than the control, likely due to the color of KGM or its interaction with other ingredients. As the concentration of KGM increased from 3% to 5%, there was a general trend of decreasing L* values, indicating that higher levels of KGM led to darker patties. This could be due to the increased amount of KGM absorbing lighter or changing the way light was scattered within the patty matrix. This finding was in agreement with the results of Huang et al. (2023), who discovered that increased KGM content resulted in lower L* values in cubic fat substitutes. Their study on pork balls also indicated that a higher KGM gel ratio led to a decrease in L* values.

The highest lightness values were observed before cooking across all formulations. The lightness (L*) of the samples is significantly higher before cooking than after cooking. Among the untreated formulations, 75:25 has the highest lightness both before and after cooking. The findings also revealed that adding KGM and MC impacted the lightness and redness of the patties, with specific ratios and types of additives significantly impacting the color of the samples before and after cooking. Additives like KGM and MC influence the lightness, especially with 3% KGM in the 75:25 formulation maintaining the highest lightness after cooking.

As the concentration of MC increased, the L* value of the patties rise. This was because the transparent characteristics of the MC colloidal gel allowed for greater light refraction. The gel became more prominent within the meat patty matrix, permitting more light to pass through and resulting in a higher L* value, which indicates a lighter color (Sothornvit, 2021).

Then, the redness (a*) increases after cooking suggesting that Maillard reaction or caramelization occurred. The highest redness values were generally observed after cooking across most formulations. An increase in the a* value was observed as the levels of KGM and MC substitution increased. The trend shows that formulations with KGM additives tend to have higher redness values after cooking compared to those with MC additives but no clear difference was observed between the different levels for each concentration.

The increases in the b* value were linked to the presence of FBPI and DMP, which imparted a yellow hue to the patties. In shellfish, color changes are frequently a result of complex reactions, including protein denaturation, lipid oxidation, and the Maillard reaction. Specifically, an increase in the b* value, indicating a shift toward yellow, was linked to lipid oxidation in seafood. This process led to the formation of compounds that absorb light in the blue region of the spectrum, thus making the food appear more yellow (Thanonkaew et al., 2006).

The color of meat or seafood analogues was significantly influenced by the raw materials and their moisture content, as well as the chemical reactions that took place during cooking, including water loss, protein denaturation, Maillard reactions, and pigment breakdown, all of which contributed to modifications in the final color of the product (Zhou et al., 2022).

Table 9. Color characteristics of plant and seafood blended patties with different concentrations of polysaccharides. Means with different superscript letters in the same row were significantly different at $P \leq 0.05$.

Ingredient (w/w%)	**75:25**							**50:50**							**C. A**	**C. B**
Con. (w/w%)	**Ctrl**	**KGM 3%**	**KGM 4%**	**KGM 5%**	**MC 2%**	**MC 3%**	**MC 4%**	**Ctrl**	**KGM 3%**	**KGM 4%**	**KGM 5%**	**MC 2%**	**MC 3%**	**MC 4%**		
L*before cooking	52.8 ± 0.30 ^{j}	42.95 ± 0.62 ^{cde}	47.56 ± 0.39 ^{g}	49.94 ± 0.26 ^{i}	42.12 ± 0.10 ^{c}	45.57 ± 0.29 ^{f}	47.81 ± 0.42 ^{h}	49.45 ± 0.60 ^{hi}	42.91 ± 0.20 ^{cde}	43.45 ± 1.03 ^{cde}	43.93 ± 0.25 ^{de}	35.38 ± 0.35 ^{a}	36.68 ± 0.61 ^{ab}	37.78 ± 0.27 ^{b}	42.4 ± 1.25 ^{cd}	44.53 ± 0.94 ^{ef}
a* before cooking	1.39 ± 0.20 ^{a}	5.97 ± 0.32 ^{ef}	5.46 ± 0.40 ^{de}	3.35 ± 0.18 ^{b}	5.39 ± 0.40 ^{de}	6.01 ± 0.20 ^{ef}	4.79 ± 0.08 ^{cd}	1.45 ± 0.14 ^{a}	4.12 ± 0.13 ^{c}	4.31 ± 0.47 ^{c}	6.94 ± 0.40 ^{gh}	7.21 ± 0.90 ^{h}	6.82 ± 0.16^{gh}	6.34 ± 0.56 ^{fg}	5.66 ± 0.29 ^{ef}	7.99 ± 0.14 ^{i}
b* before cooking	14.79 ± 0.30 ^{j}	6.52 ± 0.93^{abcd}	8.82 ± 0.71 ^{fg}	8.78 ± 0.20 ^{fg}	7.11 ± 0.20 ^{cde}	6.05 ± 0.10 ^{ab}	10.06 ± 0.11 ^{h}	15.15 ± 0.68 ^{j}	7.89 ± 0.14 ^{ef}	9.19 ± 0.15 ^{gh}	6.39 ± 0.10 ^{abc}	7.53 ± 0.13 ^{cd}	8.79 ± 0.23 ^{fg}	6.89 ± 0.19 ^{bcde}	5.66 ± 0.59 ^{a}	13.67 ± 0.28 ^{i}
L* after cooking	37.91 ± 0.40^{cd}	39.58 ± 0.58 ^{de}	41.02 ± 0.98 ^{ef}	43.91 ± 1.74 ^{g}	36.42 ± 0.10 ^{abc}	37.40 ± 0.15 ^{bcd}	44.53 ± 0.50 ^{g}	35.26 ± 0.62 ^{ab}	36.07 ± 0.85 ^{abc}	37.95 ± 0.21 ^{cd}	37.99 ± 1.89 ^{cd}	34.61 ± 0.29 ^{a}	34.61 ± 0.29 ^{a}	37.12 ± 0.60 ^{bc}	40.52 ± 0.91 ^{ef}	42.65 ± 0.63 ^{fg}

a* after cooking	0.89 ± 0.30 ^{a}	5.88 ± 0.52 ^{bcd}	6.54 ± 0.17 ^{bcdef}	5.40 ± 1.53 ^{bc}	7.80 ± 0.15 ^{def}	7.73 ± 0.16 ^{def}	7.01 ± 0.30 ^{cdef}	1.46 ± 0.60 ^{a}	8.46 ± 0.14 ^{i}	6.30 ± 0.31 ^{bcde}	6.63 ± 1.89 ^{bcdef}	8.35 ± 0.30 ^{ef}	8.32 ± 0.31 ^{ef}	5.63 ± 0.60 ^{bc}	4.82 ± 1.02 ^{b}	6.36 ± 0.46 ^{bcde}
b* after cooking	11.50 ± 0.10 ^{a}	16.05 ± 0.62 ^{de}	16.09 ± 0.20 ^{de}	17.32 ± 0.30 ^{e}	11.79 ± 0.24 ^{a}	14.34 ± 0.32 ^{bc}	18.89 ± 0.16 ^{f}	13.63 ± 0.42 ^{b}	15.63 ± 0.47 ^{cd}	13.22 ± 0.16 ^{b}	14.46 ± 0.87 ^{bc}	11.07 ± 0.40 ^{a}	11.04 ± 0.40 ^{a}	13.36 ± 0.17 ^{b}	11.00 ± 0.45 ^{a}	17.31 ± 0.76 ^{e}

*Average ± Standard deviation

L*: Lightness; a*: red-green; b*: yellow-blue; Con: concentration; Ctrl : control patties without polysaccharides; C.A : commercial plant based patty A; C.B : commercial plant based patty B.

4.3.4. Cooking loss analysis

The denaturation and contraction of myofibrillar proteins during cooking meat or seafood lead to the release of liquids, including drippings and volatile compounds. This phenomenon, referred to as cooking loss, had a substantial impact on yield and was strongly associated with the sensory attributes of meat products, such as juiciness and tenderness, along with other essential qualities (Cao et al., 2016). In this study, the weight change of the patties before and after cooking was measured to evaluate the effects of various protein ratios and polysaccharide additions on the cooking characteristics of blended patties.

The results related to cooking loss for the samples containing 3, 4, and 5 (w/w%) KGM, along with 2, 3, and 4 (w/w%) MC, were presented in **Figure 24**. The figure demonstrated that the concentration of both konjac glucomannan (KGM) and methylcellulose (MC) significantly affected cooking loss. Specifically, the inclusion of these polysaccharides, particularly MC, reduced cooking loss by enhancing water-holding capacity and gelation properties. The study also pointed out that increasing the KGM content improved the water retention and cooking qualities of the patties, since KGM was effective in holding and absorbing moisture.

Additionally, the results demonstrated that the control sample exhibited significantly higher cooking loss compared to those containing polysaccharides, indicating the effectiveness of polysaccharides in minimizing cooking loss. These findings aligned with studies by Xie et al. (2022) and Bohrer (2019), which have also highlighted the role of polysaccharides in reducing cooking loss and improving the texture of plant-based meat substitutes. Additionally, the study observed that the specific ratios and types of additives significantly impacted the cooking loss of the samples, providing valuable insights into the optimal protein mixture for the development of high-quality analogue patties. The underlying mechanism for these observations can be connected to the interactions between polysaccharides and proteins in plant-based meat alternatives. These interactions likely contribute to improved water retention and a more stable gel network, which helps to minimize moisture loss during cooking. Polysaccharides, such as starches and fibres, can form a matrix that helps to retain moisture during cooking. This matrix acts as a barrier, reducing the amount of liquid that can be expelled from the product. This approach helps preserve the juiciness and tenderness of the hybrid patties (Yang et al., 2020).

The figure indicated that lower cooking loss resulted from increasing the KGM concentration from 3% to 5% in both the 75:25 and 50:50 formulations. This showed that KGM effectively improved the water retention and cooking quality of the patties. This effect can be attributed to KGM's high water-holding capacity, allowing it to absorb substantial moisture. By forming a gel-like network within the plant-based meat matrix, KGM served as a barrier that minimized moisture loss during cooking (Zhuang et al., 2021). The study found that the 50:50 formulation with the addition of methyl cellulose (MC) demonstrated a cooking loss that closely resembled that of the commercial sample. This indicates that the incorporation of MC in the 50:50 formulation effectively reduced cooking loss, resulting in a cooking loss profile similar to the commercial sample. The addition of MC enhanced the texture and reduced cooking loss by improving water-holding capacity and gelation properties, contributing to the similarity in cooking loss between the 50:50 formulation with MC and the commercial sample.

Moreover, it can be seen clearly that when polysaccharides were present, cooking loss decreased compared to the control without added polysaccharides. This decrease in cooking loss happens as polysaccharides interact with proteins to establish a more cohesive network, which acts as a barrier to prevent water loss and enhances the hardness of the gel (Kazemi-Taskooh & Varidi, 2023). The study found a relationship between cooking loss and the hardness of the patties, as indicated by the observed trend in **Figure 22** and **Figure 24**. As the polysaccharide concentration increased, a reduction in cooking loss was observed, leading to a related increase in the hardness of the samples.

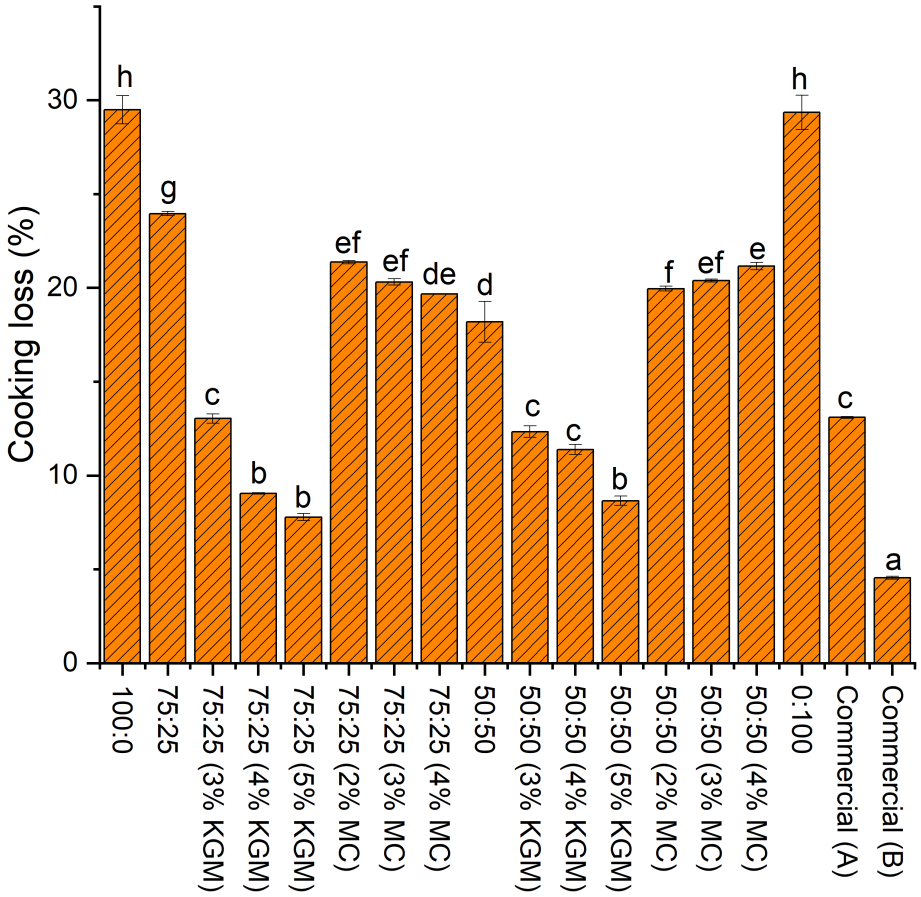


Figure 24. The cooking loss (%) of various samples includes different formulations and additives, as well as commercial samples labeled as A and B. Different letter indicate statistically significant differences between the samples.

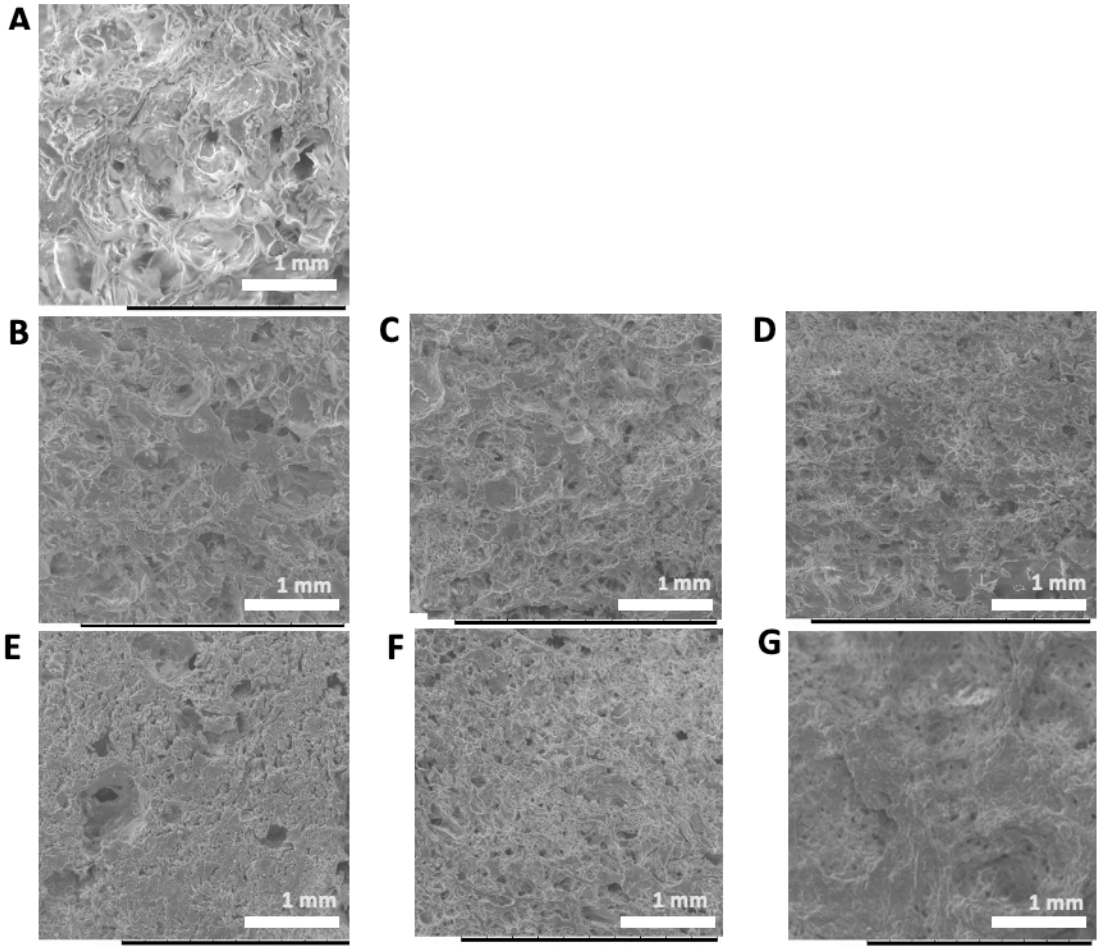
4.3.5. Microstructure

SEM proved to be an effective technique for analysing the structural composition of food, providing detailed insights into the configuration of its texture and revealing the relationships between physical properties. SEM enabled the detailed visualization of food samples' surface topography and microstructure at high magnification, offering valuable information about their physical characteristics. (Tan et al., 2024). **Figure 25** illustrates the microstructure of the cooked seafood analogue patties. For both ratios, increasing the KGM content seems to make the structure more cohesive. This could be due to KGM's gelling properties, which might help in binding the components more effectively, leading to a denser and more uniform microstructure. KGM, being a highly viscous polysaccharide, forms a gel-like structure when hydrated, filling in the gaps between protein and other particles, creating a smoother, denser matrix and reducing pore size (Ren et al., 2024).

Similar to KGM, the addition of MC also resulted in a more cohesive structure. MC was known for its gel-forming ability and stabilizing properties, which likely contributed to the observed improvements in the microstructure of the plant-based meat matrix, enhancing its texture and integrity during processing and cooking (Meng et al., 2018). The research revealed that higher levels of KGM led to greater structural cohesiveness, likely because KGM's gelling properties enhanced the binding of components, leading to a denser and more uniform microstructure. On the other hand, the addition of MC also results in a more homogeneous and compact structure, reducing pore size and surface roughness. The study also found that the presence of both KGM and MC enhanced the binding of ingredients, causing a more porous and less cohesive texture when these polysaccharides were absent. Overall, the addition of KGM and MC at varying concentrations influences the microstructure of the cooked patties, resulting in changes in texture and consistency. In plant-based meat analogues, the incorporation of MC has been shown to enhance the binding of ingredients, contributing to better texture and consistency (Bakhsh, Lee, Lee, Sabikun, et al., 2021).

The base patties (A and H) demonstrated distinct textural variations, attributable to the differing protein ratios of the base ingredients. Specifically, the patty with a 75:25 protein ratio exhibited a more irregular structure, while the patty with a 50:50 protein ratio presented a more uniform texture. The differences noted can be attributed to the gel-forming and water-binding properties of KGM and MC. Without these additives, the mixture of FBPI and DMP did not

have additional structural support, resulting in a more porous and less cohesive texture.



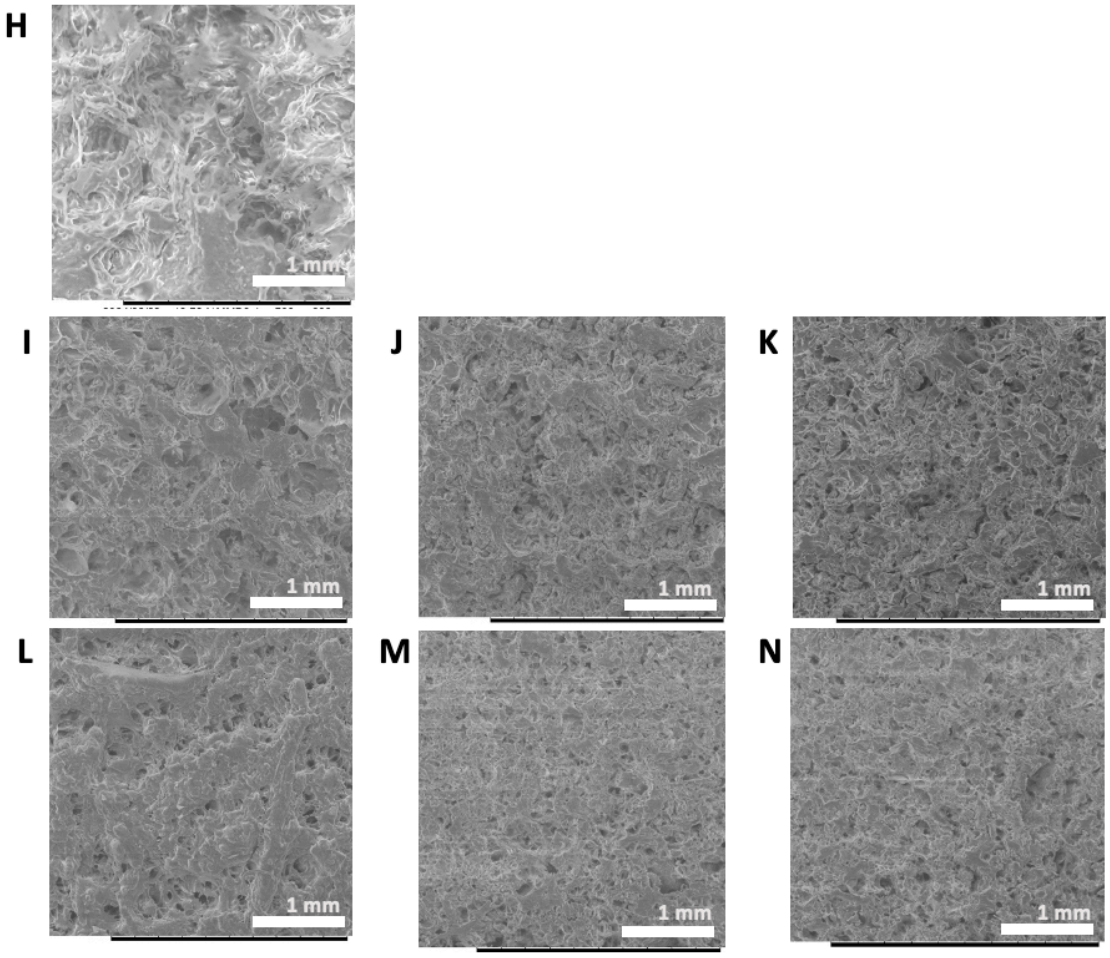


Figure 25. SEM images; (A) patty with only 75:25, (B) 75:25 with 3% KGM, (C) 75:25 with 4% KGM, (D) 75:25 with 5% KGM, (E) 75:25 with 2% MC, (F) 75:25 with 3% MC, (G) 75:25 with 4% MC, (H) patty with only 50:50, (I) 50:50 with 3% KGM, (J) 50:50 with 4% KGM , (K) 50:50 with 5% KGM, (L) 50:50 with 2% MC, (M) 50:50 with 3% MC and (N) 50:50 with 4% MC.

4.4. Conclusion

This study was focused on optimizing protein-polysaccharide mixtures for the concept development of seafood analogue patties using a combination of FBPI and DMP. The research was aimed to examine the effects of different protein ratios and polysaccharide additions on the texture, color, and physicochemical characteristics of the patties. The study utilized various analytical methods, including texture profile analysis, color measurement, cooking loss analysis, and microstructure analysis, to assess the effects of different protein ratios and polysaccharide additions. The results showed that incorporating polysaccharides enhanced the texture of the patties, reduced cooking loss, and enhanced shape retention and structural integrity after cooking. The color analysis revealed changes in color attributes before and after cooking, with higher DMP content leading to increased redness and yellowness. The microstructure analysis demonstrated that polysaccharides created a denser and more uniform matrix within the patties, enhancing their overall quality. Additionally, the study found that the concentration of both KGM and MC notably influenced cooking loss, with the inclusion of MC demonstrating a reduction in cooking loss by enhancing water-holding capacity and gelation properties. Additionally, increasing KGM content enhanced the water retention ability and cooking attributes of the patties. Adding polysaccharides, particularly MC and KGM, had a substantial impact on the texture, color, and physicochemical characteristics of the blended patties.

The findings may provide valuable insights for the food industry in formulating sustainable and nutritious meat alternatives that meet consumer expectations for nutrition, taste, texture, and visual appeal. By identifying the optimal protein ratio and polysaccharide combination, this research seeks to enhance the development of high-quality blended patties. The findings of the study were consistent with earlier research, emphasizing how polysaccharides helped to minimize cooking loss and enhance the texture of plant-based meat patties. In summary, the study's detailed analysis of the effects of protein ratios and polysaccharide additions on the characteristics of plant and seafood blended patties marks it as an important advancement in the future creation of eco-friendly and nutritious meat substitutes.

5.0. Chapter 5: Conclusion and future works

This study investigates the development of blended seafood products incorporating faba bean protein isolate (FBPI) and defatted green-lipped mussel powder (DMP). It investigates the unique gelation properties of these two protein sources and how they can be combined to replicate the texture and functional qualities of conventional protein-rich processed foods. The study aims to create a sustainable and nutritious alternative to conventional meat or seafood products by leveraging the health benefits of both plant-based and animal-derived proteins. This strategy not only addresses the growing consumer preference for eco-friendly food choices but also helps to lower the overall environmental footprint of food production.

It is mainly divided into two parts; the first part of the study is about characterization of protein gels formed from varying proportions of faba bean protein isolate (FBPI) and defatted green-lipped mussel powder (DMP), exploring their microstructural and rheological properties. This part helps us understand how these proteins work together to make the gels. The second section of the study concentrates on the practical implementation of using FBPI, DMP and polysaccharides to create seafood analogue patties. It aims to find the best combination of these ingredients to develop a product that replicates the texture of commercial patties.

The study investigated the impact of varying ratios of FBPI and DMP on the formation and characteristics of protein gels. The study found that the storage modulus (G') increased with rising temperature for all samples, indicating a gel formation. The findings indicated that a protein ratio of 50:50, meaning an equal mixture of FBPI and DMP resulted in the strongest gel, with improved water-holding capacity compared to gels made from either protein alone. As the proportion of DMP increased, the WHC decreased, and the gel became less firm. Smaller particles were linked to a denser gel network and higher storage modulus values. The color of the gels became redder and yellower with the incorporation of DMP, and heating reduced the overall lightness. CLSM analysis showed that FBPI formed a denser gel structure than DMP. The study suggests that blending FBPI and DMP in a 50:50 ratio could be beneficial for developing nutritious and sustainable food products with desirable functional properties.

In addition, the study explored different protein ratios and polysaccharide concentrations. The protein ratios evaluated were 100:0, 75:25, 50:50, and 0:100 (w/w%), with the 50:50 ratio being identified as optimal for WHC and gel strength in the previous study. The polysaccharide concentrations used in the study were 3, 4, and 5 (w/w%) for KGM, and 2, 3, and 4 (w/w%) for MC. The findings indicated that higher concentrations of both KGM and MC contribute to better shape retention and structural integrity after cooking. Specifically, the 50:50 (w/w%) protein ratio generally showed better structural integrity and shape retention compared to the 75:25 (w/w%), especially at higher concentrations of KGM and MC. The study also observed that the addition of MC was particularly effective in reducing cooking loss, and improving water-holding capacity, and gelation properties. Increasing KGM content was found to enhance the water retention ability and cooking attributes of the patties.

According to the results of this study, the future direction is proposed as below:

- Future studies should explore how pH and salt (e.g. NaCl, $CaCl_2$) concentrations affect the functional properties of the FBPI and DMP gels. This could include their influence on gelation, texture and overall nutritional value.

- Furthermore, future studies should explore the impact of different processing methods, such as high pressure and sonication, on enhancing the gelation properties of these protein ingredients.

- The study focused on optimizing protein-polysaccharide mixtures for the development of seafood analogue patties using FBPI and DMP, with the incorporation of polysaccharides konjac glucomannan (KGM) and methylcellulose (MC). The study utilized various analytical methods, including texture profile analysis, color measurement, cooking loss analysis, and microstructure analysis. Future research should conduct comprehensive sensory analysis to assess the organoleptic properties of the patties, including taste, aroma, and overall acceptability, to ensure they meet consumer preferences.

- Additionally, microbial analysis should be conducted to ensure food safety, addressing any potential risks associated with the patties' production and storage.

- Moreover, exploring the development of other products from these ingredients, such as seafood-flavored snacks or protein-enriched sauces, could expand the range of sustainable food options available to consumers.

- Conducted in vitro protein digestibility experiments to evaluate and compare the digestibility of various samples, offering valuable insights into the nutritional quality and absorption characteristics of the patties.

6.0. References

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7.0. Appendix

Appendix A

Figure 1. FBPI used in this study.

Figure 2. DMP used in this study

Appendix B

Table 1. Amino Acids Composition of FBPI and DMP mixtures.

AMINO ACIDS mg/100mg protein	FBPI : DMP			
	100 : 0	**75 : 25**	**50 : 50**	**0 : 100**
Aspartic Acid	11.87	11.14	10.42	8.96
Threonine	3.87	3.82	3.76	3.65
Serine	5.16	4.69	4.23	3.31
Glutamic Acid	16.81	14.69	12.58	8.35
Proline	4.62	4.16	3.71	2.79
Glycine	4.48	5.19	5.90	7.33
Alanine	4.34	4.04	3.76	3.17
Valine	5.14	4.76	4.38	3.63
Methionine**	0.95	0.71	0.48	-
Isoleucine	4.33	4.05	3.78	3.23
Leucine	7.99	7.08	6.17	4.35
Tyrosine	3.98	3.68	3.39	2.81
Phenylalanine	4.56	4.28	3.90	3.44
Histidine	2.42	2.13	1.85	1.29
Lysine	6.88	6.71	6.56	6.23
Arginine	9.53	8.51	7.49	5.46
Taurine	-	1.40	2.80	5.60
Cysteine	-	0.29	0.58	1.15
Methionine***	-	0.57	1.15	2.29

Methionine** refers to the amino acid profile includes cysteine and methionine, with the methionine result coming from performic acid oxidation.

Methionine*** refers to the amino acid profile using HCl hydrolysis, however, there may be about a 10% loss due to the hydrolysis process.

Appendix C

Ingredients of Commercial Products

Product A Ingredients

The following is the ingredients used in Commercial Product A:

Water, plant based proteins (wheat, soy, pea, color (caramel)), canola oil, thickeners (methylcellulose, guar gum, xanthan gum), flavor, yeast extracts, maize starch, yeast, maltodextrin (maize),salt, vegetables fibres, sugar, hydrolysed vegetable protein (maize), onion, garlic, smoke flavor, food acid, pepper, mineral zinc, vitamin (b12.)

Product B Ingredients

The following is the ingredients used in Commercial Product B:

Water, vegetable protein (soy, wheat), vegetable oil, oats, onion, seasoning (soy, wheat, barley), starch (tapioca, potato), vegetable fibre, yeast extract, natural flavor, vegetables gum (carrageenan), natural color (caramel), garlic, mineral (zinc,iron), spices, vitamin (B12).

Appendix D

An overview of classifications, properties of food polysaccharides and their links to applications in improving food textures
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